

Heterogeneity of mast cells and T cells in the nasal mucosa

Ruby Pawankar, MD, PhD, and Chisei Ra, MD, PhD Tokyo, Japan

Allergic diseases like atopic rhinitis, bronchial asthma, and urticaria are prevalent and on the rise. The need to better understand the pathophysiology of these diseases is therefore crucial to the development of newer and more effective modes of treatment. We hypothesized that in inflammatory diseases like allergic rhinitis and asthma characterized by profound local clinical manifestations and inflammation of the relevant mucosae, the most important immunopathological findings must occur locally. Although studies on the cellular elements and mediators in the peripheral blood compartment may provide useful information, they may not accurately reflect events occurring within the target organ itself. Even in the normal mucosa there is a resident population of lymphocytes and mast cells. Taking perennial allergic rhinitis (PAR) and chronic infective rhinitis (CIR) as representative chronic airway inflammatory diseases we investigated the phenotypic and functional characteristics of mast cells and lymphocytes in the nasal mucosa of patients with PAR and CIR during the natural course of the disease. We further compared the characteristics of lymphocytes in the nasal mucosa with that in the peripheral blood compartment. Our results demonstrated heterogeneity of mast cells and T cells in the nasal mucosa. Furthermore, the mucosal changes at the site of allergic inflammation were characterized by an increase in the proportion of CD4⁺ CD45RO⁺ T cells (memory cells); oligoclonal expansion and activation of V γ 1/V δ 1⁺ T cells; an increased number of Fc ϵ RI⁺ cells; an increased proportion of T_{H2}-type cytokine expressing mast cells and lymphocytes and of very late antigen-4 and very late antigen-5 expressing nasal mast cells, independent of alterations in CIR; and autologous peripheral blood. These findings strongly suggest heterogeneity of lymphocytes and mast cells in the nasal mucosa based on the underlying inflammatory disease, and compartmentalization of inflammatory cells in the nasal mucosa and peripheral blood. (J Allergy Clin Immunol 1996;98:S248-62.)

Key words: Perennial allergic rhinitis, mast cells, mucosal lymphocytes, $\gamma\delta$ T cells, adhesion molecules, T_{H2} cytokines

The nose is a potential site for various inflammatory diseases such as perennial allergic rhinitis (PAR) and chronic infective rhinitis (CIR). PAR is an IgE-mediated atopic disease characterized by perennial symptoms of sneezing, rhinorrhea, and nasal obstruction, an increased level of serum-specific IgE, and nasal eosinophilia. CIR is a bacterium- or virus-induced IgE-independent inflammatory disease characterized by mucopurulent

nasal discharge, nasal obstruction, and often associated paranasal sinusitis. Most of the earlier studies aimed at elucidating the pathophysiologic mechanisms of these diseases were limited to examination of the cellular elements and mediators in the blood compartment or the epithelial lining fluid.¹⁻⁵ Although much information is available from studies conducted on the cellular elements and mediators in the epithelial lining fluid, these elements are not totally representative of those in the nasal mucosa.²⁻⁶ Furthermore, the cellular events in the peripheral blood compartment may also not be representative of that in the nasal mucosa. With the availability of monoclonal antibodies (mAbs) and the application of sensitive techniques such as immunohistochemistry in situ hybridization, fluorescence-activated cell-sorter analysis (FACS), and the polymerase chain reaction (PCR), we have been able to achieve a better

From the Department of Immunology, Juntendo University School of Medicine.

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Reprint requests: Chisei Ra, MD, PhD, Department of Immunology, Juntendo University School of Medicine 2-1-1, Hongo, Bunkyo-ku, Tokyo 113, Japan.

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Abbreviations used

CIR:	Chronic infective rhinitis
FACS:	Fluorescence-activated cell-sorter analysis
FITC:	Fluorescein isothiocyanate
GM-CSF:	Granulocyte-macrophage colony-stimulating factor
ICAM:	Intercellular adhesion molecule
IEMC:	Intraepithelial mast cell
IFN- γ :	Interferon- γ
LEMC:	Lamina propria mast cell
LT:	Leukotriene
mAB:	Monoclonal antibody
NMC:	Nasal mast cell
NML:	Nasal mucosal lymphocyte
PAR:	Perennial allergic rhinitis
PBL:	Peripheral blood lymphocyte
PCR:	Polymerase chain reaction
PE:	phycoerythrin
PG:	Prostaglandin
TCR:	T-cell receptor
TNF- α :	Tumor necrosis factor- α
VCAM-1:	Vascular cell adhesion molecule-1
VLA:	Very late antigen

understanding of the cellular and molecular events that occur in the target organs.

