The role of cytokines in both the normal and malignant ovary

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Abstract

Normal ovarian tissue is rich in cytokines. Cytokines and chemokines are important in the physiology of ovarian function and of ovulation. Cytokines and chemokines may recruit cytokine-producing lymphocytes to the site of a developing follicle, and cytokines appear to play an important role in pre and post follicle development.

Most of the same cytokines that are found in normal ovarian tissue are also found in association with malignancy in contrast to their functions in normal tissues. It is reasonable to assume that the functions of cytokines associated with malignancy may serve to promote the unregulated growth if tumor cells and metastasis. It is also likely that cytokines produced by tumors will modulate immune responses that favor tumor progression.

In the following review, we have highlighted those functions of cytokines that have been identified as having the most significant impact on tumor growth and development. By examining activities of these cytokines in normal and in malignant ovarian tissues, it is hoped that future possible avenues for investigation may be opened up and that the results of these investigations will lead to strategies that can modulate the production or the activity of the cytokines leading to the growth of tumors or their metastases. Such strategies now fall under the general discipline of bioimmunotherapy. This is an expanding discipline as more is learned about growth regulation in cancer, and with the availability and rapid development of new molecules for therapeutic approaches.

Introduction

Cytokines, produced by virtually all types of cells, are proteins with the ability to stimulate or inhibit cell growth, regulate cell differentiation, induce cell chemotaxis, and modulate the expression of other cytokines. There is increasing evidence that cytokines may play a significant role in the progression of ovarian cancer. Because many cytokines are multifunctional, it is difficult to assess the role played by any particular cytokine in the progression of cancer without knowing which other cytokines, growth factors, hormones, etc. are also active at the concomitant tumor site. In this review we will briefly summarize what is known about the functions of certain cytokines in the normal ovary, focus on how those functions are aggressively utilized by ovarian cancer, and discuss several ways that cytokines are being used in the treatment of ovarian cancer.

Cytokines involved in the growth of the normal ovary

Many cytokines are expressed in the normal ovary. In one recent study transcripts for 16 different cytokines common to normal and tumor bearing ovaries were identified (Burke et al. 1996). The chemokines RANTES, macrophage inflammatory protein (MIP)-1α and MIP-1β were also detected in ovarian biopsy specimens (Burke et al. 1996). We will discuss primarily interleukin (IL)-1, IL-2, IL-6, IL-8, IL-10, IL-11, granulocyte-macrophage colony-stimulating factor (GM-CSF), macrophage colony-stimulating factor (M-CSF), tumor necrosis factor-α (TNF-α), transforming growth factor-β (TGF-β), interferon-γ (IFN-γ), and monocyte chemoattractant protein-1 (MCP-1). The function of these cytokines is not defined in every case, but existing data allow us to speculate about it. The physiological functions of these
Nash et al.: Ovarian cytokine expression

Table 1 Cytokines expressed in the tumor environment of ovarian cancer patients: effect on tumor growth

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Source</th>
<th>Putative effect on tumor cell growth</th>
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<tbody>
<tr>
<td>IL-1</td>
<td>Tumor cell</td>
<td>Promotes tumor cell growth by increasing production of IL-6</td>
</tr>
<tr>
<td>IL-2</td>
<td>T-cell</td>
<td>?</td>
</tr>
<tr>
<td>IL-6</td>
<td>Tumor cell</td>
<td>Promotes tumor cell attachment and migration</td>
</tr>
<tr>
<td>IL-8</td>
<td>Tumor cell, macrophage</td>
<td>Induces migration of lymphocytes, promotes tumor angiogenesis</td>
</tr>
<tr>
<td>IL-10</td>
<td>Monocyte</td>
<td>Down regulates HLA expression on tumor cells and APCs, blocks expression of IL-2 by T-cells, increases TLR expression on tumor cells</td>
</tr>
<tr>
<td>TGF-β</td>
<td>Tumor cell, monocyte</td>
<td>Down regulates HLA expression on tumor cells and APCs, inhibits T-cell proliferation, induces T-cell apoptosis, down regulates IL-2R expression on NK cells and monocytes, promotes tumor angiogenesis</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>Tumor cell</td>
<td>Promotes tumor cell growth (up regulates cyclin D1 expression)</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumor cell</td>
<td>Promotes tumor cell growth by increasing production of IL-6</td>
</tr>
<tr>
<td>M-CSF</td>
<td>Tumor cell</td>
<td>?</td>
</tr>
<tr>
<td>IAP</td>
<td>Tumor cell, monocyte</td>
<td>Down regulates CD4+ surface expression on T-cells, impairs NK cell response</td>
</tr>
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<td></td>
<td></td>
<td>IFNγ</td>
</tr>
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Cytokines include directing cytokine-producing lymphocytes to the ovary and regulating certain stages of ovarian follicle development.

