

Review article

Endocrine disrupters – potential modulators of the immune system and allergic response

Endocrine disrupters (EDs) are environmental pollutants of industrial or agricultural origin (e.g. herbicides, fungicides, insecticides, industrial chemicals) that may influence health of wildlife and human. Endocrine-disrupting effect is obtained by mimicking the action of the steroid hormones and has been associated with several reproductive disorders as well as cancerogenesis both in animals and humans. EDs can also influence synthesis of cytokines, immunoglobulins, and cell mediators as well as immune cell activation and survival. Modulation by EDs of interleukin-4 production, Th1/Th2 balance and IgE production suggest their potential effect on allergic immune responses. The aim of this review was to summarize data indicating a potential effect of EDs exposure on the immune system and allergic responses.

M. Chalubinski, M. L. Kowalski

Department of Immunology, Rheumatology and Allergy, Chair of Immunology, Medical University of Lodz, Lodz, Poland

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Marek L. Kowalski MD, PhD
Department of Immunology, Rheumatology and Allergy
Medical University of Lodz
251 Pomorska Str.
92-213 Lodz
Poland

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General information

Endocrine disrupters (EDs) are exogenous industrial and agricultural agents that are able to interfere with the delicate balance of the endocrine system. It is believed that these compounds consequently cause adverse health effects in an intact organism, or its progeny.

Endocrine disrupters are present in the human and wildlife environment and have the intrinsic property to modulate or even disturb the endocrine system of a variety of organisms (1, 2). They include numerous chemicals used in the production of paper, pulp and pesticides as well as ubiquitous and persistent organochlorines, plasticizers, pharmaceuticals, and natural hormones (3). These chemical agents are often by-products of toxic waste incineration. As they are characterized by a spread variety of actions and origin as well as chemical structure, they are also called: hormone or estrogen mimickers, endocrine modulators, endocrine-disrupting chemicals, volatile organic chemicals or persistent organic pollutants.

Colborn et al. (4) compiled numerous chemicals with widespread distribution in the environment reported to have endocrine-disrupting effect in wildlife and humans (Table 1). The main groups are: pesticides, fungicides, insecticides and industrial compounds such as dioxins, polychlorinated biphenyls (PCBs), polybrominated

bisphenyls (PBBs). They also include phytoestrogens, i.e. naturally occurring plant substances and metals, e.g. arsenic compounds (5, 6).

Endocrine disrupters can be found in every part of environment: water (rainwater, lakes, rivers, oceans), air, and soil. As they accumulate in the fat tissue, they can also enter the animal and human organisms through the alimentary system. Several endocrine modulators that have estrogen-like activity are contaminants of marine and terrestrial food products (4, 5). They bio-accumulate and magnify in level up the food chain. Thus, the highest concentrations are found in humans who ingest them with their daily diet. In human adipose tissue, breast milk and urine it is common to find: PCBs, dioxins, DDT (dichlorodiphenyltrichloroethane), or soy isoflavones (7–9).

Influence on the mammalian organism

It has been suggested that EDs are responsible for apparent changes seen in human health patterns over recent decades. Epidemiological and laboratory studies have shown that a variety of hormone mimickers can disturb endocrine development. EDs exposure during early life may cause numerous long-term general health consequences (4). It has been shown that the outcome of

Table 1. Main groups and examples of endocrine-disrupting chemicals (based on Ref. 4)

Group	Examples						
Herbicides	Alachlor	Nitrofen	Trifluralin				
Fungicides	Hexachlorobenzen	Maneb	Zineb				
Insecticides	Carbaryl	Dieldrin	Methoxychlor	Toxaphene			
Industrial chemicals	Bisphenol A	Dioxins	PBBs	PCBs	PCP	Phthalates	
Phytoestrogens	Genistein	Daidzein					

PCBs, polychlorinated biphenyls; PBBs, polybrominated biphenyls.

their action may be manifested in embryo, fetus, and neonate even with the exposure on parents in their early life. Chemicals have also the ability to influence the development of offspring even until it reaches maturity or middle age. Unfortunately, majority of these effects are often delayed and thus the dose-response as well as cause-effect relationship are very difficult to be established, sometimes even impossible (4, 10).

