

Identification of a basophil leukocyte interleukin-3-regulated protein that is identical to IgE-dependent histamine-releasing factor

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This study aimed to identify basophil leukocyte proteins associated with interleukin (IL)-3 and/or anti-IgE activation by two-dimensional (2-D) gel electrophoresis. We noticed one particular protein showing increased synthesis after recombinant human (rh)IL-3 and, to a lesser extent, anti-IgE stimulation. The protein was also present in the culture medium in increased amounts after rhIL-3 stimulation. On the basis of comigration with proteins in published 2-D gel electrophoresis databases and immunoblotting with a specific monoclonal antibody, we identified this protein as translationally controlled tumor protein (TCTP), also known as p23 or IgE-dependent histamine-releasing factor. The antibody was shown to be specific for TCTP/IgE-dependent histamine-releasing factor by blotting on 2-D gels of proteins from human lymphocytes and the human basophilic cell line KU812, followed by N-terminal amino-acid sequencing of the bound protein. Densitometric analysis of the gels showed that the synthesis of IgE-dependent histamine-releasing factor in human basophil leukocytes was dose dependent upon rhIL-3 stimulation with an optimum of 100 ng/ml. The level of the protein in the medium was also highest at an optimal rhIL-3 concentration of 100 ng/ml. Supernatants from cultured basophils were able to stimulate histamine release from other basophils. This histamine release was decreased by precipitation of TCTP/IgE-dependent histamine-releasing factor from these supernatants.

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Basophil leukocytes are involved in the late-phase reaction of allergy, as they invade the inflamed tissue hours after the initial allergic reaction (1-3). The mechanism of the activation of basophil leukocytes in this phase is not known, but a number of cytokines and an IgE-dependent histamine-releasing factor may play an important role.

The histamine-releasing factors are divided into two groups: the IgE-independent group and the IgE-dependent group, of which the first is normally considered to belong to the chemokines. The IgE-dependent group is characteristically dependent on a special type of IgE called IgE+. One of these factors has been purified, N-terminally sequenced as well as cloned from the human macrophage cell line U937 (4). It showed exten-

sive similarity to the mouse protein p21 but was identical to the human protein p23 (5). The protein p23 is also known as translationally controlled tumor protein (TCTP), which was first identified in tumor cells in the exponential growth phase (6) and later found in a number of normal human cell types (7-9).

The IgE-dependent histamine-releasing factor stimulates secretion of histamine (4) and interleukin (IL)-4 (10) by basophil leukocytes, whereas their differentiation and viability are strongly supported by IL-3 (11, 12). Furthermore, IL-3 enhances the secretion of histamine (13), IL-4 (14), and leukotriene (15) from these cells. Finally, IL-3 seems to upregulate the secretion from the basophil leukocytes induced by known secretago-

gues, even though they may initiate different signal transduction pathways. Therefore, it is thought that IL-3 regulates the secretion on central points in the signal transduction pathways (16).

A direct stimulation of histamine secretion from the basophil leukocytes by IL-3 has been demonstrated in some donors (17). Therefore, it is of considerable interest that in a subset of subjects who are responsive to IgE-dependent histamine-releasing factors, IL-3 directly stimulates histamine secretion from the basophil leukocytes (18).

Here we report that the basophil leukocytes synthesize and externalize IgE-dependent histamine-releasing factor. This synthesis and externalization are increased after IL-3 stimulation.

Material and methods

The KU812 cells were supplied by Dr Lars K. Poulsen, Allergy Unit, National University Hospital, Copenhagen, Denmark; Dynabeads were bought from Dynal, Oslo, Norway; and the monoclonal antihuman antibodies CD8, CD19, CD8, CD4, CD14, CD16, and CD3, and monoclonal anti-IgE and HRP (horseradish peroxidase)-conjugated rabbit antimouse antibody and mouse indifferent antibody were bought from Dakopatts, Glostrup, Denmark. Polyclonal goat antihuman anti-IgE was bought from Behringwerke, Marburg, Germany. Recombinant human (rh)IL-3 was obtained from Genzyme, USA. ^{35}S -Cys/Met (promixTM) was supplied by Amersham, UK. Percoll, Ficoll-Hypaque, Dextran T-70, and Ampholines pH 8–10, pH 7–9, and pH 3.5–10 were acquired from Pharmacia Biotech, Uppsala, Sweden, and Ampholines pH 5–7 from Serva, Heidelberg, Germany. Films for autoradiography were Kodak X-Omatic. Nitrocellulose paper and PVDF membrane were from MSI, Westboro, MA, USA. ECL Western blotting detection reagents were from Amersham, UK. All other reagents used were of analytic grade.

Purification of the basophil leukocytes

The basophil leukocytes were purified with Dynabeads in a negative or positive selection procedure. The negative procedure was performed by the method previously described (19). Blood (100 ml) was obtained by venopuncture from healthy volunteers. The blood was layered on Percoll gradients and centrifuged at 400 g for 20 min at room temperature. The rest of the procedures were performed at 4°C. The interface was harvested and washed, and the basophil leukocytes were counted. Contaminating nonbasophil cells were removed by

negative selection with Dynabeads (CD8, CD19, CD4, CD14, CD16, and CD3). After negative selection, the cells were counted and washed in RPMI 1640 without sera. Only samples with more than 80% basophil leukocytes were used for experiments. The average yield of these samples was 1.3×10^6 basophils.

For positive selection, the cells in the interface were incubated with Dynabeads coated with mouse monoclonal antihuman IgE antibodies (ratio one basophil : four Dynabeads) for 1 h with gentle rolling. Dynabeads with bound basophil leukocytes were selected with a magnet. The purities of these samples were above 96%, with a total average yield of 0.9×10^6 basophils.

Purification of mononuclear cells

For purification of mononuclear cells, blood was layered on Ficoll-Paque according to the instructions of the manufacturer. After centrifugation (400 g, 25 min, 22°C), interface cells were harvested, washed three times in sterile phosphate-buffered saline (PBS), and reconstituted in RPMI 1640 without serum.

