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Gene expression and production of tumour necrosis factor by a rat basophilic leukaemia cell line (RBL-2H3) with IgE receptor triggering

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SUMMARY

This study evaluated the gene expression of tumour necrosis factor (TNF) and the molecular weight of the cytotoxic factor in a subline of a rat basophilic leukaemia cell line, RBL-2H3. After IgE receptor triggering with a specific antigen that was associated with histamine release, cytotoxic activity in the cell lysates and supernatants increased for 2 hr during the culture of RBL-2H3 cells. Furthermore, calcium ionophore A23187 could induce release of histamine and cytotoxic activity from RBL-2H3 cells. However, compound 48/80, lipopolysaccharide (LPS) and phorbol 12-myristate 13-acetate (PMA) were unable to induce the release of either histamine or cytotoxic activity from the cells. These data suggested that, at least in part, there was a common pathway in histamine release and production of cytotoxic activity. A protein synthesis inhibitor, cycloheximide, did not affect histamine release, but inhibited the induction of cytotoxic activity. This cytotoxic activity from RBL-2H3 cells was completely neutralized by anti-mouse TNF rabbit serum. With Northern blot analysis, mouse TNF cDNA probe could hybridize with RNA isolated from RBL-2H3 cells. TNF mRNA was induced as early as 1 hr after stimulation with specific antigen and decreased by 4 hr. Moreover, the molecular weight (MW) of the released cytotoxic factor from RBL-2H3 cells triggered with IgE receptors was approximately 17,000 by SDS–PAGE, which was compatible to that of TNF. Thus, it is concluded that the gene expression and production of TNF occurred in RBL-2H3 cells after IgE receptor triggering in association with histamine release, suggesting that TNF produced by basophils and mast cells may play an important role in allergic reaction through its wide range of biological activity.

INTRODUCTION

Mast cells and basophils play a major role in immediate allergic reaction by releasing chemical mediators such as histamine, serotonine and arachidonate metabolites after triggering of IgE receptors with specific antigen (Wasserman, 1980). In addition, it has been reported that in response to cross-linkage of IgE receptor, cultured mast cells produce various cytokines such as interleukin (IL)-3, IL-4, IL-5, IL-6 and granulocyte–macrophage colony-stimulating factor (GM-CSF), but not tumour necrosis factor (TNF) (Plaut *et al.*, 1989; Wodnar-Filipowicz, Heusser & Moroni, 1989).

TNF was originally identified in the serum of mice infected with *Mycobacterium bovis* strain BCG (Bacillus Calmette–Guerin) and later injected with endotoxin (Carswell *et al.*, 1975), and shown to be 17,000 MW by SDS–PAGE (Haranaka *et al.*, 1985). It could elicit haemorrhagic necrosis of some tumours in recipient animals and was cytotoxic to tumour cells *in vitro* without killing normal cells (Carswell *et al.*, 1975). Activated monocytes/macrophages were known to be the major cellular source of TNF (Carswell *et al.*, 1975). Recently, from the results of neutralization of the cytotoxic activity with anti-mouse TNF (mTNF) antibodies, it has been suggested that a cytotoxic factor released from RBL-2H3 cells after triggering IgE receptors is related to TNF (Ohno *et al.*, 1988; Richards *et al.*, 1988). Mast cells were also shown to produce a TNF-like factor after stimulation with phorbol 12-myristate 13-acetate (PMA) or lipopolysaccharide (LPS), although its activity was partially blocked by anti-TNF antibody, and the molecular weight of the factor was 50,000 in SDS–PAGE under reducing condition, unlike reported TNF (Young *et al.*, 1987). Moreover, mast cells were reported to express TNF mRNA spontaneously or after stimulation with PMA (Steffen *et al.*, 1989; Ohno *et al.*, 1990). However, neither the gene expression nor the identification of a TNF-related factor released from mast cells/basophils after IgE receptor triggering were evaluated.

In this paper, first, in order to evaluate TNF gene expression, Northern blot was carried out and TNF mRNA analysed in

Abbreviations: DNP, 2,4-dinitrophenyl; OVA, ovalbumin; PMϕ, peritoneal macrophage; RBL, rat basophilic leukaemia cell line; TNF, tumour necrosis factor.

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RBL-2H3 cells after stimulation with specific antigen. Second, to determine the molecular weight of the cytotoxic factor, SDS–PAGE and Western blot analysis with anti-mTNF antibody were performed.

