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Endobronchial secretion of interleukin-13 following local allergen challenge in atopic asthma: relationship to interleukin-4 and eosinophil counts

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Endobronchial secretion of interleukin-13 following local allergen challenge in atopic asthma: relationship to interleukin-4 and eosinophil counts. C. Kroegel, P. Julius, H. Matthys, J-C. Virchow Jr, W. Luttmann. ©ERS Journals Ltd 1996.

ABSTRACT: We investigated the secretion of interleukin (IL)-13 into the airways in 10 mild allergic asthmatics by employing local allergen challenge, and compared the data both to IL-4 levels and eosinophil numbers obtained by bronchoalveolar lavage (BAL).

Appropriate allergen or saline were endoscopically instilled into different airway segments, which were lavaged 10 min and 18 h after allergen or sham challenge. IL-4 and IL-13 were measured in unconcentrated BAL fluid using a double sandwich enzymes-linked immunosorbent assay (ELISA).

Endobronchial allergen challenge induced a highly significant increase in the numbers of eosinophils after 18 h in the allergen exposed segment. Ten minutes following allergen exposure, low levels of IL-4 and IL-13 could be detected, whilst concentrations of both cytokines were significantly raised 18 h following local allergen exposure. In contrast to IL-4, the concentration of IL-13 strongly correlated with the eosinophil numbers found 18 h post-allergen challenge.

The results suggest that interleukin-13 is actively secreted during the late asthmatic response in mild asthmatic subjects. In view of its action on eosinophils and other cell types, we conclude that interleukin-13, in addition to interleukin-4, may play an important role in the pathogenesis of eosinophil-related inflammation, such as bronchial asthma.

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Bronchial asthma is now widely-accepted as a chronic inflammatory disease of the airways and characterized by eosinophil infiltration of airway tissue (for review see [1]). A large body of evidence suggests that eosinophils represent a central effector cell at the distal end of the immune response, determining severity and chronic course of asthma *via* the release of a spectrum of cytotoxic mediators and proteins [2]. In addition, the number of eosinophils in sputum and airways has been shown to correlate with the airway damage and severity of bronchial obstruction, as measured by forced expiratory volume in one second (FEV_1) as well as airway conductance [3–5], and inversely correlates with the sensitivity of the airways to methacholine [6]. Similarly, a recent study [6] demonstrated that the number of eosinophils in the blood, bronchoalveolar lavage (BAL), and bronchial mucosa correlates with the degree of asthma, assessed by a clinical scoring method.

Although the precise mechanism underlying eosinophil tissue accumulation and activation remains to be elucidated, there is increasing evidence that T-helper cells with a type 2 (Th2) cytokine phenotype, mediate and maintain the allergic airway response in asthma, including eosinophil function and survival [7–12]. For example, eosinophil infiltration and disease intensity was found to be associated with the production of Th2 cell type cytokines, such as interleukin (IL)-4 and IL-5 [19]. However, not all the functions of eosinophils could be explained by these cytokines [8, 10, 11].

Recently, IL-13 has been identified as representing another cytokine produced by Th2 cells in mice [12, 13]. The homologue human IL-13 is a pleotropic cytokine, which modulates the functions of several cell types implicated in the pathogenesis of allergic inflammation, including B-cells, endothelial cells, mast cells and eosinophils [14–21]. However, to our knowledge, only little information on the production of the biological activity of IL-13 in atopic asthma is available. Thus, in the present study, we investigated the secretion of IL-13 into lavage fluid following endobronchial allergen challenge in asthmatic subjects. In addition, the relationship between IL-13 and IL-4, as well as airway cell differential counts, was assessed.

