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Tumour necrosis factor-alpha stimulates invasiveness of T-cell hybridomas and cytotoxic T-cell clones by a pertussis toxin-insensitive mechanism

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SUMMARY

Tumour necrosis factor-alpha (TNF-α) stimulated invasion by mouse T-cell hybridomas and cytotoxic T-lymphocyte clones into rat embryo fibroblast monolayers. The effect on these highly invasive cells was limited: invasion was stimulated maximally to 130% of controls. However, when cells were pretreated with pertussis toxin (PT), which inhibits invasion to ±20% of controls, a clear-cut effect was observed: 400 U TNF-α per ml stimulated invasion usually two- to threefold, and sometimes even up to 10-fold. Therefore, experiments were done with PT-pretreated cells. Stimulation was dose dependent and maximal at 200–400 U TNF-α per ml. An anti-TNF-α monoclonal antibody completely abolished TNF-α-induced invasion. The effect was maximal 30 min after addition of cells and TNF-α to the monolayer and then declined. TNF-α preincubation of T-cell hybridoma cells, but not of fibroblasts, had a similar stimulatory effect, which was also maximal after 30 min. This shows that TNF-α acts directly on the T-cell hybridoma cells. Invasive T-cell hybridomas colonize many tissues from the blood similarly as normal T cells. Our data thus suggest that TNF-α can stimulate migration of normal T lymphocytes into inflamed tissues and can promote metastasis of malignant T lymphomas. The signals involved are transmitted via a pertussis toxin-insensitive pathway.

INTRODUCTION

T lymphocytes invade inflamed tissues in order to participate in immune responses.[1] In addition, T cells recirculate continuously between blood and lymphoid tissues,[2] and memory T lymphocytes migrate from the blood into non-inflamed, healthy tissues.[3] Haematopoietic tumour cells can be considered immortalized leukocytes, and the ability of some of these tumour types to metastasize may be ascribed to the invasive potential of the type of leucocyte they arose from. This notion is supported by the finding that T-cell hybridomas, prepared by fusion of a non-invasive, non-metastatic T lymphoma with invasive, activated normal T lymphocytes are invasive *in vitro* and metastatic *in vivo*.[4–6] This suggests that invasiveness is a prerequisite for colonization of tissues from the blood, a process similar to extravasation of normal T cells. Elucidation of the mechanisms involved in invasion by T-cell hybridomas will thus provide insight both in the migratory behaviour of normal T lymphocytes and in the metastatic properties of malignant T lymphomas.

Abbreviations: CTL, cytotoxic T lymphocyte; PT, pertussis toxin; REF, rat embryo fibroblast; TNF-α, tumour necrosis factor-alpha.

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Previously, we have shown that invasiveness of T-cell hybridomas *in vitro* and metastasis formation *in vivo* was strongly inhibited by pertussis toxin (PT).[7] Because PT irreversibly inactivates certain G proteins,[8] this implied that a G protein is involved which transmits an invasion-enhancing signal over the plasma membrane. This extracellular ligand could be a cytokine produced by the T-cell hybridoma cells themselves.

Tumour necrosis factor-alpha (TNF-α), is a candidate for the following reasons: first, because activated T lymphocytes produce TNF-α[9,10] and express high affinity TNF-α binding sites,[11,12] second, because a PT-sensitive G protein can be involved in TNF-α signal transmission,[13–15] and finally, because TNF-α can influence leucocyte migration.[16,17] This is thought to be due to its chemotactic/chemokinetic activity[18–20] and to its effect on adhesiveness of endothelial cells and fibroblasts.[21–24]

Here we report that TNF-α stimulates invasion by mouse T-cell hybridomas and cytotoxic T-lymphocyte (CTL) clones into rat embryo fibroblast (REF) monolayers *in vitro*. This was, however, PT insensitive. In fact, the effect of TNF-α was more pronounced when invasion had been reduced by PT-pretreatment of T-cell hybridoma cells. This shows that TNF-α is not the PT-sensitive autocrine invasion-enhancing factor and that TNF-α does not transmit its signal via a PT-sensitive pathway. Furthermore, these results indicate that TNF-α may enhance T-cell influx in inflamed tissues and metastasis formation by malignant T lymphomas by enhancing their invasive potential.

MATERIALS AND METHODS

Cells and culture conditions

T-cell hybridomas have been prepared by fusion of BW5147 T-lymphoma cells with allo-antigen stimulated T lymphocytes and with unstimulated T-cell-enriched spleen cells. They were cultured in Hybridoma medium.[4] Each cell line was cloned at least once by limiting dilution. Invasive and metastatic properties were routinely assayed and have been stable for several years.[4,5]

Mouse cytotoxic T-cell clones, CTL 23.21 and CTL 16N, were kindly donated by Dr H. Prahl (F. Hoffman-La Roche, Basel, Switzerland). They were isolated from spleens of AKR mice immunized with B10BR spleen cells.[25] Cells were cultured in Hybridoma medium[4] in the presence of irradiated B10BR spleen cells as stimulator cells and 12·5% Concanavalin A (Con A) supernatant as interleukin-2 (IL-2) source. Con A supernatant was derived from a 2-day culture of 5×10^6/ml rat spleen cells with 2 μg/ml Con A type IV (Sigma, St Louis, MO). CTL clones were restimulated every 2–3 weeks.

