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Cancer immunotherapy: potential involvement of mediators

S. Ben-Efraim

Department of Human Microbiology, Sackler Faculty of Medicine Tel-Aviv University, Tel-Aviv 69978, Israel
Fax: (+972) 3 6409160
Tel: (+972) 3 6416682

The description of a cell-free soluble anti-tumour factor by Carswell *et al.* in 1975 (*Proc Natl Acad Sci USA*, 72: 3666–3670) was followed by a long series of experimental and clinical investigations into the role of cell-free mediators in cancer immunotherapy. These investigations included research on the effects of macrophage-derived eicosanoids (cycloxygenase and lipoxygenase derivates of arachidonic acid) and of monokines such as tumour necrosis factor-α, interleukin-1 and granulocyte–monocyte–macrophage-colony stimulating factor) and of lymphocyte products: interleukins and interferons. The investigations yielded information on the effects of various factors on macrophage and T-cell activation *in vitro*, determination of direct anti-tumour properties on animal and human tumour cells *in vitro* and on therapeutic effectiveness in tumour-bearing individuals either alone or in combination with other therapeutic factors and their production by tumour cells. During recent years much effort has been dedicated towards the use of the tumour cells transfected with cytokine genes in the preparation of cancer vaccines. Cycloxygenase products (prostaglandins) were usually assumed to inhibit expression of anti-tumour activity by macrophages and an increase in their production in cancer patients was considered as a poor prognostic index. Lipoxygenase (leukotrienes) products were assumed to exhibit anti-tumour activity and to induce production of IL-1 by macrophages. Interleukins 2, 4, 6, 7, 12 and the interferons were extensively tested for their therapeutic effectiveness in experimental tumour models and in cancer clinical trials. The general conclusion on the use of cell-free mediators for cancer immunotherapy is that much still has to be done in order to assure effective and reproducible therapeutic effectiveness for routine use in the treatment of human neoplasia.

Key words: Cancer immunotherapy, Cytokines, Eicosanoids, Interferons, Interleukins, Leukotrienes, Prostaglandins

Introduction

The field of cancer immunotherapy began approximately 100 years ago with rather 'naive' attempts to use anti-tumour antibodies raised in various animals for treatment in human sarcoma patients.[1,2] At around the same time Coley was probably the first (1891, cited in reference 3) to suggest that cell-free filtrates of bacteria might posses anti-tumour activities. Macrophages are known to produce and secrete derivates of arachidonic acid and cytokines (such as TNF-α and Interleukin-1 (IL-1). T and B cells produced and released a long series of interleukins; both macrophage and T and B-cell products are involved in the interaction between the immune system and tumour cells in a number of ways: direct anti-tumour activity, activation of immunocompetent cells *in vivo* and/or *in vitro*, and changes in their production *in vivo* in tumour-bearing hosts. Finally, it was shown that tumour cells themselves might secrete some of the above mentioned products and develop a means to resist the anti-tumour activity of various biologically active products.

The data accumulated on the activities of macrophages and T-cell products against tumour cells helped to devise certain immunotherapeutic protocols first in experimental animal tumour systems and afterwards in human neoplasia. The immunotherapeutic protocols were based on either treatment with cell-free products alone or in combination with other treatments. They also helped in devising ways to activate *in vitro* immuno-competent cells for therapeutic uses *in vivo*.

The aim of this review is to discuss the potential involvement of cell-free mediators in cancer immunotherapy. On reviewing their anti-tumour activities it should be mentioned that some of these mediators are also involved in inflammatory processes and that their production is closely interelated.

Eicosanoids and Cancer

Cycloxygenase (prostaglandins) and lipoxygenase products have been used in experimental tumour systems and are involved in human neoplasia.

