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Interactions between macrophage cytokines and eicosanoids in expression of antitumour activity

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CYTOKINES and eicosanoid products of macrophages play an essential role in expression of antitumour activity of macrophages either in a cell-to-cell contact system between the effector and the target cell or as cell-free soluble products. In this review the relationship between three main monokines, namely TNF-α, IL-1 and IL-6 and the interrelationship between these monokines and eicosanoids (PGE_2, PGI_2, LTB_4, LTC_4) in their production and in expression of antitumour activity is discussed. Emphasis is given to the effect of tumour burden on production of the monokines and of the eicosanoids and on the production of these compounds by the tumour cells. Finally, the therapeutic implications drawn from animal studies and clinical trials is discussed.

Key words: Antitumour activity, Cancer immunotherapy, Cytokines, Eicosanoids, IL-1, IL-6, Leukotrienes, LTB_4, LTC_4, Macrophages, Monokines, PGE_2, Prostaglandins, TNF-α

Introduction

Since the pioneering work of Metchnikoff,[1] it became more and more evident that macrophage cells play an essential role in a wide array of biological activities. This includes among others the events of nonspecific resistance against invasion of foreign cells (including tumour cells), their function as a crucial mediator in the development of immune response and participation in the process of inflammation (for review see Ref. 2).

The various functions of macrophages are exerted either in a cell-to-cell contact set-up with the target cells or by various biologically and pharmacologically active factors released by these cells. Among the most salient factors released by macrophages are various monokines and products of arachidonic acid (prostaglandins and leukotrienes).

An extensive amount of work has been done on the pharmacological and biological effects of macrophage cytokines and eicosanoids. The present review is by no means intended to provide a full coverage of all the activities of macrophage cytokines and eicosanoids. The aim is to discuss only the topic of interrelation between certain macrophage cytokines and eicosanoids in the context of their expression of antitumour activity. The main emphasis will be on the role of macrophage cytokines tumour necrosis factor-α (TNF-α) interleukin-1 (IL-1) and interleukin-6 (IL-6) and on the role of prostaglandin E_2 (PGE_2), leukotriene B_4 (LTB_4) and leukotriene C_4 (LTC_4) and their interrelation in the production and expression of antitumour activity. Another aspect to be discussed is the effect of tumour burden on their production by macrophages, production of these compounds by tumour cells and their therapeutic effectiveness in experimental tumour models and in cancer patients.

The first indications on induced release of certain antitumour factors by bacteria-free filtrates came for the early work of Coley (for review see Ref. 3). Later on, Carswell *et al.*[4] reported the occurrence of an antitumour cytotoxic factor in the serum of mice which had undergone treatment with bacterial lipopolysaccharide (LPS), coined the term tumour necrosis factor (TNF) and suggested that TNF is produced by activated macrophages in response to LPS. The occurrence of a lymphocyte activated factor produced by macrophages was first reported in 1972[5,6] and was named lymphocyte activating factor (LAF). In 1979, the nomenclature of various cytokines was revised[7] and the former LAF was given the name of IL-1 which is still used today. IL-6 was initially described as a factor derived from fibroblasts with antiviral activity.[8] The term IL-6 was first suggested by Poupart and colleagues[9] and its production by human monocytes was reported.[10]

Interactions in the production of macrophage cytokines and eicosanoids

Interactions in production between TNF-α, IL-1 and IL-6: It has been reported that production from blood mononuclear cells and from peritoneal macrophages of TNF-α, IL-1 and IL-6, can be induced by the same stimulatory agents. This applies to LPS,[11–13] phytohaemagglutinin (PHA)[13]

and *Staphylococcus epidermis*.[13] It should be noted also that the same monokine can be induced by different mechanisms. Thus, it was reported that *Mycoplasma capricolum* membranes induced TNF-α by a mechanism different from induction by LPS.[14]

In spite of the similarities in stimulation of the production of the monokines, some differences were reported in the context of their production. These differences indicate that mechanisms of their production may be different. Thus, human monocytes stimulated with pneumococcal cell surface components produced IL-1 but not TNF-α.[15] Both protein kinase C (PKc) and calmodulin (CaM) kinase dependent pathways were found to be involved in the induction of IL-1 mRNA by LPS, whereas TNF-α expression seemed to be PKc dependent but not CaM kinase dependent.[16] Other authors reported that TNF-α and IL-1 production and secretion by mononuclear phagocytes can be modulated differentially.[17] Differences between the kinetics of production of IL-1α, IL-1β and TNF-α by murine peritoneal macrophages during the peritoneal exudative response, were also described in relation to the optimal culture conditions and sequence of appearance.[18] The production of TNF-α and IL-1 in alveolar human macrophages was found to be regulated differentially by LPS.[19] A different pattern of regulation was also observed in the case of human macrophages: during the initial phase of maturation of human blood monocytes (up to 7 days in culture), IL-1β and IL-6 were down-regulated whereas TNF-α levels markedly increased.[20] A synergistic effect of interferon-τ (IFN-τ) and LPS was observed in relation to the release of IL-1β, IL-6 and TNF-α from human macrophages.[20] However, the LPS-induced levels of these cytokines differed during prolonged cultivation of macrophages (up to 28 days).[20] Differences in levels of production of IL-1α, IL-1β and TNF-α versus lower levels of IL-6 were also reported following stimulation by various agents of human blood mononuclear cells.[13]

