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# Sex hormones and the immune response in humans

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**In addition to their effects on sexual differentiation and reproduction, sex hormones appear to influence the immune system. This results in a sexual dimorphism in the immune response in humans: for instance, females produce more vigorous cellular and more vigorous humoral immune reactions, are more resistant to certain infections, and suffer a higher incidence of autoimmune diseases. Disease expression is also affected by the reproductive status of the female. As sex steroids—estrogens, progesterone and testosterone—differ between gender and within different reproductive stages, a lot of research has focussed on the effects of sex hormones on immune responses. Although there is also a vast literature on the effects of sex hormones on immune responses in animals, in this review we will focus on the most intriguing effects and mechanisms by which sex hormones affect different components of the immune system in humans.**

*Key words*: cytokines/immune response/leukocytes/reproductive condition sex hormones

## Introduction

### *Sex hormones*

The ovary produces three classes of sex steroids: estrogens, progestins and androgens. Production of sex hormones fluctuates with ovarian activity. Hormonal fluctuations in the menstrual cycle include increasing 17β-estradiol ($E_2$), but low progesterone plasma concentrations in the follicular phase, and high plasma 17β-$E_2$ and progesterone concentrations in the luteal phase. If pregnancy occurs, luteolysis is prevented and 17β-$E_2$ and progesterone levels remain high. Later in life (menopause), with the depletion of follicles, sex hormone concentrations drop to very low levels. In oral contraceptive (OCC) users, the progestin component suppresses luteinizing hormone secretion, while the estrogenic component suppresses FSH secretion preventing selection and emergence of a dominant follicle and ovulation. Therefore, naturally 17β-$E_2$ and progesterone plasma concentrations are low during OCC use, however, at the end of the pill free period the 17β-$E_2$ concentration is comparable with the concentration, which characterizes the early follicular phase.

### *Immune system*

There are two arms of the immune system: the non-specific (innate or natural) immune system and the specific (acquired or adaptive) immune system. The non-specific immune response is the first line of defence against infections. It recognizes structures specific for microbes. The effector cells of the non-specific immune response are monocytes, macrophages, granulocytes (neutrophils, eosinophils and basophils), dendritic cells and natural killer (NK) cells. These cells attack microbes that have entered the circulation. They do so by phagocytosing the microbe (neutrophils, monocytes and macrophages), by lysis of infected cells (NK cells) or by producing cytokines to enhance non-specific immune and specific immune responses (all cells).

The cellular components of the specific immune response are T lymphocytes and immunoglobulin producing B lymphocytes. T lymphocytes express the T cell receptor (TCR). Two forms of TCRs are recognized: the αβ-TCR, which is responsible for major histocompatibility complex (MHC) restricted antigen recognition, and the γδ-TCR, which does not recognize MHC associated antigens and is not MHC restricted. Within the T lymphocyte population expressing the αβ-TCR, helper T lymphocytes (Th or $CD4^+$ cells) provide help to other immune cells by producing cytokines. Cytotoxic/suppressor T lymphocytes (Tc/Ts or $CD8^+$ cells) produce cytokines and can also directly kill foreign or infected cells.

### *Relation reproduction and immune system*

Different reproductive processes, including ovulation, menstruation, are influenced by the immune system. A reproductive condition in which immunology plays a pertinent role is pregnancy. It is for instance postulated that cytokines are important in

creating an optimal environment for successful implantation (Laird *et al.*, 2003; Chaouat *et al.*, 2004) and parturition (Keelan *et al.*, 2003). However, in the present review we will focus on the effects of the reproductive condition and sex hormones on the immune system. Mostly studied in this respect is the effect of pregnancy on the immune response. As recently reviewed (Veenstra van Nieuwenhoven *et al.*, 2003b), the ratio of type 1 and type 2 cytokine production of lymphocytes is decreased during pregnancy, while monocytes and granulocytes are activated.

Also a sexual dimorphism in the immune response in humans is obvious; females produce more vigorous cellular and more vigorous humoral immune reactions, they are more resistant to certain infections, such as bacterial infections, and suffer a higher incidence of autoimmune diseases as compared with males (reviewed by Ansar *et al.*, 1985).

Also disease expression is affected by the reproductive status; patients with immune-based diseases, such as multiple sclerosis, asthma or systemic lupus erythematosis (SLE), may have exacerbations during specific periods of the menstrual cycle or pregnancy (Skobeloff *et al.*, 1996; Case and Reid, 1998; Whitacre, 2001). It has been suggested that changes in disease expression during pregnancy may be due to a decrease in Th1/Th2 ratio of cytokines during pregnancy (Ostensen, 1999).

These observations suggest not only gender differences in immune responses, but also differences in immune responses between different female reproductive phases. Since differences in immune responses between sexes and reproductive phases are accompanied by variations in sex hormones, the variations in immune responses are usually suggested to be due to these hormonal variations. In the present review we will describe the most intriguing effects and mechanisms by which 17β-$E_2$, progesterone and testosterone affect immune responses in humans. We will do so by focussing on the effects of sex hormones on the different human immune cells.

## The influence of 17β-$E_2$, progesterone and testosterone on the specific immune response

### *T lymphocytes numbers*

In the peripheral blood about 30% of the white blood cells are lymphocytes. About 85–90% of the lymphocytes are T lymphocytes. Of these T lymphocytes, about 95% are expressing the αβ-TCR, while 5% are expressing the γδ-TCR. Of the αβ-TCR expressing T lymphocyte population about 60% are Th cells, while about 30% of the T lymphocytes are Tc and Ts cells. Many investigators have studied the counts of blood cell subsets at different reproductive stages, in order to explain differences in immune responses between gender and within different reproductive phases. Although not much is known about the variation in numbers of γδ-TCR expressing T lymphocytes, it has been shown that numbers of γδ T cells are increased during pregnancy (Polgar *et al.*, 1999).

Much research has been done on the numbers and subsets of αβ-TCR expressing T lymphocytes. Although total lymphocyte count in males is similar to females (Giltay *et al.*, 2000; Bouman *et al.*, 2004b), the percentage of T lymphocytes within the total lymphocyte population in males is lower as compared to females (Bouman *et al.*, 2004b). No difference was found in Th/Tc ratio between males and females (Giltay *et al.*, 2000). The decreased T lymphocyte counts in males as compared to females may be due to the increased testosterone concentrations, since testosterone may increase apoptosis in T cells (McMurray *et al.*, 2001).

