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SYSTEMIC FEATURES OF IMMUNE RECOGNITION

Bartlomiej Swiatczak

IFOM-IEO Campus, Milan

Matricola n. R07404

Supervisor: Prof. Mark Bedau

Visiting Professor

IFOM-IEO Campus, Milan

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Table of contents

List of Abbreviations

AC	Adenylyl cyclase
AID	Activation induced cytidine deaminase (AID).
APC	Antigen presenting cell
ATG16L1	Autophagy-related protein 16-1
APRIL	A proliferation-inducing ligand
ATP	Adenosine-5'-triphosphate
BAFF	B-cell activating factor
CAMP	Commensal-associated molecular pattern
CCL20	Chemokine (C-C motif) ligand 20
CD	Crohn's disease
CS	Conditioned stimulus
DAMP	Damage-associated molecular patterns
DC	Dendritic cell
ECP	*E. coli* common pilus
EGF	Epidermal growth factor
ERK1/2	Extracellular signal-regulated kinase 1/2
FimH	FimH adhesin (a component of type 1 fimbriae)
Foxp3	Forkhead box P3
GALT	Gut associated lymphoid tissue
GRR	General Reduction-Replacement Model
HNF1α	Human hepatocyte nuclear factor 1 α
HSP	Heat-shock protein

hTM5	Human tropomyosin isoform 5
IBD	Inflammatory bowel disease
IEC	Intestinal epithelial cell
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
IκB	Inhibitor of NF-κB kinase
IRGM	Immunity-related GTPase family, M
LPS	Lipopolysaccharide
M-cell	Microfold cell
MAPK	Mitogen-activated protein kinase
MAMP	Microbe associated molecular patterns
MDP	Muramyl-dipeptide
MHC	Major histocompatibility complex
MyD88	Myeloid differentiation primary response gene 88
NFκB	Nuclear factor kappa B
NLR	NOD-like receptor
NOD	Nucleotide-binding oligomerization domain
PAMP	Pathogen-associated molecular pattern
PGE2	Prostaglandin E2
PGN	Peptidoglycan
PKA	Protein kinase A
PP	Payer's patches
PPARγ	Peroxisome proliferator-activated receptor-γ
PRR	Pattern recognition receptor
SCFA	Short chain fatty acids

SFB	Segmented filamentous bacteria
STAT3	Signal transducer and activator of transcription 3
T3SS	Type III secretion system
TCT	Tracheal cytotoxin
TdT	Deoxynucleotidyl transferase
TGF-β	Transforming growth factor β
Th1 cell	T helper 1 cell
Th17 cell	T helper 17 cell
TLR	Toll-like receptor
TNF-α	Tumor necrosis factor α
Treg cell	Regulatory T-cell
TSLP	Thymic stromal lymphopoietin
UC	Ulcerative colitis
US	Unconditioned stimulus
UPR	Unfolded protein response
XBP1	X-box binding protein 1

Figure index

Author's Declaration

Some of the material in this thesis has been previously published in the following papers:

- Swiatczak B., Rescigno M., Cohen I.R. 2011, Systemic Features of Immune Recognition in the Gut. *Microbes and Infection*, forthcoming, doi:10.1016/j.micinf.2011.06.011
- Swiatczak, B. 2011, Conscious Representations. An Intractable Problem for the Computational Theory of Mind. *Minds and Machines* 21: 19-32
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Fragments of the part 2 have been included in a recently submitted paper:

- Swiatczak B., Cohen I.R., Rescigno M., Pathogenesis of chronic immune misconduct in the gut (in review).

All of the work presented in this thesis describes my original contributions, except otherwise stated and referenced.

Abstract

The gut is home to a great number of microbes. The immune system, to protect the body, must discriminate between the pathogenic and non-pathogenic microbes and respond to them in different ways. How the mucosal immune system manages to make this distinction is poorly understood. Here, we explore whether the decision to respond in a certain way to a microorganism is made by single types of cells and molecules or by the collective activity of various kinds of cells and molecules in a given anatomical compartment. As we shall see, single types of cells and single types of receptors can recognize and become activated by motifs common to both pathogenic and non-pathogenic agents. Indeed, we show here that the distinction between pathogenic and non-pathogenic microbes is made by an integrated system rather than by single types of cells or single types of receptors.

Since immune recognition is constituted by a complex network of molecular and cellular level interactions, complete understanding of this process requires knowledge of these interactions. However, is it possible to explain immune recognition in molecular and cellular terms if this process is multiple realizable? Indeed, given the number and dynamics of elements involved in the recognition, it is hardly possible that their exact configuration could ever be reproduced even in the same individual.

We argue that it is practically impossible to reduce immune recognition to its *actual* molecular and cellular realization. (This would require making reference to an infinitely long disjunction of lower level processes and each disjunct would be endlessly complex). Instead, the recognition is reducible to the *approximation* of molecular and cellular level processes. We suggest that the same strategy that was used by us to explain immune recognition in terms of lower level approximations is commonly applied by molecular biologists and systems biologists to explain complex biological processes.

Introduction

In this thesis I investigate the problem of immune recognition in the gut and try to understand if this process can be explained, reductively, in terms of constituent cells and molecules and their interactions. In this way I hope to shed light on certain aspects of explanatory practice in systems biology and immunology.

The thesis consists of three parts:

- In the first part, I propose that the immune system distinguishes a pathogen from a non-pathogen through the integration of "detection modules" that measure a number of parameters, such as the structure of the microbe, its position, alterations in the environment and the state of the host. I discuss the fact that single modules, such as recognition of microbial patterns, are not sufficient to discriminate pathogens from non-pathogens, as both types of microbe share many structures.
- In the second part, an attempt is made to explain how defects in the complex recognition system described in the first part of the thesis can lead to chronic immune misrecognition in the form of inflammatory bowel disease (IBD). I suggest that IBD almost always involves coincident alterations in several detection modules because the architecture of immune recognition is robust and alterations in one detection module are often compensated by the activity of other modules.
- In the third part of the thesis I try to understand if an explanation of immune recognition in terms its molecular and cellular parts and their complex interactions can succeed given heterogeneity of lower level realizers. There is a sense in which our explanation in terms of constituent modules and molecular and cellular parts of these modules can be considered as reductive. I suggest that one of the limitations

of this reductionist approach is that it is based on approximations of the molecular and cellular level processes rather than representations of the actual goings-on.

Immune recognition

According to the present conceptual framework in immunology, an immune reaction develops in three stages: recognition, decision making (tuning of the response) and the effector response. Although partially overlapping, each of these three steps involves characteristic cells in particular anatomical compartments (Pulendran et al. 2001). The ideas of "immune recognition", "decision making" and "response" are metaphors (Tauber 1997), but in non-metaphorical terms they refer to the early, intermediate and late stages of immune activation in reaction to different kinds of antigens or microorganisms. Each stage is believed to determine the following stage. Consequently, immune recognition can be identified with initial calibration of the immune response to an antigen or a microorganism. From this point of view, studies of immune recognition are in fact identical to studies of *determinants* of immune system response. Therefore, when we ask the question "how does the intestinal immune system recognize pathogenic bacteria?" we are really asking what determines that such-and-such responses are induced against these bacteria.

It has been proposed that the determinants of the immune response are single types of cells and single types of receptors: epithelial cells, macrophages, dendritic cells, neutrophils, Toll-like receptors (TLRs), Nucleotide-binding oligomerization domain-like receptors (NOD-like receptors) or antigen receptors of B-cells and T-cells[1]. We contrast this classical reductionist view with a systemic view, according to which immune

[1] For example, it is said that "Toll-like receptors (TLRs) expressed on immune cells trigger inflammatory responses" (Szajnik et al. 2009, p. 4353) or "[E]pithelial cells elicit inflammatory responses only against pathogenic bacteria that invade into the basolateral compartment from the apical side" (Takeda et al. 2003, p. 350) or "[M]ucosal [dendritic cells] DCs elicit innate effector responses that lead to secretion of distinct patterns of cytokines" (Iwasaki 2007, p. 399).

responses reflect the actual state of the immune system in a given environmental niche rather than being determined by single types of cells or molecules.

In general, if the immune response towards a microorganism is non-inflammatory, it is said that the microorganism is recognized by the immune system as safe. If the immune response is inflammatory, it is said that the microorganism is recognized by the immune system as pathogenic (Akira et al. 2006). By studies of immune recognition, we mean an attempt to understand the cellular and molecular mechanisms leading to inflammatory or non-inflammatory responses to different kinds of stimuli. There are potentially two types of mechanisms that could be involved in immune recognition:

1. A single type of receptor or a single type of cell performs immune recognition of a microorganism as pathogenic or safe. In this case, understanding immune recognition would rely upon studies of receptor activation or cell activation.
2. Immune recognition of a microorganism as pathogenic or safe is performed by an integrated activity of molecules, receptors and cells in their environmental context. Thus understood, immune recognition cannot be explained at the level of single cells and molecules and instead requires reference to the joint action of cells and molecules.

In the thesis an attempt is made to understand which of these two interpretations correctly represents the actual mechanism of immune recognition. Do single types of cells and receptors perform the recognition or is it performed by the *whole* immune system in a given context? Single types of cells and receptors have been proposed to act as sentinels: "Sentinel cells, including epithelial cells, luminal macrophages, and intraepithelial dendritic cells, continuously sense the environment and coordinate defenses for the protection of mucosal tissues" (Rumbo et al. 2004, p. 16). The sentinel concept implies that

certain types of immune cells discriminate between “friends” and “foes”. If a microorganism is pathogenic, it is recognized as being so first by epithelial cells, then by dendritic cells, then by macrophages, then by neutrophils and finally by B-cells and T-cells. In other words, the recognition of a given microbe is performed numerous times by numerous cells. But do immune cells really behave as solitary sentinels?

We shall argue that it is necessary to zoom out to larger scales to understand immune recognition. In everyday contexts, zooming out means getting visual access to a broader area. The scale of the perceived objects is no longer in millimeters but in meters or even kilometers. This look from a distance often entails loss of attention to many details of the visual field. This is not the kind of zooming out we would like to achieve to understand immune recognition. We would like to zoom out without losing track of molecular and cellular details. Knowledge of these details and their role in the broader picture is needed to design molecular agents that could restore immune recognition to health. Thus, what we are looking for is a *high resolution* picture of the *broad physiological area* involved in the calibration of an immune response. The higher the resolution and the broader the picture the better the understanding of the recognition.

PART I

Immune recognition as an integrated activity of cells and molecules

The healthy gut houses about 100 trillion (10^{14}) microorganisms (Ley et al. 2006). Commensal gut microbes help digest complex polysaccharides, stimulate the development of the mucosal immune system and protect the body from pathogens (Hentschel et al. 2003). However, when confronted with disease-causing microbes, the gut immune system can distinguish them from the commensals and mount a response that protects the host. Defects in the capacity of the mucosal immune system to discriminate between pathogenic and commensal microorganisms can cause pathology: On the one hand, non-inflammatory responses to pathogenic microbes can foster infection; on the other hand, inflammatory responses to non-pathogenic agents can damage the gut and lead to inflammatory bowel disease (IBD) (Podolsky 2002). The question of how the immune system manages to recognize pathogenic microbes is one of the most important issues of mucosal immunology. Here, we shall explore whether discrimination between pathogenic and commensal bacteria can be reduced to the level of single immune cells or whether this discrimination is performed by integrated cellular activities at the systems level.

According to the classical view, this discrimination is performed by single types of cells or single types of molecules (Murphy et al. 2008, p. 48). This classical view is contrasted here with a context-sensitive view in which discrimination between pathogenic and non-pathogenic microbes is the outcome of a complex exchange of information between various types of immune-system cells and non-immune cells (Cohen 2000a, p. 159-162; Cohen 2000b).

Single types of cells and molecules cannot distinguish between pathogenic and non-pathogenic microbes

Two classes of innate receptors crucially involved in intestinal immune responses are Toll-like receptors (TLRs) and Nucleotide-binding oligomerization domain-like receptors (NOD-like receptors) (Fritz and Girardin 2005). Both types of receptor molecules are

sometimes considered as sensors of pathogens. However, this idea faces empirical problems. There are at least 11 kinds of TLRs and all of them can become activated by motifs common to pathogenic and non-pathogenic microbes (Sandor and Buc 2005, p. 149). In addition, some of the TLRs can recognize the body's own molecules such as fibrinogen or heat-shock protein (HSP). Consider TLR4: TLR4 recognizes lipopolysaccharide (LPS), a component of the cell walls of gram-negative bacteria, independently of whether these bacteria are pathogenic or not. In addition, TLR4 can become activated by self-motifs found in healthy vertebrates such as HSP60 (Cohen-Sfady 2009). TLR5, too, can recognize motifs found in both pathogenic and non-pathogenic agents. One of its ligands is flagellin, a protein expressed by attenuated as well as by pathogenic strains of Salmonella (Newton et al. 1989). TLR9 is stimulated by unmethylated repeats of the dinucleotide CpG, independently of whether they originate from pathogenic or commensal bacteria (Hemmi et al. 2000). More recently, it has been suggested that TLR9 recognizes DNA in a sequence-independent manner (Haas et al. 2008). It is clear that TLRs recognize and become activated by motifs common to pathogenic and commensal agents. In addition, the outcome of TLR ligation is subject to modulation in the intestine. The same type of TLR activated by the same type of ligand in the same type of cell can promote inflammatory or non-inflammatory type responses, depending on the context: "A problem with TLR agonists that has not been fully appreciated is that they can generate suppressive as well as inflammatory responses in innate immune cells and can promote the induction of regulatory as well as effector T cells" (Conroy et al. 2008, p. 168).

Another important class of innate immune receptors active in the intestine are NOD-like receptors (NLR). But they too appear to lack the power to discriminate between pathogenic and commensal microbes. The ligands of NOD1 and NOD2 are muramyl-tripeptide and muramyl-dipeptide (MDP) that are components of bacterial peptidoglycan (PGN) (Girardin et al. 2003a; Girardin et al. 2003b). PGN is a constituent of the cell walls of both pathogenic and non-pathogenic, gram-positive and gram-negative bacteria (Guan

and Mariuzza 2007). Therefore NLRs, like TLRs, can become activated by intestinal agents independently of their commensal or pathogenic identity.

Contrary to expectations, the antigen receptors of both B cells and T cells lack the capacity to distinguish between pathogens and non-pathogens, outside of particular contexts. First, the specificity of antigen recognition by lymphocyte receptors is the result of collective interactions with antigen-presenting and other types of cells, and not by B-cell or T-cell receptors in isolation (Cohen 1992; Cohen 2000a). Secondly, it is well established that lymphocyte receptors can recognize non-virulent as well as virulent forms of pathogenic microbes. Therefore virtually any type of lymphocyte receptor can bind equally well to pathogenic and non-pathogenic agents. Collectively, these data indicate that immune receptors can become activated by motifs common to pathogenic and nonpathogenic agents and therefore have no power on their own to recognize microorganisms as pathogenic or safe.

In the face of empirical data demonstrating that *single types of immune receptors* cannot distinguish between pathogenic and commensal microbes, some researchers have turned to the idea that immune recognition is performed by *single types of cells*. For example, Aderem formulated a "barcode model of immune recognition". He argues that different types of TLRs expressed by an immune cell jointly read a "barcode" on a pathogen and induce immune responses accordingly (Figure 1): "For example, if microbe 1 activates TLR4 and TLR5, it is likely to be a flagellated gram-negative organism, whereas TLR2 and TLR6 together with TLR5 will detect a flagellated gram-positive bacterium" (Aderem 2003, p. 344).

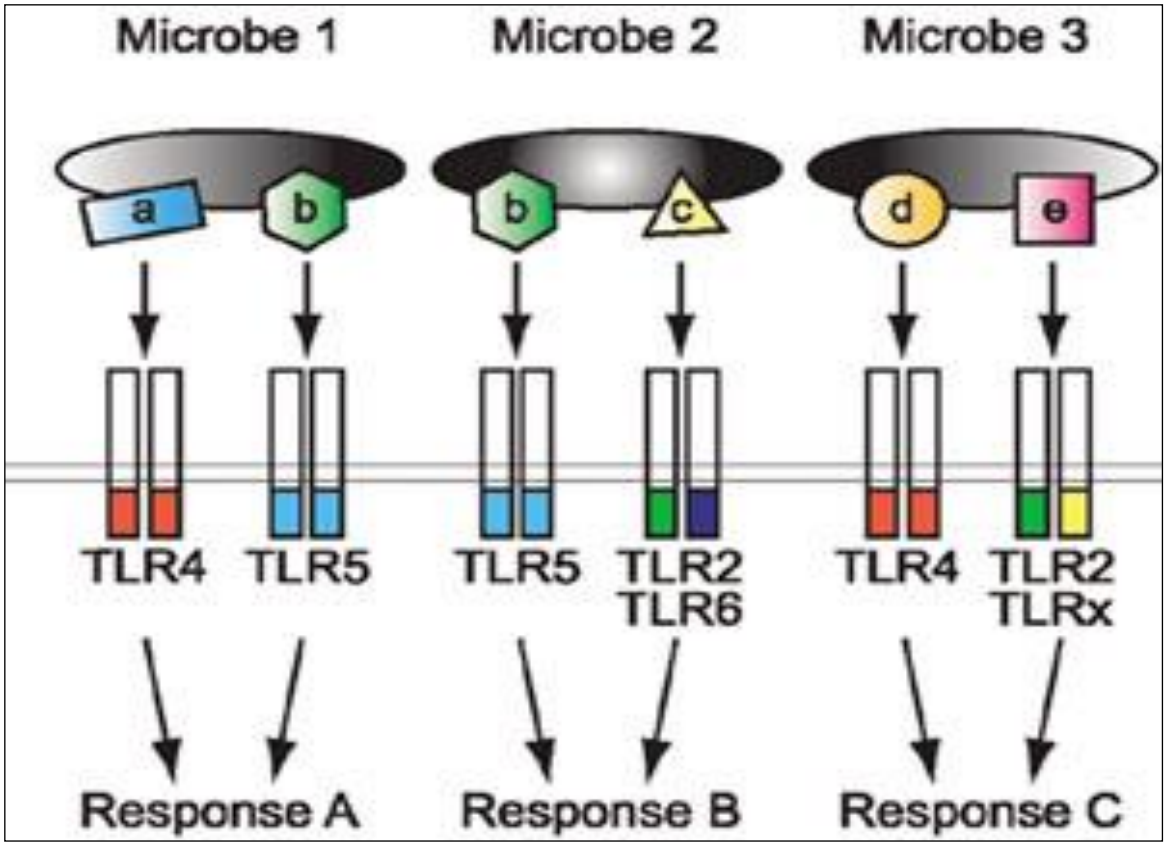


Figure 1. *According to the "barcode model", immune recognition is not performed by single types of immune receptors. Instead, it is performed jointly by several pattern recognition receptors (PRRs). The mosaic of PRR activations of an immune cell triggers an immune system response of a specific kind (Brown 2001). Source: The Scientist 2003, 17: 34).*

Netea and colleagues have formulated an "integrated model of immune recognition", which was originally designed to explain immune recognition of *Candida albicans*. However, the authors emphasize that that model can be "extrapolated to immune recognition of any microorganism" (Netea et al. 2008, p. 75). According to this model, the immune response to a microorganism depends on a mosaic of PRR activations. It is assumed, exactly as in the "barcode model of immune recognition", that it is an integrated activation of various PRRs rather than a single type PRR that can produce a uniquely tailored response. Despite similarities with the barcode model, the integrated model by Netea and colleagues is more advanced and takes into account that each type of immune

cell has its own characteristic set of PRRs. Thus, immune responses to various kinds of microorganisms is "cell-type specific" (Netea et al. 2008).

Vance and colleagues have formulated another model of immune recognition. They suggest that pathogen recognition requires molecular detection of three characteristic features that make a microorganism pathogenic: microbial growth, cytosolic access and the potential to hijack and disrupt normal host cytoskeletal function. There are adaptations that make recognition of these non-structural aspects of pathogenicity possible, namely, the same types of pattern recognition receptors that have been shown to initiate different signaling pathways depending on their cytosolic or membrane-based localization (Barton and Kagan 2009). Activation of a cytosolic receptor, in contrast to the activation of the same type of membrane receptor, can be a sign of pathogen-induced tissue damage. Thus, in this new model of immune recognition, it has been pointed out that topology of a PRR plays an important role in discrimination between pathogenic and non-pathogenic agents (Vance et al. 2009, p. 13).

All of the above attempts to fix the paradigm are based on the same general assumption: they take it for granted that immune recognition is performed by a *single type of* cell. Intestinal epithelial cells (IECs), for example, have been proposed to be able to recognize pathogens (Sansonetti 2004), acting as sentinels for certain types of microbes. The crucial feature enabling pathogen detection by IECs seems to be cell polarization (Lee et al. 2008): The apical and basolateral surfaces of IECs feature different types of TLR molecules (Schmausser et al. 2004). For example, it has been suggested that IECs do not express TLR5 on the apical surface thus making it impossible for commensal agents resident in the lumen, to activate this receptor (Gewirtz et al. 2001). However, further experiments demonstrated that TLR5 is expressed on both surfaces of the epithelium (Bambou et al. 2004).

Selective distribution of pattern recognition receptors (PRRs) on the apical and basal layers is only one aspect of IEC polarization. There is empirical evidence suggesting

that signaling pathways activated by a receptor of a given type may depend on its apical or basolateral localization. For example, activation of TLR9 on the basolateral surface has been suggested to lead to degradation of IκB, which is a necessary condition for NFκB translocation into the nucleus and for subsequent production of proinflammatory cytokines. On the other hand, activation of TLR9 on the apical surface seems to inhibit the activation of NFκB. (In this latter case, IκB is phosphorylated and ubiquitinated but not degraded) (Lee et al. 2006). In addition, the types of activated signaling pathways seem to depend on cytosolic or membrane-based localization of the receptors (Vance et al. 2009). Taking into account this compartmentalization of PRRs, it has been proposed that IECs can promote pro-inflammatory responses only against those bacteria that penetrate the epithelium and activate intracellular or basolateral PRRs (Mueller and Macpherson 2006) Thus, tissue damage produced by intestinal microbes is suggested to be a necessary condition for activation of inflammatory type responses. However, empirical evidence indicates that luminal agents can promote pro-inflammatory responses without penetrating the epithelium. For example, non-invasive *Salmonella typhimurium* has been shown to stimulate TLR5 on the apical surface of IECs leading to the production of the proinflammatory mediator CCL20 (Bambou et al. 2004; Rimoldi et al. 2005a). Similarly, activation of apical TLR2 has been shown to induce production of the proinflammatory cytokine IL-8 (Lee et al. 2006). Moreover, luminal bacteria are known to induce production of IEC-derived factors such as TGF-β. TGF-β has been shown to promote protective as well as regulatory responses against bacteria (Mangan et al. 2006; Strobl and Knapp 1999; Veldhoen 2006). The fact that intestinal microflora are able to trigger IBD suggests that IECs do not always downregulate proinflammatory responses against gut microflora (Sartor 2008).

Dendritic cells (DCs) are another class of cells believed to be decisive regarding the phenotype of immune responses; “Pathogen recognition by Toll-like receptors (TLRs) on dendritic cells (DCs) leads to DC maturation and the initiation of adaptive immunity”

(Münz et al. 2005, p.203). Interactions between intestinal microbes and dentritic cells are facilitated by microfold cells (M-cells), which transport luminal agents from one side of the follicle-associated epithelium of the Payer's patches (PPs) to the subepithelial dome area (Wassef et al. 1989). Thus transported microorganisms and antigens can be sampled by DCs located in close proximity to the epithelial cell layer. Moreover, intestinal DCs can sample luminal agents directly through the epithelial cell layer. DCs have been shown to express tight junction-like structures that allow them to extend their dendrites between adjacent IECs into the lumen and take up antigens or microorganisms (Rescigno et al. 2001).

DCs recognize microorganisms by means of their PRRs. However, the PRRs of DCs have been shown to lack the capacity to recognize pathogenic microbes specifically; "TLR agonist interaction with their receptors on DCs has the capacity to induce or expand Treg as well as pathogenic T cells" (Mills 2008, p. 518). Moreover, the course of immune responses induced by DC depends on conditioning by IEC-derived factors (Iliev 2007; Iliev 2009a; Iliev 2009b). Depending on conditioning by cytokines, intestinal DCs can promote the differentiation of T cells into regulatory T cells or effector T cells.

In addition to DCs and IECs, macrophages and neurophils have been proposed to be able to distinguish between pathogenic and nonpathogenic microbes. However, there is evidence suggesting that the type of response induced by a macrophage or a neutrophil depends on detection of common microbial constituents and environmental conditions more than the actual status of the recognized microorganism as pathogenic or safe. For example, intestinal macrophages, in contrast to other kinds of macrophages, have been shown to be resistant to the induction of inflammatory responses despite retaining their phagocytic and bactericidal functions (Smythies et al. 2005). This resistance helps prevent induction of active responses against non-pathogenic bacteria that may enter the subepithelial tissues because of tissue damage. Neurophils have been shown to target pathogenic microorganisms, non-pathogenic microorganism and the tissues of the host.

Thus, despite expectations, neutrophils do not manifest the power to discriminate on their own between pathogenic and commensal agents. These cells can contribute to accurate pathogen detection only in very specific environmental conditions (Nathan 2006). Collectively, these data suggest that single types of immune cells cannot recognize microbes *as* pathogenic or safe.

Single types of cells and molecules, in principle, cannot recognize pathogens because pathogens lack structures unique to them

As we have discussed above, empirical evidence indicates that single types of receptors or single types of cells lack the power on their own to distinguish between pathogenic and non-pathogenic agents. However, this empirical argument does not exclude the possibility of a future discovery of a class of pathogen-detecting-cells or pathogen-detecting-molecules. Here, we argue that discovery of such a class is unlikely; there are fundamental reasons why no single type of cell or single type of receptor can recognize pathogens.

Immune cells sense their environment by means of their receptors. These receptors recognize molecular motifs in the microenvironment. Taking this into account, we can say that an immune cell would be able to discriminate between pathogenic and non-pathogenic microbes only if pathogenic and non-pathogenic (commensal) microbes would have molecular motifs unique to each type; "In general, the immune system distinguishes self from infectious non-self by sensing structures unique to pathogens" (Bauer 2006, p. 13). The structures thought to be unique to pathogens are often referred to as "pathogen-associated molecular patterns" (Janeway and Medzhitov 2002). "Microbial pathogens possess specific molecular patterns called "pathogen-associated molecular patterns" (PAMPs). The host innate immune system recognizes these PAMPs by germline-encoded pattern recognition receptors (PRRs) to elicit immune responses, such as the production of proinflammatory cytokines" (Kumagai et al. 2008, p.86). Accordingly, markers thought to

be unique to commensals have sometimes been referred to as "commensal-associated molecular patterns" (CAMPs) (Cario et al. 2002).

Do microbes really express such discriminatory molecular motifs? The question, however, is hypothetical because the same microorganism can be pathogenic in one circumstance and commensal or beneficial in another (reviewed in Casadevall and Pirofski 2001). For example, *Helicobacter pylori* colonizes the gastric mucosa of 80–90% of people in less developed countries (Falkow 2006, p. 702), but it causes symptomatic disease in only 15–20% of infected individuals (Ferrero 2005). The Myxoma virus is extremely pathogenic to European rabbits (*Oryctolagus cuniculus*), but non-pathogenic to genetically resistant American rabbits (*Sylvilagus audubonii*) (Kerr and McFadden 2002). The same strains of uropathogenic *Escherichia coli* clones can be commensal in one condition and pathogenic in another (Klemm et al. 2007). One could provide a very large number of examples of microbes whose pathogenic potential is context-dependent. Obviously, the pathogenic potential of a microbe varies with the colonization site and depends on the state of the host immune system. "The question 'what is a pathogen' cannot be separated from the question 'what is a host'" (Casadevall and Pirofski 2002, p. 2).

As we have already mentioned, the pathogenesis of an *H. pylori* infection depends on both host and bacterial factors (Ferrero 2005; Viala et al. 2004). However, this does not exclude the possibility of *H. pylori* being recognized as pathogenic by epithelial cells on the basis of a single marker: PGN (Viala et al. 2004). Similarly, it has been suggested that the Myxoma virus, the causative agent of rabbit myxomatosis, can be detected as pathogenic on the basis of the "recognition of a viral pathogen-associated molecular pattern, such as Myxoma virus DNA or newly synthesized viral RNA" (Vilček 2004, p. 1206). The difference between permissive and non-permissive hosts of the virus has been said to depend on the potential to induce mitogen-activated protein kinase (MAPK) – mediated production of interferon α/β (IFNα/β) (Wang et al. 2004). In the case of uropathogenic *E. coli*, the commensal-to-pathogenic shift seems to be mediated by the

acquisition of the potential to adhere to the epithelial cells of the urinary tract (Edén et al. 1976). It has been suggested that *E. coli* can use type 1 fimbriae to attach to the epithelium (Klemm et al. 2007). There is a component of type 1 fimbriae, adhesion molecule FimH, which has recently been demonstrated to function as a ligand of TLR4 (Mossman et al. 2008). As one can see, it has been suggested that simple molecules are the only triggering factors that initiate defense responses against the microorganisms.

Even if we agree that pathogens can be recognized on the basis of a single molecular marker, the marker can hardly be classified as a pathogen-associated molecular pattern. For example, peptidoglycan is a component of cell walls of pathogenic and non-pathogenic, gram-positive and gram-negative bacteria (Guan and Mariuzza 2007). TLR9 in the intestine are continuously stimulated by the dinucleotide CpG independently of their pathogenic or commensal origin. TLR4 can also be activated by ligands whose expression is not an exclusive domain of pathogenic microbes (Sandor and Buc 2005).