The relationship in production between TNF-α, IL-1β and IL-6 is expressed by the findings that each one of these monokines can affect the production of the other monokines. Thus TNF-α was found to induce release of IL-1 *in vitro*[21,22] and *in vivo*.[23] TNF-α and IL-1 induced IL-6 production *in vivo*.[24] Stimulation of human monocytes by IL-1 caused a rapid down-regulation of IL-6 mRNA levels and concomitant enhancement of IL-6 mRNA expression.[24] IL-6 itself was found to suppress the IL-6-R at high concentrations.[25] IL-6 suppressed IL-1β and TNF-α production induced by LPS or PHA in human blood mononuclear cells.[13] Inhibition of LPS-induced TNF production by IL-6 in cultured human monocytes was also reported by other authors.[26] By working with bone marrow derived mouse macrophages it was found that LPS induces secretion of both TNF-α, IL-1 and IL-6.[27] IL-1 was able to stimulate IL-6 synthesis in human blood monocytes but not in monocyte derived macrophages whereas TNF-α had no effect on IL-6 synthesis in monocytes or macrophages.[28] Human alveolar macrophages and blood monocytes produced large amounts of IL-6 in response to LPS and monocytes produced lesser amounts of IL-6 in response to rIL-1.[29] Monocytes aged *in vitro* produced little detectable IL-6 in response to LPS or rIL-1, which might suggest that release of IL-6 under stimulus is correlated to the degree of maturity of macrophage cells.[29] TNF-α or rIL-6 itself did not modulate IL-6 production in human peripheral blood mononuclear cells.[30] No evidence was found that TNF-α acts to amplify the production of IL-6 or IL-1 by murine macrophage cell lines.[31] It was also claimed that synthesis and secretion of IL-1 either by human monocytes[32] or by mouse bone marrow derived macrophages[33] are two different biological events.

Similarly, LPS-induced production of TNF-α[34] and IL-1[35] was also reported in human peritoneal macrophages collected from patients on continuous ambulatory peritoneal dialysis (CAPD) during an episode of infectious peritonitis.

The conclusion from the above-mentioned data is that TNF-α, IL-1 and IL-6 can mutually affect their production. The production and release of these cytokines are also affected by other agents (including other cytokines) but these findings are beyond the scope of the present review. A schematic representation of the interrelationship in production between the three cytokines is given in Fig. 1.

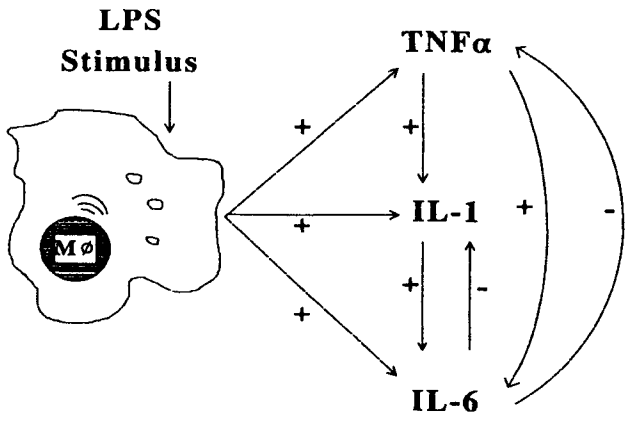

FIG. 1. Schematic representation of interrelationship in production by macrophages between TNFα, IL-1 and IL-6. +: enhancement; −: inhibition.

Interactions in production between cytokines and eicosanoids: A series of findings indicated that production of TNF-α by macrophages can be regulated by both endogenous and exogenous PGE_2. Thus, in response to LPS, murine peritoneal macrophages release concomitantly increased amounts of TNF-α and PGE_2.[36,37] However, addition of exogenous PGE_2 strongly suppressed the release of TNF-α by macrophages.[36,37] PGE_2 down-regulated the expression of TNF-α gene in human blood monocytes.[38] In another work it was reported that low amounts of PGE_2 enhance release of TNF-α from macrophages whereas high doses of PGE_2 suppress its release.[39] Some authors reported recently that the gene expression of TNF-α was enhanced by low doses of PGE_2 and by cGMP, and suggested that cGMP may represent one of the positive signals for TNF-α synthesis.[40]

Suppression of LPS-induced TNF-α production by PGE_2 was also reported by other authors.[41,42] The inhibitory effect of PGE_2 on TNF-α production was correlated with induced augmentation of cAMP in macrophage cells.[36,39] In contrast to PGE_2, leukotrienes induce increases in TNF-α release from macrophages. Thus, human monocytes exposed to graded concentrations of LTB_4 release high amounts of TNF-α.[43] The enhancing effect of leukotrienes on TNF-α may be related to increases in cGMP levels by leukotrienes[44] and is also supported by findings that lipoxygenase inhibitors suppress formation of TNF-α *in vitro* and *in vivo*.[45] Treatment of macrophages with LPS enhanced the increase of a lipoxygenase product which counteracted the suppression of TNF-α synthesis by a lipoxygenase inhibitor when added to macrophages exogenously.[46] Recent data suggest that endogenous prostaglandins and leukotrienes do not play a role in the regulation of TNF-α production.[47] In addition indomethacin (IND) (a cyclooxygenase inhibitor), exogenous arachidonate and MK-886 (a novel inhibitor of 5-lipoxygenase product formation) do not affect TNF-α production.[47] The interrelationship between TNF-α and PGE_2 production is also supported by the finding that TNF-α stimulates PGE_2 production in murine resident peritoneal macrophages.[22] Other data indicate that enhancement of TNF-α activity may be independent of PGE_2 production. Thus IFN-γ in combination with LPS enhanced TNF-α production but addition of IFN-γ to LPS had no effect on PGE_2 levels produced in human monocytes.[48]

