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Review

# The role of environmental estrogens and autoimmunity

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ABSTRACT

The prevalence of autoimmune diseases has significantly increased over the recent years. It has been proposed that this epidemiological evidence could be in part attributable to environmental estrogens, compounds that display estrogen-like activity and are ubiquitously present in the environment.
Environmental estrogens can be found in a wide variety of foods: phytoestrogens occur in plants such as clover and soy, while mycoestrogens are food contaminants produced by fungi. Meat, eggs and dairy products from animals given exogenous hormones contain relatively high concentration of estrogens. Among xenoestrogens, industrial estrogens are synthetic chemicals produced for specific purposes (pesticides, plastics, surfactants and detergents) while metalloestrogens are found in heavy metals. Estrogens can be also administered through medications (contraceptive pill, hormone replacement therapy, genistein, cimetidine, creams).
There is a considerable burden of evidence in vitro and in animal models that these compounds may exert immunotoxic effects. However, to date there is no convincing data that exposure to environmental estrogens can be regarded as a risk for human health. In particular, there is no consensus whether prolonged exposure to relatively low concentrations of different estrogenic chemicals can affect the human immune system and induce clinically evident diseases in real-life scenario. Moreover, the effects on human health of the synergistic interactions between natural, medical, dietary and environmental estrogens have not been fully elucidated yet. Here we provide an extensive review of the in vivo and in vitro effects of environmental estrogens on the immune system, focusing on the evidences of association between exposure and autoimmune disorders.



**Contents**



## 1. Introduction

Sex hormones display profound effects not only on the reproductive system but also on other cell types. In particular, they may affect the immune system by modulating cytokine production, their receptor expression and ultimately regulating the responses of different effector cells [1, 2].

Such an effect can be displayed on physiological immune responses against not only exogenous antigens but also self-components. This represents the rationale for the interference of sex hormones on autoimmunity and on its clinical manifestations [1, 2]. Autoimmune diseases are, in fact, a prototypical class of illness that displays high female-to-male (F/M) ratios. Although the reason of the F/M ratios in autoimmune diseases is still a matter of research, there is evidence for the influence of estrogenic hormones. Moreover,

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sex hormones, and in particular estrogens, may contribute to disease activity and to comorbidities influencing also the clinical management of the disease.

In addition to the endogenous source of estrogen (17β-estradiol, E2), the immune system can be targeted by different chemical molecules present in the environment and displaying an estrogen-like activity, the so called environmental estrogens [3].

The real impact of both endogenous and environmental estrogens on the immune system is still under investigation. The estrogens from exogenous sources may display a synergic/additive effect with that of endogenous molecules and theoretical may display sensitive effects on the immune responses. Because of the predominance of females in autoimmune diseases, it should also be taken into consideration the role of estrogens of therapeutic sources, i.e., oral contraceptives and hormone replacement therapy.

We will address the potential role of estrogens from exogenous sources and in particular from the environment after a schematic introduction of the potential effects of these hormonal molecules on the immune system.

## 2. Role of estrogens in the immune responses

There is evidence that estrogens may affect several effector cells of the immune system. Specific receptors for estrogens (ER) have been found on the immune cells, supporting a direct effect. Two kinds of estrogen receptors (ERs) have been identified in human beings, called ER-α and ER-β. The same estrogen can produce different effects depending on which receptor it binds and the ultimate effect of the hormones is closely related to the receptor subtype present on a given cell type. For example, increased expression of ER-β versus ER-α in several inflammatory conditions may account for the pro-inflammatory effect of estrogens [1, 2].

On the other hand, estrogens display different effects on several effector cells of the immune system depending on their levels and on the type of the target cells. For example, high levels, such as during pregnancy, may inhibit pro-inflammatory cytokines (i.e. tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6) in monocytes and dendritic cells (DCs), while anti-inflammatory cytokines (i.e. IL-10 and transforming growth factor (TGF)-β) are increased. A similar inhibition of TNF-α with increase of IL-4, IL-10, interferon (IFN)-γ and TGF-β is found in T cells . On the other hand, lower levels, as during menopause, are associated with stimulation in TNF-α, IFN-γ and TGF-β secretion by T cells and macrophages/DCs. Natural killer (NK) cell activity is inhibited by high and stimulated by low levels. This dichotomy of E2 at high versus low concentrations is not observed for B cells, because antibody production is stimulated throughout the concentration range [2].

As a whole these findings support a direct role of estrogens in tuning the immune responses and underline how many variables are involved in such a regulation. The complexity of this regulation is also related to the presence of ERs on cells of both the innate and the adaptive immunity.

## 3. Role of estrogens in autoimmunity

The key role of estrogens in autoimmunity is illuminated by the example of systemic lupus erythematosus (SLE) [4, 5].

There are several indirect facts supporting a role for sex hormones in SLE pathogenesis. For example, the incidence of SLE increases after puberty and diminishes after menopause. The severity of the disease itself varies with physiological modifications of the estrogen levels such as during the menstrual cycle and with pregnancy. Both male and female patients with SLE display higher quantities of estrogens and lower levels of androgens in comparison with the normal population. A chronic hyperestrogen state has been reported in women with SLE due to an abnormal metabolism, with an increase in the production of 16-hydroxyestrone and estrol metabolites.

The abnormally high levels of E2, together with the low levels of testosterone, dihydrotestosterone, DHEA and DHEA sulfate were suggested not only to facilitate the disease start but also to be responsible for the exacerbation of the lupus disease. Conversely, men with androgen deficiency, such as in the Klinefelter's syndrome, display a greater incidence of autoimmune diseases associated with the Th2 response (including SLE) than healthy men.

