The possible role of the immune system in the aetiopathogenesis of ovarian hyperstimulation syndrome

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This review examines recent evidence suggesting a role for the immune system, in particular cytokines, in the pathogenesis of ovarian hyperstimulation syndrome (OHSS). Ovarian tissue is known to contain cells capable of producing a range of immunological mediators and the concentrations of these have been shown to be elevated in serum and ascitic fluid from women with established OHSS. Available evidence points to a role for vascular endothelial growth factor and interleukin-2, possibly acting through other intermediary cytokines, in the pathogenesis of OHSS. However, each individual has a unique cytokine profile and several cytokines may share biological actions, making it difficult to interpret data on isolated cytokine concentrations from relatively small numbers of patients. Improved understanding of the role of the immune system in the development of OHSS may have implications for the prediction, prevention and management of this iatrogenic condition.

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Introduction

Ovarian hyperstimulation syndrome (OHSS) is the most important cause of short-term morbidity in women undergoing supra-physiological ovarian stimulation, with a reported incidence of between 0.6 and 14% of in-vitro fertilization (IVF) cycles (Rizk, 1994). Attempts accurately to predict or prevent OHSS have been disappointing (Morris et al., 1995; Mathur et al., 1996) due mainly to a lack of understanding of the underlying cause of OHSS. In the light of what is known of the pathophysiology of OHSS and the cytokine family of proteins this review examines recent work suggesting immune activation and cytokine release as important pathogenic factors for the development of OHSS.

Pathophysiological features of OHSS

For a recent detailed review of the pathophysiology of OHSS, see Elchalal and Schenker (1997).

Supra-physiological ovarian stimulation and the occurrence of luteinization mediated by human chorionic gonadotrophin (HCG) or luteinizing hormone (LH) are almost universal requirements for the development of OHSS. The dominant pathophysiological features of OHSS include increased capillary permeability, arteriolar vasodilation and variable ovarian enlargement. The rise in capillary permeability encourages a shift of fluid out of the vasculature leading to intravascular dehydration, haemoconcentration and their sequelae, including hypercoagulability and impaired renal function (Bergh and Navot, 1992). Evidence of the occurrence of systemic arteriolar vasodilatation comes from studies by Balasch et al. (1994) demonstrating lower peripheral vascular resistance, tachycardia and increased cardiac output in 31 consecutive cases of severe OHSS. It is not clear whether the occurrence of vasodilatation precedes or follows the increase in capillary permeability or whether there is indeed any relation between the two. Arteriolar dilatation by itself could promote increased transudation of fluid into the extra-vascular space by increasing the capillary surface area and hydrostatic pressure, although the transudated fluid would be expected to have a relatively lower protein content than one derived from an increase in capillary permeability.

The cause of these haemodynamic changes remains unknown. Plasma renin activity and plasma aldosterone are elevated in OHSS and are related to the severity of the condition (Navot et al., 1987; Balasch et al., 1994). However, this may represent a homeostatic response to the underfilling of the diluted vascular compartment rather than a primary cause of the syndrome (Balasch et al., 1994). A similar compensatory role is ascribed to increased concentrations of antidiuretic hormone (ADH) and vasoconstrictor amines, while high urine concentrations of vasodilator prostaglandins probably represent a homeostatic response to maintain renal function in the face of reduced perfusion in women with severe OHSS (Balasch et al., 1994). It would therefore appear likely that mediators other than these are involved in the pathophysiological changes of OHSS.

Classification and actions of cytokines

Cytokines are a family of low molecular weight proteins that play important roles in the regulation of immunological and non-immunological homeostatic responses, exerting effects on cellular proliferation, differentiation, activation and motility. They are active in extremely low concentrations and can exert their effects in an autocrine, paracrine or endocrine fashion by binding to specific receptors on target cells (Cohen and Cohen, 1996).

