Cytokines and immuno-endocrine factors in recurrent miscarriage

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TABLE OF CONTENTS

Introduction 469
Endocrinology 469
Immunology 470
Cytokines 472
Cytokines in endocrine aetiologies of recurrent miscarriage 473
Cytokine production and idiopathic recurrent miscarriage 476
Conclusions 478
References 478

Introduction

Recurrent miscarriage remains an enigma. The main aetiologies are endocrinological, immunological and unexplained. With the growth in molecular biology, it is now possible to look at the effect of these aetiologies in more detail, allowing greater understanding of the underlying pathogenesis.

Key words: cytokines/recurrent miscarriage

Endocrinology

Abnormal systemic endocrine disorders have been suggested as being associated with recurrent miscarriages, including insulin-dependent diabetes mellitus (Coulam and Stern, 1994), thyroid disorders (Moghissi, 1982; Glinoer et al., 1991), luteal phase defects (Lee, 1987) and hypersecretion of luteinizing hormone (LH) with polycystic ovaries (Regan et al., 1990).

Few studies have examined specifically the role of diabetes mellitus in recurrent miscarriage, although there have been studies on the relationship of diabetes mellitus and sporadic spontaneous miscarriage. A review of more than 50 such studies from 1950 to 1986 found no correlation between spontaneous miscarriage and pre-conceptual or gestational diabetes (Kalter, 1987). However, other authors have shown a significant increase in the rate of spontaneous miscarriage in pregnant women with poorly controlled insulin-dependent diabetes mellitus, who had a miscarriage rate of 45% in comparison with 15% in pregnant diabetic women with good control (Miodovnik et al., 1984). This negative effect of poorly controlled diabetes on pregnancy
The diagnosis of luteal phase defect is made by treatment, achieve successful pregnancies (Crosignani, 1988). Thyroid disorder as a cause of recurrent miscarriages is also controversial. The suggestion that hypothyroidism is contributory to recurrent miscarriage is derived from studies in the 1950s and 1960s. A recent study of 219 women with recurrent miscarriage failed to detect the presence of any thyroid disease (Tho et al., 1979). However, there is some evidence that thyroid autoimmunity may be associated with recurrent miscarriage. Antithyroid antibodies have been suggested to be a predictor of pregnancy loss in randomly chosen obstetric populations since their presence had been observed to be correlated with a higher rate of spontaneous miscarriage (Stagnaro-Green et al., 1990; Kilpatrick and Liston, 1995). This effect is thought to be related to abnormally activated autoimmunity rather than to overt thyroid endocrine dysfunction. The value of routine testing of thyroid function in asymptomatic women with recurrent miscarriage is questionable (Clifford et al., 1994).

The term ‘luteal phase defect’ refers to the functional inadequacy of the corpus luteum to produce appropriate amounts of progesterone. This results in inadequate endometrial maturation and probable functional defects in the early maintenance of the implanting embryo (Crosignani, 1988; Serle et al., 1994). An adequately functional corpus luteum is necessary for implantation and early growth of the embryo. Removal of the corpus luteum prior to week 7 of gestation results in induction of abortion in most cases. The significance of progesterone is illustrated in women without functional ovaries undergoing oocyte donation who, after receiving oestrogen and progesterone treatment, achieve successful pregnancies (Crosignani, 1988). The diagnosis of luteal phase defect is made by mid-cycle LH-timed biopsy (McNeely and Soules, 1989; Serle et al., 1994), dating the endometrium according to Noyes’ criteria (Noyes et al., 1950) and correlating the result to LH timing. The endometrial morphology allows dating according to the functional status, and assesses the maturation status of the secretory glands, stromal and vascular proliferation and morphology of the endometrial epithelial cells. If there is a maturation delay of two or more days compared with the chronological dating of the endometrium from the mid-cycle LH surge, it would be considered indicative of luteal phase defect (Lee, 1987; Coulam and Stern, 1994). A recent controlled study investigated the incidence of luteal phase defect using Noyes’ criteria for endometrial morphology dating and correlation to mid-cycle LH surge chronological timing. Specimens were taken on day LH + 7 for histological analysis from 25 women with unexplained recurrent miscarriage and 14 control women; 15 of the women with recurrent miscarriage (60%) and none from the control group had a retarded endometrium/luteal phase defect (Serle et al., 1994).