With regard to IL-1, it was found that the same stimulator, namely PHA, induced production of both IL-1 and prostaglandin E in human monocyte monolayers. Products of the cyclooxygenase pathway of arachidonate metabolism seem not to be involved in the mechanism by which IL-1 stimulates thymocyte proliferation, whereas products of the lipoxygenase pathway may mediate the thymocyte proliferative response induced by IL-1. Similarly, with TNF-α, it seems that an arachidonate lipoxygenase product is important in the sequence of events leading to the production of IL-1. Additionally lipoxygenase inhibitors affected production of IL-1 in human peripheral blood monocytes.[49] An enhancing role of leukotrienes in production of IL-1 was also advocated by other authors.[50,51] Recently, it was reported that addition of exogenous LTB_4 to monocytes stimulates IL-1β transcription and mRNA accumulation.[52] A self-regulatory mechanism of IL-1 production was suggested by data showing that exogenous IL-1 induces increases in the levels of PGE_2 in murine macrophage cultures, whereas exogenous PGE_2 or prostacyclin (PGI_2) (measured as its stable metabolite 6-keto prostaglandin $F_{1\alpha}$) suppressed macrophage IL-1 production.[53] The inhibitory effect of PGE_2 was correlated to induce increases in cAMP levels.[54] IL-1 was found, on the other hand, to stimulate 5-lipoxygenase activity and in this way induces increases in PGE_2 synthesis.[55] In contrast to these data, it was also reported[56] that PGE_2 had no effect on IL-1 synthesis in murine resident peritoneal macrophages but rather had a direct inhibitory effect on thymocyte proliferation.[57]

In spite of the similarities in the stimulating conditions for production of TNF-α and IL-1 some differences were also reported. Thus, it was claimed that PGE_2 suppresses expression of cell-associated TNF-α in murine peritoneal macrophages but had no effect on cell-associated IL-1 activity.[58] PGE_2 suppressed accumulation of TNF mRNA but not of IL-1α and IL-1β mRNA accumulation.[58] The conclusion from these experiments was that synthesis of TNF appears to be regulated at the level of transcription whereas synthesis of IL-1α and IL-1β is regulated post-transcriptionally.[58] Recently, it was shown that PGE_2 inhibits release of TNF-α but not of IL-1β from human peritoneal macrophages.[42] Interrelation between production of prostaglandins and of IL-1β in human peritoneal macrophages was recently reexamined.[59] It was found that PGI_2 (measured as its stable metabolite 6-keto-$PGF_{1\alpha}$) declined sharply during episodes of peritonitis both in the presence or absence of LPS in the culture medium of human peritoneal macrophages.[59] On the other hand, PGE_2 was released in the same amounts in cultures of macrophages collected during peritonitis and during an infection-free period.[59] These results suggest that PGI_2 and PGE_2 may play a different role in the regulation of IL-1β production by human macrophages.[59]

The production of IL-6 concomitantly to production of PGE_2 in LPS-stimulated rat Kupffer cells was examined.[60] IL-6 production increased in

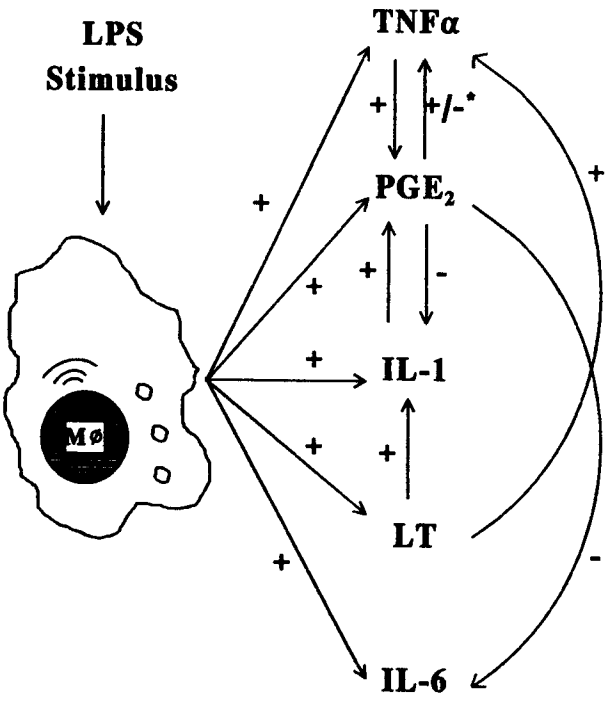

FIG. 2. Schematic representation of interrelationship in production by macrophages between cytokines (TNFα, IL-1 and IL-6) and eicosanoids (PGE_2; LT: leukotrienes); +: enhancement; −: inhibition; *+ at low doses, − at high doses.

parallel with PGE_2 before decreasing as PGE_2 continued to rise.[60] Blocking of PGE_2 production by IND increased IL-6 levels significantly thus showing that PGE_2 produced by Kupffer cells down-regulates IL-6 secretion.[60] However, cyclooxygenase inhibitors inhibited production of IL-6 by human peripheral blood mononuclear cells,[61] but no direct relationship between inhibition of IL-6 and production of PGE_2 was found.[61] Leukotrienes stimulate production of IL-6 in cultures of human monocytes.[62,63] LTB_4 stimulates production of IL-6 and induces accumulation of IL-6 mRNA.[62,63] Finally, regarding the interrelation between the production of IL-1 and PGE_2, it was reported that IL-1 and PGE_2 are produced by separate subsets of human monocytes.[64] The interrelationship between the production of cytokines and eicosanoids is given in Fig. 2.

Effect of tumour burden on production of macrophage cytokines and eicosanoids

The functions of tumour associated macrophages (TAM) have been investigated extensively (for reviews see Refs. 65, 66). It was suggested that *in situ* macrophages may affect the biology of neoplastic tissues in various ways besides tumour killing, by producing growth factors, by interaction with haemostatic mechanisms, by release of mutagenic reactive oxygen intermediates and neutral proteinases, and by their capacity to induce angiogenesis.[65]

The effect of tumour burden on production of macrophage cytokines and eicosanoids was examined in a series of experimental systems and in cases of human cancer.