MATERIALS AND METHODS

Cells

TNF-sensitive cell line L929, RBL-2H3 and mouse monocyte/macrophage cell line J774A.1 obtained from the Japanese Cancer Research Resources Bank, Tokyo were grown in RPMI-1640 (Gibco Laboratories, Grand Island, NY), except for J774A.1 which was grown in a serum-free medium, NYSF404 (Kohjin Co., Tokyo), supplemented with 100 U/ml penicillin (Meiji Seika Kaisha Ltd, Tokyo) 100 μg/ml streptomycin (Meiji) and 10% heat-inactivated fetal calf serum (FCS; Flow Laboratories Inc., Mclean, VA) at 37° in a humidified atmosphere of 5% CO_2, 95% air. Rat peritoneal macrophages (PMϕ) were isolated from pertoneal cells of male Sprague–Dawley rats, 200–250 g, purchased from Shizuoka Laboratory Animal Center, Hamamatsu. Peritoneal cells obtained by washing of the peritoneal cavity were allowed to adhere to 12-well tissue culture plates (Coastar, Cambridge, MA) at 37° for 2 hr and, after removing floating cells, the purity of macrophages was >95%, as determined by Wright–Giemsa stain. The peritoneal cells were suspended so that 10^6 rat PMϕ would adhere to each well.

Stimulation of cells

J774A.1 cells were harvested from adherent culture by scraping before stimulation. RBL-2H3 cells were harvested from adherent culture by 5 min treatment with trypsin-EDTA (Gibco) and allowed to adhere to a 12-well culture plate at 10^6 cells per well by overnight incubation. 10^6 rat PMϕ in a well were obtained as mentioned above. 10^6 cells were incubated with 1 ml of stimulant in each well of a 12-well culture plate. As stimulants, LPS (*Escherichia coli*; Sigma Chemical Co., St Louis, MO) was used at a final concentration of 10 μg/ml, PMA (Sigma) at 5 ng/ml, calcium ionophore A23187 (Sigma) at 5 μg/ml and compound 48/80 (Sigma) at 0·5 μg/ml in RPMI-1640 containing 2% of FCS, and medium alone as control. These experiments were performed in duplicate in a 12-well culture plate. After incubation for various periods, the culture supernatants were harvested. Cell lysates were obtained by freezing, thawing, sonication and centrifugation (Young *et al.*, 1987).

IgE receptor triggering

Anti-ovalbumin (OVA) mouse serum was obtained from 6- to 8-week-old female C3H/HeN mice, which were bred in Institute for Experimental Animals, Tohuku University School of Medicine, Sendai, injected at 3-week intervals with 5 μg of OVA (Sigma) in 0·5 mg of aluminium hydroxide (Wako Pure Chemical Industries Ltd, Osaka) (Ida, Siraganian & Notkins, 1983). Anti-DNP monoclonal mouse IgE was kindly provided by Creative Products Research Laboratories, Kissei Pharmaceutical Co. Ltd, Matsumoto, which was secreted in a serum-free culture medium of a mouse hybridoma generated by fusion of P3X63–Ag8.653 (ATCC CRL 1580; Rockville, MD) mouse myeloma cells and spleen cells from DNP–*Ascaris*-hyperimmunized mice and screened with passive cutaneous anaphylaxis (PCA) technique in rats. The PCA titres of this monoclonal antibody were 2^{13}. Passive sensitization and antigen challenge of RBL-2H3 cells were performed as described previously (Barsumian *et al.*, 1981). Briefly, the adherent cells in each well of a 12-well culture plate were passively sensitized with 1 ml of RPMI-1640 containing 5% of anti-OVA mouse serum or 20% of anti-DNP mouse IgE at 37° for 1 hr. After sufficient washing with RPMI-1640, the cells were incubated with 1 ml of OVA or DNP conjugated to OVA (DNP–OVA) at various concentrations as protein and harvested as described above. In some experiments, anti-OVA mouse serum and anti-DNP IgE were incubated at 56° for 4 hr before passive sensitization.