Incubation of basophil leukocytes

Shortly after purification, the basophil leukocytes were used for experiments. The cells were incubated at a concentration of $5 \times 10^6/\text{ml}$ in serum-free RPMI 1640 deficient in methionine, cysteine, and cystine with 0.3 mCi/100 μl ^{35}S -met/cys (specific activity: 1000 Ci/mM). One stimulation experiment consisted of paired samples (two or four) subjected to different stimulation. The total number of cells in an experiment varied from 0.7×10^6 to 1.8×10^6 . Within an experiment, each sample consisted of equal amounts of basophil leukocytes. Anti-IgE and recombinant human (rh)IL-3 were added as indicated. The cells were incubated for 6 h at 37°C, 5% CO_2 . The viability of the cells was assessed after incubation with trypan blue exclusion and consistently exceeded 95%. After incubation, the cells were pelleted (400 g, 4 min), and the supernatant was removed. The pellet was dissolved in lysis buffer (9.8 M urea, 2% (w/v) NP40, 2% Ampholines, pH 7–9, 100 mM DTT) and applied to the two-dimensional (2-D) gels. The supernatant was lyophilized, reconstituted in the lysis buffer, and put on the gel system.

In previous experiments, it was observed that rhIL-3 did stimulate histamine release from basophil leukocytes at an optimal concentration of 100 ng/ml (20); therefore, we chose to use this level in the initial experiments. The amount of anti-IgE used was the optimal for histamine

secretion from the donors, as determined by earlier experiments. Buffer was added as control. Parallel samples were run for the histamine measurements.

2-D gel electrophoresis

2-D gel electrophoresis was performed as described by Celis et al. (21), except that we used the Protean xi 2-D Cell from Bio-Rad. MW and pI was assigned according to comigration with 2-D-gel protein standards.

Autoradiography

Gels were fixed in 50% methanol and 10% acetic acid for 4 h and washed several times in 30% ethanol. They were dried in a Bio-Rad gel dryer and were exposed to radiographic film in a cassette at room temperature. The exposure time varied from 5 to 60 days. Gels from the same experiment were exposed an equal amount of time.

Gel analysis

Gels were analyzed both visually and with a tabletop scanner (Mustek MFS-6000CX). Spot volumes were quantified with a 2-D-gel densitometry software program from Phoretix, UK. The spot volume was defined as the summation of the value of the intensities of each pixel in the spot. It was presented according to the following formula:

$$\text{spot \% of total gel volume} = (\text{spot volume}) / (\text{total gel volume}) \times 100\%$$

Immunoblotting

For immunoblotting of TCTP/IgE-dependent histamine-releasing factor, proteins in the gel were transferred to PVDF membranes in Tris (50 mM)-boric acid (50 mM) buffer at 130 mA overnight.

After transfer of the proteins, the blot was blocked in a PBS-T (0.1% Tween) buffer with skim milk (5%) for 1 h, followed by incubation overnight at room temperature with the mouse monoclonal antihuman TCTP antibody. This antibody was produced in the laboratory of two of the authors (Sanchez and Hochstrasser). It was raised against rhTCTP and was shown to be specific for TCTP/IgE-dependent histamine-releasing factor in many different human cell types (9). The HRP-conjugated secondary rabbit antimouse antibody was used in a dilution of 1:1000 for 1 h at room temperature. Detection of the HRP-conjugated secondary antibody was performed with a chemiluminescence system (ECL).

Precipitation of TCTP/IgE-dependent histamine-releasing factor from basophil culture medium and stimulation of dextran-sedimented basophils

Mouse anti-human TCTP antibody or a mouse-indifferent (control) antibody were coupled to rat antimouse Dynabeads by incubation of 15 μ l of the antibody with 6 mg of Dynabeads in 200 μ l PBS for 1 h at 4°C with rolling followed by extensive washing. Precipitation of TCTP/IgE-dependent histamine-releasing factor was performed on supernatants from purified basophils grown overnight in the presence of rhIL-3 (100 ng/ml), as described above. After centrifugation (400 g, 4 min, 4°C), supernatant was collected and 400 μ l of PIPES buffer was added. A volume of 100 μ l of these diluted supernatants was mixed with 50 μ l of either the anti-TCTP Dynabeads solution or control-antibody Dynabeads solution with rolling for 1 h at 4°C. The Dynabeads were then removed from the solution with the aid of a magnet.

After treatment with Dynabeads, the supernatants were used to stimulate heterologous basophils in dextran-sedimented leukocyte suspensions. The percentage of basophils in these preparations was approx. 2%. The dextran-sedimented leukocytes were resuspended in 100 μ l PIPES buffer to a final concentration of 10 000 basophils/100 μ l. A volume of 150 μ l of the Dynabeads-treated supernatants was added and left to be stimulated for 120 min at 37°C, 5% CO₂. Parallel samples were run without cells to correct for the stimulation by IL-3 and the influence of histamine in the supernatant on the final measurement of histamine release from the dextran-sedimented basophils.

Amino-acid sequencing

To characterize the protein recognized by the anti-TCTP antibody, amino-acid sequencing was performed on samples from both human mononuclear blood cells and the human basophilic cell line KU812. The proteins in the gels were transferred to PVDF membranes, as described above, and stained with amido black (0.1% amido black, 45% MeOH, 7% acetic acid). The spots on the gel of interest were excised and kept moist at -20°C until amino-acid sequencing.

