Effect of TNF, IL-4 and IL-6 on histamine release from rat mast cells

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Abstract

It is well established that cytokines may influence biological functions of mature mast cells. The aim of our study was to determine the capacity of TNF, IL-4 and IL-6 to induce direct mediator release from rat peritoneal mast cells as well as the action of these cytokines on mast cell secretory activity. We have found that these cytokines did not activate rat mast cells to histamine secretion. However, we have noticed that TNF, IL-4 and IL-6 caused statistically significant decrease of spontaneous histamine release from mast cells. Moreover, we have also stated that the treatment of mast cells with TNF significantly inhibited compound 48/80 - and ConA-induced histamine release. The preincubation of mast cells with IL-4 caused significant decrease of rat mast cell reactivity to stimulation with compound 48/80, and IL-6 treatment practically did not exert any effect on mast cell releasability to activation with compound 48/80, ConA or anti-IgE. These results indicate that TNF, IL-4 and IL-6 may modulate mast cell secretory activity.

Key words: *mast cells, tumor necrosis factor, interleukin 4, interleukin 6, mast cell releasability*

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Introduction

Mast cells are known to be of major importance in the pathophysiology of immediate-type allergic reactions and other chronic inflammatory disorders [1-3]. These cells also participate in many other diverse pathological processes [4, 5]. Moreover, mast cells are thought to be involved in many physiological processes [6]. Evidence has accumulated that they may be key players in the induction of innate immunity [7, 8], especially against bacteria [9]. What is more, mast cells can influence the development of adaptive immunity [10]. Taking into account that mast cells play such diverse roles in organism it seems to be of great importance to expand our knowledge of the factors influencing their growth, proliferation and differentiation. It also seems vital to recognise the factors regulating the activity of mature mast cells in tissues.

Cytokines are undoubtedly the most important humoral factors regulating many biological processes in the organism. These factors also affect the functions of various cell populations. It is well documented that cytokines regulate mast cell growth, proliferation and differentiation [11, 12]. More and more data prove that cytokines also affect the biology of mature mast cells by influencing expression of surface receptors and other molecules, by regulating mast cell adhesion and migration and by modulating their survival and apoptosis [13]. It has been also found that cytokines may have a significant impact on the secretory activity of mast cells having either direct stimulating or modulating effect [13, 14].

Cytokines are produced and secreted by various cell populations. Nowadays, it is well established that the very important and unique source of many pleiotropic cytokines are mast cells [14-16]. Mast cells release cytokines from two distinct pools; a preformed pool, which accounts for most of the product released during the first 10 min after stimulation, and a newly synthesized pool, which accounts for the later, sustained release of cytokines. Tumor necrosis factor (TNF), interleukin (IL)-4 and IL-6 are ones of these important cytokines, secreted both as preformed and newly synthesized ones. These cytokines are characteristic for its wide-ranging immune regulatory effects, including influencing inflammatory processes [5, 14, 15], in which mast cells participate as well. For these reasons we have decided to study whether TNF, IL-4 and IL-6 could directly

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induce rat mast cell degranulation and histamine release or whether they could modulate the response of these cells to different activation.

Material and methods

Animals

Mast cells were obtained from peritoneal cavities of female albino Wistar rats weighting 200-250 g.

Mast cell isolation

Mast cells were collected from peritoneal cavities by lavage with medium containing 137 mM NaCl, 2.7 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES buffer, and 5.6 mM glucose supplemented with 1 mg/ml bovine serum albumin (BSA) (pH of the medium was adjusted to 6.9). The peritoneal cell suspension was washed twice by centrifugation (150 g, 5 min, 4°C). After being washed, mast cells were purified by recovery of the pellet fraction after sedimentation through Percoll density gradient after the method of Rossow et al. [17]. Mast cells were counted and resuspended in appropriate volume of the medium to obtain mast cell concentration 4 x 10^5 cells/ml. Mast cells were prepared with purity over 90%, as determined by metachromatic staining with toluidine blue.

In vitro experiments on rat peritoneal mast cells

The cell suspensions were carefully divided into 90 μl aliquots and incubated for equilibration at 37°C for 5 min.