LH hypersecretion in women with polycystic ovaries as measured by plasma LH (>10 IU/l) or early morning urine LH (Watson et al., 1993) has been implicated in recurrent miscarriages. The exact reason why this should be so is unknown, although various mechanisms have been proposed. One hypothesis is that a raised follicular phase LH causes premature resumption of meiosis in the oocyte (by antagonizing the action of oocyte maturation inhibitor), resulting in premature maturation of the oocyte at ovulation (Jacobs, 1991). Further supporting evidence from a study of polycystic ovary patients acting as oocyte donors demonstrated a decreased implantation rate when LH hypersecretion had not been suppressed compared to when it was suppressed with gonadotrophin-releasing hormone analogues (Ashkenazi et al., 1995). It is also probable that the high serum LH acts on the theca cells of the ovarian stroma to increase production of androgens which may be responsible for pregnancy loss (Watson et al., 1993; Tulpalla et al., 1993).

Furthermore, high LH may exert a direct effect on the endometrium, causing an endometrial defect that results in suboptimal implantation. The presence of LH receptors in the endometrium (Reshef et al., 1990) and the observation that women with polycystic ovaries have altered endometrial function (Bonney et al., 1992) support this view. However, there is evidence to the contrary in patients with unexplained infertility and premature ovarian failure, who had high LH but demonstrated no adverse effects of a high LH on endometrial development (Li et al., 1993).

Immunology

Immunological factors in recurrent miscarriage can be divided into two groups, autoimmune and alloimmune. Autoimmunity refers to the formation of antibodies against self, cellular or subcellular components. The antiphospholipid antibodies [anticardiolipin (ACA) and lupus anticoagulant (LA)] with or without systemic lupus erythematosus are recognized autoimmune immunological abnormalities associated with recurrent miscarriages (Lubbe et al., 1984; Lockshin et al., 1985; Gatenby, 1989). These antibodies are also associated with an increased risk of intrauterine growth retardation, pre-eclampsia and preterm delivery (Branch et al., 1990a, 1992).
Table I. The aetiology and prevalence of recurrent miscarriage

<table>
<thead>
<tr>
<th>Aetiology</th>
<th>Type of defect</th>
<th>Prevalence</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetic</td>
<td>aneuploidy, translocations, mosaics, single gene abnormality</td>
<td>6%</td>
<td>Coulam, 1986; Hatasaka, 1994</td>
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<tr>
<td>Anatomical uterine defects</td>
<td>unicornuate, bicornuate, didelphys, septate uterus, Asherman's syndrome (intrauterine synechiae), submucous fibroids</td>
<td>6–10%</td>
<td>Coulam, 1986; Hatasaka, 1994; Patton, 1994</td>
</tr>
<tr>
<td>Infective</td>
<td>AIDS (acquired immuno-deficiency syndrome)</td>
<td>&lt;1%</td>
<td>Summers, 1994</td>
</tr>
<tr>
<td>Endocrine</td>
<td>luteal phase defects (LPD), luteinizing hormone (LH) hypersecretion, thyroid dysfunction, diabetes mellitus</td>
<td>8–29%</td>
<td>Coulam, 1986; Hatasaka, 1994; Coulam and Stern, 1994</td>
</tr>
<tr>
<td>Immunological</td>
<td>autoimmune</td>
<td>1–40%</td>
<td>Lubbe et al., 1984; Lockshin et al., 1985; Out et al., 1991; Silver et al., 1994</td>
</tr>
<tr>
<td>Idiopathic</td>
<td>unknown</td>
<td>15–79%</td>
<td>Coulam, 1986; Hatasaka, 1994</td>
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Table II. General properties of cytokines

<table>
<thead>
<tr>
<th>Physiological roles of cytokines in multicellular organisms</th>
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<tr>
<td>Control of cell proliferation</td>
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<td>Control of cell differentiation and phenotype</td>
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<tr>
<td>Control of cytotoxic and phagocytic cells</td>
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<tr>
<td>Regulation of immune responses</td>
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<td>Regulation of haematopoiesis</td>
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<tr>
<td>Regulation of inflammatory response and pyrexia</td>
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<tr>
<td>Involvement in endocrine system modulation</td>
</tr>
<tr>
<td>Wound healing</td>
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<tr>
<td>Tissue remodelling and bone formation</td>
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<td>Influences on cellular metabolism</td>
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</tbody>
</table>

The mechanism by which antiphospholipid antibodies cause recurrent miscarriage is unknown but thought to be due to a thrombotic tendency resulting in decidual vasculopathy and placental infarction (Nilsson et al., 1975; Lubbe et al., 1984; Lockshin et al., 1985; Out et al., 1991; Silver et al., 1994). It is possible that a direct immune mechanism may be involved. Phospholipids are functional in cell adhesion, and deficiency caused by anti-phospholipid antibodies may lead to a breakdown in the cellular adhesion mechanism of the maternal–conceptus interface, allowing increased exposure of fetal cells to the maternal circulation and easier invasion of the conceptus by the maternal immune system (Branch et al., 1990b).