Prostaglandins

It has generally been assumed that prostaglandins (especially PGE_2) inhibit anti-tumour activity of macrophages. Thus a PGE_2 inhibitor, indomethacin, enhanced the macrophage cytostatic activity *in vitro* against MOPC-315 murine plasmacytoma cells.[4] Indomethacin stimulation of macrophage cytostasis was inhibited by PGE_2[5,6] and this enhancement of anti-tumour activity was also reported *in vivo* against Ehrlich murine tumour cells.[7–10] The effect of endogenous and exogenous prostaglandins on macrophage functions was described: culture conditions that caused increased PGE_2 production by activated macrophages resulted in an inhibition of their tumoricidal activity whereas production of high levels of PGE_2 by resident and elicited macrophages was associated with an increase in their tumoricidal activity.[11] In another work,[12] it was reported that a subcutaneous injection of polyacrylamide beads in mice induced a population of immature macrophages which became fully cytostatic to syngeneic P815 plasmacytoma when stimulated *in vitro* by LPS. Blocking of PGE synthesis by indomethacin prevented the effect of LPS and addition of PGE_2 did not reverse the indomethacin effect but inhibited the macrophage-mediated cytostatic activity.[12] Suppression of macrophage-mediated tumour cytotoxicity was correlated with an increase in secretion of prostaglandin from the macrophages of breast cancer patients.[13,14] Elevated prostaglandin production in human breast cancer was considered a marker of high metastatic potential for neoplastic cells, the increase in PG production occurred early in the course of breast cancer and decreased later in the course of tumour development.[15] It seems that PGE_2 production by human monocytes is by a subset of cells other than the cells which produce IL-1.[16] It should also be mentioned that cancer cells also produce PGE_2 and in this context it was reported that the amount of PGE_2 released by cancer cells which metastasized into the liver of tumour-bearing rats was higher than that of cells metastasizing into the kidney.[17] An increase in prostaglandin levels in cancer patients was also reported:[18] of plasma prostaglandin F levels in cases of tumours of the female genital tract and of plasma 6-oxo-prostaglandin $F_{1\alpha}$ in cases of gynecological tumours.[19,20]

On the assumption that prostaglandins can suppress the development and expression of effector cells, clinical studies were initiated to determine the effect of piroxicam (a prostaglandin antagonist) in patients with recurrent unresectable squamous cell carcinoma or lymphoepithelial carcinoma of the head and neck.[21] Although some improvement in immune reactivity was noted, more studies to correlate the improvement in immune reactivity with therapeutic effectiveness of either prostaglandin antagonists alone or in combination with other treatments are needed.[21–23] A paradoxical effect of indomethacin on lymphokine-activated killer cell (LAK) activity in cancer patients was described: indomethacin enhanced LAK activity in patients with no distant metastases but depressed LAK activity in patients with such metastases.[24] Apparently, these effects of indomethacin are not related to the PGE inhibiting property of this compound.[24] Increased synthesis of prostaglandin by macrophages from breast cancer patients was assumed to inhibit macrophage mediated cytotoxicity.[25]

Leukotrienes

Leukotrienes (lipoxygenase pathway of arachidonic acid) are usually considered to enhance the potential of the immune response.[26,27] Thus, leukotriene and indomethacin enhance additively the macrophage anti-tumour cytostatic function.[4,28] Leukotriene B4 (LTB4) was reported to augment human monocyte cytotoxic activity and enhance monocyte production of hydrogen peroxide, IL-1 and TNF.[26] 5-Lipoxygenase activation was also described to facilitate

an IL-1 transduction signal.[29] Augmentation by leukotrienes of IL-1 production by human monocytes was also described in other reports.[30,31] Lipoxygenase specifically inhibited indomethacin stimulation of anti-tumour macrophage cytostasis[32] and reversed macrophage cytostasis, induced by the calcium ionophore A23187, towards P815 tumour cells *in vitro*.[31] Leukotriene C4 was reported to be an essential 5-lipoxygenase intermediate in A23187-induced macrophage cytostatic activity against P815 tumour cells.[33] Products of the lipoxygenase pathway were involved in human natural killer cell cytoxicity.[34]

Cytokines

Immunocompetent cells reported to produce and secrete cytokines involved in the reaction of the organism to tumour cells were macrophages, T and B cells.