Experimental studies: It was reported that during tumour growth in rats (subcutaneous implantation), cyclooxygenase or thromboxane synthase is inhibited, whereas C5 and C12-lipoxygenases of the alveolar macrophages are activated.[67,68] A transient increase of 12-HETE and LTC_4 production in murine peritoneal macrophages was also reported in mice implanted subcutaneously with B16 melanoma cells.[69] Tumour–host derived macrophages were found to suppress a series of events including activation of T cells, natural killing (NK) cells and lymphocyte activated killer (LAK) cells, and generation of tumoricidal activity in normal syngeneic murine splenic macrophages cultures in the presence of LPS.[70] The suppressive effects were correlated with an increase in PGE_2 secretion by tumour-bearing host macrophages.[70]

The role of prostaglandin secretion by macrophages from mice bearing syngeneic tumours was also supported by other authors. Thus, splenic and peritoneal macrophages collected from mice bearing different syngeneic tumours secreted large amounts of PGE_2 as a result of their interaction with the tumours.[71] These macrophages were immunosuppressive and their suppressive activity was significantly reduced by IND thus proving that the suppressive effect was due to increased release of prostaglandin.[71] In another study, it was shown that inhibition of spleen cell cytotoxic capacity toward murine Lewis carcinoma was due to an increase in PGE_2 levels.[72] The inhibitory effect was prevented by pretreatment of tumour-bearing mice with IND.[72]

The effect of tumour burden on the ability of macrophages to secrete cytokines was also examined. Continuous alveolar macrophage (AM) and tumour infiltrated (TIM) cell lines were generated from C57BL/6J mice and tested for their potential to secrete IL-1, TNF-α or IL-1 following exposure to rMulFN-γ and LPS.[73] Neither cell line secreted substantial amounts of IL-1 or TNF-α but secreted large amounts of IL-6.[73] Peritoneal macrophages from sarcoma-bearing mice produced progressively less IL-1 as tumour burden increased.[74] Administration of LPS to tumour-bearing mice early after tumour transplantation, resulted in reduced tumour growth and prevented the down-regulation of *in vitro* IL-1 production by peritoneal macrophages.[74] Thus, it seems that a specific defect in IL-1 production was associated with increasing tumour

burden.[74] In another study it was concluded that murine tumour-infiltrating macrophages isolated from the lungs of mice bearing lung B16F10 metastases responded as normal alveolar macrophages to biological response modifiers in relation to induction of tumoricidal activity and to secretion of IL-1 and TNF-α.[75] However, tumour associated macrophages (TAM) isolated from five cases of murine sarcomas showed a limited capacity to produce and release IL-1 as compared to peritoneal macrophages upon stimulation with LPS.[76]

The role of TNF-α release by TAM in specific defence against the inoculated tumour was questioned by various authors. Thus TAM from mice bearing EMT6 tumours exhibited high anti-WEHI-164 activity[77,78] due to release of TNF-α[78] but was not effective against the EMT6 tumour.[77,78] Similarly, TAM in a murine fibrosarcoma model produced TNF-α but this production did not affect the tumour growth.[79]

Human studies: Indirect indication of the role of prostaglandin production by macrophages from cancer patients was provided by data showing that monocyte-derived macrophages isolated from blood of cancer patients can be rendered cytotoxic by treatment with IND.[80,81] Apparently not all macrophages collected from cancer patients could be rendered cytotoxic against allogeneic or autologous tumour target cells, presumably because they were nonresponsive and/or because of the presence of a plasma inhibitory factor.[81] In another work, it was reported that peripheral and bone marrow enriched fraction of monocytes produce high levels of PGE_2.[82] However, the increased release of PGE_2 was not correlated to clinical or pathological findings.[82]

The production of IL-1 and TNF-α by tumour-associated mononuclear leukocytes (TAML) and peripheral blood mononuclear leukocytes (PBML) in cancer patients was determined. Stimulation by LPS induced production of similar levels of TNF-α in TAML and PBML but production of IL-1 was markedly suppressed in LPS-stimulated tumour-associated mononuclear leukocytes (TAML).[83] In another study the release of IL-1 and IL-6 by TAM from ascites and solid tumours of human ovarian carcinoma was investigated. It was found that TAM release spontaneously or upon LPS stimulation high amounts of IL-6 whereas they were poor producers of IL-1.[84] LPS-induced production of TNF-α by peripheral blood macrophages was impaired in cases of breast cancer.[85] On the other hand, secretion of TNF-α by monocytes from patients with malignant brain tumours was significantly greater by comparison with monocytes from normal individuals.[86] Increase in secretion of TNF-α and of IL-1 was also reported by alveolar macrophages from patients with lung cancer as compared with secretion by peripheral blood monocytes from the same patients or by alveolar macrophages from patients with nonmalignant disorders.[87] However, alveolar macrophages from lung cancer patients were found to be impaired in their ability to develop antitumour cytotoxic activity compared with either the peripheral blood monocytes from the same patients or alveolar macrophages from patients with nonmalignant lung disorders.[87] An increase in the level of TNF-α was also found in tumour-infiltrating macrophages in human colorectal adenocarcinoma.[88] The increase in TNF-α levels correlated with an increase in the size of the primary tumour.[88]