As we have seen many PRR ligands are expressed by both pathogenic and nonpathogenic agents. However there is a class of features that might be exclusive domains of pathogenic agents. These are the products of the so called "pathogenicity genes"; "Genetic analyses have shown that bacterial pathogens differ from their nonpathogenic relatives or commensal bacteria by the presence of specific pathogenicity genes" (Magalhaes et al. 2007). Toxins, polysaccharide capsules, secretion systems and IgA proteases seem to be exclusive to pathogenic agents. The fact that immunosuppression of the host can lead to a change in host-parasite relationship from symbiosis to pathogenesis challenges the pathogenicity gene concept (Rubin 1993). Surprisingly, the opposite can also happen; an initially pathogenic microorganism can become symbiotic if the immune system succeeds in controlling microbial expansion and tissue damage (Hentschel et al. 2000). If the status of a microbe depends on the immune competence of the host, expression of a virulence gene by the microbe cannot serve as a reliable marker of its pathogenicity. If the potential to produce damage to the host is host-dependent, the host

may have to "self-reflect" in order to learn whether a microbe is pathogenic (cf. Cohen 2007).

Indeed, no clear-cut distinction can be made between virulence factors and symbiosis factors. A standard example of a virulence gene that belongs to a pathogenicity island is a gene coding for the type III secretion system (T3SS). T3SS is a microhomologue of a syringe that is used by many gram-negative bacteria such as *Salmonella* to transfer into the cytosol of the host proteases that can interfere with the physiology of the host cell (Galán and Collmer 1999). One might think that T3SS would serve as a dependable context-independent marker of pathogenicity. However, it has been demonstrated that, depending on the context, T3SS can be used by the same bacterial species as a virulence factor or as a symbiosis factor (Galán and Wolf-Watz 2006; Silver et al. 2007). Moreover, type III and type IV secretion systems have been shown to be expressed by many commensal bacteria (Nagai and Roy 2003; Tampakaki et al. 2004). Tracheal cytotoxin (TCT), a fragment of a bacterial PGN, is known to be a powerful tissue-damaging factor (Goldman et al. 1982). However, it has also been shown to be an important symbiotic factor enhancing tissue development (Koropatnick et al. 2004). Apart from genes that allow subjugation of host cell machinery, other genes enable pathogenic agents to adhere to the epithelial cell wall. Enterohemorrhagic *E. coli* has been found to express an adherence factor called the *E. coli* common pilus (ECP). Pili are traditionally considered as virulence factors (Jonson et al. 2005); however, it has also been demonstrated that ECP is critical for colonization by both pathogenic and commensal *E. coli* (Rendón et al. 2007).

As one can see, the products of the so-called "pathogenicity" genes cannot be reliable markers of pathogenicity. This is because there are non-pathogenic microbes that express these genes and there are microbes that are pathogenic despite lacking them. However, the most unexpected support for the thesis that the property of being pathogenic is not just a matter of having certain unique structural or biochemical features comes from

studies showing that, in certain conditions, persistent bacterial infections have long-lasting beneficial consequences for the host (Falkow 2006). For example, it has been pointed out that the coordinated balance between *H. pylori* infection and the host immune response has beneficial effects for the host (Blaser and Atherton 2004). Thus, from a wider perspective, a putative pathogen can establish its role as a symbiont.

As one can see, cytotoxins, secretion systems, fimbriae and others can be virulence factors in some conditions, colonization factors in other conditions and symbiosis factors in yet other conditions. Pathogenic and commensal microorganisms appear to employ similar or even identical molecular mechanisms to express their pathogenic or symbiotic potential (Hentschel et al. 2000). In particular, both pathogenic and symbiotic bacteria must actively "manipulate" the host immune system to make it possible for them to colonize the body. In short, no microorganism is *just* pathogenic or *just* commensal. Being pathogenic or commensal is not a pre-established, context-independent and host-independent property. Any microorganism is pathogenic or commensal in a *given* context, under *given* conditions. It is the interplay between the context and the intrinsic features of a microbe that make it pathogenic or safe. Thus, in addition to the genetic predilections of a microorganism, environmental factors play an important role in defining a microorganism as pathogenic or not (Rescigno et al. 2008, p. 669). Since the identity of a microbe as pathogenic or safe does not depend on its structural features exclusively, a single type of receptor or a single type of cell is not in the position to recognize the microbe as pathogenic or safe in principle.

Return to basics: What makes a pathogen a pathogen?

Structure is an important factor influencing the pathogenic or commensal function of a given microbe. However, as we have seen, structurally identical agents can be pathogenic or commensal depending on the context. This implies that structure is not the only factor.

In the gastrointestinal tract, another important pathogenicity making-factor is the composition of the commensal flora.

There are at least four ways in which commensal bacterial communities can influence the status of a microorganism as pathogenic or commensal. First, luminal bacteria can provide a degree of protection by occupying environmental niches needed by pathogens or by producing antimicrobial peptides (Corr et al. 2007). The damaging potential of a microorganism can thus be modulated by competition with other microbes. Secondly, bacteria are equipped with quorum-sensing mechanisms whose activation can promote expression of genes that mediate attachment, invasion, dissemination and survival in the host. Induction or inhibition of these genes can affect the capacity of a microorganism to invade the body (Sperandio et al. 2003; Walters and Sperandio 2006). Thirdly, commensal-derived metabolites such as butyrate have been shown to inhibit the production of proinflammatory cytokines and promote secretion of immunoregulatory mediators such as IL-10 (Saemann et al. 2000). This IL-10 production can in turn indirectly affect the pathogenic properties of microbes. Finally, alterations in the composition of commensal communities can affect the balance between immunity and tolerance and thus facilitate or impede the power of bacteria to establish a site of infection. For example, one commensal species, *Faecalibacterium prausnitzii*, has been shown to induce the production of IL-10 and downregulate secretion of TNF-α and IL-12 by an epithelial cell line (Sokol et al. 2008). This suggests that *F. prausnitzii* has the capacity to attenuate immune responsiveness to other bacterial species in the intestine. This down-regulation of responsiveness can assure integrity of the mucosal tissues and enhance the non-pathogenic properties of some agents.

Studies by Ivanov and colleagues provide another example of how alterations in the composition of gut microflora can influence pathogenic or commensal properties of intestinal microbes. The specific composition of commensal communities has been shown to regulate the balance between interleukin-17 producing $CD4^+$ T-cells (Th17 cells) and

Foxp3^{+} regulatory T-cells (Tregs) (Ivanov et al. 2008). Disregulation of the balance between these two classes of cells can influence the pathogenic potential of many kinds of intestinal microorganisms (Belkaid and Tarbell 2009). Everything being taken into account, it is becoming clear that the microbiota of the human gut produce complex and powerful mechanisms for shaping the pathogenic or symbiotic potential of any intestinal microbe (Foxman et al. 2008; O'Hara and Shanahan 2006). Therefore, regulated control of the composition of the microflora has emerged as a promising therapeutic opportunity for the treatment of acute and chronic intestinal diseases (Borchers et al. 2009).

In addition to the biochemical and structural composition of the gut and the microbial environment, the very act of immune recognition is another important determinant of the pathogenicity of a given intestinal agent. Sometimes, the recognition of a microbe as pathogenic makes it pathogenic and the recognition of the microbe as safe makes it safe. For example, *Shigella* is an opportunistic bacterium responsible for dysentery that invades the colonic and rectal mucosa. As a gram-negative bacterium, *Shigella* expresses LPS. Fernandez et al. reported that dimeric IgA produced by B-cells in subepithelial tissues colocalizes to LPS in the apical recycling endosome compartment of the IEC, thereby preventing LPS-induced NFκB translocation and a subsequent proinflammatory response. This colocalization makes it possible for the immune system to shut itself down and so avoid recognizing *Shigella* as pathogenic. This natural down-regulation of the response assures that the microbe remains non-pathogenic. On the other hand, in conditions of IgA deficiency, bacterial-derived LPS can successfully induce secretion of TNF-α by IECs and promote pro-inflammatory responses. The inflammation, in turn, can lead to mucosal damage, enabling *Shigella* to colonize the subepithelial tissues and so become pathogenic. As one can see, in the case of IgA deficiency, it is the recognition of the bacterium as pathogenic that makes it pathogenic (Fernandez et al. 2003).

Apart from cases where recognition of a microorganism as pathogenic or safe makes it pathogenic or safe respectively, there are also opposite examples. Recognition of

a microbe as safe can sometimes grant it the power to invade the body and become pathogenic. Many microorganisms have developed strategies to hijack host cellular mechanisms to assure that non-inflammatory responses will be induced against them. The most straightforward example is that of *Salmonella typhimurium*. *Salmonella* expresses a type III secretion system that allows it to transfer effector proteins into host cells. One of these effector proteins is a deubiquitinase that prevents polyubiquitination of IκB. This mechanism inhibits NFκB-mediated TNF-α production (Le Negrate et al. 2008; Ye et al. 2007).

As one can see, the property of being pathogenic is not a pre-established feature of a microorganism. The property of being pathogenic or commensal is complex, context-dependent and host-dependent. There are many environmental and structural factors influencing the pathogenic behavior of a microorganism, and we have mentioned only a few of them. Figure 2 enumerates some additional pathogenicity-making factors. It is very important to note that, regardless of their mode of action and number, pathogenicity-making factors do not act *in isolation*; there is always a *causal interplay* between them. In other words, the potential to produce damage by a given microbe is realized by *causal interactions* between pathogenicity-making factors, and not by the *collective sum of individual factors*. The same pathogenicity-making factors interlinked by different kinds of causal connections can determine the host-damaging potential of a microorganism in various ways.

It is impossible to make a list of *all* the causal connections between pathogenicity-making factors and explain how their mutual interactions might jointly affect the status of a microorganism as pathogenic or safe. Some examples can help us understand this causal interplay. For example, the composition of commensal communities and their immune recognition have been cited here as examples of pathogenicity-making factors. There is a strong interaction between them, so they can be said to determine pathogenic or commensal properties of a given microorganism *jointly*. For example, it has been proposed

recently that *Lactobacillus paracasei* can downregulate the production of proinflammatory cytokines by DCs. By inhibiting these cytokines, *L. paracasei* can affect the recognition of other bacteria by the immune system. Indeed, it has been demonstrated that this probiotic microbe can inhibit differentiation of T helper 1 cells (Th1) in response to *Salmonella typhimurium.* Thus, a modified immune recognition of *Salmonella* can restrain the damaging potential of this bacterium (Mileti et al. 2009).

There are also studies showing that immune recognition of intestinal microbes can be modulated by a combination of specific microbial species. For example, specific microbial compositions can induce regulatory responses to *S. typhimurium* by inhibiting proinflammatory NFκB activation following infection. This inhibition increases the frequency of $CD4^{+}CD25^{+}FoxP3^{+}$ regulatory T-cells (Tregs) in the intestinal lamina propria. Thus, induced regulatory type responses prevent *S. typhimurium*-derived inflammatory tissue damage, thereby limiting the potential of these bacteria to invade the tissues (O'Mahony et al. 2008). In other words, the specific composition of commensal communities has been shown to make *S. typhimurium* safe and this limits the pathogenic potential of the bacterium. As one can see, immune recognition and commensal composition jointly influence the status of a microorganism as pathogenic or safe. However, one should be aware that the ultimate status of a microbe does not depend on the joint action of just *two* factors but on a complex causal interplay between *a number* of factors. Some of these causal interactions are shown in Figure 2.

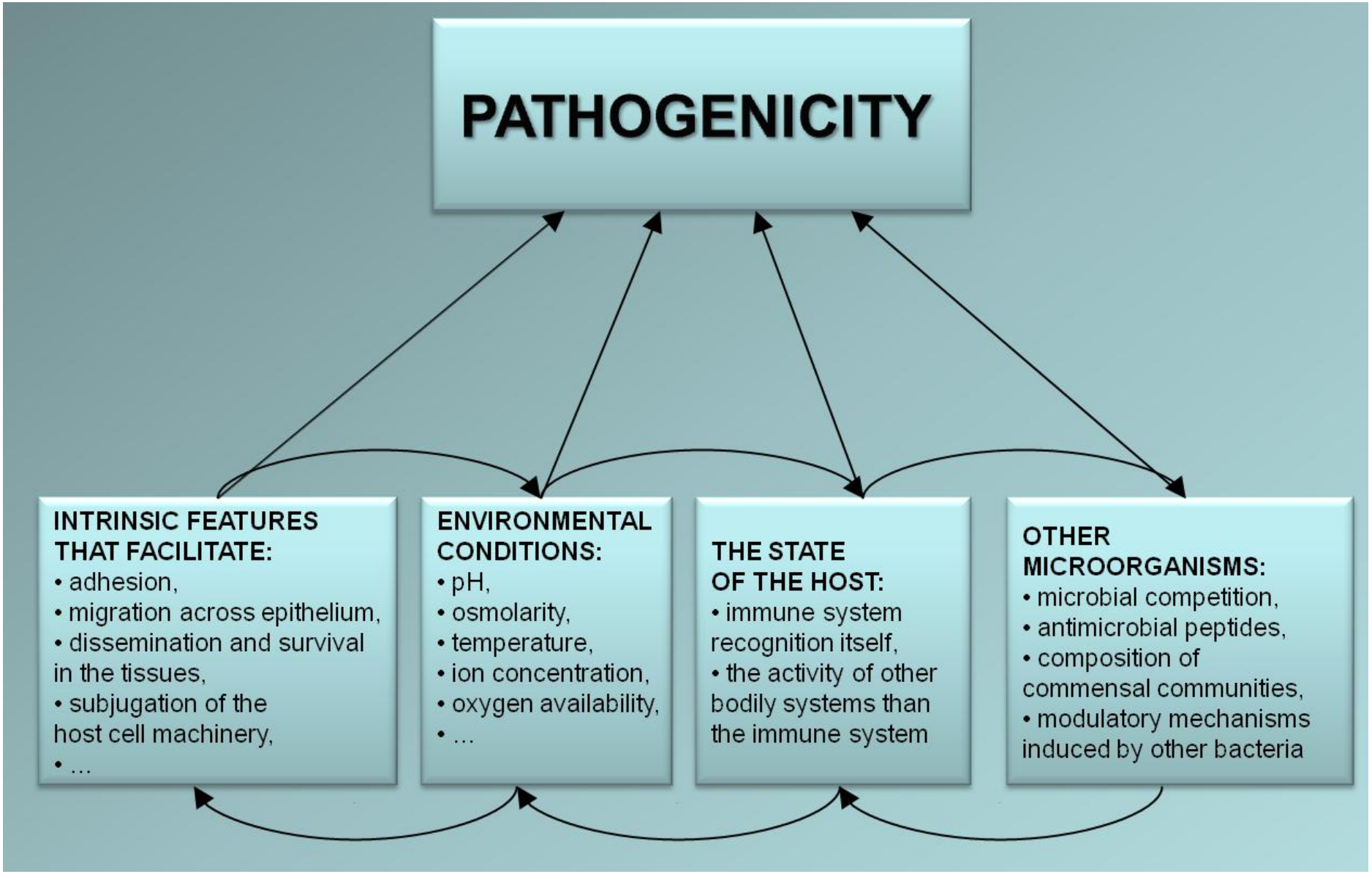

PATHOGENICITY
INTRINSIC FEATURES THAT FACILITATE:
• adhesion,
• migration across epithelium,
• dissemination and survival in the tissues,
• subjugation of the host cell machinery,
• …
ENVIRONMENTAL CONDITIONS:
• pH,
• osmolarity,
• temperature,
• ion concentration,
• oxygen availability,
• …
THE STATE OF THE HOST:
• immune system recognition itself,
• the activity of other bodily systems than the immune system
OTHER MICROORGANISMS:
• microbial competition,
• antimicrobial peptides,
• composition of commensal communities,
• modulatory mechanisms induced by other bacteria

Figure 2. *The potential to produce damage to the host (pathogenicity) is constituted by various intrinsic and extrinsic features of a given microbe. It is crucial to note that the property of being pathogenic is constituted by these features jointly. In other words, the status of a microorganism as pathogenic (or safe) results from interplay between pathogenicity-making factors and not from their collective impact. Perhaps the simplest example of the interplay is the interaction between environmental conditions and the expression of colonization factors. Some "virulence" factors are believed to be expressed only in certain pH, temperature, ion concentration or oxygen concentration. For example the expression of type 3 secretion system by Shigella flexeri is controlled by local concentration of O_2 (Marteyn et al. 2010). On the other hand, the expression of the colonization factors by a microorganism can change environmental conditions directly or indirectly. For example, Helicobacter pylori secrete urease that increases pH in its microenvironment (Celli et al. 2009).*

How does pathogen/non-pathogen discrimination take place?

If a single type of receptor or a single type of cell is not able to tell whether a given microorganism is pathogenic or safe, then how is recognition of the difference performed by the immune system? If the status of a microorganism depends on a number of proximal and distant environmental factors, then reliable recognition of the microorganism as pathogenic or safe requires recognition of all of these factors. Therefore, we propose here that immune recognition of a microorganism as pathogenic or safe requires the engagement of multiple *detection modules* specialized in the detection of individual pathogenicity-making factors.

One such detection module is a mechanism dedicated to the identification of the exact anatomical localization of a given microbe. Accurate evaluation of the potential

danger inherent in an intestinal bacterium requires detecting where the bacterium is located in the host. For example, in the context of the gut associated lymphoid tissue (GALT), if a bacterium is located in the lamina propria, the bacterium is invasive. If, in contrast, the bacterium is retained in the intestinal lumen by a mucus layer, IgAs and tight junctions between IECs, it is likely to be harmless.

There are a number of adaptations in the intestinal immune system that help to determine the exact localization of a microorganism, one of which is polarization of IECs that has been mentioned above. The kind of signaling pathway initiated by a given type of immune receptor may depend on its apical, cytosolic or basolateral localization. However, information about the apical or basolateral localization of a microorganism alone is not sufficient to determine its status as pathogenic or safe. For example, *Helicobacter pylori* do not normally penetrate the epithelium, but rather express their pathogenic potential by altering epithelial cell functions (Ferrero 2005, p. 880). Moreover, commensal non-pathogenic bacteria may find themselves in the submucosal tissues because of tissue damage. If the anatomical localization of a microorganism would be a reliable marker of its status as a pathogen or commensal, it would be advantageous for the host to express PRRs exclusively on the basolateral surface of the epithelium. This possibility was investigated extensively but turned out to be wrong (Coombes and Maloy 2007).

Another important piece of information about the status of a microorganism as pathogenic or safe comes from a detection module specializing in the recognition of microbial structural features. These features are sensed directly by antigen-sampling DCs and IECs. Recognition of such structural features is translated into signals that can influence the type of induced response.

Detecting structural motifs and topology provides very important information to help the immune system identify a microorganism as pathogenic or safe. However, as discussed above, the potential to produce damage to the host by a given agent does not only depend on its localization and structure, but also on the composition of the intestinal

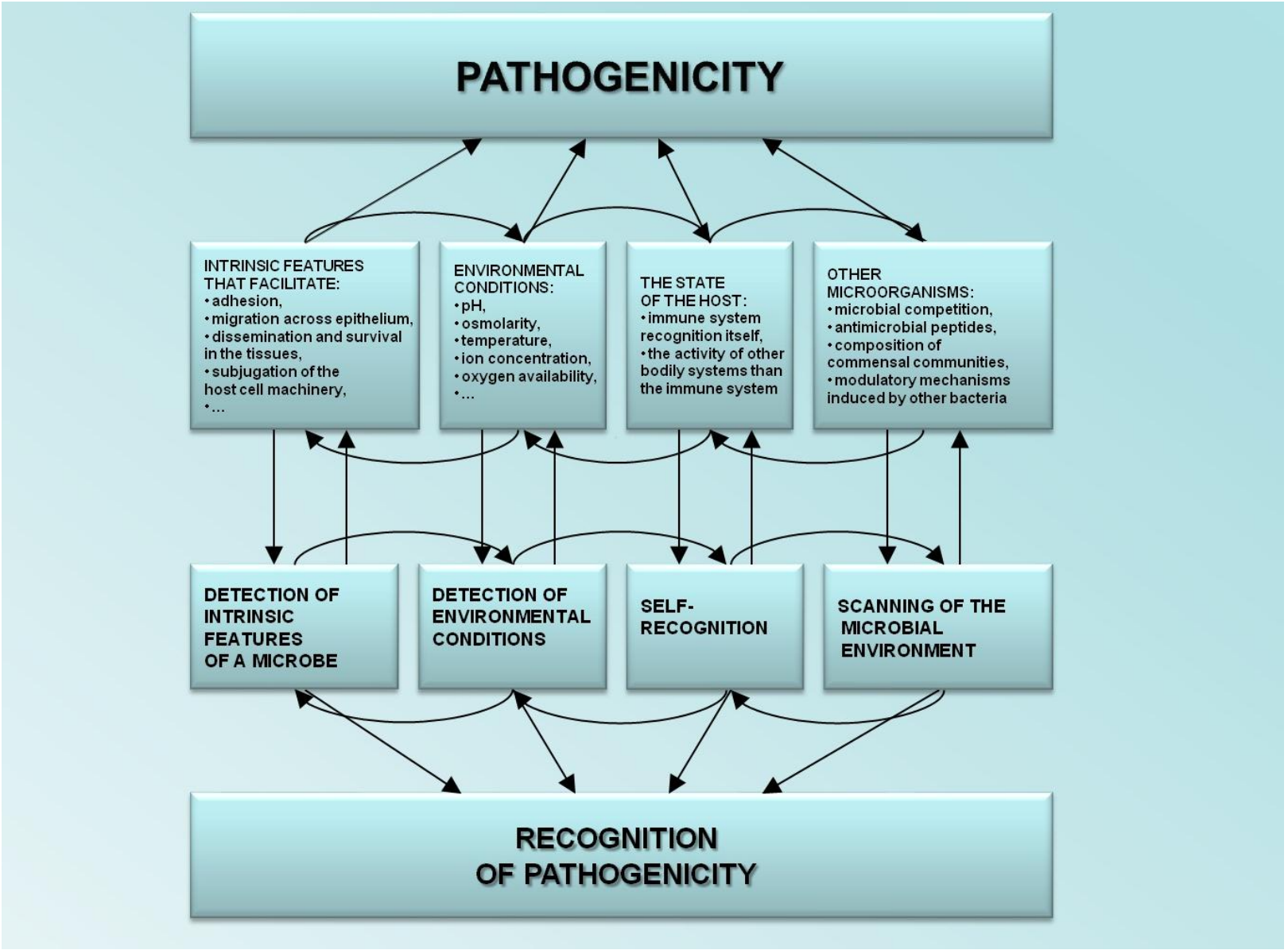
flora (cf. Figure 2). Consequently, there is a specialized detection module whose role is to recognize the composition of commensal communities; this module involves receptors on the apical surface of the intestinal epithelium. Activation of these receptors is translated into signals that can modulate immune responses accordingly. Different bacterial products can activate different signaling cascades that are translated into secreted cytokines, chemokines and other mediators. Apart from being able to activate various intracellular signaling pathways, commensal bacteria are able to suppress signaling cascades at different levels of their activation. For example, it has been shown that *Yersinia spp*. blocks the NFκB pathway at the level of IκB phosphorylation, whereas *Bacteroides spp*. can inhibit transcriptional activity of NFκB by activating peroxisome-proliferation-activated receptor-γ pathways (Artis 2008; Rescigno 2009). Inhibition of specific signaling pathways at different levels of activation results in transcriptional responses correlated with the presence of specific bacterial products in the intestine

As one can see, no single detection module alone has the capacity to distinguish between pathogenic and non-pathogenic microbes. Instead, each module provides important information about the damaging potential of a given microorganism. All these pieces of information have to be integrated to empower the immune system to decide about the type of response is appropriate for dealing with the microbe. The immune system cannot achieve this object by behaving as a passive collector of cues about various aspects of a microorganism; the system has to *integrate* all the collected information. Just as pathogenicity is the outcome of a multiplicity of pathogen and host factors, the recognition of pathogenicity requires the integrated recognition of a multiplicity of factors (Figure 2). In other words, the immune system must be able to recognize the *interactions of pathogenicity-making factors, and not only their mere presence or absence*.

This "holistic" recognition requires an exchange of information between detection modules for individual pathogenicity-making factors. How is this exchange of information possible? It is difficult to answer this question because of the complexity of the integration

processes and the scarcity of knowledge about them. However, one can get some idea of how bits of information about pathogenicity-making factors are integrated by looking at the relationship between *two* well-characterized detection modules for microbial structure and for the composition of the environment. These two cues have to be integrated. DCs detect information about the structure of the microbe, and IECs detect information about the composition of the luminal flora. Integration of the information results from a constant dialogue between these two classes of cells. Activated IECs produce factors that can modulate the activity of DCs and oher antigen presenting cells in the submucosal tissues. IEC-derived products have been shown to condition DCs to promote certain types of immune responses (Rescigno et al. 2008). Thus, immune responses induced by activated DCs depend on the sampled antigens as well as on IEC conditioning mediators (Iliev et al. 2007; Iliev 2009a; Iliev 2009b). For, example, it has been demonstrated that luminal bacterial products can activate IECs to produce thymic stromal lymphopoietin (TSLP). TSLP, in turn, has the capacity to induce the production of IL-10 and downregulate the production of IL-12 by DCs (Rimoldi et al. 2005b). Activation of naïve T-cells in the presence of IL-10 promotes their conversion into IL-10-producing T-cells. IL-10-producing T-cells, in turn, have been shown to be able to cure and protect from colitis in a T-cell transfer model. In addition to TLSP, there are many other mediators produced by IECs in response to different kinds of luminal content, including TGF-β, prostaglandin E2 (PGE2), B-cell activating factor (BAFF) and proliferation-inducing ligand (APRIL) (He et al. 2007; Sansonetti and Medzhitov 2009). Production of these factors by IECs depends on gene expression. Gene expression in IECs, in turn, is influenced by the composition of commensal-bacterial communities. As one can see, informational crosstalk between a module dedicated to recognition of commensal bacterial composition and a module specializing in detection of microbial structure expresses itself in the conditioning of dendritic cells.

Figure 3 lists observations indicating that recognition of a microorganism as pathogenic or safe is mediated by detection of factors that influence the status of a given microorganism as pathogenic or safe. It also illustrates the idea that fragments of information about various properties of a microorganism must be *put together* to produce a unified and properly adjusted immune response. This unified representation of a microorganism in the immune system is what we call *immune recognition*. The mere binding of a ligand to a receptor may activate the receptor, but it does not constitute functional recognition, which, as we have discussed here, depends on the integration of information collected from various sources. In order to reflect the dynamic interaction between pathogenicity-making factors, dynamic crosstalk between individual detection mechanisms has to take place. We refer to this crosstalk as "co-respondence" (Cohen 2000a). Co-respondence is a complex exchange of signals between cells and molecules leading to a decision at the level of the interacting population. In our case, it is an inter-cellular and inter-molecular "dialogue" whose outcome is recognition of a microorganism as pathogenic or commensal (Cohen 2000b). Since there is no information center in a strict topological sense (no brain-like controller), "co-respondence" itself should be regarded as a central processing unit integrating pieces of information about pathogenicity-making factors (Cohen 2006). Representation of a microorganism as pathogenic or safe is not encoded in a single type of receptor activation or cell activation, but is a distributed image unfolding over time and involving the dynamic actions of a great number of signals (cf. Cohen 2000a, pp. 174-181, on immunological images).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DETECTION OF INTRINSIC FEATURES OF A MICROBE
DETECTION OF ENVIRONMENTAL CONDITIONS
SELF-RECOGNITION
SCANNING OF THE MICROBIAL ENVIRONMENT
RECOGNITION OF PATHOGENICITY

Figure 3. *There are many factors that jointly determine the status of a microorganism as pathogenic or commensal. The Immune system has evolved detection modules specializing in recognition of these factors. Intrinsic features, environmental conditions, state of the host and other microorganisms influence pathogenic or commensal properties of a given microbe. Therefore there are detection modules specializing in recognition of the intrinsic features, environmental conditions, state of the host and the composition of commensal communities respectively. All these detection modules have to be integrated to give rise to immune recognition of a certain kind. It is interesting to note that one of the detection modules is self-recognition. If the property of being pathogenic is host-dependent, the immune system has to be able to recognize itself in order to produce suitably tailored immune responses (Cohen 2000a; Cohen 2007).*

Conclusions: A systems biology approach is needed to understand immune recognition

The theory that single types of cells and molecules act as sentinels (Appendix 1), when originally formulated, was a good representation of the available data. However, more recent experimental findings challenge this paradigm; discrimination between pathogens and non-pathogens is the outcome of a complex exchange of signals between cells and molecules, both of the host and the bacteria. Why then is this paradigm still maintained?

One possible reason is methodological. Contemporary immunology tends to favor molecular and cellular methods of analysis. Such reduction allows one to study single types of cells and their local interactions, and permits tracing a linear sequence of consecutive events from cause to effect. However, immune recognition is barely visible at this reduced level of analysis. Discrimination between pathogenic and non-pathogenic microbes does not involve single types of cells but many types of cells, but complex causal networks (Figure 3). Most importantly, immune recognition requires *integrated exchange*

of signals and it is the *exchange* that represents the greatest obstacle to the classical paradigm of the discriminating sentinel.

Biologic processes that go beyond direct signaling within or between cells can be termed systemic processes. Here, we show that immune recognition, is not only a cellular and molecular process but is also a systemic process performed by the system as a system. A bird's eye view is needed to grasp systemic processes. One has to zoom out to larger scales in order to see and understand how the system makes its decisions about how to respond to a microorganism (Cohen and Harel 2006). Systems biology, the study of a system as a whole, is a new field that promises to explain higher-level biological processes. We propose that immune recognition is a property of the system domain (Benoist et al. 2006; Gardy et al. 2009; Merbl et al. 2009).