These clinical observations in humans are strongly supported by experimental studies in SLE animal models. For example, the disease predominates in both MRL/*lpr* and (NZB × NZW) F1 female mice, the two prototype murine lupus models. The link is also supported by gonadectomized murine lupus models, being ovariectomized female (NZB × NZW) F1 mice less affected than castrated males. Consistently, the administration of the estrogen antagonist increases survival and normalizes immune parameters. Although, ER-α gene modulation shows different (opposite) results in (NZB × NZW) F1 and MRL/*lpr* mice, it is generally accepted that estrogen and ER-α play a key role in the onset of the experimental lupus-like disease.

There is a clear evidence for an enhancing effect of estrogens on B cells. In particular, E2 induces a polyclonal activation in vitro and is associated with increased production of autoantibodies both in patients and in lupus mice. Such a mechanism is likely to be crucial in a prototype of an autoantibody-mediated autoimmune disease as SLE.

Both androgens and estrogens have an impact on the Th1/Th2 balance. Estrogens promote autoimmune diseases with a type 2 cytokine profile, like SLE, whereas androgens promote autoimmune diseases with a Th1 profile. Th1 type diseases are improved when Th1 cytokines decrease or when Th2 are rising (i.e. by increased estrogens, as in pregnancy). Conversely, Th2 type diseases improve when Th2 are diminished (by decreased estrogen, as in post-partum period) or when type 1 response is stimulated.

So, sex hormones appear to modulate the effector cells of the immune system and consequently the immune responses both by interacting directly with their specific cell membrane receptors and indirectly by affecting the production of soluble mediators (cytokines) eventually acting on the immune cells.

However, it should be stressed that sex hormones seem to be secondary players rather than the trigger mechanisms for autoimmunity. In other words they can help to downregulate a process that is triggered by other events and strongly sustained by a genetic background.

## 4. Environmental estrogens

Environmental estrogens are ubiquitously present, being found in food, soil, air, water and household products. Therefore, environmental exposure to estrogenic chemicals can occur via oral, aerosol, dermal and subdermal routes. Many of these compounds can accumulate in adipose tissues, being released from body fat during starvation. They could also be passed on to the progeny during pregnancy and lactation.

Environmental estrogens can be of natural origin, being isolated from plants (phytoestrogens) and fungal products from *Fusarium sp* (mycoestrogens). Moreover, a number of synthetic chemicals have estrogen-like activity, referred to as xenoestrogens. Chemically-synthetized environmental estrogens can be found in plastics, detergents, surfactants, pesticides and industrial chemicals. In addition, a new class of environmental estrogens – the metalloestrogens – has been identified. It should then be mentioned that some pharmacological compounds found in medications may also exert an estrogenic activity [3].

Nowadays, environmental estrogens present a global distribution: high levels of dichlorodiphenyl-trichloroethane (DDT) have been found even in the arctic, remotely from industrialization [6, 7]. Considering their ubiquitous nature, it is clear that human beings as

well as domesticated and undomesticated animals are unavoidably exposed to these compounds. The exposure to environmental estrogens is believed to have increased over the years: while archiestrogens (phytoestrogens and mycoestrogens) have been part of the environment even before the existence of the human race, the use of synthetic estrogens in manufacturing and farming has undergone a dramatic spread in the 20th century.

Some of these chemicals resemble the structure of natural estrogens (Fig. 1), while many others interfere with the action and production of natural hormones. Although less potent than E2, these compounds have been shown to bind to ER-α and ER-β; it has been proposed that they could bind also to novel estrogen or non-estrogen receptors. Once bound, environmental estrogens presumably induce translocation of the receptor–ligand complex from the cytoplasm to the nucleus, stimulating the synthesis of estrogen-regulated proteins. Moreover, environmental estrogens could display their pharmacological effects by antagonizing endogenous hormones, altering the pattern of synthesis and metabolism of natural hormones and modifying hormone receptor levels [8]. Most of these effects are reversible once the exposure ceases, as seen in cases of occupational exposure to estrogenic pesticides. In contrast, the consequences of developmental exposure during organogenesis may result in irreversible deleterious effects.

Concerns about environmental exposure to endocrine disruptors have first been raised in the 70s, when emerged that in utero exposure to diethylstilbestrol (DES), a synthetic estrogen, was associated with somatic effects in adulthood, including female genital abnormalities, vaginal cancer, and male urogenital disorders. DES had been largely used from 1948 to 1971 in the management of conditions as threatened abortions, pre-eclampsia, prior premature labor, prostatic and breast cancer, pregnancy complications in diabetic women [9–11]. It is estimated that 2 to 4.8 million human offspring were exposed to DES [12].

Even if it is no longer used during pregnancy, it has been demonstrated that exposure to DES could occur through consumption of meat and milk products from animals that received DES as food additive, while farmers handling DES presented occupational exposure to the compound. Further, a link between prenatal DES exposure and autoimmune disease has been suggested: women prenatally exposed to DES appeared to have a higher incidence of rheumatic diseases [13, 14]. Moreover, mice given DES during the neonatal period have an impaired immune system (particularly T cells), including atrophy of the thymus, reduced proliferative response to T-cell mitogens, diminished antigen-specific delayed-type hypersensitivity, graft-versus-host reaction, cytotoxic response to mammary viruses, and NK cell function [15].