Endocrine disrupters interfere with the endocrine system and endogenous hormones, especially steroids, through various mechanisms. Firstly, they can influence hormone synthesis. For instance, xenobiotics may modulate release, transport, metabolism, and excretion of steroids. Phytoestrogens (daidzein, genistein), medications (diethylstilbestrol, tamoxifen) and industrial chemicals [DDT, bisphenol A (BPA)] may also interfere at the receptor level (5). Thus they have the potential to mimic or block sex hormones or even affect the thyroid and adrenal function. This shows that numerous chemicals act as estrogens, anti-estrogens and anti-androgens, in spite that they often have no structural similarities, because of the entirely distinct origin (3) (Fig. 1).

Endocrine disrupters interfere with the endocrine system and other organs in various ways, generally resulting in either an increase or decrease in the serum hormone levels. A number of adverse effects have been observed in which EDs could play a significant role. This includes increase in incidences of certain types of cancer, that are known to be hormone-related, observed in the Western countries and in part of the world adopting Western lifestyles (11). In women it includes breast (12–15) and ovarian cancer (16, 17), while in men prostate (18) and testes tumors (19).

As steroids have been identified as playing a major role in regulating the process of organogenesis, there is a risk that EDs may lead to congenital malformations in children. In recent years, an increase in the incidence of hypospadias and cryptorchidism in human male neonates has been observed. Some authors believe that diethylstilbestrol (DES) should be taken under the consideration as a cause of doubling of the cryptorchidism incidence in the UK (20, 21). Other argue that there is little evidence for a relation between the risk of hypospadias and maternal occupation or occupational exposure to potential EDs, that is why such findings should be interpreted with caution (22).

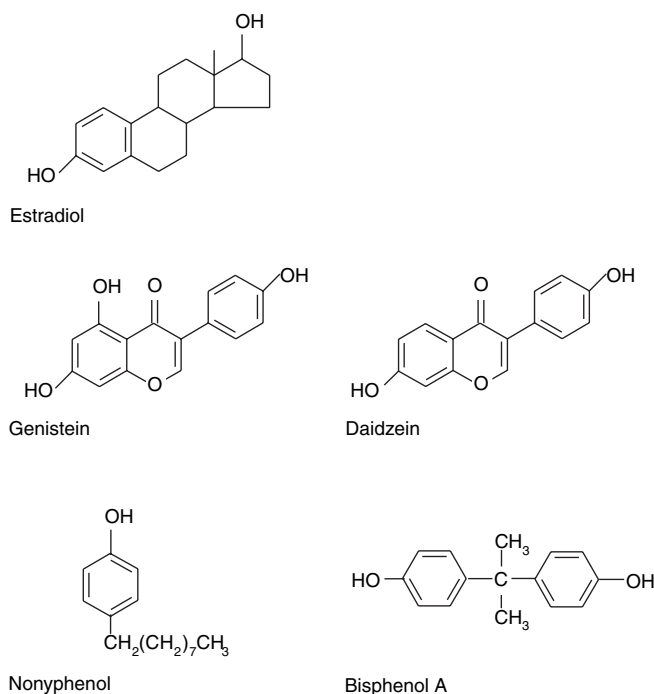


Figure 1. Chemical structures of estradiol and endocrine disrupters with estrogen activity.

In animals EDs may be a serious cause of disturbances in fertility (23, 24) and decreased hatching success (25–27). Colborn et al. draw a conclusion that decrease in sperm count in human males in some geographical regions over the last 50 years (28) may be a reflection of the increase in estrogenic pollutants in the environment. Infertile human couples with male factor infertility (low sperm counts) reported more long-term exposure to numerous insecticides and other pesticides than couples with female infertility factor (29). A significant role of those estrogen mimickers in the increase in ectopic pregnancies has been also suggested (30).

Endocrine disrupters may interfere with sex hormones. Pesticides such as toxaphene may cumulate in adrenal glands in rats and inhibit ACTH-stimulated corticosterone production (31). In wildlife exposure to hormone mimickers has been associated with abnormal thyroid function (32), as well as demasculinization and feminization (33, 34). Moreover, daughters whose mothers took DES suffered reproductive organ dysfunction, a reduction in fertility, abnormal pregnancies and vaginal adenocarcinomas (35, 36). Besides, links have been suggested with impairment of EDs in neurobehavioral development and sexual behavior (37).