Alloimmunity refers to the existence of immunological differences among individuals of the same species. The concept of alloimmunity arises from the observation by Medawar (1953) that the feto–placental unit is a semi-allograft due to the paternal genetic contribution. This suggests that the fetus is immunologically incompatible and should be rejected by the maternal immune system. However, this does not occur in normal pregnancy.

It can be postulated that in recurrent miscarriage there may be a derangement or imbalance of alloimmune factors. The frequency of alloimmune factors as a cause of recurrent miscarriage varies from 1–40% (Coulam, 1986; Hatasaka, 1994). This variation arises because there are no good, reliable tests or investigations enabling the accurate diagnosis of alloimmune factors, giving rise to controversy about the validity of alloimmune factors as a cause of recurrent miscarriage. Coulam (1986) included alloimmunity in the 40% frequency of immune factors responsible for recurrent miscarriage but Hatasaka (1994) in his study did not, resulting in a decrease to 1% of the frequency of immune factors.

Various mechanisms have been postulated for the role of alloimmunity in recurrent miscarriage. These include: (i) sharing of human leukocyte antigen (HLA); (ii) maternal antifetal blocking antibody deficiency; (iii) immune mediators and suppressor cells mechanism.

HLA antigens are found in all humans, located on the short arm of chromosome 6 as part of the major histocompatibility complex (MHC) antigens. The MHC antigens are divided into three classes, I, II and III, distinguished by differences in their expression and interaction with cells of the immune system, in particular T cells. Class I genes include HLA A, B and C, class II genes comprise HLA DP, DQ and DR, and class III genes code for complement and also certain cytokines. It was thought that parental HLA heterozygosity was important for successful reproduction, but studies have failed to confirm the benefit of genetic heterogeneity in maintaining pregnancy (Balasch et al., 1989; Coulam, 1992).

There are two possible mechanisms by which the mother may become sensitized against fetal antigens, either via transplacental fetal cell leakage into the maternal circulation or through abnormal induction of MHC class I.
expression on trophoblasts by cytokines (Feinman et al., 1987). It has been proposed that certain substances known as blocking factors (antibodies) produced by the mother are necessary for pregnancy to progress, although humans who are incapable of generating normal antibody responses still manage to reproduce normally (Rodger, 1985). This hypothesis presumes that an antifetal cell-mediated immune response would cause all pregnancies to abort if the response was not modified. The blocking factors (antibodies) camouflage fetal-trophoblast antigens or cover maternal lymphocyte receptors to interfere with the maternal antifetal cell-mediated immune response.

Although these factors/antibodies have been identified, absence of these antibodies lacks a predictive value in identifying pregnancy failure (Neppert et al., 1989; Coulam, 1992). There are also technical difficulties in interpreting the results of assays for the antibodies. It is hard to ascertain their presence or absence since detection relies technically on several indirect reactions (Park et al., 1990).

A wide variety of immune cells and soluble mediators has been found in the endometrial decidua. The population of the immune cells in decidua of women subject to recurrent miscarriages has shown a lack/deficiency of suppressor lymphocytes (Daya et al., 1985). In normal endometrial biopsies, T helper cells outnumber T suppressor cells throughout the menstrual cycle, except for the late secretory phase and onset of menstruation, when T suppressor cells predominate. This ratio of T helper to T suppressor cells in the late secretory phase is increased in endometrial biopsies from women with idiopathic recurrent miscarriages, in comparison with women who had other known causes of recurrent miscarriage (Hill and Anderson, 1988, 1990). Macrophage activation and function, which are normally suppressed in pregnancy, have been observed to be enhanced in decidua of women with spontaneous miscarriage, compared with those having elective termination (Hill, 1989). Decidual suppressor cell deficiency has been observed in biopsy specimens from women with failing biochemical pregnancies after in-vitro fertilization (IVF) and embryo transfer (Nabel, 1989) and has been postulated to be involved in recurrent miscarriages (Daya et al., 1985; Clark et al., 1986). It has been reported that leukocytes of women with idiopathic recurrent miscarriages produce cytotoxic factors in response to trophoblast antigen stimulation and this may be the reason why there is reproductive failure (Hill et al., 1992; Yamoda et al., 1994). A further study by Hill et al. (1995) showed the same cytotoxic effect but, in addition, showed derangements of certain cytokines in the serum of those who had embryotoxicity. This suggests a derangement of the immune response in recurrent miscarriages, specifically an imbalance between the cell-mediated and humoral response (Hill et al., 1995).