Therefore, concerns about possible effects of environmental estrogens on the immune system have been raised. Few peculiar characteristics make it particularly vulnerable to chemical insults. First, the evidence that immune system develops rather late in life: the thymus development continues at least until puberty. Moreover, bone marrow-derived immune components are continuously being renewed. Lastly, immune surveillance and responses to pathogens demand a delicate tuning [16].

Chemicals can adverse the immune system in several ways, not only by inducing immunosuppression but also by enhancing immune and inflammatory responses. It has been proposed that environmental estrogens may mediate immunologic changes directly by binding to immune cells or indirectly by acting on several other tissues, modulating cytokine production. The molecular mechanisms responsible for immunotoxicity have been progressively elucidated: carbamates and organophosphate insecticides inhibit acetylcholinesterase, a serine hydrolase which plays a pivotal role in several immune functions. Conversely, some organophosphorous compounds are thought to derive their immunotoxicity from induction of oxidative stress, while modulation of nuclear factor-κB (NF-κB) activation could explain some of the immunotoxic effects described for dithiocarbamates and propanil [17, 18].

### *4.1. Effects of environmental estrogens on the immune system*

Hereafter we provide an extensive review of the evidences of the effects of environmental estrogens on immunity in vitro, in animal models and in humans.

Environmental estrogens can be found in a wide range of foods; their immunotoxic effects are summarized in Table 1.

Phytoestrogens are naturally-occurring plant compounds structurally and functionally similar to mammalian estrogens, and are widely believed to provide an array of beneficial effects: being antioxidants, they exert preventive and therapeutic actions in carcinogenesis,

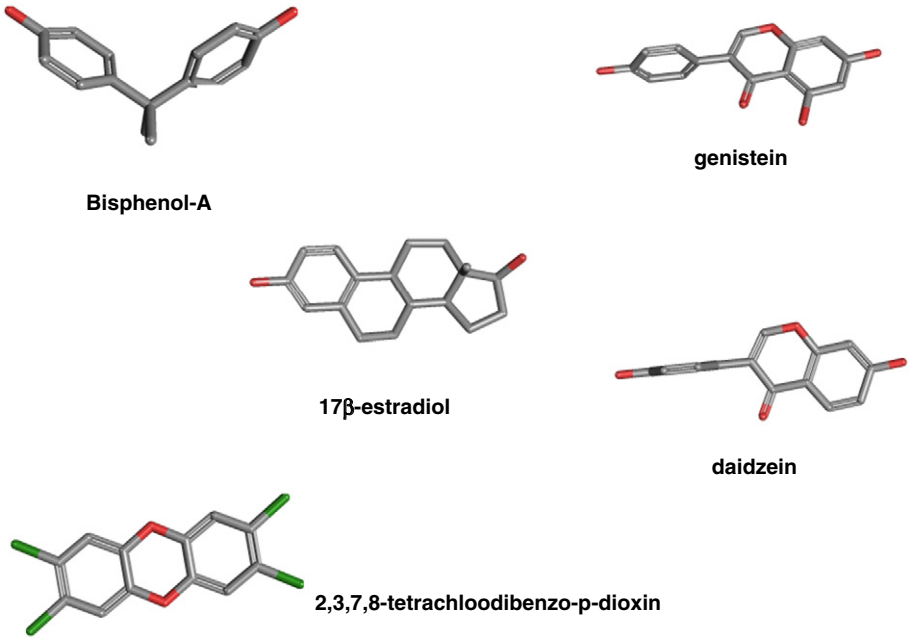


**Fig. 1.** Similarities between the chemical structures of 17β-estradiol and environmental estrogens.

**Table 1**
Immunomodulatory effects in vitro and in animal models of environmental estrogens found in food.

| Food | Compound | Effects in animal models | In vitro effects |
|---|---|---|---|
| Soybeans<br>Clover<br>Whole grains Fruits<br>Vegetables | Daidzein | – Mice: increased phagocytic response of peritoneal macrophages and thymus weight [21] | – Macrophages: reduced activation of STAT-1 [22]<br>– DCs: reduced expression of CD40, CD80, CD86, class II HLA molecules; reduced synthesis of IL-12p40, IL-6, TNF-α, [23] |
| | Genistein | – Mice: reduced thymic weight, numbers of peripheral CD4+ and CD8+ cells, suppressed antigen-specific immune response [89]<br>– Mice: improvement of collagen-induced arthritis [91]<br>– Mice: decreased IFN-γ production [90] | – Decreased lymphocyte proliferation response induced by mitogen or alloantigen [89]<br>– NKs: reduced RANTES and MIP-1 production [92]<br>– Block of the progression of IgG-R to lysosomes [92]<br>– Pulmonary endothelial cells: increased eNOS activation [93] |
| Corn<br>Grains | Zearalenone | – Suine: decreased TNF-α, IL-8 and Ig levels [24] | – Increased lipid peroxidation in lymphocyte membranes [25]<br>– Increased IL-2 and IL-5 secretion by EL4 thymoma cells [26]<br>– Reduced human and rat peripheral blood lymphocyte proliferation [27] |
| Pork meat<br>Poultry meat Cattle meat<br>Eggs<br>Dairy products | 17β-estradiol | No data | No data |

DCs: Dendritic cells; IL: interleukin; TNF: tumor necrosis factor; IFN: interferon; NKs: Natural Killer cells; MIP: macrophage inflammatory protein; IgG-R: immunoglobulin G Receptor; Ig: immunoglobulin.

atherosclerosis and osteoporosis. Lignans, one of phytoestrogen major classes, are components of plant walls, being found in many fiber-rich foods such as berries, seeds (particularly flaxseeds), grain, nuts and fruit. Most phytoestrogens, however, are phenolic compounds belonging to the isoflavone class.