A number of investigations suggest no changes in total circulating numbers of lymphocytes and no variation in percentage of lymphocyte subtypes during the menstrual cycle (Mathur *et al.*, 1979; Faas *et al.*, 2000; Bouman *et al.*, 2001b). This may indicate that neither progesterone nor estrogen affect lymphocyte numbers in the short-term. However, post-menopausal women showed a reduction of the number of total lymphocytes in comparison to fertile women (as a result of decreased B and Th lymphocytes) (Giglio *et al.*, 1994; Yang *et al.*, 2000). This may be due to long-term withdrawal of progesterone and 17β-$E_2$. However, other mechanisms involved in controlling lymphocyte counts in women cannot be excluded. One of these factors may be related with ageing (Miller, 1996; Chakravarti and Abraham, 1999).

Also synthetic hormones in OCC preparations do not affect absolute numbers or percentages of lymphocytes, T cells and subsets of T cells (Baker *et al.*, 1985; Yovel *et al.*, 2001). On the other hand, HRT in post-menopausal women did affect lymphocyte subtypes: total lymphocyte count (Burleson *et al.*, 1998), the percentage of T cells (Burleson *et al.*, 1998; Yang *et al.*, 2000) and the percentage of Th lymphocytes (Burleson *et al.*, 1998) were found to be decreased.

### *T lymphocyte function*

One of the main functions of Th lymphocytes is the production of cytokines. Although Tc lymphocytes also produce cytokines, they have a major function in cell cytotoxicity as well. One of the major advances in our understanding of the regulation of immune responses has been the description T helper 1(Th1)/T helper 2 (Th2) paradigm (Mosmann *et al.*, 1986). Th1 cytokines, are for instance interferon-γ (IFN-γ), interleukin (IL)-2, and generally promote cellular immune responses, whereas the Th2 cytokines, IL-4, IL-5, IL-9, IL-10 and IL-13, provide optimal help for humoral immune responses (Mosmann *et al.*, 1986). In general, type 1 and type 2 cytokines are reciprocally regulated; IFN-γ inhibits the proliferation of Th2 cells, whereas IL-10 inhibits that of Th1 cells (Swain *et al.*, 1991). Also Th0 and Th3 lymphocytes exist (Mosmann and Sad, 1996). Th0 lymphocytes are the precursors of Th1 and Th2 cells and secrete both Th1 cytokines and Th2 cytokines. Th3 cells produce TGFβ, but do not produce IFN-γ, IL-2, IL-4 or IL-10 (Mosmann and Sad, 1996). Although this paradigm helped us to understand how the immune response is directed towards different types of pathogens and stimuli, it may be an over-simplification of regulation of immune responses (Kelso, 1995). However, for the sake of clarity, we will use the Th1/Th2 paradigm in our review. In this part of the review we will focus on Th1 and Th2 cytokines, since most studies have been performed on these cytokines.

#### *IFN-γ production*

One of the cytokines playing a prominent role in specific immune responses is IFN-γ. IFN-γ, a 25 kDa polypeptide, acts

to control cellular immunity mainly by promoting the effector functions of lymphocytes and activating monocytes and macrophages, while it also has an antiviral activity. Combining various studies in the literature, we conclude that there are no major effects of sex hormones on lymphocyte IFN-γ production: increased IFN-γ production (Giron-Gonzalez *et al.*, 2000) as well as similar IFN-γ production (Bouman *et al.*, 2004b) by male lymphocytes as compared to female lymphocytes was found; no effect of the menstrual cycle upon IFN-γ production was found (Faas *et al.*, 2000), and no effect of synthetic hormones on lymphocyte IFN-γ production was found (Agarwal and Marshall, 1999; Berg *et al.*, 2002). These *in vivo* results are in-line with *in vitro* experiments in which neither progesterone, 17β-$E_2$ nor testosterone altered IFN-γ production (Piccinni *et al.*, 1995; Giron-Gonzalez *et al.*, 2000; Posma *et al.*, 2004).

*IL-2 production*

IL-2, a 15 kDa polypeptide is the major cytokine responsible for T lymphocyte activation and proliferation. Furthermore, IL-2 has important growth promoting functions in relation to B lymphocyte development. Contradictory results have been published for lymphocyte IL-2 production between gender and within reproductive phases, which makes it difficult to draw any conclusions. First of all, IL-2 production was found to be similar (Giron-Gonzalez *et al.*, 2000) or decreased in stimulated male—as compared to female lymphocytes (Bouman *et al.*, 2004b). Secondly, a decline in IL-2 production as well as no difference in IL-2 production in luteal phase as compared to follicular phase was found (Faas *et al.*, 2000; Trzonkowski *et al.*, 2001). Also for the effects of synthetic hormones the results are conflicting: OCC use did not affect (Bouman *et al.*, in preparation), while HRT reduced the production of IL-2 by lymphocytes (Jenkins *et al.*, 1994). IL-2 production was shown to be increased by lymphocytes of post-menopausal women as compared to fertile women (Kamada *et al.*, 2001a), while no effect of pregnancy was shown on lymphocyte IL-2 production (Veenstra van Nieuwenhoven *et al.*, 2002).

*IL-4 production*

IL-4 is a 20 kDa polypeptide released predominantly by Th2 lymphocytes. It is an important growth promoting factor for Th2 lymphocytes and a stimulus for B cell switching to immunoglobulin E (IgE) production, which is important in parasitic infection and asthma. In males and after menopause IL-4 production is similar to fertile women (Giron-Gonzalez *et al.*, 2000; Kamada *et al.*, 2001a; Cioffi *et al.*, 2002), suggesting no effect of sex hormones on the production of IL-4. In contrast with this suggestion, however, the production of IL-4 is significantly increased in Th cells in the luteal phase as compared with the follicular phase of the ovarian cycle (Faas *et al.*, 2000). Whether this increased IL-4 production in the luteal phase is due to increased progesterone or 17β-$E_2$ concentrations remains uncertain, since *in vitro* experiments showed no effect of 17β-$E_2$, or progesterone in physiological and supraphysiological concentrations on IL-4 productive capacity of T lymphocytes in whole blood (Bouman *et al.*, in preparation). One study demonstrated an increase in IL-4 production of Th lymphocytes after incubation with progesterone. However, they used Th1 cell clones rather whole blood and incubated only with supraphysiological concentrations of progesterone (Piccinni *et al.*, 1995). Also synthetic hormones do not affect lymphocyte IL-4 production (Giron-Gonzalez *et al.*, 2000; Kamada *et al.*, 2001a; Berg *et al.*, 2002).

