**Modulation of immune functions by oestrogens. Part II**

*Immunomodulacyjna rola estrogenów. Część II*

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**Summary**

One of the most important functions of oestrogens is modulation of the immune system. By interactions with specific oestrogen receptors ERα and ERβ these sex steroid hormones are capable of regulating many aspects of both natural and adaptive immunity. Aging and the hypoestrogenic state can therefore influence immunological functions. Use of drugs belonging to SERM or isoflavons could also modify immune response in post-menopausal women. All those phenomena might influence the risk of cancer initiation and the natural course of tumour growth.

**Key words:** oestrogens, immunology

**Streszczenie**

Jedną z najważniejszych funkcji estrogenów jest zdolność modulowania działania układu immunologicznego. Estrogeny, poprzez interakcje ze swoistymi receptorami estrogenowymi ERα i ERβ, są zdolne do regulowania zarówno elementów odporności naturalnej, jak i swoistej. Diagnosta starzenie się i towarzyszący mu stan hipoestrogenizmu mogą wpływać na funkcje immunologiczne. Podobnie zastosowanie u kobiet po menopauzie leków z grupy selektywnych modulatorów receptora estrogenowego (selective estrogen receptor modulator – SERM) i izoflawonów może modyfikować odpowiedź immunologiczną. Zjawiska powyższe mogą być mechanism wpływający zarówno na ryzyko powstania, jak i na charakter wzrostu nowotworów.

**Słowa kluczowe:** estrogeny, immunologia

**Influence of oestrogens on the immune function**

**Oestrogens and adaptive immunity**

Generally oestrogens stimulate adaptive immunity, but in detail their function is multidirectional and depends on hormone concentration [1]. Cytotoxic activity of T lymphocytes inside reproductive organs is oestrogen-dependent [2]. However, a high level of oestrogens is capable of suppressing both IL-2 secretion and IL-2 receptor expression in peripheral blood T CD4+ lymphocytes. This would prevent IL-2 mediated T cell expansion following antigen- or mitogen-induced activation [3]. From another point of view, oestrogens preserve proper T lymphocyte function by decreasing T lymphocyte apoptosis and Fas ligand expression, as it was shown in postmenopausal women receiving oestrogen therapy [4]. Lymphocytes T CD4+CD25+Foxp3+ are a population of natural or peripherally induced regulatory T cells (Treg), which seem to be crucial to the maintenance of tolerance, response towards transplants and pregnancy, or cancer growth [5].

Experiments performed on a mouse model indicated that estradiol increased both the number of Treg and expression of Foxp3 on their surface and that this mechanism could be responsible for protection against experimental autoimmune encephalomyelitis [6]. Not only...
the number and function of T cells could be modified by oestrogens, but also their circulation in the body. Females have increased expression of T cell C-C motif chemokine receptors (CCR)1 to CCR5, which influences the chemotactic responses of T cells [7].

The effects of oestrogen on Th1/Th2 cytokine balance also depend on hormone concentration. While in low doses estradiol triggers Th1 activity and IFN-γ secretion, in higher doses it appears to be a potent Th2 activity enhancer through stimulation of IL-10 secretion [8, 9]. Investigations performed in vivo indicated that IL-4 secretion by peripheral blood mononuclear cells correlated significantly with the oestrogen level and changed during the menstrual cycle, however, these correlations disappeared post menopause [10]. Negative influence of aging and hypoestrogenic environment on cytokine secretion was shown on mice subjected to injury. Higher serum IL-6 levels were connected with increased mortality in hypoestrogenic animals, while oestrogen substitution improved the outcome of supplemented animals [11]. Evaluation of cytokine levels in elderly women indicated the presence of hyper-inflammatory state characterized by elevated circulating levels of pro-inflammatory cytokines (IL-1β, IL-6, TNF-α, and prostaglandin E₂) [12]. Post-menopausal decline of oestrogens could contribute to these changes as oestrogen therapy improved the immune status of treated women [13]. This would rather suggest that physiological levels of oestrogens have anti-inflammatory functions. One of the possible explanations for the oestrogen role in immunoregulation is direct inhibiting properties of oestrogen on NK-kB transcription factor, which usually promotes pro-inflammatory cytokine secretion [14, 15]. Another possibility is increased production of ROI, which is secondary to an age-dependent oestrogen drop. Excessive concentration of ROI could inhibit NK-kB transcription factor [16]. Also age-dependent increase in visceral fat could account for increase in pro-inflammatory cytokines [17].

Oestrogens are engaged in activation and survival of B lymphocytes, and protect them from B cell receptor mediated apoptosis by regulation of Bcl-2 expression [18, 19]. It was also shown that oestrogen milieu and B cell receptor signalling shaped the differentiation pathway of B lymphocytes [20]. Oestrogens cause increase in immunoglobulins (IgG and IgA) in human uterine cervical mucous in the proliferative menstrual phase [21], as well as rise in total IgG and IgM production by human peripheral blood lymphocytes [22, 23].