Before the sequence analysis was carried out, the membrane piece was washed twice in 50% methanol, including a few minutes of sonication during the first wash. Then the membrane piece was transferred to the blot cartridge of the Procise protein sequencer (model 494A, Perkin-Elmer ABD, Foster City, CA, USA) and analyzed by

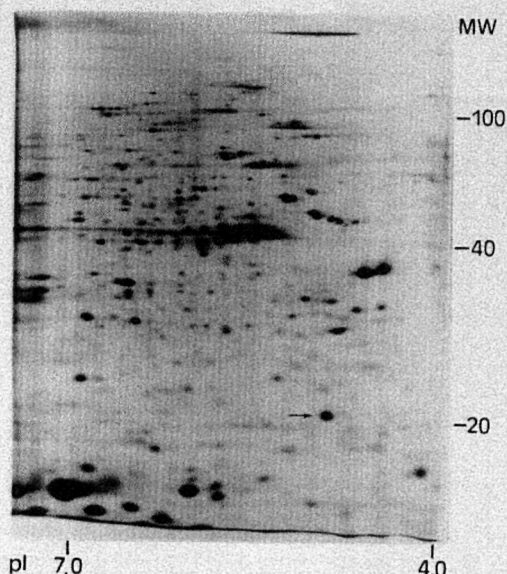


Fig. 1. 2-D gel of radiolabeled cellular basophil leukocyte proteins. Some 420 000 basophil leukocytes were grown for 6 h in labeling media with rhIL-3 (100 ng/ml) added. Purity of basophil leukocytes used for this experiment was 94%. Arrow indicates IgE-dependent histamine-releasing factor.

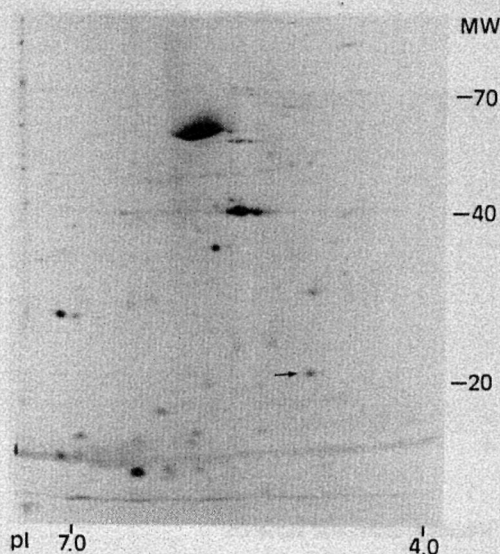


Fig. 2. 2-D gel of radiolabeled proteins recovered from culture medium of 400 000 basophil leukocytes. Basophil leukocytes were grown for 6 h in labeling media with rhIL-3 (100 ng/ml) added. Purity of basophil leukocytes used for this experiment was 97%. Arrow indicates IgE-dependent histamine-releasing factor.

essentially the preprogrammed "blot" cycles. All solvents and reagents were obtained from Perkin-Elmer ABD, except methanol and solvent B for the HPLC separation of the PTH amino-acid derivatives.

Histamine measurement

Histamine was measured in the pellet and the supernatant according to the method previously described (22). Histamine release was expressed as percentage of the total content of histamine. All measurements were made in triplicate.

Statistics

Data were analyzed by Wilcoxon's signed rank test for matched pairs. The results on the dose-response relationship were analyzed by Friedman's signed rank ANOVA and the Student-Newman-Keuls test.

Results

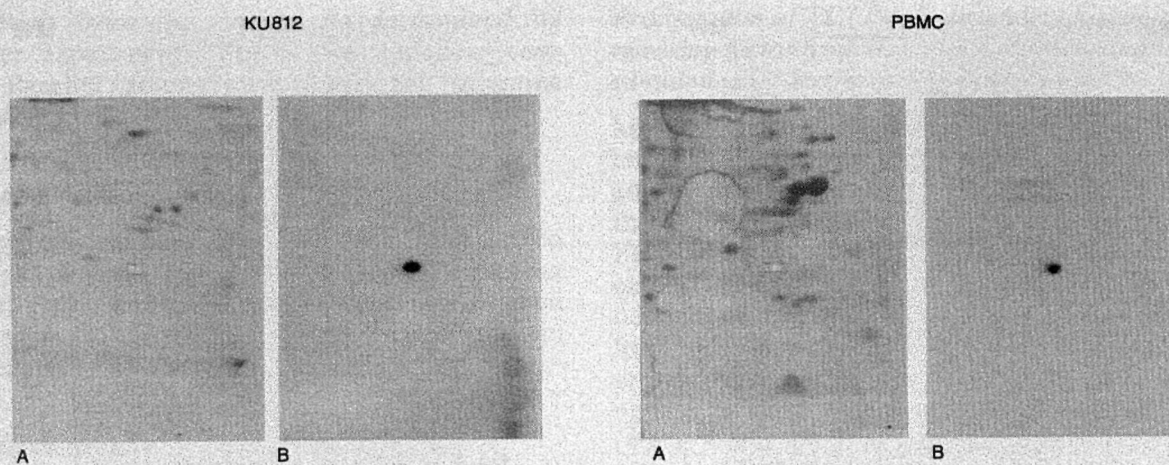
Identification of TCTP/IgE-dependent histamine-releasing factor on the gels

During our experiments, one protein was found both in the cells and in the culture medium with an apparent mass of 22 kDa and a pI of 4.7. It is indicated by arrows in Figs. 1 and 2. It is of interest that the same migration pattern was obtained with

the TCTP as shown in a published database of human keratinocytes (8).

Therefore, a 2-D immunoblot was performed with a monoclonal antibody raised against rhTCTP (9). This antibody recognized only one spot after immunoblotting on the 2-D gels of proteins from mononuclear cells of human peripheral blood or the human basophilic cell line KU812 (Fig. 3). The protein bound by the antibody was subjected to N-terminal amino-acid sequencing. The identified N-terminal amino acids of the blotted proteins from both the mononuclear cells and the KU812 cells were identical to the amino acids found on the same positions in the TCTP/IgE-dependent histamine-releasing factor. Immunoblotting with the anti-TCTP antibody on 2-D gels of the proteins from purified human basophil leukocytes was then performed in order to identify the TCTP/IgE-dependent histamine-releasing factor in basophils (Fig. 4).