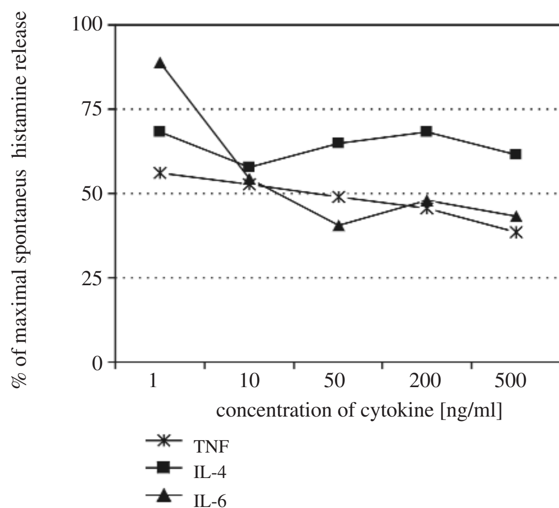

Fig. 1. Effect of TNF-, IL-4- and IL-6-treatment on spontaneous histamine release from rat mast cells. Mast cells were incubated with different concentrations of cytokines or buffer alone (control mast cells) for 30 min. Each point represents the mean ± SEM of seven experiments, with two replicates in each experiment

Subsequently, 10 μl of a stimulating agent (TNF, IL-4, IL-6, compound 48/80, concanavalin A (ConA) or mouse anti-rat IgE (anti-IgE)) was added in different concentrations as specified in the results. Incubation was carried out in a waterbath with constant stirring for different period of time, as stated in the results. In some experiments, mast cells were preincubated with cytokine at concentrations 1 ng/ml, 50 ng/ml or 100 ng/ml for 60 min (the control mast cells were preincubated at the same conditions but without cytokine) and, after washing with the medium, were challenged with compound 48/80 (at concentration of 5 μg/ml for 10 min), with ConA (at concentration of 50 μg/ml for 30 min) or with anti-IgE (at concentration of 5 μg/ml for 30 min). In every experiment appropriate controls for the determination of spontaneous histamine release in the absence of stimulating agent were included. The reaction was stopped by adding 0.9 ml of cold medium. Next, the cell suspension was centrifuged (150 g for 6 min at 4°C), and the supernatants were decanted into other tubes for histamine determination (released histamine). A total of 2 ml distilled water was added to each tube with a cell pellet (residual histamine). The histamine content was determined in both cell pellets and supernatants by spectrofluorometric method by use of o-phthaldehyde (OPT) [18]. Histamine release was expressed as a percentage of the total cellular content of this amine after correction for the spontaneous release found in controls.

Reagents

NaCl, KCl, $CaCl_2$, $MgCl_2$, buffer HEPES, glucose, (BSA), toluidine blue, OPT, compound 48/80 and ConA were obtained from Sigma Chemical Company. Percoll was obtained from Pharmacia, anti-IgE and recombinant rat TNF from Serotec, recombinant rat IL-4 and recombinant rat IL-6 from R&D Systems.

Statistical analysis

Statistical parametheers included mean value, standard error of the mean (SEM) and Student's test for „small groups". Values of P<0.05 were considered as statistically significant.

Results

To investigate the histamine releasing activity of TNF, IL-4 and IL-6, rat peritoneal mast cells were incubated with each of the cytokines at concentrations 1 ng/ml, 10 ng/ml, 50 ng/ml or 500 ng/ml for 20 min. We have stated that neither TNF nor IL-4 and IL-6, in any concentration used, directly activated mast cells to histamine release. For comparison, at the same experimental conditions rat mast cells were activated and released up to 64.3±0.6% of histamine to the challenge with compound 48/80 at concentration 5 μg/ml, 23.8±0.7% of histamine to the challenge with ConA at concentration 50 μg/ml and

15.7±1.0% of this amine to the challenge with anti-IgE at concentration 5 μg/ml (data not shown).

We have noticed, however, that the incubation of mast cells with TNF, IL-4 or IL-6 influenced mast cell releasability (fig. 1). The treatment of mast cells with different concentrations of TNF (from 1 ng/ml to 500 ng/ml) for 60 min resulted in decrease of spontaneous histamine release, as compared to that of control mast cells incubated without the cytokine. The inhibition of spontaneous histamine secretion was statistically significant ($p<0.01$), and the highest decrease of spontaneous histamine release was up to 38.6±0.2% of maximum value at the concentration of TNF 500 ng/ml. IL-4 treatment of rat mast cells also resulted in the statistically significant ($p<0.01$) reduction of spontaneous histamine secretion, up to 61.4±0.3% of maximum value, at the concentration of the cytokine 500 ng/ml. The incubation of mast cells with IL-6 for 60 min reduced spontaneous histamine release as well and this decrease was statistically significant ($p<0.01$). At the concentration of IL-6 500 ng/ml spontaneous histamine secretion was up to 43.2±0.5% of maximum value.