Mast cells are known to play a central role in IgE-mediated allergic diseases.⁷⁻¹⁰ Recent studies have demonstrated that mast cells are a potential source of multifunctional cytokines, indicating that mast cells may play important roles in allergic inflammation not only through the release of inflammatory mediators but also through the release of cytokines.¹⁰⁻¹³ In addition, previous studies have shown an increase in the number of mast cells in the epithelial compartment of the nasal mucosa of patients with allergic rhinitis, in good correlation with the severity of disease.¹⁴⁻¹⁶ However, the responsibility for the initiation and perpetuation of allergic inflammation does not lie with any single cellular element and occurs as a result of a cascade of events involving a variety of cells including mast cells, lymphocytes, eosinophils, basophils, epithelial cells, and fibroblasts.

In this article, we devote attention to the phenotypic and functional characteristics of mast cells and lymphocytes in the nasal mucosa of patients with PAR and their possible role in the perpetuation of allergic inflammation. To understand the specificity of these cellular elements in allergic diseases, we compared the characteristics of lymphocytes and mast cells in the nasal mucosa of

patients with PAR during the natural course of the disease with those in patients with CIR. To elucidate the target-organ specificity, we compared the characteristics of nasal mucosal lymphocytes (NML) in patients with PAR and CIR with those of autologous peripheral blood lymphocytes (PBL).

PHENOTYPES, DISTRIBUTION, AND STAGES OF ACTIVATION OF NML IN PAR DIFFER FROM THOSE IN CIR AND ARE UNRELATED TO THOSE IN THE PERIPHERAL BLOOD COMPARTMENT

T lymphocytes are the principal factors that regulate and coordinate immune responses. Most of the earlier studies to characterize nasal lymphocytes were limited to immunohistochemical analysis of CD4⁺ T cells, CD8⁺ T cells, CD25⁺ T cells, and B cells.¹⁷⁻²¹ By fluorescence-activated cell sorter analysis, immunohistochemistry, and PCR, we elucidated the proportions, phenotypes, stages of differentiation, distribution, and cytokine profile of lymphocytes in the nasal mucosa and peripheral blood compartments of patients with PAR and CIR.²² In both groups of patients, CD3⁺ (T) lymphocytes constituted >70% of total NML; B lymphocytes constituted about 20%; and natural killer cells constituted <5%. Although no difference was detected in the proportion of nasal mucosal T cells between PAR and CIR, B cells were significantly increased in NML of PAR patients, and natural killer cells were modestly increased in the nasal mucosa of CIR patients (Fig. 1). Second, in agreement with previous studies we observed a modest increase in the proportion of nasal mucosal CD4⁺ (helper/inducer) T cells in PAR patients.²¹ When we examined the T-cell receptor (TCR) expression in the nasal mucosa of both groups of patients, we observed that the majority of NML expressed the $\alpha\beta$ TCR, whereas $\gamma\delta$ TCR⁺ cells formed a relatively minor population. Yet, by comparison, the proportion of $\gamma\delta$ T cells was significantly increased in PAR.²²

Mucosal lymphocytes are classified into two subsets on the basis of their distribution in the tissue: those infiltrating the epithelial compartment are known as intraepithelial lymphocytes, and those infiltrating the lamina propria are known as lamina propria lymphocytes. We asked whether there were any characteristic differences in the phenotypes and proportions of lymphocytes between intraepithelial and lamina propria lymphocytes. In PAR and CIR patients, CD8⁺ (cytotoxic) T cells predominated in intraepithelial lympho-