Patients and methods

Ten nonsmoking mild-to-moderate atopic asthmatics (seven males and three females), 26–41 yrs of age (mean± SEM age 29±6 yrs) with a minimum duration of asthma

Table 1. – Clinical details of the subjects studied

Age yrs	29±6 (26–41)
Sex M/F	7/3
Duration of asthma yrs	12±9 (2–27)
Other atopic manifestations n	
Rhinitis/rhinoconjunctivitis	8
Atopic dermatitis	1
Food allergy	1
FEV_1 % pred	103±12 (92–130)
IVC % pred	97±14 (75±113)
FEV_1/VC	82±6 (75–98)
Skin-prick test (+++ positive) n	
Birch pollen	8
Grass pollen	2
Total IgE $kU \cdot L^{-1}$	475±509 (98–2217)
Specific IgE# $kU \cdot L^{-1}$	>3.5 in all subjects
Current medication	
β_2-agonists *p.r.n.*	6

M: male; F: female; FEV_1: forced expiratory volume in one second; IVC: inspiratory vital capacity; IgE: immunoglobulin E. #: Kabi Pharmacia CAP system (Uppsala, Sweden).

of more than 2 yrs (mean±SEM duration 12±9 yrs) were studied. Allergic asthma was defined as described previously [22]. The patients were selected on the basis of: 1) a history of intermittent wheezing, chest tightness, cough and sputum production either spontaneously or exercise-induced; 2) a positive skin-prick test to common inhalant allergens (birch pollen n=8, or grass pollen n=2; Abelló, Bornheim, Germany) and a cutaneous late reaction within 6 h after challenge; 3) increased specific immunoglobulin E (IgE) for the relevant allergen (Kabi Pharmacia CAP system, Uppsala, Sweden); 4) baseline FEV_1 >90% of predicted value; 5) increased bronchial responsiveness as determined by a modified bronchoprovocation test with carbachol and total or partial reversibility following administration of β_2-agonists as described previously [9]; 6) stable clinical conditions, with no acute asthma attack and no respiratory tract infection in the past 2 months; and 8) no treatment other than rescue β_2-adrenergic drugs, which were discontinued at least 24 h before the study (table 1). All patients were informed about the nature and the scope of the study and gave their written informed consent. The study was approved by the Ethics Committee of the University of Freiburg.

Bronchoscopy, segmental allergen challenge and BAL were performed as described previously [23]. In short, allergen (250 PNU in 2.5 mL saline) was instilled into the medial basal segment of the right lower lobe (B7 right) and into the medial segment of the right middle lobe (B5 right), whilst sham challenge (2.5 mL saline) was performed in the inferior lingular bronchus (B5 left) and one segment of the left lower lobe. Both the allergen- and sham-challenged segments were lavaged using 100 mL of prewarmed saline after 10 min and 18 h. BAL fluids (BALF) from antigen and sham challenge sites were collected separately and processed for differential counts and cytokine measurement.

Cytological examination of BALF was performed after cytocentrifugation and staining with May-Grünwald Giemsa. The relative proportions of the various leucocyte subpopulations were determined by a cell differential count of 1,000 cells.

IL-13 was quantified using commercially available enzyme-linked immunosorbent (ELISA) assay (Bender and Co., Vienna, Austria) according to the manufacturers instructions. Standard curves were constructed in pooled BALF and detection limits of 1 $pg \cdot mL^{-1}$ established by spiking wells with recombinant cytokine. No cross-reactivity with other cytokines was observed. Measurements were performed in duplicate and expressed as means from both determinations.

For determination of IL-4 in the BALF, ELISA plates (Maxisorp, Nunc, Denmark) were coated with 2 $\mu g \cdot mL^{-1}$ of a monoclonal mouse anti-human IL-4 serum (Clone IL-41; PharMingen, San Diego, CA, USA) in coating buffer (0.1 M $NaHCO_3$, pH 8.0) over 6 h at room temperature, washed with phosphate-buffered saline (PBS) containing 0.05% Tween, and saturated with 3% bovine serum albumin (BSA) dissolved in PBS, pH 7.5. Serial dilutions of peritoneal lavage fluid plus recombinant IL-4 (PDH, Hannover, Germany) were then applied to each well and incubated overnight at 4°C. A peroxidase conjugated monoclonal rat anti-human IL-4 antibody (Clone MP4-25D2, PharMingen, San Diego) was added for 45 min at room temperature. After extensive washings with PBS/Tween, bound antibodies were revealed by application of 200 µL tetramethylbenzidine (TMB)-H_2O_2-substrate solution containing 20 mM TMB, and 50 mM H_2O_2 dissolved in 30 mM citrate buffer, pH 4.1, to each well. Following incubation for 15 min at room temperature, the reaction was stopped by adding, 1 M sulphuric acid and colour intensity read at 450 nm, photometrically. The results were derived from a standard curve established with human recombinant IL-4. The sensitivity of the assay was 10 $pg \cdot mL^{-1}$. There was no cross-reactivity with other cytokines. The assay was log linear from 0.010 to 500 $ng \cdot mL^{-1}$ using recombinant interferon (IFN-γ). The r-value of the standard curve was >0.95 (n=13). The intra-assay variation was 3–7%, whilst interassay (day-to-day) reproducibility was 5–15%.