In another set of experiments, nonradioactive proteins from KU812 cells or purified mononuclear cells were mixed with radiolabeled proteins from basophil leukocytes and subjected to 2-D gel electrophoresis. The gels were stained with Coomassie brilliant blue, and autoradiography was performed. The results showed that this KU812 and mononuclear cell protein identified as TCTP/IgE-dependent histamine-releasing factor did comigrate with the protein from basophil leukocytes that was blotted with the monoclonal antibody (results not shown).



N-terminal amino-acid sequence:

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
TCTP/HRF:	Met	Ile	Ile	Tyr	Arg	Asp	Leu	Ile	Ser	His	Asp	Glu	Met	Phe	Ser	Asp	Ile	Tyr	Lys	Ile
PBMC(1):	(Met)	Ile	Ile	Tyr	(Arg)	Asp	Leu	Ile	Ser	X	Asp	Glu	Met	Phe	X	(Asp)	Ile	(Tyr)	Lys	-
PBMC(2):	(Met)	Ile	Ile	Tyr	(Arg)	X	Leu	Ile	X	X	-	-	-	-	-	-	-	-	-	-
KU812:	(Met)	Ile	Ile	Tyr	Arg	(Asp)	Leu	Ile	X	X	X	Glu	Met	Phe	(Ser)	X	(Ile)	(Tyr)	(Lys)	Ile

Fig. 3. Immunoblotting of TCTP/IgE-dependent histamine-releasing factor on proteins from either human basophil cell line KU812 or mononuclear cells from human peripheral blood (B). Antibody used was mouse monoclonal antihuman antibody raised against recombinant IgE-dependent histamine-releasing factor/TCTP. Blotted protein in amino black-stained 2-D gels of proteins from same cells run in parallel (A) was excised and subjected to N-terminal amino-acid sequence analysis. Amino-acid sequence was done once from KU812 cells and twice from human peripheral blood mononuclear cells. N-terminal amino-acid sequences obtained are shown. Parentheses indicate tentative assignments, and X indicates that no assignments were possible. Re-examination of unidentified residues showed no conflicts with expected amino acids, which all normally give low yields and/or are masked by background at these levels.

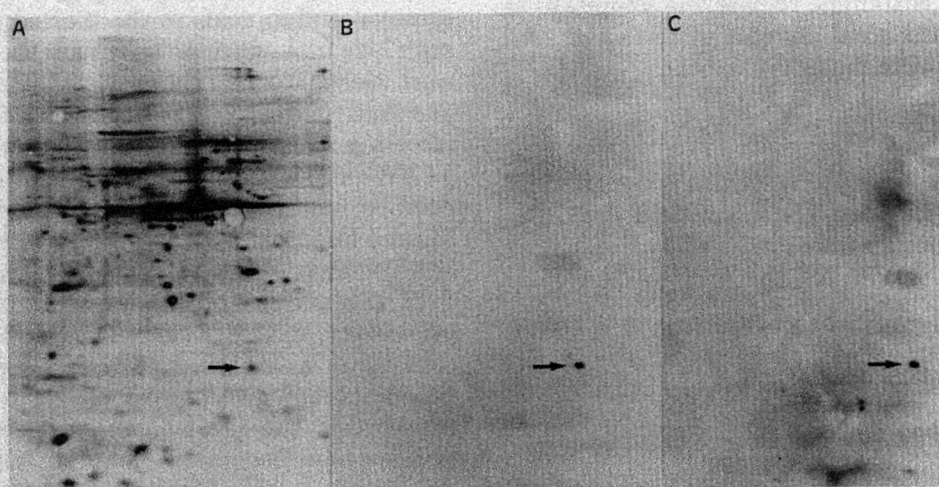


Fig. 4. Immunoblot of IgE-dependent histamine-releasing factor obtained from basophil leukocyte. Blot of radiolabeled basophil proteins obtained from 500 000 basophils with purity of 97% (A) was incubated with mouse monoclonal anti-human TCTP/IgE-dependent histamine-releasing factor antibody used in Fig. 3. HRP-labeled secondary antibody was visualized in chemiluminescence system after exposure to radiographic film for 10 (B) and 30 s (C). Arrow indicates IgE-dependent histamine-releasing factor/TCTP.