Macrophage-derived cytokines

Biologically active products described as being involved in the interaction with tumour cells were tumour necrosis factor-α (TNF-α) interleukin-1 (IL-1) and granulocyte–macrophage colony stimulating-factor (GM-CSF).

TNF-α

The first description of TNF-α as a macrophage product was made by Carswell *et al*.[35] Since then, several reviews have appeared which have been concerned with the characterization and properties of this cytokine.[3,36–38] The mechanism and activity spectrum of TNF-α has been the topic of several investigations: it was reported that exposure of human cervical carcinoma cells to Concanavlin A (ConA) increased the total number of binding sites for rTNF-α but blocked the transduction of the signal for the cytotoxic response.[39] MethA sarcoma cells were found to be sensitive *in vitro* to human tumour recombinant TNF-α and expressed low numbers of TNF-α receptors.[40] TNF-α (unlike INF-γ or IL-1α), induced regression of subcutaneous MethA implants.[40] It was assumed that the primary lesion induced by TNF-α is vascular and the mechanism(s) involved in generation of specific cell-mediated anti-tumour immunity induced by the TNF-α treatment was not clear.[41] It was claimed that there are multiple pathways leading to resistance to TNF-α induced tumour cell cytotoxicity, among them production of transforming growth factors by tumour cells and amplified expression of certain oncogenes.[41] Apparently, viable activated monocytes produce other lytic factors in addition to TNF-α and IL-1 because TNF-α and IL-1 associated with plasma membranes of activated human monocytes lyse only monokine-sensitive tumour cells whereas viable activated monocytes lyse both monokine-sensitive and monokine-resistant tumour cells.[42] The killing of tumour cells by TNF-α was assumed to involve internalization of this ligand in the target cells.[43] The anti-tumour effect of TNF-α and of Interferon-γ against MmB16 murine melanoma was potentiated by macrophage colony-stimulating factor.[44] The anti-tumour activity of recombinant human TNF-SAM1 was enhanced by connecting the TNF compound to thymosin β4.[45] TNFα acted synergistically with IL-1 in inhibiting the growth of A375 cells.[46] An experimental study carried out in nude mice showed that intratumoral injection of an adenoviral vector containing radiation inducible DNA sequence of the *Egr-1* promoter linked to a cDNA encoding TNF-α (Ad.Egr-TNF) enhanced the tumoricidal action of ionizing radiation in a human epidermoid carcinoma xenograft.[47] By another experimental approach it was shown that murine tumour cells transduced with the gene for TNF-α, regressed unlike non-transduced tumour cells after an initial phase of tumour growth.[48]

The results *in vitro* and *in vivo* from experimental tumour models which indicated the anti-tumour activity of TNF-α, prompted initiation of clinical trials devised to determine the therapeutic effectiveness of TNF-α in human neoplasia. Intravenous injection of TNF-α in 18 cancer patients led to some clinical improvement in three lymphoma patients.[49] Recombinant TNF-α was given in combination with TNF-γ in phase I trials in 36 patients with solid tumours. Side-effects such as fatigue, fever and chills occurred: in one patient with melanoma there was a mixed response and in one patient with mesothelioma there was transient clearance of ascites from malignant cells.[50] Disappointing results were reported in a phase II study of recombinant human TNF-α in 127 cancer patients. The conclusion of the authors was that: "rhuTNFα does not appear to have significant anti-tumour activity".[51] A similar conclusion was made in a phase II trial of rTNF-α in 22 patients with adenocarcinoma of the pancreas: "No objective responses were observed".[52] A phase II study of recombinant TNF-α was also performed in a group of 26 renal cell carcinoma patients. The conclusion was: "rTNF given as described, has only modest anti-tumour activity in renal carcinoma and produces considerable toxicity. We plan no further studies of rTNF in this disease".[53] In a phase I study including 16

evaluable patients with various types of metastatic cancer, there was evidence for anti-tumour effect in two patients.[54] The property of TNF-α as an immunomodulator was reported:[55] pretreatment of monocytes with IFN-α, IFN-γ, IL-1 or TNF-α resulted in enhanced human monocyte toxicity.