All data were analysed nonparametrically using Mann-Whitney U-test for paired and unpaired variates, respectively. Statistical significance was claimed when p-value was less than 0.05. The data in the text and figures are expressed as median and range unless otherwise stated. Correlations between continuous variables were calculated using Spearman's rank correlation test and expressed as the correlation coefficient r_s.

Results

Clinically, the deposition of allergen into two different segments of the right lung was well tolerated by all subjects. Although mean FEV_1 in % of predicted dropped from 102±12% before allergen challenge to 89±11% 10 min after allergen challenge, this change was not statistically significant and dyspnoea was not reported in any of the subjects. Instillation of the allergen-containing solutions through a bronchoscope induced an immediate bronchoconstriction with swelling of the airway mucosa, leading to partial airway closure in all patients studied, which had disappeared after 18 h.

BALF recovered from the sham-challenged segments ranged 26–66 mL (41±12.6 mL; mean±SEM) at 10 min and 35 to 63 (49±3.2) mL at 18 h. In the allergen challenged segment which was lavaged 10 min after challenge, BALF recovery ranged 22–55 mL (39±3.5 mL),

and in the segment lavaged 18 h after allergen challenge the recovery was 39–72 (53±3.9) mL. There was no statistically significant difference between the percentage of BALF recovered in the three different segments.

At 10 min postchallenge, no change was observed either in the differential count or in the absolute cell number between antigen and control sites (data not shown). In contrast, after 18 h, total cell recovery increased from (median) $75\times10^3\cdot mL^{-1}$ (range 14–289) and $69\times10^3\cdot mL^{-1}$ (17–159) in the 10 min control and allergen segment, respectively, (NS), to $171\times10^3\cdot mL^{-1}$ (87–431) in the sham-challenged (NS) and $469\times10^3\cdot mL^{-1}$ (165–780) in the antigen-challenged site ($p<0.01$). This increase was mainly due to a highly significant increase in eosinophils at 18 h (19-fold compared to control) after segmental allergen provocation (10 min control: $0.9\times10^3\cdot mL^{-1}$ (0–11); 10 min allergen: $1.1\times10^3\cdot mL^{-1}$ (0–13), (NS); 18 h control: $5\times10^3\cdot mL^{-1}$ (0–24); 18 h allergen: $93\times10^3\cdot mL^{-1}$ (18–516) ($p<0.0005$, compared both to the control and 10 min values). Other cell types also increased at 18 h postchallenge but not to the extent observed with eosinophils (neutrophils sixfold ($p<0.002$); lymphocytes fourfold ($p<0.01$); and macrophages twofold (NS)).

In order to determine whether IL-13 is produced in the airways following allergen exposure, the concentration of this cytokine was measured in unconcentrated BALF 10 min and 18 h following local allergen challenge and 18 h after sham saline challenge, employing a new IL-13-specific sandwich ELISA. As depicted in figure 1, IL-13 concentrations in the BALF of all 10 subjects obtained from both allergen-exposed and sham-challenged segments at 10 min were close to the lower detection level (control 0.58 (0–2.6) $pg\cdot mL^{-1}$; antigen 0.55 (0–2.5) $pg\cdot mL^{-1}$). A comparable low IL-13 concentration was observed in the lavage fluid obtained from the sham-challenge segment at 18 h postchallenge (0.5 (0–0.9) $pg\cdot mL^{-1}$). In contrast to both the 10 min antigen and 18 h control segments, IL-13 levels were significantly raised to 7.4 (2.5–85.6) $pg\cdot mL^{-1}$ in BALF recovered from the 18 h allergen-exposed segment (fig. 1). A close correlation between the absolute number of eosinophils and the BALF, IL-13 concentration 18 h after allergen provocation ($r_s=0.87$; $p<0.002$) could be calculated (fig. 2), which was not observed immediately after allergen exposure or in the sham-challenged segments. There was also a weak correlation between IL-13 and lymphocyte numbers ($r_s=0.66$; $p<0.05$), whereas no correlation existed between the cytokine level and any other cell type (neutrophils $r_s=0.44$; macrophages $r_s=0.14$; $p>0.05$).