Interleukins (IL) are a subset of cytokines, originally thought...
to be lymphocyte products involved in interactions between leukocytes (Anonymous, 1986). It is now known that these mediators are not exclusively produced by lymphocytes and have a variety of actions on endothelium, fibroblasts and granulosa/luteal cells (Schmidt et al., 1982; Bevilacqua et al., 1984; Wang et al., 1991). Cytokines important in inflammatory responses include tumour necrosis factor-α (TNF-α), TNF-β, IL-1α, IL-1β and IL-6 and are mainly derived from macrophages (Cohen and Cohen, 1996). IL-2 is a product of activated T lymphocytes which itself promotes the proliferation and/or activity of T and B lymphocytes, natural killer (NK) and lymphokine-activated killer (LAK) cells and the release of other cytokines such as IL-1, IL-4, IL-6 and TNF-α (Ortaldo et al., 1984; Doi et al., 1989; Deehan et al., 1994). Establishing the importance of cytokines in any given disease process is complicated by several factors, not least of which is the redundancy and pleiotropy of an ever-increasing number of identified cytokines (Cohen and Cohen, 1996). Redundancy is a feature whereby different cytokines exert the same effects, while pleiotropy refers to the property of an individual cytokine having multiple effects. Cytokine activity is also influenced by the functional status of cytokine receptors, the presence of cytokine inhibitors, soluble receptors and binding proteins, the concentration of other inhibitory or synergistic cytokines and the activation state of the target cells. It is therefore not surprising that contradictory cytokine results may be obtained in even an apparently homogeneous condition such as OHSS, particularly when only a small number of the aforementioned variables are examined at a single point in time. It is important to bear these points in mind when scrutinizing the literature on cytokines in relation to OHSS.

Immunocompetent cells are widely present in the female reproductive system and several cytokines are believed to be important in reproductive physiology (Ben-Rafael and Orvieto, 1992). Immunocytochemistry reveals the presence in the ovary of T lymphocytes capable of secreting IL-2 and as much as 10% of the cellular population of the human corpus luteum may be composed of macrophages, particularly concentrated at the junction of the theca and granulosa lutein cells (Wang et al., 1992). In-vitro studies have demonstrated the synthesis of TNF-α by cultured human granulosa cells (Terranova et al., 1993). The presence in the ovary of cells capable of secreting a wide variety of cytokines and evidence that cytokines can exert effects on granulosa cells and other ovarian components suggests a role for these substances in folliculogenesis, ovulation and other physiological ovarian phenomena (Wang et al., 1991; Ben-Rafael and Orvieto, 1992; Terranova et al., 1993; Best and Hill, 1995).

**Similarities between vascular leak syndrome and OHSS**

Important clues regarding the aetio-pathogenesis of OHSS may be gained from vascular leak syndrome (VLS), which has a remarkably similar pathophysiology to OHSS (Ettinghausen et al., 1988). Patients with VLS demonstrate a generalized decrease in peripheral vascular resistance and an increase in capillary permeability leading to extravasation of fluid from the vascular system and effusions. Adult Respiratory Distress Syndrome (ARDS) can develop in severely affected cases (Ferro et al., 1989). Although most often associated with recombinant IL-2 (rIL-2) administration as treatment for advanced cancer, VLS has also been observed following treatment with IL-4 and granulocyte macrophage-colony stimulating factor (Vial and Descotes, 1995). Based on murine work, the common factor may be immune activation of killer cells, which promote endothelial permeability by direct cell-to-cell contact. While IL-2 can induce the release of several pro-inflammatory cytokines such as TNF-α and IL-6, it has been suggested that TNF-α is the key mediator of the increased capillary permeability that manifests as VLS (Deehan et al., 1994). Certainly, TNF-α is known to enhance vascular permeability, possibly by altering intracellular cytoskeletal structure and increasing the expression of intercellular adhesion molecules (ICAM) (Stolpen et al., 1986; Deehan et al., 1994).

Orvieto et al. (1995) measured IL-2 concentrations in follicular fluid obtained at the time of oocyte retrieval from 40 women thought to be at risk of OHSS. Follicular fluid IL-2 concentrations were significantly higher in the seven women who went on to develop OHSS than in seven age-matched controls who did not develop this complication. Importantly, there was no significant difference in the pre-HCG oestradiol concentrations and number of eggs collected between the two groups. Plasma IL-2 concentrations were not measured in this study. A positive correlation has previously been demonstrated between follicular fluid and plasma concentrations of IL-2 in women undergoing oocyte retrieval for IVF (Wang and Norman, 1992).

Despite the similarity with VLS the precise significance of IL-2 in the pathogenesis of OHSS remains unclear. Peritoneal fluid from 12 women with established severe OHSS showed undetectable concentrations of IL-2 (Revel et al., 1996). However, the possibility remains that IL-2-induced release of other mediators, such as TNF-α, may be important in the development of OHSS.