Interleukin-1

The production, characterization and properties of IL-1 have been summarized in several reviews.[56,57] It was stated that IL-1 is a key mediator of host response to microbial invasion and that it acts as a true hormone produced during infection and inflammation.[57] Human recombinant IL-1 (hrIL-1) induced proliferative responses of T cells in the presence of sub-optimal concentrations of mitogen and doubled the response to higher concentrations.[57] Human recombinant IL-1 induced release of IL-2 by T cells and acted as a potent inflammatory agent by inducing dermal fibroblast PGE_2 production *in vitro* and of fever in rabbits and mice.[57] The overall conclusion from these data was that IL-1 possesses both immunological and inflammatory properties.[57] Human Interleukin-1 acted as a cytocidal factor for several tumour cell lines[58] and promoted human monocyte-mediated tumour cytotoxicity.[59] Human monocytes stimulated with pneumococcal cell surface components produced IL-1 but not TNF, thus showing independence of production of the two cytokines.[60] As mentioned already[46] IL-1 acted synergistically with TNF-α against tumour cells. IL-1α enhanced carboplatinum anti-tumour activity against human ovarian cells *in vitro* and *in vivo*.[61] In an *in vitro* study with human melanoma cell lines it was shown that tumour cells which secrete IL-1 exhibited increased adhesion to endothelial cells.[62]

Granulocyte–macrophage colony stimulating factor (GM-CSF)

The effect of intravenous and intraperitoneal administration of GM-CSF was examined in a phase I trial of 13 cancer patients refractory to standard chemotherapy.[63] Administration of MG-CSF was well tolerated but no data were provided on clinical improvement.[63] In another study, it was reported that administration of GM-CSF in 24 patients with solid tumours enhanced monocyte cytotoxicity against a human colon carcinoma line but no data are given on the effect on clinical course of the disease.[64]

The partial and somehow disappointing results obtained in clinical trials with TNF-α may be due to several factors:

- TNF-α can be administered only in small amounts because higher amounts are toxic and induce severe side effects.
- TNF-α is a relatively small molecule and as such, is rapidly cleared after injection.
- It is possible that some human cells are resistant to TNF-α or develop mechanism(s) of defence against the anti-tumour activity of TNF-α.
- Anti-tumour activity *in vivo* requires more than one anti-tumour factor.
- TNF-α *in vivo* does not occur sufficient concentration in its contact with tumour cells.

In view of the results obtained in clinical trials with TNF-α, experiments were undertaken to use activated human macrophages or activated human peripheral blood for therapy. It was assumed that macrophages can act indiscriminately against immunogenic or non-immunogenic tumours, and that they might release monokines continuously *in vivo* (in addition to TNF-α and/or IL-1) which would be active against the tumour cells. It should be mentioned in this context that activated macrophages might release anti-tumour cytostatic products unrelated to IL-1, TNF-α and INF-α/β.[65] Results of *in vitro* experiments showed that peritoneal human macrophages obtained from renal patients on continuous ambulatory peritoneal dialysis (CAPD) can be activated *in vitro* by LPS to express anti-tumour activity and as such they acted *in vivo* against a human tumour implanted subcutaneously in nude mice.[66] Similarly, human peripheral blood cells from cancer patients, activated *in vitro* by IFN-γ and LPS, reacted against a human tumour growing in nude mice.[67] These results prompted clinical trials with autologous human peripheral blood monocytes activated *in vitro* and reinjected in to the cancer patients.[68,69] The therapeutic effectiveness of this procedure was limited[68,69] and probably more clinical trials are required in order to improve conditions for therapy with activated macrophages.