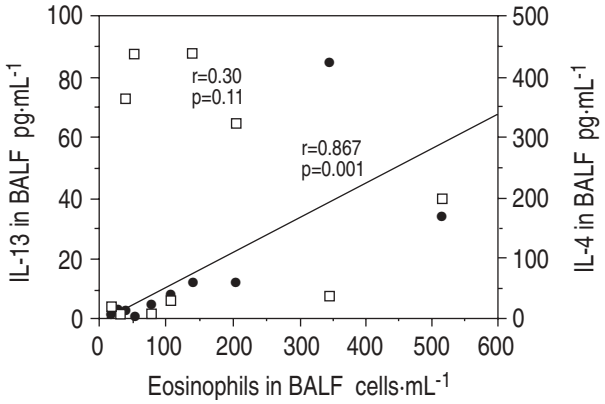

Fig. 2. – Correlation between eosinophil numbers and the concentration of IL-13 (●) as well as IL-4 (❑) measured in the BALF 18 h following segmental allergen challenge. Values represent the individual data obtained from the 10 subjects studied. The regression line for IL-13 is shown. IL-4: interleukin-4; IL-13: interleukin-13: BALF: bronchoalveolar lavage fluid.

In order to validate the data obtained on IL-13, the concentration of IL-4 in BALF were assessed. As summarized in figure 3, low levels of this cytokine were measured in the 10 min and 18 h saline sham-challenged segments as well as in the 10 min allergen-exposed segments (10 min control: 0 (0–35) $pg\cdot mL^{-1}$; 10 min allergen: 0 (0–52) $pg\cdot mL^{-1}$; 18 h control: 0 (0–45) $pg\cdot mL^{-1}$. As with IL-13, however, significantly raised levels of IL-4 to 117.2 (median) (range 0–439) were detected in

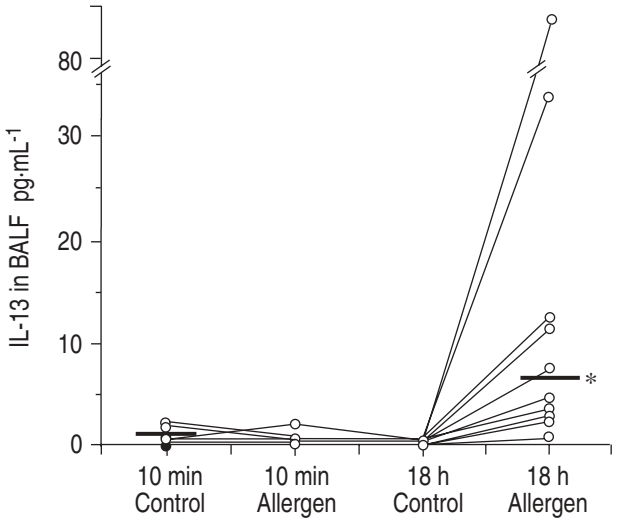

Fig. 1. – Concentrations of IL-13 in the BALF obtained 10 min and 18 h after segmental allergen provocation (Allergen) or saline control challenge (Control). Data represent the individual concentration of the cytokine. The horizontal black bar denotes the median calculated from the individual data shown. *: $p<0.001$ for the three comparisons. IL-13: interleukin-13; BALF: bronchoalveolar lavage fluid.