Isoflavones are present in berries, wine, grains and nuts, being however most abundant in soybeans and other legumes. It is worthy to underline that soy is found in up to 60% of processed food, being a food additive and a meat substitute. Genistein and daidzein are the two most well characterized isoflavones [19, 20].

*Daidzein* has been reported to exert a stimulatory effect on non-specific immunity in mice: at high dose, it increased the phagocytic response of peritoneal macrophages and thymus weight, in a dose-dependent manner. Augmentation of spleen immunoglobulin (Ig) M-producing cells against sheep red blood cells (SRBC) demonstrated an activation of humoral immunity [21].

In vitro, daidzein has been demonstrated to increase the proliferation of splenocytes and the activity of peritoneal macrophages, NK and cytotoxic T cells. In macrophages, it has also been shown to inhibit the activation of the signal transducer and activator of transcription (STAT)-1, an important transcription factor in the production of nitric oxide (NO) [22]. In DCs, daidzein significantly and dose-dependently inhibited the expression levels of maturation-associated cell surface markers (CD40, CD80, CD86) and class II major histocompatibility complex molecules. Daidzein also suppressed pro-inflammatory cytokine production such as IL-12p40, IL-6 and TNF-α, whereas didn't affect IL-10 and IL-1β expression. Furthermore, daidzein enhanced endocytosis and inhibited the allo-stimulatory ability of LPS-stimulated DCs on T cells, suggesting that daidzein treatment can inhibit the functional maturation of DCs [23].

*Zearalenone* is the best characterized mycoestrogen, a resorcyclic acid lactone produced by several *Fusarium* species. It is a fungal contaminants of corn and grains. Recently, the effects of zearalenone on the suine immune system have been reported: it induced a decrease in Ig levels, TNF-α and IL-8 synthesis [24]. In vitro, zearalenone has been shown to cause pronounced abnormalities in lymphocyte membranes through increased lipid peroxidation [25] and to augment the ability of EL4 thymoma cells to secrete IL-2 and IL-5 [26]. Moreover, zearalenone induced a dose-dependent reduction of human and rat peripheral blood lymphocyte proliferation [27].

Finally, it should be remembered that animal-derived foods possibly contain estrogens: these could be of endogenous origin, contaminants in animal feed or artificially administered. Application of estrogens in anabolic preparations to increase live weight gain is indeed licensed in several countries except the European Union. Estrogens are artificially administered also to dairy animals to increase milk production. Hormones are generally introduced into animals by an ear implant, inducing a 7-to-20 fold increase in hormone levels. Estrogens may therefore be constituents of eggs, dairy products and meat of pork, poultry and cattle. Their consumption might affect human circulating steroid hormone concentrations either directly or by influencing indirectly endogenous hormonal production through their cholesterol components. At present, the potential relevance of animal estrogens on human health is still obscure [28].

Xenoestrogens are synthetic chemical contaminants in the environment that represent a structurally diverse group of hydrocarbons with an estrogen-like activity. Many xenoestrogens contain one or two aromatic rings and some xenoestrogens are chlorinated being negatively charged.

Estrogen-mimicking chemicals can be found in many household products, as plastics, detergents and surfactants.

*Bisphenol-A* is a component of resin used in dentistry and in the plastic industry. A recently published paper reviewed more than 5000 safety-related studies, concluding that exposure to bisphenol-A represents no noteworthy risk to the health of the human population [29]. However, in vitro data suggested that bisphenol-A could display some effects on the immune system: it has been shown to inhibit the synthesis of monocyte-chemoattractant protein (MCP) –1 in a tumor cell line, with a potency three times greater than that of E2 [30]. Moreover, bisphenol-A inhibited adherence of macrophages, an essential pre-requisite for their phagocytic function [31].

*Octylphenol* is a surfactant additive widely used in the manufacture of a variety of detergents and plastic products. Reported to exhibit weak estrogenic activity, octylphenol promoted lymphocyte cell death, through a mechanism mediated by ERs [32, 33]. Moreover, octylphenol blocked the estrogen-induced activation of IL-1β gene activation in a monocytic cell line [34].

Several pesticides have been shown to exert an estrogenic activity (Table 2). The term “pesticide” defines a variety of agents used to kill living organisms. They include insecticides, herbicides and fungicides. It is estimated that annually approximately 7000 metric tons (mt) of herbicides, 8000 mt of insecticides and 9000 mt of fungicides are used worldwide.

*DDT* is a chlorinated insecticide; being highly lipid-soluble, it is stored in lipid-rich tissues, as liver, brain and adipose tissue. DDT was first synthesized in 1874, but its pesticide properties were discovered in 1939. It was used in wartime to control typhus and malaria, and put into agricultural use in the U.S. in 1945, to be banned from in 1972. However, it was still manufactured and then sent to other countries, often as part of U.S. agricultural aid. The diet is the