IL-6 is found in the normal ovary, where it may participate in follicle development by reducing the follicle-stimulating hormone binding capacity of granulosa cells (Machelon et al. 1994). However, porcine granulosa cells were shown not to make IL-6. Machelon and colleagues (1994) suggested that intraovarian IL-6 may be produced by lymphocytes, which moderate granulosa cell function. IL-6 is also made by human ovarian epithelial cells (Lidor et al. 1993).

TGF-β has a major role in follicle development and may also be involved, along with IL-6, in the post-ovulation healing process as well. The concentration of two isoforms of TGF-β, TGF-β1 and TGF-β2, were shown by immunohistochemical staining to vary in a cyclical manner in the maturing follicle (Chegini & Flanders 1992). TGF-β1 was seen in oocytes, small primary follicles, theca, granulosa cell layers, and luteal tissue. Oocytes stained intensely for TGF-β2 in the nuclear periphery, and staining of theca and granulosa increased in larger follicles during follicle maturation. Luteal tissue staining for TGF-β1 decreased in intensity by the late luteal phase, and staining of the corpora albicans was very weak. Endothelial cells, smooth muscle cells and stroma also stained for TGF-β1.

TGF-β2 was found only in theca cell layers and in small luteal cells. Staining for TGF-β2 was not seen in oocytes or the granulosa layer of small follicles, and there was only weak staining in the theca layer of small follicles. Thecal staining increased in larger follicles, and staining of the granulosa layer was also seen in large follicles.

Early-phase luteal tissue stained weakly, and this diminished during the mid and late phases. The endothelium and smooth muscle of small arterioles also stained for TGF-β2. There was little staining of large arterioles, and only weak staining for TGF-β2 was seen in ovarian stroma (Chegini & Flanders 1992, Gordinier et al. 1998). Gordinier and colleagues (1998) observed strong staining of follicles and Mullerian rests and weak staining of stroma for both TGF-β1 and TGF-β2.

Other cytokines play important roles in follicle development. In the newly formed corpus luteum, TNF-α and IL-1 inhibit the secretion of progesterone. TNF-α may also be involved in the regression of corpora lutea (Terranova & Rice 1997). IL-11 has been found in follicular fluid and may also have a role in follicle development (Braniasteam et al. 1997). GM-CSF may also be involved in follicle development since both transcripts and protein have been detected in ovarian follicles (Zhao et al. 1995, Jasper et al. 1996).

Atresia is a hormonally controlled form of apoptosis of ovarian follicles and cytokines play a role in its regulation. TNF-α, acting together with Fas ligand, and androgens have been shown to induce atresia (Kaipia & Hsueh 1997). In contrast, gonadotropins, IL-1β, insulin-like growth factor-I, epidermal growth factor, TGF-β, basic fibroblast growth factor and estrogens all act during follicle development to prevent atresia.

IL-8 is a chemokine that can induce a chemotactic response from lymphocytes (Taub et al. 1996). Ovarian follicles produce IL-8, and it is believed that this chemokine may target leukocytes to the ovary (Runesson et al. 1996). In another study, lymphocytes in the ovaries were shown to increase in number during follicle develop-
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ment (Vinatier et al. 1995). One may speculate that these lymphocytes release cytokines in response to hormonal and cytokine stimulation from the ovary and contribute to the events that occur during follicle development. Possibly TGF-β up regulates ovarian expression of IL-8 as it has been shown to do in endometrial stroma (Arici et al. 1996).

Arici and colleagues (1997) have shown that a large number of macrophages are also present within the ovarian follicle as well as within the ovarian stroma at ovulation. They suggested that these macrophages migrate to the preovulatory follicle under the influence of hormonal regulation of MCP-1. They also demonstrated that ovarian stromal and granulosa-lutein cells increased expression of MCP-1 RNA and protein in vitro when exposed to IL-1α and TNF-α.

Cytokines involved in tumor growth

There are at least two ways in which cytokines can aid the growth of tumor cells. (1) They can enhance tumor growth directly by functioning as growth factors, promoting metastasis by increasing cell adhesiveness and/or enhancing tumor angiogenesis. (2) Cytokines can also be powerful modulators of the immune system, enhancing tumor growth by blocking cell-mediated mechanisms for identifying and destroying the tumor. A list of the cytokines involved in ovarian cancer, along with their
putative activities in promoting tumor growth, is given in Table 1.

**Cytokines directly enhancing tumor growth**

Virtually all of the cytokines (and leukocytes) found in the normal ovary are also found in ovarian tumors. However, there is a shift in the balance of expression of these cytokines, with significantly increased expression of certain growth-stimulating and immunoinhibitory cytokines observed in ovarian cancer.