Part 2

Chronic immune misrecognition:

Inflammatory bowel disease

The above hierarchical model of immune recognition can be used to elucidate the fundamental basis of inflammatory bowel disease (IBD). IBD is a group of conditions characterized by chronically inflamed intestinal tissue. It includes two major forms: Crohn's disease (CD) and uncreative colitis (UC). Symptoms of IBD include diarrhea, abdominal pain, rectal bleeding and fever. The primary etiology of IBD is unknown as many different combinations of genetic, microbial and environmental factors have been shown to support it. The diversity of mechanisms supporting IBD prevents development of an effective treatment strategy that would target integrated cause of this disease and restore homeostasis in the gut. However, after several decades of studies, a unified picture of IBD is emerging. Here, we propose that the shared proximal cause of various forms of IBD is a loss of robustness in the immune recognition machinery at the systems level.

Factors that support IBD may be clustered into several different categories

Inflammatory bowel disease (IBD) is a group of conditions characterized by chronically inflamed intestinal tissue. It includes two major forms: Crohn's disease (CD) and uncreative colitis (UC). The primary etiology of IBD is unknown as many alternative combinations are known to support it. Here we catalogue alterations associated with IBD according to the biological function or biological domain they alter.

1. *Structural changes in microbes.* Intestinal microbes are sensed by pattern recognition receptors (PRRs) that bind conserved microbe-associated molecular patterns (MAMPs). Structural changes in commensal bacteria may allow them to access epithelial PRRs, change the pattern of their activation and promote inflammatory-type responses. Acquisition of virulence factors by commensal bacteria like *E. Coli* has been found to be associated with many instances of IBD (Martin et al. 2004; Martinez-Medina et al. 2009). The colitogenic strains of *E Coli*

are highly adherent and thus stimulate apical PRRs directly (Darfeuille-Michaud et al. 1998).

2. *Specific alterations in the chemical and physical environment of the intestinal lumen*. These alterations include (but are not limited to) changes in the thickness and quality of the mucus layer, changes in the level and type of antimicrobial molecules (defensins, cathelicidins), alterations in the concentration of fermentation products (short chain fatty acids, butyrate), products of microbial metabolism (ATP), alterations in the peristalsis, changes in luminal pH and shifts in the concentration of ions and compounds. These chemical and physical changes can promote intestinal inflammation characteristic of IBD in many different ways; for example, short chain fatty acids (SCFA) have been shown to attenuate colitis by means of GPR43 receptor signaling (Maslowski et al. 2009). Therefore changes in the concentration of SCFA and polymorphisms of GPR43 are IBD risk factors (Sina et al. 2009).

3. *Imbalances in the activity of various bodily systems of the host.* For example, an elevated sympathetic nervous system tone, psychological stress, anxiety and trauma have been shown to be contributing factors in IBD (Mackner et al. 2011).

4. *Alterations in the composition of intestinal microbes (dysbiosis).* Several types of changes in the intestinal microbes have been found to be associated with various forms of IBD. They include decreases in the bacterial load (Frank et al. 2007), loss of microbial diversity (Ott 2004) and change in the ratios of bacterial species occupying the lumen (Frank et al. 2007). There is no consensus regarding the question whether the microbial imbalances are primary or secondary to alterations in the immune activity (Kaser et al. 2010). Regardless of its exact source, dysbiosis

has proved to be a powerful factor promoting aberrant responses in IBD (Garrett et al. 2007).

5. *Defects in the ability to detect structural features of intestinal microbes by the immune system.* Individuals suffering from IBD often have their PRR genes mutated. One example is polymorphism in the NOD2 gene in patients suffering from CD (Barrett et al. 2008). Mutated NOD2 affects the ability to sense muramyl dipeptide MDP, which is a component of a bacterial cell wall peptidoglycan (PGN) (Rescigno and Nieuwenhuis 2007). It has also been suggested that a polymorphism of TLR4 can contribute to the development of both CD and UC (Franchimont et al. 2004). Other studies indicate that NOD1, TLR1, TLR2, TLR3 and TLR6 each can contribute to the pathogenesis of IBD (Yamamoto-Furusho and Podolsky 2007).

6. *Alterations in intestinal epithelial cells (IECs) function.* To maintain their secretory, barrier and signal transduction functions, IECs must keep their metabolism under control. Disturbances in the endoplasmic reticulum (ER) activity require a cytoprotective reaction in the form of an unfolded protein response (UPR). The special role of the UPR pathway in IECs is illustrated by the fact that these cells express isoforms of UPR molecules that have not been found in other cells (Kaser et al. 2010). Mutations in genes coding for components of the UPR signaling pathway, like XBP1, have been found to be associated with various forms of IBD (Kaser et al. 2008). Another form of reaction to ER stress (and other forms of stress) is autophagy. Autophagy helps to restore homeostasis in a cell by clearing foreign material including microbes and toxins. It is well established that mutations in autophagy genes such as ATG16L1 or IRGM are associated with an increased risk for CD (Hampe et al. 2007). IBD is also associated with defects that compromise specific functions of IECs. These defects include abnormalities in tight junction

proteins (Vetrano et al. 2008) or in proteins involved in epithelial regeneration. For example IBD is known to be associated with a reduction in HNF1α, a transcription factor playing an important role in the maintenance of intestinal epithelial integrity (Ahn et al. 2008).

7. *Alterations in the physiology of immune self-recognition*. Apart from defending the host, the immune system is also engaged in self-maintenance (Cohen 2000). This requires a certain degree of self-reactivity (Cohen 2007). This aspect of immune functioning is often found defective in IBD. In particular, UC involves a strong autoimmune component. Anti-neutrophylic cytoplasmic antibodies cross-reacting with colonic bacteria have been found in 60%–75% of patients suffering from UC (Duerr et al. 1991). Moreover, it has been found that a population of IgG antibodies in UC patients reacts with a cytoskeletal microfilament protein, tropomyosin 5 (hTM5), an isoform of the protein predominantly present in the colon (Das and Biancone 2008).

8. *Molecular defects altering communication between gut microflora and cells of the gut associated lymphoid tissue (GALT).* Luminal microbes (in particular segmented filamentous bacteria, SFBs) can stimulate PRRs from the apical surface. Different species of these bacteria have been shown to modulate signaling pathways in IECs in different ways (Artis 2008). This varied modulation determines the repertoire of IEC-derived products which condition antigen presenting cells (APCs) to promote conversion of naïve T-cells into regulatory or effector phenotypes (Illiev et al. 2009a; Iliev et al. 2009b). This IEC-mediated modulation of immune responses by specific composition of luminal microbes is often impaired in IBD. For example, *Bacteroides thetaiotaomicron* can normally influence NF-κB signaling in IECs by PPAR-γ-dependent removal of an active transcriptional subunit RelA from the

nucleus (Kelly et al. 2004). However, there is some evidence that PPAR-γ signaling in IECs can be impaired in UC (Dubuquoy et al. 2003) making it difficult for this commensal species to modulate responses via the PPAR-γ pathway.

9. *Defects downstream of the PRRs.* There are many different ways in which the mucosal immune system can respond to intestinal microbes. T_H1, T_H2, T_H17 type responses are recognized as distinct categories, but there is evidence for many more intermediate T helper types (Matzinger and Kumala 2011). Tailored immune responses require not only functional PRR signaling but also a reliable cytokine network. Key agents of the network system include IL-23, Il-22, IL-6 and IL-10, each playing a different role in the modulation of immune responses in the gut. This modulation is often found impaired in IBD. For example, the capacity of IL-23 to promote differentiation of Th17 cells has been shown to be impaired in IBD because of IL-23R polymorphisms (Duerr et al. 2006). CD and UC can also involve mutations in STAT3, a component of the pathway mediating IL-6 and IL-10 signaling (Barrett et al. 2008; Franke et al. 2008).

Hence, at least nine different classes of IBD-related risk factors can be identified. As mentioned above and in agreement with the view of Kaser et al. that understanding pathogenesis of IBD requires considering factors supporting this disease in their *totality* (2010), we now try to consider synergistic role of different risk factors in affecting the activity of the immune system, the recognition of microbes and the potential pathogenicity of otherwise innocuous commensals, thus leading to IBD. After having segregated IBD risk factors into classes according to the domain or function they alter, we can now group them further into those that influence the pathogenicity of a microbe and thus affect the immune system indirectly and those that affect it directly (Table 1).

Table 1 Domains and functions whose alterations are associated with IBD

Nr	Altered domain in IBD	Integrated role of the affected domains
1	Microbial structure	Pathogenicity-making factors
2	Chemical environment	
3	Activity of various bodily systems	
4	Microbial composition	
5	Pattern detection	Detection of pathogenicity-making factors, integration of information about these factors and calibration of an immune response
6	IEC functions	
7	Self-recognition	
8	Communication between microbes and immune cells	
9	Immune processes downstream of PRRs	

IBD is correlated with alterations in factors that define the pathogenic potential of gut microbes

We first focus on those risk factors that influence the pathogenicity of a microbe. They include structural features of gut microbes, the chemical environment of the intestine, the activity of host bodily systems and the composition of the microbiota. What is the integrated role of these factors? The answer is that they jointly define the disease-causing potential of intestinal agents:

1. *Structure* is an important pathogenicity-making factor in that it allows microbes to adhere, disseminate and penetrate the tissues. For example, flagellum can equip a microorganism with the capacity to penetrate.

2. *Chemical and physical environment* in the gut may influence the pathogenicity of microbes by inducing expression of virulence factors or by changing the phenotype of the immune response. For example. pH can control conversion of commensal bacteria into pathogens (De Bernardis et al. 1998). The chemical environment in the lumen can also influence virulence of gut microbes by promoting certain types of immune responses. One example is environmental control of Th17-mediated responses (Quintana and Weiner 2009). This type of response influences pathogenicity of gut microbes by, among other things, controlling the release of antimicrobial peptides by IECs and by influencing the integrity of the epithelium (Liang et al. 2006).

3. *The activity of various bodily systems of the host* determines the pathogenic potential of microbes by changing their chemical environment or by modulating the defense system. For example, psychological stress related to maternal separation has been shown to change the composition of gut microbes. This stress established the pathogenic status of otherwise opportunistic bacteria and promoted inflammation (Bailey and Coe 1999).

4. *The composition of the microbiota* is a well known pathogenicity-making factor. One, obvious mechanism by which the pathogenic potential of microbes is modulated by other microbes is through competition for attachment sites and for nutrients. Other mechanisms include quorum sensing, which involves release of molecules by bacteria that influence the gene expression of other microbes. In addition, some bacteria produce antimicrobial peptides that may restrain the proliferative capacities of other microbes.

Together, these observations indicate that being pathogenic or commensal is not only a matter of having a particular structure, but is determined by a complex interplay between various environmental factors and the host. IBD involves alterations in these pathogenicity-making factors. Indeed a shared feature of various forms of IBD is a shift in the status of microbes from commensal to pathogenic because of changes in their structural or contextual features (Chassaing and Darfeuille-Michaud 2011).

IBD is correlated with alterations in modules involved in the detection of pathogenicity-making factors

Secondly, we focus on those factors that affect the immune system directly. These can interfere with the capacity of the immune and non-immune system to monitor pathogenicity-making factors and to integrate information about them.

5. *MAMP detection by innate immune cells is required to adjust the host response.* Structural features of microbes are sensed directly by APCs and this detection influences the course of immune responses. As we have seen, this function is impaired by mutated PRRs in IBD.

6. *Metabolic functions of IECs have to be maintained to allow these cells to adequately respond to fluctuations in the concentration of luminal chemicals and to provide information about the topology of intestinal microbes.* Since chemical the environment in the gut influences disease-causing power of gut microbes, it is important for the IECs to respond to these microbial changes accurately. Defective responses to stress characteristic of IBD may interfere with this function (Garrett et al. 2010). IECs are functionally and structurally polarized; they promote different types of responses depending on their apical or basolateral stimulation (Lee et al. 2006). This polarization allows the immune system to promote different types of

responses depending on the localization of the microbe relative to the epithelium. In IBD, epithelial integrity may be compromised by mutations in genes coding for tight junction proteins and other proteins involved in barrier functions. This defect may facilitate access of non-pathogenic bacteria to the basal layer and so misinform the immune system about their invasive potential.

7. *Self-recognition is needed to evaluate the body's potential to control invasive gut microbes.* The host immune system itself profoundly influences the disease-causing potential of intestinal microbes. The capacity of the immune system to adjust its responses relative to its own defense resources is illustrated by the fact that adaptive immunity can compensate for deficiencies in host innate immunity (Slack et al. 2009). For example, IL-$10^{-/-}$, $MyD88^{-/-}$ mice, despite crippling of the innate system, are protected from colitis (Rakoff-Nahoum and Medzhitov 2008). One means by which the immune system can acquire information about the disease-causing potential of intestinal microbes by self-recognition is through detection of endogenous damage-associated molecular patterns (DAMPs) (Seong and Matzinger 2004; Quintana and Cohen 2011). Autoimmune factors involved in certain forms of IBD distort the self-recognition abilities of the immune system.

8. *Normal communication between gut microflora and GALT is needed to provide accurate information about the composition of the microflora.* The composition of gut microbes is a powerful pathogenicity-making factor. Since different groups of bacteria modulate IEC signaling in different ways, their composition is reflected in the pattern of IEC-derived products, promoting different types of immune responses (Rimoldi et al. 2005; Iliev 2009a; Iliev 2009b). The secretory functions of Paneth cells and enteroendocrine cells are controlled by direct recognition of bacterial cell products by receptors on the apical surfaces of these cells (Vaishnava

et al. 2008). Similarly, phagocytosis by M cells is modulated by microbial sensors (Kyd and Cripps 2008) and could be receptor-mediated (Hase et al. 2009). As we have mentioned, genetic defects in IBD often entail alterations in the modulation of signaling pathways in IECs by specific types of bacteria. These alterations interfere with the capacity to acquire information about the microbial composition of the gut and lead to inappropriate responses.

9. *Processes downstream of the PRRs* allow integration of information about factors that detect the pathogenic potential of microbes and tailor responses accordingly. Defects in genes coding for cytokines, chemokines and their receptors downstream of PRR signaling characteristic of IBD may perturb the capacity to integrate information about pathogenicity-making factors and lead to inappropriate responses.

All in all, defects in IBD alter the capacity of the system to acquire (or integrate) pieces of information about factors that define the disease-causing potential of intestinal microbes.

IBD viewed as chronic immune misrecognition at the systems level

Realization that inadequate responses in IBD are caused by failures to monitor changes in factors that define the disease-causing potential of intestinal microbes challenges the classical view of immune recognition and response. According to the classical view, immune responses are triggered by single types of receptors or single types of cells: "It is now generally accepted that recognition of foreign organisms or damaging circumstances by cells of the innate immune system, i.e. antigen-presenting cells (APC), triggers immune response initiation" (Barr et al. 2007).

However, studies of IBD bring us to realize that the decision to respond in a certain way is made collectively by many types of cells and molecules engaged in the global detection of pathogenicity-making or inflammatory-inducing factors. These cells and

molecules exchange signals and integrate information about structural and environmental features of microbes to tailor responses accurately (Fig 4A). Thus, immune recognition of a microbe as pathogenic or commensal is expressed by a complex, collective signaling process rather than by a single receptor ligation or cell activation. We refer to this systemic process of information integration and signal exchange as "co-respondence" (Cohen 2000a).

The systemic character of immune recognition has two advantages to the host. Firstly, it allows tailoring the immune response precisely to the conditions developing in the gut. This permits the maintenance of selected groups of bacteria, and restrains or eliminates the others. Secondly, it grants robustness. Since the decision to respond in a certain way is a product of a process of "deliberation" composed of many cellular and molecular agents, damage to one of these components has a limited impact on the overall response (Fig 4B,C). If information about the presence of MDP in the cell wall of a bacterium is missing, the immune system may still adjust its responses to the invasive capacities of the microbe by integrating information about other structural features of the microbe, its position relative to the intestinal tissues and the microbial, chemical and host environment. For example, the occurrence of a defect in NOD2, one of the most important risk factors in CD it is neither necessary nor sufficient for the disease. It is not sufficient because *Nod*$^{-/-}$ mice do not develop spontaneous colitis and most individuals with mutated NOD2 never develop IBD (Hugot et al. 2007). It is not necessary because fewer than 20% of CD patients are homozygous for the NOD2 susceptibility gene variants (Podolsky 2002). All the other genetic factors have been associated with very law frequencies. Only when the accumulation of genetic and environmental defects reaches a certain threshold, does immune recognition lose its robustness and promotes the chronic immune misrecognition and misbehavior we call IBD (Fig. 4D-F).

All things considered, IBD is a disease of the immune recognition system. Alteration in this system is difficult to restore because of its complexity and robustness.

The number of alternative defects sufficient for the expression of the disease may supersede the number of molecular elements involved in the system because *combinations* of molecular alterations are needed to compromise the recognition system. Thus, the resistance to therapy of IBD may have a similar basis to that of cancer (Kitano 2004).

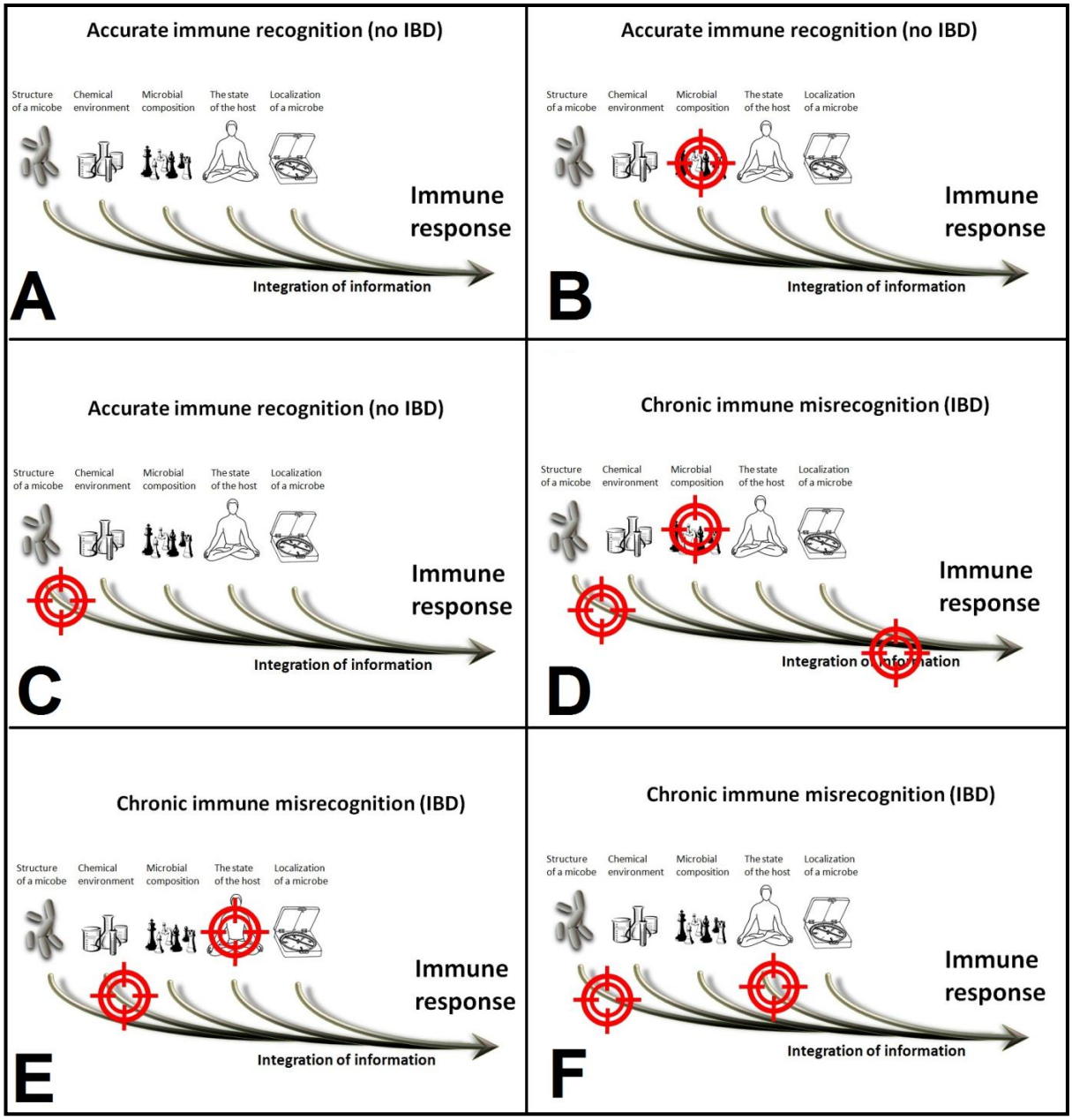

*Figure 4. Inflammatory bowel disease as a loss of robustness in the immune recognition system. (**A**) The power of the immune system to distinguish between pathogenic and nonpathogenic microbes depends on the capacity of the system to integrate information about factors that define the pathogenic potential of intestinal agents (they include structural features of the microbes, their localization and their chemical, microbial and host environment). (**B**) Alterations in one pathogenicity-making factor (e.g. a change in the microbial composition) can increase the invasive potential of intestinal agents. However, the immune system monitors these changes and adjusts its responses accordingly. (**C**) Genetic defects that alter the capacity to detect a single pathogenicity-making factor may not interfere with the overall potential to produce accurate responses; information about the remaining pathogenicity-making factors may be sufficient to estimate pathogenic potential of the microbes accurately (**D-F**) A coincidence of defects that alter the capacity to detect several pathogenicity-making factors or a single defect correlated with profound alterations in a pathogenicity-making factor may perturb the whole recognition system and lead to chronic immune misrecognition in the form of IBD.*

Conclusions

The capacity of the immune system to maintain homeostasis with the intestinal microbes depends on the ability of the system to adjust to changing chemical, microbial and self-induced alterations. Since these alterations modify the invasive potential of intestinal microbes, it is critical for the system to monitor these changes by means of built-in mechanisms that translate information about environmental conditions into tailored responses.

This complex architecture grants robustness. Loss of information about one pathogenicity-making factor may be compensated by unaffected pathways that measure other parameters of the intestinal agents. In IBD this robustness is lost because of defects

in *several* genes or because of profound environmental perturbations accompanied by defects that have already compromised the system.

Part 3

Systems level understanding of immune recognition and reduction

Our analysis in the first part of the thesis led us to conclude that systems level approach is needed to understand immune recognition. Here, an attempt is made to clarify this statement. Systems level approach is often contrasted with reductionist approach in the literature. However, there is also sense of reduction from the point of view of which systems biology approach is reductive.

Thus our aim in the rest of the thesis is to understand sense in which our explanation of immune recognition is reductionist and sense in which it is anti-reductionist. This clarification may not only expose the "anatomy" of our own explanation of immune recognition in the first part of the thesis but also elucidate the character of standard explanatory practices of systems biology and immunology.

Our analysis in the remaining part of the thesis consists of three steps. First, we clarify the concept of reduction by discussing some of the most influential models of reduction in recent philosophy of biology. Second, we explain sense in which systems biology approach counts as reductive. Third, we analyze reductionist aspects of systems level approach to understanding immune recognition and reveal some limitations of this approach.

Chapter I

The idea of reduction in philosophy of biology

In order to understand sense in which systems level approach to understanding immune recognition is reductionist and sense in which it is not reductionist, it is first necessary to make the idea of reduction possibly clear. In the present chapter we analyze various notions of reduction in philosophy of biology and explain what motivates them. This will help to find and motivate the notion of reduction best characterizing our own attempt to explain immune recognition in molecular and cellular terms.

Reduction: first approximation

Complex biological processes can be analyzed at distinct levels of granularity. For example, memory can be studied at the molecular level, in terms of signaling pathways controlling strength of synaptic connections between neurons (Kandel 2001). It may be studied at the cellular network level as a process distributed along various brain regions (Miyashita 2004). It can also be considered as a psychological process, whose identity is constituted by its relationship to other mental states such as mental images, believes and desires (Neisser 1997). Realization that a given process can be studied at various levels provokes the question of the relationship between these levels. Are they independent from one another? For example, are there any relevant aspects of memory storage and retrieval that could not be captured by most complete molecular knowledge of these processes? Reduction is an important concept helping to answer this and other related kinds of questions.

In the most general sense, reduction refers to an explanation in which one unit of knowledge (usually about higher level features) is subsumed under (or included in) another

unit of knowledge (usually about lower level features). Even though this definition is very broad, it does not cover all the usages of the term in philosophy. For example some authors consider a type of reduction that is not an explanation. Nickles takes into account, what he calls "domain preserving" reduction which is a relationship between historically earlier and historically later theories. This kind of successional reduction does not seem be a subspecies of explanation because historically later theories often do not explain earlier theories (cf. Nickles 1973; Sarkar 1998, p. 45). However, even if we limit our discussion to reductionist explanation, the above general characterization will give us only a vague idea of what reduction is because it does not specify what the relevant units of knowledge are and how the subsumption or inclusion should be understood. Here we discuss some of the most important notions of reduction in philosophy of biology to understand better what reduction is.

Theory reduction: Nagel

According to some scholars, philosophical debate about reduction in biology was initiated by Aristotle (Brigandt and Love 2008). Other authors suggest that it has rather began as late as in the seventeenth century (Sarkar 1998, p. 16). However, there is no doubt that the modern version of the reductionism/antireductionism debate was initiated by Ernest Nagel in the middle of the XX century. Ernest Nagel worked in the context of post-positivist philosophy of science. Post-positivist philosophy of science, as well as its predecessor, logical neopositivism, emphasized the need for formal, logical analysis of the scientific discourse. One of its tenets was the idea that physics is an ideal, prototype science. Thus also Nagelian account of reduction was focused on formal aspects of reduction and issues particularly relevant for physics.

According to Nagel, reduction is a relationship between two theories, T_1 and T_2 such that T_2 is logically derivable from T_1. In particular, reduction requires *laws* of the theory T_2 to be deducible from the *laws* of T_1. For example, thermodynamics can be said to

be reducible to statistical mechanics if the laws of thermodynamics can be derived from the laws of statistical mechanics. For Nagel, reduction was also a form of intertheoretic explanation (1961): reducing theory (T_1) *explains* the reduced theory (T_2).

From the logical point of view, one theory cannot be derived from another theory unless the vocabulary of the reduced theory is already included in the language of the reducing theory. Having this in mind, Nagel considered two forms of reduction: homogenous and heterogeneous. In homogenous reductions, predicates of the reduced and the reducing theory have the same meanings and thus their vocabularies are already reconciled. In heterogeneous reductions, reduced theory contains terms (predicates) that do not appear in the language of the reducing theory (for example "heat" is part of the language of thermodynamics but not of the language of statistical mechanics even though thermodynamics is reducible to statistical mechanics).

According to Nagel, heterogeneous reductions require statements that connect the vocabularies of the reduced and the reducing theories. These statements must *fix* for example that heat is mean molecular kinetic energy in the language of statistical mechanics. Otherwise, laws concerning heat in thermodynamic cannot be derived from statistical mechanics. Thus, in heterogeneous reduction, the reducing theory reduces the reduced theory but only with such statements (Nagel 1961, pp. 353-354). This condition was labeled by Nagel "condition of connectability". Statements linking languages of the reduced and the reducing theory have been traditionally referred to as bridge principles or reduction functions.

The idea of bridge principles connecting vocabularies of the reduced and the reducing theory initiated an intense debate in philosophy of science. Many authors have attempted to specify character of these statements. Some claimed that they should be considered as analytical (definitional), others argued that they are synthetic (factual). Nagel himself did not specify exactly how these bridge principles should be interpreted. Instead he considered three different possibilities. First, that they are logical connections linking

meanings of expressions of two theories (this includes the relations of synonymy). Second, that they are conventions, established in some historical contexts. Third, that they are factual statements that must be discovered empirically (Nagel 1961, p. 354). In order to decide which of the three interpretations reflects the actual connections between vocabularies of the reduced and the reducing theory in the scientific practice, Nagel considered the relationship between "temperature" in classical thermodynamics and "mean kinetic energy of molecules" in statistical mechanics. He found that the relationship between these two statements cannot be analytical because these expressions are not synonymous. However, he did not solve the problem which of the remaining two interpretations of connectability statements is applicable in this case.

Ernest Nagel focused on formal issues related to reduction. However, as Kenneth Waters emphasized, he was well aware of the importance of the non-formal aspects of reduction (Waters 1990, p. 126). In particular, Nagel claimed that the conditions of reduction such as derivability and connectability do not by themselves assure that the reduction is scientifically fruitful. He speculated that non-trivial reductions may involve formulation of laws with a broader scope and unexpected associations between already established laws (Nagel 1961, p. 358; Waters 1990, p. 126).

Sarkar mentions three possible reasons why theory reduction by Nagel was an attractive option for post-positivist philosophers. Firstly, it was relatively simple. Secondly, it was a modification of a deductive-nomological notion of explanation that was widely accepted in the neopositivist circles. Thirdly, it was focused on the epistemic aspect of reduction which reflected antimetaphysical approach of neopositivism (Sarkar 1998, p. 26).

Theory reduction: Schaffner

One of the arguments against the model of reduction by Nagel was that it cannot accommodate the fact that the reduced theory often contains false statements (Feyerabend 1962). Indeed, one reason why reduction of a historically earlier by a historically later

theory takes place is that the former is often less accurate than the later. It has been argued that even in the context of interlevel reduction, it is often impossible to derive the exact laws of the reduced theory from the more fundamental reducing theory. If the reducing theory is assumed to include statements that are true, it cannot logically entail a theory containing false statements. Such a derivation is impossible because the relation of deduction is truth-preserving.