Histamine assay

At 30 min after stimulation, the culture supernatants were harvested and assayed for histamine content with an automated fluorometric technique. The total histamine content of each well was determined by adding 6% of perchloric acid. Spontaneous histamine release was obtained from the cells stimulated with medium alone, and less than 10% of total histamine content. The experimental results were expressed as the percentage of histamine release calculated from the following formula:

$$\text{histamine release}(\%) = (E - S) \times 100/(T - S)$$

where E was the histamine content of experimental sample, T was the total histamine content, and S was the spontaneously released histamine content (Siraganian & Hook, 1980).

Assay for TNF activity

The cytotoxic activity of the culture supernatants and the cell lysates was measured on L929 cells with actinomycin D (Sigma) (Warren & Ralph, 1986). Briefly, L929 cells were incubated with the test samples containing 1 μg/ml of actinomycin D in RPMI–1640 containing 2% FCS in 96-well plastic tissue culture plates (Corning Glass Works, Corning, NY). After incubation for 18 hr, the culture medium was discarded and the L929 cells were further incubated with 0·006% of Neutral Red (Gibco) for 75 min. Neutral Red in the viable L929 cells was eluted, and the absorbance of each well was read at 540 nm with Titertek Multiskan (Flow Laboratories) (Borden & Leonhardt, 1977). A total of 100% lysis of cells was achieved with 100 μl of RPMI-1640 containing 5% Triton X-100 (Sigma), and 0% lysis was determined from wells incubated only with reagents used for stimulation of cells. Percentage cytotoxicity was calculated from the following formula:

$$\text{cytotoxicity}(\%) = [A(0) - A(S)] \times 100/[A(0) - A(100)]$$

where A(0), A(100) and A(S) were A_{540} of each well of 0% lysis, 100% lysis and samples. Data were averaged from four wells. In addition, recombinant human TNF (hTNF) (5×10^5 U/ml, provided from Asahi Chemical Industry Co. Ltd, Tokyo), recombinant mTNF (>99% pure, 4×10^7 U/mg on L929 cells; Genzyme Corporation, Boston, MA) and some samples were also assayed after incubation for 30 min at 37° with a polyvalent rabbit antiserum against recombinant mTNF-α (approximately 10^6 neutralizing units per ml; Genzyme) or normal rabbit serum (Tago Inc., Burlingame, CA).

Effect of a protein synthesis inhibitor on the induction of cytotoxicity

In some experiments, cells were incubated with stimulants in the

presence of cycloheximide (Sigma) at 2 μg/ml. Histamine was assayed after 30 min and the cytotoxic activity after 24 hr, as described above.

RNA preparation and Northern blot analysis

Extraction of cellular RNA and evaluation of TNF mRNA were performed as described previously (Yamauchi, Martinet & Crystal, 1987; Chirgwin *et al.*, 1979). 10^7–10^8 cells of each J774A.1 or rat PMϕ adherent to tissue culture dishes (150 × 25 mm style; Becton-Dickinson Labware, Lincoln Park, NJ) were stimulated with LPS at 10 μg/ml in RPMI-1640. Confluent RBL-2H3 cells in the dishes were stimulated with A23187 at 5 μg/ml in RPMI-1640 or with DNP–OVA at 1 μg/ml in RPMI-1640 after passive sensitization with anti-DNP mouse IgE. After stimulation for 1–4 hr, the cells were lysed in 5·2 M guanidine isothiocyanate (Wako) solution. The lysates were mixed with cesium chloride (Mitsuwa's Pure Chemicals, Osaka) (1 g/2·5 ml), layered on a cushion of 5·6 M cesium chloride, 0·1 M EDTA, pH 7·6, in a polyallomer tube (Hitachi Koki Co. Ltd, Tokyo) and centrifuged at 20° in a RPS50-2 roter (Hitachi) at 100,000 *g* for 12 hr. The pellet was dissolved in 100 mM NaCl, 10 mM Tris-HCl, pH 8·6, 1 mM EDTA, 1% (w/v) SDS (Sigma), precipitated with ethanol, redissolved in autoclaved water, and stored in REVCO freezer (Rheem Manufacturing Company, Asheville, NC) at −80°. TNF mRNA in the cellular RNA was evaluated using Northern analysis. Twenty micrograms of each RNA were fractionated in 1% agarose gel (International Biotechnologies Inc.) under denaturing conditions with 6% of formaldehyde, and transferred to nitrocellulose filter (Bethesda Research Laboratories Life Technologies Inc., Gaithersburg, MD) at 4°, overnight. The nitrocellulose filter was hybridized with mTNF cDNA (provided by Asahi Chemical Industry Co. Ltd) (Shirai *et al.*, 1988) or human β-actin cDNA (Oncor Inc., Gaithersburg, MD) labelled with multiprime DNA labelling system using [^{32}P]dCTP-α (Amersham International plc., Amersham, U.K.). In this paper, a mTNF cDNA probe was used with rat PMϕ stimulated with LPS as positive control cells. Hybridization was performed at 42°, for 18 hr followed by washing of the filter four times (20°, 5 min each) in 2 × SSC, 0·05% SDS and four times (60°, 30 min each) in 0·1 × SSC, 0·05% SDS. Autoradiogram was performed by exposure to RX X-ray film (Fuji Photo Film Co. Ltd, Tokyo).