One of the most widely studied cytokines in ovarian cancer is IL-6 (Offner et al. 1995, Rabinowich et al. 1996, Asschert et al. 1997, Merogi et al. 1997). Two major sources of IL-6 production at the tumor site are peritoneal mesothelial cells and cancer cells (Offner et al. 1995). It is interesting that a significant percentage of ovarian cancers overexpress the p53 protein, which can regulate expression of IL-6 in vitro (Asschert et al. 1997). Also, a correlation was demonstrated between elevated levels of IL-6 in the cystic fluids of ovarian cancer patients and decreased hemoglobin levels (van der Zee et al. 1995), which is another poor prognostic factor for this disease. However, IL-6 does not enhance tumor growth directly, as in vitro treatment of tumor cells with neutralizing anti-IL-6 antibody fails to affect cell proliferation (Watson et al. 1990). Obata and colleagues (1997), studying attachment of ovarian cancer cell lines to Matrigel, concluded that IL-6 may promote tumor growth by its effects on cell attachment and migration.

TGF-β is a multifunctional cytokine with a multiplicity of effects on tumor growth in vivo. It was recently demonstrated that freshly isolated ovarian tumor, as contrasted with tumor cell lines, produced high levels of TGF-β (Santin et al. 1996). Some investigators have reported that TGF-β inhibits the growth of ovarian cancer cells in vitro (Hurteau et al. 1994), while others showed that there is no in vivo growth inhibition, and even growth enhancement, of prostate tumors treated with TGF-β (Barrack 1997). TGF-β is also thought to have angiogenic properties (Roberts et al. 1986). Whereas TGF-β normally inhibits the growth of epithelial cells, it also stimulates the growth of mesenchymal cells (Roberts et al. 1985). Thus, whether TGF-β stimulates or inhibits the growth of epithelial cancer cells may depend on a number of factors including the state of dedifferentiation of the tumor cells.

In epithelial cells, TGF-β blocks cell proliferation mainly by down regulating the expression of cyclins (Satterwhite & Moses 1994, Ko et al. 1995, Ravitz et al. 1995). Expression of GM-CSF can overcome the TGF-β-induced block on cell division by up regulating the expression of cyclin D2, as was shown recently in human leukemia cells (Ohtsuki et al. 1997). Therefore, it may be significant that GM-CSF transcripts have been found in ovarian cancer cells (Merogi et al. 1997).

Signal transduction is performed by heterodimer formation of TGF-β receptors 1 and 2 (TβR1 and TβR2), which are serine/threonine kinases (Wrana et al. 1994, Wrana & Attisano 1996). Mutations in TGF-β receptor genes have been implicated in the development and/or spread of various neoplasms including gastric (Park et al. 1994) and colon (MacKay et al. 1995) cancers. A recent report has shown that inactivating mutations of the TβR2 gene are also not uncommon in ovarian cancer (Lynch et al. 1998). The intracellular pathway of signal transduction by TGF-β has until recently been largely unknown. It was reported that TβR1 may phosphorylate the alpha subunit of p21-ras farnesyl transferase (Kawabata et al. 1995). This is interesting because H-ras is involved in regulating expression of both TβR1 and TβR2 and it also blocks internalization of the activated receptors (Zhao & Buick 1995). Also, H-ras transcription is up regulated by the p53 protein, which is over expressed in about 40% of ovarian tumors (Zachos & Spandidos 1998). Therefore, it seems likely that in those ovarian tumors over expressing p53, up regulated H-ras could modulate the tumor cell’s response to TGF-β, and TGF-β could, perhaps, regulate the expression of H-ras through the farnesylation pathway.

More recently, it was shown that internalized TβR1 phosphorylates a member of a family of proteins known as SMAD. The phosphorylated SMAD then forms a heterodimer with SMAD4, which moves into the nucleus and binds to DNA (reviewed by Massagué et al. 1997). Other SMAD proteins can block this pathway. Mutations in the SMAD proteins are only rarely seen in tumors (Riggins et al. 1997). SMAD4 has been described as a tumor suppressor gene (De Winter et al. 1997, Liu et al. 1997, Yingling et al. 1997), as has TβR2 (Parsons et al. 1995). A generalized pathway for TGF-β expression in the cell is depicted in Fig. 1.

**Possible role of TGF-β2 in the development of ovarian cancer**

It is possible that the risk of ovarian cancer is proportional to the number of ovulations that occur before the onset of the menopause (Schildkraut et al. 1997). However, it is also possible that it is the cyclic surges of cytokine expression occurring in the ovary during ovulation that are more directly involved in tumor promotion. TGF-β2 would be one obvious candidate cytokine.