In order to solve some of the problems of the Nagelian notion of reduction, Kenneth Schaffner proposed General Reduction-Replacement (GRR) model. This model can be considered as a development of the original Nagelian model in that it incorporates many fundamental assumptions about reductionist relationship elaborated by Nagel. For example, GRR model inherits the idea that reduction requires derivability as well as connectability. One important difference between the GRR model and the Nagelian model is that the reducing theory (T_1) it not assumed to entail the original reduced theory (T_2) but its corrected version (T_2*). The corrected version does not contain false statements from the point of view of T_1. (Thus, it allows to formulate more accurate predictions than T_2).

The idea that the reducing theory entails a corrected version of the reduced theory rather than its original version, helped to understand why reduction occurs between the two theories even though the reduced theory contains false statements. An important question in this context was one of the relationship between the reduced theory and the corrected version of the reduced theory. Schaffner emphasized that the relationship between T_2 and T_2* must be of a strong analogy. This analogy allows one to maintain, for example, that the theory T_2 is reducible to T_1 even though it is really the theory T_2* that is reducible.

Another contribution of Schaffner to the reductionist debate was clarification of the concept of bridge principles. He referred to this principle as reduction function and claimed that it is a function that makes predicates of the reduced (in fact corrected version of the reduced) and the reducing theory coextensional (Schaffner 1993, p. 443). In particular, he believed that reduction functions have to express synthetic identities, like those between a

gene and a fragment of the DNA molecule coding for a protein or RNA in molecular biology (Schaffner 1976, pp. 614-615).

Schaffner's version of the theory reduction is more appealing than the original model by Nagel because it is more realistic. It takes into account that the reduced theory often incorporates false statements. It also clarifies many concepts that have not been fully developed by Nagel.

Even though theory reduction by Schaffner was more realistic, it still faced some problems. One of the problems was that it focused on formal issues related to reduction, such as the logical form of reduction, rather than substantial issues. It was also limited by the fact that it considered relationship between theories rather than other epistemic units. Finally, it did not explicate the relation of strong analogy that was central to this model of reduction.

The above and other problems of theory reduction by Nagel and Schaffner prevented from their application to biology. In fact, there is a consensus in the field of philosophy of biology that reduction in the sense of theory reduction normally does not take place (Brigandt and Love 2008). Firstly, it is difficult (if not impossible) to reconstruct reasoning patterns in biology formally. Secondly, biology does not employ theories in the sense presupposed by theory reduction. Thirdly, biologists do not seem to bother to establish relationships of strong analogy between different epistemic domains to explain biological phenomena.

Even Schaffner, in his recent papers, admits that the requirements imposed on reduction by these early models were too stringent (2006). He proposes now that reduction in biology is fragmentary; it does not involve entire theories but rather simpler epistemic units such as models or representations of mechanisms. In addition, he suggests that biological explanations are usually interlevel in the sense that they incorporate elements of different scales (Schaffer 2006, p. 384). Indeed, as we have seen in the first part of the thesis, immunological explanations appeal to interactions between cells and molecules,

even though cells are themselves constituted by molecules and thus belong to a different scale than the molecules. For example, cytokines are said to change the behavior of cells and cells are said to release cytokines.

Explanatory reduction: Wimsatt

Problems of theory reduction inspired scholars to search for alternative notions of reduction. In particular, it became clear that the notion of reduction has to take into account relationships between epistemic units other than theories. The pioneer of this new way of thinking about reduction was William Wimsatt. For him, reduction was an explanation of higher-level entities in terms of the properties and relationships between lower-level entities. The lower-level entities are constituents of the higher level ones (Wimsatt 2007 (1976), p. 249). Wimsatt rejected deductive-nomological account that appeals to explanations in terms of laws (Hempel and Oppehneim 1948). Instead, he took it for granted that scientific explanation may well appeal to causal mechanisms or causal factors (Wimsatt 2007 (1976), p. 249).

The idea of reduction by Wimsatt was inspired by Wesley Salmon's statistical relevance model of explanation. This model is based on the intuition that only those properties that are statistically relevant are explanatory. Property C is statistically relevant to a property B if the probability of B given C and A is different from the probability of A: $P(B|A.C) \neq P(B|A)$. To put it simply, property C is statistically relevant and explanatory if it makes a difference (Salmon 1971).

Important for the Salmon's account of explanation is the notion of homogenous partitioning. Roughly, property A is homogenously partitioned with respect to B if its subclasses (C_1, C_2, …, C_n) are mutually exclusive and exhaustive and no other statistically relevant partition of the subclasses can be made with respect to B. Intuitively, there should be no other factors statistically relevant to B than the factor under consideration. For example, partition of a reference class into smokers and non-smokers is homogenous with

respect to lung cancer, if no other factors are involved in the risk of developing this type of cancer (Papineau 1989).

The notion of homogenous partitioning helps to avoid Simpson's paradox. Simpson's paradox is one in which correlation between certain groups seems to be different when the groups are combined. One illustration is the case of Berkley university which was once accused of gender bias. The accusation was based on the fact that the percentage of women admitted to the university was found to be smaller than the percentage of admitted men. However, after homogenous partitioning of candidates into groups according to the faculty they were applying to, it became clear that there was no gender bias because women were choosing more competitive departments.

Another important notion in the statistical relevance model of explanation was "screening off". Salmon introduced this notion to distinguish between cases of mere statistical correlation and causation. He gave the following example. Atmospheric pressure A explains the occurrence of the storm S. If this is the case, there must be a statistically relevant association between S and A. However, there appears to be also similar association between S and the reading of a barometer B. How do decide which property is explanatory, A or B? In order to solve this problem, one should note that $P(S|A.B) = P(S|A)$ (B is not relevant to P) but $P(S|A.B) \neq P(S|B)$ (A is relevant to P). In this case A is said to *screen off* B.

Wimsatt applied the model of explanation by Salmon to *reductive* explanations. In reductive explanations, homogenous partitioning of the reference class is a partitioning of lower-level realizations that constitute a given phenomenon. Reductive explanation should be understood as demonstration of how a given phenomenon "is a product of causal interactions at lower levels" (Wimsatt 2007 (1976), p. 258). According to this interpretation, microstate C is explanatory in regard to the macro-state B if the probability of B given C and a macro-law A is different from the probability of the macro-law A.

Thus, according to Wimsatt, there is no need of reduction if there is no deviation from regularity described by a macro law. For example, the ideal gas law can serve as a sound explanation of behavior of some concrete gas sample if this gas sample obeys this law. However, if we consider case of gas that does not obey the ideal gas law (there is some upper level anomaly), we have to appeal to lower level description to explain it. In the former case the probability of a macro-state given the macro-law is the same as the probability of the macro-state given the micro-state realization. In the latter case the probability of the macro-state given the macro-law is different than the probability of the macro-state given the micro-state realization. In this case, micro-state description *screens off* the macro-state description.

One should note that on the grounds of the statistical relevance model of explanation, a given property can be explanatory even if the probability of making a difference by this property is very small. This is a feature of the Salmon's model that Wimsatt tried to improve. He argued that if the improvement in the characterization of the macro-state B by microstate description C in comparison with the macro-state description A is very small, than A effectively screens of C. Thus, even if C *screens off* A, A *effectively screens off* C. Another case in which A *effectively screens off* C is when the information provided by C is much more expensive to get than the information provided by A (Wimsatt 2007 (1976), p. 258). According to Wimsatt reduction occurs if C *effectively screens off* A.

There are many reasons why Wimsatt's account of reduction can be considered as more appealing than the models of theory reduction. Firstly, it permits other relata of the reductionist relationship than theories and thus extends the range of possible applications. Secondly, the model of reduction by Wimsatt focuses more on substantial issues than formal ones.

Despite many advantages, Wimsatt's model of reduction is still hard to accept because it is based on a controversial model of explanation. Many different arguments

have been put forward against statistical relevance model of explanation. For example, it has been argued that in biomedical sciences it is practically impossible to partition a reference class homogenously to compare difference-making power of various factors. It has also been suggested that the theory does not provide sufficient means to distinguish between correlation and causation (reviewed in: Woodward 2010).

In immunology, an explanation in terms of statistical relevance is not satisfactory. For example, finding that functional specialization of APCs in the intestine of mice depends on whether the mice come from Charles River Laboratory or Jackson Laboratory even though statistically relevant (Denning et al. 2011) is not by itself explanatory. An explanation would be provided if the *causal* factors responsible for the functional difference of APCs between these two groups of mice were given. Thus, crucial for immunological explanation is finding causal explanations for statistically relevant associations between properties involved in immunological processes.

Explanatory reduction: Sarkar

Sahotra Sarkar has developed a detailed model of reduction that seems to reflect the actual explanatory practice in life sciences (1998). He explicitly focused on substantial issues of reduction to deepen the understanding of explanation in classical genetics and molecular genetics.

His analysis of reduction is based on the intuition that many forms of reduction are explanations of wholes in terms of their parts (cf. Beatty 1990, p. 201-202). In addition, representations of the wholes are assumed to belong to a different epistemic domain than the representations of the constituent parts. Thus, by reducing the wholes to their parts, one makes a step towards unification of knowledge (Sarkar 1998, pp. 39-40).

These general observations about reduction lead Sarkar to distinguish between three features of reductionist explanations: fundamentalism, abstract hierarchy and spatial hierarchy. Fundamentalism is an appeal to more fundamental properties to explain some

phenomenon. For example, an adaptive immune response towards an antigen can be explained in terms of more fundamental process of clonal selection of antibodies. Abstract hierarchy means that a system to be explained is represented as hierarchical according to some criterion, but the lower levels are *not necessarily* mereological parts of the higher levels in the hierarchy. For example, it is commonly distinguished between four levels of protein structure, even though lower levels are not component parts of the higher levels. Even though the sequence of the amino acids (primary structure) determines specific pattern of hydrogen bonds (secondary structure), it is not, by itself, part of the pattern. Finally, spatial hierarchy is a representation of a system as being made up of compositional levels. That is, each lower level is assumed to be made up of parts of the higher level. For example, an organism can be considered as belonging to one level, cells that make up this organism can be considered as belonging to another level, molecules that constitute all of these cells can be considered as belonging to yet another level (Sarkar 1998, pp. 43-44).

The above three features of reductionist explanations, fundamentalism, abstract hierarchy and spatial hierarchy are used by Sarkar as criteria for distinguishing between five different types of reduction: weak reduction, approximate abstract hierarchical reduction, abstract hierarchical reduction, approximate strong reduction and strong reduction. Each of these different types of reduction satisfies one or more of the three criteria and some of these criteria can be satisfied only approximately. For example, weak reduction for Sarkar is a form of fundamentalist explanation that is neither abstract-hierarchical nor spatial- hierarchical explanation. For other types of reduction by Sarkar and the criteria they satisfy see table 2.

Table 2 Types of reduction by Sarkar (1998, pp. 44-45)

Criterion	Fundamentalism	Abstract hierarchy	Spatial hierarchy

Type of reduction			
Weak reduction	Satisfied	Not satisfied	Not satisfied
Approximate abstract hierarchical reduction	Approximately satisfied	Satisfied	Not satisfied
Abstract hierarchical reduction	Satisfied	Satisfied	Not satisfied
Approximate strong reduction	Approximately satisfied	Satisfied	Satisfied
Strong reduction	Satisfied	Satisfied	Satisfied

All in all, the model of reduction by Sarkar provides a framework that allows one to classify various forms of reductionist explanations in biology according to the character of the *representations* involved in the explanation and the form of the *relationship* between these representations. Sarkar himself applied this framework to understand the character of explanation in classical genetics and molecular biology. He came to conclusion that classical genetics involves abstract hierarchical reductions and molecular biology involves spatial hierarchical reductions (1998).

Explanatory reduction: Waters

Kenneth Waters considers the problem of reduction in the context of the relationship between classical genetics and molecular genetics (1990; 1994). He points out that there is a consensus according to which classical genetics is irreducible to molecular genetics. One reason why these two branches of genetics are considered irreducible is that the concept of gene employed by classical genetics seems to be radically different from the one in molecular genetics (Waters 1990, p. 126).

However, according to Waters, the classical concept of gene is often misunderstood by many philosophers. For example, David Hull suggests that the relationship between genes and phenotypic features was considered as relatively simple and direct by classical geneticists (1994, p. 29). However, in fact, geneticists working at the beginning of the twentieth century were aware that a single phenotypic feature may be determined by many genes. They were also aware that a single gene may affect many different phenotypic features. Thus, against some interpretations, it was taken for granted in classical genetics that the relationship between genotype and phenotype was many-many and not one-one (Waters 1990, p. 129). In addition, genes were understood as abstract rather than concrete biochemical entities and no real attempt was made by classical geneticists to explain the contribution of genotype to the phenotype (Waters 1994, pp. 169-171).

Even though the material character of genes was not known, it was accepted in classical genetics that gene differences cause phenotypic differences. In other words, it was assumed that genes are difference-making factors. Since this general view of genes, as difference-makes, has not changed for a long time, it can be considered as a conceptual link connecting classical genetics with the modern, molecular genetics. Specific contribution of molecular genetics to the idea of gene was that it introduced molecular level understanding of it. All of this lead Waters to suggest that the relationship between classical genetics to molecular genetics is that of an explanatory reduction (Waters 1990; Waters 1994, pp. 182-184).

In his work on genetics, Waters does not define the notion of reduction explicitly. However, one can try to reconstruct this notion on the basis of his analysis of the relationship between classical genetics and molecular genetics. First of all, what Waters considers as a proof of reduction of classical genetics to molecular genetics is the fact that "the behavior of specific Mendelian genes *has been explained by identifying them* with relatively short segments of DNA which function as units to influence the course of chemical reactions within a biochemical system" (Waters 1990, p. 130). This fragment

seems to suggest that according to Waters reduction is an *explanation by identification*. Indeed, the intuitive cases of reduction such as "Water is H_2O" or "Temperature is mean molecular energy" seem to be of that type. Another important feature of reductionist explanations according to Waters is a conceptual link between the reduced and the reducing level. For example, classical genetics and molecular genetics have been argued by Waters to be linked by fundamental assumptions about a gene, such as the one that says that gene is a phenotypic difference-maker. If there was no such conceptual link, the reduction of one level of understanding to another would be impossible (cf. Waters 1994). Finally, Waters allows other units of reductionist explanations than theories. In particular he considers reduction as a relationship between *levels of understanding.* By so doing, he rejects Nagel-Schaffner model of reduction and embraces a version of explanatory reduction. He also criticizes post-positivist emphasis on formal, syntactical aspects of reduction (Waters 1990, p. 130).

Apart from applying an informal model of reduction to elucidate the relationship between classical genetics and molecular genetics, Waters also attempted to challenge some interpretations of the fundamental scientific aims of life sciences. In particular, he questioned a widespread philosophical view that research programs within biology apply, extend and validate some central theory. Indeed, it has often been suggested that classical genetics attempted to apply, extend and validate certain explanatory theory of gene transmission. However, detailed historical analysis made Waters to conclude that classical genetics rather aimed to study many different phenomena and the theory of inheritance and gene transmission served as *means* for advancing knowledge about these other phenomena. Thus, the central aim of classical genetics was not to explain inheritance patterns but, instead, it was an open-ended aim of developing new knowledge about many different phenomena by *means* of knowledge about inheritance patters (Waters 2004, p. 792). The observation that classical genetics was not focused on developing and testing one central theory is in agreement with an attempt of Waters and other authors to change the focus of

contemporary philosophy of science from theories to the actual investigative and explanatory patterns in life sciences. It also supports the view that reduction can be considered as a relationship between epistemic units other than theories (Waters 2004, p. 807).

Methodological reductionism

Each model of reduction is an attempt to formulate the notion of reduction in the way that reflects the actual explanatory practice of science (in our case, biology). However, it is one thing to claim that a given domain of biological knowledge is reducible (in some sense) to another domain and another thing to actually pursue this kind of reduction. For example, we could easily imagine a person claiming that psychological studies of memory storage and retrieval are in principle reducible to neuroscience but the reduction should not be pursued because the potential benefits from such a reduction would be incomparably smaller than the effort that would have to be taken to actually execute it.

The idea according to which certain model of reduction should be followed as a fruitful explanatory practice is referred to as methodological reductionism (Brigand and Love 2008). For each model of epistemic reduction there is a methodological counterpart claiming that this particular version of the reduction should be followed.

Selection of and motivation for the model of reduction that suits the case of immune recognition

So far, we have described various models of epistemic reduction. We have distinguished between theory reduction and explanatory reduction. We have also mentioned methodological reductionism, a recommendation to apply certain form (or forms) of reduction in the scientific practice. Now, we are ready to choose and motivate the idea of reduction that may be considered against our own attempt to explain immune recognition in the first part of the thesis.

First, consider theory reduction. We have already pointed out that there are serious obstacles to apply this version of reduction to biology in general. Theory reduction is based on the deductive-nomological account of explanation. From the point of view of this account, the *explanandum* is an event to be explained and the *explanans* are laws and conditions that precede the event in question (Hempel and Oppehneim 1948). Hempel and Oppenheim considered this model of explanation as ubiquitous in science. However, our account of immune recognition does not incorporate laws. Instead it appeals to generalizations of limited scope. For example, in our discussion of IBD we have emphasized that CD is often correlated with polymorphisms in the NOD2 gene. However, fewer than 20% of CD patients are homozygous for the NOD2 susceptibility gene variants (Podolsky 2002). On the top of that, there is no evidence for a correlation between NOD2 polymorphisms and IBD in patients of Asian and African origin (Cho and Abraham 2007). Nevertheless, "The identification of NOD2 (CARD15) as a susceptibility gene for CD was a highly significant advance in our understanding of CD pathogenesis and in complex disease genetics as a whole" (Brant and McGovern 2005).

Hence, models of explanatory reduction seem to be better suited to our case. First consider the model of reduction by Wimsatt. We have already mentioned that this model of reduction can be applied to epistemic units other than theories. Thus, potentially, it can be applied to our account of immune recognition. However, problems with statistical relevance model of explanation prevent from considering the model of reduction by Wimsatt as correct representation of the explanatory practice in biology in general and immunology in particular.

Even if the statistical relevance model of explanation proved to be correct, there are still some fundamental problems that prevent from embracing Wimsatt's model of reduction. In particular, it is not clear whether the version of reduction by this author is ontological or epistemic. Wimsatt writes that for him reduction is a relationship between levels of *organization*, features of the *world.* He emphasizes that higher levels of

organization are composed of lower level parts. (Wimsatt 2007 (1976), p. 249). All of this could suggest that Wimsatt focuses on *ontological* reduction rather than *epistemic* one. On the other hand, however, the levels of organization are defined by him in quasi-epistemic terms as "local maxima of regularity and predictability in the phase space of different modes of organization of matter" (Wimsatt 2007 (1976), p. 249). This peculiar fusion of ontology and epistemology is also present in the Wimsatt's characterization of complex systems (Wimsatt 2007 (1994)). He claims that levels of organization are made up of patterns of causal networks. However, he also maintains that the very same levels (ones that are made up of causal patterns) are also special cases of *perspectives* that, he admits bluntly, have subjective character (cf. Wimsatt 2007 (1994), p. 200, 227, 229; Wimsatt 2007, p. 358). Thus, perspectives for Wimsatt are ontological structures (cf. Wimsatt 2007 (1994), p. 205) with subjective, epistemological features like "being from a point of view" (Wimsatt 2007 (1994), p. 222, 227).

Epistemic and ontological questions of reduction and reducibility of immune recognition can potentially give entirely different answers (Sarkar 1998, p. 22). Therefore, these two domains have to be carefully separated. As we have mentioned at the beginning of the thesis, immune recognition is taken to be metaphysically identical to molecular and cellular level processes. What is unsettled in our context is the question if our idea of immune recognition can be reduced to the knowledge of lower level processes. Since the model of reduction by Wimsatt does not distinguish sharply between levels of analysis and levels of organization, we cannot apply his model of reduction to our case.

Consider now the models of reduction by Waters and Sarkar. Waters applies a notion of reduction that is largely informal. For him reduction is an *explanation by identification.* In particular, what is identified in reduction are levels of understanding. At the first glance, or account of immune recognition is of that type. One level of understanding consists of our general observations regarding initial stages of immune activation. Another level is the level of detection modules. The third level is the level of

interacting cells and molecules. We have explained the immune recognition by identifying it, first with a dynamic network of detection modules and second, with a network of complex interactions between cells and molecules. An important ingredient of the notion of reduction by Waters is the need for a conceptual link between the reduced and the reducing level of understanding. What links together the systems level notion of immune recognition and the molecular level notion of the same process is the idea that they are involved in the calibration of an immune response and that they are in fact determinants of this response. Similarly as in the problem of genetics considered by Waters, the molecular notion of immune recognition somehow modifies the original, systems level notion, nevertheless there seems to be some core idea of immune recognition that is preserved in the molecular level and systems level analysis.

Also, the idea of explanatory reduction by Sarkar seems to be applicable to our example of immune recognition. As we have mentioned, for Sarkar, reduction includes explanations of wholes in terms of their constituent parts. In addition, the wholes and the parts are taken to belong to different epistemic domains. Our account of immune recognition seems to be of that type. Immune recognition is a complex process and interacting detection modules can be considered as parts of this process. Moreover, immune recognition is assumed to be explainable in terms of interaction between these modules. However, more complete explanation would be provided by an explanation in terms of interacting cells and molecules. This is because the interacting modules are constituted by nothing but cells and molecules and their interactions. Again, there is nothing at the level of modules and immune recognition itself that would not be non-cellular or non-molecular.

Conclusion

In the above chapter, we have analyzed some of the most influential notions of reduction developed in philosophy of biology for the last 60 years. This analysis brought us to repeat

after many authors that the initial models of reduction, especially those concerned with theory reduction are not applicable to biological sciences. They have been developed in the context of physics and they invoke exceptionless laws and theories. In contrast, biological sciences do not employ theories in the sense presupposed by these early models (Kitcher 1984). In addition they do not involve laws in the strict sense but rather generalizations of a limited scope. All of these fundamental problems in applying models of theory reduction to biology also prevent from considering them as reflections of immunological explanation of immune recognition in the first part of the thesis.

However, recent developments in the reductionist debate have come out with models of reduction that focus on the actual explanatory patterns in life sciences. Nevertheless, only those models that distinguish between ontological and epistemic aspects of reduction can be considered against our case of immune recognition. It is because questions about ontological and epistemic reducibility of immune recognition could potentially give entirely different answers. At the first glance, our own attempt of immune recognition is well reflected by the notion according to which reduction is an explanation of one level in terms of another level.

Chapter II

Reducibility of systems level understanding of biological phenomena

In the first part of the thesis we emphasized need for systems level understanding of immune recognition. Here we clarify that there is nothing in this statement that would suggest that molecular and cellular level understanding of this process fails. Quite the contrary, systems level understanding *is* an understanding in terms of complex interactions between constituent cells and molecules.

Thus, taking into account some of the notions of reduction discussed in the previous chapter, we argue that there is a sense in which systems level understanding of immune recognition is *reducible* to molecular and cellular level understanding of this process. Indeed, we suggest here that reductionist explanations are ubiquitous in systems biology. This in turn brings us to realize that there is no fundamental methodological difference between systems biology and molecular biology. Indeed, as we shall see, the core explanatory approach of systems biology and molecular biology is similar. In particular, both branches of biology explain wholes in terms of their constituent parts and dynamic interactions between these parts. The difference lies in the extend of dynamic interactions and the number of parts that are included in the explanation. The observation that systems biology is no less reductionist than molecular biology will help us understand character of the postulated systems level approach to understanding immune recognition in the last chapter.

The idea of reduction in philosophy and systems biology

The statement that systems level understanding of immune recognition reduces to molecular and cellular level understanding of this process might come as a surprise to some readers. We have spent a lot of time arguing for complexity of immune recognition to end up promoting reductionism about the recognition. Is not it self-contradiction?

Many biologists consider reductionist approach and systems approach as opposite. The reason is that they employ a different notion of reduction and non-reduction from a philosophical one. As we have seen, most philosophers understand reduction as a relationship between two domains of knowledge (one more fundamental than the other) such that the more fundamental domain is used to explain phenomena the other domain refers to (Brigandt and Love 2008). In contrast, biologists often understand reduction as a form of oversimplification:

> "By grasping simple causes, we free ourselves of the annoyance of the complex. Reductionism is the principle of replacing perplexity (complex questions) with what passes for understanding (simple answers)" (Cohen 2000, p. 11).

> "A major problem is that the reductionist approach can promote overly simple thinking, with a focus on the single connection under study that ignores the multiplicity of other influences impinging on the pathway in question and the modulation of distant network properties when the chosen element is manipulated" (Benoist et al. 2006).

> "Biological systems are extremely complex ... The reductionist approach—although successful in the early days of molecular biology—underestimates this complexity ... " (Van Regenmortel 2001, p. 1016).

More specifically, biologists often understand reduction as an explanation of a complex biological phenomenon in terms of *isolated, individual* parts without taking into account complex interactions between these parts.

> "Biology has traditionally followed reductionist approach in which individual components of a living system are studied *separately*. It is becoming clear that we need to reverse the process and to study how these components *interact* to form complex systems using an integrative approach" (Palsson 2000, p. 1147) (Emphasis mine).

> "In the reductionist approach, researchers attempted to understand a complex entity (e.g. a cell, an organ or a disease) by breaking it down into smaller, more tractable units for study, such as genes, complexes or pathways. The reductionist approach was characterized by the concept that a system is the sum of its parts and simply identifying and characterizing these parts would be sufficient to generate predictions about the system's behavior" (Gardy et al. 2009, p. 251).

> "It became apparent that a holistic rather than a reductionism approach for understanding in biology is imperative: not only how many genes there are or even how they are connected but how they interact to result in observed behaviour of the overall system" (Mesarovic et al. 2004, p. 19).

Thus, in the light of the discussion in the previous chapter, reduction in the biological sense appears to be a subcategory of reduction in the philosophical sense. Explanatory reduction says that a representation of higher level phenomena can be explained by a representation of lower level phenomena (Brigandt and Love 2008). This also includes cases considered by systems biologists when higher level phenomena are explained in terms of single molecular parts or many static individual parts.

The philosophical concept of reduction is broader because it embraces not only cases of explanation in *simple* terms but also explanations in *complex* terms. Indeed, as we shall see, many philosophers accuse reductionist approaches for making things *too* complicated (cf. "gory details" objection against reductionism by Kitcher 1984, cf. also discussion in Waters 1990). All in all, biological concept of epistemic reduction is narrower than the similar concept in philosophy.

What about the corresponding ideas of non-reduction, such as holism and emergence? Are these concepts also understood by biologists differently? Analysis of systems biology literature reveals that these non-reductionist concepts often fall under the scope of the concepts of reduction in philosophy. For example, the idea of emergence in systems biology includes many forms of epistemic reduction in the philosophical sense. Systems biologists often understand emergence as unpredictability or unexplainability of the whole on the basis of the knowledge of *isolated*, *individual* parts;

> "The holistic approach is based on the idea that complex wholes cannot be understood by a study of the *isolated* parts. It is argued that when many components are put together, especially with interactions that are nonlinear, there are new emergent properties which can only be comprehended in the context of the whole system" (Brenner 2010, p. 208) (Emphasis mine).

> "Complex systems display properties, often called 'emergent properties' that are not demonstrated by their *individual* parts and cannot be predicted even with the full understanding of the parts *alone*" (Zak and Aderem 2009, p. 265) (Emphasis mine).

> "Significant efforts are underway to understand key pathway and organism-level responses by relying on the emergent properties of global gene and protein expression data (that is, the properties of the system as a whole that cannot be predicted from the parts)" (Butcher et al. 2004, p. 1254).

So understood concepts of emergence are broad enough to incorporate not only corresponding philosophical concepts but also many ideas of epistemic reduction that require a whole to be explained in terms of many parts and *interactions* between these parts. Emergence in philosophy means something different. It is often defined as "a failure of any possible explanation of a whole in terms of its parts and their *relations*" (Schaffner 2006, p. 382) (emphasis mine). This failure can have different sources, which is illustrated

by a diversity of various philosophical stances about reduction (cf. Bedau and Humphreys 2007).

All things considered, the concept of epistemic reduction in biology is narrower than the similar concept in philosophy. On the other hand, anti-reductionist concepts in biology such as holism or emergence are understood broader by biologists and incorporate cases of reduction considered by many philosophers. In the remaining part of this chapter we focus on the actual method of systems biology to understand if it is only the *concept* or also *the actual practice* of systems biology that can be considered reductionist from a philosophical point of view.

Methodology of molecular biology and systems biology

As we have seen, systems biologists and philosophers of biology tend to understand concepts of reduction and non-reduction in different ways. We have seen that instances of explanations in terms of complex interactions between parts of a given process are often considered as anti-reductionist by biologists. On the other hand, the same types of explanations are often considered as hardcore reductionist by some philosophers. How does this understanding of "reduction" and "non-reduction" translates into the actual practice of biology?

Systems biology promises to give an antireductionist account of biological systems. It also promises to explain how emergent properties arise in these systems (Zak and Aderem 2009). Here we look at the actual practice of systems biology to understand if systems biologists *really mean* methodological antireductionism (in the philosophical sense) when they insist on the antireductionist approach. In order to understand it, we shall compare systems biology approach with molecular biology approach because systems approach is often represented as reductionist by systems biologists and contrasted with the systems approach that is allegedly non-reductionist (or even anti-reductionist).

Methodological approach of molecular biology (a case study)

Systems biologists often distance themselves from molecular biologists by claiming that their aim is to understand the *structure* and *dynamics* of biological systems. They promise to provide an explanation of how parts of a system dynamically interact to give rise to various systemic properties (Kitano 2002). Is this aim of systems biology truly different from the one of molecular biology? It appears that molecular biologists also attempt to provide an account of the *structure* and *dynamics* of biological systems. They also try to explain how parts of a system dynamically interact to give rise to systemic properties (even though they do not label these properties as "systemic") (cf. De Backer et al. 2010 pp. 34, 40).