Western blot analysis

Confluent RBL-2H3 cells in tissue culture dishes were passively sensitized with 2% of anti-OVA mouse serum followed by stimulation with OVA at 10 ng/ml in RPMI-1640. After incubation for 24 hr, 50 ml of the culture supernatants were harvested and concentrated with Diaflo membrane (PM 10; Amicon corp., Lexington, MA) to 0·1 ml. Twelve microlitres of the sample and recombinant mTNF (10 ng/ml; Genzyme) were loaded to a 10–20% polyacrylamide gradient gel containing 0·1% SDS under reducing conditions (Laemmli, 1970), and electrophoretically transferred to filter (Millipore filter, type GV; Nihon Millipore Kogyo K.K., Yonezawa). Molecular weight standards were purchased from Bio-Rad Laboratories (Richmond, CA). After saturation in phospate-buffered saline (PBS) containing 3% of low fat milk overnight at 4°, the blot was incubated with anti-mTNF rabbit serum (Genzyme) for 2 hr at room temperature. After washing with PBS buffer containing 0·05% of Tween 20 (Sigma), anti-rabbit IgG sheep IgG antibodies conjugated to horseradish peroxidase (Serotec, Oxford, Oxon, U.K.) were added for 1 hr at room temperature and, after extensive washing, peroxidase activity was revealed by the H_2O_2-chloronaphtol substrates (Konika Co. Ltd, Tokyo).

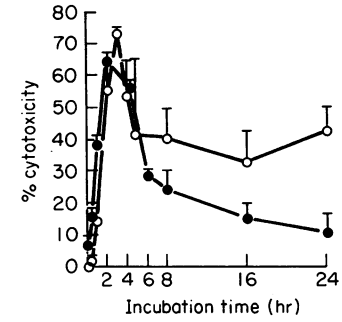

Figure 1. Kinetics of cytotoxic activity from RBL-2H3 cells. RBL-2H3 cells were passively sensitized with anti-OVA mouse serum followed by incubation with OVA at 10 ng/ml. After incubation for various periods, the culture supernatants (○) and cell lysates (●) were assayed for cytotoxic activity and expressed as mean ± SE of four to eight samples.

Table 1. Effect of heat treatment of anti-DNP IgE on histamine release and cytotoxicity induction

Sensitization*	Histamine release†	Cytotoxicity†
Anti-DNP IgE	18·2 ± 33	44·7 ± 6·6
Heat-inactivated anti-DNP IgE	2·8 ± 0·9	0·1 ± 0·1
Medium	0·0 ± 0·0	0·0 ± 0·0

* RBL-2H3 cells were incubated with anti-DNP mouse IgE, heat-inactivated anti-DNP mouse IgE or medium alone at 56° 30 min.

† Histamine was assayed after 30 min of stimulation with 1 μg/ml of DNP–OVA and cytotoxicity after 4 hr in independent culture wells. Data are expressed as mean ± SEM of four samples.

RESULTS

Kinetics of cytotoxic activity in culture supernatants and cell lysates of RBL-2H3 cells

RBL-2H3 cells were sensitized with anti-OVA mouse serum and incubated with OVA at 10 ng/ml, which induced the release of histamine (31·9 ± 7·1%, $n = 8$) after 30 min. After various incubation periods, the culture supernatants and cell lysates were harvested and assayed for cytotoxicity (Fig. 1). After 30 min, low levels of cytotoxics activity (2·2 ± 0·6%) were detected in the culture supernatants. However, afterwards the cytotoxic activity continued to rise and reached a plateau after 2 hr. On the other hand, in the cell lysates, cytotoxic activity was at a low level at the starting point (6·6 ± 2·4%), increased significantly during 2 hr (1 hr, 38·6 ± 2·9%; 2 hr, 64·6 ± 2·4%), and then decreased until 6 hr (29·1 ± 1·1%). Moreover, to certify that the release of cytotoxic activity was mediated through IgE receptors, RBL-2H3 cells were passively sensitized with anti-DNP monoclonal IgE antibody (Table 1). After stimulation with