All lines used were mycoplasma free, as shown by regular screening using a Mycoplasma rapid detection system (Gen-Probe Inc., San Diego, CA).

Reagents

Mouse recombinant TNF-α (4×10^7 U/mg) was obtained from Genzyme (Boston, MA). Iodinated mouse recombinant TNF-α (specific activity $45{\cdot}6 \times 10^3$ c.p.m./μg) and anti-TNF-α monoclonal antibody, 1F3F3, were kindly donated by Dr P. De Baetselier (Free University, Sint Genesius-Rode, Belgium). A 1 : 10^6 dilution of 1F3F3 neutralizes the cytotoxicity of 1 U TNF-α for L929 cells. Pertussis toxin (List Biol. Lab., Campbell, CA) was dissolved in 0·1 M sodium phosphate, 0·5 M NaCl, pH 7. Cells (10^6/ml) were incubated with 200 ng PT/ml in Hybridoma medium[4] for 2–3 hr at 37° and 5% CO_2, washed free of toxin and then used for invasion assays.

Invasion assays

Invasiveness in REF monolayers was determined as described previously.[5] Briefly, cells, $2{\cdot}5 \times 10^6$ in Earle's balanced salt solution containing 20 mM Hepes, 0·9 mM $CaCl_2$ and 1 mM $MgCl_2$ with or without cytokine and/or monoclonal antibody, were added to confluent REF monolayers in 10 cm^2 wells and incubated at 37° and 5% CO_2. Non-infiltrated cells were then removed by repeated washing and the monolayers were fixed. Infiltrated cells, visible as dark and flattened cells in the phase-contrast microscope, were counted in 10 randomly distributed fields of 0·27 mm^2. From this, the number of infiltrated cells per well was calculated and invasion was expressed as percentage of added cells.

In some experiments invasion was quantitated using ^{51}Cr-labelled cells. Cells were labelled with ^{51}Cr,[7] and used for invasion assays as described above, except that $1{\cdot}3 \times 10^5$ labelled cells were used per 2 cm^2 well. Cultures were washed, lysed with 1 N NaOH and radioactivity was counted. Invasion was expressed as the percentage of label associated with the monolayer. This was feasible because most adherent cells can be washed off the fibroblasts,[26,27] so that radioactivity is largely derived from invaded cells. With this method, the background is higher, but the TNF-α effects seen were similar (Table 1 and data not shown).

TNF-α binding

5×10^5 T-cell hybridoma cells per well, in V-bottom microtitre plates (Greiner, Nürtingen, Germany), were incubated with 10 ng/ml ^{125}I-TNF-α in phosphate-buffered saline containing 0·9 mM $CaCl_2$, 1 mM $MgCl_2$ and 0·1% BSA for 30 min at 4°, washed in the same, ice-cold buffer, transferred to vials and counted. Non-specific binding was determined in the presence of 200 ng/ml unlabelled TNF-α and was less than 10%.

RESULTS

TNF-α effect on invasion is PT insensitive and transient

The invasive T-cell hybridoma TAM2D2.2.6 was used to analyse the effect of TNF-α on invasion of rat embryo fibroblast (REF) monolayers. TAM2D2.2.6 is a clone from the previously described T-cell hybridoma TAM2D2.2.[4,5] TNF-α increased invasion in a dose-dependent manner when it was present during the invasion assay [Fig. 1 (□)]. In this experiment TNF-α stimulated invasion to 127% of the control. In a large number of similar experiments we found that TNF-α enhanced invasion, but this effect was not always as clear. This might be due to the high level of invasiveness of these cells, which can possibly not be stimulated much further.

We had previously shown that pertussis toxin (PT) inhibited invasion by TAM2D2.2 cells into REF monolayers to 30% or less.[5] To test the PT sensitivity of the observed TNF-α effect, we assayed invasion of PT-pretreated TAM2D2.2.6 cells (200 ng PT per ml for 2 hr) in the presence of TNF-α. As shown in Fig. 1 (■), PT did not inhibit TNF-α-induced enhancement of invasion. On the contrary, the effect of TNF-α was now much more evident: invasiveness of PT-pretreated cells was stimulated 10-fold, whereas invasiveness of untreated cells was stimulated only