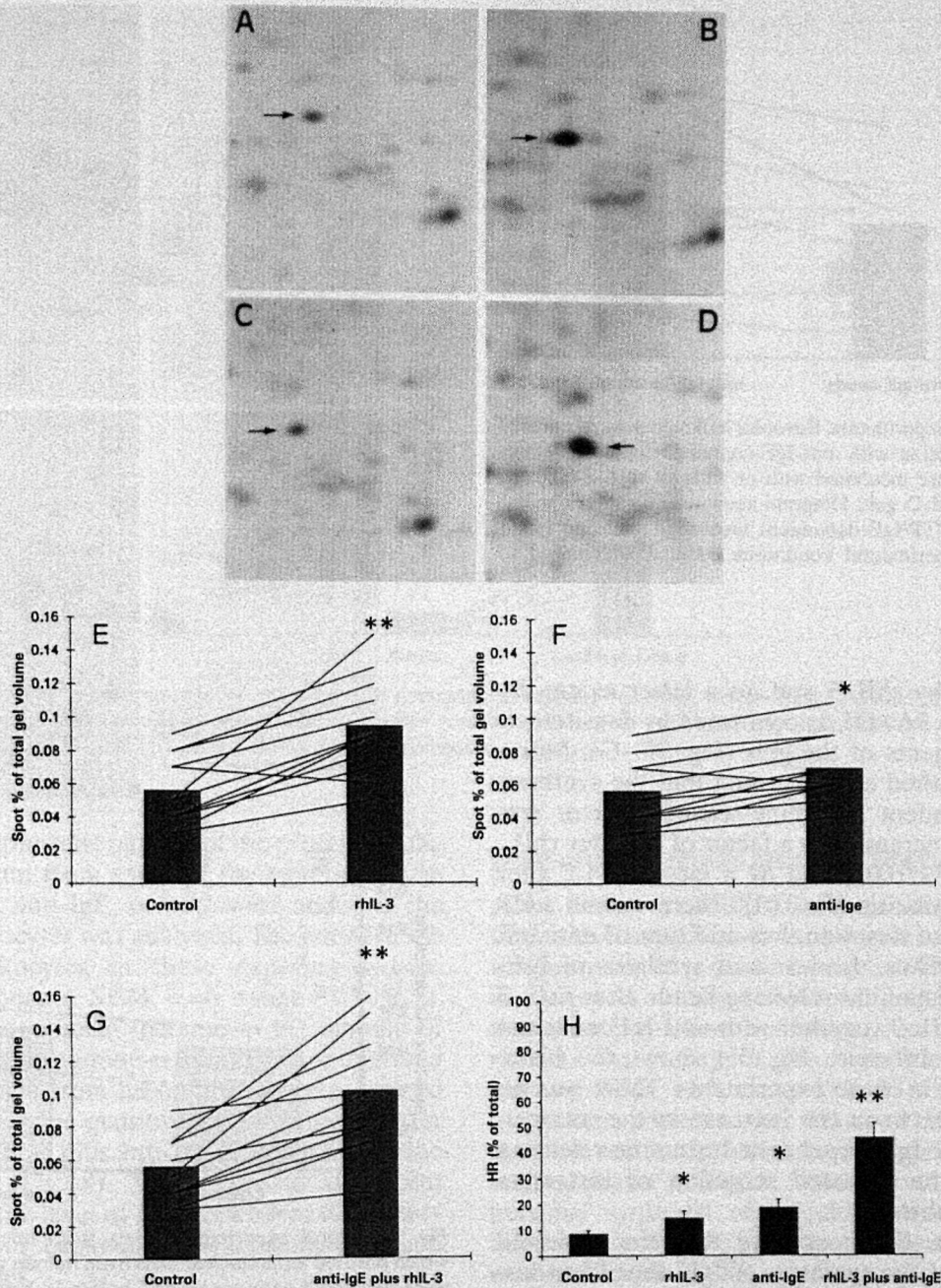


Fig. 5. Intracellular expression of IgE-dependent histamine-releasing factor in purified human basophils. Panels A–D show 2-D gel electrophoretic patterns from one experiment in which basophils were incubated with media and buffer (control) (A), rhIL-3 100 ng/ml (B), anti-IgE 50 IU/ml (C), or anti-IgE 50 IU/ml plus rhIL-3 100 ng/ml (D) for 6 h. Arrow indicates IgE-dependent histamine-releasing factor. Experiment was repeated 13 times; graphs E–G show densitometric measurements of IgE-dependent histamine-releasing factor at different experimental conditions compared to controls: with rhIL-3 100 ng/ml (E), with anti-IgE 50 IU/ml (F), with rhIL-3 100 ng/ml plus anti-IgE 50 IU/ml (G). Histamine release (mean $\pm$ SEM) in samples of all 13 experiments is shown as well (H). * P < 0.01; ** P < 0.001 compared to control.

Stimulation of the synthesis of IgE-dependent histamine-releasing factor

A number of experiments were conducted to investigate the effect of either rhIL-3, polyclonal goat antihuman anti-IgE, or both on the synthesis of the

TCTP/IgE-dependent histamine-releasing factor. The purity of the basophil leukocytes in these experiments was $93.3 \pm 1.1\%$ (mean $\pm$ SEM, $n=13$, range 82.3–96.1).

Visual inspection of the gels revealed that the IgE-dependent histamine-releasing factor was

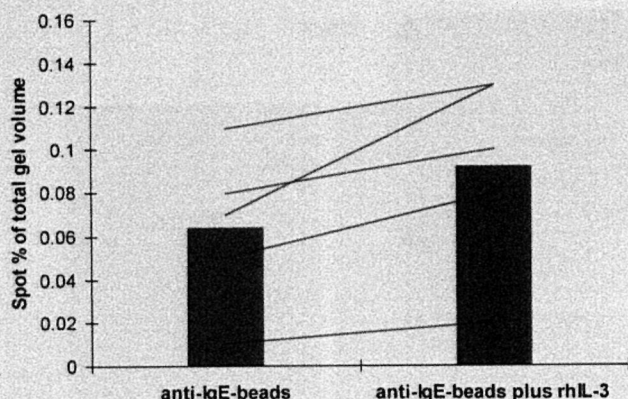


Fig. 6. Control experiments. Basophil leukocytes were purified by positive selection with anti-IgE-coated Dynabeads. Subsequently, they were incubated with or without rhIL-3 (100 ng/ml) and put on 2-D gels. Diagram shows densitometric measurements of TCTP/IgE-dependent histamine-releasing factor at different experimental conditions. * $P < 0.05$ compared to control.

upregulated by rhIL-3 and, to a lesser extent, by anti-IgE (Fig. 5A–D), as confirmed by densitometric measurements of the gels (Fig. 5E–G). Moreover, this method demonstrated that the synthesis of IgE-dependent histamine-releasing factor was significantly increased by a factor of 1.8 after rhIL-3 addition ($P < 0.001$) and by a factor of 1.2 after anti-IgE stimulation ($P < 0.01$). There was no additional effect of simultaneous addition of anti-IgE and rhIL-3. Thus, the levels of synthesis of IgE-dependent histamine-releasing factor after rhIL-3 alone and rhIL-3 together with anti-IgE were not significantly different. Fig. 5H shows the histamine release in these experiments. There was no correlation between the increase in the intracellular level of IgE-dependent histamine-releasing factor and the elevated secretion of histamine (results not shown).

To exclude the possibility that the observed stimulation of the synthesis of IgE-dependent histamine-releasing factor was due to contaminating cells, human basophil leukocytes were purified by positive selection. The cells were subsequently incubated with rhIL-3 as described. The purity of these samples was $97.6 \pm 0.5\%$ (mean $\pm$ SEM, $n = 5$, range: 96.0–99.0). Densitometric measurements (Fig. 6) showed a significant increase in the synthesis of IgE-dependent histamine-releasing factor by a factor of 1.5 ($P < 0.05$) after rhIL-3 stimulation.