Molecular biology is sometimes represented as an enterprise whose aim is to *catalogue* biological parts (Powell 2004) or to analyze complex phenomena in terms of *single* or *static* molecular constituents (Noble 2008; Smaglik 2000, p. 828). However, even if molecular biologists sometimes catalogue parts they do so only to provide a dynamic explanation of how these parts interact to give rise to a given phenomenon. It is impossible to study interactions between molecules without first constructing a library of molecular components, interactions and responses (Ideker 2004). Systems biology itself contributes to this step by helping to complete the list of molecular components and responses (Ge et al. 2003) and by providing a catalogue of molecular interactions (Milo et al. 2002).

In order to see how molecular biology explains systemic processes in terms of molecules and their dynamic interactions consider molecular studies of classical conditioning in a marine sea slug, *Aplysia*. Classical conditioning is a kind of learning in which an animal learns to associate weak conditioned stimulus (CS) with a strong unconditioned one (US). After repeated, sequential activation of CS and US, the response to the conditioned stimulus is significantly enhanced in *Aplysia*. This process of learning is systemic in the sense that it is constituted by the whole system of interacting molecular parts.

Classical conditioning in *Aplysia* has been studied in a neural circuit consisting of two sensory neurons (one receiving information about CS from the siphon and one receiving information about US from the tail), a facilitating interneuron and a motor neuron. Molecular components of the process of learning include ions, ion channels, neurotransmitters, genes, receptors, transcription factors, protein kinases and many other. The dynamics between molecular constituents jointly contributing to the process of learning is the following. CS applied to the siphon leads to an increase in the flow of Ca^{2+} ions into the sensory neuron innervating the siphon. The ions bind to the intracellular protein called calmodulin. Activated Ca^{2+}/calmodulin binds to adenylyl cyclase (AC), which is an enzyme converting ATP into the second messenger cAMP. Importantly, binding of Ca^{2+}/calmodulin to AC potentiates its response to serotonin. Now, if an unconditioned stimulus is applied to the tail, facilitating interneurons are activated. They use serotonin as their neurotransmitter. When released by the presynaptic neuron, serotonin binds to the receptors embedded in the membrane of the postsynaptic sensory neuron. This in turn leads to an increase in the activation of AC (Fig. 5B). Since activation of AC has already been increased by the conditioned stimulus, the response of AC to serotonin will become much greater than the one that could be produced if no conditioned stimulus was previously applied. The enhanced activation of AC further augments the production of cAMP molecules. cAMP binds to the regulatory subunit of the protein kinase A (PKA), causing it to release its active catalytic subunit.

The active, catalytic subunit of PKA acts along several biochemical pathways. During long-term training, PKA recruits mitogen activated protein kinase (MAPK), and they both translocate into the nucleus. PKA phosphorylates a transcription factor called CREB-1 (cAMP response binding protein-1) which binds to promoter elements CRE, thus inducing a transcription of genes coding for proteins important for neurotransmitter release and the synthesis of new synaptic connections (Kandel 2001). As one can see, adenylyl cyclase responds to both the molecular representation of a conditioned stimulus

(Ca^{2+}/calmodulin binding) and the representation of an unconditioned stimulus (the activation of G protein after a pulse of serotonin). Thus at the molecular level, adenylyl cyclase establishes association between representations of the two stimuli: conditioned and unconditioned ones (Kandel 2000, pp. 1247–1279).

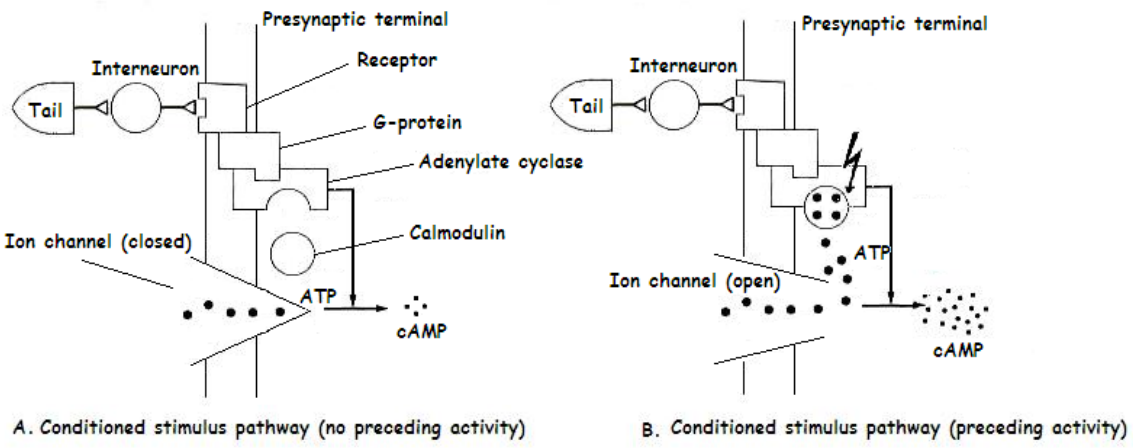

Figure 5. Classical conditioning in *Aplysia* from a molecular point of view.

As the above example suggests, molecular biology studies of classical conditioning are not based on the analysis molecular constituents *in isolation*. They are not limited to *cataloguing* molecular parts involved in the learning process. Instead, they explain how molecular components and their dynamic interactions constitute the process. (The explanation includes various temporal and kinetic aspects of the process). In addition, molecular studies often do not limit themselves to explanation in terms of molecules exclusively. They make reference to organized aggregates of molecules such as aggregates of cells (e.g. neural circuits), cells (e.g. neurons) and organelles (e.g. cell nuclei). All in all, taking into account the aims of molecular biology and its actual explanatory practice it would be unfair to claim that it applies reductionist approach in the sense defined by systems biologists. As we have seen, systems biologists define reductionist approaches as attempts to explain complex phenomena in terms of single, static, isolated constituents. However, many examples of molecular biology explanations appear to deny this claim.

If molecular biology and systems biology share their aims and adopt the same general strategy (explanation of complex phenomena in terms of molecular parts and their dynamic interactions), what is a difference between these two fields? The answer seems to be that systems biology attempts to take into account *more* molecular parts and *more* dynamic interactions than molecular biology (this includes also forms of dynamic interactions that have not been fully recognized so far). To achieve this end, systems biologists apply high-throughput technologies, computational tools and computer-aided models (Ideker 2004). These models are based on *molecular* data and their ultimate aim is to refine and complete missing *molecular* data (Palsson 2000).

Methodological approach of systems biology (a case study)

So far we have argued that from the point of view of its aims and aspirations, systems biology is no less reductionist from molecular biology. The difference is in the scope and the form of research methods. Systems biologists do not differ from molecular biologists when they claim that "To understand how a particular system functions, we must first examine how the individual components dynamically interact during operation" (Kitano 2002, p. 1662). Now, we shall look more closely at two representative studies in systems biology to see how this reductionist agenda works in practice.

The study of galactose utilization pathway in yeast by Ideker et al. (2001) is iconic of systems approach. The galactose pathway is a metabolic pathway that converts galactose into glucose-6-phosphate. The pathway is an example of a genetic regulatory switch because enzymes needed for catabolism of galactose are expressed only in the presence of this sugar (and in the absence of repressing sugars such as glucose). In their experimental work, the authors deleted single genes coding for individual components of the pathway to study the effects of these deletions on the transcription and translation of other genes. They have discovered that a single perturbation of the pathway (single deletion of a gene coding for a molecular constituent of the pathway in the presence or

absence of galactose) changes transcription of almost 1000 other genes. This observation confirmed the model of molecular interactions previously elaborated by molecular biologists and helped to get an insight into previously unknown molecular processes that regulate the pathway.

To obtain these results, the authors applied a systems approach. They designed this approach to consist of the following four consecutive steps. The first step is to "define *all* of the genes in the genome and the subset of genes, proteins, and other small molecules constituting the pathway of interest. If possible define an initial model of molecular interactions" (Ideker et al. 2001, p. 929). The second step is to perturb the pathway by single gene knock-outs and measure the level of mRNA and protein responses. The third step is to compare the results of mRNA and protein expression measurement with the available model of molecular interactions. The fourth step is to formulate new molecular hypotheses to explain phenomena unpredictable by the original model (Ideker et al. 2001; Ideker 2004).

Another representative example of systems biology studies is modeling of the dynamics of the MAPK pathway by Schoeberl et al. (2002). In general, the authors developed a computational model that reconstructs signaling cascade downstream of EGF (epidermal growth factor) receptor. This allowed them to formulate testable predictions that have confirmed the accuracy of the model.

MAPK signaling pathway is one of the most fundamental signaling cascades in mammals. It is involved in many different functions including development and tumor progression. The consecutive events in the pathway have been well characterized in molecular biology. Before the studies, 94 molecular components have been identified and described and many complex relationships between these components have been analyzed at the biochemical level. This also includes the studies of individual phosphorylation steps.

One of the aims of the mathematical model by Schoeberl et al. was to deepen the understanding of the kinetics of the signaling network. What was meant by the kinetics of

the pathway was the change in concentration of the individual components of the pathway over time following the receptor activation within one cell. The model provided mathematical tools to measure this change. Modeling required making use of around 100 differential equations. The accuracy of the model was confirmed by experimental verification of the predictions formulated within the model about the concentration of phosphorylated ERK1/2 (one of the components of the pathway) and the level of expression of *c-fos* (the gene whose expression is controlled by the pathway).

As the above examples indicate, systems biologists exactly as molecular biologists, explain complex phenomena in terms of their parts and their interactions. As in reaction engineering, thus also in systems biology, components of chemical reaction, their dynamic features, topology and interactions between components are determined by a detailed empirical observations (cf. Sorger 2005). Systems biology helps to give a broader picture of life processes on the basis of this knowledge.

The study of galactose utilization pathway was based on the model of the pathway already elaborated by molecular biologists. It also required deletions of individual genes and thus interventions at the molecular level. The effects of these perturbations on individual components of the pathway have been observed and compared with the initial conceptual model. Also the study by Schoeberl et al. is entirely dependent on the detailed molecular knowledge. The authors based their model on the information about each molecular component of the pathway and the knowledge about biochemical reactions between interacting molecules (2002, fig. 1). This information as well as kinetic parameters were derived from the molecular biology literature and from published time-dependent quantitative observations (2002, p. 373-374). They integrated all these pieces of data to develop a model of the pathway.

The observation that systems biologists build models on the basis of molecular data in order to complete missing molecular data suggests that no emergent biological properties in a *strong* philosophical sense are foreseen. Strongly emergent properties are

those that are in principle unpredictable on the basis of molecular level knowledge (cf. Bedau 2002, Chalmers 2006). The idea that the aim of systems biology is to understand and predict emergent properties of a system on the basis of a knowledge of interactions between all elements (Ahn et al. 2006; Hood and Perlmutter 2004, p. 1215; Hood et al. 2004, p. 640) suggests that systems biologists do not understand emergence in the strong ontological sense. Instead, they seem to embrace the idea of *weakly* emergent properties, unpredictable and unexplainable on the basis of molecular level knowledge except by simulation (Bedau 2002). Timothy Galinski from the Institute of Systems Biology says "I'm interested in having the computer do that to get me quickly and systematically to a level in the data where I can extract insights [about the system]" (Powell 2004, p. 302). A derivation of weakly emergent properties require knowledge of all micro-facts of the system that constitute this property. There is no short-cut in a molecular level explanation that would be used to predict accurately the behavior of the system: "The behavior of weakly emergent systems cannot be determined by any computation that is essentially simpler than the intrinsic natural computational process by which the system's behavior is generated" (Bedau 2002; cf. also Bedau 2008). Thus many biologists argue that it will be impossible to simulate and model biological processes accurately until each individual component of the system is identified and its local interactions precisely determined (Palsson 2000, p. 1148; Sorger 2005, p. 10). We shall return to this issue later.

All things considered, systems biologists take it for granted that once knowledge of molecular parts and their dynamic interactions is completed, *all* other features of a biological system will be derived from this knowledge in one way or another (Kitano 2002). It becomes clear that what systems biologists mean by an antireductionist, holistic approach should rather be considered as a thoroughly reductionist stance from a philosophical perspective (Kellenberger 2004, p. 547). "Just as traditional molecular biologists were reductionists without being sure of what reductionism entailed, so are these

systems biologists similarly holists by declaration rather than practice" (Gatherer 2010, p. 7).

Systems biology approach does not meet philosophical requirements for an antireductionist approach

Methodological reductionism is the idea that "biological systems are most fruitfully studied at the lowest possible level" (Brigandt and Love 2008). (In the case of cell biology and immunology it is the level of cells and molecules). This definition is broad enough to include not only structural *parts* of a system but also their interactions. From this point of view, systems biology approach trying to explain systems level phenomena in terms of molecules and their interactions can be classified as a methodological reductionism.

Methodological reductionism in the sense defined above has often been subject of criticism by philosophers of biology and mind. Now, we shall try to understand arguments against this approach. We shall also try to get an insight about the conditions that are expected to be met by an approach to count as antireductionist. This will help us to understand what is missing from the systems biology approach that it falls under the philosophical category of methodological reductionism.

For many philosophers of biology, reductionist approach is biased by its focus on irrelevant details. For example, Hilary Putnam formulated an interesting argument against methodological reductionism in the context of philosophy of mind. This argument is also sometimes invoked in the context of philosophy of biology (e.g. Kitcher 1984; Rosenberg 1994; 2007; Sober 1999). Putnam starts with the observation that the following statement is often taken for granted: "If materialism is true, one should seek for physical explanation of everything". He gives the following example to argue that this statement is wrong. Imagine a cubical peg that can pass through a square hole but not though a circle hole. In principle there are two ways in which one could attempt to explain why the peg cannot pass through the circle hole. The first strategy is to provide a detailed explanation of this

situation in molecular terms, in terms of a rigid lattice of atoms, electrical potential energy and so on. The second strategy is to ignore the details and focus on the higher level structure. This strategy would explain why the peg cannot pass through the hole by making reference to the geometry of the peg and the hole and their rigidity. According to Putnam it is only the second explanation that brings out the "relevant structural features of the situation" and therefore counts as an explanation. The first one is either a "terrible explanation" or "no explanation".

Is not it a matter of subjective opinion and the explanatory context whether the second explanation is better than the first one? (cf. similar discussion in Rosenberg 2007). According to Putnam, the non-reductionist explanation is better *objectively*. The reason is that the second explanation is more general than the first one and there is a rule in science according to which "an explanation is superior if it is more general" (Putnam 1975, p. 132). This putative rule stems from the fact that the science looks for laws.

In line with the arguments by Putnam, Philip Kitcher has argued that molecular explanation of why genes on non-homologous chromosomes assort independently in principle fails. According to him, this kind of explanation, if provided, would require making reference to irrelevant details. This detailed explanation would *blur* and *disguise* relatively simple facts of cytology, such that chromosomes line up with their homologues during meiosis. It would also *decrease explanatory power* of the simpler, more straightforward cytological story according to which genes on non-homologous chromosomes assort independently because these chromosomes are transmitted independently at meiosis (Kitcher 1984, pp. 347-348).

From the point of view of Kitcher, an antireductionist approach is characterized by the capacity to *ignore* gory molecular details to explain a biological process. This power to ignore irrelevant details is crucial in explaining various biological processes. Indeed, the antireductionist approach appears to be a good explanatory strategy in many contexts. For example, an explanation of price drop of beer in a supermarket does not require making

references to molecular processes in a sales manager's brain. Indeed, it many contexts (especially in everyday ones), the ability to ignore details is crucial for understanding and communication. However, systems biology is not one of these contexts. For systems biology no molecular detail of a biological process is *irrelevant* (Hood and Perlmutter 2004, p. 1215; Ideker 2004, p. 2738). In fact, a detailed knowledge of the components is a starting point for a systems biology study (Powell 2004, p. 300; Strange 2005, p. C968; De Backer et al. 2010, pp. 19, 40). According to the founder of the Institute for Systems Biology, Leroy Hood "Systems biology defines and analyses the interrelationships of *all* of the elements in a functioning system in order to understand how the system works" (quote after Mesarovic et al. 2004, p. 19; Hood et al. 2004, p. 640). Zak and Aderem define systems biology as "the comprehensive and quantitative analysis of the interactions between *all* components of biological systems over time" (Zak and Aderem 2009, p. 264) Thus the aim of systems biology can be defined as an attempt to link detailed molecular knowledge with higher level properties: "Ultimate goal of biology is to understand *every detail* of and *principle* of biological systems… Systems biology… links the behaviors of molecules to system characteristics and function" (Kitano 2001, p. 1).

Apart from those who claim that reductionist approach unnecessarily *complicates* things, there are also those who claim that it exceedingly *simplifies* things (Dupré 1993, pp. 87-88; Powell and Dupré 2009; Wimsatt 1985). Contrary to appearance, there may be no contradiction between these two claims. Reductionist approach, even the one, philosophically understood, can be considered as complicating one aspect of reality while simplifying another one. According to Powell and Dupré "At the core of all accounts of reductionism lies a seductive vision of the simplicity of phenomena. … Any one approach, or any exclusive focus on one ontological level (in so far, indeed, as there really are such things) will almost certainly be inadequate to all aspects of the task" (2009, p. 62). There are reasons to believe that biological systems are hierarchically organized and a reductionist approach gives a simplified outlook by focusing on one level and only one

level of reality. In addition, there seem to be distinctly systemic aspects of biological processes that cannot get a proper treatment from the molecular perspective. This is why O'Malley and Dupré (2005) call for a systems theoretic approach in systems biology. An approach that would perceive biological systems as *systems* and would emphasize system principles. Similarly Mesarovic et al. complain that systems biology uses only a fraction of the concepts and results of system theory (2004). Also Cornish-Bowden et al. claim that "systems biology" is "often little more than a euphemism for gathering ever more details on an ever larger scale" (2004, p. 713).

Above, we have seen that methodological reductionism is often criticized for its focus on irrelevant details, its lack of explanatory power or ignorance of higher levels of organization. However, the most powerful argument against methodological reductionism is the multiple realizability argument. It has been invoked in many versions and contexts by many authors including Putnam, Kitcher, Fodor. According to this argument, the same functional role can be realized by a diversity of molecular processes and therefore studying these roles at the molecular level would lead to an unmanageable disjunction of alternatives. Methodological reductionism would unavoidably overlook what these different disjunctions have in common (cf. Rosenberg 2007). Thus, for example, if somebody attempted to provide an extremely detailed molecular explanation of antigen presentation in terms of chemical bonds and physical forces, the person would run the risk of overlooking other ways the presentation can be performed by means of other kinds of APCs, other classes of major histocompatibility complex (MHC) molecules and other kinds MHC molecules within each class, other configurations of the antigen, other forces and so on and so forth. Even if the person collected data about the exact molecular interactions involved in each particular instance of the antigen presentation to cover all possible instances of antigen presentation the person would overlook what all of them have in common. All of it implies, that one should ignore the gory details and remain at the cytological level of analysis. This is how the argument goes or so.

There is no doubt that most (if not all) higher-level biological properties are multiple realizable at the molecular level. Cell organelles, genomes, gene assortments, antigen presentations, immune recognitions, all can be supported by different molecular constituents and different molecular interactions. In fact, finding two identical cell organelles or genomes is as unlikely as finding two identical fingerprints. Nevertheless, molecular studies of both the genomes and the organelles allowed to formulate many Nobel prize-winning generalizations (e.g. The discovery of the structure of the ribosome, decoding the DNA sequence and so on). We have already seen how systems biologists formulated generalizations about galactose utilization pathway and MAPK pathway despite the fact that the exact components of these pathways and the amino acid sequences of these components can vary.

As the above discussion indicates, at least one of the following conditions have to be met by a scientific method to be considered antireductionist.

- It must focus on higher level properties and their causal roles while ignoring some lower level details even though the higher level properties are *ontologically reducible* (metaphysically identical) to the lower level properties.
- It must identify and include in the explanation those higher level properties and their interactions that are *ontologically irreducible* to (metaphysically distinct from) the lower level properties and their interactions.
- It must be able to identify instances of the same natural higher level type, even though these instances appear heterogeneous from the lower level perspective.

As we have argued above, systems biology does not meet these requirements and therefore does not adopt methodological antireductionism in the sense defined above. We can repeat after De Backer et al. that "methodological reductionism is methodologically maintained in systems biology" and that "the richness of system's biology practice … shows the

flexibility and strength of the 'reductionist paradigm'" (2010, pp. 40, 41). We are not going to evaluate this approach here. It should be obvious however, at this stage, that the aptness of this approach depends on whether there are relevant aspects of a biological system activity that cannot be derived from the most complete knowledge about the molecular level details even in principle.

Discussion

The above analysis of various concepts of reduction and non-reduction led us to conclude that the idea of systems biology approach falls under the philosophical concept of methodological reductionism. We have also argued that it is not only the concept but also the actual practice of systems biology that can be considered as reductionist from a certain point of view. How to reconcile this latter observation with the arguments of those who believe that systems biology will explain the behavior of parts in terms of the whole? (cf. Gilbert and Sarkar 2000; Cornish-Bowden 2004).

According to Gilbert and Sarkar, "we are now at the point where the bottom-up approach is meeting the top-down approach" (2000, p. 7). Throughout their paper, the authors emphasize the need for this top-down approach, which they call "organicism". They argue that complex biological systems cannot be explained in terms of their parts and their properties. Instead their explanation requires making reference to upper-level factors.

At first glance, the idea of top-down approach by Gilbert and Sarkar seems to go up against methodological reductionism. After all, methodological reductionism always insists on lower-level explanation, not on some upper-level one (Brigandt and Love 2008). However, what do the authors mean by the upper-level? Closer look at the argument by Gilbert and Sarkar helps to understand that the upper-level they make reference to is just the context of the system (in particular, the environmental context): "When we try to

explain how the whole system behaves we have to talk about the context of the whole and cannot get away talking only about parts. This philosophical stance is variously called *wholism, holism,* or *organicism*" (2000, p. 1). Association of the upper level with the environmental context is also evident in the statements like: "the upper level (the environment) selects the phenotype" (2000).

Is the need to embrace the environment in an explanation sufficient to reject methodological reductionism in biology? There are versions of methodological reductionism clearly incompatible with this claim (e.g. unifactorialism). However, methodological reductionism, broadly understood, only requires an explanation to be in lower-level terms (Brigandt and Love 2008). Thus, methodological reductionism is perfectly safe if the environment the explanation refers to is lower-level.

The examples of top-down biological explanations by Gilbert and Sarkar help to realize that the environment they appeal to is indeed lower level rather than higher level. What has been cited as an example of top-down influence calling for top-down explanation is the process of generation of lymphocyte antigen receptors (2000, p. 6). This process allows the immune system to generate antigen receptors that can recognize any foreign antigen. It is clear that antigen receptors cannot be encoded in full in the genome because human genome contains around $3x10^4$ genes and the antigen receptors can recognize 10^{11} different antigens (Janeway et al. 2005, p. 136). Susumu Tonegawa discovered that DNA codes for separate segments (fragments) of lymphocyte receptor molecules (Tonegawa 1983). There are different types of these segments and they are located on different chromosomes. During the development of a lymphocyte, antigen receptor gene segments are joined together in different combinations. This process is coordinated by recombinase protein complex which includes products of recombination-activating genes and DNA-modifying proteins. Further events in the process of the antigen receptor generation involve terminal deoxynucleotidyl transferase (TdT) and, in the case of the B-cells, activation induced cytidine deaminase (AID). In a nutshell, the process of generation of

antigen receptors involves complex chemical interactions between various enzymes and the DNA molecule. The enzymes act in concert to produce nucleolytic cleavage of the DNA in places marked by conserved sequence signal motifs, rearrange the DNA fragments, reconstitute the chromosome and generate more diversity by subtracting, adding and replacing nucleotides.

Taking into account the example of antigen receptors generation, we have to agree with Gilbert and Sarkar that "in situations such as these context is all important" (p. 6). However, we also have to realize that this context is molecular though and trough. As we have seen, all the events of antigen receptor gene generation take place at the same (molecular) level. (Apparently, no extra-molecular factor is involved in the process of antigen receptor generation). The same can be said about other examples by Gilbert and Sarkar.

All in all, the arguments by Gilbert and Sarkar do not seem to provide the case for upper-level explanation. Instead, they emphasize the importance of lower-level context. Indeed, limitations of the experimental setup in molecular biology do not allow to include many aspects of the context. We fully agree with the authors that emerging new techniques such as high-throughput technologies will allow one to broaden the view of the context and thus provide more adequate explanation of biological development.

Similarly, Denis Noble emphasizes the importance of higher level approach in biology in general and in systems biology in particular. What he means by this approach is the need to perceive a behavior of molecular parts in the context of its relationship to other levels of biological world organization. In particular, one has to take into account the influence of higher level phenomena on the lower level phenomena (downward causation or feedback control of a lower level by the higher level). On the other hand however, he admits that "the concept of level in biology is metaphorical" and that "there is no literal sense in which genes and proteins lie underneath cells, tissues and organs" (2008, p. 21).

Thus, in the literal sense, there is only one level at which causation operates and the higher levels should be understood as in Gilbert and Sarkar as the proximal same-level influences.

As an example of downward causation from an organism to DNA, Noble gives "methylation of cytosine bases and control of by interactions with the tails of histones around which the DNA is wound" (p. 19). Both processes have been described in molecular terms in the literature and do not require making reference to irreducible non-molecular factors (Cedar and Bergman 2009). Such reference would be required if the processes were instances of interaction between two distinct ontological levels. Another example of a feedback between a higher-level property and a lower-level property is the dependence of the kinetics of the ion channels activation on the cell potential. In this case it is not clear why and in which sense cell potential is qualified as a higher level property and not as the same level property as the kinetics of the channel. If the cell potential is at a higher level than the kinetics of an ion channel it cannot be in a mereological sense because the channel kinetics is not part of the potential. A given cell potential seems to be causally determined rather than supervenient upon the kinetics of the channel.

Conclusions

One striking conclusion from our analysis in this chapter is that philosophers and systems biologists often understand reductionism and antireductionism in quite different ways. In fact, what is meant by methodological antireductionism in systems biology is a brand of methodological reductionism in philosophy.

Systems biology declares itself as an *antireductionist* alternative to molecular biology. However, from a certain philosophical perspective, systems biology is a deeply reductionist approach. The difference is that systems biology attempts to provide explanation of phenomena whose complexity was an obstacle for traditional molecular studies. Traditional molecular studies can focus only on the analysis of few molecular parts

and their local interactions. Instead, system biology applies methods having potential to embrace more parts and more interactions to explain complex phenomena.

Chapter III

Reducibility of systems level understanding of immune recognition

So far we have analyzed some of the most influential notions of reduction in philosophy of biology. We have also distinguished between philosophical and biological notions of reduction and argued that, from a philosophical point of view, standard approach in systems biology counts as reductionist. In particular, systems biology seeks for explanations of complex biological phenomena at the lower, molecular and cellular levels.

We have also mentioned that our own account of immune recognition seems to be reductionist. In the present chapter we try to understand sense in which systems level understanding of immune recognition is reducible to molecular and cellular level understanding of this process. This analysis will reveal that our representation of immune recognition is not reducible to the actual representation of the *actual* molecular and cellular processes. Instead, it is reducible to the approximation of these processes. This observation may help to understand the character of reductionist explanations in biology in general.

Systems level account of immune recognition is reductionist

As we have already mentioned, many different models of reduction have been formulated for the last 60 years. Some of these models, especially those formulated in the context of post-positivist philosophy of science, are not applicable to biology. However, there are also models elaborated on the basis of detailed analyses of explanatory practices in biology

which may be considered against our case of immune recognition. One of the models of explanatory reduction that seems to reflect our own approach to immune recognition is the model by Sahotra Sarkar. As we have already mentioned, he distinguishes between three criteria of reductionist explanations (table 2). These criteria may serve as the basis for classification of various types of reduction and include fundamentalism, abstract hierarchy and spatial hierarchy (1998, pp. 43-44).

Fundamentalism is an idea that an explanation of a systemic feature requires making reference to factors that belong to a different realm. This criterion seems be satisfied in the case of our account of immune recognition. We invoked different realms to explain immune system recognition. Firstly, we made reference to the realm of interacting modules. The realm of interaction modules includes dynamic aggregates of cells and molecules whose behavior depends on structural features of intestinal microbes, chemical and physical conditions in the lumen and the state of the host. Secondly, we made reference to the realm of interacting cells and molecules. For example, we pointed out that the module involved in detection of the microbial composition in the gut is constituted by a pattern of activation of PRRs located on the apical surface of IECs. It also includes signaling pathways downstream of these PRRs and a particular repertoire of cytokines and chemokines released by IECs as a result of the activation of these pathways.

The second criterion of a reductionist explanation by Sarkar is abstract hierarchy. Abstract hierarchy means that a system under consideration consists of levels that can be distinguished on the basis of some independent principle. From the point of view of this principle, reductionist explanation appeals only to the entities and properties at the lower level. Again, this criterion also appears to be satisfied by our explanation of the recognition. We have argued that immune recognition involves three distinct levels: the level of molecules (receptors, signaling molecules and the like), the level of functional modules (the level of virulence factors detectors) and the level of recognition itself (decision-making level determining the type of immune response) (Figure 6).

