Immunology 1988 **65** 647–649

Tumour necrosis factors modulate the affinity state of the leukotriene B_4 receptor on human neutrophils

J. BROM, J. KNÖLLER, M. KÖLLER & W. KÖNIG *Lehrstuhl für Medizinische Mikrobiologie und Immunologie, Arbeitsgruppe für Infektabwehrmechanismen, Ruhr-Universität Bochum, FRG*

Accepted for publication 18 August 1988

SUMMARY

Pre-incubation of human polymorphonuclear granulocytes with recombinant human tumour necrosis factors (TNF) revealed a time- and dose-dependent reduction of the expression of leukotriene B_4-receptor sites. Analysis of the binding data by Scatchard plots showed a shift from a heterologous receptor population (indicating high- and low-affinity subsets) to a homologous population. From the results it is considered that TNF can influence host defence through the modulation of leukotriene B_4 receptor affinity.

Tumour necrosis factor (TNF) was reported as a serum factor from endotoxin- and bacillus Calmette–Guerin-injected animals that caused the necrosis of certain tumours (Carswell *et al.*, 1975; Aggarwal *et al.*, 1985). The major cellular source of TNF-α (cachectin) is the monocyte–macrophage lineage, whereas TNF-β (lymphotoxin) is a product of T lymphocytes and B-lymphoblastoid cell lines. The genes for TNF-α and TNF-β have been cloned and an approximately 30% sequence homology was demonstrated. Both cytokines share a common receptor and show similar biological properties (reviewed by Beutler & Cerami, 1988). Aside from cytotoxicity, TNF mediates multiple aspects of the host inflammatory response such as activation of polymorphonuclear granulocytes (PMN). In this way, TNF augments the phagocytic activity, the degranulation and the neutrophil adhesion to endothelial cells (Shalaby *et al.*, 1985; Klebanoff *et al.*, 1986; Ferrante *et al.*, 1988).

Neutrophils provide a major role during host defence against bacterial infections. In this way, PMN generate and release leukotriene B_4 (LTB_4) which expresses profound chemotactic activity (Bray, 1983). In addition PMN respond to LTB_4 (Goldman & Goetzl, 1984) and metabolize LTB_4 (Brom, Schönfeld & König, 1988) via specific receptor sites. Binding to the high-affinity receptor results in chemotactic migration of the cells, binding to the low-affinity receptor transmits the release of granular enzymes. Recently, it has been shown that TNF influences the binding of the chemotactic peptide f-met-leu-phe to its receptor on granulocytes (Atkinson *et al.*, 1988).

Abbreviations: f-met-le-phe, *N*-formyl-methionyl-leucyl-phenylalanine; HPLC, high-performance liquid chromatography; LPS, lipopolysaccharides; LTB_4, leukotriene B_4; NDGA, nordihydroguaiaretic acid; PBS, phosphate-buffered saline; PMN, polymorphonuclear granulocytes; TNF, tumour necrosis factor.

Correspondence: Professor W. König, Lehrstuhl für Medizinische Mikrobiologie und Immunologie, Arbeitsgruppe für Infektabwehrmechanismen, Ruhr-Universität Bochum, Universitätsstraße 150, Postfach 10 21 48, D-4630 Bochum, FRG.

It was the purpose of the study to analyse the effects of recombinant human TNF on the specific binding of LTB_4 to human neutrophils as an example of the interaction between various cell populations via mediator release.

TNF-α and TNF-β were generously provided by Dr Adolf, Boehringer–Bender Institut, Vienna, Austria. The concentrations of TNF were 2×10^8 U/mg, as determined by the cytotoxicity for mouse L-M cells. The contamination of the TNF preparation with endotoxin was less than 0·1 ng/mg protein. [14, 15-^{3}H(N)]LTB_4 (specific activity: 0·74–2·22 TBq/mmol) was supplied by New England Nuclear, Dreieich. Unlabelled LTB_4 was kindly provided by Dr Rokach, Merck-Frosst, Pointe Claire, Canada, Ficoll 400 was from Pharmacia, Uppsala, Sweden; Macrodex (6%) was from Schiwa, Glandorf; metrizoate (75% w/v) was obtained from Nycomed, Oslo, Norway. Polymyxin B and nordihydroguaiaretic acid (NDGA) were purchased from Sigma, Deisenhofen.