Table 2. Cytotoxic activity in culture supernatants of RBL-2H3 cells with various stimulants

Cells	Stimulants*				
	Medium	LPS	PMA	OVA	A23187
J774A.1	0·0±0·0 (4)†	79·1±8·5 (4)	18·1±5·3 (4)	ND	ND
rat PMϕ	18·9±5·8 (4)	89·9±3·4 (6)	54·7±0·4 (4)	ND	10·5±1·2 (4)
RBL-2H3	2·0±0·7 (8)	0·6±0·6 (4)	0·4±0·3 (4)	64·3±4·7 (8)	81·4±3·5 (4)

* One millilitre of the stimulants was added to 10^6 of the cells. As stimulants, LPS was used at 10 μg/ml, PMA at 5 ng/ml, OVA at 10 ng/ml and A23187 at 5 μg/ml.

† After incubation for 24 hr, cytotoxic activity was assayed and expressed as mean±SEM (*n*). ND, not done.

DNP–OVA, these cells released histamine and cytotoxic activity. In contrast, heat-inactivation of anti-DNP IgE abolished not only histamine release but also induction of cytotoxicity. These data suggested that triggering of IgE receptor could induce the cytotoxic activity from RBL-2H3 cells.

Cytotoxic activity in culture supernatants of RBL-2H3 cells with various stimulants

TNF has been known to be produced mainly by macrophages/monocytes (Carswell *et al.*, 1975). In this experiment, cytotoxic activity of RBL-2H3 cells was compared with that of J774A.1 cells and rat PMϕ when stimulated with LPS, PMA or A23187 (Table 2). The cytotoxic activity in the supernatants from J774A.1 cells was detected when stimulated with either LPS or PMA. The supernatants from rat PMϕ incubated with LPS showed cytotoxic activity. Furthermore, the cytotoxic activity from rat PMϕ was detected when stimulated with either PMA or A23187. In contrast, when RBL-2H3 cells were stimulated with either LPS or PMA, neither histamine release nor cytotoxic activity in the supernatants and cell lysates was detected. No cytotoxic activity was detected even after stimulation for 4 hr under these conditions (data not shown). Compound 48/80, which has been known to induce histamine release from mast cells but not basophils (Lichtenstein, 1975), also could induce the release of neither histamine nor cytotoxic activity from RBL-2H3 cells (data not shown). However, by either IgE receptor stimulation or A23187, histamine (56·8±6·4% and 59·8±2·3%, respectively) was released after 30 min of stimulation, and, after 24 hr, cytotoxic activity (64·3±4·7% and 81·4±3·5% respectively) was accumulated in the supernatants. Moreover, when J774A.1 cells were incubated with LPS and sensitized RBL-2H3 cells with OVA in the presence of cycloheximide, which is an inhibitor of protein synthesis, the cytotoxic activity of the supernatants from each cell did not show any increase compared with control (data not shown). The data suggested that *de novo* protein synthesis was required for the induction of cytotoxic activity. In addition, from the result that anti-mTNF rabbit serum completely blocked the cytotoxic activities, not only from J774A.1 cells and rat PMϕ as well as recombinant mTNF but also in the supernatants and the lysates of RBL-2H3 cells (data not shown), it was suggested that the cytotoxic factors obtained from RBL-2H3 cells had the same immunoreactivity to anti-mTNF-specific serum as TNF.

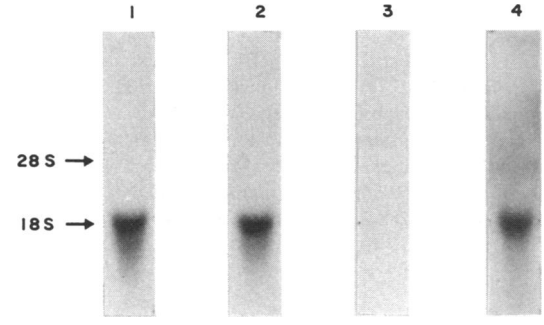

Figure 2. TNF gene expression by RBL-2H3 cells with A23187. Twenty micrograms of RNA were isolated from the cells 4 hr after stimulation: lane 1, J774A.1 cells with LPS; lane 2, rat PMϕ with LPS; lane 3, RBL-2H3 cells with medium alone; and lane 4, RBL-2H3 cells with A23187. TNF mRNA on nitrocellulose filters was hybridized with ^{32}P-labelled mTNF cDNA probe. The migration positions of 28S and 18S ribosomal RNA are indicated to the left.