Lymphocyte-derived Interleukins

Interleukin-2

Interleukin-2 (IL-2) is one of the main products of T cells and was extensively studied in the context of its anti-tumour activity. IL-2 was reported to increase human natural killer (NK) cell activity *in vitro* and this activity was partially reduced by monocytes due to PGE_2 production.[70] Murine lymphocytes cultured in

the presence of IL-2 lysed syngeneic murine tumour cells.[71] The lytic activity was attributed to the occurrence of lymphocyte activated killer (LAK) cells Thy-1 + Lyt^{-}1-2+.[71] The described effects of IL-2 in enhancing anti-tumour activity promoted a series of clinical trials devised to determine the therapeutic effects of IL-2 in cancer patients. It was found that of 106 patients with metastatic cancer receiving LAK cells plus IL-2, eight had complete responses, 15 had partial responses and ten had minor responses.[72] The same group of researchers reported that autologous tumour infiltrating lymphocytes (TIL), cultured in the presence of IL-2, lysed melanoma tumour cells.[73] Melanoma-specific cytolytic tumour lymphocytes derived from TIL grown in the presence of IL-2, and injected together with IL-2, induced tumour rejection *in vivo* possibly by reacting against the gp100 epitope.[74] A phase I trial in a total of 31 evaluable patients with metastatic cancer of the breast, gastric cancer, colorectal cancer, melanoma, non-small cell lung cancer, osteosarcoma or renal cancer, received a combined treatment of IL-2, followed by TNF-α and indomethacin:[75] two partial responses were seen (in breast and renal cancer).[75] In another clinical trial, 16 patients with advanced renal cell cancer (stage IV) received a combined treatment of cyclophosphamide, INF-α and IL-2.[76] Two patients had a partial response, two had a minor response and three patients achieved stable disease.[76] The effect of IL-2 with or without LAK cells was assayed in another group of 71 patients with advanced renal cell carcinoma.[77] A low level of anti-tumour response was detected and the addition of LAK cells did not improve the response.[77] IL-2 administration was found to prolong survival of some metastatic renal cell carcinoma patients with no or moderate HLA-II expression and/or no or moderate macrophage presence in the primary tumour, but was not effective in patients with both high HLA-II and high macrophage expression.[78] Some potential uses of IL-2 treatment have been described: IL-2 and IL-7 augmented the cytolytic activity and the anti-tumour killing spectrum of αCD3-induced activated killer cells, and such cells were suggested for immunotherapy of non-immunogenic tumours.[79] Cytotoxicity of induced LAK cells against human leukaemia was augmented in the presence of either IFN-α, IFN-γ or TNF-α in cultures, and as such they might be more effective in treating human leukaemia.[80] Adoptive therapy of established pulmonary metastases with LAK cells and recombinant IL-2 has been reported.[81] Adoptive therapy with highly enriched NK cells was assumed to have a potential use in leukaemia.[82] It should be also mentioned that LAK cells are apparently not a unique cell type but a function of various types of cells.[83] Finally, one of the problems of IL-2 therapy is the toxicity of the compound: in this context, it was suggested that the oxygen free-radical scavenger, dimethythiourea, ameloriates pulmonary permeability and vascular leak syndrome associated with multiple-dose IL-2 therapy without inhibiting IL-2 induced anti-tumour cytotoxicity.[84] On the other hand, the tumour-associated antigen 90K and the soluble IL-2 receptor were associated with poor prognosis in human ovarian cancer.[85] Another way of promoting the anti-tumour activity of IL-2 was by gene transfer into tumour cells.[86–89] The use of a human renal carcinoma line transfected with IL-2 and/or INF-α gene has been suggested for the preparation of live cancer vaccines.[90] Combined treatment with granulocyte colony stimulating factor and IL-2 increased the survival time of nude mice bearing human ovarian cancer cells.[91]

Interleukin-4

Interleukin-4 was first described as B-cell stimulatory factor. IL-4 was reported to inhibit tumour growth of syngeneic mammary adenocarcinoma, plasmacytoma,[92] and renal carcinoma[93] by using cytokine gene transfer into the murine tumours. Repeated injections of small amounts of IL-4 around the tumour draining nodes of mice resulted in growth inhibition of poorly immunogenic and non-immunogenic tumours (cited in reference 94). Transfection of IL-4 into tumour cells induced release of this cytokine and it was effective, *in vivo*, against a wide range of tumour cells implanted in nude mice.[92] Similarly, treatment of established murine renal cancer by tumour cells engineered to secrete IL-4, induced specific T-cell dependent systemic immunity against the non-transfected tumour.[93] To my knowledge, IL-4 therapy has not yet been tested in human neoplasia.

