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Cytokines in tumour therapy

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Cytokines are low molecular weight proteins released by cells of the immune system that have therapeutic potential in cancer. They include the interleukins, the interferons, tumour necrosis factor and the colony-stimulating factors. Cytokines are capable of producing significant and sustained responses against a number of tumours. Clinically, the highest response rates to cytokine immunotherapy have been seen in melanoma and renal cell cancer. Current efforts aim to reduce treatment-related toxicity while maintaining the efficacy of cytokines. The therapeutic potential of these agents may be increased with genetic manipulation by introducing genes encoding cytokines into tumour-infiltrating lymphocytes and certain tumour cells. However, immunotherapy remains time consuming and expensive, and further developments are necessary before it can have a definitive role in tumour management.

Despite advances in surgical technique and improvements in chemotherapy, the ability to cure many patients with cancer still remains an ideal rather than a reality. There is a consensus that the three classic approaches to cancer treatment, i.e. surgery, radiotherapy and chemotherapy, have reached a plateau[1], although further developments in these will probably lead to refinements in the management of malignant disease. It has long been recognized that the immune system plays a pivotal role in the patient's response to disease. Recently, cancer therapies have therefore been directed at modulation of components of the immune system. In particular, 'immune messengers' or cytokines may play a vital role in regulating host antitumour defence mechanisms. Knowledge of cytokines and their functions is expanding rapidly[2]; this article reviews the cytokines and discusses their potential role in the treatment of malignant disease.

Cytokines are low molecular weight (10–50 kDa) proteins released by cells of the immune system. They bind with extreme specificity and great affinity to receptors on target cells and regulate their proliferation, differentiation and functional activation[3]. Cytokines produced by lymphocytes are called lymphokines; those produced by mononuclear phagocytes are called monokines. Cytokines are different from endocrine hormones in that they are produced by a number of cells rather than by specialized glands. They are not usually present in detectable levels in the serum and generally act on target cells in a paracrine (i.e. locally near the producing cells) or autocrine (i.e. directly on the producing cells) manner. Different cytokines display considerable overlap in their biological activities. The elucidation of various *in vitro* and *in vivo* properties of these proteins has led to their introduction into clinical medicine as therapeutic agents in the treatment of infection and cancer. The cytokines that have been shown to have therapeutic potential in treating cancer include the interleukins, the interferons, tumour necrosis factor (TNF) and the colony-stimulating factors.

Interleukins

The interleukins are a family of protein molecules that are fundamental to both cellular and humoral immune responses. At least 11 different types have been described, many with a potential antineoplastic role.

Interleukin 1

Interleukin 1 (IL-1) was originally described in the 1940s as 'endogenous pyrogen' because of its ability to produce fever when injected into experimental animals[4]. Two polypeptide forms are now recognized: IL-1α and IL-1β. IL-1α is cell associated and thought to be involved in antigen presentation[5]. IL-1β is the predominant form and is readily secreted from macrophages. Although they have only limited amino acid sequence identity, IL-1α and IL-1β recognize the same receptor and share the same biological properties. IL-1 is synthesized predominantly by mononuclear phagocytes, but can also be derived from a variety of other cells including endothelial cells, keratinocytes, neutrophils and B lymphocytes[6].

The actions of IL-1 have been elucidated in recent years. T cells proliferate at accelerated rates in the presence of IL-1 in response to mitogen[7]. IL-1 stimulates T cell proliferation by inducing production of interleukin 2 (IL-2) as well as increasing the number of IL-2 receptors on the T cell. It acts as an endogenous pyrogen[8] by stimulating local release of prostaglandins in the anterior hypothalamus and induces anorexia by acting on the central satiety centre. Furthermore, IL-1 attenuates the perception of pain after injury or inflammation by increasing the release of β-endorphins and increasing the number of opiate-like receptors in the brain[8]. It also protects against the effects of lethal radiation[9]. The metabolic effects of IL-1 include induction of hepatic acute-phase proteins, remodelling of connective tissue and bone during injury and inflammation, stimulating the activity of osteoclasts in bone, and degradation of collagen and proteoglycan in connective tissue[4].