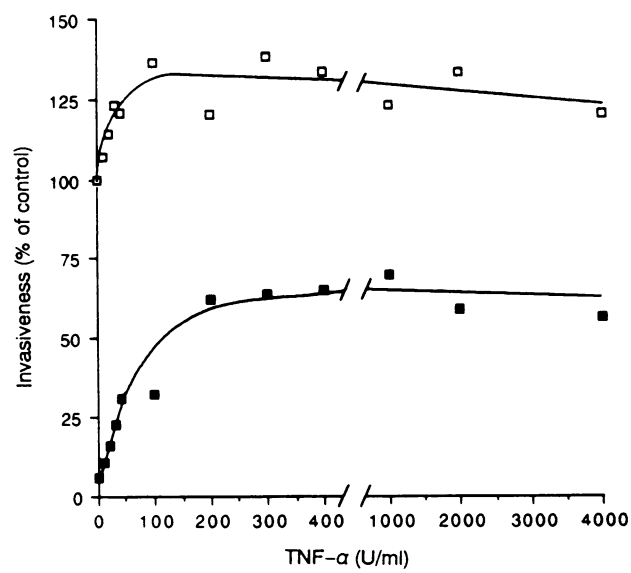

Figure 1. The effect of TNF-α on invasion is dose dependent. Invasion by untreated (□) and PT-pretreated (200 ng/ml, 2 hr) (■) TAM2D2.2.6 cells in the presence of increasing concentrations of TNF-α, added at $t = 0$. The assay was terminated after 30 min. Invasion is expressed as the percentage of control values. Invasion by untreated cells without TNF-α was 8·1% and by PT-pretreated cells without TNF-α 0·5% of added cells.

1·3-fold. This shows that TNF-α does not influence invasion via a PT-sensitive mechanism. Because the effect of TNF-α was more evident after PT pretreatment, all further experiments were performed with PT-pretreated cells.

In nine similar experiments using 400 U TNF-α per ml, invasion was stimulated, however, to a variable extent. Usually, invasion was enhanced two- to threefold (Figs 2–4, Table 1 and data not shown), but in some experiments, an example of which is shown in Fig. 1, even higher stimulation was seen. Because of this variation, results of single experiments representative of at least four are shown.

Maximal stimulation of invasion was observed with 200–400 U TNF-α per ml (Fig. 1) and 30 min after addition of T-cell hybridoma cells and TNF-α to the monolayer (data not shown). The effect of TNF-α on invasion was transient, because it disappeared after longer incubation. For this reason, subsequent invasion assays were terminated after 30 min.

TNF-α pretreatment of T-cell hybridoma cells, but not of fibroblasts, stimulates invasion

TNF-α was present during the invasion assay and thus the results obtained might be due to an effect on either the T-cell hybridoma cells or on the fibroblasts or both. To test this, TAM2D2.2.6 cells and REF monolayers were preincubated with the same concentration of TNF-α that was used for invasion assays (400 U/ml), washed free of cytokine and then immediately used for invasion assays. As can be seen in Fig. 2, invasion by PT-pretreated TAM2D2.2.6 cells was stimulated twofold when TNF-α was present during the invasion assay (Fig. 2, control), and a similar effect was observed when the cells were preincubated with TNF-α for 15 and 30 min (Fig. 2, preincubation). Moreover, the time-course of stimulation following preincubation was identical to that observed when TNF-α was present during the invasion assay: stimulation was maximal after 30 min and then declined. In contrast, TNF-α preincubation of fibroblast cultures, even up to 24 hr, did not alter invasion (data not shown). PT pretreatment of TAM2D2.2.6 cells inhibited invasion to approximately 20% of controls, and TNF-α stimulated invasion approximately twofold in all cases. Taken together, these data show that TNF-α directly acts on the T-cell hybridoma cells to stimulate their invasiveness, and that it does not influence invasion by up-regulation of cellular adhesion molecules, that mediate T-cell binding,[1,2] on the cell surface of the fibroblasts.

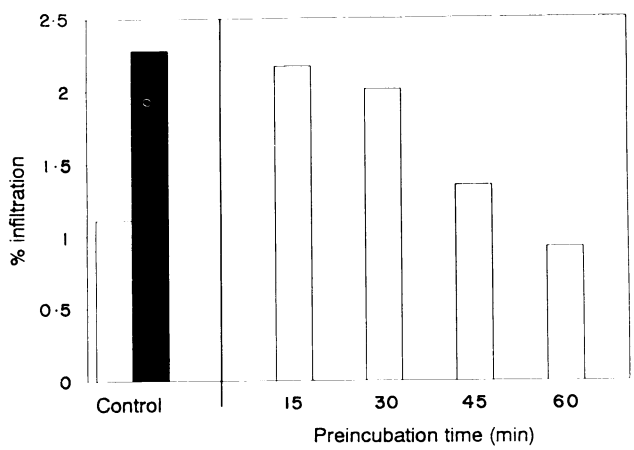

Figure 2. TNF-α-pretreatment of T-cell hybridomas stimulates invasion. TAM2D2.2.6 cells were pretreated with PT (200 ng/ml, 2 hr), washed, and incubated with 400 U TNF-α per ml for 15, 30, 45 and 60 min, respectively, washed twice, and then immediately used to determine invasion in a 30-min assay without TNF-α [preincubation (□)]. As a control, invasion by TAM2D2.2.6 cells pretreated with PT, but not with TNF-α, was measured in the presence [control (■)] or absence [control (□)] of 400 U TNF-α per ml, added at the start of the invasion assay. Invasion is expressed as the percentage of added cells.