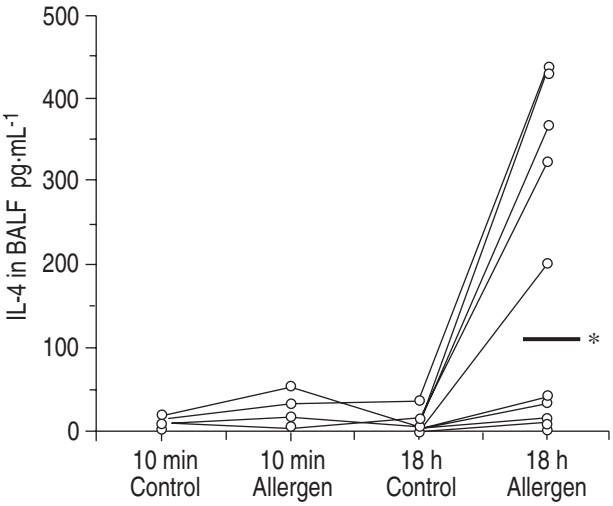

Fig. 3. – Concentrations of IL-4 in the BALF obtained 10 min and 18 h after segmental allergen provocation (Allergen) or saline control challenge (Control). Data represent the individual concentration of the cytokine. The horizontal black bar denotes the median calculated from the individual data shown. *: $p<0.001$ for the three comparisons. IL-4: interleukin-4; BALF: bronchoalveolar lavage fluid.

the 18 h post-allergen challenged segment in 8 out of the 10 patients studied ($p<0.002$). In contrast to IL-13, there was no correlation between IL-4 and eosinophils numbers in BALF obtained 18 h following segmental allergen challenge ($r_s=0.30$; $p>0.05$) (fig. 2) or between IL-4 and IL-13 ($r_s=0.21$; $p>0.05$). In addition, no significant correlation occurred between IL-4 and lymphocytes ($r_s=0.44$), neutrophils ($r_s=0.49$) and macrophages ($r_s=0.27$).

Discussion

The technique of segmental allergen challenge has been demonstrated as representing a useful model to investigate inflammatory processes underlying bronchial asthma. Employing this technique we and others have demonstrated increased levels of IL-5, IL-2, IL-1, tumour necrosis factor-α (TNF-α), IL-6, IL-8 and granulocyte/macrophage colony-stimulating factor (GM-CSF) during the late asthmatic response [24–26]. The data presented here extend these findings in three important aspects. Firstly, in contrast to a previous study [24], a significant increase in IL-4 was observed, 18 h following allergen challenge. Although the reason for this discrepancy is not entirely apparent, the detection of IL-4 in the present study may relate to the use of a new, more sensitive assay which did not require any further concentration of lavage fluid, thus, excluding the loss and degradation of IL-4 during the concentration process. Secondly, and even more importantly, the results presented above demonstrate that IL-13 is secreted in the course of allergic inflammation in atopic subjects with mild-to-moderate asthma. Thirdly, IL-13, but not IL-4, strongly correlates with the eosinophil numbers detected 18 h following local allergen exposure. Thus, in addition to IL-4, the data presented here implicate IL-13 in the immune response underlying allergic inflammation.

IL-13 is a recently cloned [12] protein secreted by activated T-cells [13]. It shows a 60% amino acid homology with a previously cloned mouse Th2 cell product, designated P600 [12, 13]. Whilst there has been no biological activity reported so far for P600, human IL-13 selectively induces vascular cell adhesion molecule-1 (VCAM-1) expression on endothelial cells *in vitro* [14, 15], and modifies cytokine secretion as well as surface antigen expression on monocytes [17–19]. In addition, IL-13 the immediate-early response gene (*c-fos*) and surface antigen expression in human mast cells [20], promotes both human eosinophil survival and activation [21], enhances CD23/FcεRII expression on resting B-cells [27], and induces the production of IgE and IgG_4 by B-cells [16]. Although these actions resemble those of Th2-like cytokines in humans, only limited evidence has so far been presented as to whether IL-13 is produced during allergic inflammation.

At the time of submitting this manuscript, an excellent paper by HUANG *et al*. [28] has reported a significant enhancement both of IL-13 transcripts and secreted protein in allergen-challenged BAL compared to sham-challenged control sites of allergic asthmatics and rhinitic patients 18–24 h following allergen provocation. In contrast, they could not find a change in the BAL IL-13 level in two nonatopic patients. Although our data on the increases of IL-13 protein within the BAL compartment are in good agreement with the findings reported by HUANG *et al*. [28], there are a number of differences which deserve attention. For instance, in addition to measuring the cytokine level at 18 h postchallenge, we also assessed the secretion of IL-13 at the time of the immediate asthmatic response. In addition, we observed a strong correlation between IL-13 and eosinophil counts, which was not mentioned in the paper by HUANG *et al*. [28]. Furthermore, whilst we did not investigate the cellular source of IL-13, HUANG *et al*. [28] have convincingly shown that IL-13 is produced exclusively by mononuclear cells within the airways.