Transcription of TGF-β2 is regulated by gonadotropin (Mulheron et al. 1991), and it consequently undergoes rather dramatic localized changes in concentration during follicle maturation (Chegini & Flanders 1992). It is commonly believed that the failure of epithelial cells to halt proliferation in response to TGF-β occurs late in tumor development (Cui et al. 1994). Therefore, the repeated localized expression of high levels of TGF-β2 during numerous cycles of follicle development may
eventually condition precancerous epithelial cells gradually to lose their sensitivity to TGF-β, and this may be a factor in the development of ovarian cancer. One argument against this hypothesis is that the majority of ovarian cancer cases occur in post-menopausal women. However, because it is present in the ovary and in ovarian tumor cells in a relatively high concentration (Chegini & Flanders 1992, Gordinier et al. 1998), and because of its role in regulating growth and development (Sanford et al. 1997), TGF-β2 may yet be a candidate for tumor promotion late in ovarian cancer development.

TGF-β2 was also shown to inhibit cell-mediated immune function (Takeuchi et al. 1997). It, along with other immunoinhibitory cytokines such as TGF-β1 and IL-10, can provide a status of immune privilege at the tumor site, allowing the developing tumor to escape immune surveillance. TGF-β2 is expressed in a number of other cancers, including breast cancer (Auvinen et al. 1995), melanoma (Reed et al. 1994), and prostate cancer (Barrack 1997). It was recently reported that normal differentiation gene products functioning as tumor associated antigens (TAA s, e.g. MART-1, MAGE, GAGE) can be effective targets for immunotherapy (reviewed by Rosenberg 1997). Because TGF-β2 can function as a differentiation gene and exhibits a wide-spread presence in tumor cells but is not commonly expressed in most normal tissues (notable exceptions being the ovary and the eye), it has all the hallmarks of a TAA and thus may be a potential target for ovarian cancer immunotherapy.

The role of immunoinhibitory cytokines in ovarian cancer
Using nomenclature based on observations in the mouse (Mosmann & Coffman 1989), Th1 type cytokines (IL-2

**Figure 2** Pathway for CTL activation. As depicted in this figure, APCs activate CD4+ (helper) and CD8+ (lytic or CTL) cells by presenting MHC-associated tumor-specific antigen to the lymphocyte while simultaneously stimulating the CD28 pathway. A single mature APC can activate both a CD4+ and CD8+ T-cell as shown, or the two activation steps can be accomplished separately by different mature APCs. The activated CD4+ cells produce Th1 cytokines which co-stimulate CD8+ lysis of the tumor cell. If the T-cell receptor (TCR) of the T-cell binds to MHC-presented antigen on the surface of the tumor cell without CD28 stimulation, then a state of anergy is induced.
and IFN-γ) produced by CD4+ helper T-lymphocytes, appear to facilitate cell-mediated immunity (see Fig. 2). On the other hand, the Th2 type cytokines IL-4, IL-5, and IL-10 are produced by CD4+ helper cells that assist in humeral immunity (reviewed by Lanzavecchia 1993). Obviously, conditions favoring a Th2 response would block an effective cell-mediated response to a tumor. It has been suggested that T-cells associated with ovarian cancer exhibit a Th2 phenotype (Schondorf et al. 1997), probably because of the presence of immunoinhibitory cytokines, i.e. IL-10. However, we have observed that T-cells at the tumor site frequently express transcripts for IL-2, but only rarely IFN-γ transcripts (Nash et al. 1998). To us these data suggest that a failed attempt at T-cell activation has occurred at the site of the tumor. Also, we detected no expression of IL-10 by T-cells at the tumor site in untreated patients, further evidence of a lack of a tumor-induced Th2 response. However, we did detect IL-10 transcripts in a subpopulation of monocytes at the tumor site (Loercher et al. 1998).

Lack of immunogenicity of the tumor in the absence of co-stimulation by B7 and accessory molecules in antigen presenting cells may contribute to a state of T-cell anergy in vivo. Decreased immunogenicity may result from human leukocyte antigen (HLA) gene deletions or from impaired presentation of the antigen epitopes as a result of down regulation of HLA class I or HLA class II antigens. This can be due to immunosuppressive cytokine activity that can regulate these antigens in tumors or on antigen presenting cells. Therefore, the cytokine microenvironment is pivotal in setting the stage for anergic or agonistic T-cell responses. Although we detected many monocytes at the tumor site, we observed poor expression of accessory molecules such as B7.1 on professional antigen-presenting cells (APCs) (Melichar et al. 1998). This could be a significant factor in the failure of T-cells to recognize and attack the tumor because poor recognition of the tumor by T-cells can lead to T-cell anergy (Schwartz 1996). In a recent review, Pardoll (1998) presented a model in which inappropriate antigen presentation by the tumor results in T-cell tolerance. Because we have frequently shown that transcripts for IL-2 but much less frequently transcripts for IFN-γ occur in leukocytes obtained from the peritoneal cavity of ovarian cancer patients, our findings could suggest a state of tolerance or anergy (Nash et al. 1998).

