Alterations in regulatory T-cells: Rediscovered pathways in immunotoxicology

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Abstract

In addition to the effector T-cells subsets, T-cells can also differentiate into cells that play a suppressive or regulatory role in adaptive immune responses. The cell types currently identified as regulatory T-cells (T_{regs}) include natural or thymic-derived T_{regs}, T-cells which express $Foxp3^+CD25^+CD4^+$ and can suppress immune responses to autoreactive T-cells, as well as inducible T_{regs}, that are generated from naïve T-cells in the periphery after interaction with antigens presented by dendritic cells. Inducible T_{regs} include T_H3 cells, T_r1 cells, and $Foxp3^+$-inducible T_{regs}. T_{regs} have been shown to be critical in the maintenance of immune responses and T-cell homeostasis. These cells play an important role in suppressing responses to self-antigens and in controlling inappropriate responses to non-self-antigens, such as commensal bacteria or food in the gut. For example, depletion of $CD4^+CD25^+$ T_{regs} from mice resulted in the development of multi-organ autoimmune diseases. $CD4^+CD25^+$ T_{regs} and/or IL-10-producing T_r1 cells are capable of suppressing or attenuating T_H2 responses to allergens. Moreover, adoptive transfer of $CD4^+CD25^+$ T_{regs} from healthy to diseased animals resulted in the prevention or cure of certain autoimmune diseases, and was able to induce transplantation tolerance. Clinical improvement seen after allergen immunotherapy for allergic diseases such as rhinitis and asthma is associated with the induction of IL-10- and TGFβ-producing T_r1 cells as well as FoxP3-expressing IL-10 T-cells, with resulting suppression of the T_H2 cytokine milieu. Activation, expansion, or suppression of $CD4^+CD25^+$ T_{regs} *in vivo* by xenobiotics, including drugs, may therefore represent a relevant mechanism underlying immunotoxicity, including immunosuppression, allergic asthma, and autoimmune diseases.

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Declaration of interest

Keywords

Regulatory T-cells; immunosuppression; allergy; autoimmunity

Introduction

The existence of suppressor T-cells and their role in the maintenance of immune homeostasis was first proposed in the early 1970s (Gershon and Kondo, 1971). The rediscovery of suppressor T-cells as regulatory T-cells (T_{regs}) took place in the 1990s, and it is now fully accepted that distinct $CD4^+$ T-cell subtypes, discriminated on the basis of phenotype or cytokine secretion, play a critical role in maintenance of self-tolerance (Bloom et al., 1992; Sakaguchi and Powrie, 2007). T_{regs} are a specialized subpopulation of T-cells critical in the modulation of immune responses and in the maintenance of T-cell homeostasis and self-tolerance (Figure 1). There are several types of T_{regs}, including natural, T-helper (T_H)-3 and T-regulatory (T_r)-1 subsets (Bach, 2003) as well as $CD8^+$ T_{regs} (Filaci, 2007; Funatake et al., 2008; Dinesh, 2010).

Natural T_{regs} are generated in the thymus as a part of thymic selection as well as in the periphery in response to antigen exposure. Natural T_{regs} account for ≈5–10% of the total $CD4^+$ T-cells in the periphery; they express CD4, high levels of CD25 and are also positive for the transcriptional repression factor fork-head box P3 (FoxP3). They are essential for the prevention of autoimmunity and keeping the immune response under control (Sakaguchi et al., 2006) as their absence results in multi-organ autoimmune disease in both mice and humans. Adaptive or induced T_{regs} are regulatory cells that develop in the periphery, and include T_r1 and T_H3 cells that are $CD4^+CD25^-FoxP3^-$. These T_{regs} secrete interleukin (IL)-10 and transforming growth factor (TGF)-β, and regulate immune responses. It has been suggested that in contrast to naturally occurring $CD4^+CD25^+$ T_{regs}, these cells represent altered states of differentiation rather than a unique cell lineage (Jonuleit and Schmitt, 2003). Tr_1 cells are defined by their ability to produce large amounts of IL-10 and low to moderate levels of TGFβ, whereas T_H3 cells produce preferentially TGFβ. *In vitro* stimulation of $CD4^+CD25^-FoxP3^-$ cells with TGFβ induces them to express FoxP3 and secrete TGFβ. They contribute to the development of antigen-specific immunosuppressive responses (Roncarolo and Levings, 2000). Immunoregulatory $CD8^+CD28^-$ T_{regs} are present and functional in many human tumors, being able to inhibit both T-cell proliferation and cytotoxicity (Filaci, 2007). Data are accumulating showing the potential role of $CD8^+$ T_{regs} in other diseases, including chronic infection and autoimmunity (Dinesh, 2010).