*IL-10 production*

IL-10 is an 18 kDa polypeptide. Th2 cells are the predominant source of IL-10. IL-10 inhibits pro-inflammatory cytokine production (i.e. IL-1β, TNF-α) by monocytes and macrophages (Howard and O'Garra, 1992), T cells and NK cells (de Waal *et al.*, 1991). The net result of these actions is to down-regulate T cell immune responses. No difference in IL-10 production between both males or post-menopausal women and premenopausal women could be demonstrated (Giron-Gonzalez *et al.*, 2000; Bouman *et al.*, 2004b). Also during the menstrual cycle lymphocyte IL-10 production after stimulation is stable (Maskill *et al.*, 1997; Faas *et al.*, 2000), suggesting no effect of sex hormones on IL-10 production. The results with OCC, which do not influence IL-10 production (Agarwal and Marshall, 1999) and *in vitro* experiments which show no effect of 17β-$E_2$, progesterone or testosterone on IL-10 production (Piccinni *et al.*, 1995; Giron-Gonzalez *et al.*, 2000; Posma *et al.*, 2004) corroborate this suggestion.

In conclusion, the effects of gender and reproductive conditions on lymphocytes are not very obvious. However, in males the decreased T lymphocyte count as compared to females may play a role in the differences in immune responses between sexes. Thus far no differences in Th2 cytokine production (IL-4 and IL-10) could be found between gender and within reproductive phases, which is in-line with lack of effect of the sex hormones *in vitro* on the production of these cytokines. No effects of gender and reproductive phases upon the production of IFN-γ could be found, while the literature on the effects of gender and the reproductive phase on the production of IL-2 are inconclusive. It remains thus uncertain at this moment whether differences in immune responses between sexes and within reproductive phases are due to (direct) effects of sex steroids on lymphocyte cytokine production.

***B lymphocytes numbers***

B lymphocytes are antibody producing cells and reconstitute 5–15% of circulating lymphocytes. Conventional B cells (B2 cells) present internalized antigens to T cells through which they get activated and develop into antibody-producing plasma cells (Fagarasan and Honjo, 2000). In contrast, the other subset of B cells, B1 cells, produce antibodies in a T cell independent manner (Fagarasan and Honjo, 2000). These B1 cells are suggested to be responsible for autoantibody production (Kasaian and Casali, 1993).

Reports of B cell counts are more scarce than reports on cell counts of the other leukocytes and no data are available on differences in B lymphocyte count between males and females. No differences could be demonstrated in B lymphocyte count within the menstrual cycle (Lopez-Karpovitchs *et al.*, 1993; Auerbach *et al.*, 2002), and OCC use did not affect B cell count (Auerbach *et al.*, 2002). After menopause, B cell numbers were shown to be similar to (Yang *et al.*, 2000) or decreased from the numbers in fertile women (Giglio *et al.*, 1994). Although after

1 month and 6 months of HRT use B cell count did not alter (Gronroos and Eskola, 1984; Yang *et al.*, 2000), prolonged HRT use (>12 months) induced a significant increase in B cell numbers (Porter *et al.*, 2001). Estrogens may affect B lymphocyte subsets. It appeared that B1 subsets remained stable after menopause and were not affected by HRT, while the B2 subset decreased after menopause and increased after HRT (Kamada *et al.*, 2001b). Also studies in animals have shown an effect of estrogens on B cell development: estrogens increase bone marrow progenitor B cells in mice by protecting the progenitor cells from apoptosis (Medina *et al.*, 2000; Grimaldi *et al.*, 2002), and increase survival in splenic B cells (Grimaldi *et al.*, 2002). These estrogen effects on B cell development may decrease negative selection in naïve immature B cells and enhance the survival of autoreactive B cells (Grimaldi *et al.*, 2002) and may, therefore, be involved in the higher incidence of autoimmune diseases in women.

#### *B lymphocyte function*

*Antibody production*

Since one of the main functions of B lymphocytes is the production of antibodies, differences in B cell function between sexes can be derived from differences in plasma levels of antibodies. Since women produce more vigorous humoral immune reactions, it seems likely that B lymphocyte function differs between males and females. Indeed, women have higher serum levels of total IgM and IgG (Butterworth *et al.*, 1967; Lichtman *et al.*, 1967; Eidinger and Garrett, 1972; Grundbacher, 1972; Giltay *et al.*, 2000). However, they showed no changes throughout the menstrual cycle in serum immunoglobulin levels (Gomez *et al.*, 1993; Lopez-Karpovitchs *et al.*, 1993). Conflicting results have been reported for OCC users: immunoglobulin levels and immunoglobulin production in OCC users are unaltered as compared to females not taking OCC (Bisset and Griffin, 1988a,b), while others found immunoglobulin levels to be decreased (Klinger *et al.*, 2000) or even increased (Lali *et al.*, 1996) in females using OCC as compared to females not using OCC. The higher serum levels of immunoglobulin in females may suggest a stimulating effect of female sex hormones and or an inhibiting effect of testosterone upon this parameter.

This suggestion is corroborated by various studies. *In vitro* it has been shown that estrogen induces polyclonal activation of B cells in humans: it increased IgG and IgM production of PBMCs both from males and females (Weetman *et al.*, 1981; Kanda *et al.*, 1999a). Testosterone inhibited immunoglobulin IgG and IgM (Kanda *et al.*, 1996). In-line with these results, it has also been shown that estrogen increased and testosterone decreased autoantibody production of PBMC in patients with SLE (Kanda *et al.*, 1997, 1999b). From animal data, it appears that estrogen not only up-regulated total antibody production and autoantibody production, it may also induce a switch in antibody isotype: in mice treated with estrogens, autoantibody production was increased and the antibodies were mainly of the IgG isotype, and the main subisotype were IgG2b and IgG1 (Verthelyi and Ansar Ahmed, 1997; Latham *et al.*, 2003).

Present evidence points towards an important role for estrogen and testosterone in antibody production. This is in-line with clinical evidence for an involvement of these hormones in the pathogenesis of SLE and experimental SLE (Verthelyi, 2001; Askanase and Buyon, 2002). We need a better understanding of the gender and sex hormone influences on B function and counts in humans, in order to produce, novel therapeutic approaches for humoral autoimmune diseases. Since more is known about the effects of estrogen on B cells in animals then in humans (as reviewed by Verthelyi, 2001) there is much to learn from this animal work for the human situation. However, it is, outside the scope of this review to discuss this animal work.