There is new evidence that patients with ovarian cancer may benefit from intraperitoneal immunotherapy (Freedman et al. 1998). Surgically documented responses have occurred following intraperitoneal treatment with recombinant (r)IFN-γ, rIFN-α, rIL-2, LAK cells and T cells with monoclonal antibodies (mAbs). However, it is also evident that the ovarian tumor and its immediate environment produce factors that can block T-cell activation at different levels. Two of these are the immunoinhibitory cytokines TGF-β and IL-10 which have been detected in significant amounts at the tumor site in ovarian cancer.

There have been several reports of increased IL-10 expression in ovarian cancer (Pisa et al. 1992, Rabinowich et al. 1996, Merogi et al. 1997, Nash et al. 1998). Although tumor-associated T-cells could be a source of IL-10 production in ovarian cancer (Rabinowich et al. 1996), we have shown that a subpopulation of monocytes is the main producer of IL-10 in patients who have not been treated with immunotherapy (Loercher et al. 1998). Similar immunoinhibitory monocytes have been identified in murine tumors that are activated by tumor-derived GM-CSF (Young et al. 1996). As stated above, GM-CSF transcripts are present in ovarian cancer cells (Merogi et al. 1997).

IL-10 down regulates expression of human HLA class I and HLA class II on tumor cells thus effectively interfering with HLA associated epitope mediated activation of T-cells (Matsuda et al. 1994). Interleukin-10 also down regulates HLA expression on APCs (Fiorentino et al. 1991, de Waal Malefyt et al. 1991) thus preventing cross-priming of T-cells and it can also block post-transcriptional expression of other cytokines, such as IL-2 (Fiorentino et al. 1991, Tg & Tosato 1992). It has been suggested, moreover, that IL-10-induced prostaglandin E2 (PGE2) is involved in the deactivation of peritoneal macrophages in mice (Strassmann et al. 1994). Interestingly, we have observed that ovarian tumor cells...
treated with IL-10 exhibit increased expression of TGF-β receptors (A Loercher, M A Nash, and R S Freedman, unpublished results), whereas the expression of HLA class I and HLA class II is decreased. As shown in Fig. 3, both TβR1 and TβR2 showed a substantial increase in expression. These data further suggest that the growth of tumor cells in vivo is probably not inhibited by TGF-β since IL-10 is frequently detected at the tumor site (Gotlieb et al. 1992, Rabinowich et al. 1996, Merogi et al. 1997, Loercher et al. 1998).

Expression of all three mammalian isotypes of TGF-β has been identified in ovarian tumors (Henriksen et al. 1995) and high levels of TGF-β that are inhibitory to LAK cells have been identified in ovarian cancer ascites (Hirte & Clark 1991). TGF-β isotypes are known inhibitors of immunological processes, although precise differences between the isotypes in immunological suppression have not been characterized. It can down regulate expression of the major histocompatibility complex (MHC) (Czarniecki et al. 1988, Marth et al. 1990, Donnet-Hughes et al. 1995), inhibit certain T-cell functions (Vánky et al. 1997), induce apoptosis in T-cells (Weller et al. 1994), and redirect the immune response from Th1 to a Th2 response (Maeda & Shiraishi 1996). TGF-β can also down regulate expression of IL-2 receptor gamma (IL-2Rγ) on monocytes and, perhaps, NK cells (Bosco et al. 1994), thus rendering them incapable of IL-2-stimulated, MHC-independent lysis of tumor cells. In addition, TGF-β was shown to up regulate IL-10 production by macrophages in mice (Maeda et al. 1995) and to inhibit IL-2-, IL-4- and IL-12-induced cytotoxic T-lymphocyte (CTL) responses (Herrmann & Abdi 1996). The relevance of TGF-β expression to tumor growth was demonstrated recently in a rat glioma model. Not only were rats treated with TGF-β anti-sense oligonucleotides protected from challenge with glioma cells, but also established tumors could be eliminated (Fakhrai et al. 1996). This was shown to be an immunological response in that mononuclear leukocytes taken from treated animals exhibited enhanced killing of tumor cells in cell-mediated cytotoxicity assays.
Another immunosuppressive cytokine reported in ovarian cancer is immunosuppressive acidic protein (IAP). This protein, which can be up regulated by IL-6 (Yoshizawa et al. 1996), has two immunosuppressive activities: (1) it down regulates expression of the CD4+ surface molecule on T-cells (Yamaguchi et al. 1995) and (2) it impairs the response of NK cells to IFN-γ (Aso et al. 1992).

A general overview of the immunoinhibitory mechanisms active in ovarian carcinoma is depicted in Fig. 4.

Cytokines as tumor markers in ovarian cancer patients

Given the role of cytokines in the biology of ovarian tumor cells, it is not surprising that attention has also been focused on the clinical application of cytokines for diagnosis, clinical monitoring, and prediction of clinical outcome. In particular, IL-6, M-CSF, and IAP appear to provide additional information that could help in the prognostic characterization of ovarian cancer patients.