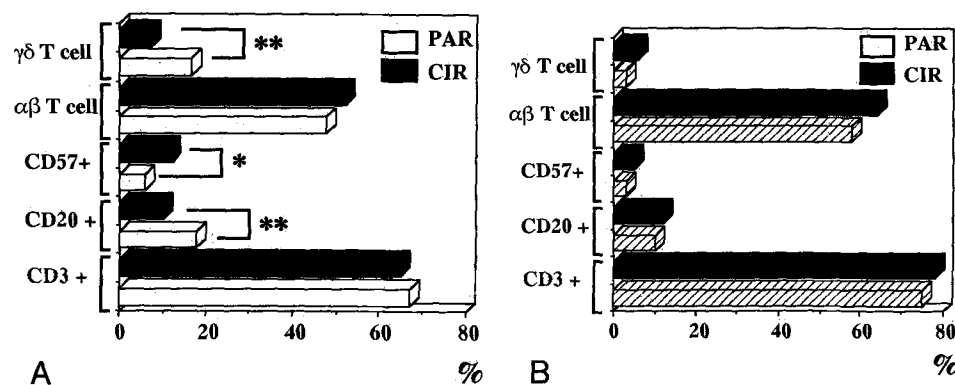


FIG. 1. Comparison of lymphocyte subpopulation in nasal mucosa of PAR and CIR patients. **A**, Flow cytometric analysis of CD3⁺ (T cells), CD20⁺ B cells, CD57⁺ (natural killer cells), and $\alpha\beta$ and $\gamma\delta$ T cells in the nasal mucosa of PAR and CIR patients was carried out by FACS after staining of isolated NML with relevant FITC/PE-conjugated mAbs, as previously described.²² CD20⁺ (B cells) and $\gamma\delta$ T cells were significantly increased in NML of PAR patients. By contrast, natural killer cells were modestly increased in NML of CIR patients. **B**, Flow cytometric analysis of CD3⁺, CD20⁺, CD57⁺, and $\alpha\beta$ and $\gamma\delta$ T cells in PBL of PAR and CIR patients was carried out by FACS after staining of isolated PBL with relevant FITC/PE-conjugated mAbs, as previously described.²² No significant differences were detected in proportion of lymphocyte subsets or natural killer cells between PAR and CIR. PAR, $n = 13$; CIR, $n = 16$. * $p < 0.05$; ** $p < 0.01$.

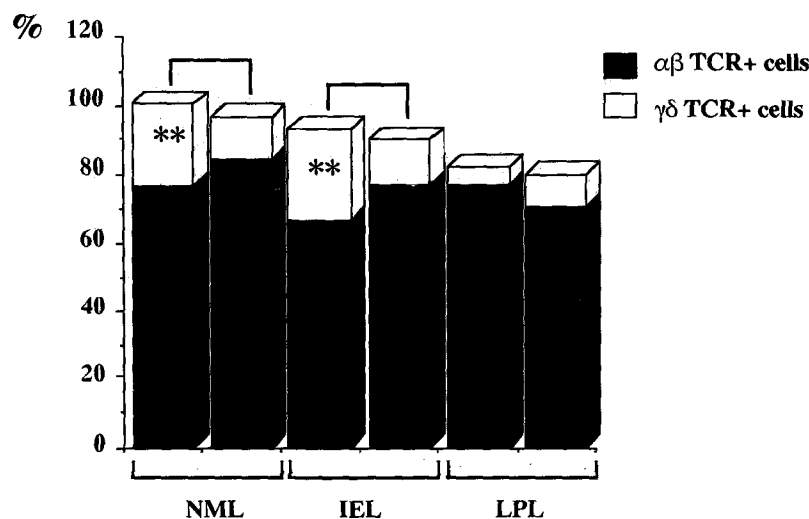


FIG. 2. Comparison of $\alpha\beta$ and $\gamma\delta$ TCR expression in nasal mucosa, nasal epithelium, and lamina propria of PAR and CIR patients. Flow cytometric analysis of $\alpha\beta$ and $\gamma\delta$ TCR expression in nasal mucosa, nasal epithelium, and lamina propria of PAR and CIR patients was carried out by FACS after staining of isolated intraepithelial lymphocytes (IEL), lamina propria lymphocytes (LPL), and NML with relevant FITC-conjugated mAbs, as previously described.²² $\gamma\delta$ T cells were selectively increased in IEL and NML of PAR patients. However, no difference was detected in $\gamma\delta$ T cell population in LPL or in $\alpha\beta$ T-cell population in NML, IEL, or LPL between PAR and CIR. NML, $n = 30$; IEL, $n = 22$; LPL, $n = 22$. ** $p < 0.01$.

cytes whereas CD4⁺ (helper/inducer) T cells were predominant in lamina propria lymphocytes. What was of much interest was the preferentially distribution of $\gamma\delta$ T cells in the epithelial compartment, even though $\alpha\beta$ T cells were equally distributed in both compartments (Fig. 2).

T cells expressing the CD45RO surface antigen represent a subset of primed T cells that react more easily to recall antigens and produce more lymphokines upon restimulation than their naïve counterparts (CD45RA⁺ cells).²³ To elucidate the stages of activation of NML, we examined the