To investigate the effect of TNF, IL-4 and IL-6 on rat mast cell reactivity, the cells were preincubated with each of the cytokines at concentrations 1 ng/ml, 50 ng/ml or 100 ng/ml for 60 min, and then challenged with compound 48/80, ConA or anti-IgE. We have noticed that TNF- and IL-4-pretreatment (cytokines at concentration 500 ng/ml) significantly inhibited compound 48/80-stimulate release of histamine with the percent release decreasing up to 58.0±1.8 and 65.0±4.1 of the control value (mast cells preincubated without cytokine and then challenged with compound 48/80), respectively. Pretreatment of mast cells with IL-6 did not affect compound 48/80-induced histamine release (fig. 2).

Preincubation of rat mast cells with TNF also resulted in statistically significant decrease of ConA-dependent histamine release (at concentration of TNF 100 ng/ml up to 41.0±2.2% of the control value). Pretreatment of mast cells with IL-4 did not change their reactivity to ConA challenge, and preincubation with IL-6 resulted in decrease of ConA-induced histamine secretion only when IL-6 was used at concentration 100 ng/ml (fig. 3).

As it is shown in fig. 4, mast cells pretreatment with TNF, IL-4 and IL-6 exerted insignificant effect on anti-IgE-induced histamine release. Only preincubation with TNF at concentration 100 ng/ml caused statistically significant decrease of histamine secretion up to 90.0±1.6% of the control value.

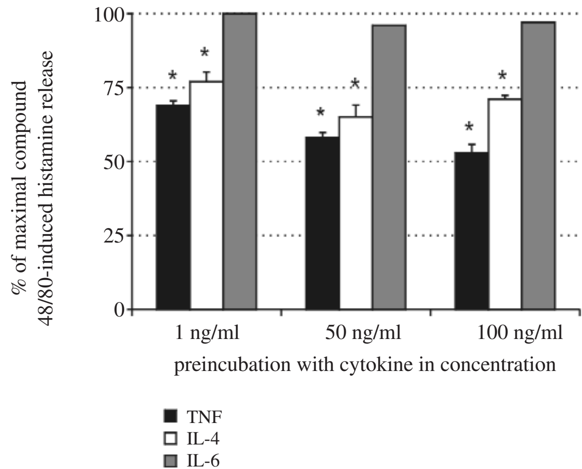

Fig. 2. Effect of pretreatment with TNF, IL-4 and IL-6 on mast cell reactivity to compound 48/80-stimulation. Mast cells were preincubated with cytokines in concentrations 1 ng/ml, 50 ng/ml or 100 ng/ml (control mast cells were preincubated without cytokine) for 60 min, then washed and challenged with compound 48/80 at concentration 5 μg/ml for 10 min. The results are the mean ± SEM of five experiments, with two replicates in each experiment. *$p<0.01$

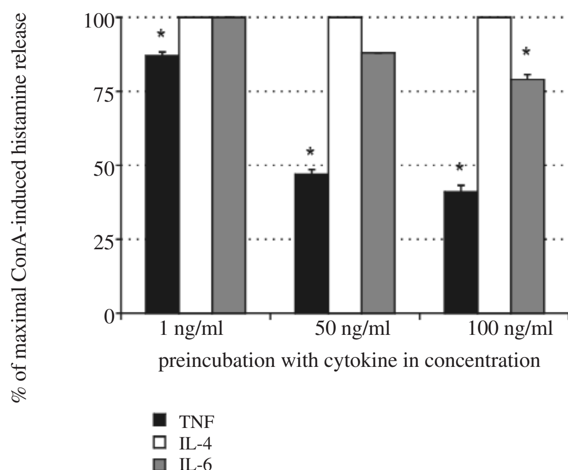

Fig. 3. Effect of pretreatment with TNF, IL-4 and IL-6 on mast cell reactivity to ConA-stimulation. Mast cells were preincubated with cytokines in concentrations 1 ng/ml, 50 ng/ml or 100 ng/ml (control mast cells were preincubated without cytokine) for 60 min, then washed and challenged with ConA at concentration 50 μg/ml for 30 min. The results are the mean ± SEM of five experiments, with two replicates in each experiment. *$p<0.01$

Discussion

Many authors have proven that only a few cytokines can directly activate mast cells to degranulation and preformed mediators release. Coleman et al. [19] have observed histamine release from stem cell factor (SCF)-stimulated mouse peritoneal mast cells. Nakajima et al. [20] have noted that SCF directly activate rat peritoneal mast cells to histamine secretion. It has been also found that nerve growth