Expression of macrophage colony-stimulating factor-1 (M-CSF-1) and its receptor in metastases may be a poor prognostic factor for ovarian cancer (Chambers et al. 1997). Interestingly, however, high expression of M-CSF-1 in the stroma of primary tumors is associated with low grade tumors. Elevated levels of IL-1 (Huleihel et al. 1997) and TNF (Sarandakou et al. 1997) have also been observed in ovarian cancer specimens, and may play a role in tumor cell growth by up regulating expression of IL-6 (Offner et al. 1995).

The clinical and biological relevance of IL-6 in ovarian carcinoma is suggested by several in vitro observations. Moreover, higher serum IL-6 levels have been found in ovarian cancer compared with other gynecological malignancies: in one study 53% of primary epithelial ovarian tumors and 35% and 10% respectively of endometrial and cervical cancer patients were found to be IL-6-positive (Scambia et al. 1994). These findings suggest the direct interaction and cytokine-promoted crosstalk between host immune cells and tumor cells.

In several studies, serum IL-6 levels were shown to be higher in ovarian cancer patients than in healthy controls (Erroi et al. 1989, Berek et al. 1991, Gastl et al. 1993, Moradi et al. 1993, Scambia et al. 1994, Tempfer et al. 1997). However, IL-6 does not appear to be as sensitive or useful as a tumor marker as CA 125 (Scambia et al. 1994, Tempfer et al. 1997). Although the proportion of patients with an elevated IL-6 level is higher in CA 125-positive than in CA 125-negative patients (Berek et al. 1991), the studies by Scambia et al. (1994) and Gastl et al. (1993) showed no linear correlation between IL-6 and CA 125. The combination of serum IL-6 with serum CA 125 values only slightly increased the overall sensitivity of CA 125. Ninety-two percent of the serum samples containing both IL-6 and CA 125 showed a positive reaction in at least one test, as compared with 87% of the samples containing CA 125 alone (Scambia et al. 1994). In addition, even though preliminary observations by Berek et al. (1991) suggested that serial determination of serum IL-6 levels could be useful in monitoring the clinical course of disease during chemotherapy, more recent data from a larger series of cases failed to find any relevant association between serial assessment of IL-6 and early detection of ovarian cancer progression (Scambia et al. 1994, Tempfer et al. 1997). However, it must be considered that IL-6 is involved in the recovery of hematopoiesis following chemotherapy (Baiocchi et al. 1993); therefore it is conceivable that the IL-6 level may be influenced by the effects of chemotherapy on myelopoiesis. No correlation between IL-6 serum levels and findings from second-look surgery were demonstrated in two different studies (Moradi et al. 1993, Scambia et al. 1994). Postoperative IL-6 levels were shown to be less sensitive than CA 125 levels in detecting residual disease after surgery (Scambia et al. 1994).

While there seems to be a general consensus that IL-6 determination cannot provide any useful information as a tumor marker, the negative prognostic role of a high IL-6 level has been emphasized in studies of different tumor types (Blay et al. 1992, Seymour et al. 1997, De Vita et al. 1998). As far as ovarian cancer is concerned, IL-6 status has been found not to be related to classical clinical-pathological parameters such as stage, grade, and histopathology (Gastl et al. 1993, Scambia et al. 1994). On the other hand, serum IL-6 levels were found to be associated with the extent of residual disease since 89% of patients with a residual tumor larger than 2 cm were shown to have a higher IL-6 level than that of patients with a residual tumor smaller than 2 cm (66%) and microscopic disease (13%) (Berek et al. 1991). This finding has, more recently, been confirmed in a larger series of cases (Scambia et al. 1995).

It has been reported that a high IL-6 level is associated with an unfavorable clinical outcome (Berek et al. 1991, Scambia et al. 1995), even in the subgroup of patients who experienced complete or partial response to chemotherapy. These results have recently been confirmed by Tempfer et al. (1997), who reported a significant association between elevated IL-6 levels and poor overall and progression-free survival. The possibility that a high IL-6 level in ovarian cancer patients is associated with a worse prognosis in that it reflects the presence of a large tumor burden and macrophage activation with subsequent release and accumulation of the cytokine in the serum and ascites, seems to be unlikely. Multivariate analysis demonstrated that the International Federation of Gynaecology and Obstetrics (FIGO) stage and IL-6 retained an independent negative prognostic role, suggest-
CA 125 has been found by several authors (Xu et al. 1991) even though no definite correlation between M-CSF and sensitivity of CA 125 status has also been investigated.