Recombinant IL-1 is cytostatic towards human melanoma tumour cells *in vitro*[10]. Human peripheral blood monocytes stimulated with lipopolysaccharide release a substance with an activity that is cytotoxic for the A375 melanoma cell line. Biochemical and immunological characterization of this substance indicate that it is IL-1β. Finally, human recombinant IL-1β, purified to homogeneity, is directly cytotoxic for the A375 cell line[10].

IL-1 is identical to 'endogenous pyrogen' and its administration induces severe pyrexia, an unacceptable side-effect[11]. Although Lachman *et al.* have demonstrated that it has antitumour properties[10], IL-1 has not been employed in clinical trials because of these side-effects.

Interleukin 2

IL-2 was first characterized in 1976 by Morgan *et al.*, who reported the discovery of a protein, produced by normal peripheral blood lymphocytes, that induced proliferation of stimulated T lymphocytes[12]. This 15·5-kDa glycoprotein was

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initially called T cell growth factor but subsequently renamed IL-2. It is the pivotal molecule in the maintenance of an intact immune system and stimulates both T and B cell immunity. IL-2 is a vital cofactor in the development of cytotoxic T lymphocyte activity against tumours. In addition to its T cell growth-promoting effects, which allow maintenance of T cell lines and clones, IL-2 participates in tumoricidal activity[13,14] through growth of natural killer (NK) and lymphokine-activated killer (LAK) cells[15]. IL-2 also augments B cell growth and immunoglobulin production[16], and enhances interferon gamma (IFN-γ) production[17].

A number of studies using recombinant interleukin 2 (rIL-2) in both experimental animals and humans have shown that it has some activity against a variety of cancers[18,19]. Clinical immunotherapy protocols with IL-2 were initiated in 1980 in the Surgery Branch of the US National Cancer Institute when cultured peripheral blood lymphocytes were safely infused into three patients[20]. Early efforts at adoptive immunotherapy[21] dealt with NK cells activated either by the lectin phytohaemagglutinin or by crude culture supernatants containing IL-2. Subsequent studies used purified homogenous natural IL-2 derived from the Jurkat high-producer cell line[22]. When bacterially produced rIL-2 became available this was used in clinical trials. More recent studies[23–25] have utilized either high doses of rIL-2 alone or the adoptive transfer of LAK cells generated using rIL-2. In 1985 the first report was published documenting tumour regression in patients with melanoma following administration of IL-2 with LAK cells[24]. LAK cells are peripheral blood lymphocytes that can be generated *in vitro* by incubation with high-dose IL-2. They possess the ability to kill cancer cells but not normal cells. IL-2 is used in combination with LAK cells to produce a more potent antitumour response[25,26].

Cytotoxic T lymphocytes are a subset of T cells that kill target cells expressing specific antigen. To recognize and respond to a foreign antigen, a T cell must be presented in a complex with a self major histocompatibility complex (MHC) molecule. The expression of MHC proteins on tumour cells may be critical for their immunological recognition and destruction. NK cells are effector cells of natural and acquired immune responses to tumours. They utilize the same lytic mechanisms as cytotoxic T lymphocytes to kill tumour cells; however, they do not express T cell antigen receptors and they kill targets in an MHC-unrestricted manner. This difference in mechanism of action may explain why melanoma responds to cytotoxic T lymphocyte-specific therapy whereas other tumour types tend not to respond.

Tumour-infiltrating lymphocytes are lymphoid cells that infiltrate solid tumours and can be grown[20] by culturing suspensions of lymphoid cells from tumours in IL-2. Tumour-infiltrating lymphocytes obtained from some human melanomas have unique lytic specificity for autologous tumours[27,28] and the use of tumour-infiltrating lymphocytes and IL-2 together can mediate substantial tumour regression in some patients with advanced malignant melanoma[29,30]. Kradin *et al.*[31] reported objective tumour responses lasting 3–14 months in 29 per cent of patients with renal cell cancer and 23 per cent of those with melanoma treated with tumour-infiltrating lymphocytes and IL-2.