TNF gene expression by RBL-2H3 cells

RNA extracted from the cells after stimulation was evaluated for TNF gene expression using mTNF cDNA probe (Figs 2 and 3). J774A.1 cells and rat PMϕ treated with LPS for 4 hr expressed a hybridizing band at the position of 18S ribosomal RNA, corresponding to TNF mRNA (Fig. 2). From this result, a mTNF cDNA probe was capable of hybridizing with rat TNF mRNA in these conditions. While TNF mRNA from RBL-2H3 cells incubated with medium alone was not detected, in contrast the cells treated with A23187 for 4 hr expressed TNF mRNA (Fig. 2). The RNA from RBL-2H3 cells stimulated with DNP–OVA following passive sensitization with anti-DNP mouse IgE was extracted at time-points 0, 1, 2 or 4 hr after stimulation, and probed for mTNF and human β-actin. As shown in Fig. 3, TNF mRNA was not detected before stimulation, but appeared and peaked as early as 1 hr after exposure to DNP–OVA, and declined thereafter. As control, β-actin mRNA was present at any time-point in this time-course (Fig. 3). Northern analysis demonstrated that A23187 and IgE receptor stimulation increased TNF mRNA level in RBL-2H3 cells.

Western blot analysis of the culture supernatants from RBL-2H3 cells

To evaluate the molecular weight of the cytotoxic factors released from RBL-2H3 cells that had immunoreactivity to anti-

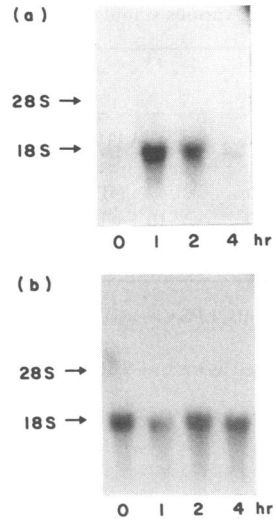

Figure 3. TNF gene expression by RBL-2H3 cells with IgE receptor triggering. RBL-2H3 cells were passively sensitized with anti-DNP monoclonal IgE and exposed to DNP–OVA. RNA was extracted at the indicated time-points after stimulation and loaded at 20 μg to each lane. The nitrocellulose filters were hybridized with ^{32}P-labelled mTNF cDNA probe (a) or ^{32}P-labelled human β-actin cDNA probe (b). The positions of 18S and 28S are indicated, as in Fig. 2.

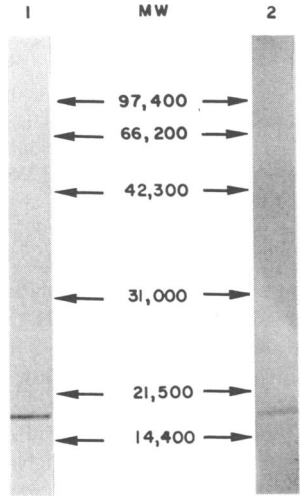

Figure 4. Western blot analysis of the cytotoxic factor released from RBL-2H3 cells. RBL-2H3 cells were passively sensitized with anti-OVA mouse serum and incubated with OVA. After 24 hr, the culture supernatants were harvested and concentrated as described in the Materials and Methods. Recombinant mTNF (lane 1) and the concentrated culture supernatants (lane 2) were loaded to SDS–PAGE and electrophoretically transferred to filters. The filters were incubated with anti-mTNF rabbit serum and developed using anti-rabbit IgG sheep IgG conjugated to horseradish peroxidase. The positions of standard molecular weight are indicated between the lanes.

mTNF, the culture supernatants were examined by Western blot analysis using anti-mTNF rabbit serum. The Western blot shown in Fig. 4 revealed that a cytotoxic factor released from RBL-2H3 cells with IgE receptor triggering was an approximately 17,000 MW protein which co-migrated with recombinant mTNF.