In the context of chemotherapy resistance, it is worth noting that IL-6 expression is considered a resistance factor for chemotherapeutic agents in different experimental models in vitro. For example, high IL-6 levels have recently been related to resistance to cisplatin in non-small cell lung cancer (De Vita et al. 1998). In primary ovarian cancer patients exhibiting a partial or complete response to chemotherapy, the percentage of IL-6 positivity was significantly lower (31%) than in patients with no response (71%) (Scambia et al. 1994). Similarly, Gastl et al. (1993) demonstrated that IL-6 concentrations in the ascites of chemotherapy-unresponsive patients was higher than in the ascites from chemotherapy-responsive patients. The biochemical mechanisms responsible for IL-6-induced chemotherapy resistance are far from being clarified; induction of metallothionin expression has been suggested to play an important role (Johnson et al. 1992). Also, modulation of the anti-apoptotic gene bcl-2 has also been suggested.

Since the preliminary observations by Kacinski et al. (1989) suggesting that M-CSF could be a useful marker in gynecological tumors, several investigators have reported the presence of increased levels of this cytokine in the serum of ovarian cancer patients compared with that in the serum of healthy controls (Xu et al. 1991, Suzuki et al. 1993). In particular, M-CSF positivity was found to be approximately 60-70% in ovarian cancer patients compared with 4% in healthy women. As reported for IL-6, serum M-CSF levels were higher in ovarian cancer patients compared with cervical and endometrial cancer patients (Suzuki et al. 1995), emphasizing the peculiar characteristics of ovarian tumor cell/peritoneal environment interactions.

The possible role of M-CSF in improving the sensitivity of CA 125 status has also been investigated. Even though no definite correlation between M-CSF and CA 125 has been found by several authors (Xu et al. 1991, Scholl et al. 1994; Suzuki et al. 1993, 1995), it seems that the combined determination of these two markers can lead to an improvement in overall sensitivity, especially in stage I ovarian cancer patients (Suzuki et al. 1993).

No significant changes in serum M-CSF levels were observed in ovarian tumor tissue samples collected both prior to and at the end of surgery (Scholl et al. 1994). On the other hand, significant decreases in M-CSF levels were observed in serial determinations during chemotherapy. The relationship between M-CSF and response to chemotherapy has only been marginally investigated. Xu et al. (1991) reported that patients with clinical evidence of disease had a higher M-CSF serum level than did patients whose disease was detected at second-look surgery.

The prognostic role of both serum and ascitic M-CSF concentrations has also been analyzed: M-CSF serum levels were shown to be higher in patients with advanced disease (Suzuki et al. 1993, 1995) although Price et al. (1993) failed to find any association between the ascitic M-CSF levels and the FIGO stage. Interestingly, M-CSF levels showed a trend of being associated with the extent of residual tumor after surgery because higher cytokine levels were detected in patients with suboptimally debulked tumors (Price et al. 1993). Elevated M-CSF levels in both the serum and ascites have been reported to be associated with a worse prognosis in ovarian cancer patients. In particular, Price et al. (1993) reported that in stage III-IV cases high ascitic M-CSF levels together with the presence of residual tumor after surgery retained an independent negative prognostic role in multivariate analysis. Similarly, in a series of 82 ovarian cancer patients, high serum M-CSF levels were associated with an unfavorable clinical outcome after adjustment for stage, grade, and extent of cytoreduction (Scholl et al. 1994).

Among cytokines with possible clinical relevance in ovarian carcinoma, mention should be made of IAP, a glycoprotein mainly produced by activated macrophages that was first detected by Tamura et al. (1981) in the sera of cancer patients and found to exert a powerful immunosuppressive activity both in vitro and in vivo (Shibata et al. 1983). High serum levels of IAP have been detected in ovarian cancer patients (Sawada et al. 1983, Castelli et al. 1991). IAP positivity was found in 70% of ovarian cancer patients but in only 4.5% of controls (Castelli et al. 1991).

A direct correlation between serum IAP level and CA 125 was found in a study of IAP in advanced ovarian cancer (Scambia et al. 1996). Moreover, in early-stage ovarian cancer the combined determination of CA 125 and IAP increased the assay sensitivity by about 30% compared with the determination of CA 125 alone (Castelli et al. 1991). IAP levels were found not to be associated with stage, histopathology, grade of differentiation and response to chemotherapy, suggesting that IAP determination could provide additional information about the prognostic characterization of ovarian cancer patients. High IAP levels were found to be associated with an unfavorable clinical outcome in both overall and progression-free survival. Moreover, the negative prognostic role of IAP was also observed when its level was used as a continuous variable in the Cox hazard regression analysis; the relationship between IAP level and outcome...
was found to be linear, suggesting that the risk of death or progression increased linearly as the IAP level increased (Scambia et al. 1996). Finally, the association of IAP with a poor prognosis was strengthened by the demonstration that the negative role of IAP was retained in multivariate analysis, taking into account several clinicopathological factors.

Several pieces of evidence emphasize the possible clinical role of macrophage-derived cytokines in the management of ovarian tumor patients. While their usefulness as tumor markers seems to be limited, their significance as new prognostic factors able to define high-versus low-risk patients offers new tools in individualizing treatment.