Cytokines

The immune system discriminates between self and non-self, attacking and rejecting non-self foreign antigens/immunogens. There are two levels of immune response, innate and adaptive (acquired) immunity. Innate immunity is present from birth and is non-specific, forming a first line defence against immunogens. The cells involved are primarily leukocytes and natural killer (NK) cells. Adaptive immunity is a specific response, forming the second line response to antigens. The cells involved in this are the lymphocytes (T and B lymphocytes) and macrophages-monocytes. The adaptive immune response consists of cell-mediated and humoral immunity. The cells involved in cell-mediated immune response are T cytotoxic and T helper 1 (TH-1) cells whereas those involved in humoral immune response are B and T helper 2 (TH-2) cells.

T helper cells play an important role in deciding the predominant nature of an immune response. If there is a predominance of TH-1 cells then the immune response will favour a cytotoxic or cell mediated response, whereas a predominance of TH-2 cells would lead to a predominantly humoral or antibody-mediated response. The differentiation of naïve T cells into either TH-1 or TH-2 cell is dependent on the presence of cellular factors known as cytokines.

Cytokines are polypeptides involved in the control of local and systemic events of the immune response, inflammatory reactions, healing and haematopoiesis. They are produced mainly by cells of the immune system, although they are increasingly found to be produced by virtually every nucleated cell type in the body. The general properties are shown in Table II.

Cytokines are pleiotropic, expressing features of ‘redundancy’ and ‘overlap’. The effects of each polypeptide are not exclusive but may be produced by others and have overlapping functions rather than a distinct function. Currently identified cytokines include the interleukin family (IL-1 to IL-17), tumour necrosis factor (TNF-α, β), colony stimulating factors (M-CSF, G-CSF, GM-CSF), transforming growth factors (TGF-α, β) and the interferon family (IFN). There is now increasing evidence that cytokines are also involved as autocrine, paracrine and endocrine factors to modulate cell functions ranging from proliferation and differentiation to metabolic effects on a variety of cell types (Imura et al., 1991).
Within the field of reproduction, there is emerging evidence that cytokines play an increasingly important role in the regulation of hormonal effects, acting as the cellular mediators as well as the cellular link in the interaction between the endocrine and immune systems. There is evidence of this in the hypothalamic–pituitary system (Bateman et al., 1989; Kalra et al., 1990), the ovary (Adashi, 1990; Andreani et al.; 1991; Hurwitz et al., 1991) and the endometrium (Romero et al., 1989; Tabibzadeh, 1990, 1991a; Kauma et al., 1990; Tabibzadeh and Sun, 1992). In these areas, cytokines are not produced mainly by the immune cells but by other cells including the epithelial, endothelial and mesenchymal cells of the endometrium.

Cytokines in endocrine aetiologies of recurrent miscarriage

Hypothalamus, pituitary and ovary

Hypersecretion of LH has been shown to be an aetiological factor in recurrent miscarriages (Regan et al., 1990; Reshef et al., 1990; Jacobs, 1991; Bonney et al., 1992; Tulpalla et al., 1993; Watson et al., 1993). The mechanism of hypersecretion of LH and its mode of action in recurrent miscarriage is unknown, although it may involve abnormal cytokine secretion. Cytokines may be directly or indirectly involved, via the hypothalamus, in modulating the secretion of LH from the pituitary, as well as modulating the LH effect on the ovarian functions of steroidogenesis, maturation and ovulation.

In the animal model, IL-1 inhibits the release of hypothalamic luteinizing hormone-releasing hormone (LH-RH) (Kalra et al., 1990), while TGF-α increases the release of LH-RH (Ojeda et al., 1990). Pituitary cell function is also influenced by cytokines. These cells express cytokine receptors for IL-1, IL-2 and IL-6 (Marquette et al., 1990). The secretion of pituitary hormones including growth hormone (GH), prolactin (PRL), thyroid stimulating hormone (TSH) LH and follicle stimulating hormone (FSH) are under the influence of cytokines such as IL-1α and β, IL-6, TNF-α and IFN-γ (Scarborough, 1990). IL-1 inhibits production of serum prolactin and the ovarian steroid-induced LH surge (Kalra et al., 1990) while causing a significant release of adreno-corticotrophic hormone (ACTH), GH, FSH and TSH (Marquette et al., 1990; Yamaguchi et al., 1990a).