Interleukin-6 (INF-β$_2$)

Interleukin-6 is induced in T cells by antigen stimulation. Purified human recombinant (rIL-6) mediated substantial reduction in the number of tumours.[95] Recombinant human IL-6 produced in *Escherichia coli* inhibited the growth of human breast carcinoma and leukaemia/lymphoma cell lines *in vitro*.[96] Anti IL-6 receptor antibody prevented muscle atrophy in Colon-26 adenocarcinoma bearing mice, thus suggesting that this antibody could be a potential agent against muscle atrophia in cancer cachexia.[97] IL-

6 gene transfer into murine syngeneic tumours inhibited growth of lung carcinoma[98] and of sarcoma.[99] IL-6 gene transfected into Lewis Lung Carcinoma tumour cells suppressed the malignant phenotype and was effective against parental metastatic cells.[98] Mice rejecting murine fibrosarcoma cells, transduced with retroviral vectors containing the murine IL-6 gene and secreting IL-6, exhibited a later resistance to challenge with wild tumour cells.[98] Aquisition of the ability to synthesize endogenous IL-6 markedly accelerated the growth of weakly tumorigenic rat urothelial cells, but did not induce a tumorigenic phenotype in non-tumorigenic cells.[100]

Interleukin-7 (B/T maturation factor)
Murine plasmacytoma cells transfected with the IL-7 gene produced IL-7 *in vivo* and were completely rejected in syngeneic mice by a T-cell dependent process.[101]

Interleukin-12
IL-12 has received considerable attention during recent years as a strong anti-tumour agent: IL-12 was reported to react *in vivo* against B16F10 tumour-bearing mice and its anti-tumour effect was inhibited by anti-IFN-γ antibody;[102] IL-12-transfected fibroblast cells admixed with the murine melanoma, BL-6, showed that local IL-12 expression suppressed tumour growth and promoted development of specific anti-tumour immunity;[103] IL-12 engineered dendritic cells were effective *in vivo* against murine tumour cells.[104] In another report, it was shown that anti IFN-γ antibody blocked IL-12-mediated tumour regression in mice.[105] In view of the results obtained from IL-12 therapy against murine tumours, clinical trials were initiated to determine the effect of IL-12 in human cancer. Phase I clinical trials of IL-12 gene therapy were done by direct injection of tumours with genetically engineered autologous fibroblasts.[106,107] Out of 13 cancer patients (six breast, five melanoma, and two head and neck), significant reduction in tumour size was observed in three patients with melanoma and one with head and neck cancer.[108]

Interferon-α_{2b}
A clinical trial was done in renal cancer and melanoma patients by treatment with INF-α_{2b}. Of 12 melanoma patients, four patients showed a partial response whereas eight patients progressed.[109] In the case of 35 patients with advanced renal cancer, an increase in immune response potential was observed.[109] The conclusion was that more studies are required to determine the therapeutic effectiveness of INF-α_{2b}.[109] A human renal carcinoma line transfected with the IL-2 and/or the IFN α gene was suggested for use in preparation of live cancer vaccines.[90]