### Inlfuence of 17β-$E_2$, progesterone and testosterone on the non-specific immune response

#### *Monocyte numbers*

Monocytes constitute between 5 and 10% of circulating white blood cells and have a short half-life, spending approximately 24 h in the blood. One of the best known effects of sex hormones on monocytes is the effect on monocyte count. During menopause and in males an increase in blood monocyte number has been demonstrated as compared to females in the follicular phase (Ben Hur *et al.*, 1995; Bouman *et al.*, 2004b). Moreover, during menopause the monocyte counts decline following estrogen replacement therapy (Ben Hur *et al.*, 1995). These findings suggest that estrogen, and possibly also progesterone, decrease monocyte numbers. This decreasing effect of estrogen and progesterone on monocyte numbers may be due to sex hormones inducing mitotic arrest and apoptosis in monocytes (Thongngarm *et al.*, 2003).

Others, however, demonstrated an increase in monocyte count in the luteal phase and during pregnancy as compared with the follicular phase (Bain and England, 1975; Mathur *et al.*, 1979; Bouman *et al.*, 2001b; Elenkov *et al.*, 2001). Interestingly, this has also been suggested to be due to increased 17β-$E_2$ or progesterone concentrations; these sex hormones induce the release of monocytes from the bone marrow (Bain and England, 1975). Since monocytes play an important regulatory role in immune responses (they produce cytokines and clear pathogens from the circulation) by affecting monocyte numbers, sex hormones may play an important role in the differences in immune responses between gender and within reproductive phases.

#### *Monocyte function*

While monocytes are able to ingest and kill micro-organisms by the process of phagocytosis, a very important function of monocytes is to direct immune responses by the production of cytokines. Important cytokines in this respect are: IL-1β, tumour necrosis factor-α (TNF-α), IL-12, IL-18 and IL-6. The effects of sex hormones on this function of monocytes have been studied extensively and will be discussed in the next sections.

*TNF-α production*

TNF-α, a 25 kDa polypeptide hormone, secreted by activated macrophages and monocytes, has pleiotropic actions and has emerged as an especially important mediator in pro-inflammatory responses and activation of T cells (Beutler *et al.*, 1985).Various *in vivo* observations suggest that sex hormones may influence monocyte TNF-α production. In males endotoxin-stimulated monocytes produce more TNF-α as compared to

females (Schwarz *et al.*, 2000; Asai *et al.*, 2001; Bouman *et al.*, 2004b). Whether this is due to increased testosterone concentrations remains uncertain since *in vitro* studies showed no effect of testosterone upon monocyte TNF-α production (Posma *et al.*, 2004).

Also the female reproductive phase influences monocyte TNF-α production. Higher plasma levels of TNF-α have been observed during the luteal phase as compared with follicular phase, while endotoxin-stimulated monocytes of luteal phase women produce more TNF-α as compared with the follicular phase (Brannstrom *et al.*, 1999; Bouman *et al.*, 2001b). Although this suggests a role for the female sex hormones in increasing monocyte TNF-α production, preliminary experiments in our own lab suggest that after menopause, i.e. a situation with very low levels of sex hormones, monocyte TNF-α production is increased, rather then decreased. Moreover, HRT in post-menopausal women and OCC use did not affect TNF-α production by monocytes (Rogers and Eastell, 1998; Bouman *et al.*, 2004a). These observations indicate that other factors, apart from 17β-$E_2$ and progesterone may affect monocytes.

Various papers describe *in vitro* experiments in which stimulated and unstimulated monocytes were incubated with 17β-$E_2$ or progesterone. Conflicting results have been published. First some authors claim a down-regulation of male monocyte endotoxin-induced TNF-α production by 17β-$E_2$ at both physiological and supraphysiological levels (Asai *et al.*, 2001), whereas others demonstrated no effect of either 17β-$E_2$ or progesterone upon TNF-α production in stimulated monocytes of males and post-menopausal women (Ralston *et al.*, 1990; Rogers and Eastell, 2001; Bouman *et al.*, 2004a). Also an increase in TNF-α mRNA from stimulated luteal peripheral blood monocytes at respectively physiological levels and a decrease at supraphysiological serum levels of both 17β-$E_2$ and progesterone levels was found (Loy *et al.*, 1992); Similar controversial results have also been described for the effects of 17β-$E_2$ and progesterone upon cytokine production of unstimulated monocytes (Ralston *et al.*, 1990; Asai *et al.*, 2001; Rogers and Eastell, 2001).

*IL-1β*

IL-1β, a 17 kDa polypeptide produced by peripheral monocytes and macrophages, mediates a wide variety of immune responses. As for TNF-α, differences in IL-1β synthesis at different reproductive stages have been demonstrated; in the luteal phase an increased IL-1β plasma concentration and IL-1β mRNA after endotoxin stimulation and an increased percentage IL-1β producing stimulated monocytes was demonstrated as compared to the follicular phase (Cannon and Dinarello, 1985; Polan *et al.*, 1994; Bouman *et al.*, 2001b). This suggests a 17β-$E_2$ and/or progesterone effect on monocyte IL-1β production. However, in males a higher percentage IL-1β producing stimulated monocytes was demonstrated as compared with females in the follicular phase, suggesting that other mechanisms may be present (Bouman *et al.*, 2004b). No difference in percentage IL-1β-producing monocytes in OCC users between the OCC use and the OCC free period (Bouman *et al.*, 2004a) was found, suggesting no effect of synthetic hormones upon this parameter.

As *in vivo* situations and experiments suggest various mechanisms, it is important to evaluate the effect of progesterone and 17β-$E_2$ on IL-1β production *in vitro*. Again the results are contradictory; no effect of both sex hormones *in vitro* on endotoxin-stimulated monocytes IL-1β production (Rogers and Eastell, 2001; Bouman *et al.*, 2004a), an inhibition of IL-1β production and IL-1β mRNA by endotoxin-stimulated monocytes by both 17β-$E_2$ and progesterone at supraphysiological concentrations and a stimulation of IL-1β mRNA and IL-1β production in endotoxin-stimulated monocytes by sex hormones was observed (Polan *et al.*, 1988, 1989; Morishita *et al.*, 1999). As far as *in vitro* studies on IL-1β production by unstimulated monocytes are concerned, conflicting data have also been published, varying from little to no effect of 17β-$E_2$ or progesterone on IL-1β production (Stock *et al.*, 1989; Morishita *et al.*, 1999; Rogers and Eastell, 2001). The effect of testosterone upon monocyte IL-1β production was tested. Although we showed that incubation of whole blood with physiological concentrations of testosterone increased monocyte IL-1β production (Posma *et al.*, 2004), this was contradictory to the work of Morishita *et al.* (1999).