In humans as well as animal models, IL-6 is produced by anterior pituitary cells, functioning as an intrapituitary releasing factor. It controls the production, secretion and release of FSH, LH and PRL (Marquette et al., 1990; Yamaguchi et al., 1990a). Animal model in-vitro studies have shown a rapid release of LH, PRL and ACTH when cells are perfused with TNF-α (Yamaguchi et al., 1990b). The synthesis of TNF-α by macrophages is inhibited by hypophysectomy, which can be overcome by administration of GH or IFN-γ (Edwards et al., 1991). It is clear that cytokines interact with the secretion of pituitary hormones, producing a knock-on effect on the functions of the ovary and endometrium.

There is evidence in the animal model that IL-1 may play an important role in ovulation by serving as a local mediator for LH action (Adashi, 1990; Brannstrom et al., 1994). IL-6 is also involved in the modulation of steroidogenesis in the ovary. It has no significant effect on progesterone production by granulosa cells but it inhibits, in a dose-dependent fashion, FSH-stimulated progesterone production by these cells. In turn, FSH stimulates the release of IL-6 by granulosa cells (Gorospe et al., 1992). It has been shown that prior to and at the time of ovulation in the animal model, concentrations of IL-6 are increased (Robertson et al., 1992), suggesting its role in the molecular mechanism of ovulation. TGF-β is present in rat granulosa cells and is secreted by theca cells (Skinner et al., 1987; Bendell and Dorrington, 1988; Thompson et al., 1989), enhancing FSH induction of LH receptors as well as increasing the FSH stimulated secretion of oestradiol, progesterland and inhibin (Adashi and Resnick, 1986; Dorrington et al., 1988; Mulheron and Schomberg, 1990).

Endometrium

Luteal phase defects have been shown to be a contributory factor in recurrent miscarriage, although the exact mechanism of endometrial retardation resulting in recurrent miscarriage is unknown. LH hypersecretion is thought to exert a direct adverse effect on endometrial function, supported by the finding of LH receptors in the endometrium (Reshef et al., 1990; Bonney et al., 1992). However, the exact mechanism is again unknown, although there is some suggestion that cytokines may be involved. The endometrium can be divided into epithelial and stromal cell layers. These cells are hormonally responsive, express steroid receptors, produce cytokines and express cytokine receptors (Tabibzadeh, 1991a; Tabibzadeh and Sun, 1992).

Interleukin-1

IL-1 consists of two distinct but related molecules, termed IL-1α and IL-1β, which bind to the same receptor and mediate similar actions (Dinarello, 1986; Kilian et al., 1986; Lomedico et al., 1986; Oppenheim et al., 1986; Dower and Urdal, 1987; Mizel et al., 1987). The genes for human IL-1α and IL-1β are located on the long arm of chromosome 2 (Webb et al., 1986; Lagage et al., 1987).
IL-1 is primarily produced by peripheral blood mononuclear cells (Dinarello, 1991), which act mainly as inflammatory mediators, inducing hypotension, shock and fever (Smith et al., 1992).

IL-1α and β have been demonstrated to be present in the late secretory phase of the human menstrual cycle (Kauma et al., 1990). IL-1β has been shown to be present in the epithelium and stromal cells throughout the menstrual cycle, with progressive expression from the proliferative to the secretory phase of the menstrual cycle (Simón et al., 1993). The endometrium also expresses IL-1β receptor throughout the menstrual cycle, again increasing as the cycle progresses (Simón et al., 1993). Cultured decidual cells secrete IL-1 (Romero et al., 1989) and it has been implicated in human embryo implantation (Simón et al., 1994a). IL-1 is present in human implantation sites, the villous trophoblast, maternal–trophoblast interface and maternal decidua/endometrial glands (Simón et al., 1994b). Together with previous evidence of IL-1 in implantation, this suggests that IL-1 has a definite role in implantation (Kauma et al., 1990; Simón et al., 1993). Furthermore, in animal models, the blockade of IL-1 receptors by antagonist during the period before implantation, results in inhibition and prevention of embryonic implantation (Simón et al., 1994a). It has been shown in humans that cytotrophoblast is the main producer of IL-1 during the first trimester of pregnancy, and that the concentration is proportional to the degree of trophoblast invasion (Paulesu et al., 1991; Simón et al., 1995) (Figure 1).