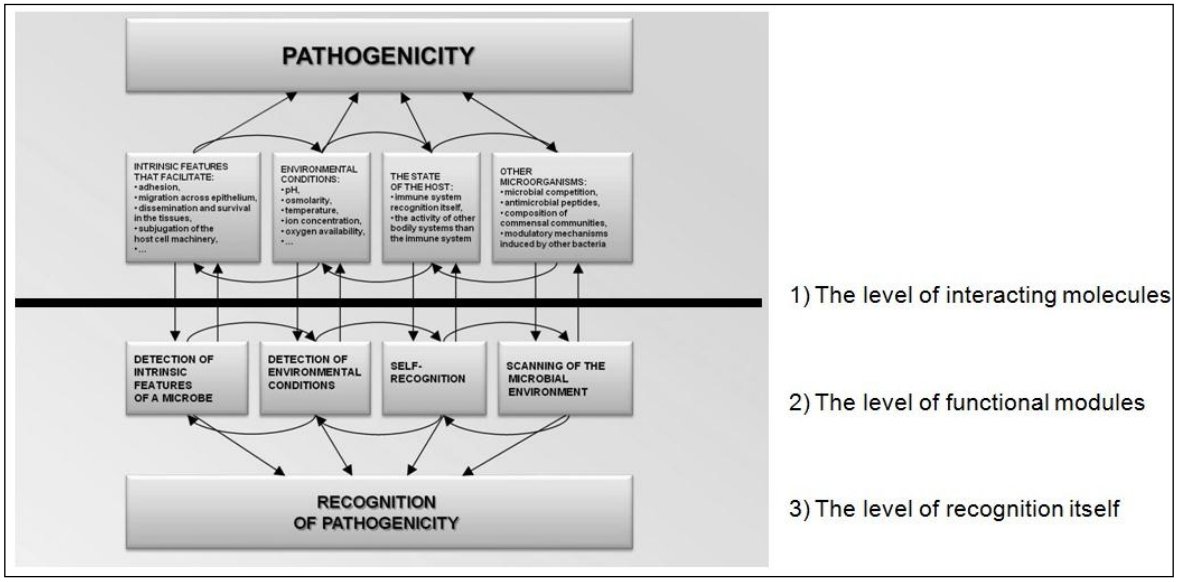

Figure 6. Hierarchical architecture of immune recognition.

Each level consists of elements that respond to a different type of environmental stimuli. Therefore, a response to a given type of stimulus can be considered as an independent principle on the basis of which we distinguish between these levels. The level of recognition itself responds to various forms and degrees of pathogenicity of a microbe, the level of functional modules responds to different kinds of pathogenicity-making factors and the level of interaction molecules responds to biochemical and cellular signals in the immediate environment of the constituent cells and molecules of the immune recognition process.

In order to explain the activity at each level we have referred to the elements and their properties at the lower level exclusively. We have appealed to the interaction between detection modules (level number 2) to explain immune recognition itself (level number 3) and we have appealed to complex interaction between cells and molecules (level number 1) to explain the interaction between the modules (level number 2). Even though our list of detection modules may not be complete (we predict that some, yet undiscovered modules will be found in the course of the experimental work in immunology), we have taken for

granted that a complete list of modules and complete understanding of their causal interplay would make up complete knowledge about immune recognition in the gut.

How can we know that complete understanding of detection modules can provide explanation of immune recognition? This hypothesis cannot be based on empirical evidence because, as we have mentioned, the list of detection modules may be incomplete. It is based on a rational argument. Pathogenicity of a microbe depends on a finite number of intrinsic and extrinsic factors (we call them "pathogenicity-making factors"). Detection of at least some of these factors is absolutely necessary to protect the host. It is also sufficient because nothing else than pathogenicity making factors is informative about disease causing power of a microbe.

Hence, the immune recognition has been explained in terms of modules that detect pathogenicity-making factors. The activity of the modules and relationships between them have been explained, in turn, in terms of lower level molecular and cellular parts and interactions between them. For example, module involved in detection of the localization of a microbe relative to the epithelium has been explained in terms of polarization of IECs. This polarization allows divergent effects of the ligation of apical and basolateral PRRs. As an example of our explanation of an interplay between modules we can give the account of the interaction between the module involved in detection of structural features of a microbe and the one engaged in detection of microbial composition in the lumen. This relationship was explain purely cellular and molecular terms as a process of conditioning of DCs by IEC-derived factors.

The third criterion of a reductionist explanation distinguished by Sarkar is spatial hierarchy. Spatial hierarchy refers to the physical organization of a system, such that each level consists of constituent parts of the higher level. This criterion also seems to be satisfied by our explanation of immune recognition because each level in the hierarchical architecture of immune recognition consists of parts of the higher level (Figure 6). Detection modules and their interactions are parts of the immune recognition process. Cells

and molecules at the lower level are parts of detection modules and their interactions and thus they are also parts of the immune recognition process itself.

As one can see, our explanation of immune recognition satisfies criteria of a reductionist explanation formulated by Sarkar. Thus, at least from his perspective, our explanation of immune recognition is reductive. However, one should note that on the grounds of Sarkar's model of reduction there may be different degrees in which criteria are satisfied and therefore there may be different strengths of a reductionist explanation itself (1998).

Apart from Sarkar's model of reduction, we have also mentioned Kenneth Waters' model of reduction as apparently applicable to the systems level account of immune recognition. We have also mentioned that Waters does not explicitly provide a model of reduction. Instead, his notion of reduction can be reconstructed on the basis of his analysis of the relationship between classical genetics and molecular genetics (Waters 1990; 1994; 2004). Roughly, reductionist explanation for Waters requires identification of a phenomenon with something more fundamental. In addition, there must be a conceptual link between higher level understanding of the phenomenon and lower-level understanding of this phenomenon.

How are these two reductionist conditions satisfied by our explanation of immune recognition? First of all, we have indeed identified immune recognition with something more fundamental. We took it for granted that immune recognition is nothing but a complex molecular and cellular interaction that determines an immune response. By no means immune recognition was considered as a distinct process, independent from the lower level interactions. In addition, we have assumed that there is a strong conceptual link between the immune recognition, traditionally understood, and the analyzed network of interacting cells and molecules. Both, the idea of immune recognition and the network of underlying cells and molecules are assumed to refer to the very same process of determination of an immune response. We have modified the traditional concept of

immune recognition by revealing that the process involves not only the immune system (traditionally understood as a system of cells and molecules of hematopoietic origin) but also other bodily systems and enteric bacteria. However, we have preserved the notion according to which immune recognition is the process that determines an immune response.

All things considered, our explanation of immune recognition appears to satisfy conditions of reductionist explanation formulated by Sarkar and Waters. In the remaining part of the thesis we make reference to one of the most powerful arguments against reductionism to see how our account of immune recognition can possibly sustain it.

Multiple realizability argument against reduction

Any defense of reductionist approach must respond to antireductionist arguments. We focus here on one of the most powerful of them, the so called "multiple realizability argument". This argument is largely responsible for the current antireductionist consensus in philosophy of biology (Sober 1999, p. 542). It starts with the observation that the same type of higher level biological process can be realized by many different alternative molecular and cellular level processes. If this is the case, the higher level process cannot be explained reductively in terms of a single kind of molecular and cellular realization. Instead, it must be explained in terms of a disjunction of alternative realizations that can support the process. This, however may be impossible to achieve because the list of alternative realizations of a given process may be very long. The classical example is pain (Putnam 1967). Pain is irreducible to a pattern of neural activation because different patterns of neural (and, possibly, non-neural) activation can support it. (It is empirically proved that pain may be realized by many different neural patterns in different species, in different individuals and even in the same individual in two different moments of time). If pain is multiple realizable in this sense, its reduction requires making reference to all its possible realizations. This, however, may be difficult to achieve in practice because realization of pain is not even well described for a single species.

As we have already mentioned in chapter 2, multiple realizability argument against theory reduction was originally formulated by Putnam and Fodor in the context of philosophy of mind (Putnam 1975a; Fodor 1974). We focus here on the Fodor's presentation of this argument. He appeals to multiple realizability to argue against many physicalists that theories formulated within special sciences are irreducible to physical theories.

Fodor considers reduction in the sense defined by Nagel. He assumes that reduction is primarily a relationship between laws and that it requires each predicate of the reduced theory to be either included in the vocabulary of the reducing theory or connected with the vocabulary of the reducing theory by means of bridge principles (he calls them "bridge laws"). Bridge laws are defined by Fodor as expressions of identities between *events* (Fodor 1974, p. 100). He also emphasizes that predicates of the reduced and the reducing theory must refer to the same things (Fodor 1974, p. 99). Therefore, he finds it legitimate to consider bridge laws as statements expressing co-reference of predicates of the reduced and the reducing theories.

The central assumption of theory reduction of special sciences is that each natural kind predicate in a special science is co-extensional with a predicate in the physical science. However, according to Fodor, predicates of special sciences are rarely co-extensional with single predicates of physical sciences. Instead, they are co-extensional with many alternative predicates of the physical science. This observation implies that bridge principles must establish connections not between single predicates (one from the special science and the other from the physical science), but instead link predicates of a special science to a wild disjunction of predicates of the physical science. This, in turn, suggests that there is no type-type correlation between a special science and the physical science.

One could argue that the failure of type-type correlation does not undermine reduction of a special science to the physical science. Instead it shows that the description in the physical science can be reduced to many alternative descriptions in the physical

science. However, as we have mentioned, reduction for Fodor is a relationship between *laws*. And the laws are assumed to involve natural kind predicates. Natural kind predicates in special sciences do not correspond to natural kind predicates in the physical science. Instead they correspond to a disjunction of natural kind predicates in the physical science, which by themselves, are not natural kinds. All of this demonstrates that laws of special sciences are irreducible to laws of physics.

As one can see, central for the multiple realizability argument by Fodor is the observation that a predicate term in the special science is co-extensional with many alternative predicates of the physical science. This observation is based on the fact that there are many generalizations formulated within special sciences that cannot be formulated in terms of the lower-level physical science. In fact, one of the reasons why special sciences are still there is that they look for generalizations that cannot be formulated in physical terms because of their heterogeneity at the physical level (Fodor 1974, p. 103). Fodor gives an example from the field of economics to illustrate this point; a law of economics cannot be reduced to a law of physics because many different alternative physical entities and relationships between these entities support an economical law. This implies that each predicate of economics is co-extensional with a wild disjunction of physical predicates (Fodor 1974, p. 103-104).

Multiple realizability argument in the context of philosophy of biology has been first formulated by David Hull. Hull used this argument to argue that classical genetics is irreducible to molecular genetics. He pointed out that the relationships between predicate terms in classical genetics and molecular genetics are not simple one-one relationships (Hull 1974, pp. 37-39). First of all, a single predicate term in classical biology often does not correspond to a single predicate in molecular genetics. Instead, it corresponds to a description of many alternative molecular mechanisms. For example, it is impossible to find a single predicate in molecular genetics that could play the role of the term "dominance" in classical genetics. Instead, conceptual counterparts of this term in

molecular genetics would be complex descriptions of various mechanisms. On the other hand, a single predicate term in molecular biology can be linked to many predicate terms in classical biology. For example, the term "enzyme-synthesizing system" would have to be characterized by many different predicates of classical genetics (cf. Schaffner 1976, p. 619). These considerations lead Hull to conclude that it is practically impossible to establish reductive functions (bridge principles) that could link vocabularies of the classical genetics and molecular genetics. This, in turn, implies that it is impossible to derive generalizations of classical genetics from molecular genetics (Hull 1974, pp. 37-39). All things considered, the relationship between classical genetics and molecular genetics must be considered as a case of theory replacement rather than reduction.

Philip Kitcher also makes reference to the multiple realizability argument in the context of the debate about reduction in classical genetics. He follows Nagel and Schaffer claiming that reduction of classical genetics to molecular genetics requires laws of the former to be derivable from the latter. He also assumes that this derivation requires bridge principles linking vocabularies of molecular genetics and classical genetics. Kitcher considers the example of "gene" to show that bridge principles connecting vocabularies of these two versions of genetics are not forthcoming. He points out that the central notion of classical genetics, that is the notion of gene, cannot be reformulated in the language of molecular genetics. It is because there is a great variety of alternative molecular structures and complex molecular processes (some of the unknown) that could be considered as equivalent to the concept of gene in classical genetics (Kitcher 1984).

Kitcher further argues that even if it was possible to reconcile the vocabularies of the classical genetics and molecular genetics by making reference to a long disjunction of molecular processes that realize gene in the sense of classical genetics, it would be impossible to derive laws about gene transmission from molecular genetics. This observation is reminiscent of the Fodor's argument for the autonomy of special sciences discussed above.

Even though the above versions of the multiple realizable argument have been formulated to undermine theory reduction specifically, they might be modified to serve as cases against other models of reduction. (This point has already been made by Fodor 1974, p. 114). In particular, multiple realizability argument can be used against different versions of explanatory reduction. Explanatory reduction says, among other things, that a complex biological process can be explained in terms of its constituents and their interactions. If many types of constituents and their interactions can support the same type of higher level process, reduction of this process may be difficult to achieve because of the necessity of making reference to all the alternative types of underlying constituents and their interactions.

The antireductionist argument seems to threaten our explanation of immune recognition. If the same type of immune recognition can be implemented by many alternative cellular and molecular level processes, it is a mistake to claim that the recognition reduces to such and such molecular and cellular level process. Instead, it is necessary to make reference to all possible molecular and cellular level processes that can support this kind of recognition. In other words, if immune recognition is multiple realizable and if reduction is an explanation by identification, it cannot be identified with any single molecular and cellular level process. This could not be a problem if a given type of immune recognition had few possible realizations. However, if the number of possible molecular and cellular level realizations of immune recognition is very big, reduction may be practically unachievable. Now, we try to estimate the number of possible realizations for a given type of immune recognition.

Immune system recognition is multiple realizable

Multiple realizability argument can be used as an argument against reduction of those higher level processes that are constituted by many alternative lower level processes. There are reasons to believe that immune recognition is multiple realizable in this sense. Given

the complexity and the number of constituent molecules and cells involved in the recognition, it is highly unlikely that their exact dynamic configuration could ever be reproduced even in the same individual. This observation is trivially true and does not require any evidence. No two instances of immune recognition are the same because they always involve different microbes, different initial number of immune and non-immune cells, different chemical and physical environment in the gut, different composition of the gut microbes and so on. Also the exact mechanism of immune recognition of a given type may vary depending on the available resources of an individual. For example, we have already mentioned that there is evidence showing that deficiencies in the innate immune recognition system can be compensated by the adaptive immunity.

One obvious manifestation of multiple realizability is robustness[2]. Robustness is the ability of a process to maintain performance despite perturbations at the molecular level. (Alon et al. 1999; Kitano 2004; Kitano 2007; Stelling et al. 2004; Wagner 2005). Robustness is a feature of most biological processes. For example, it is estimated that single deletion of 80% of genes in yeast do not affect the overall viability of the organism (Cornish-Bowden 2004, p. 715). This property is often ascribed not only to biological processes but also to biological *functions*, *systems*, *states*, *reactions*, *modules*, *pathways*, *traits* and other features (Kitano 2004).

From the point of view of complex biological processes, robustness is a feature that allows these processes to retain their functional identities despite radical changes at the molecular level. It is important to realize that molecular perturbations may lead to radical changes in the underlying mechanism while leaving the higher level function intact. One example of such change is a diauxic shift, a change from anaerobic to aerobic respiration

[2] It is interesting to note that Wimsatt refers to robustness in the above sense as "dynamical autonomy" and considers it as an instance of multiple realizability (Wimsatt 1994, p. 217-220). (What he calls "robustness", is something different; it is the feature of properties, objects or states of affairs that allows multiple, independent means of cognitive access).

(cf. Kitano 2004, p. 828). A bacterium can maintain respiration process despite radical changes in the environment by changing the supporting molecular level mechanism.

Immune recognition is also robust. Mutations in genes coding for receptors involved in detection of microbial structural motifs may not affect the capacity of the system to distinguish pathogenic and nonpathogenic agents. We have already made reference to strong scientific evidence showing that single molecular or cellular defect is not sufficient to perturb accuracy of the immune recognition process. The best known example is that of NOD2, specialized in detection of PGN. Mutations in the gene coding for NOD2, even though alter the function of the receptor do not lead to immune misrecognition unless they are accompanied by other organic or environmental defects (Cho and Abraham 2007). NOD2 knockout mice do not have any phenotypic defects. The same is true about other single environmental defects. Alterations in the integrity of epithelial cell barrier in the intestine lead to IBD only in the minority of cases (May et al. 1993). Thus even though one could expect that mutation of a crucial receptor gene or a defect in the integrity of the epithelial surface would automatically make the immune system unable to distinguish between pathogenic and commensal agents, this does not have to be the case. For more examples of studies showing that the mucosal immune system does not lose its capacity to produce suitable responses despite serious defects at the molecular and cellular levels compare our discussion on IBD in the first part of the thesis.

Systems level understanding of immune recognition does not reduce to understanding of the *actual* lower level processes.

As one can see, there is strong body of evidence showing that the same type of immune recognition can be realized by almost infinitely many alternative molecular and cellular level processes. This fundamental fact suggests that systems level understanding of immune recognition cannot be reduced in practice to a representation of the *actual* molecular and cellular level process. In other words, it seems impossible to explain

immune recognition in terms of the genuine interaction between cells and molecules because different types of interactions can support the same type of recognition.

Mark Bedau discussed the problem of irreducibility in practice in a number of papers (1997; 2002; 2008). He distinguished a class of macro-level properties that cannot be explained in terms of micro-level properties because these micro-level properties are involved in a complex network of interactions. According to Bedau, knowledge about the macro-level properties could be derived, at least in principle, from the knowledge about the underlying micro-level properties, but given the complexity of the micro-level, the derivation would be extremely complex (Bedau 2002; 2008, p. 445). He uses the concept of explanatory incompressibility to express this thought. Incompressibility is a feature of reductive explanations that require detailed analysis of the microcausal web and cannot be replaced by simpler short-cut explanations (Bedau 2008). Since epistemic reduction requires identification of a macro-level property with some micro-level properties the complexity of the micro-level properties may exclude the possibility of reduction. This epistemic irreducibility, in turn, grants the macro-level properties apparent epistemic autonomy referred to as "weak emergence" (2002).

One of the central assumptions by Bedau is that there are macro-level properties, derivation of knowledge of which must involve *representations* whose complexities mirror the *actual* complexities at the lower-level. For example, in the context of simulations that could be used to facilitate derivation of the knowledge of macro-level properties from the knowledge of micro-level properties; he writes "It is an especially "long-winded" derivation because it mirrors each individual step in the system's micro-level causal dynamics" (Bedau 2002). He contrasts such derivations with "short-cut" (or compressible) derivations that, he admits, are very frequent in science.

One could argue that there is no evidence for the first kind of derivations in biology. Derivation of knowledge of macro-level properties from the knowledge of micro-level properties (in some informal sense of derivation) is a standard explanatory procedure in

immunology as well in medicine and does not involve representations reflecting the *actual* complexity at the micro-level. A doctor can infer the course of a complex macro-level pathology by performing a simple genetic test even though the pathology is realized by extremely complex lower level process. One of the goals of clinical immunology is to develop methods that allow acquisition of accurate knowledge about complex diseases (their causal powers) on the basis of simple, uninvasive molecular tests.

On the other hand, however, it is important to realize that standard derivations of knowledge about macro-properties in biology and immunology have limited accuracy. It is most probably because they are based on a *fragmentary* representation of the micro-level. There are reasons to believe that reductionist explanations involving representations mirroring the *actual* complexity at the lower level would be much more accurate. This is why systems immunologists look for integration of knowledge about *all* molecular and cellular parts and their interactions: "A key aim of postgenomic biomedical research is to systematically catalogue all molecules and their interactions within a living cell. There is a clear need to understand how these molecules and the interactions between them determine the function of this enormously complex machinery, both in isolation and when surrounded by other cells" (Barabasi and Oltivai 2004). All things considered, we agree with Bedau that *full* knowledge about the macro-facts requires *full* knowledge about micro-facts and the complexity of the latter is directly proportional to the complexity of the micro-facts and their relationships. Knowledge about macro-facts standardly acquired in biology on the basis of fragmentary knowledge about micro-facts is also often fragmentary and limited in scope.

Even though the primary concern of Bedau is reducibility of those macro-properties that are complex at the micro-level, his conclusions can be extended to include also multiple realizable macro-level properties. Even if it was possible in practice to derive knowledge about certain macro-level properties on the basis of the knowledge about micro-level interactions, knowledge about the macro-level properties could be irreducible

to the knowledge about lower-level interactions because of the number of possible alternative lower-level realizations.

This is exactly the problem we are facing while discussing multiple realizability of immune recognition. Empirical evidence for the robustness of the recognition and the observation that the same type of recognition can be implemented by structurally diverse systems prevent from reducing knowledge about immune recognition to only one type of molecular and cellular level interaction. One would have to make reference to *all possible* lower level interactions to perform this kind of reduction successfully. This is achievable only *in principle* but not *in practice* (cf. the discussion on the distinction between reducibility in principle and in practice in: Bedau 2008, p. 449).

Systems level understanding of immune recognition reduces to an *approximation* of the lower level processes

Throughout this thesis we have argued that systems level understanding of immune recognition is in a sense reducible. However, multiple realizability argument seems to deny this claim. It seems to demonstrate that systems level understanding of immune recognition is at best reducible *in principle* but not *in practice* to the molecular and cellular knowledge of the process. Indeed, there seems to be no way out from the evidence showing that different molecular and cellular level explanations may apply to the same type of immune recognition.

However, one should note that multiple realizability argument undermines the possibility of reducing higher level representation of a given type of process to the lower level representation of the *actual* process. It takes it for granted that reductionist explanation must reflect, like a mirror, the exact configuration of the constituent elements of a given process. This however, rarely occurs in biology.

If researches sought to explain a complex process in terms of its *actual* components they would end up with an explanation applicable to a single occurrence of the process.

They would have to note for example, that a molecule X located at a position Y at time *t* is responding to a molecule Z and so on. Their aim, instead, is to formulate explanations that are applicable to possibly large number of molecular and cellular cases. Having this goal in mind they have to abstract from many lower level idiosyncrasies and focus on those lower level features that are shared by many members of a given biological category.

In order to formulate reductionist generalizations applicable to a possibly large number of cases, researchers use *approximations*. Instead of trying to reproduce bit by bit the exact topology, shape and movement of each and every constituent molecule and cell of a given process, they sketch a simplified picture of the components and their interactions and use it further as a basis for prediction and derivation of knowledge about higher level processes. Even those biologists who aspire to complete knowledge about *all* cellular and molecular components of a process do not mean the *actual* cellular and molecular components that can be found in each and every individual. Instead they refine already existing approximation to draw a picture of lower level interactions that comes closer to the actual complexity at the lower level in each member of a given category. All in all, approximations serve as tools helping to deal with multiple realizability. They allow to omit idiosyncrasies of a particular data sample.

What are approximations though? Approximations can be defined simply as "inexact description of a target system" (Norton, unpubl.). The description of lower level processes constituting the process of recognition in the first part of the thesis counts as approximation because it is inexact, that is, it does not include all the elements and interactions involved in the recognition and it does not map accurately the exact dynamics and structure of the actual processes.

Sarkar distinguishes between different types of approximations in biology on the basis of several different criteria. Particularly important from the point of view of our analysis is a distinction between *corrigible*, *incorrigible in practice* and *incorrigible in principle* approximations (1998, p. 49). As we have already mentioned, one of the central

assumptions of the systems biology approach in immunology is that standard lower level approximations in immunology will be corrected if more molecular and cellular details will be included in the explanation. Ideally, number of molecular details should reflect the actual complexity at the lower level. For the reasons we have mentioned in the previous section, the complete correction of an approximation would narrow the scope of an explanation to a single case. Thus, approximations seem to be inevitably embedded in immunological explanations, whether systemic or molecular (cf. Sarkar 1998, pp. 48-52). According to Rosenberg, exceptionless laws apply only to the most fundamental level and therefore generalizations at the higher levels have unavoidably approximate character (1994, p. 37).

Another important distinction by Sarkar is that between the *estimable*, *not estimable in practice* and *not estimable in principle* effects of approximation. Since the approximations involved in the immunological explanations are mostly implicit, their effects are rarely estimated. It is not even clear how these effects could be measured given that they are inseparately intertwined with scientific explanation (cf. Wimsatt 2007a, pp. 16-17).

All things considered, realization that lower level explanations in biology are doomed to refer to lower level *approximations* rather than representations of the actual goings on suggests that our own explanation of immune recognition does not reduce to the lower level representation of the lower level processes but to the approximation of the latter.

Is the reduction to approximation a reduction still?

The above analysis lead us to conclude that systems level understanding of immune recognition does not reduce to a description in terms of actual interaction between molecular and cellular parts but rather the approximation of this interaction. However, there is a problem of whether reduction to approximation can be considered reduction.

Think about the following argument by John Dupré. He argues that individuals that are postulated by some higher level theory are not identical to those that are postulated by the lower level theory. He considers an ecological model, in which foxes are represented as having propensities to eat hares. However, on the grounds of a lower level analysis the same individuals may be represented differently, as for example eating in some situations hares, in other situations rabbits. Similarly, an idealized rabbit that is a subject of studies by physiology is a different individual than an idealized rabbit that is a pray for an idealized fox that is a subject of studies by ecology. Thus, a defining features ascribed to certain individuals at the higher level may not be exactly the ones that are ascribed at the lower level. This is considered by Dupré as the evidence that reductionism of the higher level to the lower level fails (Dupré 1993, p. 116).

We face similar problem in the case of immune recognition. Properties of molecular and cellular constituents of immune recognition may look differently when perceived from the immune system recognition level perspective and when studied independently from molecular or cellular one. We suggest that the molecular and cellular properties as postulated by the systems level of understanding are approximations of those that could be discovered by molecular biology or cellular biology at the lower level. This, in turn lead us to conclude the systems level understanding of immune recognition reduces to these approximations.

A detailed lower level reconstruction of the interactions between cells and molecules at the mucosal tissues might not allow one to isolate immune recognition as a distinct process. It is because distinct levels of analysis focus on different kinds of properties (Dupré 1993). Properties that are visible for immunology are isolated on the basis of some broader theoretical framework; "the properties of concern are determined by the macrotheory we are trying to construct" (Dupré 1993, p. 116). For example, immune recognition process is isolated as a distinct process on the grounds of the cognitive paradigm that perceives the immune system as a cognitive system. For a competitive

framework, for example of the one that considers the relationship between microbes and the immune system in terms of a homeostatic balance, immune recognition might not be isolated as a distinct process.

All things considered, systems level understanding of immune recognition can be considered as reducible to lower level approximations. These approximations however, are themselves formulated from the perspective of the broader theoretical framework at the higher level. Approximations of the same lower level phenomena but formulated from the lower level perspective are different. Whether this weakened version of reduction to approximations formulated from the higher level point of view is a genuine reduction is an open question and depends on the employed notion of reduction.

Conclusions

In the first two parts of the thesis we argued that the classical view of immune recognition fails to explain how the mucosal immune system manages to produce accurate responses to pathogenic and non-pathogenic microbes. According to the classical view, pathogen/non-pathogen discrimination is performed by single types of cells and molecules. However, there is evidence enough to conclude that immune recognition is performed collectively by many different types of cells and molecules. Indeed it is the job of the immune *system* as a *system* to design responses in accordance with disease causing powers of microbes. From this perspective, all the past research projects aiming to explain how DCs, macrophages, TLR4s or other single units discriminate between pathogenic and nonpathogenic microbes turn out to be based on wrong assumptions.

Recent studies of the immune system help to realize that the systemic recognition involves specialized detection modules engaged in processing information about factors that influence disease causing power of intestinal microbes. Understanding immune recognition requires analysis of these functional modules and their complex interactions.

Since immune recognition appears to be modular and performed collectively by many different types of cells of molecules, we suggest that a systems biology approach is needed to explain the recognition. Systems biology approach is often contrasted with a reductionist approach. However, these two approaches can be reconciled if reduction is understood as an explanation in terms of complex interactions between constituent molecular and cellular parts. This notion of reduction has been elaborated in philosophical circles for the last several decades and differs from the similar notion in biology.

Reductionist approaches of various sorts are confronted with a powerful antireductionist argument, the so called "multiple realizability argument". According to this argument, a process of a given type cannot be reduced to a given type of lower-level process if it can be realized by many different types of lower level processes. One has to make reference to a disjunction of the alternative types of lower level processes that realize a higher level process to reductively explain this higher level process. This kind of explanation may not be available if the number of possible realizations of the higher level process is very big.

Immune recognition is no doubt multiple realizable. Each instance of immune recognition may involve different lower level interactions. However this does not mean that the systemic explanation of this process is not reductive. Quite the contrary, it reduces to a single *approximation* of many alternative types of lower level processes that realize the recognition. Indeed, multiple realizability argument is based on the assumption that reduction requires making reference to the exact representation of lower level processes. However, researches tend to refer to *approximations* of these processes. Lower level approximations differ from the exact lower level representations in that they abstract from molecular idiosyncrasies and focus on molecular features that are shared by many different instances of lower level realizations of a given process. Some could argue that reduction to approximation is not a *real* reduction. Whether reduction to approximation can be

considered as *real* reduction is an open question and the answer seems to depend on the exact requirements one imposes on reduction.

All the analysis of systems level understanding of immune recognition allows one to answer the initial question about the sense of reduction involved in our explanation of immune recognition. Systems level understanding of immune recognition is reducible to *approximation* of the lower level processes. It is however irreducible to representation of the *actual* lower level processes.

Supplement 1

Classical paradigm of immune recognition (examples)

A theory according to which single types of cells and molecules can discriminate between pathogenic and nonpathogenic agents was originally formulated as a good reflection of available data. However, now, in the light of new data, there is a discrepancy between the paradigm and available evidence. The table quotes some recently published, important immunological papers in order to show that the paradigm according to which single types of cells and molecules discriminate between pathogenic and nonpathogenic agents is still maintained.

Nr	**Paradigmatic pathogen recognizing cells and molecules**	**Corresponding reference**
1	**Epithelial cells**	"[Intestinal epithelial cells] IECs emerge as sentinel cells that are not only able to discriminate between 'friends' (the commensal microorganisms) and 'foes' (the pathogens) but are also able to translate this recognition process into signals to the mucosal innate immune system that tip the balance towards tolerance in the presence of commensal microorganisms and inflammation aimed at microbial destruction in the presence of pathogens." (Sansonetti 2004, p. 962). "IECs show qualitatively distinct responsiveness to commensal and pathogenic bacteria species." (Coombes and Powrie 2008, p. 440).