The above-mentioned deleterious health effects were observed mostly in animals within the areas with the presence of multiple man-made chemicals, such as by-products of industrial chemical synthesis and pesticides. One should remember that endocrine-disrupting chemicals are in most cases neither mutagens nor acute

toxicants in ambient concentrations. There is limited information based on prospective research and a little causal association with chemical exposure has yet been established. The importance of numerous other confounding environmental and lifestyle factors should be considered in the cause–effect relationship, especially in human population (Fig. 2).

Influence on the immune system and allergic response

Endocrine disruptors affect not only the endocrine or reproductive, but also markedly influence the immune system. As steroids have been identified as playing a significant role in development and regulation of the immunity, one must consider that EDs, which mostly mimic steroids, may influence immune processes and mechanisms in various ways. A few research have been reported on hormone mimickers impact on the immune system, and majority of them were carried out *in vitro* or *in vivo* in animal models (Table 2).

Endocrine disruptors may modulate cytokine synthesis. For example, Concanavalin A (Con A)- activated splenocytes from mice treated with genistein, a soy isoflavone, or alpha-zearanol, release less interferon (IFN)- γ into supernatants (38). Other studies have demonstrated that IFN- γ synthesis was only marginally higher in genistein-exposed than in genistein-free rat males (39). Both zineb, a pesticide used to protect fruit and vegetable crops from foliar and other diseases, and alachlor, an aniline herbicide used to control annual grasses and broadleaf weeds in field corn, soybeans and peanuts, enhanced lipopolysaccharide (LPS)-induced tumor necrosis factor (TNF)- α production by mouse peritoneal macrophages *ex vivo*. Nitrofen, a herbicide used in vegetable crops and simazine (algae destroyer), as well as benzyl butyl phthalate inhibited LPS-induced TNF- α production by mouse macrophage cell line RAW 264 *in vitro* (40).

Some endocrine-disrupting chemicals have been shown to affect immunoglobulin synthesis. DES and BPA enhanced autoantibody IgM production by murine B1 cells *in vitro* and *in vivo* (41). Those chemicals also