Some work was dedicated to determining the interrelationship between the production of TNF-α and PGE_2 by monocytes from cancer patients. LPS-incubated monocytes from cancer patients with malignancies of the digestive tract, produced high levels of TNF-α and PGE_2 when cultured in medium with foetal bovine serum.[89] Addition of cancer-patient plasma to the medium suppressed markedly TNF production but induced a prominent enhancement of PGE_2 production.[89] Plasma of cancer patients did not exhibit TNF-α activity but such plasma contained increased levels of PGE_2.[89] However, although low amounts of exogenous PGE_2 suppressed TNF-α production by normal monocytes, addition of 10% plasma-containing PGE_2 did not induce suppression of TNF-α production, thus indicating that some unidentified factor(s) in the plasma of cancer patients modulates the TNF-α and PGE_2 production in these patients.[89] Correlation between production of TNF-α and PGE_2 by peripheral blood monocytes was also studied in patients with bladder cancer. It was found that these patients had either higher TNF-α production or higher PGE_2 production.[90] A shift in macrophage population was due to tumour growth in BALB/c mice: immunosuppression in the tumour-bearing host was caused at least in part to the inability of Mac-1^+ and/or Mac-3^+ to control production of PGE_2 by Mac-2^+ macrophages.[91] Certain tumour-cell membrane constituents were found to activate human monocytes for TNF-α synthesis.[92]

In view of the wide variation in the results on release of macrophage cytokines and prostaglandin by monocyte-macrophages and tumour-associated macrophages from cancer patients, it still seems difficult to draw final conclusions on the role of this release in clinical settings of neoplasia. It seems that increased release of PGE_2 is usually found in TAM and may be correlated to an increase in the severity of the disease. Thus, high prostaglandin production in tissues surrounding human breast tumours is

correlated with high metastatic potential for neoplastic cells.[93] Successful therapy with IND and with a combination of IND and IL-2 was explained by abrogation of prostaglandin-mediated suppression of NK activity and IL-2 production.[94-97]

Production of eicosanoids and cytokines by tumour cells

In view of the role played by eicosanoids and cytokines in expression of antitumour activity it seems likely that intrinsic production of these compounds by tumour cells may affect the resistance to tumour development in tumour-bearing animals and in human neoplasia.

First reports indicated that BP8/P_1 murine ascitic tumour and, to a less extent a subcutaneously implanted S180 rat tumour, produced PGE_2.[98] However, the role of PGE_2 production in the development of the tumour remains uncertain because induction of a decrease in PGE_2 levels by IND did not affect appreciably the tumour growth.[98] In more recent work it was shown that certain murine tumours produce prostaglandins and that their response to IND therapy was directly related to their ability to produce prostaglandin.[99] Production of PGE_2 by EL4 leukaemia cells from C57BL/6 mice was also correlated to the extent of migration and dissemination of the tumour.[100] Prostaglandin biosynthesis was also found to occur in established cell lines derived from human lung, colorectal adenocarcinoma, and ovarian adenocarcinoma.[101] A difference in the amount of PGE_2 released was found between cancer cells metastasizing into rat liver or rat kidney.[102] It was assumed that this difference may be related to the mechanism of cancer metastases or to selection of the organ in which metastases occur.[102]

Tumour cells were reported to also produce cytokines. Thus, tumours from cachectic mice produced both TNF-α and IL-1α *in vivo* as documented by the presence of TNF-α and IL-1α mRNA and immune-reactive protein for IL-1α.[103] The tumour cells also produced TNF-α and IL-1α in long-term cultures but not IL-6.[103] Secretion of TNF-α by human leukaemic cells was also reported.[104] A myeloma cell line established from the pleural effusion of a myeloma patient secreted both TNF-α and IL-6 and these cytokines induced proliferation of the cell line.[105] In IL-6 production a dual effect was described: the murine MH134 tumour cells produced high amounts of IL-6 whereas the murine CSA1M tumour produced only marginal levels of IL-6.[106] However, both tumours induced production of IL-6 by T cells in the tumour-bearing host.[106] Leukaemic cells from patients with acute myeloid leukaemia produced both IL-6 and IL-1.[107] Prostatic carcinoma cell lines expressed the IL-6 receptor and secreted IL-6.[108] Squamous cell carcinoma cell lines produced both IL-1 and IL-6.[109]

An interesting situation was described with the murine P815 mastocytoma: this line produces TNF[110] but, at the same time, this was one of the first tumour cell lines which was found to be sensitive to exogenous TNF.[3,4]

It seems likely that production of prostaglandins and macrophage cytokines as well as induction of their production by macrophages in tumour-bearing hosts plays a role in the development of the tumour *in vivo*. However, the direct relationship between neoplasia and the ability of tumour cells to produce and/or induce production of macrophage eicosanoids and cytokines is not clear yet.

Interactions between macrophage cytokines and eicosanoids in expression of antitumour activity

Interactions between TNF-α, IL-1 and IL-6: TNF-α alone is cytostatic or cytotoxic for a wide range of murine and human tumour-cell lines.[111,112] On the other hand, TNF had no effect on a wide variety of murine and human tumour-cell lines and enhanced the growth of various normal cell lines.[111,112] Moreover, a heterogeneous cytotoxic response of TNF-α was described for various cell lines isolated from the same single neoplasm of human colorectal carcinoma[113] or renal cell carcinoma.[114] Membrane-associated TNF was shown to be the lytic principle of activated macrophages cytotoxic for TNF susceptible tumour cells.[115] The complexity of TNF antitumour activity is also shown by findings that TNF-α mediates the enhanced cytotoxicity induced in monocytes by IFN, IL-1 and by TNF itself.[116] Treatment of human monocytes with TNF-α increased their cytostatic ability in a dose-dependent manner against P815 murine mastocytomas.[117] IL-1 was also reported to be cytocidal for several tumour-cell lines.[118] However, IL-1 can act also as an autocrine growth factor for acute myeloid leukaemia cells.[119]

The fact that TNF-α and IL-1 are both produced by activated macrophages, and that TNF-α itself is an inducer of IL-1 production, prompted investigations devised to determine possible synergistic and additive antitumour effects of combinations of the two cytokines. Combination of TNF-α and IL-1 synergistically[120] or in additive manner[121] inhibited the growth of human A-375 melanoma cells. In another study, it was claimed that enhancement of antitumour human monocyte activity by combined TNF-α and IL-1β was less than additive.[122] We found that a combination of TNF-α and IL-1 had an additive effect on antitumour cytostasis against WEHI-3B murine

tumour cells.[123] It should be noted that TNF-α and IL-1 represent only a fraction of a wider spectrum of macrophage cytokines involved in expression of antitumour activity of macrophages.[124,125]

Antitumour activity of IL-6 against human breast carcinoma and leukaemia/lymphoma cell lines[126] and *in vivo* against four murine metastatic tumours[127] has been reported. On the other hand, autocrine generation and requirement as a growth factor for human multiple myelomas[128] and for human renal cell carcinomas[129] has also been described.