As the contaminating cells in the purified samples were mainly lymphocytes, control experiments were conducted in which purified mononuclear cells were obtained and stimulated as already described (Fig. 7A–D). The content of basophil leukocytes in these samples was below 2%. As

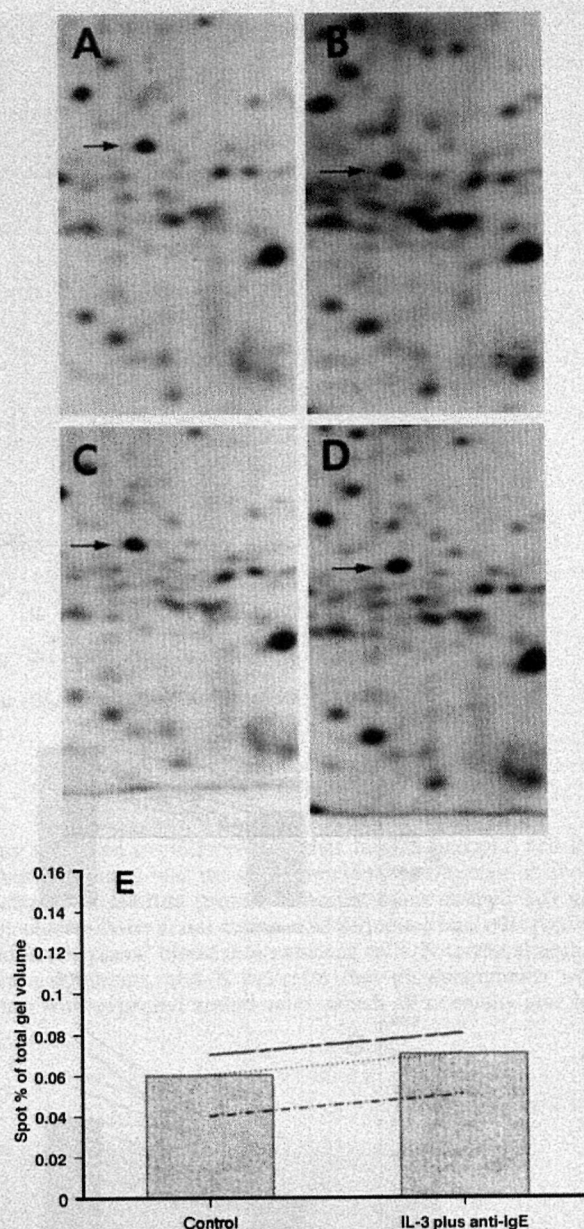


Fig. 7. Control experiments. Gels from one experiment in which purified mononuclear cells from human peripheral blood were incubated with media and buffer (A), rhIL-3 100 ng/ml (B), anti-IgE 50 IU/ml (C), or rhIL-3 100 ng/ml plus anti-IgE 50 IU/ml (D) for 6 h. Arrow indicates TCTP/IgE-dependent histamine-releasing factor. Diagram (E) shows densitometric measurements of TCTP/IgE-dependent histamine-releasing factor in control cells and cells stimulated with anti-IgE and rhIL-3.

shown in Fig. 7E, there was a slight increase in IgE-dependent histamine-releasing factor synthesis in these samples during rhIL-3 and anti-IgE stimulation, but not nearly to the same extent as seen in the basophil samples. Stimulation with rhIL-3 or anti-IgE alone did not increase the synthesis of IgE-dependent histamine-releasing factor in these samples (results not shown).

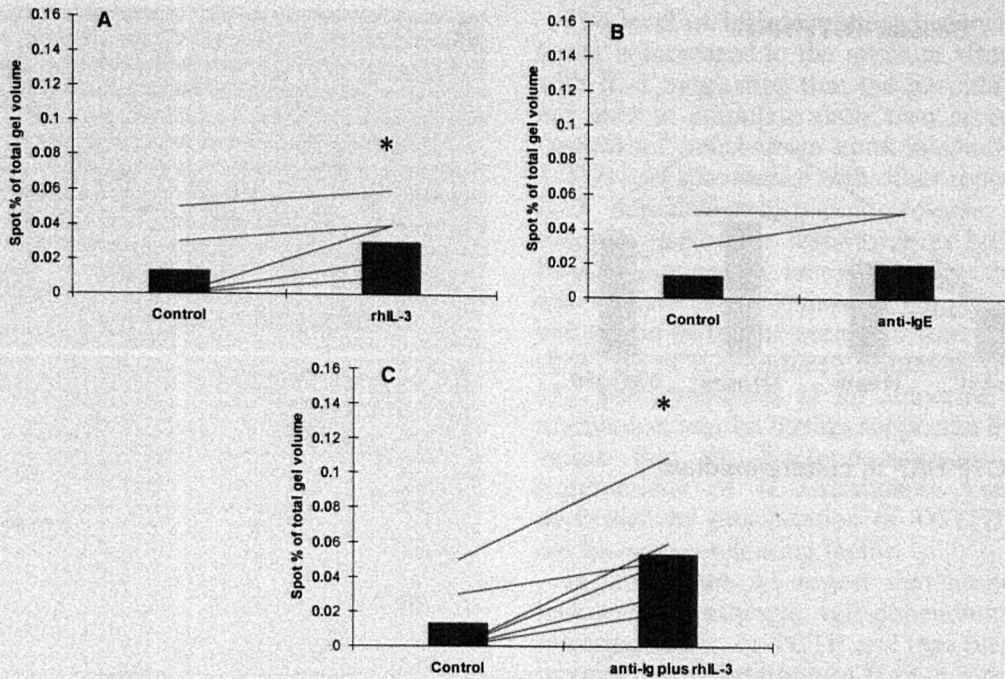


Fig. 8. Densitometric measurements of secreted IgE-dependent histamine-releasing factor from purified human basophil leukocytes. Basophils were incubated with media and buffer (control), rhIL-3 100 ng/ml (A), anti-IgE 50 IU/ml (B), and rhIL-3 100 ng and anti-IgE 50 IU/ml (C) for 6 h ($n=6$). * $P<0.05$ compared to control.