Concentrations of TNF-α were shown to be significantly higher in peritoneal fluid from women with severe OHSS in comparison with healthy controls undergoing diagnostic laparoscopy or tubal ligation (Revel et al., 1996). Friedlander et al. (1993) failed to find elevated plasma TNF-α concentrations in the peripheral circulation of women with OHSS. A recent study using high sensitivity enzyme-linked immunoabsorbent assay showed significantly higher TNF-α concentrations in plasma from seven women admitted with severe OHSS in comparison with control groups consisting of healthy female volunteers not undergoing any treatment and women undergoing ovarian stimulation without developing OHSS (Abramov et al., 1996). Further, TNF-α concentrations declined in parallel with improvement in the patients’ clinical condition and resolution of OHSS. In this regard TNF-α promotes endothelial leukocyte interaction by up-regulating both E- and P-selectin expression by endothelial cells and subsequently ICAM-1 and vascular cell adhesion molecule-1 (VCAM-1) expression (Sterner-Kock et al., 1996; Luscinskas et al., 1996; Haraldsen et al., 1996). Of interest, albumin flux across cultured endothelial monolayers could be inhibited by antibodies against
lymphocyte function-associated protein-1 (LFA-1) and ICAM-1 (Damle and Doyle, 1989).

**Vascular endothelial growth factor and OHSS**

The main vascular permeability agent in ascitic fluid from women with severe OHSS may be vascular endothelial growth factor (VEGF), also known as vascular permeability factor (VPF). VEGF/VPF belongs to a family of heparin-binding proteins that induce angiogenesis and increase vascular permeability, allowing the passage of large molecular weight substances through the vessel walls (Senger et al., 1983; Ferrara et al., 1992). Synthesis of VEGF/VPF by cultured human granulosa cells has been observed (Neulen et al., 1995) and VEGF may be involved in the regulation of intrafollicular oxygen concentrations and oocyte maturation. A recent study found a correlation between the extent of perifollicular angiogenesis, as measured by colour Doppler ultrasound, and follicular fluid concentrations of VEGF (Van Blerkom et al., 1997).

McClure et al. (1994), in a study of three patients with ascites secondary to severe OHSS and three female patients with chronic liver failure, found similar peaks of permeability activity in OHSS ascites and liver failure ascitic fluid spiked with recombinant human VEGF. Specific anti-serum directed against human VEGF inhibited 70% of the vascular permeability-enhancing effect of OHSS ascites. Peritoneal fluid VEGF concentrations at the time of egg collection in eight women at risk of OHSS were similar to serum concentrations and approximately 100-fold lower than follicular fluid concentrations (Krasnow et al., 1996), suggesting an ovarian origin for this mediator. In the same study serum VEGF concentrations 14 days after HCG administration were significantly higher in patients who developed OHSS than in those who did not. There was no significant difference between the groups on the day of HCG administration or on day 7 after HCG, at which point all subjects exhibited abdominal discomfort but no significant OHSS. Patients of severe OHSS who achieved pregnancy had significantly higher VEGF/VPF concentrations than pregnant oocyte recipients of similar gestational age (Krasnow et al., 1996).

**Cytokines, VEGF and OHSS: a synthesis**

It is interesting to note that the ovary has cellular components capable of producing TNF-α and VEGF/VPF following luteinization. TNF-α is produced by macrophages, which are abundantly distributed amongst lutein cells (Wang et al., 1992) and may be subject to gonadotrophin influence (Terranova et al., 1993). Cultured granulosa cells are capable of producing VEGF and the relevant mRNA expression is enhanced in a time- and dose-dependent fashion by HCG (Anthony et al., 1994; Neulen et al., 1995). OHSS is conditional upon the occurrence of luteinization mediated by HCG or LH and increases in frequency and severity with increasing exposure to HCG (MacDougall et al., 1992; Dahl Lyons et al., 1994; Mathur et al., 1995). Gonadotrophin-induced cytokine release may initiate activation of other pro-inflammatory products and cells, the combined effects on the vascular endothelium and coagulation system manifesting as OHSS (see Figure 1).