Table 1. Anti-TNF-α monoclonal antibody neutralizes TNF-α-induced invasion

Antibody dilution	Invasion (% of added cells)	
	+PT−TNF-α	+PT+TNF-α
—	8·1±0·3	16·0±1·9
1:2500	8·1±0·5	11·0±0·1
1:1000	ND	11·7±0·2
1:400	9·4±0·3	8·9±0·6

^{51}Cr-labelled TAM2D2.2.6 cells were preincubated with PT (200 ng/ml, 3 hr) and invasion in the presence or absence of 400 U TNF-α per ml and/or anti-TNF-α monoclonal antibody 1F3F3 at indicated dilutions was measured in a 20-min assay. Control invasion by untreated cells was 48·1% (±1·6) of added cells.

ND, not determined.

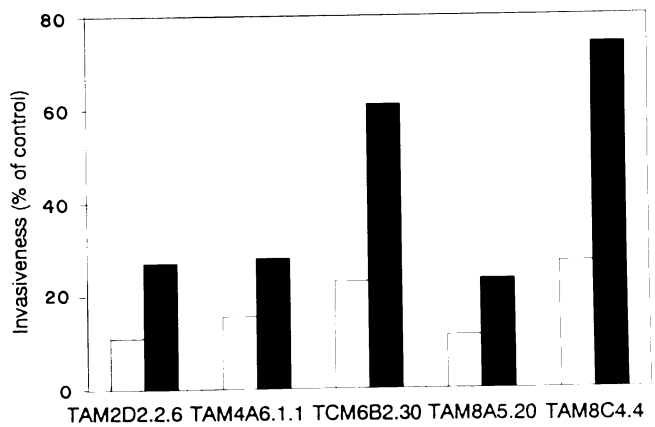

Figure 3. TNF-α stimulates invasion by five invasive T-cell hybridomas. Cells were preincubated with PT (200 ng/ml, 2 hr) and invasion in the presence (■) or absence of (□) 400 U TNF-α per ml was measured in a 30-min assay. Invasion is expressed as percentage of controls. Control invasion without PT and TNF-α was: TAM2D2.2.6, 11·5%; TAM8C4.4, 16·4%; TAM4A6.1.1, 16·1%; TCM6B2.30, 6·4%; TAM8A5.21, 9·9% of added cells.

Anti-TNF-α antibody neutralizes TNF-α-induced invasion

To demonstrate that the observed stimulation was in fact due to TNF-α, we assayed invasion by PT-pretreated TAM2D2.2.6 cells in the presence of TNF-α and the anti-TNF-α monoclonal

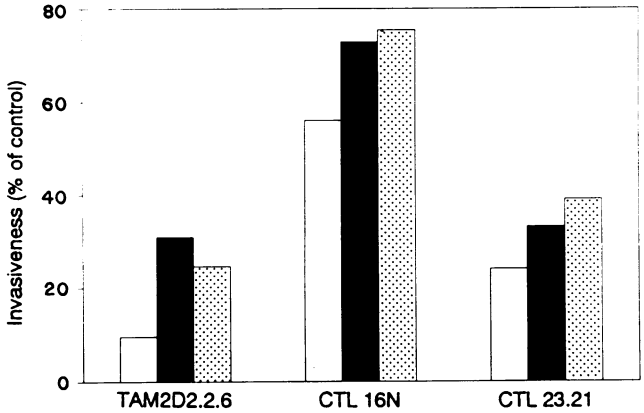

Figure 4. TNF-α stimulates invasion by two CTL clones. Cells were preincubated with PT (200 ng/ml, 2 hr), and invasion in the absence (□) or presence of 400 (■) and 1000 (▨) U TNF-α per ml was measured in a 30-min assay. Invasion is expressed as the percentage of controls. Control invasion without PT and TNF-α was: TAM2D2.2.6, 14·6%; CTL16N, 67·6%; CTL23.21, 43·3% of added cells.

antibody, 1F3F3. The results of a representative experiment are shown in Table 1. 1F3F3 (1:400–1:2500) alone did not inhibit invasion by either PT-pretreated (Table 1) or untreated cells (data not shown). TNF-α stimulated invasion by PT-pretreated cells twofold and this was completely abolished by a 1:400 dilution of 1F3F3.