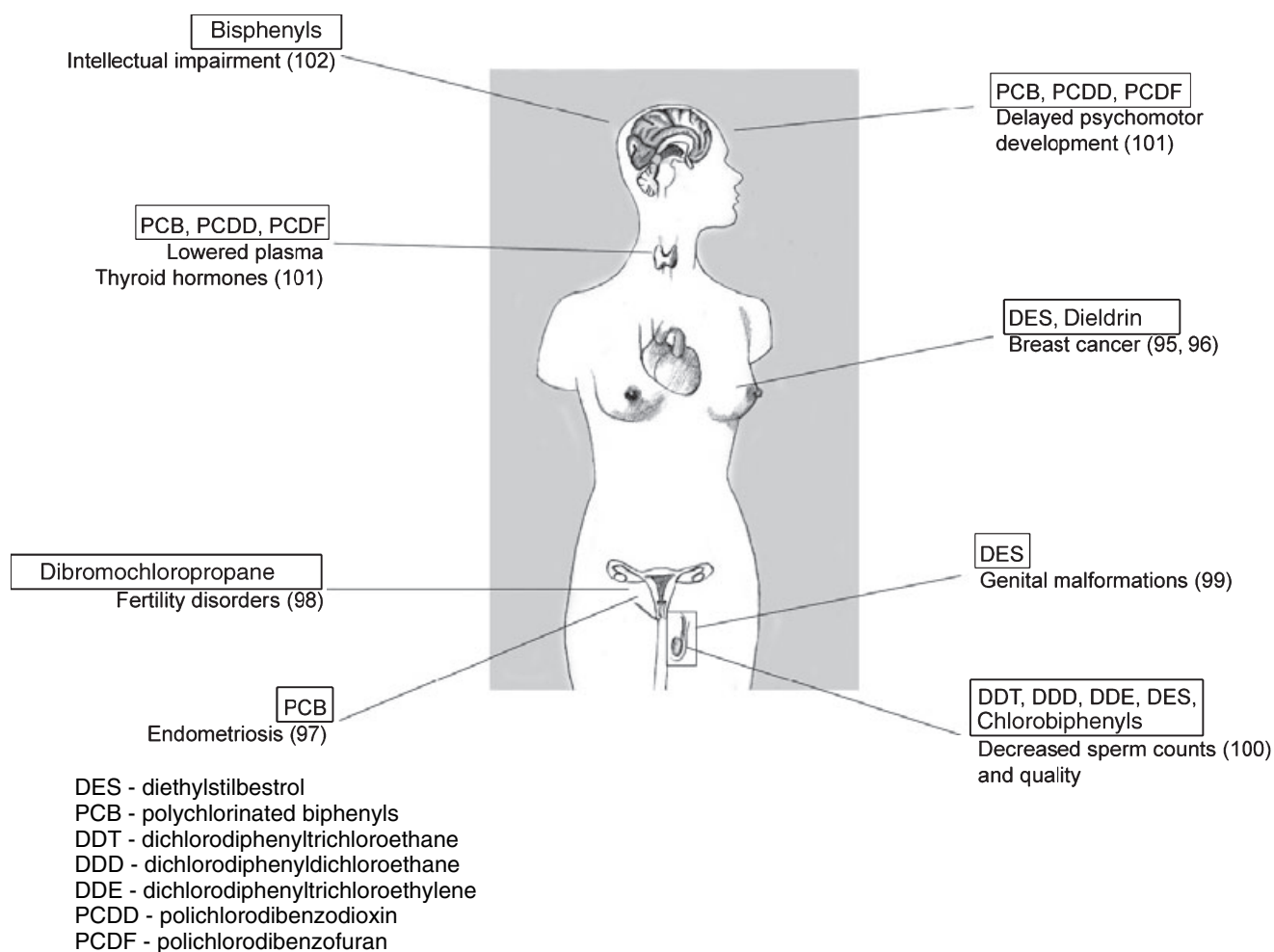


Figure 2. Endocrine disruptors implicated in human pathology.

Table 2. Endocrine disrupters affecting immune response – cytokine synthesis and T cells profile (from *in vitro* and animal studies)

Endocrine disrupter	IL-4	IFN- γ	IgE	Th1	Th2
BPA	+		+	++	+
NP	+		+	–	+
OP	+			–	+
DEHP	+		+		
DINP	+		+		
Alpha-zearanol		+			
Genistein		+			
Hydroquinone	+		+		
Daidzein			+		
DES		+			
References	(46–50)	(38, 39)	(43, 46, 48, 49)	(42, 50)	(42, 50)

+, increase; ++, strong increase; –, decrease; BPA, bisphenol A; NP, nonylphenol; OP, octylphenol; DEHP, diethylhexyl phthalate; DINP, diisononyl phthalate; DES, diethylstilbestrol.

increased estrogen receptor (ER) expression in B1 cells from mice with lupus nephritis, which suggests that DES and BPA may be associated with the development of autoimmune diseases. Prenatal exposure to BPA was followed by significant increase in anti-HEL (hen egg lysozyme) IgG as well as antigen-specific cell proliferation in mice offspring (42). In mice exposed to daidzein, a soy isoflavone, an activation of spleen cells producing IgM antibodies against sheep erythrocytes occurs, indicating an augmentation of humoral immunity by phytoestrogens (43).

An *in vitro* study with human cells has shown that 17 β -estradiol (E₂) enhanced IgM and IgG production by peripheral blood mononuclear cells from men and women, which could be an effect of increased level of monocyte-derived IL-10 (44). On the other hand, genistein, another soy isoflavone, produced suppression of humoral immunity (45). These results indicate that EDs may act as important modulators for human humoral immunity. Some endocrine-disrupting chemicals have been shown to affect nitric oxide (NO) synthesis. Carbaryl, an insecticide, alachlor, nonylphenol (NP) and octylphenol (OP) – break-down products from both domestic and industrial detergent use, as well as tributyltin, and triphenyltin – highly toxic biocides used to protect the hulls of large ships – inhibited, while 2,4-dichlorophenoxy acetic acid and BPA enhanced LPS-induced NO production by mouse macrophages (40). Thus, some EDs exert modulatory activity on non-specific immune response (e.g. endotoxin-induced macrophage activation) acting either positively or negatively.