*IL-12*

IL-12 is produced by monocytes, macrophages and B cells and plays a primary role in the induction of cell-mediated immunity; i.e. together with IFN-γ it is a major inducer of Th1 differentiation; it stimulates the functional activity of Tc, NK cells and activated macrophages, which are the major components of cellular immunity (Trinchieri, 1995).

Although IL-12 is an important cytokine that links the non-specific immune system to the specific immune system, not many studies have focussed on the effect of the reproductive condition on this cytokine. The regulation of the IL-12 production by reproductive phase is shown by the fact that IL-12 production may change during pregnancy: stimulated IL-12 production was shown to be decreased (Elenkov *et al.*, 2001; Sakai *et al.*, 2002; Veenstra van Nieuwenhoven *et al.*, 2003a), or increased (Sacks *et al.*, 2003). We have shown no difference in the IL-12 productive capacity of monocytes when comparing the luteal phase with the follicular phase, while in males this IL-12 productive capacity of lipopolysaccharide (LPS)-stimulated monocytes was increased as compared with women (Bouman *et al.*, 2001b, 2004b). This may suggest that testosterone stimulates monocyte IL-12 production. Indeed, physiological levels of testosterone increased IL-12 production by LPS-stimulated monocytes (Posma *et al.*, 2004). *In vitro*, no effect of 17β-$E_2$ (Elenkov *et al.*, 2001) or a decreasing effect (Matalka, 2003) of 17β-$E_2$ on IL-12 production was found, while progesterone did not affect the production of IL-12 (Elenkov *et al.*, 2001; Matalka, 2003).

*IL-6*

IL-6 is a pleiotropic cytokine, which stimulates B lymphocyte and T lymphocyte differentiation, and activates macrophages and NK cells. IL-6 also possesses anti-inflammatory properties. It is produced by a variety of cells, among others monocytes and macrophages in response to microbes and to other cytokines. Since IL-6 has direct actions on osteocytes and plays a major role in bone remodelling, which is important after menopause, many studies have been performed as to the effect of sex hormones on IL-6 production. It has been shown that plasma IL-6 levels are increased after menopause (McKane *et al.*, 1994;

Kania *et al.*, 1995; Cioffi *et al.*, 2002; Rachon *et al.*, 2002); these increased IL-6 levels were decreased by HRT (Straub *et al.*, 2000; Rachon *et al.*, 2002). Further evaluation indicated that the decrease in plasma IL-6 levels are due to the estrogenic component in HRT (Rogers and Eastell, 1998; Rachon *et al.*, 2002). This is in-line with a study of Angstwurm et al, who showed decreased plasma IL-6 levels during the luteal phase compared with the follicular phase (Angstwurm *et al.*, 1997). However, others showed no variation in plasma IL-6 levels or leukocyte IL-6 production during the menstrual cycle (Jilma *et al.*, 1997; Brannstrom *et al.*, 1999; Al Harthi *et al.*, 2000; Konecna *et al.*, 2000; Verthelyi and Klinman, 2000; Abrahamsen *et al.*, 2003). Still, the general idea is that female sex hormones, especially estrogens, decrease plasma IL-6 concentration.

Although there appears to be general consensus about the role of estrogens in spontaneously produced IL-6, conflicting results have been found for stimulated IL-6 production. No difference (Angstwurm *et al.*, 1997; Abrahamsen *et al.*, 2003), an increase (Konecna *et al.*, 2000) or a decrease (Schwarz *et al.*, 2000) in stimulated IL-6 production was found between the follicular and luteal phase when whole blood was stimulated with LPS.

Also studies into the influence of HRT in post-menopausal women upon stimulated IL-6 production yielded conflicting results: stimulated IL-6 production was either decreased (Berg *et al.*, 2002) or not affected (Rogers and Eastell, 1998; Brooks-Asplund *et al.*, 2002) by the estrogenic compound in HRT, while one of these studies showed that the prostagens in the HRT up-regulates stimulated IL-6 production (Brooks-Asplund *et al.*, 2002).

*IL-18*

IL-18 was originally described as an IFN-γ inducing factor (Lebel-Binay *et al.*, 2000). It is a relatively newly discovered cytokine, which can stimulate both Th1 and Th2 responses. IL-18 together with IL-12 can shift specific immune responses towards type 1 responses, but in the absence of IL-12, IL-18 can stimulate TH2 responses, while IL-18 also participates in innate immune responses (Nakanishi *et al.*, 2001). Today, not much is known about variations in plasma IL-18 concentrations in various reproductive conditions. It has been shown that plasma IL-18 is increased in post-menopausal women (Cioffi *et al.*, 2002). Also during pregnancy, serum levels of IL-18 were increased (Ida *et al.*, 2000). Both these studies suggest that the sex hormones may modulate IL-18 production; the exact mechanism, however, remains to be elucidated, since no further studies have been done into the effects of estrogen and progesterone on IL-18 production.

*Leukaemia inhibiting factor*

From a reproductive biology point of view, leukaemia inhibiting factor (LIF) is an interesting cytokine, since it is well-known for its role in embryo implantation and development (Piccinni, 2002). LIF is a pleiotropic cytokine, which is a member of a family structurally and functionally related cytokines that also include IL-6. LIF has been shown to stimulate bone marrow production of blasts and megakaryocytes (Metcalf *et al.*, 1991; Verfaillie and McGlave, 1991) and to inhibit the differentiation of embryonic stem cells (Smith *et al.*, 1988). In immune responses LIF has been shown to possess both anti-inflammatory (Ulich *et al.*, 1994; Tang *et al.*, 1996) as well as pro-inflammatory (Waring *et al.*, 1993, 1994) properties. Because of its role in reproduction and the fact that LIF has been implicated in the pathogenesis of inflammatory conditions that vary with the reproductive condition, such as rheumatoid arthritis (Waring *et al.*, 1993), it seems likely that the production of LIF is influenced by sex hormones. However, few studies have investigated the role of sex hormones in LIF production. *In vitro* it has been shown that high concentrations of progesterone can up-regulate LIF production (Piccinni *et al.*, 2001), while others have shown that estrogens may down-regulate LIF production (Bamberger *et al.*, 1997).