Intravascular dehydration increases the risk of endothelial adhesion of leukocytes and platelets, leading to contact activation of the coagulation system. VEGF is capable of up-regulating both procoagulant factors such as tissue factor (Pepper et al., 1991) and those such as tissue plasminogen activators and plasminogen activator inhibitor-1 which affect fibrinolysis (Clauss et al., 1990). While these effects may be theoretically important there is no direct evidence that they influence intravascular coagulation in OHSS. However, VEGF stimulates the release of von Willebrand factor (vWF) by endothelial cells (Brock et al., 1991) which may encourage platelet adhesion leading to intravascular thrombus formation. Additionally, VEGF-induced capillary hyperpermeability may encourage the extravasation of fibrinogen and other coagulation proteins (Dvorak et al., 1995). These become activated in the extravascular space to form a fibrin gel, which may inhibit tissue oxygenation and nutrition. The fibrin gel may encourage coagulation in capillaries in which the endothelium has been activated by TNF-α and in which up-regulation of cell adhesion molecules has produced sluggish blood flow. Finally, the extracellular coagulation may trap pro-inflammatory cytokines and VEGF, amplifying their effects.

It has been suggested that OHSS occurs in two distinct clinical forms (Dahl Lyons et al., 1994). 'Early' OHSS presents in the first week of the luteal phase and may reflect the magnitude of preovulatory ovarian stimulation and response, while 'late' OHSS appears to relate to implanting pregnancies.
Prediction of OHSS

Accurate prediction of the likelihood of development of OHSS in an individual treatment cycle remains a difficult task (Mathur et al., 1996). Ideally, such prediction is best made prior to the administration of the ovulatory HCG trigger, allowing cancellation of truly high-risk treatment cycles whilst allowing other cycles, where the ovarian response to stimulation may have been above average but which will not result in OHSS, to continue. A retrospective study of patients undergoing ovarian stimulation for IVF failed to find any significant difference between pre-HCG concentrations of IL-1 receptor antagonist, IL-6 and TNF-α between 10 women who went on to develop OHSS and matched controls who did not (Loret de Mola et al., 1996). Both groups had higher average follicular phase and pre-ovulatory concentrations of IL-6 than healthy volunteers not undergoing any treatment. Krasnow et al. (1996) did not find any significant difference in pre-ovulatory plasma VEGF/VPF concentrations between women who subsequently developed OHSS and matched controls who did not, although the number of patients was small.

VEGF is known to induce the release of vWF from vascular endothelial cells (Brock et al., 1991) and this glycoprotein may turn out to be a marker for VEGF action in patients at risk of OHSS. In a study of 37 ‘high-risk’ women, Todorow et al. (1993) found elevated serum concentrations of vWF in all 18 women who developed OHSS in comparison with those who did not. The rise in vWF concentrations preceded the appearance of clinical symptoms of OHSS in all cases, but no significant difference was apparent between OHSS and control groups until the day before oocyte retrieval.

Follicular fluid IL-2 concentrations were found by Orvieto et al. (1995) to be significantly higher in women who went on to develop OHSS than controls. This preliminary study was restricted to women felt to be at high risk of developing OHSS on the basis of ‘conventional’ criteria (peak oestradiol concentrations and/or number of oocytes collected). Significant OHSS can develop in the absence of these risk factors and the occurrence of pregnancy, particularly if it is multiple, may be the crucial determining factor in its occurrence (Dahl Lyons et al., 1994; Morris et al., 1995; Mathur et al., 1995).

Once the treatment cycle has reached the stage of egg collection, options shown to minimize the risk of OHSS are at present restricted to those directed to the avoidance of further HCG exposure, either by elective cryopreservation of all embryos or by using progesterone for luteal support. Embryo cryopreservation with transfer in a subsequent cycle has not been shown to reduce the incidence of OHSS, though severe forms may be less likely (Wada et al., 1993). Luteal support with progesterone rather than HCG is less likely to be associated with OHSS (Soliman et al., 1995), but the protection is not absolute (Mochtar et al., 1996). Progesterone suppresses IL-2-induced mononuclear cell cytotoxicity in a dose dependent fashion (Feinberg et al., 1991), though whether this bears any relevance to the observed lower risk of OHSS is unclear.