The constitutive expression of CD25 has been considered to be a characteristic feature in the identification of T_{regs}; however, activated T-cells also up-regulate CD25. $CD4^+CD25^-$ T-cells that express FoxP3 have been demonstrated during immune responses associated with bacterial infection (Fontenot et al., 2005). The expression of FoxP3 is therefore the most definitive marker to identify T_{regs}. CD25 and FoxP3 are also expressed by $CD8^+$ T_{regs}. Down-regulation of CD127, a subunit of the IL7R, is another mechanism to identify T_{regs} as it is generally accepted that the $CD25^{high}/CD127^{low/-}$ phenotype is specific to these cells (Liu et al., 2006).

The balance between activated effector cells and T_{regs} is important for efficient immune responses. Just as important as mounting an immune response, organisms have to control the magnitude and duration of the response. Suppression of T_{reg} function and/or number may lead to unchecked immune responses resulting in autoimmune diseases, allergies, transplant rejection, maternal intolerance of fetal alloantigens, septic shock and chronic inflammation (Kondelkova et al., 2010). Enhancement of regulatory T-cell functions is a therapeutic goal

for the treatment of many autoimmune diseases, and therapeutics such as rapamycin and its derivatives, and corticosteroids have been shown to expand T_{regs} (Smith and Kumar 2008; Powell and Delgoffe, 2010; Stary et al., 2011). Murine T_{regs} are more resistant than effector T-cells to the apoptotic effects of dexamethasone *in vitro* and treatment of mice with dexamethasone results in an increased proportion of T_{regs} in immune tissues (Chen et al., 2004). A T_{reg} suppression of inflammatory responses can be both a desired and undesired effect depending on the disease. There is growing evidence that the suppressive effects of T_{regs} may contribute to tumor growth (Colombo and Piconese, 2007), and to poor responses to infectious agents and vaccines (Rouse et al., 2006). Therefore, the ability of xenobiotics to inhibit or activate/expand T_{regs} may represent a relevant mechanism underlying chemical-induced immunotoxicity (Fort and Narayanan, 2010).

As part of the 49th Annual Meeting of the Society of Toxicology in 2010, a symposium entitled 'Alterations in Regulatory T-Cells: Novel Pathways to Immunotoxicology' was convened to explore the role that T_{regs} may play in chemical-induced immunotoxicity. The Symposium started with an 'Introduction to the role of T_{regs} in immunity' by Mohamed Oukka, and was followed by presentations on 'Role of immunoregulatory cells in chemical and protein allergy' by Raymond Pieters, 'Induction of AHR-dependent T_{regs}: A novel pathway for TCDD immunotoxicity' by Nancy Kerkvliet, and 'Safety assessment of immunomodulatory biologics post-TGN1412: The promise and challenges of regulatory T-cell modulation' by Rafael Ponce. The purpose of this review article is to highlight the role of regulatory T-cells in allergy and in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-induced immunosuppression; a separate paper will deal with the pharmacological modulation of regulatory T-cells.

Role of immunoregulatory cells at sites of exposure

The identification of T_{regs} capable of suppressing responses mediated by T_H1 and T_H2 cells has prompted a paradigm shift in our understanding of the regulation of immune responses. Regulatory T-cells, including T_r1 and T_H3, are crucial for the induction of tolerance to food antigens, and in the maintenance of tolerance at mucosal surfaces (Battaglia et al., 2006; Lan et al., 2007). The skin, the respiratory and the gastrointestinal tract are continuously exposed to a large number of antigens, representing a relentless challenge to the immune system. An appropriate immune response must be able to differentiate between harmful and innocuous antigens. To protect the host, mechanisms must be in place to prevent deleterious reactions, such as immune responses to self-antigens, immune response to food antigens in the intestine, or exaggerated immune responses to benign environmental antigens. One key adaptation to the mucosal microenvironment is the generation within, or recruitment to the mucosal surface of T_{regs} (Allez and Mayer, 2004). Disturbance of normal immunoregulatory processes at mucosal surfaces, i.e., the breaking of tolerance, may occur, resulting in sensitization to chemicals, certain pharmaceuticals and food or airborne proteins.