The effects of gender and the reproductive condition upon monocyte cytokine production are obvious. The most important and consistent effects are: plasma IL-6 levels appear to be decreased by estrogens; stimulated TNFα and IL-1β production is increased in males as compared to females, and also increased in the luteal phase as compared to the follicular phase of the ovarian cycle; stimulated monocyte IL-12 production is only increased in males and not affected by the ovarian cycle. These differences in monocyte function may play a role in the differences in immune responses between gender and reproductive condition. Whether these differences are due to sex hormones variations remains uncertain, since *in vitro* experiments in which monocytes were incubated with sex hormones revealed conflicting results upon monocyte cytokine production. Further studies are needed to evaluate the exact effects of sex hormones on monocyte cytokine production. Moreover, future studies also need to focus on important cytokines such as IL-18 and LIF.

***Granulocyte numbers***

The granulocytes constitute approximately 65% of all white blood cells and can be divided in basophils (0.5–1%), eosinophils (3–5%), and neutrophils (90–95%). Since neutrophils constitute 90–95% of the granulocytes, they are the best investigated granulocyte population as far as effects of sex hormones are concerned. We will, therefore, here focus on these cells. Neutrophils are the first cells recruited from the bloodstream to sites of infection. They are terminally differentiated cells, incapable of cell division, and synthesize only very low levels of RNA and protein. Neutrophils are an essential component of the acute inflammatory response and the resolution of microbial infection.

A significant increase in granulocyte numbers was found during pregnancy and in the luteal phase as compared to the follicular phase of the normal ovarian cycle (Northern *et al.*, 1994; Apseloff *et al.*, 2000; Faas *et al.*, 2000; Bouman *et al.*, 2001b; Veenstra van Nieuwenhoven *et al.*, 2002). This suggests a role for progesterone and estrogen in increasing granulocyte numbers. This may be due to recruitment of new granulocytes from the bone marrow (Bain and England, 1975) as well as to delayed apoptosis (Molloy *et al.*, 2003). However, this does not explain the fact that male granulocyte count did not differ from females in their menstrual cycle (Yovel *et al.*, 2001; Bouman *et al.*, 2004b). The effects of synthetic hormones is not clear, since depending on the type of OCC used, OCC may or may not increase granulocyte numbers (Klinger *et al.*, 2000; Yovel *et al.*, 2001).

### *Granulocyte function*

The function of neutrophils is mainly phagocytosis. To be able to effectively phagocytose bacteria or other agents, the neutrophils need to be able to respond to chemotactic stimuli and produce factors, such as free radicals, in order to kill the phagocytosed cells. The effects of sex hormones on these functions have been investigated in a few studies. It has been shown that progesterone enhanced chemotactic activity of neutrophils, while estrogens decreased this activity (Miyagi *et al.*, 1992). The effects of progesterone and estrogen on free radical production by neutrophils have also been investigated by various groups. Neutrophil free radical production has been shown to be increased (Molloy *et al.*, 2003), decreased (Bekesi *et al.*, 2000) or not affected (Cassidy, 2003) by estrogen or progesterone incubations *in vitro*.

Other effects of estrogen and progesterone on neutrophils are the effects of these hormones on nitric oxide (NO) production via NO-synthase. NO production by neutrophils has been shown to have anti-inflammatory effects since it prevents neutrophil adhesion to the endothelium (Kubes *et al.*, 1991). It has been shown that *in vivo* NO-synthase in neutrophils varies with the reproductive condition, being highest in the presence of estrogen (Garcia-Duran *et al.*, 1999), which is in-line with the fact that *in vitro* estrogen can up-regulate NO-synthase expression in neutrophils (Garcia-Duran *et al.*, 1999; Stefano *et al.*, 1999).

In summary, although much more research is needed, it appears that both gender and reproductive condition affect neutrophil numbers and function. As far as neutrophil numbers are concerned the exact mechanism remains illusive. However, 17β-$E_2$ seems to have anti-inflammatory effects on neutrophils, while progesterone seems to have pro-inflammatory effects on these cells. Therefore, sex hormones can affect non-specific immune responses by modulating neutrophil numbers and function.

### *NK cell numbers*

Approximately 5% of the leukocytes are NK cells. Peripheral blood NK cells can be recognized by the fact that they express $CD16^+/CD56^-$ or $CD16^+/CD56^+$. NK cells are capable of killing virus-infected cells or tumour cells in the absence of prior immunization and without MHC restriction. They are able to lyse target cells by direct contact with them (in the absence of antibody) or by antibody dependent cellular cytotoxicity. Besides their role in early immunity against certain viruses, intracellular bacteria and parasites, the role of NK cells in human reproduction has been extensively investigated. NK cells in the endometrium, which is a specific subset of NK cells (i.e. $CD16^-/CD56^+$), play an important role in implantation of the blastocyst and in placentation (King, 2000). Because of their role in reproduction, it is important to investigate the effects of sex hormones on peripheral NK cells.

No difference could be demonstrated in NK cell count between males and females (post-menopausal or fertile) and OCC users (Baker *et al.*, 1985; Giglio *et al.*, 1994; Scanlan *et al.*, 1995; Giltay *et al.*, 2000; Yovel *et al.*, 2001). This suggests no effect of testosterone on NK cell count. However, other studies show an influence of female sex hormones upon NK cell number. Within the menstrual cycle peripheral blood NK cells increase in the late secretory phase of the menstrual cycle as compared with the late proliferative phase (Flynn *et al.*, 2000; Bouman *et al.*, 2001a; Yovel *et al.*, 2001), while NK cell count and percentage is decreased when administering estrogens plus anti-androgen to transsexual males (Giltay *et al.*, 2000). Also during pregnancy, the numbers of peripheral NK cells are decreased (Watanabe *et al.*, 1997; Kuhnert *et al.*, 1998; Veenstra van Nieuwenhoven *et al.*, 2002). Together, these data suggest that NK cell counts are decreased by estrogen.