Prevention of OHSS

It may become possible in the future to use pharmacological manipulation of the immune system to prevent OHSS. Orvieto and Ben-Rafael (1995) raise the possibility of modifying the immunological homeostasis of the woman at risk of OHSS by the administration of intravenous immunoglobulins (IVIG), with the object of preventing the abnormal immune response to supra-physiological ovarian stimulation that may underlie OHSS. In the rabbit model of OHSS, pre-treatment with IVIG was associated with a lower ascites response than pre-treatment with bovine serum albumin (Orvieto et al., 1997). In-vitro studies with IVIG reveal inhibition of T-cell derived lymphokines and TNF-α. Production of monokines such as IL-1α, IL-1β, IL-6 and IL-8 is either unaffected or increased (Andersson et al., 1996). Glucocorticoids are potent inhibitors of cytokine synthesis (Lew et al., 1988). In a randomized controlled trial Tan et al. (1992) did not find a protective effect for corticosteroids administered from just after oocyte retrieval for a period of 10 days to women believed to be at risk of OHSS. At the time of oocyte retrieval follicular fluid IL-2 concentrations in high-risk women who went on to develop OHSS were already significantly higher than in high-risk women who did not develop OHSS (Orvieto et al., 1995), indicating that therapy to be effective may need to be started before this time. Paradoxically, low concentrations of corticosteroids induce the production of the pro-inflammatory cytokine macrophage migration inhibitory factor (MIF), which can overcome their inhibitory effects on lipopolysaccharide-induced cytokine production (Calandra et al., 1995). It is unclear whether this has any relation to the observed lack of protective effect of post-oocyte retrieval corticosteroids in preventing OHSS. Albumin administered intravenously at or around the time of oocyte retrieval may reduce the incidence of OHSS in women believed to be at risk on the basis of peak oestradiol concentrations and numbers of oocytes retrieved (Shalev et al., 1995), although its efficacy remains uncertain (Ng et al., 1995; Lewitt et al., 1996). However, the relationship, if any, of this to the activation of the immune system that appears to underlie OHSS remains unclear.

Management of OHSS

There may be a future role for plasma cytokine concentrations in the monitoring of patients with OHSS. Serum inflammatory cytokines are elevated in cases of severe OHSS and decline with improvement in the clinical status of the patient (Abramov et al., 1996). These investigators noted statistically significant correlations between serum concentrations of IL-1 and IL-6 and haematocrit and serum concentrations of TNF-α and white
cell count in seven women admitted with severe OHSS. Similar correlations have recently been described for VEGF (Abramov et al., 1997). It remains to be seen whether cytokine assays would have any advantages over the use of more conventional monitoring techniques, such as haematocrit which directly reflects plasma volume changes caused by the major pathophysiological process of increased vascular permeability (Bergh and Navot, 1992).

Specific anti-cytokine measures may become available in the future to treat established OHSS. The recognition of the key roles of these immunological mediators in the pathogenesis of a variety of conditions has led to the development of several cytokine antagonists, some of which have undergone clinical trials in human subjects (Ferrara, 1995). Four major points of attack have been identified – inhibition of cytokine synthesis, release, action or intracellular signalling pathways. Some antagonists may have more than one mode of action – IL-1 receptor antagonist (IL-1ra) inhibits IL-1-induced cytokine synthesis and the binding of IL-1 to its receptor on human monocytes (Granowitz et al., 1992). However it remains to be seen whether the complex effects of a number of cytokines acting in concert can be blocked by measures directed against a single mediator (Henderson, 1995). The efficacy of therapeutic paracentesis in improving the clinical status of patients of severe OHSS (Jenkins et al., 1995) may relate to the removal of large amounts of vasoactive products from the peritoneal cavity. In the light of present knowledge it would seem reasonable to advise against auto-transfusion of aspirated fluid into the patient’s circulation, for fear of worsening or prolonging the course of the disease.

Conclusions

There is growing evidence for a role in the immune system and, in particular, cytokines, as mediators of the clinical and pathological manifestations of OHSS. In interpreting data regarding immune activation it is important to keep in mind that healthy subjects with a balanced immune system have a unique cytokine profile and activation state of their immune cells. Each person will in addition respond in a unique way to any in-vivo stimulus owing to the multitude of factors that act on the host’s immune system including concentration of stress, nutritional state and recent infections. Cytokine measurements before HCG administration may show considerable variability as the state of activation of each individual’s corpus luteum macrophages and systemic capillary endothelium is likely to be different. The change in the cytokine profile with exposure to HCG may therefore be more important than the precise concentration of each cytokine. Further research, taking these factors into account, is needed to elucidate the cytokine profile prior to the onset and during the course of OHSS and its relationship, if any, with the severity of the disease and the occurrence of pregnancy. It is unlikely that measurement of a few cytokines on infrequent occasions will uncover the complexities of the inter-relationship between OHSS and disturbance of immune function. Nevertheless, as long as supra-physiological ovarian stimulation remains a treatment for infertility it is important that this area is investigated in depth, with the hope of gaining information to solve the enigma of OHSS leading to improved patient management.

References


Cytokines and ovarian hyperstimulation syndrome