Key components of the mucosal microenvironment include the luminal surface, epithelial cells, the lamina propria, and mucosal-associated lymphoid tissues (MALT), which can be found within the mucosal layers. The mucosal epithelium forms a tight protective barrier facilitated by integral membrane proteins and adhesion molecules, while the thick mucous secreted by specialized epithelial cells such as goblet cells provides another important barrier. Beneath the epithelial barrier, the lamina propria plays a critical role in host defense with its network of capillaries and lymphatics.

Communication is constantly established between the external environment and the host immune system. Mucosal epithelial cells actively participate in mucosal immunity through production of anti-microbial peptides, chemokines, and cytokines, and are equipped with pattern recognition receptors (PRRs) including Toll-like receptors and nucleotide-binding

oligomerization domain (NOD) receptors: such cells also have antigen-presenting capability (Kunisawa et al., 2007).

Functional and customized T_{reg} cells

Naïve CD4$^+$ T-cells can differentiate into many different subsets of T_H effector cells, notably T_H1, T_H2, T_H9, and T_H17 cells. The functional specialization of these various CD4$^+$ T-cell subsets is due to the differential expression of 'master' transcription factors, namely retinoic acid receptor-related orphan receptor-γt (RORγt), T-bet, and GATA-binding protein 3 (GATA3), which turn on distinct programs of gene expression that control T-cell function and migration (Littman and Rudensky, 2010). However, each of these responses is pro-inflammatory and, in some circumstances, can damage tissues and lead to autoimmunity. Furthermore, T_H1, T_H2, and T_H17 cells express various chemokine receptors that help guide these cells into appropriate tissues in which these cells are needed to fight various pathogens.

Recently, several groups demonstrated that T_{reg} cells are not all created equal; various natural-occurring T_{reg} subsets exist and express transcriptional factors and chemokine receptors commonly found in either T_H1 or T_H17 cells (Chaudhry et al., 2009; Koch et al., 2009; Zheng et al., 2009). These T_{regs} are rather specialized in preferentially suppressing T_H1 or T_H17 cells. T-bet is the master transcription factor controlling the differentiation, migration, and function of interferon (IFN)-γ-producing T_H1 cells (Szabo et al., 2000). Interestingly, T-bet is also expressed by a subset of T_{reg} cells, and is required for T_{reg} cell homeostasis and function during polarized T_H1-type immune response (Koch et al., 2009). Finally, deletion of signal transducer and activation of transcription-3 (STAT3) in T_{reg} cells results in the development of a spontaneous fatal intestinal inflammation that is characterized by excessive IL-17 production, but normal levels of T_H1-associated inflammatory cytokines; this indicates that there is selective dysregulation of T_H17-type responses in the absence of STAT3-expressing T_{reg} cells (Chaudhry et al., 2009). The mechanisms by which T-bet and STAT3 control T_{reg} cell activity during T_H1, T_H2, and T_H17 cell-mediated responses are still unclear, but are likely to involve a combination of influences on T_{reg} cell migration, function and homeostasis. Additionally, loss of these transcription factors may affect the functional properties of T_{reg} cells.

From these data, a model emerges in which selective expression or activation of transcriptional regulators associated with T_H1, T_H2, and T_H17 cells drives the phenotypical and functional specialization of T_{reg} cells, and the expression of subset of specific genes needed to restrain these different types of CD4$^+$ T-cell responses.

Role of immunoregulatory cells in chemical and protein allergy

As early as 1970, studies in guinea pigs demonstrated that dermal sensitization to 2,4-dintrochlorobenzene (DNCB) is subject to tolerance induction by oral pre-exposure to DNCB (Lowney, 1971). In rodents, tolerance induction could be prevented by cyclophosphamide, which has been shown to inhibit T_{regs} (Ikezawa et al., 2005). Subsequently, in the 1980s and 1990s, the mechanisms regulating sensitization became clearer through studies using multiple rat strains to examine autoimmune phenomena, particularly following exposure to mercuric chloride (Pelletier et al., 1988, 1990; Mathieson et al., 1991; Kosuda et al., 1994). Brown Norway rats recovering from mercury induced autoimmunity became resistant to disease induction upon re-exposure to the same compound. A similar phenomenon was observed in rats treated with and then re-exposed to D-penicillamine (Masson et al., 2004; Seguin et al., 2004) and nevirapine (Shenton et al., 2003). In mice, similar immunoregulatory processes were found to occur in response to procainamide (Layland et al., 2004), nickel (Roelofs-Haarhuis et al., 2004), and contact

sensitizers such as DNCB (Ju et al., 2003). Adoptive transfer and/or depletion of specific T-cell populations in these rodent models of allergy and autoimmunity reinforced the concept that active regulation by CD4^{+} and CD8^{+} T-cells is one of the key mechanisms for the maintenance of self-tolerance and protection from sensitization and self-reactivity (Field et al., 2003; Roelofs-Haarhuis et al., 2004; Seguin et al., 2004; Shenton et al., 2005).