Human neutrophils were isolated from heparinized (15 U/ml) peripheral blood of healthy donors using a Ficoll–metrizoate gradient and dextran sedimentation (Böyum, 1968). The purified PMN were adjusted to a cell concentration of $2{\cdot}4 \times 10^7$/1·2 ml of phosphate-buffered saline (PBS, pH 7·4) and incubated with TNF in the presence of calcium (0·5 mM) at 37°. After the pre-incubation period the cells were washed by short-term centrifugation, resuspended in calcium-free buffer and placed on ice. The binding assays were carried out using 96-well filtration plates with 5-μm pore-size polyvinylidene fluoride membranes (Millipore, Eschborn). Each well contained [^{3}H]LTB_4 (1·6 nM) and 40 μg of bovine serum albumin. Non-specific binding was determined in the presence of 220 nM of unlabelled LTB_4. Neutrophils at a concentration of 4×10^6 cells/200 μl PBS were added; after 45 min at 4° the incubation was terminated by rapid filtration using the Millititer vacuum holder. The filters were transferred to scintillation vials; methanol (0·5 ml) and 4 ml of Rotiszint 2211 (Roth, Karlsruhe) were added; the radioactivity was measured by liquid scintillation counting (Rack beta

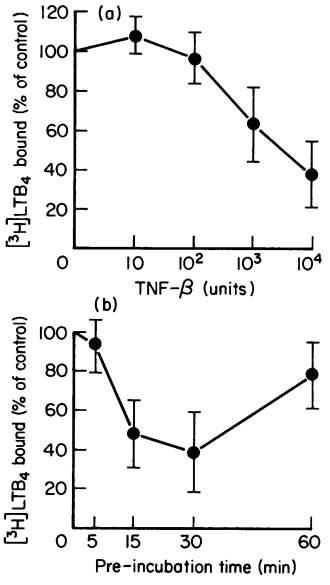

Figure 1. Dose- and time-dependent effects of TNF-β on [^{3}H]LTB$_4$ binding to human neutrophils. Purified human PMNs were pre-incubated with various concentrations of TNF-β (10^1–10^4 U) for 30 min (a) or with TNF-β (10^4 U) for the indicated periods at 37° (b). As control served cells pre-incubated with PBS which was defined as 100%. Each value represents the mean ± SD of four individual experiments.

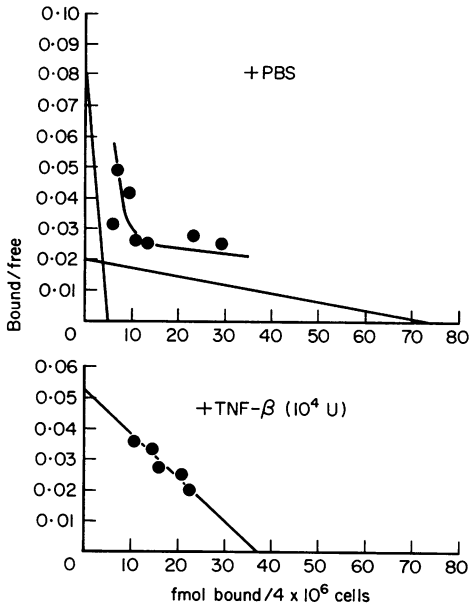

Figure 2. Scatchard plot analysis for the binding of [^{3}H]LTB$_4$. PMNs were incubated with TNF-β (10^4 U) or PBS for 30 min at 37°, washed and the specific binding of various concentrations of [^{3}H]LTB$_4$ was determined. (a) Neutrophils stimulated with PBS; (b) neutrophils stimulated with TNF-β. The data represent a characteristic result of two individual experiments.

1209, LKB, Turku, Finland). All experiments were carried out in triplicates. Specific binding was expressed as total binding minus non-specific binding. Scatchard plot analysis was carried out using various concentrations of [^{3}H] LTB$_4$. The binding data were analysed according to Rosenthal (1967).

Generation of leukotrienes were determined using reverse-phase high-performance liquid chromatography (HPLC) as was described recently (Knöller *et al.*, 1988).

Pre-incubation of PMN with recombinant human TNF-β using a concentration range between 10^1 and 10^4 units for 30 min at 37° resulted in a decreased expression of specific LTB$_4$-binding sites (Fig. 1a). Time-course experiments demonstrated that the maximal inhibitory effect was found after a pre-incubation period of 30 min with TNF-β (Fig. 1b).

Preparations of TNF may be contaminated with bacterial lipopolysaccharides (LPS). To exclude that the inhibition of the LTB$_4$-binding was due to the presence of LPS, the cells were pre-incubated with TNF-β in the presence of polymyxin B (1 mg/ml), which is a potent inhibitor for the biological activities of LPS. No differences were obtained when the specific LTB$_4$-binding was studied using cells prestimulated with TNF-β or TNF-β in the presence of polymyxin B. In contrast, boiling of TNF-β for 30 min resulted in the loss of the inhibitory effect (data not shown). Therefore, we conclude that the results are due to TNF itself and not to LPS.