**Table 2**
Immunomodulatory effects of estrogenic pesticides in vitro, in vivo in humans and in animal models.

| Class | Compound | In vivo effects in humans | Effects in animal models | In vitro effects |
|---|---|---|---|---|
| Insecticides | DDT | – Prenatal exposure: increased risk of otitis media [35]<br>– Postnatal exposure: increased upper respiratory tract infections [36]<br>– Immune response to diphtheria vaccine not impaired [37]<br>– Increased rate of asthma in children [38] | – (NZB X NZW) F1 mice: increased incidence of albuminuria, reduced uterine weight [41] | – Diminished neutrophil functions and mitogen-induced lymphocyte proliferation response [36]<br>– Increased rate of mast-cell degranulation [39, 40] |
| | Methoxychlor | | – Male rats: increased splenic weight, decreased thymus weight and Abs forming cells; female rats: thymic atrophy [3]<br>– (NZB X SWR:J) F1 mice: splenomegaly, increased splenocyte numbers, expression of CD95 and CD54 on lymphocytes [3]<br>– Female rats: decreased bone marrow CFU-GM, CFU-M AND IL-3 [45] | |
| | Chlordane | – Increased incidence of sinusitis, bronchitis, migraine [42]<br>– Increased frequency of ANA [43, 44], anti-dsDNA and anti-smooth muscle Abs [43]<br>– Increased frequency of cortical thymocytes, decreased T suppressors [43] | | |
| Herbicides | Hexachlorbenzene | – Increased serum IgM and IgG levels [46]<br>– Increased autoimmune conditions [47]<br>– Decreased neutrophil functions [48]<br>– Decreased IFN-γ production [49] | – Mice: depressed production of antibody-forming cells, serum IgG, IgM, IgA levels, delayed hypersensitivity, lymphocyte responses, Tand B-cell functions [50]<br>– Lewis rats: increased serum IgG levels and Abs positivity, lymphoid organ weights, T- and B-cell specific mitogen blastogenesis [50] | |
| Fungicides | Pentachlorphenol | – Decreased lymphocyte proliferative response to mitogens [57,58]<br>– Decreased lymphocyte proliferative response to mitogens, blood monocytes, serum IL-8, CD4/CD8 ratio, CD4 cells [46]<br>– Decreased lymphocyte proliferative response to mitogens, serum Ig [55]<br>– Increased anti-smooth muscle Abs, CD10 cells, NK cell activity [55]<br>– Increased prevalence of low-grade infections or inflammations of the skin, subcutaneous tissues, eyes and upper respiratory tract mucosa [56]<br>– Decreased T cells, CD8 cells, NK cells, lymphocyte proliferative response to mitogens [57] | | – Decreased NK cell activity [59] |
| Nematocides | Aldicarb | – Increased ANA positivity [51]<br>– Decreased CD4/CD8 ratio [51] | | |

DDT: dichlorodiphenyl-trichloroethane; Abs: antibodies; ANA: anti-nuclear antibodies, anti-dsDNA: anti-double strand DNA antibodies, IL: interleukin; Ig: immunoglobulin; IFN: interferon; NK: natural killer.

major source of exposure to DDT: the largest amount of dietary DDT comes from meat, poultry, dairy products and fish. DDE, DDT's main metabolite, is one of the most ubiquitous toxins in foods.

Prenatal exposure to DDT increases the risk of otitis media [35]; post-natal exposure to DDT has been linked to impairment of the upper respiratory tract immunity as evidenced by increased upper respiratory tract infections. It has been demonstrated that DDT diminished neutrophil functions and reduced mitogen-induced lymphocyte proliferation response [36]. On the other hand, a study enrolling children from Brazil exposed to DDT showed that the immune response to diptheria vaccine was not impaired [37]. Prenatal DDE exposure increases also the rate of asthma in children (relative risk of 2.6) [38]; in vitro studies showed that DDT induces an increase in the rate of mast-cell degranulation therefore augmenting the risk of allergy [39, 40]. In (NZB × NZW) F1 lupus prone mice, DDT exposure markedly increased the incidence of albuminuria, reduced uterine weight but had no measured effects on immunity or mortality [41].

*Methoxychlor* is a synthetic organochlorine used as an insecticide; it was intended to be a replacement for DDT, but has since been banned based on its acute toxicity, bioaccumulation and endocrine disruption activity. It has been demonstrated that male rats exposed to methoxychlor during perinatal and prepubertal life had decreased thymus weights and antibody forming cells with an increase in splenic weight. Conversely, the only immunological change of note in females was thymic atrophy when exposed to the higher dose of methoxychlor. The reported differences between genders suggest that endogenous sex hormones may modify the response to environmental chemicals. Lupus-prone (NZB × SWR:J) F1 mice receiving methoxychlor had splenomegaly, increased splenocyte cell numbers and increased expression of CD95 and CD54 molecules on lymphocytes [3].

*Chlordane* is an organochlorine insecticide, whose effects in humans have been investigated by few studies. In a non-random study involving individuals concerned about exposure to chlordane, an increased incidence of sinusitis, bronchitis and migraines was reported in exposed subjects [42]. In another study, 27 patients exposed to chlordane reported multiple immune impairments compared to controls: increased frequency of cortical thymocytes with decreased T-suppressors. Sera of chlordane-exposed individuals also had an increased frequency of autoantibodies against nuclear (ANA), DNA and smooth muscle antigens [43]. The increased incidence of ANA has been confirmed by an epidemiological study conducted in a rural population exposed to agricultural chemicals (including chlordane, dieldrin, heptachlor, lindane) [44]. Chlordane has been shown to display immunosuppressive effects in prenatal exposed mice: females, but not males, had a decrease in the numbers of bone marrow colony forming units-granulocyte/macrophage (CFU-GM), CFU-IL-3, and CFU-macrophage (CFU-M) [45].