TNF-α stimulates invasion by five invasive T-cell hybridomas

Next, we tested whether TNF-α also stimulated invasion by other invasive T-cell hybridomas. The cell lines used are clones of the hybrids that have been described previously[4,5] and are a representative sample from the group of highly invasive, highly metastatic T-cell hybridomas. Invasion by PT-pretreated cells was assayed in the presence and absence of 400 U TNF-α per ml (Fig. 3). TNF-α stimulated invasiveness of each cell line to a similar extent, that is 1·8- to 2·7-fold. In this experiment, invasion of TAM2D2.2.6 cells was stimulated 2·4-fold.

TNF-α stimulates invasion by two CTL clones

To test whether TNF-α affected invasion by normal T cells, two mouse cytotoxic T-lymphocyte (CTL) clones were used. Both CTL clones were very highly invasive: in this experiment 68% and 43% of added cells invaded the monolayer in case of CTL 16N and CTL 23.21, respectively, compared to 15% of added cells for TAM2D2.2.6 (Fig. 4). PT pretreatment inhibited invasion by both CTL clones, but less than T-cell hybridomas. In four experiments, invasion by CTL clones was reduced to on average 45% of controls (data not shown), whereas PT inhibited invasion by T-cell hybridomas to on average 10% of controls (Figs 1, 3 and 4, Table 1). In the experiment shown, PT pretreatment inhibited invasion by CTL 16N to 56% and by CTL 23.21 to 24% of controls. TNF-α enhanced invasion by PT-pretreated cytotoxic T lymphocytes 1·3- to 1·6-fold. This effect is less impressive than for T-cell hybridoma cells: the same concentrations of TNF-α stimulated invasion by PT-pretreated TAM2D2.2.6 cells 2·6- to 3.2-fold. However, in absolute numbers the increase in invasion for CTL was substantial: 11·4% and 3·8% of added cells by 400 U TNF-α per ml, and 13·1% and 6·4% by 1000 U TNF-α per ml for CTL 16N and CTL 23.21, respectively, compared to 3·1% (400 U) and 2·2% (1000 U) for TAM2D2.2.6.

TNF-α does not stimulate invasion by non-invasive T-cell hybridomas

In addition to invasive T-cell hybridomas, we have tested the effect of TNF-α on non-invasive T-cell hybridomas, that we have obtained previously.[4,5] Invasion by neither untreated- nor PT-pretreated cells was affected by TNF-α, whereas in the same experiments the usual results were obtained with invasive TAM2D2.2.6 cells, so both PT and TNF-α were active (Table 2). To test whether this was due to absence of TNF-receptors, we measured ^{125}I-TNF-α binding to non-invasive cells. Cells were incubated with 10 ng/ml ^{125}I-TNF-α, the same concentration that was used in invasion assays. As expected, ^{125}I-TNF-α bound to invasive TAM2D2.2.6 cells (1816 ± 16 c.p.m./500,000 cells), but also to the non-invasive T-cell hybridoma TAS1ONI14.31 and the non-invasive BW5147 T lymphoma (1549 ± 45 c.p.m. and 788 ± 23 c.p.m./500,000 cells, respectively). Thus, although non-invasive cells have TNF-α binding sites on their cell surface, TNF-α cannot induce invasiveness of these cells.

DISCUSSION

The results presented here demonstrate that TNF-α stimulates invasion by T-cell hybridomas into fibroblast monolayers *in vitro*. We have used T-cell hybridomas as a model for invasion by normal T lymphocytes, rather than freshly isolated spleen T cells, because the spleen contains a heterogeneous mixture of T cells in different maturation stages and the level of invasiveness of different isolates is variable (G. La Rivière, J. Klein Gebbinck, C. Schipper and E. Roos, unpublished results). The advantage of T-cell hybridomas is that they are cloned cell lines, consisting of a single population of cells and that their invasive potential is stable.[4,5] TNF-α not only enhanced invasion by five independent, representative invasive T-cell hybridomas, but also by two mouse cytotoxic T-cell clones. This indicates that at least part of the invasive machinery is identical and that T-cell hybridomas are an appropriate model to study invasion by normal T lymphocytes.

For T-cell hybridomas invasiveness *in vitro* correlates with widespread metastasis *in vivo*,[4,5] indicating that invasiveness is a prerequisite for colonization of tissues from the blood, a process similar to extravasation of normal T cells. The CTL clones tested were also very highly invasive *in vitro*. Because these cells have been repeatedly restimulated,[25] they are probably comparable to memory-T lymphocytes *in vivo*. Recently, Mackay *et al.*[3] showed that memory-T cells migrate from blood into healthy, non-inflamed tissues. Taken together, this suggests that the high level of invasiveness of memory CTL is responsible for their *in vivo* migration behaviour. The effect of TNF-α on untreated T-cell hybridoma cells was not always evident, and maximal stimulation was only 1·3-fold. It is difficult to judge whether this limited effect is relevant *in vivo*, but TNF-α may contribute to migration of (memory) T cells into inflamed areas, where cytokines, among which TNF-α, are produced.[28]