A more pronounced effect was observed analysing the affinity state of the LTB$_4$ receptor. Therefore, PMN were incubated with various concentrations of [^{3}H]LTB$_4$ (0·3–4·8 nM) in the presence or absence of unlabelled LTB$_4$ (220 nM) for 45 min at 4°. The total and the unspecific binding of [^{3}H]LTB$_4$ were determined, the specific component was calculated and the data were analysed by Scatchard plots. The analysis exhibited a biphasic plot indicating high- and low-affinity receptor sites. However, in contrast to resting PMN the pre-incubation period of 30 min at 37° and the subsequent washing procedure reduced the values for the binding sites to 800 sites/cell (K_D: 0·17 nM) for the high-affinity receptor and 11·500 sites/cell (K_D: 11·0 nM) for the low-affinity state (Fig. 2a). Stimulation of the cells with TNF-β, 10^4 units, showed a conversion of the Scatchard plot characteristics (Fig. 2b). The linear curve suggested a homogenous receptor population. PMN prestimulated with TNF-β expressed 5·500 binding sites/cell with a K_D of 2·0 nM, respectively.

A similar receptor distribution pattern was obtained when PMN were incubated with NDGA (0·1 mg/ml), an inhibitor of the 5-lipoxygenase, for 10 min before TNF-β (10^4 units) was added. In addition, stimulation of PMN with TNF-β seems not to generate LTB$_4$ as measured by HPLC. Therefore, we suggest that the TNF effect on LTB$_4$ receptor distribution seems not to be the result of endogenous LTB$_4$ synthesis (data not shown).

Similar effects as with TNF-β were found when recombinant TNF-α was studied. A decreased LTB$_4$-binding and a shift in Scatchard plot characteristics were obtained.

Cytokines and lipid mediators play an important role in the regulation of the inflammatory reaction. Recently it was shown that arachidonic acid metabolites regulate the production of IL-1 by human monocytes (Kunkel & Chensue, 1985; Rola-Pleszczynski & Lemaire, 1985). Furthermore, the activation of neutrophils by a cytokine, the granulocyte–macrophage colony-stimulating factor, was described (Weisbart *et al.*, 1985). In addition, the tumour necrosis factor was shown to be a weak direct stimulus of neutrophil functions such as the respiratory burst, the phagocytic or the cytotoxic activity (Klebanoff *et al.*, 1986; Shalaby *et al.*, 1985). The effects of TNF on the chemotactic migration of human PMN were controversial (Ji

Ming, Bersani & Mantovani, 1987; Ferrante *et al.*, 1988). However, TNF seems to be a strong modulator of various stimulus-induced cellular responses. Recently, it was demonstrated that pre-incubation of neutrophils with TNF-α resulted in the modulation of f-met-leu-phe-receptor affinity. The receptor population changed from a biphasic population, including high- and low-affinity subsets, to a homogenous population with an intermediate affinity as a result of TNF-α-stimulation (Atkinson *et al.*, 1988). Our data demonstrate that a similar shift was observed measuring the affinity state of the LTB_4 receptor following stimulation of human PMN with TNF-β. These effects are due to TNF because the addition of polymyxin B excludes LPS activity. Resting PMN express two subclasses of LTB_4 binding sites mediating different functional activities of the cell. The high-affinity receptor mediates the chemotactic response, the low-affinity class induces the degranulation. It may be suggested that the shift of the LTB_4 affinity state influences the biological responsiveness of neutrophils. The affinity state of the receptor is regulated by guanine nucleotide-binding proteins and guanine nucleotides. The high-affinity receptor is converted into the low-affinity state in the presence of guanosine triphosphate (Goldman *et al.*, 1987). It is not known in which way TNF modulates the neutrophil responsiveness towards chemotactic stimuli. It may be possible that pre-incubation of the neutrophils with TNF results in a more pronounced loss of high-affinity receptors as a consequence of direct or not direct interaction with guanine nucleotide-binding proteins. Previously, it was described that small amounts of LTB_4 occupy and down-regulate the high-affinity receptor subset (Goldman & Goetzl, 1984). Such a specific desensitization of receptors following binding to minor amounts of endogenously generated LTB_4 could not be ruled out totally. However, within the limits of our test system we could not detect the generation of LTB_4 as a result of TNF stimulation. In addition, pre-incubation of neutrophils with TNF in the presence of the 5-lipoxygenase inhibitor NDGA showed similar effects compared with control stimulations without NDGA.

The presented results demonstrate a novel example for the interaction of different cell populations within the inflammatory reaction. These interactions are mediated by the release of cytokines, e.g. TNF-α and TNF-β derived from monocytes or lymphocytes, and lipid mediators such as leukotrienes, prostaglandins or the platelet-activating factor.