Endocrine disrupters may have an impact on immune cells survival. Exposure to DES significantly decreased mice thymocyte numbers, although relative percentages of thymocyte subsets were not altered (38). What is interesting, splenocytes from DES-exposed mice had considerably decreased ability to proliferate in response to Con A (38), while fetal exposure to BPA after

immunization with HEL was shown to be followed by significant increases in antigen-specific cell proliferation (42).

The above-mentioned information justify a question whether a variety hormone mimickers acting on the human organism from gestation through adolescence to the old age may become one of many environmental factors determining development and/or the outcome of allergic diseases. This hypothesis is worthy to be tested, especially when the increase in the prevalence of allergy over the last decades in 'Western lifestyle' countries is taken into consideration.

Laboratory studies demonstrated that EDs can modulate allergic reactions. Some hormone mimickers have been shown to affect IL-4 synthesis. BPA and NP considerably enhanced IL-4 production in keyhole limpet hemocyanin-primed CD4(+) T cells from mice in a concentration-dependent manner (46), and OP augmented IL-4 synthesis in antigen-primed murine T cells (47). Diethylhexyl phthalate (DEHP) and diisononyl phthalate (DINP), two commonly used plasticizers in flexible polyvinylchloride formulations, significantly enhanced IL-4 production in activated T cells from mice. Treatment with DEHP or DINP *in vivo* resulted in a significant increase in IL-4 production in T cells. Furthermore, DEHP and DINP enhanced the activation of IL-4 gene promoter in T cells (48). Similar changes in cytokine synthesis induced by hydroquinone (HQ), a major metabolite of benzene present in large quantities in cigarette tar, were observed and there is some evidence that transcription factor NF-AT may be involved in this process (49). What seems to be important is that the increase in IL-4 synthesis was parallel to the enhancement of the IgE production.

These findings suggest that BPA, NP, DEHP and DINP, environmental compounds commonly believed to be EDs, may augment or even shift immune processes towards IgE-related response by enhancement of IL-4 production by T cells, probably via stimulation of NF-AT-binding activity. Treatment with BPA and NP, DEHP or DINP and HQ *in vivo* resulted in a significant increase of antigen-specific IgE levels in sera of antigen-primed mice (46, 48, 49). This supports the hypothesis that some EDs may have the ability to augment allergic processes.

Laboratory studies demonstrated the influence of EDs on CD4(+) and CD8(+) as well as Th1 and Th2 subset. NP and OP suppressed Th1 and enhanced Th2 cells development, while other alkylphenol: *p*-*n*-dodecylphenol (DDP) failed to affect Th1/Th2 balance in animal model (50). Other animal study demonstrated that both Th1 and Th2 responses were augmented by prenatal exposure to BPA, but the augmentation of Th1 processes were greater than that of Th2 type (42). Those mice that were exposed to BPA prenatally had more splenic CD3(+) CD4(+) and CD3(+) CD8(+) cells than control animals. These results suggest that prenatal exposure to BPA may result

in the up-regulation of immune responses even in adulthood.

It should be stressed that these *in vitro* and animal data cannot be directly applied to human situation, because of unknown lifetime exposure to EDs and lack of information of their effect in human.

Phytoestrogens, gender and allergic response

In recent years there has been a growing interest in the possibility that gender and sex hormones may be an important determinants for asthma and other allergic diseases. In murine model of allergic rhinitis the levels of PLA2-specific IgE in females sensitized with PLA2 were significantly higher than in males. Castrated male mice generated more PLA2-specific IgE than sham-treated male mice. In addition, PLA2-specific IgE production decreased in castrated mice treated with testosterone. These results suggest that gender may affect antigen-specific IgE synthesis (51).