Finally, observations have been made that a relevant association exists between IL-6 and M-CSF since these two cytokines can be directly co-regulated in vitro (Schafsmo et al. 1989, Lidor et al. 1993); also, a direct correlation between these two cytokines has been observed in ovarian tumor patients (Foli E et al., unpublished observations). Therefore, the possible combination of several cytokines in improving the prognostic definition of ovarian cancer patients deserves further attention.

Cytokines in the treatment of ovarian cancer

Cytokines have been used for treating ovarian cancer patients basically in two ways: (1) to treat or prevent the myelosuppressive effects of chemotherapy and (2) directly to treat the disease itself. Cytokines have proved to be very useful in ameliorating the effects of bone marrow suppression caused by chemotherapy. G-CSF is widely used to prevent severe neutropenia in patients at risk (Klingemann 1989, Morstyn et al. 1989). Recombinant GM-CSF has also been used for this purpose. Recombinant human IL-3 may be effective combined with either GM-CSF or G-CSF in treating severe thrombocytopenia (Farber et al. 1997). Recombinant human IL-6 has also been administered to ovarian cancer patients to treat thrombocytopenia (D’Hondt et al. 1995). Recently, IL-11 has been shown to be effective in reducing thrombocytopenia (Tepler et al. 1996). The complete subject of cytokines used in treating granulocytopenia and thrombocytopenia is beyond the scope of this review.

One of the earliest cytokines to be used in cancer therapy was TNF-α. Early experiments in mice indicated that it was very effective, and intraperitoneal administration of TNF-α was used with some success in suppressing ascites in ovarian cancer patients (Kaufman et al. 1990). However, TNF-α has shown unacceptable toxicity in humans and there is evidence that it may even support tumor growth in some instances, despite the amelioration of ascites (Balkwill 1992).

Taxol, the first-line chemotherapeutic agent used in ovarian cancer, was recently shown to induce transcription of the IL-8 gene in ovarian cancer cells. This led some to speculate that IL-8 plays a role in the therapeutic effect of the drug (Lee et al. 1996), perhaps by inducing the migration of therapeutic lymphocytes to the tumor site. However, IL-8 has recently been attributed to neovascularization in ovarian cancer (Yoneda et al. 1998).

The potential for a therapeutic role for cytokines and other immunotherapy agents in the treatment of ovarian cancer could be enhanced by a better understanding of the factors in the microenvironment of the tumor that modify both the host response to the tumor and the malignant behavior of the tumor itself.

There is substantial evidence (reviewed in Freedman et al. 1998) that certain cytokines and other forms of immunotherapy have antitumor activity in ovarian cancer patients. In particular, recombinant IFN-γ has been shown to produce a number of surgically documented responses after injection into the peritoneal cavity (Pujade-Lauraine et al. 1996). To explore these powerful agents fully, it will be necessary thoroughly to understand the factors in the tumor environment that can either facilitate or inhibit the response to these agents. We are beginning to have a better understanding of the activity of immune inhibitory cytokines such as the TGF-β isotypes and IL-10, and the feasibility of developing strategies to overcome their inhibitory effects. In a recent experiment (A Loercher et al., unpublished results), ovarian cancer cell lines, following treatment with IFN-γ showed a reduction in the tumor cell expression of the immunosuppressive cytokines TGF-β1 and TGF-β2. It will be important to determine the cellular and stromal sites in the tumor microenvironment for activation of these TGF-β isotypes. Also, to discover any other molecules produced by these tumors that are important for regulating T-cell function, either upward or downward.

It is also important to realize that the immune system cannot function independently of other systems or factors in the host. Factors that are produced within the spectrum of lymphocyte activation can influence tumor behavior through other mechanisms, such as tumor adhesiveness, tumor cell migration, and neovascularization. It is only by developing an integrated approach to the problem that we will be able to develop better immunotherapeutic strategies.

Summary

Cytokines have very important roles in ovarian follicle development. They regulate the growth, differentiation, and death (apoptosis) of various cellular components of the ovary. They also regulate the number and composition
of the lymphocytes that produce many of the ovarian cytokines.

In some ways, tumor cells are able to upset the balance of cytokine expression in the ovary such that ovarian cytokine expression ultimately both augments the growth of the tumor and protects the tumor from being identified and destroyed by the immune system.

However, recent work suggests that cytokines such as IL-12 and IFN-γ may prove useful in treating ovarian cancer. Also, gene therapy approaches involving tumor cells expressing introduced cytokine genes may, some day, provide active tumor vaccines.

The purpose of this article has been to provide an overview of the important role that cytokines play in the development, spread, and treatment of ovarian cancer. We hope that it will lead to speculation regarding new treatment modalities for this highly refractory disease.

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