*Hexachlorbenzene* (HCB) was commonly used as a pesticide, herbicide and fungicide. Although its production and use have ceased in many countries, the compound is still generated inadvertently, as a byproduct in the manufacturer of various chlorinated chemicals. In a study in workers occupationally exposed to HCB, increased serum IgM and IgG levels were observed, whereas serum IgA levels were normal [46]. From 1955 to 1959, approximately 3000–5000 people in southeastern Turkey ingested HCB-treated seed grain: in follow-up studies autoimmune conditions such as dermatologic abnormalities, neurologic symptoms, enlarged thyroid and hand arthritis still persisted 25–30 years after the poisoning incident [47]. Respiratory burst and chemotaxis of neutrophils from 51 workers exposed to HCB were significantly reduced as compared to controls [48], while in 146 patients with occupational exposure to HCB, a strong negative association between HCB and IFN-γ blood levels was reported, suggesting that HCB could have a significant impact on Th1 lymphocytes [49].

In animal models, the effects of HCB on the immune system are species-dependent. Humoral and cell-mediated immune responses in adult mice are both affected by HCB. In these animals, it significantly depressed the production of antibody-forming cells, serum Ig levels (IgG, IgM and IgA), delayed type hypersensitivity, mixed lymphocyte responses, T- and B-cell functions. On the other hand, rats exposed to HCB presented a marked increase in lymphoid organ weights, T- and B-cell specific mitogen blastogenesis and antibody response. In particular, in Lewis rats, this chemical has been shown to stimulate the immune system, as evidenced by an increase in the expression of serum IgM, autoantibody positivity and in the number of intraepithelial T cells [50].

*Aldicarb* is a nematocide widely used in agriculture. An epidemiological study showed an increased incidence of positivity for ANA in a rural population exposed to aldicarb. Moreover, the chronic exposure of aldicarb via contaminated ground water has been associated with a decreased CD4/CD8 ratio [51].

*Pentachlorophenol* (PCP) is an organochlorine compound. First produced in the 1930s, it has been extensively used as a biocide in fishery, agriculture and industry. In many lakes of China, a large quantity of PCP has been sprayed to prevent a schistosomiasis epidemic. Although PCP had been banned for further use in 1990, its persistence resulted in a long-lasting contamination of aquatic environment [52]. PCP displays an anti-estrogenic effect, reducing the estrogenicity of E2 by competitive binding to the estrogen receptors [53]. The toxicological and immune effects of PCP on the immune system had been evaluated in 32 subjects who had prolonged exposure to PCP in a wood factory and in 37 controls. The findings were not suggestive of any PCP-dependent immune deficiency: there was no difference in CD3+, CD4+, and CD8+ lymphocyte subsets and in the proliferative response of peripheral blood mononuclear cells to mitogens between patients and controls [54]. Conversely, another study on 38 subjects with exposure to PCP provided conflicting results: exposed individuals had activated T-cells, autoimmunity, functional immunosuppression with decreased serum Ig levels and B-cell dysregulation. Autoimmunity was evidenced by elevation of TA1 phenotype frequencies and a 21% incidence of anti-smooth muscle antibody [55]. In another study enrolling 46 subjects with occupational exposure to PCP, an increased prevalence of low-grade infections and inflammations of the skin, subcutaneous tissues, eyes and upper respiratory tract mucosa has been reported. Strong to moderate statistical associations were observed between PCP exposure and increased immature leucocytes and basophils, raised plasma cholinesterase, alkaline phosphatase, IgG and uric acid, with decreased serum calcium [56]. Several studies have reported an association between diminished lymphocyte proliferative response to mitogens and exposure to PCP [46, 55, 57, 58]. A reduced NK cell activity has been linked to PCP exposure in vitro [59].

Although several pesticides (chlordane, HCB, PCP, chlorpyrifos [43, 45, 55, 60]) have been associated with an increased prevalence of ANA, few studies have evaluated the possible role of pesticides as risk factors for autoimmune diseases. In the Carolina Lupus Study, investigators found a trend for an association between increased prevalence of high-titer ANA and the summation of exposures to pesticides, silica, mercury, sunlight and use of hair dyes [61]. Farming and agricultural pesticide use have been associated with SLE, with an odds ratio (OR) of 7.4 [62].

A weak association has also been reported for organochlorine pesticides exposure and rheumatoid arthritis (RA, Relative Risk 2) [63, 64]. In the Women's Health Initiative Observational Study (76.861 postmenopausal women enrolled, aged 50–79 years), personal use of insecticides was associated with an increased risk of RA and SLE. Risk was also associated with long-term insecticide application (Hazard Ratio (HR) 1.85) and frequent application among women with a farm history (HR 2.73). Although these findings require replication in other populations, they support a role for environmental pesticide exposure in the development of autoimmune rheumatic diseases [65].

Estrogenic chemicals are often produced in many industries: these compounds are referred to as industrial estrogens.

*Polychlorinated biphenyls (PCBs)* are a group of 209 different chemicals which share a common structure but vary in the number of attached chlorine atoms. These compounds were once used as insulators in the electrical industry and are now banned in the U.S., Northern Europe and other countries.