Further potential for IL-2 in antitumour therapy has been established by using rIL-2 in combination with other cytokines[32]. In several murine tumour models, rIL-2 in combination with recombinant interferon alpha (IFN-α) elicits a potent antitumour response which is often greater than that which can be reached with the individual agents at non-toxic doses[33]. IL-2 *in vitro* promotes the release of cytokines, including IFN-γ and TNF, which also possess antileukaemic activity and can enhance granulocyte function. This secondary cytokine release of IFN-γ and TNF by IL-2 may contribute to the antileukaemic effects of IL-2 in patients following bone marrow transplantation or chemotherapy[34]. Bello-Fernandez *et al.* have shown that interleukin 4 (IL-4) acts as a homoeostatic regulator of IL-2-induced TNF and IFN-γ release[35]. The addition of recombinant IL-4 to IL-2-stimulated cultures leads to significant inhibition of IFN-γ and TNF production. Since both TNF and IFN-γ may contribute to the antineoplastic action of IL-2, manipulating the level of IL-4 activity *in vivo* could augment the benefits of IL-2 immunotherapy[35]. IL-2 in combination with interferon beta (IFN-β) has been shown to have a 27 per cent objective response rate in 22 patients with advanced renal cell carcinoma[36].

IL-2 has been given to patients in combination with chemotherapeutic agents in an attempt to obtain possible synergistic effects. A regimen using low-dose cyclophosphamide (350 mg m^{-2}) in combination with IL-2 in 32 patients with either malignant melanoma or renal cell carcinoma achieved only minor antitumour activity[37]. Dillman *et al.* evaluated rIL-2 plus LAK cells alternating with sequential dacarbazine chemotherapy in 27 patients with metastatic melanoma[38]. This therapeutic protocol was reasonably tolerated, but the response rate was not significantly better than that achieved with other IL-2 regimens or with chemotherapy alone[38]. Paciucci *et al.* administered IL-2 with weekly doxorubicin to 12 patients with advanced malignancy[39], demonstrating a partial response in five. However, toxicity of the regimen proved to be a significant problem.

The most significant antitumour responses associated with IL-2 therapy have been identified in the treatment of malignant melanoma[40–42] and renal cell carcinoma[43–51]. Other tumours treated with IL-2 include gliomas[52,53], bladder[54,55], ovarian[56], neuroblastoma[57], lung[58], head and neck[59], breast, lymphoma, colon and mesothelioma[19].

IL-2 therapy is associated with significant side-effects including pronounced fluid retention, anaemia, thrombocytopenia and hypotension[60]. Marked immunological changes have been observed in patients receiving this cytokine: raised levels of lymphocytes bearing IL-2 receptors, free circulating IL-2 receptors, and the presence of monocytes bearing IL-2 receptors[61]. The significance of these findings is unknown, but they occur in the presence of a marked lymphopenia which resolves within 48–72 h. Regrettably, there seems little doubt that intravenous IL-2 therapy alone is toxic. A review of 285 patients with renal cell carcinoma treated with IL-2 alone demonstrated that the antitumour activity was dependent on both schedule and intensity of the dose administered[62]. Controversy persists over the relative merits of regimens devised to reduce the toxicity; Rosenberg has described a high-dose bolus regimen[18] while West *et al.* have utilized a continuous-infusion regimen[19].

IL-2 is the first cytokine to be employed widely in clinical trials. It provides significant antitumour responses in patients with malignant melanoma and renal cell carcinoma, but its side-effects have limited greater clinical use. Usage will expand with the further development of adoptive immunotherapy using LAK and tumour-infiltrating lymphocytes as well as in combination with other cytokines.

Interleukin 3

Interleukin 3 (IL-3) is released from T cells stimulated with the mitogen phytohaemagglutinin[63]. Synonyms for this cytokine are 'colony forming unit-stimulating activity' and 'multi-colony stimulating factor'. Murine IL-3 is a polypeptide of 133 amino acids with a molecular weight[64] of 30–40 kDa. IL-3 is produced by T lymphocytes as well as by a monomyelocytic cell line (WEHI 3), and activates lymphocytes as well as mast cells[65]. Reported activities include the stimulation of mast cells, neutrophils, macrophages and megakaryocytes from isolated haematopoietic progenitors[66]. To date, no study has identified IL-3-mediated antineoplastic activity, but this cytokine has a role in increasing platelet and neutrophil counts in patients with advanced malignancy[67].

