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Role of TNF- α in Tumor Cell Destruction

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Tumor Cell-Induced TNF Production

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Clinical studies with recombinant TNF did not sustain the great hope as an anticancer agent that was placed on TNF. This hope was based on the early toxin studies by Coley et al. [1] in which tumor regression and necrosis was probably conferred by endogenous TNF [2]. Several explanations could account for this discrepancy in activity of exogenous versus endogenous TNF. For example, the amount and the localization of applied versus induced TNF and the combination with other cytokines might determine the antitumor activity. Today, TNF can no longer be regarded to be solely a tumor cytotoxic or cytostatic agent. It is now generally accepted that it exerts a multitude of effects on many different cell types and that it can act as an important immunomodulator [3]. Therefore, these considerations raise the question whether tumor cells can induce TNF production and what role such endogenous tumor-induced TNF might play for the pathophysiology of human cancer.

Material and Methods

Cell cultures: Culture methods of tumor cells and isolation procedures for human peripheral monocytes have been described recently [4]. The cell lines used in this study were K562, a proerythromyeloid cell line; Jurkat, a T cell line; CFS-1, a methylcholanthrene-induced fibrosarcoma cell line of C3H mouse origin; Lewis lung carcinoma cells kindly provided by H. Osswald, Heidelberg; ESb, a T cell lymphoma of DBA/2 mouse origin kindly provided by V. Schirmmacher, Heidelberg.

Tumor models: For tumor induction 10^6 CFS-1 cells or ESb cells were implanted i.d. on the flanks of C3H or DBA/2 mice, respectively. Lewis lung carcinoma cells were passaged from lung metastases i.m. into B6/D2 mice.

TNF assays: L929 bioassay and TNF ELISA were performed as described recently [5].

Membrane preparation and chromatography: The methods for membrane preparation and chromatography have been described recently [4]. HPLC gel permeation chromatography was performed on a Beckmann System Gold system with a Beckmann TSK 3000 SW column.

Northern blot and in situ hybridization procedures: RNA extraction and hybridizations were performed as described recently [4, 5]. cDNA and RNA probes used were: a 750-bp *EcoRI* fragment of the coding region of human TNF cDNA; a 460-bp human IL-1 *EcoRI-BamHI* DNA fragment (p3 IL-1 α) and a 530-bp human IL-1 β *BamHI-NdeI* DNA fragment (p11 IL-1 β) of the coding region, respectively, kindly provided by U. Gubler, Hoffmann-La Roche, Nutley, N.J.; a 560-bp *Sall-EcoRI* fragment of human β -actin cDNA; a TNF RNA probe consisting of a 750-bp *EcoRI* fragment of the coding region was synthesized with T7 RNA polymerase in the presence of ^{35}S -UTP.

Results

TNF Production Induced by Tumor Cells in vitro

Several reports describe the correlation of monocyte/macrophage activation and TNF production in a host with tumor burden [6–9]. We therefore investigated in vitro whether tumor cells could stimulate human peripheral blood monocytes from healthy donors to synthesize TNF and IL-1 [4]. The human tumor cell lines K562, a proerythromyeloid cell line, and Jurkat, a T cell line, were used to stimulate the adherent fraction of freshly isolated peripheral blood mononuclear leukocytes. Cultivation of Jurkat cells with monocytes for 16 h led to the release of soluble TNF which was measured by the sensitive bioassay as well as by the TNF-specific ELISA. K562 did not induce the release of measurable amounts of soluble TNF. In addition, a 17-kD TNF protein biosynthetically labeled with radioactive leucine was immunoprecipitated from the supernatant of Jurkat cells stimulated with monocytes, whereas Western blot analysis of monocytes stimulated with both Jurkat and K562 showed the appearance of 26 kD cell-associated TNF protein. Both cell lines, K562 and Jurkat, induced the expression of TNF mRNA and IL-1 mRNA in the monocytes (fig. 1a, b). This indicated that both tumor cell lines activated the monocytes and induced biosynthesis of TNF.