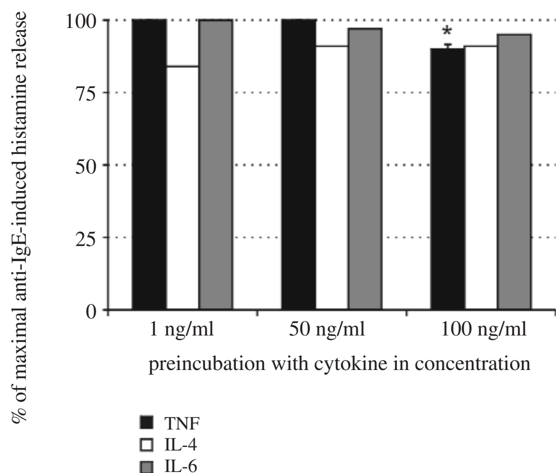

Fig. 4. Effect of pretreatment with TNF, IL-4 and IL-6 on mast cell reactivity to anti-IgE-stimulation. Mast cells were preincubated with cytokines in concentrations 1 ng/ml, 50 ng/ml or 100 ng/ml (control mast cells were preincubated without cytokine) for 60 min, then washed and challenged with anti-IgE at concentration 5 μg/ml for 30 min. The results are the mean ± SEM of five experiments, with two replicates in each experiment. *p<0.01

factor (NGF) itself can induce activation of rat mast cells to serotonin release [21], and that macrophage inflammatory protein 1 alpha (MIP-1α) stimulates murine mast cells to histamine secretion [22]. Data concerning direct effects of TNF activity are conflicting. Van Overveld et al. [23] have shown that this cytokine is a direct stimulus for human skin mast cells. Our earlier studies have indicated that human TNF stimulate histamine secretion from human adenoidal and cutaneous mast cells [24] and from rat peritoneal mast cells [25]. However, we have also proven that rat TNF does not cause degranulation and histamine and serotonin release from rat mast cells [26]. Hughes et al. [27] have not observed TNF-induced degranulation of human mast cells isolated from lungs either. In the following study we have found that none of the three cytokines tested directly activate rat peritoneal mast cells to histamine secretion. Our results confirm that TNF does not cause degranulation of mast cells. We have also documented that IL-4 does not activate rat mast cells to histamine release, what is in accord with previous observations [19, 28-31]. Our results indicate that IL-6 is not a factor directly activating mast cells either. Kikuchi et al. [32] had previously noticed that IL-6 does not influence anaphylactic histamine release from human peripheral blood-derived mast cells, and that IL-6 functions as a secretagogue for the histamine release of human mast cells only in the presence of SCF.

We have stated, however, that TNF, IL-4 and IL-6, at concentrations from 1 ng/ml to 500 ng/ml, cause statistically significant inhibition of spontaneous histamine release from mast cells, i.e. these cytokines decrease mast cell releasability. We have also established that the treatment of mast cells with TNF significantly inhibits compound 48/80- and ConA-induced histamine release. The preincubation of mast cells with IL-4 causes significant decrease of rat mast cell reactivity to stimulation with compound 48/80, and IL-6 treatment practically does not exert any effect on mast cell releasability to activation with compound 48/80, ConA or anti-IgE. Earlier studies by Takaishi et al. [31] have indicated that IL-4 does not influence mast cell reactivity, and Hughes et al. [27] have observed that TNF increase the anaphylactic histamine release from these cells. However, it should be remembered that the studies mentioned above have been conducted basing on a different experimental model, that is on human mast cells isolated from lungs.

As it has been proven, IL-4 influences mature mast cells in tissues in various ways. It enhances the expression of some adhesion molecules on mast cells [33] and regulates the adhesion of these cells to extracellular matrix proteins [34]. Moreover, IL-4 enhances the expression of FcεRI on mast cells [35] and induces mast cell apoptosis [36, 37]. IL-4 is also mast cell chemoattractant [38]. TNF modulates the expression of intercellular adhesion molecule (ICAM-1) [39] and causes the induction of MHC class II molecules on mast cell surface [40]. It also inhibits integrins expression and downregulates mast cell adhesion [41]. TNF also acts as mast cell chemoattractant [38]. IL-6 induces integrins expression and stimulates mast cell adhesion to extracellular matrix [41]. As our studies have shown, TNF, IL-4 and IL-6 influence mast cell releasability, and TNF and IL-4 change mast cell reactivity. Taking into account the results of ours and those of other authors, it may be suggested that TNF, IL-4 and IL-6, and especially the first two, are important factors modulating mature mast cell functions, probably also *via* autocrine pathway.

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