Additional regulatory mechanisms for compounds that induce hypersensitivity include NK-T cells, dendritic cells (DC), and Kupffer cells. When activated, these cells have been shown to induce and/or expand T_{reg} (Ju et al., 2003; Roelofs-Haarhuis et al., 2004). Tolerance, and induction of regulatory mechanisms to protein allergens, is dependent on route of exposure, dose, genotype, gender, and age (neonatal tolerance; Field et al., 2000, 2003). Oral exposure can induce anergic T-cells with regulatory activity (Artik et al., 2001) and the presence of commensal organisms, CD8^{+} intraepithelial lymphocytes, and DC in the gut foster a tolerogenic environment (Weiner et al., 2011). It has been suggested that low dose oral exposure to protein allergens favors the induction of T_{regs}, whereas higher doses favor the induction of anergy or deletion of reactive T-cells (Weiner et al., 2011). Low dose oral tolerance has been shown to prevent mercury induced autoimmune disease (Szeto et al., 1999; Pelletier et al., 1990; Mathieson et al., 1991) with both compound-specific and non-specific T-cell regulation described (Layland et al., 2004). For example, CD8^{+} suppressor cells from mercuric chloride-exposed rats appear to act through similar regulatory cytokines as has been described for CD4^{+} T_{regs}, i.e., TGFβ and IL-10, as well as via IFNγ- and receptormediated regulation (e.g., CTLA-4).

Orally-induced tolerance is of particular relevance in case of food allergy, where there is a lack of tolerance and proper immunoregulation to specific proteins (Mowat et al., 2003). Several mouse studies with food allergens indicate that CD25^{+} T_{regs} are at least of some importance in preventing responses to peanut (van Wijk et al., 2007) and milk proteins (Schouten et al., 2010). However, other regulatory processes may also be involved, including IFNγ-producing cells, other regulatory T-cells (Rezende, 2011), gδ T-cells (Bol-Schoenmakers et al., 2011), and regulatory mucosal DC (Smit et al., 2011).

Induction of AhR-dependent T_{regs}: A novel pathway for TCDD immunotoxicity

TCDD is an aryl hydrocarbon receptor (AhR) ligand that induces immunosuppression in experimental animals, including thymic involution, decreased antibody production and cytotoxic T-lymphocyte development, and increased susceptibility to a variety of infectious diseases (Kerkvliet et al., 2002). It has been shown that the immunosuppressive effect of TCDD in mice correlated with CD25^{+}CD4^{+} regulatory T-cell generation (Funatake et al., 2005; Kerkvliet et al., 2009).

Like natural T_{regs}, TCDD-derived CD4^{+} T-cells did not produce IL-2 and their suppressive function was contact dependent (Marshall et al., 2008). Depending on the experimental model, TCDD-derived T_{regs} tended to secrete significant amounts of IL-10 or TGFβ in response to both polyclonal and allogeneic stimuli (Marshall et al., 2008; Quintana et al., 2008). Interestingly, in one study, exposure of murine immune cells to 6-formylindolo(3,2-b) carbazole (another AhR ligand) in combination with TGFβ resulted in the formation of pro-inflammatory IL-17-secreting T-cells, that could exacerbate inflammation *in vivo* (Quintana et al., 2008).

It remains to be determined whether the generation of IL-10- or TGFβ-producing CD4^{+} T-cells is a major component of TCDD-induced immunosuppression in experimental animals. Proposed mechanisms by which AhR signaling might promote T_{reg} differentiation include reduction in CD62L expression in T-cells (Funatake et al., 2004, 2005), regulation of FoxP3 expression (Quintana et al., 2008; Hauben et al., 2008; Kimura et al., 2008), and/or

modulation of DC antigen presentation. Antigen presentation by DC plays a key role in converting naïve T-cells into adaptive T_{regs}. TCDD-induced activation of the AhR causes an increase in CTLA-4 expression in T-cells that may induce tolerogenic DC (Funatake et al., 2005). In the absence of the appropriate cytokines, DC can induce clonal deletion, anergy or tolerogenic regulatory T-cells (Vorderstrasse and Kerkvliet, 2001; Thorstenson and Khoruts, 2001; Yamazaki et al., 2006). In addition, signaling through the AhR may up-regulate TGFβ signaling, promoting the expansion and function of T_{regs} (Huber et al., 2004).