2	**Macrophages**	"To eliminate pathogens macrophages, like other cells from the immune system, need to be able to distinguish self from non-self." (Heinsbroek and Gordon 2007, p. 7) "As sentinels of the immune system, macrophages must be able to determine the nature and scope of microbial threats to mount appropriate transcriptional responses. Macrophages need to discriminate not only viral from bacterial infection, but also extracellular and possibly killed microbes from intracellular and replicating pathogens (Leber et al. 2008).
3	**Dendritic cells**	"Dendritic cells recognize pathogens through receptors that 'see' common determinants found on pathogens. The best characterized of these receptors are the Toll-like receptors. After recognizing a pathogen, dendritic cells migrate to the lymphoid organs, where they interact with T cells, transmitting information about the type of infection encountered and inducing a T-cell response." (Lehar and Bevan 2004, p.150). "Dendritic cells reside in most peripheral tissues, where they monitor the tissue environment for the presence of pathogens by using various PRRs. When a pathogen is encountered by a dendritic cell, it is taken up by phagocytosis, and its protein constituents are processed into antigenic peptides, which are presented at the cell surface by MHC class I and/or class II molecules." (Medzhitov et a1. 2007, p. 823).
4	**Neutrophils**	"Until recently, little was known about the ways in which neutrophils and macrophages, the major players in innate immunity, recognized *C.albicans* as a pathogenic microorganism,

		or how the fungal-leukocyte interaction triggers an inflammatory response" (Netea et al. 2008, p. 67).
5	**TLRs**	"Toll-like receptors (TLRs) are the archetypal pattern recognition receptors (PRRs) envisioned by as innate sensors of pathogen attack and host triggers of an adaptive immune response." (Kirk and Bazan 2005, p. 347) "Paradoxically, Toll-like receptors (TLR) control the mucosal defense against pathogens, even though the TLR recognize conserved molecules like LPS, which are shared between pathogens and commensals. This study proposes a mechanism of pathogen-specific mucosal TLR4 activation, involving adhesive ligands and their host cell receptors... TLR4 may be engaged specifically by pathogens, when the proper cell surface receptors are engaged by virulence ligands... The present study used P fimbriated *E. coli* to investigate how the TLR4 response can distinguish pathogenic from commensal strains and how the downstream signaling pathways maintain pathogen specificity." (Fischer et al. 2006, pp 267-268).
6	**NLRs**	"Cytosolic recognition of microbial factors by NLR proteins appears to be one mechanism whereby the innate immune system is able to discriminate between pathogenic bacteria ('foe') and commensal ('friendly') members of the host microflora". (Kaparakis 2007, p. 395).
7	**Antigen receptors of B-cells and T-**	"Antigen receptors are clonally distributed on T and B lymphocytes, which allows clonal selection of pathogen-specific receptors and is the basis for immunological memory. (That is,

	cells	each lymphocyte expresses antigen receptors of a single specificity, so only specific populations of lymphocytes are selected to expand in response to a pathogen.)" (Medzhitov 2007, p. 819).

Supplement 2

Philosophy of immunology

Immunology (the study of the immune system) appears to help elucidate many philosophical questions regarding complex systems, and traditional philosophical discussion on these complex systems can shed light on some fundamental immunological issues. Immunologists have always asked and attempted to answer questions that extend beyond the empirical data. By doing so, they have *pursued* philosophy. According to Robin George Collingwood, «The theory of science and the theory of history are not parts of science and of history; if scientists and historians study these things, they study them not in their capacity as scientists or historians, but in their capacity as philosophers» (Collingwood, 1933, pp. 1-2). From this point of view, any analysis of theoretical problems relevant for immunology concerns the philosophy of immunology. The philosophy of immunology in this broad sense has always been a vibrant and potent discipline. Theories such as the "germ theory of disease" or "clonal selection theory", first formulated as controversial theoretical hypotheses, established new paradigms, and therefore, became important turning points in the history of immunology (Silverstein, 1989). Philosophical methods, when applied by theoreticians of immunology, have often been used unsystematically and intuitively. However, more recently, a new way of addressing the philosophy of immunology is emerging. Immunologists as well as philosophers start applying philosophical methods methodically in an attempt to disentangle various theoretical problems in immunology. Most of these questions are not only relevant for immunology, but they are also interesting to the philosophy of science and of the mind. In this particular sense, the philosophy of immunology is a brand new subject. Everybody

who joins the field faces an enormous landscape of unexplored theoretical problems that have not yet been analyzed. Here, I discuss briefly some of the most original issues that have been analyzed recently in this new field of the philosophy of immunology.

It seems that the most important topic in the field of the philosophy of immunology has been the problem of self/non-self discrimination. Immunology in its present molecular and cellular form is a very young science; molecular and cellular immunology was only established 60 years ago. Central to this new molecular and cellular paradigm was the assumption according to which the immune system has the power to distinguish between self and non-self (Burnet and Fenner, 1949). It was assumed that the immune system ignores the self and protects an individual from the pathogenic non-self. The immune system was perceived as a system that actively defines its identity, its self (Tauber, 2009). However, the commonsensical idea, according to which the immune system distinguishes and defines between self and non-self, has been challenged by the overwhelming evidence showing that the immune system does not simply ignore the self. For example, in the intestine, commensal nonpathogenic bacteria, even though incorporated as part of the host organism, engage immune receptors and initiate immune system responses (Coombes and Maloy, 2007). Moreover, immune autoreactivity, the immune responses against the body's own cells or tissues, has proven to be part of normal, healthy functioning (Schwartz and Cohen, 2000). All of this evidence taken together reveals that immune reactivity cannot be reduced to the dichotomous recognition of self and non-self. It demonstrates that the initial dichotomous model was too simplistic (Tauber, 2009).

A number of theoretical models have been proposed to accommodate the theory of self/non-self discrimination, with the new experimental data questioning the idea according to which the immune system simply ignores the non-self. One interesting theoretical solution is the danger model by Polly Matzinger. According to Matzinger, the immune system does not have any "interest" in ignoring the self and triggering immune system responses towards the non-self. The primary role of the immune system is to protect an

organism from agents that can harm and kill it. Thus, it distinguishes between dangerous and non-dangerous agents rather than between the self and non-self. This theoretical hypothesis can be tested experimentally. It will prove to be correct if the immune system responses end up depending not only on immune receptor recognition *per se*, but also on the so-called "danger signals". These are signals in the form of molecules that indicate the presence of tissue damage (Matzinger, 2002). Another interesting attempt to formulate a general theory of when and why immune system responses occur is Pradeu and Carosella's criterion of continuity. The authors begin with the observation that immunogenic or tolerogenic responses are induced independently of whether an antigen is part of an individual (self) or comes from the outside world (non-self). They argue that immunogenic responses are triggered against antigens whose features differ from the ones with which immune receptors usually interact (Pradeu and Carosella, 2006).

Another very important topic in the field of the philosophy of immunology is the idea of cognitive immunology. Roughly, the immune system shares many features with the nervous system. Crucially, the immune system can be a subject of Pavlovian conditioning (Ader and Cohen, 1975). It learns through experience, stores memory, recognizes microorganisms and produces responses on the basis of complicated decision-making processes (Sotelo, 1999; Hershberg and Efroni, 2001). Perhaps, all of these features would not suffice to consider the immune system a cognitive system if it could not search for a particular kind of information (Cohen, 1992a). Indeed, the immune system does not only cognize whatever it interacts with, but it recognizes it. The prefix "re" means "again". This signifies that the immune system is prepared to encounter a microorganism. Thus, the immune system has been suggested to exploit internal images that precede the very act of recognition (Cohen, 2000a, pp. 174-175). It is well established that the nervous systems of higher animals do not only exploit images of their external environments, but they can also represent themselves (Penfield and Boldrey, 1958). The cortex areas that produce these internal images are referred to as neurological homunculi. Irun Cohen formulated a theory

of immunological homunculus according to which the immune system is capable of exploiting images of itself encoded in a distributed network of interacting cells and molecules (Cohen, 1992b, 2007). Cohen and his colleagues studied the immunological homunculus, the global patterns of autoantibody reactivities, by using an antigen microarray chip (Merbl et al., 2007).

Another interesting area of research in the field of the philosophy of immunology is the problem of immune specificity. It has been assumed at the early stages of the history of immunology that immunoglobulins are highly specific in their ability to recognise antigens. However, it was recently demonstrated that antibodies are relatively promiscuous. Each antibody can potentially bind to more than one antigen. Taking into account this promiscuity or degeneracy of immune receptors, it has been proposed that the specificity of an antibody is an emergent property arising from the activity of many different cellular and molecular factors (Cohen and Harel, 2007). The philosophical idea of emergence behind these considerations is close to the one originally formulated by Mark Bedau (1997). Namely, discussion on the problem of immune specificity rests on the idea that emergent properties are both ontologically autonomous and reducible to the underlying molecular and cellular processes (Cohen, 2000a, p. 27-32). Another group of problems are those related to the complexity and context-sensitivity of various aspects of immune activity. For example, Alfred Tauber has proposed an ecological approach as a conceptual framework that helps to understand the principles of immune regulation (Tauber, 2008). This new perspective permits the elimination of military metaphors according to which the immune system *defends* the body from pathogenic microorganisms. From an ecological point of view, the immune system and microorganisms interact in order to maintain an equilibrium whose disruption may lead to pathology. Systems biology appears to provide the appropriate tools for studying the immune system within the larger environmental context.

Finally, there is a group of theoretical issues related to signaling and information processing within the immune system. A good example is the problem of meaning-making

in the immune system. There is an analogy between meaning-making in cell communication and a similar process in linguistic communication. The same linguistic signs can represent different things in different communication contexts. Thus, it is necessary to define the context and explain how it contributes to meaning-making processes (Neuman, 2004). Apart from the problem of self, context-sensitivity and specificity, there are many other theoretical issues in the field of the philosophy of immunology. Most of these problems have not yet been explored. One group of such unexplored theoretical problems appears to be those related to psychoneuroimmunology, the study of the relationship between the nervous system and the immune system. Strong empirical evidence exists to demonstrate a cross-talk between these two systems. The activity of the immune system can be modulated by the nervous system and vice versa (Ader at al., 2006) Observation, according to which there is a link connecting the mind and immunity, has interesting implications for the ontology of the mind. Psychoneuroimmunology puts the mind-body problem in a new light. In addition, it helps to understand the power of the placebo effect, alternative medicine and faith healing. Furthermore, it assists in explaining these allegedly miraculous phenomena in a purely naturalistic way.

Bibliography

Ader R., Felten D.L., Cohen N. 2006, *Psychoneuroimmunology*. San Diego: Academic Press.

Ader R. and Cohen, N. 1975, Behaviorally Conditioned Immunosuppression. *Psychosomatic Medicine* 37: 333-340.

Aderem A. 2003, Phagocytosis and the inflammatory response, *The Journal of Infectious Diseases* 187: S340-345.

Ahn A.C., Tewari M., Poon C–S, Phillips R.S. 2006, The Limits of Reductionism in Medicine: Could Systems Biology Offer an Alternative? *PLoS Medicine* 3: e208.

Ahn S.H., Shah Y.M., Inoue J., Morimura K., Kim I., et al. 2008 Hepatocyte nuclear factor 4alpha in the intestinal epithelial cells protects against inflammatory bowel disease. *Inflammatory Bowel Disease* 14: 908-920

Akira S., Uematsu S., Takeuchi. O. 2006, Pathogen Recognition and Innate Immunity. *Cell* 124: 783-801.

Alon U., Surette M.G., Barkai N. et al. 1999, Robustness in bacterial chemotaxis. *Nature* 397, 168-171.

Artis, D. 2008, Epithelial–cell recognition of commensal bacteria and maintenance of immune homeostasis in the gut, *Nature Reviews Immunology* 8: 411-420.

Bailey M.T., Coe C.L. 1999, Maternal separation disrupts the integrity of the intestinal microflora in infant rhesus monkeys. *Developmental Psychobiology* 35: 146-155.

Bambou J.C., Giraud A., Menard S., Begue B., Rakotobe S., Heyman M., Taddei F., Cerf–Bensussan N., Gaboriau–Routhiau V. 2004 In vitro and ex vivo activation of the TLR5

signaling pathway in intestinal epithelial cells by a commensal Escherichia coli strain. *The Journal of Biological Chemistry* 279: 42984-42992.

Barabási A.L. and Oltvai Z.N. 2004, Network biology: understanding the cell's functional organization. *Nature Review Genetics* 5: 101-113.

Barr T.A., Brown S., Ryan G., Zhao J., Gray D. 2007, TLR-mediated stimulation of APC: Distinct cytokine responses of B cells and dendritic cells. *European Journal of Immunology* 37: 3040-3053.

Barrett J.C., Hansoul S., Nicolae D.L., Cho J.H., Duerr R.H., Rioux J.D., Brant S.R., Silverberg M.S., Taylor K.D., Barmada M.M., Bitton A., et al. 2008, Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease, *Nature Genetics* 40: 955-962

Barton G.M., Kagan J.C. 2009, A cell biological view of Toll–like receptor function: regulation through compartmentalization. *Nature Reviews Immunology* 9: 535-542.

Bauer S. 2006, Toll–erating self DNA. *Nature Immunology* 7: 13-15.

Beatty J. 1990, Evolutionary anti-reductionism: historical reflections. *Biology and Philosophy* 5: 197-210.

Bedau M.A. 1997, Weak Emergence. *Philosophical Perspectives* 11: 375-399.

Bedau M.A. 2002, Downward Causation and the Autonomy of Weak Emergence, *Principia* 6: 5-50.

Bedau M.A. 2008, Is weak emergence just in the mind? *Minds & Machines* 18: 443-459.

Bedau M.A. and Humphreys P. (Eds.) 2007, *Emergence: Contemporary Readings in Philosophy and Science*, MIT Press: London.

Belkaid Y. and Tarbell K. 2009, Regulatory T cells in the control of host–microorganism interactions. *Annual Review of Immunology* 27: 551-589.

Benoist C., Germain R., Mathis D. 2006, A *Plaidoyer* for 'Systems Immunology'. *Immunological Reviews* 210: 229-234.

Blaser M.J., Atherton J.C. 2004, Helicobacter pylori persistence: biology and disease. *Journal of Clinical Investigation* 113: 321-333.

Borchers A.T., Selmi C., Meyers F.J., Keen C.L., Gershwin M.E. 2009, Probiotics and immunity, *Journal of Gastroenterology* 44: 26-46.

Brant S.R., McGovern D.P. 2005, NOD2, not yet: con. *Inflammatory Bowel Disease* 11: 507-509.

Brenner S. 2010, Sequences and consequences. *The Philosophical Transactions of the Royal Society B* 365: 207-212.

Brigandt I. and Love A. 2008, Reductionism in biology, *The Stanford Encyclopedia of Philosophy*. E.N. Zalta (ed.), URL = <http://plato.stanford.edu/archives/fall2008/entries/reduction-biology/>

Brown P. 2001, Cinderella goes to the ball. *Nature* 410: 1018-1020.

Burnet F.M. and Fenner F. 1949, *The Production of Antibodies*. Melbourne: Macmillan and Co.

Butcher E.C., Berg E.L., Kunkel E.J. 2004, Systems biology in drug discovery. *Nature Biotechnology* 22: 1253-1299.

Cario E., Brown D., McKee M., Lynch–Devaney K., Gerken G., Podolsky D.K., 2002, Commensal–associated molecular patterns induce selective toll–like receptor–traficking

from apical membrane to cytoplasmic compartments in polarized intestinal epithelium, *American Journal of Pathology* 160: 165-173.

Casadevall A. and Pirofski L. 2001, Host–pathogen interactions: The attributes of virulence. *The Journal of Infectious Diseases* 184: 337-344.

Casadevall A. and Pirofski L. 2002, What is a pathogen? *Annals of Medicine* 34: 2-4.

Cedar H., Bergman Y. 2009, Linking DNA methylation and histone modification: patterns and paradigms. *Nature Review Genetics* 10: 295-304.

Celli J.P., Turner B.S., Afdhal N.H., Keates S., Ghiran I., Kelly C.P., Ewoldt R.H., McKinley G.H., So P., Erramilli S., Bansil R. 2009, Helicobacter pylori moves through mucus by reducing mucin viscoelasticity. *The Proceedings of the National Academy of Sciences U.S.A.* 106: 14321-14326.

Chalmers D. 2006, Strong and weak emergence, In P. Clayton and P. Davies, (eds.) *The Re-emergence of Emergence*, Oxford: Oxford University Press.

Chassaing B., Darfeuille-Michaud A. 2011, The commensal microbiota and enteropathogens in the pathogenesis of inflammatory bowel diseases. *Gastroenterology* 140: 1720-1728.

Cho J. H. and Abraham C. 2007, Inflammatory bowel disease genetics: Nod2. *Annual Review of Medicine* 58: 401-416.

Cohen I.R. 1992a, The cognitive principle challenges clonal selection. *Immunology Today* 13: 441-444.

Cohen I.R., 1992b, The cognitive paradigm and the immunological Homunculus. *Immunology Today* 13: 490-494.

Cohen I.R. 2000, *Tending Adam's garden*. *Academic Press.* San Diego, CA.

Cohen I.R. 2000a, Discrimination and dialogue in the immune system. *Seminars in Immunology* 12: 215-219.

Cohen I.R. 2006, Immune system computation and the immunological homunculus, In: O. Niestrasz, J. Whittle, D. Harel and G. Reggio, Eds. *Lectures notes in computer science 4199 MoDELS*, Springer-Verlag, Berlin, pp. 499-512.

Cohen I.R. 2007, Biomarkers, self–antigens and the immunological homunculus. *Journal of Autoimmunity*, 29: 246-249.

Cohen I.R. and Harel D. 2007, Explaining a Complex Living System: Dynamics, Multi–Scaling and Emergence. *Journal of the Royal Society Interface* 4: 175-182.

Cohen–Sfady M., Pevsner–Fischer M., Margalit R., Cohen I.R. 2009, Heat shock protein 60, via MyD88 innate signaling, protects B cells from apoptosis, spontaneous and induced, *The Journal of Immunology* 183: 890-896.

Collingwood R.G. 1933, *An Essay on Philosophical Method*. Oxford: Clarendon Press.

Conroy H., Marshall N.A., Mills K.H.G. 2008, TLR ligand suppression or enhancement of Treg cells? A double–edged sword in immunity to tumors. *Oncogene* 27: 168-180.

Coombes J.L., Maloy K.J., 2007, Control of intestinal homeostasis by regulatory T cells and dendritic cells. *Seminars in Immunology* 19: 116-126.

Coombes J.L., Powrie F. 2008, Dendritic cells in intestinal immune regulation, *Nature Reviews Immunology* 8: 435-446.

Cornish-Bowden A., Cardenas M.L., Letelier J.-C., et al. 2004, Understanding the parts in terms of the whole, *Biology of the Cell* 96: 713-117.

Corr S. C., Li Y., Riedel C. U., O'Toole P. W., Hill C., Gahan C. G. M. 2007, Bacteriocin production as a mechanism for the antiinfective activity of Lactobacillus salivarius UCC118, *The Proceedings of the National Academy of Sciences U.S.A.* 104: 7617-7621.

Darfeuille-Michaud A. et al. 1998, Presence of adherent Escherichia coli strains in ileal mucosa of patients with Crohn's disease. *Gastroenterology* 115: 1405-1413.

Das K.M., Biancone L. 2008, Is IBD an autoimmune disorder? *Inflammatory Bowel Disease* Suppl 2: S97-101.

De Backer P., De Waele D., Van Speybroeck L., 2010, Ins and outs of systems biology vis-à-vis molecular biology: continuation or clear cut? *Acta Biotheoretica* 58: 15-49.

De Bernardis F., Mühlschlegel F.A., Cassone A., Fonzi W.A. 1998, The pH of the host niche controls gene expression in and virulence of Candida albicans. *Infection and Immunity* 66: 3317-3325.

Denning T.L., Norris B.A., Medina-Contreras O., Manicassamy S., Geem D., Madan R., Karp C.L., Pulendran B. 2011, Functional Specializations of Intestinal Dendritic Cell and Macrophage Subsets That Control Th17 and Regulatory T Cell Responses Are Dependent on the T Cell/APC Ratio, Source of Mouse Strain, and Regional Localization. *Journal of Immunology* 187: 733-747.

Dubuquoy L. et al. 2003, Impaired expression of peroxisome proliferator-activated receptor gamma in ulcerative colitis. *Gastroenterology* 124: 1265-1276.

Duerr R.H., Targan S.R., Landers C.J., LaRusso N.F., Lindsay K.L., Wiesner R.H., Shanahan F. 1991, Neutrophil cytoplasmic antibodies: a link between primary sclerosing cholangitis and ulcerative colitis, *Gastroenterology* 100: 1385-1391.

Duerr R.H., Taylor K.D., Brant S.R., Rioux J.D., Silverberg M.S., Daly M.J., Steinhart A.H., Abraham C., Regueiro M., Griffiths A., Dassopoulos T., Bitton A., Yang H., Targan

S., Datta L.W., Kistner E.O., Schumm L.P., Lee A.T., Gregersen P.K., Barmada M.M., Rotter J.I., Nicolae D.L., Cho J.H. 2006, A genome-wide association study identifies IL23R as an inflammatory bowel disease gene. *Science* 314: 1461-1463.

Dupré J. 1993, *The disorder of things: metaphysical foundations of the disunity of science*, Harvard University Press, Cambridge, MA.

Edén C.S., Hanson L.A., Jodal U., Lindberg U., Akerlund A.S. 1976, Variable adherence to normal human urinary–tract epithelial cells of Escherichia coli strains associated with various forms of urinary–tract infection. *Lancet* 7984: 490-492.

Falkow S. 2006, Is persistent bacterial infection good for your health? *Cell* 124: 699-702.

Fernandez M., Pedron T., Tournebize R., Olivo–Marin J., Sansonetti P., Phalipon A. 2003, Anti–Inflammatory Role for Intracellular Dimeric Immunoglobulin A by Neutralization of Lipopolysaccharide in Epithelial Cells. *Immunity* 18: 739-749.

Ferrero R.L. 2005, Innate immune recognition of the extracellular mucosal pathogen, Helicobacter pylori. *Molecular Immunology* 42: 879-885.

Feyerabend P. 1962, Explanation, Reduction and Empiricism. *Minnesota Studies in the Philosophy of Science* 3: 28-97.

Fischer H., Yamamoto M., Akira S., Beutler B., Svanborg C. 2006, Mechanism of pathogen–specific TLR4 activation in the mucosa: fimbriae, recognition receptors and adaptor protein selection. *European Journal of Immunology* 36: 267-277.

Fodor J.A. 1974, Special sciences (or: the disunity of sciences as a working hypothesis), *Synthese* 28: 97-115.

Foxman B., Goldberg D., Murdock C., Xi C., Gilsdorf J.R. 2008, Conceptualizing human microbiota: from multicelled organ to ecological community. *Interdisciplinary Perspectives on Infectious Diseases* 2008: 1-5.

Franchimont D., Vermeire S., El Housni H., et al. 2004, Deficient host–bacteria interactions in inflammatory bowel disease? The toll–like receptor (TLR)–4 Asp299gly polymorphism is associated with Crohn's disease and ulcerative colitis. *Gut* 53: 987-992.

Frank D.N., St Amand A.L., Feldman R.A., Boedeker E.C., Harpaz N., Pace N.R. 2007, Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases, *Proceedings of the National Academy of Sciences U.S.A.* 104: 13780-13785.

Franke A., Balschun T., Karlsen T.H., Hedderich J., May S., Lu T., Schuldt D., Nikolaus S., Rosenstiel P., Krawczak M., Schreiber S. 2008, Replication of signals from recent studies of Crohn's disease identifies previously unknown disease loci for ulcerative colitis. *Nature Genetics* 40: 713-715.

Fritz, J.H., and Girardin, S.E. 2005, How Toll–like receptors and Nod–like receptors contribute to innate immunity in mammals. *Journal of Endotoxin Research* 11, 390-394.

Galán J.E., Collmer A. 1999, Type III secretion machines: bacterial devices for protein delivery into host cells. *Science* 284: 1322-1328.

Galán J.E., Wolf–Watz H. 2006, Protein delivery into eukaryotic cells by type III secretion machines. *Nature* 444: 567-573.

Gardy J.L., Lynn D.J., Brinkman F.S., Hancock R.E. 2009, Enabling a systems biology approach to immunology: focus on innate immunity. *Trends in Immunology* 30: 249-262.

Garrett W.S., et al. 2007, Communicable ulcerative colitis induced by T-bet deficiency in the innate immune system. *Cell* 131: 33-45.

Garrett W.S., Gordon J.I., Glimcher L.H. 2010, Homeostasis and inflammation in the intestine. *Cell* 140: 859-870.

Gatherer D. 2010, So what do we really mean when we say that systems biology is holistic? *BMC Systems Biology* 4: 22.

Ge H., Walhout A.J., Vidal M. 2003, Integrating 'omic' information: a bridge between genomics and systems biology. *Trends in Genetics* 19: 551-560.

Gewirtz A.T., Navas T.A., Lyons S., Godowski P.J., Madara J.L. 2001, Cutting edge: bacterial flagellin activates basolaterally expressed TLR5 to induce epithelial proinflammatory gene expression. *Journal of Immunology* 167: 1882-1885.

Gilbert S.F. and Sarkar S. 2000, Embracing complexity: organicism for the 21st century. *Developmental Dynamics* 219: 1-9.

Girardin S.E., Boneca I.G., Carneiro L.A., Antignac A., Jéhanno M., Viala J., Tedin K., Taha M.K., Labigne A., Zähringer U., Coyle A.J., DiStefano P.S., Bertin J., Sansonetti P.J., Philpott D.J. 2003a, Nod1 detects a unique muropeptide from gram–negative bacterial peptidoglycan. *Science* 300: 1584-1587.

Girardin S.E., Boneca I.G., Viala J., Chamaillard M., Labigne A., Thomas G., Philpott D. J., Sansonetti P.J. 2003b, Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection. *The Journal of Biological Chemistry* 278: 8869-8872.

Goldman W.E., Klapper D.G., Baseman J.B. 1982, Detection, isolation, and analysis of a released Bordetella pertussis product toxic to cultured tracheal cells. *Infection and Immunity* 36: 782-794.

Guan R., Mariuzza R.A. 2007, Peptidoglycan recognition proteins of the innate immune system. *Trends in Microbiology* 15: 127-134.

Guerra N., Tan Y.X., Joncker N.T. 2008, NKG2D-deficient mice are defective in tumor surveillance in models of spontaneous malignancy. *Immunity* 28: 571-80.

Haas T., Metzger J., Schmitz F., Heit A., Müller T., Latz E., Wagner H. 2008, The DNA Sugar Backbone 2′ Deoxyribose Determines Toll–like Receptor 9 Activation. *Immunity* 28: 315-323.

Hampe J., Franke A., Rosenstiel P., Till A., Teuber M., Huse K., Albrecht M., Mayr G., De La Vega F.M., Briggs J., Günther S., Prescott N.J., Onnie C.M., Häsler R., Sipos B., Fölsch U.R., Lengauer T., Platzer M., Mathew C.G., Krawczak M., Schreiber S. 2007, A genome-wide association scan of nonsynonymous SNPs identifies a susceptibility variant for Crohn disease in ATG16L1. *Nature Genetics* 39: 207-211.

Hase K., Kawano K., Nochi T., Pontes G.S., Fukuda S., Ebisawa M., Kadokura K., Tobe T., Fujimura Y., Kawano S., Yabashi A., Waguri S., Nakato G., Kimura S., Murakami T., Iimura M., Hamura K., Fukuoka S., Lowe A.W., Itoh K., Kiyono H., Ohno H. 2009, Uptake through glycoprotein 2 of FimH(+) bacteria by M cells initiates mucosal immune response. *Nature* 462: 226-230.

He B., Xu W., Santini P.A., Polydorides A.D., Chiu, A., Estrella J., Shan M., Chadburn A., Villanacci V., Plebani A., et al. 2007, Intestinal bacteria trigger T cell–independent immunoglobulin A(2) class switching by inducing epithelial–cell secretion of the cytokine APRIL. *Immunity* 26: 812-826.

Heinsbroek S.E.M., Gordon S., 2007, Macrophages, In: G.D. Brown, M.G. Netea (eds.), *Immunology of Fungal Infections*, Springer: Dordrecht, pp. 3-25.

Hemmi H., Takeuchi O., Kawai T., Kaisho T., Sato S., Sanjo H., Matsumoto M., Hoshino K., Wagner H., Takeda K., Akira S. 2000, A Toll–like receptor recognizes bacterial DNA. *Nature* 408: 740-745.

Hempel, C.G., and Oppenheim P. (1965 [1948]), Studies in the logic of explanation, In C.G. Hempel (ed.), *Aspects of scientific explanation and other essays in the philosophy of science*, New York: Free Press, pp. 245-290

Hentschel U., Dobrindt U., Steinert M. 2003, Commensal bacteria make a difference. *Trends in Microbiology* 11: 148-150.

Hentschel U., Steinert M., Hacker J. 2000, Common molecular mechanisms of symbiosis and pathogenesis. *Trends in Microbiology* 8: 226-231.

Hershberg U. and Efroni S. 2001, The Immune System and Other Cognitive Systems. *Complexity* 6: 14-21.

Hood L. and Perlmutter R.M. 2004, The impact of systems approaches on biological problems in drug discovery. *Nature Biotechnology* 22: 1215-1217.

Hood L., Heath J.R., Phelps M.E., Lin B. 2004, Systems biology and new technologies enable predictive and preventative medicine. *Science* 306: 640-643.