IL-6 production is induced by LPS which also stimulates production of TNF-α and IL-1, IL-1 induced synthesis of IL-6 in human blood monocytes[28,29] and TNF-α induced IL-6 in sera of cancer patients and tumour-bearing mice.[130] Moreover, it was found that IL-6 is involved in IL-1 induced activities as pyrogenicity and stimulation of thymocyte proliferation.[131] However, there are apparently few data on synergistic, additive or antagonistic effects of combination of IL-6 with either TNF-α or IL-1 on tumour cells. It was claimed that systemic administration of low doses of IL-6 in combination with sub-therapeutic doses of TNF to mice bearing a weakly immunogenic syngeneic tumour resulted in marked regression and some cure.[127]

Interactions between cytokines and eicosanoids: Modulation of production of various arachidonic acid derivatives is by itself related to induction of antitumour activity in macrophages. Thus, *in vitro* treatment of murine peritoneal macrophages with IND, a cyclooxygenase inhibitor, induced antitumour cytostatic activity against a murine plasmacytoma and this effect was increased when LTB_4 was added to the cultures.[132,133] The IND stimulation of macrophage cytostasis against the murine plasmacytoma was enhanced by endogenous metabolites of lipoxygenase and counteracted by PGE_2.[134] Induction of macrophage cytostasis towards P815 mastocytoma by calcium ionophore was reversed by specific inhibition of lipoxygenase.[135] In other work it was shown that LTC_4 is an essential 5-lipoxygenase intermediate in A23187-induced antitumour cytostatic activity[136] and that addition of L-serine to cultures stimulated by calcium ionophore increased both the accumulation of LTC_4 in murine macrophages as well as their antitumour activity.[137] LPS-induction of macrophage tumour killing was counteracted by PGE_2 but not by PGI_1.[138]

It should be noted that there are contradictory reports concerning the effect of prostaglandin production by macrophages tumour cells, and the direct effect of prostaglandins on tumour growth. Thus, it was reported that PGE could inhibit DNA synthesis and tumour cell replication *in vitro* and tumour growth *in vivo*.[139,140] PGA also inhibited tumour growth *in vitro* and *in vivo*.[141,142] However, in spite of the fact that induction of antitumour activity in macrophages by LPS was associated with an increase in PGE_2 and thromboxane production, these compounds did not seem essential for the expression of antitumour activity, as induction of antitumour activity took place and was even enhanced in the presence of indomethacin.[143] It has been reported also that addition of exogenous PGE_2 to murine peritoneal macrophages did not alter the ability of these cells to produce high levels of tumour-cell lysis when stimulated with LPS.[144] Finally, contrary to suggestions in Refs. 143 and 144 blockade of prostaglandin synthesis by indomethacin prevented the effect of LPS and led to a substantial resumption of target growth in the presence of activated macrophages.[145] A differential effect of PGE_2 on expression of macrophage antitumour activity was described in relation to the state of the macrophages: culture conditions that caused increased PGE_2 production by activated macrophages resulted in inhibition of their tumoricidal activity but production of high levels of PGE_2 by resident and peptone elicited macrophages was associated with an increase in antitumour activity.[146]

The contradictory results described may be due to differences in sources of macrophages, in types of target tumour cells and in the physiological state of the effector cells.

We and others have investigated the interrelation between eicosanoids and TNF-α or IL-1β in expression of antitumour activity. Human peritoneal macrophages collected from CAPD patients during an intercurrent infectious inflammation showed a sharp drop in cAMP and a decrease in production of cyclooxygenase metabolites.[147] On the other hand, they were primed in an *in vivo* inflammatory environment so that they were much more cytostatic against murine tumour cells than macrophages collected during inflammation free periods.[148] When macrophages collected during inflammation were cultured with LPS, their antitumour cytostasis against two murine tumour-cell lines was markedly increased and this increase was associated with increase in TNF-α and IL-1β release.[34,35,148]

Interrelation between eicosanoids and TNF-α or IL-1β in expression of antitumour activity was also examined by addition of the cell-free compounds to cultures of tumour cells. Interestingly, concomitant addition of PGE_2 enhanced the antitumour effect of IL-1β on a IL-1β susceptible WEHI-3B murine tumour, whereas addition of LTC_4 inhibited the antitumour effect of IL-1.[149] The synergistic effect between prostaglandins and cytokines in expression

of antitumour activity was also observed when the WEHI-3B murine tumour cells were first treated with the cytokine and afterwards with the prostaglandin: pretreatment with IL-1β rendered the tumour cells susceptible to PGE_2 or PGI_2 whereas only susceptibility to PGE_2 was increased by pretreatment with TNF-α.[123] Other authors found that Kupffer resident rat cells and Kupffer inflammatory murine liver cells produced both TNF-α and PGE_2. Upon activation with IFN-γ + LPS (for mouse resident Kupffer cells) or with LPS alone (for rat Kupffer cells and mouse inflammatory Kupffer cells) the cells produced more PGE_2 and more TNF-α.[150] However, PGE_2 did not play a role in tumour because treatment with indomethacin increased the TNF induced killing.[150]

Therapeutic implications

Work on therapeutic effectiveness was mostly concentrated on ways to affect *in vivo* prostaglandin production and on the possibility of using TNF-α either alone or in combination with other agents for therapy. The therapeutic effectiveness was examined in three systems: against animal tumours, against xenogeneic transplants of human tumours in nude mice and in clinical trials in cancer patients.