The activating agent of the tumor cells was found to be associated with the outer cell membrane. Nonadherent peripheral blood leukocytes from histoincompatible donors or proliferating T cell clones did not induce TNF mRNA expression in monocytes. Bacterial, viral and mycoplasma contaminations were excluded as causative agents. Sizing experiments on SDS con-

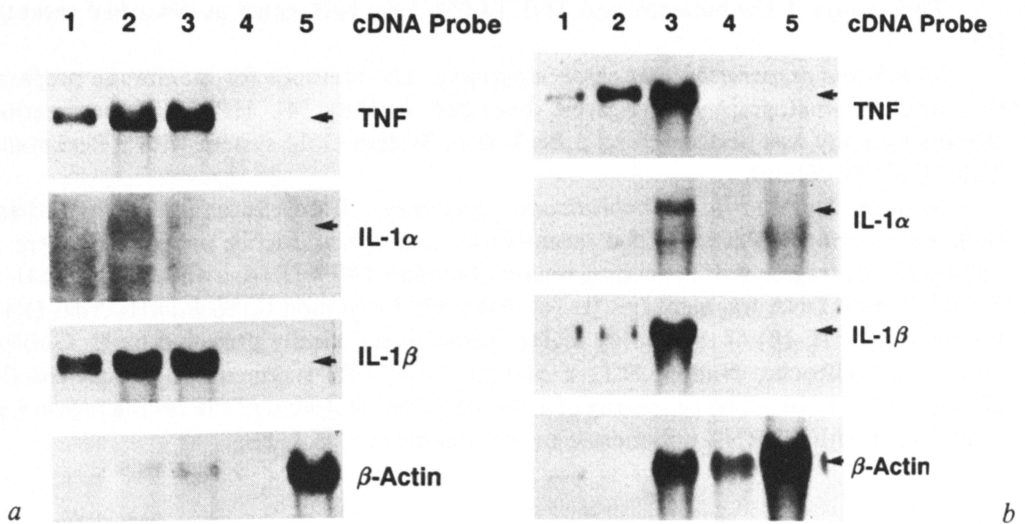


Fig. 1. TNF mRNA and IL-1 mRNA expression in monocytes stimulated by Jurkat cells (a) or by K562 (b). a RNA was prepared from monocytes cultured for 2 h in medium (lane 1); with 5×10^6 Jurkat cells (lane 2); with 5×10^6 UV-irradiated Jurkat cells (lane 3); from 5×10^6 viable Jurkat cells cultured for 2 h with UV-irradiated monocytes (lane 4), and from 5×10^6 Jurkat cells without monocytes (lane 5). The tumor cells were removed from all monocyte-containing cultures before RNA extraction. b RNA from monocytes cultured for 2 h in medium (lane 1); with *Staphylococcus aureus* 50 μ g/ml (lane 2); with 5×10^6 K562 cells (lane 3); from 3×10^6 (lane 4), and 9×10^6 (lane 5) K562 cells without monocytes. The Northern blots were sequentially probed with TNF, IL-1 α , IL-1 β and β -actin cDNA probes.

taining polyacrylamide gels and on HPLC using gel permeation chromatography revealed a Jurkat cell membrane fraction of about 40 kD with this monocyte-activating capacity. The biologically active membrane constituent was sensitive to periodate treatment and relatively resistant to treatment with protease and glutaraldehyde, thus pointing at a glycoprotein structure as the monocyte-activating agent.