ACKNOWLEDGMENTS

This work was completed in partial fulfilment of J. Brom's PhD thesis, and was supported by Deutsche Forschungsgemeinschaft (KÖ427/7-3,4).

REFERENCES

AGGARWAL B.B., KOHR, W.J., HASS P.E., MOFFAT B., SPENCER S.A., HENZEL W.J., BRINGMAN T.S., NEDWIN G.E., GOEDDEL D.V. & HARKINS R.N. (1985) Human tumor necrosis factor. Production, purification and characterization. *J. biol. Chem.* **260,** 2345.

ATKINSON Y.H., MARASCO W.A., LOPEZ A.F. & VADAS M.A. (1988) Recombinant human tumor necrosis factor-α. Regulation of *N*-Formyl-methionyl-leucyl-phenylalanine receptor affinity and function on human neutrophils. *J. clin. Invest.* **81,** 759.

BEUTLER B. & CERAMI A. (1988) The common mediator of shock, cachexia, and tumor necrosis. *Adv. Immunol.* **42,** 213.

BÖYUM A. (1968) Isolation of mononuclear cells and granulocytes from human blood. Isolation of mononuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation at 1g. *Scand. J. Clin. Lab. Invest.* **21** (Suppl. 97), 77.

BRAY M.A. (1983) The pharmacology and pathophysiology of leukotriene B_4. *Br. Med. Bull.* **39,** 249.

BROM J., SCHÖNFELD W. & KÖNIG W. (1988) Metabolism of leukotriene B_4 by activated human polymorphonuclear granulocytes. *Immunology*, **64,** 509.

CARSWELL E.A., OLD L.J., KASSEL R.L., GREEN S., FIORE N. & WILLIAMSON B. (1975) An endotoxin-induced serum factor that causes necrosis of tumors. *Proc. natl. Acad. Sci. U.S.A.* **72,** 3666.

FERRANTE A., NANDOSKAR M., BATES E.J., GOH D.H.B. & BEARD L.J. (1988) Tumour necrosis factor beta (lymphotoxin) inhibits locomotion and stimulates the respiratory burst and degranulation of neutrophils. *Immunology*, **63,** 507.

GOLDMAN D.W., GIFFORD L.A., MAROTTI T., KOO C.H. & GOETZL E.J. (1987) Molecular and cellular properties of human polymorphonuclear leukocyte receptors for leukotriene B_4. *Fed. Proc.* **46,** 200.

GOLDMAN D.W. & GOETZL E.J. (1984) Heterogeneity of human polymorphonuclear leukocyte receptors for leukotriene B_4. Identification of a subset of high affinity receptors that transduce the chemotactic response. *J. exp. Med.* **159,** 1027.

JI MING W., BERSANI L. & MANTOVANI A. (1987) Tumor necrosis factor is chemotactic for monocytes and polymorphonuclear leukocytes. *J. Immunol.* **138,** 1469.

KLEBANOFF S.J., VADAS M.A., HARLAN J.M., SPARKS L.H., GAMBLE J.R., AGOSTI J.M. & WALTERSDORPH A.M. (1986) Stimulation of neutrophils by tumor necrosis factor. *J. Immunol.* **136,** 4220.

KNÖLLER J., SCHÖNFELD W., KÖLLER M., HENSLER T. & KÖNIG W. (1988) Arachidonic acid metabolites from polymorphonuclear leukocytes of healthy donors, severely burned patients and children with cystic fibrosis. Routine monitoring by high-performance liquid chromatography. *J. Chromatogr.* **427,** 199.

KUNKEL S.L. & CHENSUE S.W. (1985) Arachidonic acid metabolites regulate interleukin-1 production. *Biochem. Biophys. Res. Commun.* **128,** 892.

ROLA-PLESZCZYNSKI M. & LEMAIRE I. (1985) Leukotrienes augment interleukin 1 production by human monocytes. *J. Immunol.* **135,** 3958.

ROSENTHAL H.E. (1967) A graphic method for the determination and presentation of binding parameters in a complex system. *Anal. Biochem.* **20,** 525.

SHALABY M.R., AGGARWAL B.B., RINDERKNECHT E., SVEDERSKY L.P., FINKLE B.S. & PALLADINO M.A. (1985) Activation of human polymorphonuclear neutrophil functions by interferon-γ and tumor necrosis factor. *J. Immunol.* **135,** 2069.

WEISBART R.H., GOLDE D.W., CLARK S.C., WONG G.G. & GASSON J.C. (1985) Human granulocyte-macrophage colony-stimulating factor is a neutrophil activator. *Nature* (*Lond.*), **314,** 361.