Experimental animal tumours: Prostaglandins have been implicated as enhancers of tumour growth and spread.[93,151] The sources of prostaglandin were tumour cells[99] and/or cells of monocyte-macrophage lineage. Accordingly, it was assumed that inhibition of prostaglandin biosynthesis by cyclooxygenase inhibitors might have a beneficial therapeutic effect.

Most of the work on the basis of this assumption was done by looking on the therapeutic effect of the cyclooxygenase inhibitor IND as downgrading PGE_2 production. Thus, it was shown that IND therapy prevents tumour metastasis of a mouse mammary carcinoma[94] cures B16F10 murine melanoma lung metastasis when given in combination with IL-2,[95] and cures murine Ehrlich ascites tumours when administered in combination with LAK cells and IL-2.[96] The effectiveness of IND therapy was correlated to the ability of murine tumours to produce prostaglandin.[99] However, it was doubted if the therapeutic effectiveness of indomethacin is due to inhibition of PGE_2 production.[152] IND treatment prolonged survival of sarcoma-bearing mice without, however, having an effect on serum concentrations of IL-6 which increased progressively with increase of the tumour.[153]

Tumour necrosis-like activities have been described since the initial observations in the 1890s on regression of tumours in patients with concomitant bacterial infections or injected with bacterial culture filtrates (for review see Ref. 3). After its first characterization as tumour necrosis factor produced by macrophages,[4] its activity against a long series of human and murine tumours was demonstrated *in vitro*.[111,112] The next obvious step was to determine the therapeutic effectiveness of TNF-α in tumour-bearing hosts. rHuTNF-α was shown to be effective against subcutaneously implanted murine MethA sarcoma but not against the same tumour injected i.p.[154] The curative effect of TNF-α was attributed to its ability to induce local haemorrhages because of its effect on the endothelial cells.[154] It should be noted also that TNF-α therapy involved generation of specific cell-mediated antitumour immunity by a still undefined mechanism.[154]

In another experimental system the antitumour effect of recombinant murine TNF-α given either by continuous i.v. infusion or by repeated i.v. injections was determined in a rat liver metastases model.[155] Only early continuous infusion had an effect on the number of liver metastases presumably because higher doses of TNF-α were tolerated by this schedule.[155] The conclusion of the authors was that TNF-α by itself is not a very efficient antitumour agent and it might be necessary to use TNF-α in combination with other antitumour agents.[155] A similar conclusion of more therapeutic effectiveness of TNF in combination with other treatments was in the case of TNF radiotherapy by comparison with TNF-α alone in rat renal-cell carcinoma,[156] or a combination of TNF-α with the interferon-inducer bropirimine in rat colon cancer.[157] Sequential use of anti-CD3, IL-2 and TNF for LAK induction and maintenance potentiated antitumour activity against a pulmonary metastatic model in mice.[158]

Another interesting additive effect was described after combined treatment with activated macrophages and a low dose of TNF-α in mice bearing Lewis lung carcinoma or EMT6 sarcoma.[159] A synergistic therapeutic effect was also described in tumour-bearing mice treated with low doses of IL-6 in combination with subtherapeutic doses of TNF-α.[127] The therapeutic use of TNF-α in tumour-bearing mice was found to be affected by an increase in the toxicity of TNF-α in tumour-bearing hosts[160] and by induction of tolerance to TNF-α.[161] A side effect of antibodies to TNF-α was reported: passive immunization with anti-TNF antibodies abrogated partially IL-2 toxicity in tumour-bearing mice.[162] Finally, the effect of TNF therapy might differ in context to the strain of mice: the curative effect of TNF was stronger against MethA sarcomas implanted in BALB/c nu/ + mice than into BALB/c nu/nu mice, when TNF was injected i.v. and similar when injected i.t.[163] A new

therapeutic approach was described recently: a novel chimera tumour necrosis factor ($TNF\text{-}S_{TH}$) constructed by connecting a modified recombinant human TNF-α (rTNF-S) with thymosin-β_4 was suggested to be a promising approach for obtaining molecules that more favourably attack tumours than conventional rTNF.[164]

Xenografts of human tumours in nude mice: TNF was also found to be effective against human malignant melanoma, human gastric cancer and nasopharyngeal carcinoma cell lines implanted in nude mice.[163] Combined therapy with IFN-γ and TNF-α was found to be more effective than TNF-α alone against human ovarian cancer cells inoculated in nude mice.[165] A similar synergistic effect between IFN (interferon-alpha) and TNF-α was described against a human tumour line causing lung metastasis and intra-abdominal carcinomatosis in nude mice.[166] In another study it was found that combined treatment with TNF-α and etoposide was efficient against a human renal cell carcinoma implanted in athymic mice.[167]