Interferon-γ (IFN-γ)
IFN-γ was first described as an antiviral agent and was among the first cytokines assayed for therapeutic effectiveness in human neoplasia. In one of the first clinical trials done in Hodgkin's disease patients it was reported that treatment with IFN-γ led to an extension of the disease-free survival time.[110] Complete or partial remission in multiple myeloma by IFN-γ treatment was also reported.[111] Remissions induced by IFN-γ were also reported in patients with lymphocytic lymphoma,[112] in cases of metastatic breast cancer,[113] non-Hodgkin's lymphoma[112] and multiple myeloma.[113] However, in another study in a group of non-small cell lung cancer patients, INF-γ treatment did not induce tumour regression.[114] A phase I trial on the effect of IFN-γ treatment was conducted in six patients with lung cancer;[115] systemic side effects such as transient fever, nausea, headaches and flu-like symptoms were noted[115] but no data on therapeutic effect were given.[115] In a recent *in vitro* study it was reported that human carcinoma cell lines cultured with IFN-γ expressed more CD80 and CD86 costimulatory molecules and that this increase in expression was inhibited by IL-10.[116] Transfer of the IFN-γ gene into murine neuroblastoma,[117] fibrosarcoma,[118] adenocarcinoma,[119] colon carcinoma[119] and lung carcinoma[120] cell lines induced tumour inhibition in syngeneic mice. Human peripheral blood monocytes collected from cancer patients were activated *in vitro* to express anti-tumour activity in the presence of IFN-γ and LPS.[67,68]

Anti-tumour effects vs other functions of cytokines

Some facts which should be considered in the context of the anti-tumour activity of cytokines are discussed below.

Relationship with inflammatory functions
Macrophage-derived cytokines such as TNF-α and IL-1 are also major inflammatory mediators.[5,27,28,37,56,57] Human peritoneal macrophages from CAPD patients collected during infectious peritonitis are 'primed' to produce and secrete more TNF-α and IL-1 when cultured in the presence of LPS.[121,122] These data indicate

that inflammation and cancer are interrelated events.

Interactions in cytokine production

Production and release of various eicosanoids and cytokines are closely interrelated.[37] For, example: production of IL-1, TNF-α and IL-6 by human mononuclear cells was induced by stimulatory agents such as LPS,[36,56] LPS-induced TNF-α production is inhibited by PGE_2,[123] endotoxin, TNF-α and IL-1 induce IL-6 production *in vivo*;[124] production of IL1 and TNF-α was induced in human blood mononuclear cells by LPS, whereas IL-6 suppressed the induction of IL-1β and TNF-α by LPS or PHA.[125]

Production of eicosanoids and cytokines by tumour cells

Production of eicosanoids and cytokines by tumour cells may have an influence on the effect of these products on tumour development: tumour cells might produce PGE_2;[126] the response of murine tumours to indomethacin therapy was directly related to their ability to produce prostaglandin;[127] tumours from cachectic mice produced both TNF-α and IL-1α *in vivo*;[128] a myeloma human cell line produced both TNF-α and IL-6;[129] leukaemic cells from patients with acute myeloid leukaemia produced both IL-6 and IL-1.[130] These are a few examples; the relationship between the ability of tumour cells to produce eicosanoids or cytokines and development of cancer is not yet clear.

Production of eicosanoids and cytokines in the tumour-bearing host

During tumour growth in rats cycloxygenase or thromboxane synthase was inhibited whereas C5 and C12-lipoxygenases of the alveolar macrophages were activated.[131] Macrophages derived from tumour-bearing animals suppressed activation of T cells, of NK cells, of LAK cells and of generation of tumoricidal activity in normal syngeneic splenic macrophages in cultures stimulated by LPS.[132] The secretion of IL-1, TNF-α but not of IL-6 was impaired in alveolar macrophages collected from tumour-bearing mice.[133] A decrease in production of IL-1 was also reported in peritoneal macrophages collected from sarcoma-bearing mice.[134] Production of IL-1 and TNF-α by tumour associated mononuclear monocytes from cancer patients was examined and showed that production IL-1 was suppressed whereas production of TNF-α was not affected.[135] LPS induced TNF-α production was impaired in macrophages from breast cancer patients,[136] but increased in patients with malignant brain tumours.[137] An example of correlation between production of TNF-α and PGE_2 by peripheral blood monocytes was seen in patients with bladder cancer: these patients had either higher TNF-α production or higher PGE_2 production.[138]