**Interleukin-6**

Human IL-6 is a 21–28 kDa glycoprotein (Hirano et al., 1986) with a 68% homology with rat IL-6. Human IL-6 is mapped to chromosome 7 (Bowcock et al., 1988) and has structural similarity to growth hormone, prolactin and erythropoietin, suggesting an evolutionary relationship between the molecules involved in the immune and hormone systems (Bazan, 1991). The IL-6 receptor consists of two molecules, α and β (Taga et al., 1989; Hibi et al., 1990). Production of IL-6 is mainly by T lymphocytes, macrophages and monocytes.

IL-1β induces the synthesis and release of IL-6 by human endometrial stromal cells (Tabibzadeh et al., 1989). IL-6 is expressed mainly in the epithelial layer (Tabibzadeh and Sun, 1992), under the regulation of oestrogen and progesterone (Jacobs et al., 1992). One could speculate that IL-6, among others, may modulate the interaction between cytokines and gonadal steroid hormones in the proposed feedback of the cytokine-steroid loop within the endometrium. The addition of oestrogen and progesterone...
to human endometrial epithelial cells in the proliferative and early secretory phase of the menstrual cycle results in an increased IL-6 production (Tabibzadeh, 1994). However, if the cells are in the late secretory phase, IL-6 production is inhibited (Laird et al., 1993). The same effect occurs in human endometrial stromal cells, as production was down-regulated by physiological concentrations of oestrogen (Laird et al., 1993). Within the lymphoid cells, oestrogen had a similar effect but progesterone and testosterone had no effect on IL-6 production (Li et al., 1992). Human trophoblasts express IL-6R and produce IL-6 which induces the production of human chorionic gonadotrophin (HCG) in an autocrine manner (Nishino et al., 1992). Human trophoblasts express IL-6R and produce IL-6 which induces the production of human chorionic gonadotrophin (HCG) in an autocrine manner (Nishino et al., 1992). Human trophoblasts express IL-6R and produce IL-6 which induces the production of human chorionic gonadotrophin (HCG) in an autocrine manner (Nishino et al., 1992). Human trophoblasts express IL-6R and produce IL-6 which induces the production of human chorionic gonadotrophin (HCG) in an autocrine manner (Nishino et al., 1992). Human trophoblasts express IL-6R and produce IL-6 which induces the production of human chorionic gonadotrophin (HCG) in an autocrine manner (Nishino et al., 1992). Human trophoblasts express IL-6R and produce IL-6 which induces the production of human chorionic gonadotrophin (HCG) in an autocrine manner (Nishino et al., 1992).

**Tumour necrosis factor-α and β**

TNF-α is a 17 kDa polypeptide (Camussi et al., 1991) also known as cachectin. TNF-β is a 25 kDa polypeptide homotrimer (Schoenfeld et al., 1991). The gene for TNF-α and β is located on chromosome 6 (Nedwin et al., 1985a,b; Wang et al., 1985). The main sources of TNF-α and β production are macrophages/monocytes, T and B lymphocytes (Ratner et al., 1987; Kronke et al., 1988; Tschachler et al., 1989; Tracey and Lowry, 1990). The functions of TNF-α are primarily pro-inflammatory, resulting in fever, anorexia, shock, chemotaxis, increased capillary permeability, mediation of IL-2 activity, enhanced natural killer (NK) cell function as well as activation of cell mediated cytotoxicity (Tracey and Lowry, 1990). TNF-β mediates cytotoxic T cell functions, inducing apoptosis, cell-mediated killing and graft rejections or manifestations of graft versus host reactions (Schmid et al., 1987).

TNF-α and its mRNA are expressed by epithelial, stromal and lymphoid cells of the human endometrium (Tabibzadeh, 1991b; Hunt et al., 1992). The concentration of TNF-α within the endometrium shows variation throughout the menstrual cycle, with progressive increase towards the late secretory phase (Philippaues and Piguet, 1993). This coincides with the fall in oestrogen values and rise in progesterone values. Indeed, TNF-α release by human blood mononuclear cells in vivo is inhibited by the administration of 17β-oestradiol (Ralston et al., 1990; Loy et al., 1992). An inverse relationship exists between progesterone and TNF-α, as TNF-α expression increases with a decrease in the concentration of progesterone (Loy et al., 1992). In humans, TNF-α is also known to inhibit trophoblast proliferation which may be of clinical importance in implantation and early pregnancy when trophoblast invades the endometrium (Eades et al., 1988; Jaattela et al., 1988; Hunt et al., 1990) (Figure 1).