Phase I and II trials with recombinant human interleukin 3 (rIL-3) expressed in yeast have been carried out in patients with advanced malignancy as well as in those with bone marrow

suppression. Subcutaneous administration of rIL-3 at doses of 30–500 $\mu g\ m^{-2}$ for 15 consecutive days in patients with advanced malignancy but normal haematopoiesis resulted in a dose-dependent increase in platelet counts, as well as a substantial increase in the number of circulating neutrophils, eosinophils, monocytes, and lymphocytes[67]. In patients with secondary haematopoietic failure caused by prolonged chemotherapy[68], radiotherapy or bone marrow infiltration by tumour cells, treatment with rIL-3 leads to a clinically significant restoration of haematopoiesis, especially of thrombopoiesis and granulopoiesis. Adverse effects of rIL-3 are minor at therapeutic doses and include fever, bone pain, headache, and neck stiffness[67]. Recombinant human IL-3 is a multilineage haematopoietic cytokine with promising effects on platelet and neutrophil count augmentation in patients with advanced malignancy.

Interleukin 4

IL-4 is a T cell-derived glycoprotein of molecular weight 20 kDa that was originally characterized as 'B cell stimulatory factor 1'[69]. It was initially discovered as a result of experiments by Howard *et al.* demonstrating its proliferative effects on small B cell subset proliferation[70]. Human IL-4 has also been cloned. It is a 129 amino acid glycoprotein secreted primarily by activated T cells that acts on T and B lymphocytes, monocytes, polymorphonuclear cells, fibroblasts and endothelial cells.

Antineoplastic functions of IL-4 include enhancement of B cell[70], T cell[71], NK cell[72] and mononuclear phagocyte[73] proliferation. IL-4 can also enhance the generation of cytotoxic T cells, mediate induction of LAK cells and synergize with IL-2 in this activity[74]. IL-4 has been shown to inhibit IL-2-induced LAK activity in normal human mononuclear cells[75], but augments LAK activity in mononuclear cells pretreated[76] with IL-2 and such cells derived from IL-2-treated patients with cancer[77]. Furthermore, IL-4 has been shown to stimulate the generation of tumour-infiltrating lymphocytes in human melanoma[72].

Functions on human monocytes and mouse macrophages have also been reported. IL-4 has been shown to increase antigen-presenting ability, surface Ia expression and tumoricidal activity in mouse macrophages[73,78]. It also appears to inhibit TNF, IL-1 and interleukin 6 (IL-6) release[79]. However, effects on respiratory burst activity are conflicting[80].

IL-4 has been demonstrated to have potent antitumour activity by experiments in which a complementary DNA for IL-4 was introduced into the J558 murine melanoma cell line[81]. IL-4-producing J558 cells fail to form tumours in mice and block tumour formation by a variety of other transplantable tumour lines when these cells are cocultured with IL-4-producing J558 cells. The effects of the transfected J558 cells can be inhibited by treating the recipient mouse with anti-IL-4 antibody. Forni *et al.* have reported that repeated injections of small amounts of IL-4 into draining lymph nodes can limit the growth of cells of several transplantable tumours[82].

The mechanism by which IL-4 limits tumour cell growth has not been completely established. Tepper *et al.*[81] reported that IL-4 failed to block the *in vitro* growth of transfected J558 cells, indicating that it was not directly toxic to them. They showed that the IL-4-producing J558 cells failed to grow in *nu/nu* mice, suggesting that T cell immunity was not essential for the elimination of the transplanted cells. Redmond *et al.* have demonstrated a beneficial role for IL-4 in upregulating macrophage and T cell cytotoxicity directed against the Lewis lung carcinoma[83].

IL-4-transfected tumour cells have been used to demonstrate the potential of lymphokine gene-transfected tumour cells as a method of cancer therapy[84]. When cells from a spontaneously arising murine renal cell tumour were engineered to secrete large doses of IL-4 locally, they were rejected in a predominantly T cell-independent manner. However, animals that rejected the IL-4-transfected tumours developed T cell-dependent systemic immunity to the parental tumour. This systemic immunity was tumour specific and mediated primarily by $CD8^+$ T cells. Established parental tumours could be cured by the systemic immune response generated by injection of the genetically engineered tumours[84].

The role of IL-4 as an antitumour agent, particularly against spontaneous tumours, remains to be more fully elucidated. None the less, these studies suggest that this cytokine may marshal endogenous host responses in a particularly potent way to limit the growth of certain types of tumour.