Oestrogen deprivation caused either by castration or by senescence has profound adverse effects on adaptive immunity. Both aged and ovariectomized animals exhibited reduced T cell response to antigens and mitogenic stimuli, as well as disturbed IL-2 secretion and chemotaxis [24-26]. Compared to the pre-menopausal period, elderly females showed lowered numbers of T and B cells, poor anti-viral immunity, decreased proliferative response and T helper cytokine secretion [27-30].

**Role of soy isoflavones and SERM in modulating the immune function**

Genistein is one of the most important soy isoflavones, which due to its structural similarity to estradiol and has similar biological effects. Genistein is a ligand both for ERα and ERβ, however, unlike oestrogens, has a greater affinity for ERβ, and may act as ER modulator possessing both estrogenic and antiestrogenic effects [31-33]. Like in the case of oestrogens, the biological effects of genistein on immune functions depend on its dose. Higher concentrations of genistein indicated immunosuppressive activity, and caused decrease in T and NK cells activity, T cell proliferation and NO production in macrophages. Those changes resulted in genistein dose-dependent inhibition of both humoral and cell-mediated immunity [31, 34]. Some authors did not confirm these results and showed that genistein induced activity of cytotoxic T cells and NK cells [35]. They later showed that genistein treatment was also associated with IFN-γ secretion and a decrease in Treg CD4+CD25+ cells, which resulted in decreased susceptibility to toxin-induced carcinogenesis [36]. The most reliable study was based on genistein concentrations usually met during dietary isoflavones intake. This study indicated that genistein was able to inhibit exaggerated IFN-γ production during bacterial infection in mice [37]. In vivo studies performed on post-menopausal women showed that genistein caused an increase in B cell population, however, did not significantly influence IFN-γ, TNF-α or IL-2 serum concentrations [38]. A commonly accepted observation is that use of soy isoflavones in post-menopausal women lowers the risk of cardiovascular disease, osteoporosis, and cancer of the breast [39-42].

Selective oestrogen receptor modulators (SERM) like tamoxifen and raloxifene could effectively bind to ER, however their function of ER agonists or antagonists depends on the drug and type of responding tissue. Tamoxifen is used for prevention and treatment of breast cancer, but it increases the risk of endometrial hyperplasia including endometrial cancer. Raloxifene is used for treatment of osteoporosis, however it also decreases the incidence of both breast and endometrial cancer [43, 44]. Modulation of ER implies that SERM could influence immune functions. It was shown that they were able to regulate anti- and proinflammatory cytokines, decrease B cell activity and diminish intensity of autoimmune reactions [45-50]. SERM were shown to antagonize oestrogens in GM-CSF mediated differentiation of DC cells, which displayed immature phenotype [51]. This mechanism was probably based on SERM ability to modify IFN-γ, IL-12, and IL-6 production as well as STAT3 activity in monocyte cultures [44].
Possible influence of oestrogen-dependent immune changes on the risk of cancer

Oestrogens could predispose to breast, endometrial and probably ovarian cancer. The mechanisms of carcinogenesis consist of oestrogen proliferative abilities directed to target tissues, induction of growth factors, stimulation of activation-induced deaminase (AID), mutagenic properties of oestrogen metabolites or direct stimulation of ER positive tumours [52-56]. However, multidirectional influence of oestrogens on immune functions could also affect tumour development. The results of some studies seem to support this possibility, as it was shown, that oestrogen treatment of mice with grafted ER-negative tumours suppressed macrophage recruitment and pro-inflammatory cytokine secretion at the tumour site, thus lowering defence mechanisms [57]. Tumour-associated macrophages (TAM) are one of the most important immune cell populations inside tumours. According to the profile of secreted cytokines, there are two macrophage populations identified, M1 cells secreting pro-inflammatory Th1 cytokines, and M2 cells producing Th2 cytokines [58, 59]. There is a possibility that oestrogens could regulate both the proportion of M1/M2 TAM and their activity, thus influencing the tumour growth. Defective M1-type functions showed by TAMs inside tumours are probably caused by disturbed activation of NF-κB in response to pro-inflammatory stimuli present in advanced tumours [60]. It cannot be excluded that similar effects on NF-κB function might follow oestrogen activity. Progressive tumours contain immature DC having pro-tolerogenic functions, and being unable to stimulate host anti-tumour cytotoxic responses [61-63]. Regulatory function of oestrogens on DC could augment their differentiation into an immature phenotype. Moreover, oestrogen-dependent expansion of CD4+CD25+Foxp3+ Treg cells could adversely modify anti-tumour responses, as Treg cells were found to accumulate in tumour and local lymphatic nodes in cancer patients with unfavourable outcome [reviewed in 64]. Positive impact of oestrogens on B cell proliferation and function could potentially alter anti-tumour response, because B lymphocytes are able to create an inflammatory environment supporting tumour growth [65]. However, decreased T and NK cells function observed after menopause could also discriminate anti-cancer response. Thus, due to multidirectional influence of oestrogens on immunity, both their presence in the reproductive period of a woman’s life, as well as their lack after menopause could in some aspects predispose to cancer growth.

Conclusion

Oestrogens are potent and multidirectional modifiers of the immune system. Our growing knowledge concerning this function could help in prophylaxis and treatment of many tumours in women. Use of selective ER agonists and antagonists in treatment of reproductive tract tumours, could be further adopted to management of other tumours met in females.

References