TNF Production during Tumor Growth in vivo

TNF production has repeatedly been reported for tumor tissues from human biopsies [10, 11]. We also found TNF mRNA signals by in situ hybridization and TNF protein stained by immunohistochemical staining in biopsies of colon and ovarian carcinomas. To systematically follow TNF synthesis in a murine tumor model, sections of fibrosarcoma biopsies from different stages of tumor growth were subjected to in situ hybridization with a TNF RNA probe and also staining with a polyclonal anti-rmTNF immuno-

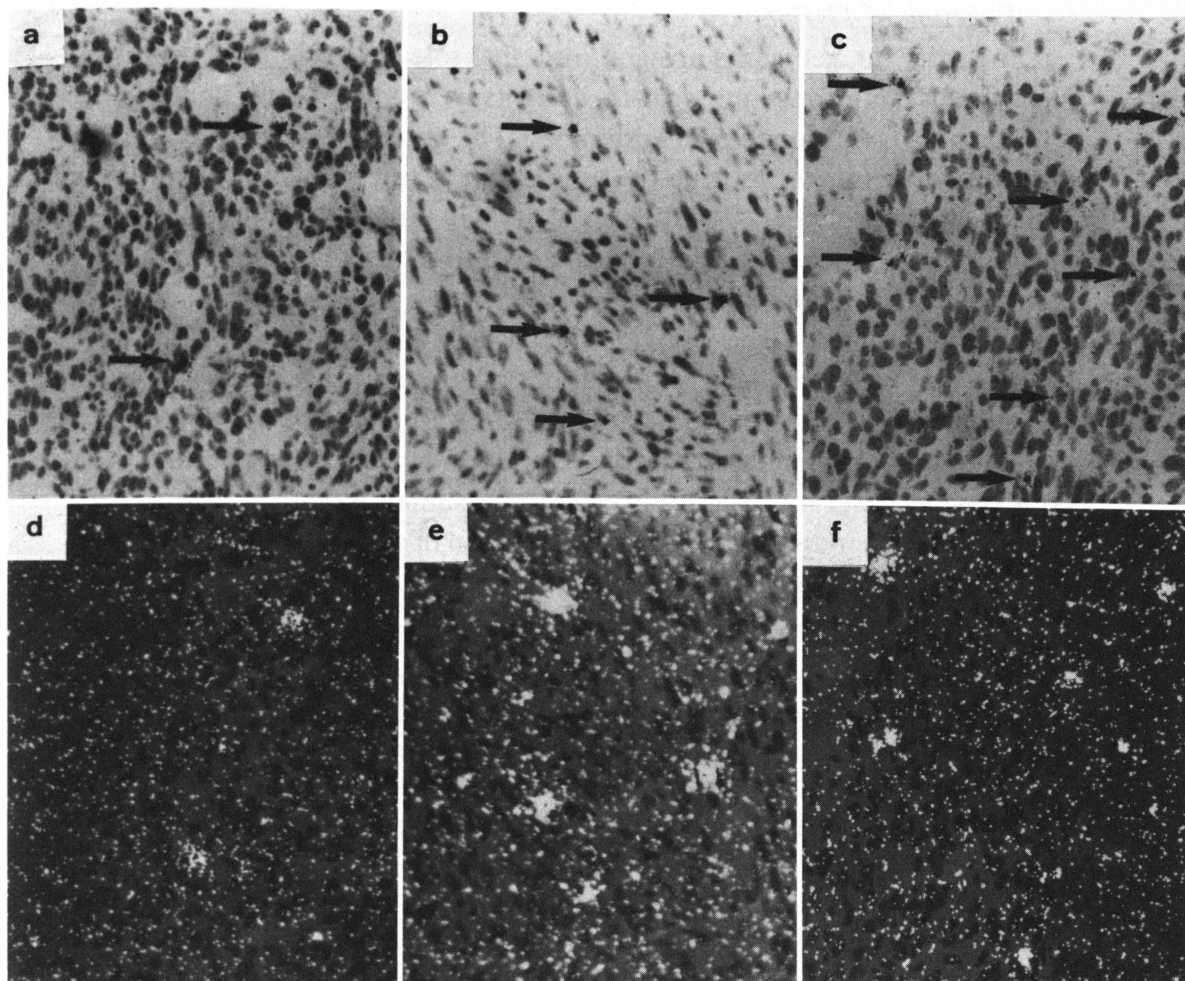


Fig. 2. TNF mRNA expression in solid tumors during tumor growth. Tumor tissue (CFS-1) from day 8 (*a, d*), day 15 (*b, e*) and day 20 (*e, f*) after tumor implantation were hybridized with a TNF RNA probe. The photographs display the same tissue area $\times 250$ as light (*a-c*) and dark (*d-f*) field, respectively.