Exposure to PCBs has been linked to RA: in a 2007 cross-sectional study, serum concentration of PCBs was positively associated with a self-reported diagnosis of RA (OR 2.2) [63]. Concerns of immune alterations in PCB-exposed humans have also been raised: in a study in Dutch children, PCB levels were tied to an increased prevalence of ear infections and chickenpox with lowered immune system function [66]. These findings have been confirmed in the Yusho and Yu-Cheng populations that experienced the highest levels of PCB exposure and least complex exposure mixture. Moreover, serum IgA, IgM and monocyte counts were decreased in the Yusho and Yu-Cheng populations [67]. Substantial evidence of the immunotoxicity of PCBs in research animals lends strong support to the human data. Particularly relevant findings in animals include reduced antibody responses and T-lymphocyte levels, which are similar to changes observed in some of the human populations. The antibody response to SRBC antigens is the immune parameter most commonly and consistently shown to be affected by PCBs in animals: reduced responses have been demonstrated in most tested species, including adult and infant monkeys, which are sensitive to chronic low PCB doses. Studies in rats, mice, guinea pigs, and rabbits showed that intermediate-duration exposures to relatively high doses of commercial PCB mixtures caused morphological and functional alterations in the immune system: thymic and splenic atrophy, increased susceptibility to infections, reduced skin reactivity to tuberculin and increased proliferation of splenic lymphocytes in response to mitogenic stimulation [68].

However, a 2003 critical synopsis of published data concluded that there is no conclusive evidence of adverse health effects of PCBs at concentrations encountered with human exposures [69].

*2,3,7,8-tetrachloodibenzo-p-dioxin (TCDD)* is the most potent among the polychlorinated dibenzo-*p*-dioxins (in short but inaccurately also called dioxin). It is formed as an unintentional by-product of incomplete combustion, being released to the environment during the combustion of fossil fuels and wood, and during the incineration of municipal and industrial wastes. It is also a contaminant in Agent Orange, a herbicide used in the Vietnam War [70].

The actions of TCDD and dioxin-like chemicals are mediated by the aryl hydrocarbon receptor (AhR), a basic helix–loop–helix transcription factor. Following ligand binding, the receptor–ligand complex is translocated to the nucleus via the AhR nuclear translocator, resulting in transcriptional activation. Target genes include cytochrome P-450 and genes involved in cellular growth, differentiation and inflammation [71]. TCDD is not mutagenic and not directly genotoxic: it promotes the carcinogenicity initiated by other compounds. However,

very high doses may, in addition, cause cancer indirectly; one of the proposed mechanisms is oxidative stress and the subsequent oxygen damage to DNA [72]. TCDD has also been shown to exert an anti-estrogenic activity in the presence of estrogens, and estrogenic property in the absence of estrogen [73].

The industrial accident that occurred in the area of Seveso (Italy) on July 10, 1976 exposed a large residential population to substantial amounts of TCDD. In the immediate aftermath, typical effects of exposure to polychlorinated hydrocarbons such as chloracne were observed mainly in children [74], an excess risk of lymphatic and hematopoietic tissue neoplasms in the most exposed zones was reported [73].

Two cross-sectional studies suggest enhanced autoimmunity in TCDD-exposed individuals: in a Vietnam and in a UK study, TCDD exposure was associated with an increased positivity of ANA, concentration of circulating immune complexes and lymphocytes [75, 76], while a larger study in a German pesticide-producing factory reported no correlation between dioxin concentration in blood lipids and autoantibody levels [77]. Two more recent reports also failed to demonstrate any induction of ANA in Japanese subjects intoxicated with TCDD [78] and in Koreans veterans exposed to Agent Orange [79]. A 2004 study showed that secondary immune responses to influenza viral pathogens were likely to be influenced by TCDD exposures occurring prior to or at the time of primary infections, as measured by virus-specific Ig levels, while secondary responses were only temporarily suppressed. It is not clear whether or not this immune alteration results in increased clinical susceptibility to viral infections [80]. Current available data from animal models suggest that TCDD exposure increases postnatal autoimmune responses.

In (SWR × NZB) F1 mice, resistant males were rendered susceptible to lupus by E2 or TCDD treatment [81]. Moreover, prenatal exposure to TCDD has been shown to cause selective enhancement of postnatal immune responses, causing an autoreactive immune phenotype in adult non-autoimmune mice and exacerbating autoimmune nephritis in adult autoimmune SNF1 animals. Indeed, TCDD crosses the placenta and alters normal development of central tolerance in the thymus and normal prenatal thymocyte maturation, T-cell receptor and thymic major histocompatability complex class II molecule expression [82].

Metalloestrogens include the heavy metals and metalloids cadmium, aluminum, antimony, arsenite, barium, cobalt, copper, chromium, lead, mercury, nickel, nitrite, selenite, tin, uranium and vanadate.

*Cadmium* is widely used in industry, principally in galvanizing and electroplating, in batteries, in electrical conductors, in the manufacture of alloys, pigments, plastics and in the stabilization of phosphate fertilizers. As a byproduct of smelters, cadmium is a prevalent environmental contaminant. In the general population, exposure to cadmium occurs primarily through dietary sources, cigarette smoking and, to a lesser degree, drinking water. Similar to E2, cadmium induces cellular proliferation, increases the transcription and expression of estrogen regulated genes in human breast cancer cells [83, 84]. The effects of the metal are mediated not only by the ERα genomic pathway but also by nongenomic ERα pathways through ERK1/2 and Akt [85]. Early studies in Sprague–Dawley rats showed that exposure to cadmium via drinking water caused immune-complex nephritis [86] and induced production of antibodies against two components of the glomerular basement membrane. After cadmium exposure, a significant induction of ANA was observed in ICR but not Balb/c mice, while exposing male MRL *lpr/lpr* mice to cadmium accelerated the age-dependent production of anti-DNA antibodies [87].

It is important to bear in mind that also active compounds found in some medications can exert, beside the main pharmacological activity, estrogenic properties.