The electrophoretic pattern of the proteins in the culture medium from some of the experiments in which rhIL-3, anti-IgE, or both were added to the basophil leukocytes was analyzed. The purity of the basophil leukocytes in these experiments was $92.4 \pm 2.3\%$ (mean $\pm$ SEM, $n=6$, range 82.3–96.2). There was a significant increase in the amount of IgE-dependent histamine-releasing factor liberated into the medium from basophil leukocytes treated with rhIL-3, but the quantity was even higher after addition of rhIL-3 plus anti-IgE as compared to the control cells (Fig. 8). There was no significant difference in the level of IgE-dependent histamine-releasing factor between anti-IgE-stimulated and control samples.

Only small amounts of IgE-dependent histamine-releasing factor were present in the supernatant as compared to the cellular amount. Thus, for discernment of spots on the autoradiograms of the supernatants, the films had to be exposed for at least 30 days, while the autoradiograms of the cellular proteins needed only 5–7 days of exposure.

In conclusion, TCTP or IgE-dependent histamine-releasing factor is synthesized by human basophil leukocytes, and it is increased after incubation with rhIL-3 and/or anti-IgE. TCTP or IgE-dependent histamine-releasing factor is also liberated into the culture media, and the amount is also increased after incubation with either rhIL-3 or rhIL-3 plus anti-IgE.

Dose-response studies after IL-3 addition

After it was established that IgE-dependent histamine-releasing factor is upregulated by rhIL-3, the presence of a dose-response relationship was investigated. Purified basophil leukocytes were stimulated for 6 h with different concentrations of rhIL-3. The purity of the cells was $91.9 \pm 3.2\%$ (mean $\pm$ SEM, $n=5$, range 87.7–94.8%). The electrophoretic patterns were obtained of the cellular proteins and the proteins in the supernatant. The results, shown in Fig. 9, demonstrated significant maximal synthesis of IgE-dependent histamine-releasing factor in the basophil leukocytes and maximal levels in the supernatant at rhIL-3 concentrations of 100 ng/ml. An example of the gels of supernatant proteins of one experiment is shown in Fig. 10.

The role of basophil-derived IgE-dependent histamine-releasing factor in basophil histamine release

The presence of IgE-dependent histamine-releasing factor in the culture medium of basophils raises the question of its role in basophil activation. Therefore, a number of experiments were done in which basophils were incubated overnight in the presence of rhIL-3 (100 ng/ml). The number of basophils producing these supernatants was, on average, 1.6×10^5 . TCTP/IgE-dependent histamine-

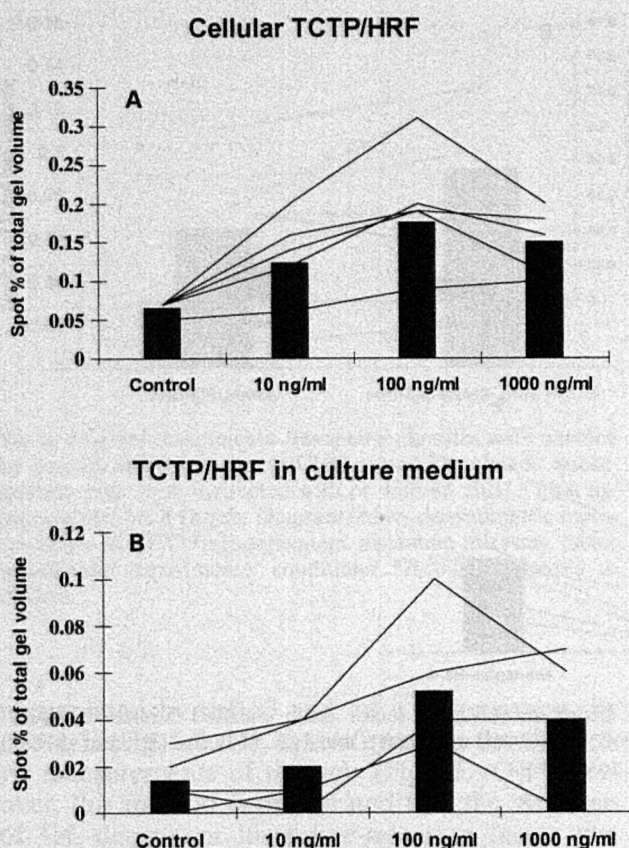


Fig. 9. Densitometric measurements of IgE-dependent histamine-releasing factor in basophil leukocytes (A) or in incubation medium (B) after incubation with different concentrations of rhIL-3 for 6 h ($n=5$). Differences in median values among groups were statistically significant ($P<0.01$). In cells (A), amount of IgE-dependent histamine-releasing factor was significantly higher after incubation with 100 ng/ml rhIL-3 than for all other stimulations ($P<0.05$). In culture medium (B), amounts of IgE-dependent histamine-releasing factor after incubation with 100 and 1000 ng/ml rhIL-3 were not significantly different, but were significantly higher ($P<0.05$) than values after incubation with 0 or 10 ng/ml of rhIL-3.

releasing factor was then precipitated, as explained in the Material and methods section. 2-D gels of the proteins bound by these Dynabeads showed that the anti-TCTP antibody also under these native conditions did bind to the TCTP/IgE-dependent histamine-releasing factor, while the Dynabeads coupled to the indifferent mouse antibody (control) did not bind to any protein. The supernatants partially depleted for TCTP/IgE-dependent histamine-releasing factor were used to stimulate dextran-sedimented basophils from another donor. Six experiments were done. The supernatant treated with anti-TCTP Dynabeads had a significantly ($P<0.05$) decreased ability to induce histamine release from the dextran-sedimented basophils compared to the supernatants treated with control Dynabeads (Table 1).