Interleukin 5

Interleukin 5 (IL-5) has a molecular weight of 18 kDa. It is a product of activated T lymphocytes and is a lineage-specific eosinophil differentiation and activation factor[85]. IL-5 acts as a costimulator for the growth of antigen-activated mouse B cells and was previously called either 'B cell growth factor 2' or 'T cell replacing factor'.

Murine IL-5 induces eosinophil colonies in human liquid bone marrow cultures and the antibody-mediated killing of tumour cells by peripheral blood eosinophils. It also enhances phagocytosis of serum-opsonized yeast cells by eosinophils[86]. A definitive role for IL-5 as an antineoplastic agent awaits evaluation.

Interleukin 6

IL-6 was initially described as 'β2-interferon' and is also known as 'B cell differentiation factor' and 'hepatocyte-stimulating factor'. It is a 19–26-kDa protein produced by a variety of cells including mononuclear phagocytes, fibroblasts, keratinocytes and endothelial cells[87]. IL-6 is released in response to endotoxin (lipopolysaccharide) and appears to be regulated by IL-1 and TNF. It is released into the circulation in response to injury[88]; its principal functions include stimulation of B cells for immunoglobulin production, promotion of the synthesis of numerous acute-phase proteins and pyrogenic activity by increasing the production of prostaglandins[89].

IL-6 has been shown to augment the growth of freshly isolated human myeloma cells, and the cells constitutively produce IL-6 and express IL-6 receptors[90]. Moreover, anti-IL-6 antibody can inhibit the *in vitro* growth of the myeloma cells[90]. This is direct evidence that an autocrine loop is operating in freshly isolated myeloma cells, and that constitutive production of IL-6 and activation of the IL-6 gene could be involved in the oncogenesis of human myeloma.

Mulé *et al.* have shown that IL-6 has antitumour activity when administered to tumour-bearing mice. Purified human rIL-6 administered to tumour-bearing mice at relatively high doses (33 μg per day) caused substantial reductions in the number of pulmonary and hepatic micrometastases. The dose regimen used was comparable to therapeutic levels of IL-2. Unlike IL-2, IL-6 injections resulted in neither observable toxicity nor death of the treated mice at the doses used[91]. To date, no human studies have been carried out to evaluate the potential antineoplastic effects of IL-6.

Interferons

The interferons are a family of regulatory glycoproteins produced by many cell types in response to viral infections, double-stranded RNA, endotoxin (lipopolysaccharide) and a variety of mitogenic and antigenic stimuli. Human and murine interferons have recently been reclassified as IFN-α, IFN-β (previously type I leucocyte and fibroblast respectively), and IFN-γ (previously type II, or 'immune' interferon). There are heterogeneities within these groups and considerable species specificity.

IFN-α has produced some remarkable results in hairy cell leukaemia[92] and in the carcinoid syndrome[93], but has failed to be of benefit in secondary disease from breast cancer, malignant melanoma and colorectal cancer[94,95]. A response rate of 15 per cent has been observed in patients with advanced renal cell carcinoma treated with IFN-α[96].

Phase II studies of high-dose IFN-α in patients with advanced colorectal carcinoma have not demonstrated any therapeutic responses[94,97]. However, *in vitro* studies have shown that IFN-α synergistically augments the cytotoxic effects of 5-fluorouracil (5-FU) against two human colonic cancer cell lines[98]. A total of 32 patients with previously untreated irresectable colorectal carcinoma received 5-FU 750 mg m^{-2} daily for 5 days by continuous infusion followed by weekly bolus therapy. IFN-α was administered subcutaneously three times a week. A 63 per cent partial response rate was achieved, compared with rates of 15–35 per cent with single-agent 5-FU therapy. These findings are encouraging, but proof of superiority over single-agent 5-FU requires controlled clinical trials, currently in progress[98,99]. The chemotherapeutic agent dacarbazine exhibits activity against metastatic melanoma. When the combination of IFN-α and dacarbazine was evaluated in a pilot study of 44 patients, a 30 per cent response rate was demonstrated. These results, however, remain to be confirmed in a controlled clinical trial[100].