Cell-free mediators as cancer therapeutic agents

In vitro activation of cells for immunotherapy

Activation of autologous human peripheral blood monocytes by culture in the presence of LPS and IFN-γ was assayed with the aim of inducing anti-tumour cytotoxic activity in the treated monocytes and to use such activated cells for immunotherapy.[68,69] Clinical trials reported limited success.[68,69] Induction of LAK cells, and later of activated tumour infiltrating lymphocytes (TIL) by culturing in the presence of IL-2 was also suggested in a series of reports.[71–74] Clinical trials were carried out in groups of cancer patients; the results obtained indicated some therapeutic benefit in melanoma and renal carcinoma with approximately 10% clinical improvement in patients treated with both IL-2 and LAK cells.[72]

Direct administration of cytokines in cancer patients

As previously mentioned the results of treatment with TNF-α were disappointing; injection of IL-2 with or without LAK cells was problematic due to the toxicity of the agent; the effect of IL-2 in patients with squamous cell carcinoma of the head and neck was limited to temporary regression of the tumours.[139] Another approach suggested was to counteract the activity of PGE_2 by using a PGE inhibitor: while some improvement in immunological functions was observed no data are yet available on the therapeutic effectiveness of this treatment.[21]

Transfection of tumour cells with cytokine genes

This approach has been extensively investigated during recent years, firstly in experimental tumour models and later in clinical trials. The transfected tumours released the appropriate cytokine *in vitro* and in some cases induced T-cell mediated specific anti-tumour immunity. Partial success was observed in terms of tumour regression and clinical improvement.[140–142] Productive transfer of the IL-2 gene was shown for

melanoma, renal cell carcinoma, neuroblastoma and acute leukaemia cell lines.[86,108,139–141] Several reviews have been published on the role of cytokines in cancer therapy: in a review published in 1989 it was concluded "The clinical results obtained with cytokines (INF-α, IL-2, TNF-α, GM-CSF, G-CSF) expected to show direct tumoristatic or tumoricidal effects have been disappointing".[143] A more optimistic view was expressed in another review: "local presence of cytokines, either injected repeatedly at the tumour site or released by cytokine-engineered tumour cells, arouses immunogenicity in apparently nonimmunogenic spontaneous tumours" and as such they might indicate "potential use of cytokines as a component of new tumour vaccines".[144] In another review, it was concluded: "Clinical applications [of cytokines] are progressing, but many trials must follow to assess precisely the multitude of potential uses of these molecules".[94] Finally, in a recent review it was concluded: "Although several years will probably be required to establish the true impact of the gene-transfer modalities … technological advances have opened prospects for the management of cancer patients".[142]

Concluding Remarks

The 'golden' era on the role of cytokines in the fight against cancer started with the description by Carswell in 1975 of tumour necrosis factor.[35] Since then, various aspects of the interaction between eicosanoids, macrophage and lymphocyte cytokines and tumour state have been described and investigated. The various topics of investigation included:

- direct effects *in vitro* and *in vivo* of eicosanoids and cytokines on tumour cells in experimental and clinical trials;
- activation of monocytes, macrophages and lymphocytes *in vitro* by cytokines for use in autologous cancer patients;
- effect of tumour state in experimental systems and human neoplasia on the production and release of eicosanoids and cytokines;
- therapeutic effectiveness of combined treatment with activated immunocompetent cells and cytokines;
- role of secretion of eicosanoids and cytokines by tumour cells in the interaction between the tumour cells and the tumour-bearing host;
- therapeutic effectiveness of tumour cells transfected with cytokine genes. It is assumed that transfected tumour cells are more immunogenic and as such they induce a T cell mediated response against the wild type non-transfected tumour. They might also secrete cytokines *in vivo* which react against the tumour cells.

Marked progress has been made during recent years on the interaction between macrophage and lymphocyte products and tumour cells, and on their possible role in immunotherapy against cancer. However, many more clinical trials are required before these agents can be used in routine therapy of human neoplasia.

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