By enhancing invasiveness, TNF-α may also enhance metastasis formation by T lymphomas. In line with this notion are the

Table 2. TNF-α does not stimulate invasion by non-invasive T-cell hybridomas

Cell line	Invasion (% of added cells)			
	−PT−TNF-α*	−PT+TNF-α†	+PT−TNF-α‡	+PT+TNF-α§
BW5147	2·1	1·5	1·5	1·7
TAS5C4.1.2.18	1·0	0·6	0·6	1·1
TAS10NI14.31	0·3	1·6	1·0	0·3
TAS9B5.49	0·9	0·8	0·3	0·5
TAM8A3.15	0·6	0·7	0·6	0·6

Invasion by untreated (*, †) and PT-pretreated (200 ng/ml, 2 hr) (‡, §) cells was determined in the presence (†, §) or absence (*, ‡) or 400 U TNF-α per ml in a 30-min assay. Parallel control experiments using invasive T-cell hybridoma cells and the same PT and TNF-α preparation showed that both TNF-α and PT were active (data not shown).

enhanced metastasis of TNF-α transfected Chinese hamster ovary cells,[29] and the increased lung colonization of human melanoma cells in nude mice caused by TNF-α.[30] Furthermore, it is conceivable that TNF-α is one of the factors causing enhanced metastasis to injured and healing tissues.[31] In using TNF-α as an 'anti-cancer' agent, this should be taken into account.

Pertussis toxin (PT) strongly inhibits invasion and metastasis by T-cell hybridomas.[5,7] This implies that an invasion-enhancing signal is transmitted over the plasma membrane via a PT-sensitive G protein. The ligand is not TNF-α, because TNF-α stimulated invasion also after PT pretreatment. Preliminary experiments have indicated that T-cell hybridomas synthesize TNF-α messenger RNA (mRNA), but this does not necessarily imply that the cells also secrete TNF-α protein, as shown by Krönke *et al.*[32] If the T-cell hybridoma cells do produce TNF-α, it could be responsible for the low level of invasiveness that remains after PT pretreatment.

The signal leading to TNF-α-mediated stimulation of invasion is not transmitted by a PT-sensitive G protein, but it is not clear which other transduction pathway may be used. TNF-α can enhance phospholipase A_2 activity,[13,33] transiently activate protein kinase C[34,35] and increase or decrease intracellular cyclic AMP levels.[20,36,37] TNF-α-induced endothelial cell retraction is mediated via phospholipase A_2, and this is inhibited by PT,[13] indicating that in T-cell hybridoma cells the invasion-enhancing signal of TNF-α is not transmitted via this pathway. In neutophils, a fall in cyclic AMP levels was induced by a synergistic action of TNF-α receptor binding and CD18 (β_2) integrin engagement, and this was accompanied by actin reorganization and cell spreading.[37] This is particularly interesting, because the CD18 integrin leucocyte function-associated antigen-1 (LFA-1) is involved in invasion by T-cell hybridomas.[38,39] It is therefore conceivable that joint action of TNF-α and LFA-1 binding to its ligand on the fibroblasts, induces a fall in cyclic AMP and promotes cell spreading, similarly as in neutrophils, thus enhancing invasion by T-cell hybridoma cells. This is in line with the very recent observations that TNF-α induces motility of lymphokine-activated killer cells apparently by decreasing cyclic AMP levels.[20]

TNF-α did not induce invasion by non-invasive T-cell hybridoma cells. This is not due to lack of TNF receptors, because the cells bound ^{125}I-TNF-α. Possible explanations are that these cells: (i) lack (part) of the post-receptor signal transduction machinery, and/or (ii) lack protein(s) that are indispensable for invasion to occur and/or (iii) contain invasion suppressors. Identification of invasion-specific proteins and elucidation of their function, should reveal which of these explanations is correct.

On endothelial cells, TNF-α induces expression of the cellular adhesion molecules (CAM), intercellular CAM-1 (ICAM-1), endothelial CAM-1 (ELAM-1) and vascular CAM-1 (VCAM-1), which mediate T-cell binding.[1,2,40] Similarly, TNF-α induces ICAM-1 on fibroblasts.[41] We found that TNF-α pretreatment of REF did not enhance invasion by either control or PT-pretreated cells, showing that the effect of TNF-α on invasion is not due to increased expression of adhesion molecules on the fibroblasts. In line with this we found that the level of ICAM-1 was not increased during a 30-min treatment with TNF-α (data not shown). In fact, preincubation of T-cell hybridoma cells stimulated invasion to the same extent and with the same kinetics as when TNF-α was present during the invasion assay. Gamble *et al.*[21] showed that TNF-α increases neutrophil adhesion to endothelial cells, by affecting both endothelial cells and neutrophils. Our data show that TNF-α can also act directly on T cells to stimulate their invasiveness. TNF-α thus plays a regulatory role, not only in T-cell growth and function,[11,28] but also in T-cell migration.