globulin. The number of cells positive for TNF mRNA (fig. 2) and for TNF protein (data not shown) increased during tumor growth. Also, in the fibrosarcoma tumor model and in a tumor model using the T cell tumor ESb, TNF mRNA was clearly present in spleen cells of tumor-bearing animals. Thus, although no circulating TNF was detected in the serum of these mice by ELISA and in the biological assay, TNF production in tumor and spleen became obvious with tumor growth.

Function of TNF in vivo

In order to get some insight into the role of such tumor-induced endogenous TNF for tumor growth, animals were treated with neutralizing anti-murine TNF monoclonal antibodies. The animals had TNF-neutralizing antibody serum levels from the time of tumor cell implantation until the end of the experiment. No difference in size or weight of the primary fibrosarcoma tumor was found when the anti-TNF-treated animals were compared to untreated tumor-bearing mice. However, a significant effect of anti-TNF treatment became obvious when the numbers of metastases were compared in tumor models using metastasizing tumors. Metastases in livers and spleens of mice inoculated with ESb, a highly metastatic T cell tumor, and in lungs of mice inoculated with Lewis lung carcinoma were counted. In both systems fewer metastases were found in animals treated with anti-TNF antibodies. This indicated a supportive function of endogeneous TNF for the development of metastases from primary tumors.

Discussion

The results described above clearly demonstrate the capability of a tumor cell constituent to activate human monocytes as shown by the induction of mRNA for TNF and IL-1 and release of TNF protein. This could account for the enhanced serum levels of TNF found in tumor patients [6, 7] and for the cytostatic activity of monocytes from cancer patients [8, 9]. It is not excluded that other cells besides monocytes could also be stimulated for TNF production. Possible candidates would be T cells, NK cells and granulocytes which all have been shown to be TNF producers [12–14].

The observation that the K562 cells in contrast to Jurkat cells did not induce the release of 17 kD TNF from monocytes seems most likely to be due to absorption of this soluble TNF by the relatively numerous TNF receptors found on the K562 tumor cells [4]. Clearly, both tested tumor cell lines induced biosynthesis of TNF. Preliminary characterization of the possible candidate for the activating agent suggested a 40-kD glycoprotein constituent on the outer cell membrane of the tumor cells in the case of Jurkat cells. Experiments to further clarify the structure of this agent are ongoing.

Even though there is conflicting evidence concerning the presence of TNF in serum from cancer patients [6, 7, 15], production in tumor tissue has been reported by several groups [5, 6, 10, 11]. In one report, TNF was probably produced by the tumor cells [11] while in the others the host cells

were the more likely producers. The results presented here confirm the finding of tumor-induced TNF production *in vivo* in two mouse tumor models. Cancer cachexia, anemia and hypercalcemia could possibly be induced by such endogenous TNF [16, 17]. It can also be assumed that such endogenous TNF interferes with tumor growth and dissemination. TNF exerts a number of biological effects that could be important in this respect, e.g. cytostatic or cytotoxic activity, induction of angiogenesis, enhanced expression of adhesion molecules, activation of monocytes/macrophages and granulocytes, enhanced expression of class I and II MHC antigen expression, and increased plasminogen activator inhibitor activity [reviewed in 3, 18]. Depending on the tumor model under investigation, different sets of parameters could be involved in the outcome of the experiment. It is important to stress that with the multitude of TNF effects in mind it is not so surprising that in the complex sequence of events leading to metastasis the net effect of TNF seems to support formation of metastases. Reports from other investigators support this notion that TNF enhances the metastatic potential of tumor cells [19–21]. It remains to be analyzed which stages of tumor growth and metastasis are influenced by TNF and whether one could make use of this influence for a patient's benefit by application of TNF or TNF antagonists, respectively.

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