DISCUSSION

Previously it has been reported that RBL-2H3 cells released a cytotoxic factor the activity of which was completely blocked by anti-mTNF antibody (Ohno *et al.*, 1988). In this paper, gene expression and production of TNF molecule using Northern and Western blot analysis are demonstrated. As TNF-related factors that were released from a basophil/mast cell lineage by IgE receptor triggering, Djeu and colleagues reported that a cytotoxic factor was released from RBL-2H3 cells or IL-3-dependent cultured mast cells by IgE receptor stimulation with specific antigen or anti-IgE receptor antibody (Okuno *et al.*, 1986), and that its activity was completely neutralized by anti-mTNF antibody (Richards *et al.*, 1988). In their report, half of the maximal cytotoxic activity was released as early as 5 min after stimulation, similar to the pattern of serotonin release. In contrast, it is observed here that not only extracellular but also intracellular cytotoxic activity rose gradually and peaked 2 hr after stimulation. However, the difference between their results and these cannot be explained. Furthermore, they reported that the molecular weight of the cytotoxic factor was 43,000 MW by gel filtration. However, this study demonstrates not only the release of a 17,000 MW molecule by Western blot analysis, but also gene expression of TNF by Northern blot analysis. In the present study cytotoxicity from RBL-2H3 cells by LPS and PMA could not be induced. However, Young *et al.* (1987) reported that, in cytoplasmic granules of mouse mast cells, a molecule functionally similar to TNF was present and produced with a combination of either Con A/PMA or LPS/PMA (Young *et al.*, 1987). Cell-associated cytotoxic activity of unstimulated RBL-2H3 cells in the experiments here was less than that of unstimulated mouse mast cells. Moreover, in contrast to the present results, the cytotoxic activity of the molecule was reduced by only 60% by anti-mTNF antibodies, and the molecular weight of the cytotoxic factor that had the same antigenic characteristics as mTNF was demonstrated to be approximately 50,000 MW by immunoblot analysis under reducing conditions. In addition, neither resting nor antigen-stimulated cultured mast cells were demonstrated to express TNF mRNA, although these cells were shown to express mRNA of various cytokines, such as IL-3, 4, 5, 6 and GM-CSF (Plaut *et al.*, 1989; Wodnar-Filipowicz *et al.*, 1989). There may be heterogeneity of basophils and mast cells in not only morphological and immunological characteristics but also cytotoxicity production. A TNF-like factor, whose activity was partially blocked by anti-hTNF antibody, was reported to be released by human basophil/mast cells from bone marrow cultures, and the presence of TNF mRNA in these cells was demonstrated using a mTNF cDNA probe (Steffen *et al.*, 1989). However, the authors did not show any data on gene expression and production of TNF after IgE receptors stimulation. Therefore, this is the first report that TNF mRNA was expressed by basophil/mast cell lineage after IgE receptor triggering with specific antigen which was important in triggering allergic reaction.

Since basophils and mast cells are thought to play a central role in allergic reaction of bronchial asthma, TNF production by these cells may be involved in the pathogenesis of asthma. After inhalation challenge with allergen, dual broncho-constriction has been often observed in asthmatic patients and allergic animal models (Pepys *et al.*, 1974; Abraham *et al.*, 1988).

Immediate bronchial response occurs after 15–30 min of allergen challenge, and late response after 2–12 hr. Mast cells and basophils are thought to initiate immediate bronchial response by releasing chemical mediators through IgE receptor triggering by inhaled allergen (Abraham *et al.*, 1988; Casale *et al.*, 1987). In the late phase, eosinophils and neutrophils are observed to infiltrate to lung tissue and are suggested to be involved in the pathogenesis of late bronchial responses after allergen challenge (De Monchy *et al.*, 1985; Murphy *et al.*, 1986). As one of the possible cytokines to mediate cellular infiltration and activation, TNF is known to be chemotactic for neutrophils and monocytes (Ming, Bersani & Mantovani, 1987) and, furthermore, to be able to activate neutrophils (Klebanoff 1986), eosinophils (Silberstein & David, 1986) and monocytes (Philip & Epstein, 1986). Therefore, the results presented here suggest that basophils and mast cells contribute not only to the immediate response by releasing chemical mediators such as histamine, serotonine and metabolites of arachidonate, but also to the late response by producing TNF to induce the migration and activation of neutrophils and eosinophils.

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