There is another significant difference between the two studies. Employing a competitive enzyme immunoassay (EIA) kit, the IL-13 concentrations detected by HUANG *et al*. [28] ranged 120–2,000 $pg \cdot mL^{-1}$, which is more than 20 times the concentrations that we measured using a novel sandwich ELISA. Since the provocation techniques employed in the two studies were identical, this discrepancy may, in part, relate to the slightly higher allergen concentration employed in the present investigation. However, we also found high IL-13 levels in our samples with an EIA kit (Cytokit Red™ 13; Genzyme, Boston, USA) comparable to that used by HUANG *et al*. [28] (data not shown), suggesting that the different cytokine concentrations most likely relate to the two detection systems used. Despite this apparent discrepancy, however, both the current study and the data reported by HUANG *et al*. [28] complement one another and provide strong evidence for IL-13 as a major cytokine produced during the course of asthmatic inflammation.

Local airway production of IL-13 may have several important pathophysiological consequences, which may contribute to eosinophil tissue infiltration during the inflammatory response in asthma. Firstly, as has been shown for IL-4, IL-13 may promote the VCAM-1/very late activation antigen-4 (VLA-4)-dependent accumulation of eosinophils during allergic and other inflammatory reactions due to its selective induction of VCAM-1 expression on human endothelial cells [14, 15]. Secondly, the capacity of IL-13 to downregulate production of IL-12 and IFN-γ [17, 29] by monocytes may favour the development of Th2 responses, thus, enhancing eosinophil infiltration and activation *via* the secretion of Th2 cell-associated cytokines, such as IL-5. Thirdly, its eosinophil survival enhancing activity [21] may represent yet another mechanism through which IL-13 may maintain the presence of eosinophils once they have arrived in the bronchial tissue. Our observation that, in contrast to IL-4, BAL IL-13 levels closely correlate with the numbers of eosinophils but not with other cell types, provides additional evidence for a possible relationship between this cytokine and eosinophil infiltration in asthmatic bronchial inflammation. Although IL-13 shows partial structural homology to and shares several biological activities as well as a common receptor component with IL-4, this latter observation, together with the finding that IL-4 does not prolong eosinophil survival, indicates that IL-13 may also have some unique properties.

From data obtained in mice, it has been suggested that human IL-13, like IL-4, may represent a Th2-associated cytokine [2–4]. However, in contrast to IL-4, IL-13 is released by human Th cells expressing not only the Th2

but also the uncommitted T-helper (Th0) or type 1 T-helper (Th1) phenotype [30]. In addition to Th cells, IL-13 is also released by $CD8^+$ cells as well as Epstein-Barr virus (EBV)-transformed B-cells [30]. In agreement with these findings, HUANG *et al*. [28] convincingly demonstrated that IL-13 is produced by the mononuclear cell fraction. Furthermore, in T-cell clones derived from either nonatopic subjects [13, 31] or from BAL of atopic asthmatics, following allergen challenge [31], IL-13 messenger ribonucleic acid (mRNA) expression was found exclusively in T-cell clones. Although we did not investigate the potential sources of IL-13, these data taken together suggest that the local production of IL-13 observed in the asthmatic subjects studied has most likely derived from various lymphocyte subsets located within the airways and activated during the course of the allergen-induced airway inflammation.

In summary, the results presented here demonstrate that exposure of asthmatic airways of atopic individuals to relevant allergen induces local interleukin-13 secretion, which parallels but does not correlate with the release of interleukin-4. Since T-cells appear to represent the major origin of interleukin-13 in humans [13, 28, 31], the study further supports the role of T-cells in orchestrating the allergic inflammation in asthma. Interleukin-13 may play a critical role in this process, since it integrates several biological actions of type 2 T-helper cell cytokines implicated in the pathogenesis of asthma. Firstly, together with interleukin-4, it is involved in regulating eosinophil migration, mast cell activation, and immunoglobulin E production. Secondly, similar to interleukin-5, interleukin-13 activates eosinophils and regulates eosinophil survival. The understanding of these overlapping functions of different cytokines is of significance as regards future approaches in the treatment of asthma involving anticytokine antibodies.

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