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REFERENCES

1. Osborn L. (1990) Leukocyte adhesion to endothelium in inflammation. *Cell*, **62**, 3.
2. Springer T.A. (1990) Adhesion receptors of the immune system. *Nature*, **346**, 425.
3. Mackay C.R., Marston W.L. & Dudler L. (1990) Naive and memory T cells show distinct pathways of lymphocyte recirculation. *J. exp. Med.* **171**, 801.
4. Roos E., La Rivière G., Collard J.G., Stukart M.J. & De Baetselier P. (1985) Invasiveness of T-cell hybridomas *in vitro* and their metastatic potential *in vivo*. *Cancer Res.* **45**, 6238.

5. La Rivière G., Schipper C.A., Collard J.G. & Roos E. (1988) Invasiveness in hepatocyte and fibroblast monolayers and metastatic potential of T-cell hybridomas in mice. *Cancer Res.* **48,** 3405.
6. Roos E. (1991) Adhesion molecules in lymphoma metastasis. *Cancer Metast. Rev.* **10,** 33.
7. Roos E. & van de Pavert I.V. (1987) Inhibition of lymphoma invasion and liver metastasis formation by pertussis toxin. *Cancer Res.* **47,** 5439.
8. Stryer L. & Bourne H.R. (1986) G proteins: a family of signal transducers. *Ann. Rev. Cell Biol.* **2,** 391.
9. Nedwin G.E., Svedersky L.P., Bringman T.S., Palladino, M.A. & Goeddel D.V. (1985) Effect of interleukin 2, interferon-γ, and mitogens on the production of tumor necrosis factors α and β. *J. Immunol.* **135,** 2492.
10. Cuturi M.C., Murphy M., Costa-Giomi M.P., Weinmann R., Perussia B. & Trinchieri G. (1987) Independent regulation of tumor necrosis factor and lymphotoxin production by human peripheral blood lymphocytes. *J. exp. Med.* **165,** 1581.
11. Scheurich P., Thoma B., Ücer U. & Pfizenmaier K. (1987) Immunoregulatory activity of recombinant human tumor necrosis factor (TNF)-α: induction of TNF receptors on human T cells and TNF-α-mediated enhancement of T cell responses. *J. Immunol.* **138,** 1786.
12. Andrews J.S., Berger A.E. & Ware C.F. (1990) Characterization of the receptor for tumor necrosis factor (TNF) and lymphotoxin (LT) on human T lymphocytes. *J. Immunol.* **144,** 2582.
13. Clark M.A., Chen M., Crooke S.T. & Bomalaski J.S. (1988) Tumour necrosis factor (cachectin) induces phospholipase A_2 activity and synthesis of a phospholipase A_2-activitating protein in endothelial cells. *Biochem. J.* **250,** 125.
14. Imamura K., Sherman M.L., Spriggs D. & Kufe D. (1988) Effect of tumor necrosis factor on GTP binding and GTPase activity in HL-60 and L929 cells. *J. biol. Chem.* **263,** 10247.
15. Brett J., Gerlach H., Nawroth P., Steinberg S., Godman G. & Stern D. (1989) Tumor necrosis factor/cachectin increases permeability of endothelial cell monolayers by a mechanism involving regulatory G proteins. *J. exp. Med.* **169,** 1977.
16. Issekutz T.B. & Stolz J.M. (1989) Stimulation of lymphocyte migration by endotoxin, tumor necrosis factor, and interferon. *Cellular Immunol.* **120,** 165.
17. Mason M.J. & van Epps D.E. (1989) *In vivo* neutrophil emigration in response to interleukin-1 and tumor necrosis factor-alpha. *J. Leuk. Biol.* **45,** 62.
18. Ming W.J., Bersani L. & Mantovani A. (1987) Tumor necrosis factor is chemotactic for monocytes and polymorphonuclear leukocytes. *J. Immunol.* **138,** 1469.
19. Wang J.M., Walter S. & Mantovani A. (1990) Re-evaluation of the chemotactic activity of tumour necrosis factor for monocytes. *Immunology*, **71,** 364.
20. Maghazachi A.A. (1991) Tumor necrosis factor-α is chemokinetic for lymphokine-activated killer cells: regulation by cyclic adenosine monophosphate. *J. leuk. Biol.* **49,** 302.
21. Gamble J.R., Harlan J.M., Klebanoff S.J. & Vadas M.A. (1985) Stimulation of the adherence of neutrophils to umbilical vein endothelium by human recombinant tumor necrosis factor. *Proc. natl. Acad. Sci. U.S.A.* **82,** 8667.
22. Cavender D., Saegusa Y. & Ziff M. (1987) Stimulation of endothelial cell binding of lymphocytes by tumor necrosis factor. *J. Immunol.* **139,** 1855.