Both murine and human IFN-γ exert their biological effects through specific saturable binding to a single class of high-affinity receptors. These receptors can be found in a number of different tissues including myelomonocytic cells, lymphoid cells, mast cells, endothelial cells, fibroblasts, neuronal cells and melanocytes[101]. Other than its antiviral activity, IFN-γ has been shown to inhibit cell growth in the presence of lymphotoxin (TNF-β)[101] and to induce MHC class I and II expression in target tissues[102–105]. IFN-γ is a cofactor in the activation of macrophages for tumour killing and enhances NK-cytotoxicity[101,106]. It plays an important role in B cell differentiation and has been shown to induce and enhance immunoglobulin secretion in resting B lymphocytes[107–109]. IFN-γ may prove to have clinically significant antineoplastic effects; it may also prove effective in the treatment of a variety of autoimmune diseases. For example, recent data suggest that it may be used to ameliorate some of the symptoms of rheumatoid and psoriatic arthritis[110].

Tumour necrosis factor

TNF is a proinflammatory cytokine that derives its name from its ability to induce necrosis in experimental animal tumours[111]. It is identical to 'cachectin' and was initially isolated during studies aimed at defining the underlying mechanisms of cancer cachexia[112]. It is produced primarily by mononuclear phagocytes in response to endotoxin (lipopolysaccharide)[113]. TNF is initially produced as an inactive propeptide and in active form is a polypeptide existing in multimers of two, three or five subunits.

There appear to be two forms of TNF, one of which (TNF-α or cachectin) is a cytotoxic factor with a molecular weight of 17 kDa released by macrophages following their activation by bacterial endotoxin (lipopolysaccharide)[112]. TNF-β, also known as 'lymphotoxin'[114], has a molecular weight of 18 kDa and is released from stimulated lymphocytes. Both forms have been produced by recombinant DNA technology and appear to have antiproliferative, cytostatic and cytolytic effects against human tumour cells either *in vitro*[115,116] or when injected into nude mice[117]. Human recombinant TNF-α also enhances the cytolytic activity of NK cells[118] and so may theoretically mediate tumour regression through this mechanism. Preliminary reports on the clinical administration of recombinant TNF-α do not indicate significant therapeutic effects and describe considerable toxicity[119]. Although initial phase I and II studies using TNF in humans have shown the expected toxicity, few antitumour effects have been observed[119]. However, a role for TNF in cancer therapy may exist when used in combination with either biological or chemotherapeutic agents. The combination of TNF-α with other biological response modifiers such as IFN-γ has theoretical advantages and appears greatly to augment TNF-mediated tumour regression in human breast and colonic cancers implanted into nude mice[120]. However, clinical trials of IFN-γ and TNF have been abandoned because of serious toxicity problems, emphasizing that toxicity from cytokines remains a major factor in limiting their use in immunotherapy.

Colony-stimulating factors

The colony-stimulating factors are a group of glycoproteins with the ability to control the proliferation and differentiation of granulocytes, mononuclear phagocytes and certain related haematopoietic cells. They include IL-3 (multi-colony-stimulating factor), macrophage colony-stimulating factor (M-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF).

M-CSF is produced by monocytes, fibroblasts and endothelial cells, and stimulates the formation of macrophage colonies[65]. G-CSF is a product of monocytes and fibroblasts that acts directly on target progenitors to induce the formation of granulocyte colonies *in vitro*[121,122]. GM-CSF is a glycoprotein secreted by activated T cells, endothelial cells, fibroblasts and macrophages[123]. This cytokine provides an effective proliferative stimulus in human marrow cultures to granulocyte, macrophage and eosinophil colonies. It is also capable of stimulating antibody-dependent cytolysis of tumour cells by mature human neutrophils and eosinophils[124]. An important pathway in antitumour defence may be provided by GM-CSF as it induces macrophage tumoricidal activity[125]. *In vivo* administration of GM-CSF decreases tumour growth in a murine Lewis lung carcinoma model[126].

Both GM-CSF and G-CSF are significantly beneficial in the treatment of leucopenia[127,128]. Studies in monkeys show that combined administration of GM-CSF and IL-3 dramatically increases circulating leucocyte levels. Furthermore, phase I and II clinical trials currently underway indicate that GM-CSF may be effective in accelerating the recovery of patients with leukaemia following autologous bone marrow transplantation[129]. GM-CSF is of benefit to patients with myelodysplastic syndrome[130] and it has been observed that neutropenic patients with acquired immune deficiency syndrome receiving daily doses for 14 days exhibit marked leucocytosis[131]. Furthermore, patients with inoperable metastatic sarcomas receiving doxorubicin, ifosfamide and dacarbazine experienced a reduction in the duration and degree of neutropenia when treated with GM-CSF[127].