Hugot J.P., Laurent–Puig P., Gower–Rousseau C., et al. 1996, Mapping of a susceptibility locus for Crohn's disease on chromosome 16. *Nature* 379: 821-823.

Hull D. 1974, *Philosophy of biological science*. New Jersey: Prentice-Hall Inc.

Ideker T. 2004, Data collection and analysis in systems biology, in: *Encyclopedia of Genetics, Genomics, Proteomics and Bioinformatics*, J. Wiley & Sons, pp. 2736-2744.

Ideker T., Thorsson V., Ranish J.A. et al. 2001, Integrated genomics and proteomic analyses of a systematically perturbed metabolic network. *Science* 292: 929-934.

Iliev I.D., Matteoli G., Rescigno M. 2007, The yin and yang of intestinal epithelial cells in controlling dendritic cell function. *The Journal of Experimental Medicine* 204: 2253-2257.

Iliev I.D., Mileti E., Matteoli G., Chieppa M., Rescigno M. 2009a, Intestinal epithelial cells promote colitis–protective regulatory T–cell differentiation through dendritic cell conditioning. *Mucosal Immunology 2*, 340-350.

Iliev I. D., Spadoni I., Mileti E., Matteoli G., Sonzogni A., Sampietro G. M., Foschi D., Caprioli F., Viale G., Rescigno M. 2009b, Human intestinal epithelial cells promote the differentiation of tolerogenic dendritic cells. *Gut* 58: 1481-1489.

Ivanov I.I., Frutos R.L., Manel N., Yoshinaga K., Rifkin D.B., Sartor R.B., Finlay B.B., Littman D.R., 2008, Specific microbiota direct the differentiation of IL–17–producing T–helper cells in the mucosa of the small intestine. *Cell Host & Microbe* 4: 337-349.

Iwasaki A. 2007, Mucosal Dendritic Cells. *Annual Review of Immunology* 25: 381-418.

Janeway C.A. Jr, Medzhitov R. 2002, Innate immune recognition. *Annual Review of Immunology* 20: 197-216.

Janeway C.A., Travers P., Walport M., Shlomchik M.J. 2005, *Immunobiology*, Garland Science.

Jonson A., Normark S., Rhen M. 2005, Fimbriae, Pili, Flagella and Bacterial Virulence, In: Russell W., Herwald H. (eds.) *Concepts in Bacterial Virulence* vol. 12, Basel: Karger, pp. 67-89.

Kandel E. 2000, Cellular mechanisms of learning and the biological basis of individuality. In E. Kandel, J. Schwartz & T. Jessell (Eds.). *Principles of neural science*, New York: McGraw-Hill, pp. 1245-1279.

Kandel E. 2001, The molecular biology of memory storage: A dialogue between genes and synapses. *Science* 294: 1030-1038.

Kaparakis M., Philpott D.J., Ferrero R.L., 2007, Mammalian NLR proteins; discriminating foe from friend. *Immunology and Cell Biology* 85: 495-502.

Kaser A., Lee A.H., Franke A., Glickman J.N., Zeissig S., Tilg H., Nieuwenhuis E.E., Higgins D.E., Schreiber S., Glimcher L.H., Blumberg R.S. 2008, XBP1 links ER stress to intestinal inflammation and confers genetic risk for human inflammatory bowel disease. *Cell* 134: 743-756.

Kaser A, Zeissig S, Blumberg RS. 2010, Inflammatory bowel disease. *Annual Review Immunology* 28: 573-621.

Kellenberger E. 2004, The evolution of molecular biology. *EMBO Reports* 5: 546-549.

Kelly D., Campbell J.I., King T.P., Grant G., Jansson E.A., Coutts A.G., Pettersson S., Conway S. 2004, Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR-gamma and RelA. *Nature Immunology* 5: 104-112.

Kerr P., McFadden G. 2002, Immune Responses to Myxoma Virus. *Viral Immunology* 15: 229-246.

Kirk P., Bazan J.F. 2005, Pathogen recognition: TLRs throw us a curve. *Immunity* 23: 347-350.

Kitano H. 2001, *Foundations of Systems Biology*, Cambridge MA: MIT Press.

Kitano H. 2002, Systems biology: A brief overview. *Science* 295: 1662-1664.

Kitano H. 2004, Biological robustness. *Nature Review Genetics* 5: 826-837.

Kitano H. 2004, Cancer as a robust system: implications for anticancer therapy. *Nature Reviews Cancer* 4: 227-235.

Kitano H. 2007, Biological robustness in complex host–pathogen systems. *Progress in Drug Research* 64: 241-263.

Kitano H. 2007, Towards a theory of biological robustness. *Molecular Systems Biology* 3.

Kitcher P. 1984, 1953 and all that. A tale of two sciences. *Philosophical Review* 93: 335-373.

Klemm P., Hancock V., Schembri M.A. 2007, Mellowing out: adaptation to commensalism by Escherichia coli asymptomatic bacteriuria strain 83972. *Infection and Immunity* 75: 3688-3695.

Koropatnick T.A., Engle J.T., Apicella M.A., Stabb E.V., Goldman W.E., McFall–Ngai M.J. 2004, Microbial factor–mediated development in a host–bacterial mutualism. *Science* 306: 1186-1188.

Kumagai Y., Takeuchi O., Akira S. 2008, Pathogen recognition by innate receptors. *The Journal* of *Infection and Chemotherapy* 14: 86-92.

Kyd J.M., Cripps A.W. 2008, Functional differences between M cells and enterocytes in sampling luminal antigens. *Vaccine* 26: 6221-6224.

Le Negrate G., Faustin B., Welsh K., Loeffler M., Krajewska M., Hasegawa P., Mukherjee S., Orth K., Krajewski S., Godzik A., Guiney D.G., Reed J.C. 2008, Salmonella secreted factor L deubiquitinase of Salmonella typhimurium inhibits NF–kappaB, suppresses IkappaBalpha ubiquitination and modulates innate immune responses. *The Journal of Immunology* 180: 5045-5056.

Leber J.H., Crimmins G.T., Raghavan S., Meyer–Morse N.P., Cox J.S., Portnoy D.A. 2008, Distinct TLR– and NLR–mediated transcriptional responses to an intracellular pathogen. *PLoS Pathogens* 4: e6.

Lee J., Gonzales–Navajas J.M., Raz E. 2008, The "polarizing–tolerizing" mechanism of intestinal epithelium: its relevance to colonic homeostasis. *Seminars in Immunopathology* 30: 3-9.

Lee J., Mo J.H., Katakura K., Alkalay I., Rucker A.N., Liu Y.T., Lee H.K., Shen C., Cojocaru G., Shenouda S. et. al. 2006. Maintenance of colonic homeostasis by distinctive apical TLR9 signalling in intestinal epithelial cells. *Nature Cellular Biology 8*: 1327-1336.

Lehar S. M., Bevan M. J. 2004, Immunology: Polarizing a T–cell response. *Nature* 430: 150-151.

Ley R.E., Peterson D.A., Gordon J.I. 2006, Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell* 124: 837-848.

Liang S.C., Tan X.Y., Luxenberg D.P., Karim R., Dunussi-Joannopoulos K., Collins M., Fouser L.A. 2006, Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. *The Journal of Experimental Medicine* 203: 2271-2279.

Mackner L.M., Clough-Paabo E., Pajer K., Lourie A., Crandall W.V. 2011, Psychoneuroimmunologic factors in inflammatory bowel disease. *Inflammatory Bowel Disease* 17: 849-857.

Magalhaes J.G., Tattoli I., Girardin S.E. 2007, The intestinal epithelial barrier: how to distinguish between the microbial flora and pathogens. *Seminars in Immunology* 19: 106-115.

Mangan P.R., Harrington L.E., O'Quinn D.B., Helms W.S., Bullard D.C., Elson C.O., Hatton R.D., Wahl S.M., Schoeb T.R., Weaver C.T. 2006, Transforming growth factor–beta induces development of the T(H)17 lineage. *Nature* 441: 231-234.

Marteyn B., West N.P., Browning D.F., Cole J.A., Shaw J.G., Palm F., Mounier J., Prévost M.C., Sansonetti P., Tang C.M. 2010, Modulation of Shigella virulence in response to available oxygen in vivo. *Nature* 465: 355-358.

Martin H.M., Campbell B.J., Hart C.A., Mpofu C., Nayar M., Singh R., Englyst H., Williams H.F., Rhodes J.M. 2004, Enhanced Escherichia coli adherence and invasion in Crohn's disease and colon cancer. *Gastroenterology* 127: 80-93.

Martinez-Medina M., Aldeguer X., Lopez-Siles M., González-Huix F., López-Oliu C., Dahbi G., Blanco J.E., Blanco J., Garcia-Gil L.J., Darfeuille-Michaud A. 2009, Molecular diversity of Escherichia coli in the human gut: new ecological evidence supporting the role of adherent-invasive E. coli (AIEC) in Crohn's disease. *Inflammatory Bowel Disease* 15: 872-882.

Maslowski K.M., Vieira A.T., Ng A., Kranich J., Sierro F., Yu D., Schilter H.C., Rolph M.S., Mackay F., Artis D., Xavier R.J., Teixeira M.M., Mackay C.R. 2009, Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. *Nature* 461: 1282-1286.

Matzinger P. 2002, The Danger Model: a Renewed Sense of Self. *Science* 296: 301-305.

Matzinger P., Kamala T. 2011, Tissue-based class control: the other side of tolerance. *Nature Review Immunology* 11: 221-230.

May G.R., Sutherland L.R., Meddings J.B. 1993, Is small intestinal permeability really increased in relatives of patients with Crohn's disease? *Gastroenterology* 104: 1627-1632.

Medzhitov R. 2007, Recognition of microorganisms and activation of the immune response. *Nature* 449: 819-826.

Merbl Y., Zucker–Toledano M., Quintana F.J., Cohen I.R. 2007, Newborn Humans Manifest Autoantibodies to Defined Self Molecules Detected by Antigen Microarray Informatics. *The Journal of Clinical Investigation* 117: 712-718.

Merbl Y., Itzchak R., Vider–Shalit T., Louzoun Y., Quintana F. J., Vadai E., Eisenbach L., Cohen I. R. 2009, A systems immunology approach to the host–tumor interaction: large–

scale patterns of natural autoantibodies distinguish healthy and tumor–bearing mice. *PLoS One* 4: e6053.

Mesarovic M.D., Sreenath S.W., Keene J.D. 2004, Search for organizing principles: understanding in systems biology. *Systems Biology* 1: 19-27.

Mileti E., Matteoli G., Iliev I.D., Rescigno M. 2009, Comparison of the immunomodulatory properties of three probiotic strains of Lactobacilli using complex culture systems: prediction for in vivo efficacy. *PLoS One* 4: e7056.

Mills K. H. 2008, TLR9 turns the tide on Treg cells. *Immunity* 29: 518-520.

Milo R., Shen-Orr S., Itzkovitz S., Kashan N., Chklovskii D., Alon U. 2002, Network motifs: simple building blocks of complex networks. *Science* 298: 824-827.

Miyashita Y. 2004, Cognitive memory: cellular and network machineries and their top-down control. *Science* 306: 435-440.

Mossman K.L., Mian M.F., Lauzon N.M., Gyles C.L., Lichty B., Mackenzie R., Gill N., Ashkar A.A. 2008, Cutting edge: FimH adhesin of type 1 fimbriae is a novel TLR4 ligand. *The Journal of Immunology* 181: 6702-6706.

Mueller C. and Macpherson A.J. 2006, Layers of mutualism with commensal bacteria protect us from intestinal inflammation. *Gut* 55: 276-284.

Münz C., Steinman R. M., Fujii S. 2005, Dendritic cell maturation by innate lymphocytes: coordinated stimulation of innate and adaptive immunity. *The Journal of Experimental Medicine* 202: 203-207.

Murphy K. M., Travers P., Walport M. 2008, *Janeway's Immunobiology*, 7th Revised Edition Taylor & Francis Inc.

Nagai H., Roy C.R. 2003, Show me the substrates: modulation of host cell function by type IV secretion systems. *Cellular Microbiology* 5: 373-383.

Nagel, E. 1961, *The structure of science: problems in the logic of scientific explanation*. New York: Harcourt, Brace & World.

Nathan C. 2006, Neutrophils and immunity: challenges and opportunities. *Nature Reviews Immunology* 6: 173-182.

Neisser U. 1997, The ecological study of memory. *Philosophical Transactions of the Royal Society of London Series B Biological Sciences*, 352: 1697-1701.

Netea M. G., Brown G. , Kullberg B. J. Gow N. A. R. 2008, An integrated model of the recognition of 'Candida albicans' by the innate immune system. *Nature Reviews Microbiology* 6: 67-78.

Neuman Y. 2004, Meaning–making in the immune system. *Perspectives in Biology and Medicine* 47: 317-327.

Newton S.M., Jacob C.O., Stocker B.A. 1989, Immune response to cholera toxin epitope inserted in Salmonella flagellin. *Science* 244: 70-72.

Nickles T. 1973, Two concepts of inter-theoretic reduction. *Journal of Philosophy* 70: 181-201.

Noble D. 2008. Claude Bernard the first systems biologist and the future if physiology. *Experimental Physiology* 93: 16-26.

Norton J. unpublished, Approximation and idealization: Why the difference matters. http://philsci-archive.pitt.edu/8622/

O'Malley M.A., Duprè J. 2005, Fundamental issues in systems biology. *Bioessays* 27: 1270-1276.

O'Hara A. M., Shanahan F. 2006, The gut flora as a forgotten organ. *EMBO Report* 7: 688-693.

O'Mahony C., Scully P., O'Mahony D., Murphy S., O'Brien F., Lyons A., Sherlock G., MacSharry J., Kiely B., Shanahan F., O'Mahony L. 2008, Commensal–induced regulatory T cells mediate protection against pathogen–stimulated NF–kappaB activation. *PLoS Pathogens* 4(8): e1000112.

Ott S.J., Musfeldt M., Wenderoth D.F., Hampe J., Brant O., et al. 2004. Reduction in diversity of the colonic mucosa associated bacterial microflora in patients with active inflammatory bowel disease. *Gut* 53: 685-93.

Palsson B. 2000, The challenges of in silico biology. *Nature Biotechnology* 18: 1147-1150.

Papineau D. 1989, Pure, Mixed, and Spurious Probabilities and Their Significance for a Reductionist Theory of Causation, In P. Kitcher and W. Salmon (eds.) *Scientific Explanation*, Minneapolis: University of Minnesota Press, pp. 307-348.

Penfield W. and Boldrey E. 1958, Somatic Motor and Sensory Representation in the Cerebral Cortex of Man as Studied by Electrical Stimulation. *Brain* 60: 389-443.

Podolsky, D.K. 2002, Inflammatory bowel disease. *The New England Journal of Medicine* 347: 417-429.

Powell A, Dupré J. 2009, From molecules to systems: the importance of looking both ways. *Studies in History and Philosophy of Biological and Biomedical Sciences* 40: 54-64.

Powell K. 2004, All systems go. *The Journal of Cell Biology* 165: 299-303.

Pradeu T. and Carosella, E.D. 2006, On the Definition of a Criterion of Immunogenicity. *Proceedings of the National Academy of Sciences U.S.A.*, 103: 17858-17861.

Pulendran B., Palucka K., Banchereau J., 2001, Sensing Pathogens and Tuning Immune Responses, *Science* 13: 253-256.

Putnam H. 1975, Philosophy and our mental life. In (Putnam 1975a, p. 291-303).

Putnam H. 1975a, *Philosophical Papers. Vol. 2: Mind, Language and Reality*. Cambridge: Cambridge University Press.

Putnam, H. 1967, Psychological Predicates, In W. Caplan and D. Merrill (eds.), *Art, Mind and Religion*. Pittsburgh: University of Pittsburgh Press.

Quintana F.J., Cohen I.R. 2011, The HSP60 immune system network. *Trends in Immunology* 32: 89-95.

Quintana F.J., Weiner H.L. 2009, Environmental control of Th17 differentiation. *European Journal of Immunology* 39: 655-657.

Rakoff-Nahoum S., Medzhitov R. 2008, Innate immune recognition of the indigenous microbial flora. *Mucosal Immunology* 1 Suppl 1:S10-4.

Rendón M.A., Saldaña Z., Erdem A.L., Monteiro–Neto V., Vázquez A., Kaper J.B., Puente J.L., Girón J.A. 2007, Commensal and pathogenic Escherichia coli use a common pilus adherence factor for epithelial cell colonization. *Proceedings of the National Academy of Science U.S.A.* 104: 10637-10642.

Rescigno M., 2009, Gut commensal flora: tolerance and homeostasis. *F1000 Biology Reports* 1: 1-6.

Rescigno M., Lopatin U., Chieppa M. 2008, Interactions among dendritic cells, macrophages, and epithelial cells in the gut: implications for immune tolerance. *Current Opinion in Immunology* 20: 669-675.

Rescigno M., Nieuwenhuis E.E. 2007, The role of altered microbial signaling via mutant NODs in intestinal inflammation. *Current Opinion in Gastroenterology* 23: 21-26.

Rescigno M., Urbano M., Valzasina B., Francolini M., Rotta G., Bonasio R., Granucci F., Kraehenbuhl J. P., Ricciardi–Castagnoli P., 2001, Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nature Immunology* 4: 361-367.

Rimoldi M., Chieppa M., Larghi P., Vulcano M., Allavena P., Rescigno M. 2005a, Monocyte-derived dendritic cells activated by bacteria or by bacteria-stimulated epithelial cells are functionally different. *Blood* 106: 2818-2826.

Rimoldi, M., Chieppa, M, Salucci, V., Avogadri, F., Sonzogni, A., Sampietro, G. M, Nespoli, A., Viale, G., Allavena, P., Rescigno, M., 2005, Intestinal immune homeostasis is regulated by the crosstalk between epithelial cells and dendritic cells. *Nature Immunology* 6: 507-514.

Rosenberg A. 1994, *Instrumental Biology or The Disunity of Science,* Chicago: University of Chicago Press.

Rosenberg A. 2007, Reductionism in biology. In D.M. Gabbat, P. Thagard, J. Woods (eds.) *Handbook of the Philosophy of Science. Philosophy of Biology*, Elsevier.

Rubin R.H. 1993, Fungal and bacterial infections in the immunocompromised host. *European Journal of Clinical Microbiology & Infectious Diseases,*12 Suppl 1: S42-48.

Rumbo M., Anderle P., Didierlaurent A., Sierro F., Debard N., Sirard J.C., Finke D., Kraehenbuhl J.P. 2004, How the gut links innate and adaptive immunity. *Annals of The New York Academy of Sciences* 1029: 16-21.

Saemann M.D., Bohmig G.A., Osterreicher C.H., Burtscher H., Parolini O., Diakos C., Stockl J., Walter H., Horl W.H., Zlabinger G.J. 2000, Anti–inflammatory effects of sodium

butyrate on human monocytes: potent inhibition of IL–12 and up–regulation of IL–10 production. *FASEB Journal* 14: 2380-2382.

Salmon W. 1971, *Statistical Explanation and Satistical Relevance*, Pittsburgh: University of Pittsburgh Press.

Sandor F., Buc M. 2005, Toll–like receptors. Structure, function and their ligands. *Folia Biologica* 51: 148-157.

Sansonetti P., Medzhitov R. 2009, Learning Tolerance while Fighting Ignorance. *Cell* 138: 416-420.

Sansonetti, P.J. 2004, War and peace at mucosal surfaces. *Nature Reviews Immunology* 4: 953-964.

Sarkar S. 1998, *Genetics and reductionism*. Cambridge: Cambridge University Press.

Sartor R.B. 2008, Microbial influences in inflammatory bowel diseases. *Gastroenterology* 134: 577-594.

Schaffner, K.F. 1976, "Reductionism in biology: Prospects and problems", in R.S. Cohen, *et al.* (eds), *PSA 1974*, D. Reidel Publishing Company, pp. 613-632.

Schaffner K.F. 1993, *Discovery and explanation in biology and medicine.* Chicago: Chicago University Press.

Schaffner K.F. 2006, Reduction: the Cheshire cat problem and return to roots. *Synthese* 151: 377-402.

Schmausser B., Andrulis M., Endrich S., Lee S. K., Josenhans C., Muller–Hermelink H.K., Eck M. 2004, Expression and subcellular distribution of toll–like receptors TLR4, TLR5 and TLR9 on the gastric epithelium in *Helicobacter pylori* infection. *Clinical and Experimental Immunology* 136: 521-526.

Schoeberl B., Eichler-Jonsson C., Gilles E.D., Muller G. 2002, Computational modeling of the dynamics of the MAP kinase cascade activated by surface and internalized EGF receptor. *Nature Biotechnology* 20: 370-375.

Schwartz M. and Cohen I.R. 2000, Autoimmunity can Benefit Self–maintenance. *Immunology Today* 21: 265-268.

Seong S.Y., Matzinger P. 2004, Hydrophobicity: an ancient damage-associated molecular pattern that initiates innate immune responses. *Nature Reviews Immunology* 4: 469-478.

Silver A.C., Kikuchi Y., Fadl A.A., Sha J., Chopra A.K., Graf J. 2007, Interaction between innate immune cells and a bacterial type III secretion system in mutualistic and pathogenic associations. *Proceedings of the National Academy of Sciences U.S.A.* 104: 9481-9486.

Silverstein A.M. 1989, *History of Immunology*. San Diego: Academic Press.

Sina C., Gavrilova O., Förster M., Till A., Derer S., Hildebrand F., Raabe B., Chalaris A., Scheller J., Rehmann A., Franke A., Ott S., Häsler R., Nikolaus S., Fölsch U.R., Rose-John S., Jiang H.P., Li J., Schreiber S., Rosenstiel P. 2009, G protein-coupled receptor 43 is essential for neutrophil recruitment during intestinal inflammation. *Journal of Immunology* 183: 7514-7522.

Slack E., Hapfelmeier S., Stecher B., Velykoredko Y., Stoel M., Lawson M.A., Geuking M.B., Beutler B., Tedder T.F., Hardt W.D., Bercik P., Verdu E.F., McCoy K.D., Macpherson A.J., Innate and adaptive immunity cooperate flexibly to maintain host-microbiota mutualism. *Science* 325: 617-620.

Smaglik P. 2000, For my next trick..., *Nature* 407: 828-829.

Smythies L.E., Sellers M., Clements R.H., Mosteller–Barnum M., Meng G., Benjamin W.H., Orenstein J.M., Smith P.D. 2005, Human intestinal macrophages display profound

inflammatory anergy despite avid phagocytic and bacteriocidal activity. *The Journal of Clinical Investigation* 115: 66-75.

Sober E. 1999, The multiple realizability argument against reductionism. *Philosophy of Science* 66: 542-564.

Sokol H., Pigneur B., Watterlot L., Lakhdari O., Bermúdez–Humarán L.G., Gratadoux J.J., Blugeon S., Bridonneau C., Furet J.P., Corthier G., Grangette C., Vasquez N., Pochart P., Trugnan G., Thomas G., Blottière H.M., Doré J., Marteau P., Seksik P., Langella P., 2008, Faecalibacterium prausnitzii is an anti–inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. *The Proceedings of the National Academy of Sciences U.S.A.* 105: 16731-16736.

Sorger P.K. 2005, A reductionis't systems biology. *Current Opinion in Cell Biology* 17: 9-11.

Sotelo J. 1999, Conspicuous Similarities Between the Nervous and Immune Systems. *Archives of Medical Research* 30: 345-346.

Sperandio V., Torres A.G., Jarvis B., Nataro J.P., Kaper J.B. 2003, Bacteria–host communication: the language of hormones. *The Proceedings of the National Academy of Sciences U.S.A.* 100: 8951-8956.

Stelling J., Sauer U., Szallasi Z., et al. 2004, Robustness of cellular functions. *Cell* 118: 675-685.

Strange K. 2005, The end of 'naïve reductionism': rise of systems biology or renaissance of physiology? *American Journal of Physiology* 288: C968-C974.

Strobl, H. and Knapp, W. 1999, TGF–β1 regulation of dendritic cells. *Microbes and Infection* 15, 1283-1290.

Szajnik M., Szczepanski M.J., Czystowska M., Elishaev E., Mandapathil M., Nowak–Markwitz E., Spaczynski M., Whiteside T.L., 2009, TLR4 signaling induced by lipopolysaccharide or paclitaxel regulates tumor survival and chemoresistance in ovarian cancer cancer. *Oncogene* 28: 4353-4363.

Takeda K., Kaisho T., Akira S., 2003, Toll–like receptors. *Annual Review of Immunology* 21: 335-376.

Tampakaki A.P., Fadouloglou V.E., Gazi A.D., Panopoulos N.J., Kokkinidis M. 2004, Conserved features of type III secretion. *Cellular Microbiology* 6: 805-816.

Tauber A. 1997, Historical and philosophical perspectives concerning immune cognition. *Journal of the History of Biology* 30: 419-440.

Tauber A. 2008, The Immune System and Its Ecology. *Philosophy of Science* 75: 224-245.

Tauber A. 2009, The Biological Notion of Self and Non–self. In: E.N. Zalta, ed. *The Stanford Encyclopedia of Philosophy.* URL = <http://plato.stanford.edu/entries/biology–self/>.

Tonegawa S. 1983, Somatic Generation of Antibody Diversity. *Nature*, 302: 575-558.

Vaishnava S., Behrendt C.L., Ismail A.S., Eckmann L., Hooper L.V. 2008, Paneth cells directly sense gut commensals and maintain homeostasis at the intestinal host-microbial interface. *Proceedings of the National* Academy *of Sciences U.S.A.* 105: 20858-20863.

Vance R.V., Isberg, R.R., Portnoy, D.A. 2009, Patterns of Pathogenesis: Discrimination of Pathogenic and Nonpathogenic Microbes by the Immune System. *Cell Host & Microbe* 6: 10-21.

Veldhoen M., Hocking R.J., Atkins C.J., Locksley R.M., Stockinger B. 2006, TGFbeta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL–17–producing T cells. *Immunity* 24: 179-189.

Vetrano S., Rescigno M., Cera M.R., et al. 2008, Unique role of junctional adhesion molecule-a in maintaining mucosal homeostasis in inflammatory bowel disease. *Gastroenterology* 135: 173-184.

Viala J., Chaput C., Boneca I.G., Cardona A., Girardin S.E., Moran A.P., Athman R., Mémet S., Huerre M.R., Coyle A.J, DiStefano P.S., Sansonetti P.J., Labigne A., Bertin J., Philpott D.J., Ferrero R.L. 2004, Nod1 responds to peptidoglycan delivered by the Helicobacter pylori cag pathogenicity island. *Nature Immunology* 5: 1166-1174.

Vilček J. 2004, Why are rabbits uniquely sensitive to myxoma virus? Cherchez l'interferon! *Nature Immunology* 5: 1205-1206.

Wagner A. 2005, *Robustness and Evolvability in Living Systems*, Princeton University Press, Princeton.

Walters M., Sperandio V. 2006 Quorum sensing in Escherichia coli and Salmonella. *Journal of Medical Microbiology* 296: 125-131.

Wang F., Ma Y., Barrett J.W., Gao X., Loh J., Barton E., Virgin H.V., McFadden G., 2004, Disruption of Erk–dependent type I interferon induction breaks the myxoma virus species barrier. *Nature Immunology* 5: 1266-1274.

Wassef J.S., Keren D.F., Mailloux J.L. 1989, Role of M cells in initial antigen uptake and in ulcer formation in the rabbit intestinal loop model of shigellosis. *Infection and Immunity* 57: 858-863.

Waters C.K. 2004, What was classical genetics? *Studies in History and Philosophy of Science* 35: 783-809.

Waters C.K. 1990, “Why the antireductionist consensus won't survive the case of classical Mendelian genetics”, in A. Fine, M. Forbes and L. Wessels (eds.), *Proceedings of the biennial meeting of the Philosophy of Science Association*, Vol. 1, East Lansing, MI: Philosophy of Science Association, pp. 125-139

Waters C.K. 1994, Genes made molecular. *Philosophy of Science* 61: 163-185.

Wimsatt W.C. 1976, Reductive explanation: a functional account”. In R.S. Cohen and A. Michalos (eds.), *Proceedings of the 1974 meeting of the Philosophy of Science Association*, Dordrecht: D. Reidel, pp. 671-710.

Wimsatt W.C. 1985, Heuristics and the study of human behavior. In: D.W. Fiske and R. Shweder (eds.), Metatheory in Social Science: Pluralisms and Subjectivities, Chicago: University of Chicago Press, pp. 492-503.

Wimsatt W.C. 1994, The ontology of complex systems: levels, perspectives, and causal thickets, in: *Biology and Society: Reflections on Methodology*, ed. M. Matthen and R. Ware, suppl. Vol. 20 of the Canadian Journal of Philosophy, pp. 207-274.

Wimsatt W.C. 2007, *Re-engineering philosophy for limited beings*, Harvard University Press, Cambridge Mass.

Wimsatt W.C. 2007a, Normative Idealizations versus the Metabolism of Error, In (Wimsatt 2007).

Woodward J. 2010, Scientific Explanation, *The Stanford Encyclopedia of Philosophy (Spring 2010 Edition)*, Edward N. Zalta (ed.), URL = <http://plato.stanford.edu/archives/spr2010/entries/scientific-explanation/>.

Yamamoto–Furusho J.K, Podolsky D.K. 2007, Innate immunity in inflammatory bowel disease. *World Journal of Gastroenterology* 13: 5577-5580.

Ye Z., Petrof E.O., Boone D., Claud E.C., Sun J. 2007, Salmonella effector AvrA regulation of colonic epithelial cell inflammation by deubiquitination. *American Journal of Pathology* 171: 882-892.

Zak D.A., Aderem A. 2009, Systems biology of innate immunity. *Immunological Reviews* 227: 264-282.