23. Thornhill M.H., Kyan-Aung U., Lee T.H. & Haskard D.O. (1990) T cells and neutrophils exhibit differential adhesion to cytokine-stimulated endothelial cells. *Immunology*, **69,** 287.
24. Piela T.H. & Korn J.H. (1987) Lymphocyte-fibroblast adhesion induced by interferon gamma and tumor necrosis factor. *Arthritis Rheum.* **30,** S90.
25. Di Pauli R. & Opalka B. (1982) Antigen-dependent, H-2-restricted cytolytic and noncytolytic T cell lines with specificity for minor histocompatibility antigens. *Eur. J. Immunol.* **12,** 365.
26. Verschueren H., Dekegel D. & De Baetselier P. (1987) Development of a monolayer invasion assay for the discrimination and isolation of metastatic lymphoma cells. *Invasion Metast.* **7,** 1.
27. Collard J.G., van de Poll M., Scheffer A., Roos E., Hopman A.H.M., Geurts van Kessel A.H.M. & van Dongen J.J.M. (1987) Location of genes involved in invasion and metastasis on human chromosome 7. *Cancer Res.* **47,** 6666.
28. Beutler B. & Cerami A. (1989) The biology of cachectin/TNF—primary mediator of the host response. *Ann. Rev. Immunol.* **7,** 625.
29. Malik S.T.A., Naylor M.S., East N., Oliff A. & Balkwill F.R. (1990) Cells secreting tumour necrosis factor show enhanced metastasis in nude mice. *Eur. J. Cancer*, **26,** 1031.
30. Giavazzi R., Garofalo A., Bani M.R., Abbate M., Ghezzi P., Boraschi D., Mantovani A. & Dejana E. (1990) Interleukin 1-induced augmentation of experimental metastases from a human melanoma in nude mice. *Cancer Res.* **50,** 4771.
31. Murphy P., Alexander P., Senior P.V., Fleming J., Kirkham N. & Taylor I. (1988) Mechanisms of organ selective tumour growth by bloodborne cancer cells. *Br. J. Cancer*, **57,** 19.
32. Krönke M., Hensel G., Schlüter C., Scheurich P., Schütze S. & Pfizenmaier K. (1988) Tumor necrosis factor and lymphotoxin gene expression in human tumor cell lines. *Cancer Res.* **48,** 5417.
33. Haliday E.M., Ramesha C.S. & Ringold G. (1991) TNF induces *c-fos* via a novel pathway requiring conversion of arachidonic acid to a lipoxygenase metabolite. *EMBO J.* **10,** 109.
34. Lane T.A., Lamkin G.E. & Wancewicz E.V. (1990) Protein kinase C inhibitors block the enhanced expression of intercellular adhesion molecule-1 on endothelial cells activated by interleukin-1, lipopolysaccharide and tumor necrosis factor. *Biochim. biophys. Res. Comm.* **172,** 1273.
35. Schütze S., Nottrott S., Pfizenmaier K. & Krönke M. (1990) Tumor necrosis factor signal transduction: cell-type-specific activation and translocation of protein kinase C. *J. Immunol.* **144,** 2604.
36. Zhang Y., Lin J., Yip Y.K. & Vilček J. (1988) Enhancement of cAMP levels and of protein kinase activity by tumor necrosis factor and interleukin 1 in human fibroblasts: role in the induction of interleukin 6. *Proc. natl. Acad. Sci. U.S.A.* **85,** 6802.
37. Nathan C. & Sanchez E. (1990) Tumor necrosis factor and CD11/CD18 (β2) integrins act synergistically to lower cAMP in human neutrophils. *J. Cell Biol.* **111,** 2171.
38. Roos E. & Roossien F.F. (1987) Involvement of leukocyte function-associated antigen-1 (LFA-1) in the invasion of hepatocyte cultures by lymphoma and T-cell hybridoma cells. *J. Cell Biol.* **105,** 553.
39. Roossien F.F., de Rijk D., Bikker A. & Roos E. (1989) Involvement of LFA-1 in lymphoma invasion and metastasis demonstrated with LFA-1-deficient mutants. *J. Cell Biol.* **108,** 1979.
40. Graber N., Gopal T.V., Wilson D., Beall L.D., Polte T. & Newman W. (1990) T cells bind to cytokine-activated endothelial cells via a novel, inducible sialoglycoprotein and endothelial leukocyte adhesion molecule -1. *J. Immunol.* **145,** 819.
41. Rothlein R., Czajkowski M., O'Neill M.M., Marlin S.D., Mainolfi E. & Merluzzi V.J. (1988) Induction of intercellular adhesion molecule-1 on primary and continuous cell lines by pro-inflammatory cytokines: regulation by pharmacologic agents and neutralizing antibodies. *J. Immunol.* **141,** 1665.