There is also an evidence that the prevalence of asthma in humans among children is higher in boys than in girls, but after puberty women are at higher risk to develop asthma as compared to men (52, 53). Some studies suggest that asthma incidence is significantly higher in postmenopausal women being on hormone replacement therapy (HRT), than in those who never used it (54). Moreover, it has been shown the association of maternal use of oral contraceptives before birth with a higher risk of atopic diseases (asthma, allergic rhinitis and atopic eczema) in the offspring compared with children of mothers who had never taken hormonal pills (55). As estrogens may modulate allergic immune responses, the influence of sex hormones on the immune system may partly explain such observation (56, 57). Natural environment contains a number of factors mimicking hormones having a strong influence on the human immune system. Considerable attention has been focused on 'environmental estrogens', among them isoflavones, as being known to modulate the immune response.

Isoflavones, a major group of phytoestrogens, which are the secondary plant components structurally analogous to human estrogens, occur predominantly in plants. Soy and soy-derived products, which recently are being consumed in increasing quantities, are potent sources of isoflavones, such as genistein and daidzein, in human diet (58, 59). For instance Japanese people consume quantities of soy that are likely to be physiologically meaningful (60) and epidemiological studies showed that serum genistein and daidzein concentrations are significantly associated with dietary intake of tofu, and slightly with intake of miso soup – products made of soy (61).

There are some data regarding the influence of isoflavones on the immune response. Isoflavones were shown to affect chemotactic factors production (60–62), oxygen

radical generation (63–66), adhesion molecule expression (67–69) and alter arachidonic acid metabolism (70–72), compatible with their anti-inflammatory effects. Dietary and injected genistein suppressed cell-mediated immunity in mice even at levels similar to those obtained in human diet (73). As cell activity recovered to normal after the treatment was stopped, it has been suggested that immune changes caused by genistein may be reversible. Genistein, when administered to ovariectomized female mice by injection or in the diet, induced thymic atrophy, decreased delayed type hypersensitivity (DTH) down to 30–40%, and reduced numbers of CD4(+) and CD8(+) T cells in popliteal lymph nodes. Authors speculated that these effects were mediated not only through ER, as it had been established earlier (74–76), but also via non-ER pathways. The above-mentioned data correspond with results showing that estradiol treatment of adult mice decreased leukocyte numbers in the draining lymph nodes from the injected limb during the DTH response (77).

Another study showed that genistein injections in adult mice resulted in dose-dependent decrease in thymic weight, decreased thymocyte number and doubled apoptosis (45). Those changes were accompanied by a systemic lymphocytopenia indicating that the mechanism of the genistein-induced loss of thymocytes may be caused partly by enhanced apoptosis. In mice, injection of genistein caused a decrease in relative percentages of both thymic CD4(+)CD8(–) and CD4(+)CD8(+) thymocytes (39, 45). As genistein is an inhibitor of tyrosine kinase – an enzyme playing a pivotal role in the activation of inflammatory cells, it has been proposed that soy isoflavones have immunosuppressive and anti-inflammatory properties (78–80). However, a few experimental data does not correspond with this assumption. In rat dams, exposure to genistein produced a significant enhancement in NK cell activity, while the percentage of helper T cells was decreased (81). The number of splenic B cells, T cells, and T cell subsets also increased. Daidzein exerted a stimulatory effect on nonspecific immunity, as shown by enhancement in the phagocytic response of peritoneal mice macrophages and thymus weight (43). Studies on an guinea-pig model of asthma demonstrated that genistein, contrary to daidzein, which has no inhibitory activity against tyrosine kinases, effectively reduced acute bronchoconstriction, pulmonary eosinophilia, and AHR induced by ovalbumine (OVA), but not histamine or metacholine (79). However, other results show that the reduction of antigen-induced eosinophilia in the lung of the asthmatic guinea-pigs being on a diet enriched with isoflavones is accompanied by a potentially detrimental increase in antigen-induced leakage of protein into the airspace (78).

As shown above, there is evidence that soy phytoestrogens exert an inhibitory effect on the mechanisms of allergic response, which might suggest their protective properties against allergy. On the other hand, *in vitro* and

animal studies suggest that soy isoflavones may also stimulate allergic reactions. Daidzein significantly enhanced IL-4 production from both CD4(+) T cells and EL4 T lymphoma cells probably by increased activation of AP-1 through the PI3-K/PKC/p38 MAPK signaling pathway (82). Daidzein and genistein induced IgE synthesis by mouse splenocytes although at high concentrations suggesting that phytoestrogens may increase allergic responses via the enhancement of IL-4 production by T cells and IgE synthesis (83).