### *NK cell function*

There are various reports on the effect of the reproductive condition and gender on the main function of NK cells, their ability to lyse other cells [NK cell activity (NKA)]. Higher NKA was found in post-menopausal women and in males as compared to females with a regular menstrual cycle and women on OCC (Souza *et al.*, 2001; Yovel *et al.*, 2001). This may suggest a suppression of NKA by progesterone or 17β-$E_2$. Accordingly, exposure to OCC showed a trend or significant reduction in NKA as compared to non-users (Baker *et al.*, 1985; Scanlan *et al.*, 1995; Yovel *et al.*, 2001). Also, *in vitro* it has been demonstrated that sex hormones affect NKA. However, this effect depends on the incubation time and dose; high dose and prolonged exposure to 17β-$E_2$ suppress NKA (Ferguson and McDonald, 1985), whereas low dose and short exposure to 17β-$E_2$ *in vitro* did not yield a significant effect (Sulke *et al.*, 1985a,b). *In vitro* no effect of progesterone on NK activity was demonstrated (Sulke *et al.*, 1985a,b; Uksila, 1985). Although, it appears that estrogen suppresses NKA, this is not always reflected in the menstrual cycle, since within the menstrual cycle results are inconclusive; varying from no difference as well as highest NKA in follicular phase, periovulatory phase or luteal phase (White *et al.*, 1982; Thyss *et al.*, 1984; Sulke *et al.*, 1985a,b; Souza *et al.*, 2001; Yovel *et al.*, 2001). Differences in results between these papers may be due to different time points in the ovarian cycle of measuring NKA.

Another function of NK cells is cytokine production. The cytokine repertoire of peripheral NK cells is mainly type 1 cytokines (IL-2, IFN-γ) (Biassoni *et al.*, 1991; Mendes *et al.*, 2000). Although there are many studies into cytokine production of uterine NK cells during pregnancy, surprisingly little is known about cytokine production by peripheral NK cells in relation to the reproductive condition or separate effects of sex hormones on peripheral NK cells. Although during pregnancy, the stimulated IFN-γ production of peripheral NK cells is decreased, no effect of the menstrual cycle upon IFN-γ production of NK cells was found (Bouman *et al.*, 2001a). It seems therefore likely that during pregnancy other mechanisms, rather than sex hormones affect NK cell IFN-γ production.

In conclusion, it seems likely that estrogen decreases NK cell numbers and NKA but, that sex hormones do not affect NK cell cytokine production. However, this remains to be confirmed in other reproductive phases and by *in vitro* experiments.

## Mechanisms of action of 17β-$E_2$, progesterone and testosterone on immune cells

The reaction of tissue/cells to sex hormones is a result from the binding of these hormones to their receptor. Due to their

lipophilic nature, steroid hormones can diffuse across the cell membrane; classical steroid hormone receptors can thus be found intracellularly, rather than on the cell membrane (Beato and Sanchez-Pacheco, 1996). Steroid binding to the intracellular steroid receptor results in translocation of this complex to the nucleus. The steroid/steroid receptor complex in the nucleus functions as a transcription factor, directly regulating expression of genes containing a binding site for this complex in the promoter region (Beato and Klung, 2000). The effects of steroid hormones upon cytokine production are suggested to be mediated by the nuclear factor-kB (NF-kB). This is an inducible transcription factor that positively regulates the expression of proimmune and pro-inflammatory genes (McKay and Cidlowski, 1999). It has been shown that the steroid/receptor complex can physically interact with NF-kB and inhibits its transactivational activity (McKay and Cidlowski, 1999). Via this mechanism estrogens, progesterone and testosterone can inhibit pro-inflammatory cytokine expression in immune cells expressing the respective receptor. The mechanism by which steroid binding with membrane receptors, such as described for estrogen and testosterone, affect immune cell function remains obscure.

### *The estrogen receptor*

Two estrogen receptors (ERs) have been identified, designated as ER-alpha and ER-beta (Kuiper *et al.*, 1996; Mosselman *et al.*, 1996). The same estrogen binding to the alpha or beta receptors can produce opposite effects in the same system (Paech *et al.*, 1997). In the lymphocyte population, ERs were only found in T cells of the suppressor/cytotoxic subset while helper lymphocytes showed no significant steroid binding, suggesting the absence of receptors for steroid hormones (Cohen *et al.*, 1983; Stimson, 1988). On the other hand, mRNA for ERs appeared to be present in both T lymphocyte populations (Suenaga *et al.*, 1998). In helper lymphocytes, this is apparently not translated to the receptor itself. Also B lymphocytes express ERs (Suenaga *et al.*, 1998; Benten *et al.*, 2002).

It has been known for many years that classical intracellular ERs are present in monocytes (Weusten *et al.*, 1986; Wada *et al.*, 1992; Ben Hur *et al.*, 1995; White *et al.*, 1995; Suenaga *et al.*, 1996, 1998). Recently, however, the expression of either ER-alpha or ER-beta and their response to estrogen in monocytes was found to be dependent on the their stage of cell differentiation, i.e. monocytes expressing ER-beta and macrophages expressing ER-alpha (Mor *et al.*, 2003). Although, very little is known about whether the expression of the ERs vary between sexes or reproductive phases, it has been shown that a significant decrease occurs in the percentage of ER-positive monocytes during menopause (Ben Hur *et al.*, 1995). There are also data emerging that demonstrate an estrogen surface receptor on monocytes (Stefano *et al.*, 1999; Stefano and Peter, 2001).

For neutrophils it has been shown that they possess both ER-alpha and ER-beta receptors (Molero *et al.*, 2002). Although there are no data available on the existence of ERs in human peripheral NK cells, murine peripheral NK cells express both the ER-alpha and ER-beta receptors (Curran *et al.*, 2001).

### *The progesterone receptor*

There is no evidence for progesterone receptors mRNA or progesterone receptor expression on resting lymphocytes (Szekeres-Bartho *et al.*, 1989a,b; Mansour *et al.*, 1994; Szekeres-Bartho, 1995; Schust *et al.*, 1996; Vegeto *et al.*, 1999, 2002). However, various studies demonstrated a pregnancy-induced appearance of progesterone binding sites in peripheral blood lymphocytes (Szekeres-Bartho *et al.*, 1989a,b; Szekeres-Bartho, 1995, 2002; Barakonyi *et al.*, 1999; Polgar *et al.*, 1999). This suggests that activated lymphocytes do express progesterone receptors and that once activated progesterone may affect lymphocyte function via binding to its receptor. It has been suggested that during pregnancy in response to binding to its receptor lymphocytes produce progesterone-induced blocking factors (PIBF) (Szekeres-Bartho *et al.*, 2001). This PIBF may induce a Th2 biased immune response, and may control NK activity, thereby exerting anti-abortive effects.

For monocytes (Schust *et al.*, 1996), neutrophils (Aerts *et al.*, 2002), NK cells and B lymphocytes, there is no evidence for the presence of progesterone receptors.