The AhR has also been reported to play a role in the development of T_H17 cells (Quintana et al., 2008; Veldhoen et al., 2008a). T_H17 cells are characterized by their secretion of pro-inflammatory cytokines IL-17 and IL-22. The ligand-activated AhR regulates expression of these cytokines in tissue culture. A role for the AhR in the regulation of T_H17 cells is supported further by the observation that the absolute number of T_H17 cells is reduced in AhR null mice upon induction of Experimental Autoimmune Encephalomyelitis (Veldhoen et al., 2008b). Because T_H17 cells promote the immune response and T_{regs} are known to decrease immune reactivity, a model has emerged suggesting that the T_{reg}/T_H17 balance distinguishes an effective immune response and self-antigen tolerance from chronic infection or autoimmunity. Preliminary evidence from multiple laboratories has suggested that the AhR modifies the T_{reg}/T_H17 cell balance through modifying the cytokine milieu. The mechanism at work may be related to the fact that TGFβ induces T_{reg} differentiation, while the presence of IL-6 leads to TGFβ-dependent T_H17 cell production. Elucidating the specific transcriptional targets of the AhR that result in the induction of T_{regs} through the used of potent and specific AhR ligands such as TCDD that may provide the most direct route to understand signaling steps by which the AhR influences the immune system.

Concluding remarks

The concept of regulatory or suppressor cells having a role in chemical-induced immune system toxicology has been somewhat understudied. However, it is now recognized that T_{regs} play a critical role in maintaining the careful balancing act that allows the immune system to respond appropriately in the face of infection or disease, resolve when the challenge has diminished, and fail to respond to self-antigens. As shown by the speakers in this symposium, various immunoregulatory T-cell subsets may be induced by environmental chemicals and protein allergens. Chemical-induced alterations in T_{regs} number or function may be of pivotal importance in the clinical outcomes following chemical exposure and further research is needed to elucidate the role of T_{regs} in chemical-induced modulation of immune function and how this may impact human health and the burden of disease.

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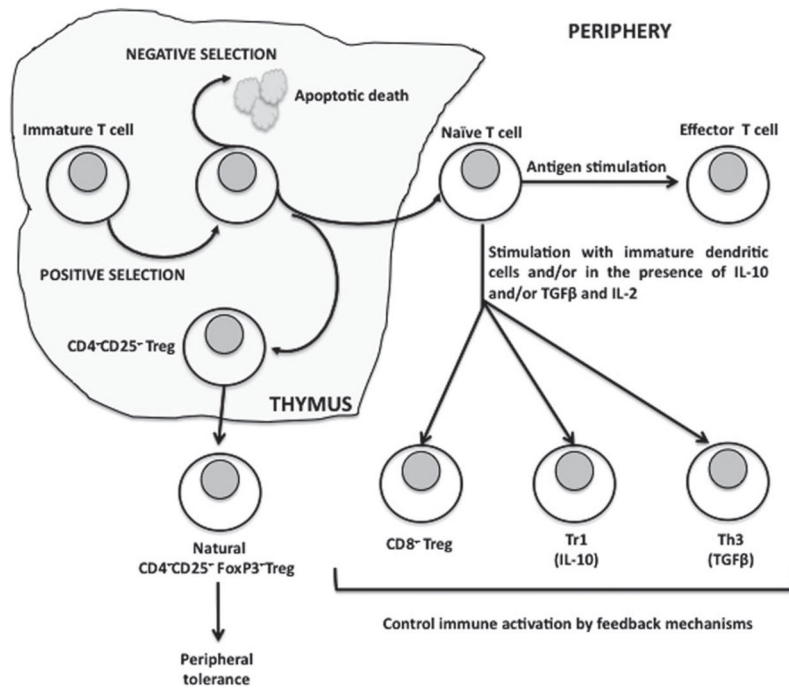
Figure 1.
Regulatory T-cells and their role in immune response.