

Nasal memory T lymphocytes capable of producing IL-4 in the allergic reaction

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This paper reports the presence of memory T cells in the nasal mucosa of allergic patients. The demonstration of CD4⁺/CD29⁺ (CD4⁺/CD45RO⁺) T lymphocytes, which are capable of interleukin-4 production, can indicate a complementary cell-mediated regulatory mechanism for mast cell proliferation and IgE synthesis in human nasal allergy. No substantial IgE production can be obtained in the absence of IL-4. Therefore, the existence of IL-4 producing cells on site in the nasal mucosa of allergic subjects probably implies a complementary interaction between cytokines and different immunocompetent nasal cells in the regulation of B cells and IgE synthesis.

Key words: nasal allergy; memory T cells; interleukin-4; cytokines.

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The role of the mast cell and IgE in the acute phase of the allergic reaction has been extensively studied. However, recent reports indicate that the late phase may be regulated by an interaction between nasal Langerhans cells, nasal T lymphocytes and cytokines (3, 4, 5, 6). The presence of activated T lymphocytes in the nasal mucosa in allergic patients has recently been demonstrated (6, 7). Human activated (and resting) T lymphocytes may not fall into strict TH1 and TH2 cells as in mice (9), but human CD4⁺ T lymphocytes are either CD4⁺ 2H4⁺ 4B4[−] cells (induce CD8 T cells to become suppressive) or CD4⁺ 2H4[−] 4B4⁺ cells (CD45RO⁺), the latter being true helper cells, and capable of providing help for Ig synthesis by B cells (8, 10).

After activation by exposure to antigen CD4⁺ T cells change their phenotype from CD4⁺/CD45RA (virgin T cells) to CD4⁺/CD29/CD45RO (memory T cells). The CD4⁺/CD29 T lymphocyte is (after another exposure to antigen) capable of producing interleukin-4 (1). Interleukin-4 is known to influence the regulation of mast cell proliferation as well as the IgE synthesis and, furthermore, to increase the vascular permeability. Thus the demonstration of nasal memory T cells may imply a new possible major pathway in the regulation of the mast cell in the nasal allergic reaction, particularly the late phase reaction and the year-to-year memory present in these patients.

Material and methods

The present study reports the results from a larger ongoing provocation study of patients allergic to birch pollen, but also some patients with isolated grass-pollen allergy. Healthy volunteers were provoked in the same way as the patients (6). Nasal biopsies were taken (in the winter season) before and after provocation with the allergen, and also during the pollen season. Fine needle biopsies from the tonsils, brush samples from the nasal cavity, and blood samples for flow cytometry were taken simultaneously. The nasal biopsies were snap frozen and double-stained for CD4⁺ and CD29/CD45RO/CD45RA, i.e. CD4⁺ T cells expressing CD29 (memory), CD45RO (UCHL1, memory), and CD45RA (virgin), respectively. This report deals with findings in immunostained nasal biopsies with regards to T-memory and T-virgin cells in provoked patients and controls. Avidin-Biotin Complex method was used for the first antibody (anti-CD4) and the second antibody was applied according to APAAP method. No counter-staining was used. Thus CD4⁺ cells were red and non-CD4 lymphocytes/monocytes expressing either CD29, CD45RO or CD45RA were blue. T lymphocytes expressing both CD4⁺ and CD29/CD45RO/CD45RA consequently stained both red and blue. The slides were examined and coded.

Table 1. Immunostaining of frozen nasal biopsies

Case/Mab	Allergics						Controls					
	1	2	3	4	5	6	1	2	3	4	5	6
CD4	0	++(+)	+++	++(+)	+++	+++	+++	+++	++	++	++	++
CD29	0	+++	++	++	++	++	++	-	++	+	+	-
CD45RO	+	+	++	++	++	++	+	+	++	++	-	-
CD45RA	-	+	++	++	++	++	+	+	++	++	-	+
CD4/CD29	-	++	++	+	++	+++	+	-	-	-	-	-
CD4/CD45RO	-	-	++(+)	-	++	++	+	-	-	-	-	-
CD4/CD45RA	-	-	+	-(+)	++	++	+	-	+	-	-	-

Antibodies CD4 and CD45RO (UCHL1) from Dakopatts; CD29 and CD45RA from Coulter Corporation. - = no positive cells; + = 1-2 positive cells; ++ = 3-5 positive cells; +++ = >5 positive cells.

0 = evaluation not possible. Evaluation performed per high power field (5, 6).

Figures given are after provocation, and figures within parentheses represent result before provocation if any difference recorded.

Results

Double-stained T lymphocytes were found in only one of the six controls whilst five of six allergics had double-stained T lymphocytes. There was no significant difference between before and after provocation, which was to be expected as the time interval was only 30-45 min. Five patients showed numerous CD4 + /CD29 + cells (Table 1). Apart from lymphocytes, monocytes and macrophages may stain positively for CD4-antibodies. Thus care had to be taken not to misinterpret some double-stained monocytes/macrophages as T-memory lymphocytes. Thus all large cells that were double-stained were disregarded as being possible macrophages, and all intermediate-sized cells were also disregarded as being possible monocytes. Since activated T lymphocytes do enlarge a little in size, great care was taken to separate lymphocytes from monocytes. However, even after excluding many cells as *not* being typical small lymphocytes, positive double-stained lymphocytes were still found in five of six allergic patients, both before and after provocation, but only in one control. The allergic patients showed numerous non-CD4 memory cells (blue cells), and to a much greater extent than the healthy subjects.

Discussion

The presence of CD4 + /CD29 + T-memory lymphocytes in the nasal mucosa in allergic patients is, to our knowledge, a new finding that has not hitherto been reported. After stimulation CD4 + /CD29 + T lymphocytes have the capacity of IL-4 production. Interleukin-4 is the essential factor for induction of human IgE synthesis *in vitro* (11). Thus, a similar regulatory mechanism of IgE synthesis may exist *in vivo*, and preliminary results of this study therefore imply that allergic patients may have a cell-mediated regulatory mechanism via memory T cells.

There was no difference in the ratio of CD4 + /CD29 + cells in the peripheral blood between allergics and controls; however, an increase of CD4/CD45RA was noted in allergic patients (Karlsson et al., unpublished data). Therefore, at this period it is difficult to conclude whether the nasal memory T cells are recruited from the blood or reside in the vicinity (nasal mucosa and in NALT/general immune system). Cytokines act locally in a paracrine fashion on B cells and mast cells and very likely play an important role in the allergic reaction. Interleukin-5 producing cells have recently been demonstrated in the nasal mucosa of allergic patients (2), and our findings indicate the presence of memory T cells capable of interleukin-4 production. These two findings, taken together, strongly suggest that allergics have a pronounced interaction between T-cell-mediated immunological reactions and cytokines in the regulation of B cells and IgE synthesis in the late allergic reaction. Due to the results presented here and other recent reports concerning cytokines (12, 13) the study has been extended and investigations of signal substances such as IL-4, IL-5, IL-6 and INF-gamma will be carried out by means of both immunohistochemistry and *in situ* hybridization.

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