*Genistein*, now used as chemotherapic agent, had been isolated from *Genista tinctoria* in 1899; it has been chemically synthetized in 1928. Genistein is a phytoestrogen that can be found in broad beans, soy, coffee and psoralea. It inhibits the tyrosine-kinase protein of the enzyme DNA topoisomerase type II [88]. Nowadays genistein is used as antineoplastic agent: it has been shown to stop G2 phase of cellular cycle. In animal models, genistein induced dose-dependent reduction in thymic weight, reduced the number of peripheral CD4+ and CD8+ cells, suppressed antigen-specific immune response [89] and decreased levels of IFN-γ protein [90]. Due to its unique effect on the immune system, genistein has been used in the treatment of collagen-induced arthritis, an animal model for RA: mice receiving genistein prior to immunization with collagen showed less frequent and less severe arthritis than controls [91]. The in vivo findings have been confirmed by in vitro experiments: genistein inhibited lymphocyte proliferation response induced by mitogen or alloantigen [89]. In NK cells stimulated with antibodies against CD43, it has been shown to inhibit the secretion of chemokines as RANTES and macrophage inflammatory protein (MIP)-1. Genistein also blocked the progression of internalized high-affinity receptor for IgG to lysosomes. Moreover, it has been shown to reverse in vitro the loss of T-cell signaling components at post-menopausal estrogen levels, with no significant effect at pre-menopausal estrogen levels [92]. In broiler pulmonary arterial endothelial cells genistein stimulated a rapid phosphorylation of eNOS activating the eNOS/NO axis; the activation of eNOS was not mediated through estrogen receptors or tyrosine kinase inhibition, but via a phosphatidylinositol 3-kinase (PI3K)/Akt-dependent signaling pathway, as the eNOS activity and related NO release were largely abolished by pharmacological inhibitors of PI3K or Akt [93].

*Cimetidine* is a histamine $H_2$-receptor antagonist; it has been shown to interfere with the metabolism of estrogens. It enhances estrogen activity by decreasing the 2-hydroxylation of E2 resulting in an increase in the serum estradiol concentration [94].

*4-methylbenzylidene camphor* is an organic camphor derivative with a weak estrogenic activity. It is used in the cosmetic industry for its ability to protect the skin against UV, specifically UV B radiation. Therefore, it is widely used in sunscreen lotions and other skincare products [95].

*Parabens* are a class of chemicals widely used as preservatives in the cosmetic and pharmaceutical industries. They can be found in shampoos, commercial moisturizers, shaving gels, personal lubricants, topical/parenteral pharmaceuticals, spray tanning solution, makeup and toothpaste. In an in vivo study, the effect of butylparaben was 100,000 times weaker than that of E2, and was only observed at a dose approximately 25,000 times higher than the concentration usually found in commercial products. The in vivo estrogenic activity of parabens has also been found to be three orders of magnitude reduced compared to in vitro activity [96].

Lastly, it should be remembered that estrogens from human urine enter the rivers from sewage; moreover, women receiving estrogens for medical purposes (contraceptive pill, hormone replacement therapy) display much higher urinary E2 concentrations.

## 5. Conclusions

Epidemiological evidence from Western countries indicates that the prevalence of autoimmune diseases is strongly increasing, being not merely attributable to improved diagnostics alone. Some authors have therefore postulated that this could be, at least, partially attributable to new or modified patterns of exposure to chemicals, including environmental estrogens.

However, despite the immunotoxic effects generated under experimental conditions, there is no convincing evidence that exposure to environmental estrogens can be regarded as a risk for human health. In particular, there is no data about the effects on human health of the synergistic interactions between natural, medical, dietary and environmental estrogens.

Therefore, the main issue is whether prolonged exposure to relatively low concentrations of different chemicals showing immunotoxicity in experimental models can affect the human immune system

in real life conditions. It also should be investigated whether these effects on the human immune system are adaptive or if they are early signs leading to clinically evident diseases.

These critical questions will receive an answer only from prospective, controlled epidemiological studies with rigorous exposure assessment in order to generate reliable exposure-response data. Immunotoxicity studies in humans should focus on those compounds for which there is convincing laboratory evidence for an effect on the immune system. These studies should be performed in large cohorts: it is possible that environmental estrogens may affect only certain subsets of the population with appropriate contributing factors, as a susceptible genetic background. Moreover, the studies should be long-term: the consequences of immune alterations by environmental estrogens may not be clinically apparent immediately after exposure.

There are a number of factors whose role should be critically addressed when establishing the effects of environmental estrogens on autoimmunity. First of all, developmental and senescent ages, both periods characterized by sub-optimal immune competence, may be more vulnerable to the effects of environmental estrogens on the immune system. Gender could also affect sensitivity to estrogenic chemicals: in animal models, male and female mice had different responses to estrogen after exposure to chlordane and methoxychlor. Studies in relevant animal models may offer more information at a mechanistic level in particular.

It must be taken into account that in real-life scenario exposure to multiple chemicals may occur, making it difficult to assess the effects of each compound.

Another critical issue is that most of the toxic effects exerted by chemicals are characterized by the presence of a threshold, under which no effect is anticipated. At this regard, it is also important to consider that the immune system also possesses functional reserve and redundancy in response to xenobiotics.

Moreover, it is likely that immune effects may vary depending on the dose: estrogens display immunostimulant or immunodepressive effects depending on the concentration. Also the duration of exposure may affect the response of immune cells, being acute exposure more critical for cellular balance.

In conclusion, the future challenge will be to unequivocally establish whether environmental estrogens may contribute to the increasing burden of immuno-related diseases.

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