The significance of gender and sex hormones in allergy still remains unclear, but soy isoflavones may modulate allergic response, including IgE production, differentially in male and female. *In utero* exposure to genistein by feeding only during gestation increased the synthesis of IgE at 84th postnatal day only in male mice (84). What seems to be interesting, in female mice serum total IgE were enhanced only when genistein was administrated continuously from gestation to 84th postnatal day. On the contrary, *in utero* exposure to genistein by gavage increased the IgE production in female mice but not in male mice. Furthermore, the enhancement in IgE production following genistein exposure in females, but not in males, was accompanied with decrease in the percentages of CD4(+)CD25(+) T suppressor cells, and increase in the NK cell activity as well as the basal splenocyte proliferation and the production of IL-2 and IL-4. One may wonder if female infants might be more susceptible to immune effects of genistein than male infants, as female mice has been shown to be more responsive to the thymic effects of genistein (85). Moreover, peak serum genistein concentrations are higher in female compared with male mice fed with the same amount of soy isoflavone (86).

There are little data from *in vitro* and *in vivo* studies on the soy isoflavones influence in humans. In cultured human cells, genistein has been shown to inhibit T-cell receptor activation-induced protein tyrosine phosphorylation and subsequent cytokine gene expression as well as cell proliferation (87, 88). Postmenopausal women showed significantly higher IL-6 levels after a high-isoflavone soy diet in comparison with control values, whereas no significant effects were seen on TNF- α generation (89).

Soy products – a source of phytoestrogens

Phytoestrogens are present in food products containing soy, e.g. soy-based infant formulas. Soy protein has been used in infant feeding for nearly 100 years (90). It has been estimated that the serum concentration of isoflavones is five to eight times higher in soy-based formula than in mother milk. Accordingly, genistein and daidzein serum levels of children fed with soy-based milk is 60–150 times higher in comparison with children fed with mother

milk (91). It has been suggested that serum genistein concentrations with potential estrogenic effects found in soy-fed infants may be capable of producing thymic and immune abnormalities and other immune impairments in soy-fed human infants which may appear even not till in adulthood.

Soy-based infant formulas are commonly used in infants with IgE-mediated cow's milk allergy, lactose intolerance, galactosemia, and as the vegetarian human milk substitute (90). However, the only concerns which have been raised with respect to soy products referred to their potential allergenicity. According to The British Dietetic Association pediatric group position statement on the use of soy protein for infants dietitians should discourage the use of soy proteins in children with atopy or cow's milk allergy in the first 6 months of life to avoid sensitization to soy protein (92). No similar concern has been raised as to the potential effect of soy-based products on development of allergic diseases.

Few studies examining the relationship between dietary soy products and isoflavone intake and the prevalence of allergic diseases have been published. The results of the cross-sectional study show that a high dietary intake of soy proteins, including genistein and daidzein, is associated with the reduced prevalence of allergic rhinitis in human, although no significant dose-response relationships were observed (60). Other study based on questionnaire of participants in a randomized clinical trial with patients with asthma revealed that increasing consumption of genistein is associated with better lung function (93).

Although it has been hypothesized that the soy consumption have a variety of human health benefits, effects of isoflavones present in food on inflammatory and allergic responses have not been established. Potentially isoflavones may have both deleterious and beneficial effect (94). Further *in vitro* and *in vivo* studies are needed to determine if widespread dietary soy phytoestrogen exposure can produce effects on the immune system and allergic response in humans.

Conclusion

No clear relationship between adverse general health effects and exposure to endocrine modulators has been established, although involvement of EDs in human pathology has been suggested in numerous studies (95–102). The influence of chemical compounds on allergic immune responses remains also unclear. Conceivably, an alteration of the immune system by environmental EDs could affect the individuals' ability to mount well-regulated immune responses to microbial and vaccine antigens, allergens, self and tumor antigens. The effects of exposure to EDs during early development and later in life on allergic sensitization and development of allergic diseases have not been studied.

Bearing in mind that the immune imbalance can be detected early in life of allergic children, cross-sectional or prospective studies addressing the effect of EDs with estrogenic activity on development of allergic responses in children seem to be warranted.

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