**Interferon-γ**

IFN-γ was originally defined for its anti-viral activity and is differentiated from other interferons by its sensitivity to extreme temperature and pH. IFN-γ is a homodimer of 166 amino acid residues, with two sites for N-linked glycosylation. The gene for IFN-γ is found on chromosome 12 (Gray and Goeddel, 1982; Devos et al., 1982). Its receptor consists of an extracellular and intracellular domain, and is found on chromosome 21 (Gibbs et al., 1991). IFN-γ is produced primarily by activated TH-1, T cytotoxic/suppressor and NK cells (Pestka and Langer, 1987) involved in the cell-mediated immune response (Mosmann et al., 1986). IFN-γ inhibits TH-2 activity (Gajewski and Fitch, 1988) and hence the humoral immune response.

IFN-γ receptor expression has been localized to the human endometrial epithelium consistently throughout the menstrual cycle (Tabibzadeh, 1990). Oestrogen markedly increases the activity of the IFN-γ promoter in lymphoid cells. Short-term exposure of murine splenic cells to oestrogen increased the IFN-γ mRNA expression (Tabibzadeh and Satyaswaroop, 1989; Fox et al., 1991). In addition to its direct effects on endometrial cell function, it is postulated that oestrogen may act indirectly via lymphoid cells, increasing IFN-γ production to further modulate endometrial cell function. However, any interaction between IFN-γ and steroid hormones such as oestrogen is intricate and involves various feedback mechanisms, the exact nature of which is still to be fully elucidated. It has been shown in a human breast carcinoma cell line that IFN-γ induces a 30–50% increase in oestrogen receptor expression (Solary et al., 1991).

**Transforming growth factor-β**

TGF-β is a member of the family of growth factor cytokines, with five different isoforms, each coded by a separate gene on different chromosomes. TGF-β binds to at least eight different types of receptor, each with different properties (Massague, 1992). It is produced by almost all nucleated cells, functioning as a growth factor as well as promoting cell differentiation, proliferation and morphogenesis (Tabibzadeh, 1991a). Within the immune system, TGF-β promotes the generation of TH-1 cells (Swain et al., 1991).

In the animal model, TGF-β mRNA has been shown by in-situ hybridization to be expressed in endometrium and decidua during the preimplantation period (Tamada et al., 1990). Furthermore TGF-β gene expression has been
localized using immunohistochemical staining to endometrial and stromal cells on days 1 to 4 of murine pregnancy (Tamada et al., 1990). In the endometrium TGF-β modulates epithelial proliferation, enhances gland formation, promotes angiogenesis, activation and proliferation of fibroblasts and deposition of extracellular matrices such as collagen (Sporn and Roberts, 1990; Casscells et al., 1990; Miller et al., 1990; Quaglino et al., 1990). In humans, TGF-β mRNA expression is up-regulated by progestogens, while oestrogens down-regulate it. (Knabbe et al., 1987; Colletta et al., 1991) (Figure 1).

Cytokine production and idiopathic recurrent miscarriage

The exact mechanism by which abnormal immune factors cause recurrent miscarriages is uncertain, but there is a suggestion that this may involve modulation or imbalance between the various cells of the immune systems, especially the T cell subgroups, as indicated by the cytokine secretion profile.

The basis for a disturbance of alloimmune factors as a cause of recurrent miscarriages arises from the observation that the fetus is a semi-allograft due to the genetic contribution from each parent. In normal pregnancy, the fetus is not rejected immunologically, despite the paternal (foreign) genetic contribution (Medawar, 1953). Numerous researchers have attempted to unravel this ‘mystery’ but it is only recently that more progress has been made.

Thomas Wegmann, who proposed the original immunotrophic hypothesis, focused attention on the role of cytokines in the feto–maternal dialogue (Wegmann, 1984). He hypothesized that ‘successful allo-pregnancy is a TH-2 phenomenon’ and demonstrated a TH-2 cytokine profile response in normal pregnancy (Wegmann et al., 1993). This TH-2 response allows the production of blocking antibodies to mask fetal trophoblast antigens from immunological recognition by maternal TH-1 cell-mediated/cytotoxic response which is responsible for rejection of allografts/semi-allografts. In the animal model, the immunological profile of early pregnancy loss is biased towards an excessive TH-1 cytokine profile (Tangri and Raghupathy, 1993, Tangri et al., 1994). When abortion-prone CBA×DBA/2 mice were given IL-10, which is a TH-2-inducing cytokine, fetal wastage was prevented (Chaouat et al., 1994) (Figures 2, 3).
This imbalance of cytokine profiles and hence immune response has been demonstrated recently in recurrent miscarriage patients. It was shown that peripheral lymphocytes of 57 idiopathic/unexplained recurrent miscarriage patients exhibited cell proliferation and cytotoxic/cell-mediated response after exposure to trophoblast antigen stimulation. These lymphocytes were found to be embryotoxic when they were subsequently exposed to mouse embryos. In contrast, the lymphocytes of 10 control women with no miscarriages did not proliferate in response to trophoblast stimulation and did not exhibit any embryotoxicity (Yamoda et al., 1994). Higher concentrations of TH-1 cytokines TNF-α and IL-2 have been demonstrated systemically in the serum of patients with idiopathic/unexplained recurrent miscarriages than normal controls (Mallmann et al., 1991). Although the evidence points to a predominant cell-mediated/cytotoxic or TH-1 immune response in idiopathic/unexplained recurrent miscarriages, there is a need to be cautious as these cytokines are found systemically in the serum and further studies on the profile of local endometrial cytokines in recurrent miscarriage need to be initiated.