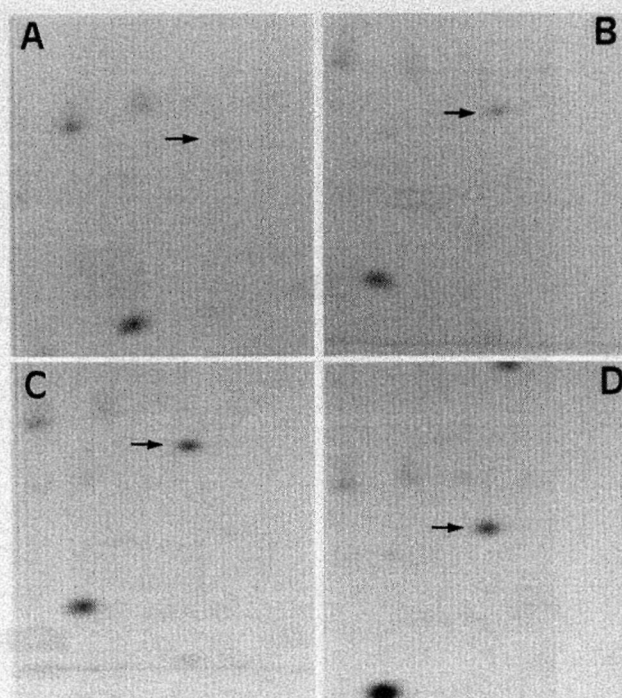


Fig. 10. Basophil leukocytes were incubated in labeling media with different concentrations of rhIL-3 for 6 h. Autoradiography of 2-D gel electrophoretic protein pattern of incubation medium was obtained (exposure time: 30 days). Concentrations of added rhIL-3 were as follows: 0 (A), 1 ng/ml (B), 10 ng/ml (C), and 100 ng/ml (D). Arrow indicates IgE-dependent histamine-releasing factor.

Discussion

TCTP/IgE-dependent histamine-releasing factor is a ubiquitous protein found in several different tumoral and normal human cells, including keratinocytes, hepatocytes, erythrocytes, platelets, and macrophages (7–9), as well as in fetal human tissue (J. E. Celis, Internet at biobase.dk/cgi-bin/celis). Proteins with strong homology to TCTP have also been described in other primates (9), seed plants (23), and yeast (24).

This report demonstrated that the IgE-dependent histamine-releasing factor is synthesized in human basophil leukocytes but also exported to the incubation medium. Moreover, IL-3 upregulates the synthesis by a factor of 1.8. It has been demonstrated that TCTP/IgE-dependent histamine-releasing factor is upregulated after stimulation with LPS and PMA in a cell line of macrophages (U937) which was analyzed by the 2-D gel electrophoretic technique (25). These results suggest that TCTP/IgE-dependent histamine-releasing factor may be involved in the activation of these cells.

It was recently reported that TCTP is a calcium-binding protein with four potential phosphorylation sites, one of which is a potential protein kinase

Table 1. Change in histamine-releasing activity of supernatants from cultured purified human basophils after precipitation of TCTP/IgE-dependent histamine-releasing factor. Purified human basophils were grown overnight with 100 ng/ml rhIL-3. TCTP/IgE-dependent histamine-releasing factor was precipitated from these supernatants by incubation with Dynabeads coupled to specific TCTP antibody. Supernatants were then used to stimulate dextran-sedimented heterologous basophils, as explained in Material and methods section. Control samples were treated with Dynabeads coupled to indifferent mouse antibody. Histamine release induced by control supernatants was set to 100%. Tables show difference induced by anti-TCTP-treated supernatants compared to control

Experiment no.	% Decrease in histamine release	
	after precipitation of	
	TCTP/HRF	
1	32	
2	15	
3	2	
4	8	
5	81	
6	31	
Average	28 ($P < 0.05$)	

C (PKC) binding site (residue 98-ser) (9). Thus, the TCTP/IgE-dependent histamine-releasing factor possesses some of the properties seen in proteins which belong to the signal transduction pathways. However, further studies are needed to determine its possible role in signal transduction in basophil leukocytes.

IgE-dependent histamine-releasing factor is present in the medium of cultured basophil leukocytes, but it is unclear how the protein is secreted, as it contains no hydrophobic signal sequence (4). One possible explanation could be that its presence in the culture media was caused by debris from dead cells or from living cells still present in the media after the final centrifugation of the samples. However, four observations make this possibility unlikely:

- 1) viability counts showed that more than 95% of the basophils were living after the 6-h incubation
- 2) we were able to detect only radiolabeled proteins; that is, proteins that were synthesized in living cells *in vitro*
- 3) analysis of the gels showed that most of the prominent spots on the gels of cellular proteins could not be recognized on the gels of the medium proteins, even after long-term (60 days) exposure
- 4) it appears unlikely that IL-3, a strong supporter of basophil viability, should increase basophil cell death and thereby promote nonspecific secretion of IgE-dependent histamine-releasing factor into the media.

The level of IgE-dependent histamine-releasing factor is increased in the medium after incubation with IL-3, suggesting that the basophil leukocytes are able to stimulate their own secretion by an "autocrine" mechanism upon incubation with IL-3. This is in accordance with other reports (18) that IL-3 stimulates histamine release only from basophil leukocytes responsive to IgE-dependent histamine-releasing factor. This mechanism may also explain the considerable lag period observed before the histamine secretion starts and the very slow rate of IL-3-induced histamine secretion (17, 18). The hypothesis of an autocrine stimulatory mechanism may be further supported by our observation that the histamine-releasing activity in supernatants of IL-3-stimulated basophils was decreased by precipitation of TCTP/IgE-dependent histamine-releasing factor.

In conclusion, we report that human basophil leukocytes synthesize IgE-dependent histamine-releasing factor, or TCTP, and that the synthesis is increased after addition of IL-3 in a dose-dependent manner. IgE-dependent histamine-releasing factor is also found to be increased in the supernatant of cultured human basophil leukocytes after IL-3 stimulation.

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