Clinical trials: The findings on cytotoxic effects of cytokines (especially TNF-α) on a wide array of murine and human tumour cell lines *in vitro* and the results on therapeutic effectiveness against murine tumours and against human tumour cells implanted in nude mice prompted the start of clinical trials in various cancer patients. Unfortunately the results in clinical trials were not very spectacular. In one of the first studies a certain improvement due to TNF-α therapy was observed in three out of 18 patients: two cases of lymphoma and one case of Hodgkin's.[168] Febrile reactions and other side effects occurred in most of the patients and they could be prevented by steroids and IND.[168] However, according to the authors, "such prophylaxis may not be desirable because its influence on possible therapeutic benefits is unknown". Some beneficial response in cases of gastric cancer and non-Hodgkin's lymphoma were also reported by other authors.[169] Side effects to TNF-α were recorded and could be ameliorated by IND or ketoprofen.[169] In cases of B-lymphoma some improvement was detected in one out of 26 patients and side effects to TNF-α were again observed.[170] In another study no antitumour response was detected in 29 cancer patients.[171] In two cases out of 16 evaluable patients with disseminated cancer, TNF-α therapy had some antitumour effect: regression of a neck lesion in one case and resolution of malignant ascites in another.[172] The results on various clinical trials with TNF-α until 1988 were summarized at a seminar of the 19th German Cancer Congress: "Although TNF as a single agent is unlikely to be of major benefit for patients with cancer, it has to be concluded that the great expectations which were particularly due to its dramatic effect in the murine sarcoma model leading to the designation 'TNF' have been disappointed".[173]

More clinical trials have been performed with TNF-α in recent years. In a phase II trial with 22 eligible patients with metastatic colorectal adenocarcinoma, treatment with TNF-α was not effective.[174] A more promising result was obtained in a clinical trial with TNF-α including 29 patients with refractory malignant ascites: out of 29 patients, 22 responded with a complete (16) or partial (6) resolution of their ascites.[175] Recently, in one out of 53 patients with advanced malignancies a partial response to TNF was observed in one patient with colorectal carcinoma.[176] In another recent study no clinical efficacy of TNF-α was found in a phase I trial with patients with advanced cancer.[177] Rather disappointing results concerning the use of human recombinant TNF-α for cancer therapy were reported recently by two groups: no objective responses were observed in 22 cases of advanced carcinoma of the pancreas[178] and no significant antitumour activity of rHTNF-α was detected in 127 eligible patients with diverse metastatic malignancies.[179] Another group concluded that rHTNF-α has only modest antitumour activity in 26 patients with renal cell carcinoma.[180]

A more promising approach for therapy was suggested by using TNF-α in combination with other interleukins.[181,182] This was suggested by results obtained *in vitro* and in experimental systems *in vivo* with such combinations. However, in only one patient with melanoma and one patient with mesothelioma (out of 36 patients), was some response observed to combined treatment of recombinant TNF-α with recombinant IFN-γ,[183] and two partial responses were seen in a study with combined recombinant IL-2 followed by recombinant TNF therapy in 31 patients with metastatic malignancies.[184]

The use of TNF-α therapy is handicapped by the toxicity of the agent and by its extremely pleiotropic biological effects. It should be also noted that in most clinical trails with TNF-α most of the patients were found refractory to other kinds of treatments and were in an advanced stage of disease. Moreover, it was reported that sera of cancer patients may contain factor(s) inhibiting TNF.[185,186] It has been also reported that treatment with TNF-α might induce decreases of NK cell activity and of monocyte production in cancer patients.[187]

The general conclusion from clinical trials until now is that therapy with TNF-α is still in its infancy. Apparently, combinations of various interleukins for cancer therapy might be more promising. A new approach was suggested in the recent years consisting of therapy by monocytes from cancer patients induced to maturate *in vitro* to macrophages

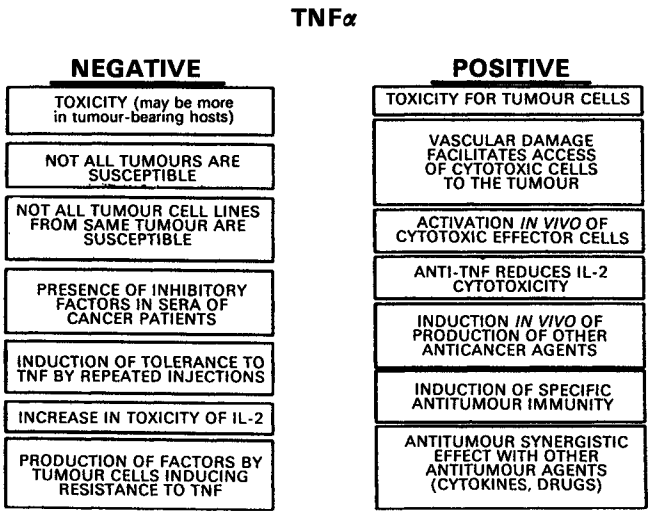

FIG. 3. Negative and positive aspects of the TNFα anticancer therapy.

possessing antitumour cytotoxic activity.[188,189] In view of the findings that such macrophages secret various cytokines,[20] it might be that the activated macrophages continue to secrete *in vivo* interleukins cytotoxic for cancer cells.

Concluding Remarks

A vaste amount of material has accumulated on the interrelationship in production between macrophages cytokines, macrophage cytokines and eicosanoids, production of these products by tumour cells and the effect of tumour burden on their production. The expression of interrelationship between macrophage cytokines themselves and between macrophage cytokines and eicosanoids was also extremely investigated. In certain instances the results obtained in *in vitro* systems were also expressed *in vivo* in various therapeutic schedules in mice bearing syngeneic tumours on xenografts of human tumour-cell lines. Unfortunately, the promising results obtained *in vitro* and in experimental systems *in vivo* have not yet been well duplicated in clinical trials. Much more work has still to be done in defining optimal conditions for the use of single interleukins and (more likely) combinations of interleukins for effective anticancer therapy. Some of the negative and positive aspects of use of one of the most commonly tested cytokine (TNF-α is schematically represented in Fig. 3). It is also possible that other approaches such as therapy with cells able to produce *in vivo* a wide array of cytokines (macrophages) or devising ways for effective induction of cytotoxic interleukins *in vivo* by a cancer patient's own cells might lead to more promising results.

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