The concept of altered TH-1 and TH-2 immune responses as aetiological factors in recurrent miscarriage has been further explored in a recent study (Hill et al., 1995). In this study, peripheral blood lymphocytes were taken from 20 patients with idiopathic recurrent miscarriage and compared with those from 13 non-pregnant women with previous pregnancies and no miscarriages, as well as from 10 men. The lymphocytes were exposed to trophoblastic tissue during culture and then exposed to mouse embryos. The supernatant of the recurrent miscarriage and control lymphocyte cultures were assayed for concentrations of IFN-γ. All the recurrent miscarriage lymphocytes exhibited both embryotoxicity and elevated values of IFN-γ, whereas none of the lymphocytes of controls exhibited embryotoxicity or elevated IFN-γ. Furthermore, the recurrent miscarriage group showed elevated values of TNF-α in all 20 cases (100%), TNF-β in 17 out of 20 cases (85%) and IFN-γ in all 20 cases (100%). None of the 13 non-pregnant control women and the 10 control men had elevation of TNF-α, TNF-β or IFN-γ. In contrast, all 13 non-pregnant control women (100%) and 9 of the 10 control men (90%) showed elevated values of IL-10, and three of the 13 non-pregnant control women (23%) had elevated IL-4, whereas only one (5%) and two (10%) of the 20 women with recurrent miscarriage showed elevated values of IL-4 and IL-10.
respectively. This suggests that some of the idiopathic recurrent miscarriages may in fact be due to immunological factors and that these women may exhibit one extreme of the TH-1 and TH-2 immune responses.

The theories regarding alloimmunity in recurrent miscarriage as reviewed earlier propose two main ideas: the formation of antifetal antibody blocking factors or an increase of cytotoxic activity against the conceptus. Formation of antibody is a TH-2-mediated response whereas cytotoxicity is a TH-1-mediated response. Current evidence suggests the derangements in cytokine profiles during pregnancy would be influential in determining a TH-1 or TH-2 immune response. There is therefore the potential for modulating the local immune system in recurrent miscarriage by exogenous topical or systemic administration of cytokines to favour a TH-2 rather than a TH-1 response.

Conclusions

The current understanding of the pathogenesis of the various aetiologies in recurrent miscarriage is limited. The expansion of research into molecular biology over the past few years has allowed us to delve more deeply into deciphering the molecular effects of the various aetiologies associated with recurrent miscarriage. Greater understanding and knowledge of the effects at the molecular level of these aetiologies will allow us to utilize and modulate them. Cytokines have been shown to have a role in the endocrine and immune systems. There seem to be some interactions between cytokines and the endocrine system in steroidogenesis and endometrial development during implantation. It is inferred that cytokines are immuno-endocrine modulatory factors which play an essential role in the pathophysiology of recurrent miscarriage.

If this hypothesis is true, attempts at immuno-modulation may be of clinical importance. Indeed, immunotherapeutic regimes involving cytokine modulation have been tried in mice with rheumatoid arthritis, using TGF-β to down-regulate the TH-1 response and promote a TH-2 response (Thorbecke et al., 1992). As rheumatoid arthritis is characterized by a predominantly TH-1 cytotoxic immune response, there are parallels to be drawn with immune or idiopathic recurrent miscarriage. Modulation of cytokine profiles have also been tried in abortion-prone CBA×DBA/2 mice using IL-10, a TH-2 cytokine, with good results (Chaouat et al., 1994). It would be interesting to see the results of an immuno-modulation trial in humans with recurrent miscarriage. There is also the potential to apply immuno-modulation to other reproductive events with the object of improving pregnancy rates in assisted reproduction as well as in contraception.

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Cytokines and immuno-endocrine factors in recurrent miscarriage


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