Similarly, G-CSF seems to shorten the period of neutropenia associated with chemotherapy in animals and humans. Monkeys treated with G-CSF exhibited a dose-dependent increase in leucocyte count, and the duration of granulocyte recovery in phosphamide-treated groups was considerably shortened[132]. Patients with transitional cell carcinoma of the bladder, treated with methotrexate, vinblastine, doxorubicin and cisplatin experienced up to a fourfold increase in neutrophil count on administration of G-CSF, with little or no toxicity observed[133]. In contrast, purified human urinary M-CSF given to leucopenic patients produced only modest effects on circulating leucocyte levels[134]. However, M-CSF may act to enhance monocyte function[135] and may yet prove to be of some therapeutic benefit.

Other cytokines

Other interleukins, such as interleukin 7, interleukin 8 (IL-8), interleukin 9, interleukin 10 and interleukin 11, have not been studied for specific antitumour activity[136]; their role in cancer immunotherapy awaits further evaluation. However, caution is needed in suggesting that all these may be useful in tumour-bearing patients; in some instances cytokines may actually harm patients. IL-8 has been characterized as a neutrophil-activating protein; its intravenous injection in rabbits leads to immediate and profound neutrophilia[137]. The potential of these cytokines to induce cancers by their effects on cellular proliferation must be addressed carefully.

Natural killer cell stimulatory factor (NKSF) is a recently discovered cytokine purified from the supernatant of an Epstein–Barr virus-transformed B lymphoblastoid cell line (RPMI 8866). It has been examined for its effect on NK- and antibody-dependent cellular cytotoxicity of human colon adenocarcinoma cell lines[138]. NKSF significantly enhanced NK cytolysis of colonic carcinoma and NK-resistant lymphoma cell lines, and on a molar basis was approximately 300 times more potent than IL-2 in generating NK cytotoxicity. Furthermore, NKSF significantly augmented lymphocyte-mediated antibody-dependent cellular cytotoxicity against colonic carcinoma targets, and the combination of NKSF with the antibody CO17-1A had an additive effect on lymphocyte tumoricidal capacity. Thus, NKSF may have a potential role in the treatment of colonic cancer[138].

Conclusions

Knowledge of cytokines and their precise physiological and therapeutic roles is still in its infancy, but understanding of their function is expanding rapidly. At present the role of many cytokines in tumour immunology is still being evaluated in animal models.

One of the more interesting developments in the use of cytokines in cancer therapy is 'adoptive immunotherapy' or cell-transfer therapy. Cells of the immune system are removed from a patient and their native cytotoxic activity enhanced *in vitro* by challenge with a cytokine (usually IL-2). The cells are then returned to the patient in combination with the cytokine[18,139]. Tumour-infiltrating lymphocytes require less IL-2 to remain active *in vivo* and the overall dose of IL-2 can be reduced significantly using this method, relieving some of the toxicity of IL-2 therapy.

The possibility of using genetic manipulation to increase the therapeutic potential of adoptive immunotherapy, by introducing genes encoding cytokines such as TNF, IFN-α or IL-2 into tumour-infiltrating lymphocytes, has recently been addressed. Rosenberg *et al.* have undertaken gene transfer therapy in humans. Using retroviral gene transduction they have introduced a marker, the bacterial neomycin resistance gene, into human tumour-infiltrating lymphocytes[140]. Following administration of the 'engineered' lymphocytes to a group of patients with metastatic melanoma, neomycin-resistant cells were present in the circulation and could be recovered from tumour biopsies for up to 2 months with no apparent adverse effects of genetic manipulation[140]. Immunotherapy with genetically engineered lymphocytes would allow delivery of significant amounts of cytokine directly to the microenvironment of the tumour. Clinical trials of such cells, genetically engineered to produce TNF, have begun at the US National Cancer Institute[141]. More recent attempts at using genetic manipulation to increase the therapeutic potential of adoptive immunotherapy include the use of gene-transfected tumour cells[84]. Established parental tumours may be cured by the systemic immune response generated by injection of genetically engineered tumours[84].

To date, immunotherapy has not fufilled its initial promise – a realistic inroad into cancer therapy. It remains time consuming and expensive, and further developments will be needed before it can become a commonplace treatment.

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