### *The androgen receptors*

Although in the past T lymphocytes were considered to be unresponsive to testosterone due to the absence of androgen receptors (Cohen *et al.*, 1983; Rife *et al.*, 1990), recent studies have demonstrated a membrane testosterone receptor on the lymphocyte, which is not identical to the classical intracellular testosterone receptor (Benten *et al.*, 1999). B lymphocytes do express the intracellular androgen receptor (Benten *et al.*, 2002). In literature we only found one study reporting the existence of androgen receptor expression in murine macrophages (Bebo *et al.*, 1999). There are no reports about the presence of androgen receptors on human monocytes, neutrophils or NK cells

### *Other mechanisms*

Although effects of progesterone, 17β-$E_2$ and testosterone upon immune cells have been shown, it appears that testosterone receptors are lacking on these immune cells, while progesterone receptors and ERs are not found on all immune cells. Therefore, it seems likely that other mechanisms must be present by which steroid hormones exert their effects on immune cells. It may be suggested that progesterone could exert its actions on immune cells by binding to the glucocorticoid receptor (Kontula *et al.*, 1981). This has been disputed by others (Lamche *et al.*, 1990; Schust *et al.*, 1996). An alternative explanation is that by their lipophilic nature, sex steroids can integrate into the membrane and alter membrane properties, such as fluidity, and thereby changing the function of the immune cells (Lamche *et al.*, 1990). Further studies into the mechanisms of action of steroid hormones upon immune cells are necessary.

## Concluding remarks

Studies on the relation between the immune system and reproduction/reproductive factors are important for several reasons. First of all, immune responses regulate various reproductive processes, so that deviations from normal immune responses may

interfere with fertility. Secondly, immune responses vary with gender and the reproductive phase, suggesting that factors associated with reproduction regulate immune responses. This was the focus of the present review. Available evidence from animal studies suggests that sex hormones regulate immune responses *in vivo* (as reviewed by Ansar *et al.*, 1985). In the present review we focussed on the effects of sex hormones on human immune cells, since they are the major parts of the immune system, and it seems likely that the sex hormones exert their effects on these cells.

In the past, the effects of gender and reproductive phase upon the specific immune response have gained much more attention than the effects on the non-specific immune response. At present, evidence points towards a role for estrogens and testosterone in (auto)antibody production; estrogen increases, while testosterone decreases antibody production. However, no effects or inconclusive effects of sex hormones were found as far as effects of sex hormones on lymphocyte cytokine production are concerned. The present review indicates that the effects of gender and the reproductive condition on the non-specific immune response may be more clear. This is not surprising, since it is the non-specific immune response that is involved in various reproductive processes, such as ovulation and menstruation. It is therefore much more important for the ovaries to regulate the non-specific immune response then the specific immune response.

It is clear that the ovaries regulate non-specific immune responses, by affecting monocyte, granulocyte and NK cell numbers, by an-as-yet unknown mechanism, but also by affecting the function of these cells. For neutrophils and NK cells it has been shown that estrogens exert anti-inflammatory effects. Progesterone, on the other hand has been shown to exert pro-inflammatory effects on neutrophils. It has also been shown that the ovaries affect monocyte function, i.e. monocyte cytokine production; whether this is due to effects of sex steroids remains unclear. Monocyte cytokine production (TNFα and IL-1β) was shown to be increased during the luteal phase as compared with the follicular phase (Bouman *et al.*, 2001b). However, other studies, including recent studies from our research group, have shown that this is most likely not due to increased sex steroid concentrations during the luteal phase: monocyte cytokine production in OCC use (in stop week and during pill intake) (Bouman *et al.*, 2004a), in males (Bouman *et al.*, 2004b; Posma *et al.*, 2004) and post-menopausal women (preliminary results from our lab) was similar to monocyte cytokine production in the luteal phase. This may suggest that neither progesterone nor 17β-$E_2$ increase monocyte cytokine production, but that other factors, specifically factors produced during the follicular phase, decrease monocyte cytokine production. Our present research is directed towards finding these anti-inflammatory factors produced during the follicular phase.

This review also shows that the data on the effects of sex hormones on the various immune cells are conflicting. Conflicting results may be due to handling of the cells for *in vitro* research, which may result in changes in expression of various receptors or in priming of the cells (Macey *et al.*, 1995; Sacks *et al.*, 1997), especially for monocytes and granulocytes. Moreover, *in vivo* effects of sex hormones may not be due to direct effects of the sex hormones on the immune cells and similar results may therefore not be found in the *in vitro* situation. On the other hand, conflicting results can also (partly) be explained by different experimental methods used: the use of whole blood assays versus isolated peripheral blood monocytes, since isolation per se may affect the leukocytes (Macey *et al.*, 1995), while the stimulation of the cells takes place in a setting, which is very different from the *in vivo* environment. Also measurement of cytokine production versus measurement of percentage of cytokine producing cells may be a reason for conflicting results. Use of different stimuli (polyclonal activators, such as phorbol myristate acetate and calcium ionophore and phytohaemagglutinin (PHA), versus activation of T cells with specific antigens), which activate cells via different pathways may result in different results. Another reason for varying results between various *in vivo* experiments may be the timing of the blood samples. For instance the timing of the blood samples in the menstrual cycle (and pregnancy) may be very important, since hormone concentration fluctuate on a daily basis; leukocytes from the mid follicular phase may respond differently then leukocytes from early or late follicular phase.

Thus in starting to unravel the mechanism by which the reproductive condition and sex hormones regulate immune responses, we should not only standardize our experiments, but we should also direct our focus towards other factors, such as the anti-inflammatory factors produced during the follicular phase. Moreover, leukocytes are not the only cells involved in immune responses. Also endothelial cells and thrombocytes play a role in immune responses, especially in the non-specific immune responses. Unfortunately, in the literature, there are no studies investigating the effects of sex hormones on these cells as far as immune function is concerned. However, it is well-known from other research disciplines, such as research into vascular tone, that ERs are present in endothelial cells and it has been shown that both estrogen and progesterone promote endothelium dependent vasodilatation (Orshal and Khalil, 2004). Finally, not only immune cells, but also a lot of other cells in the body produce cytokines, for instance trophoblast cells (Griesinger *et al.*, 2001; Sacks *et al.*, 2001), and endometrial stromal and epithelial cells (Fukuda *et al.*, 2003). It has been shown that cytokine production by these cells can be under hormonal control (Laird *et al.*, 1996; Okada *et al.*, 2000; Wira and Rossoll, 2003). Whether this cytokine production exerts only paracrine or also endocrine effects needs to be established.

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