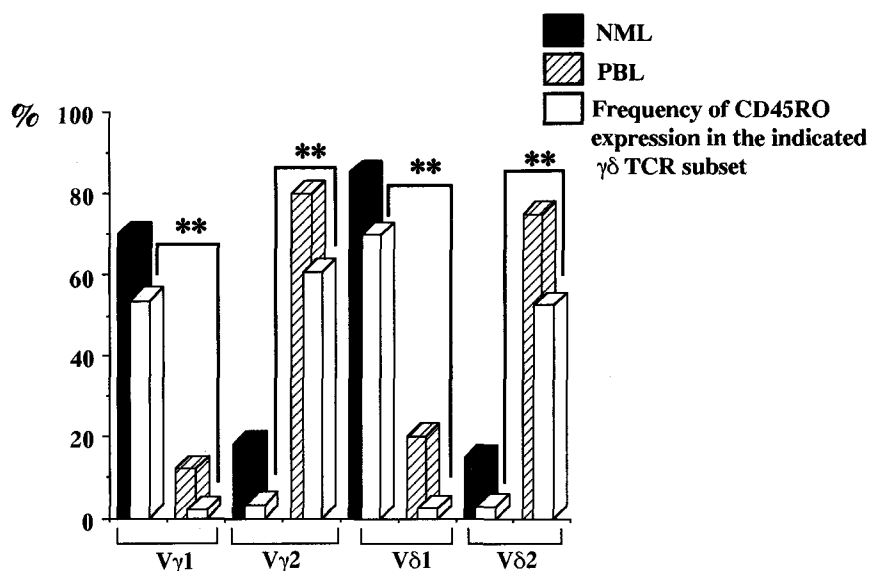


FIG. 3. CD45RO and CD45RA expression in $\gamma\delta$ T cell subsets in nasal mucosa and peripheral blood of PAR patients. Flow cytometric analysis of CD45RO and CD45RA⁺ $\gamma\delta$ T-cell subsets in isolated NML and PBL was carried out by FACS after staining with relevant FITC/PE-conjugated mAbs, as previously described.²⁴ Solid bars and hatched bars, Proportion of indicated $\gamma\delta$ T cells in NML and PBL, respectively; open bars, proportion of CD45RO and CD45RA positivity in indicated $\gamma\delta$ T cells. CD45RO expression was higher in Vδ1⁺ T cells in NML. By contrast, CD45RO expression was higher in Vδ2⁺ T cells in PBL. $n = 15$. ** $p < 0.01$.

CD45RO and CD45RA expression in NML of PAR and CIR patients. Of note, we found an increase in the proportion of CD4⁺ CD45RO⁺ NML in PAR patients. Moreover, an increased proportion of these CD4⁺ CD45RO⁺ T cells were distributed in the epithelial compartment.²² These findings of an increased distribution of CD4⁺ CD45RO⁺ T cells in the epithelial compartment of the nasal mucosa of PAR patients may have important clinical consequences because these cells are constantly exposed to environmental antigens. Furthermore, when we compared the proportion of CD45RO⁺ $\alpha\beta$ T cells and CD45RO⁺ $\gamma\delta$ T cells in NML, we found that the proportion of CD45RO⁺ $\gamma\delta$ T cells was remarkably increased in PAR, but no difference was detected in the proportion of CD45RO⁺ $\alpha\beta$ TCR⁺ cells between PAR and CIR.

$\alpha\beta$ T cells in both groups of patients expressed either the CD4 or CD8 surface antigen. By contrast, the majority of $\gamma\delta$ T cells were CD3⁺4⁺8⁻. The proportions of CD4⁺ and CD4⁺8⁻ $\gamma\delta$ T cells were selectively increased in the nasal mucosa of PAR patients but no significant difference was detected in the $\alpha\beta$ T-cell population.²⁴ We next asked whether nasal $\gamma\delta$ T cells were distinct from those in the peripheral blood. To make clear this point, we compared the phenotypic and molecular

characteristics of nasal and peripheral blood $\gamma\delta$ T cells in the same patients. Whereas the majority of nasal $\gamma\delta$ T cells were CD4⁺8⁻, those in the peripheral blood were predominantly CD8⁺. CD4⁺ $\gamma\delta$ T cells constituted about 20% of total nasal $\gamma\delta$ T cells but were undetected in the peripheral blood. Furthermore, nasal $\gamma\delta$ T cells in patients with PAR and CIR and in normal subjects dominantly expressed the Vγ1/Vδ1 genes, whereas the majority of those in the peripheral blood expressed the Vγ2/Vδ2 genes.²⁴ In addition, the expanded population of nasal $\gamma\delta$ T cells in PAR patients expressed the Vγ1/Vδ1-Jδ1 TCR, and >60% of these Vγ1/Vδ1 TCR⁺ cells were primed (CD45RO⁺) unrelated to those in autologous peripheral blood (Fig. 3). This finding indicated that nasal $\gamma\delta$ T cells are distinct from those of the peripheral blood and are oligoclonally expanded locally under specific antigenic stimulation.

To clarify whether the expansion of nasal mucosal $\gamma\delta$ T cells was antigen specific, we stimulated highly purified (magnetic activated cell sorting-purified) nasal and peripheral blood $\gamma\delta$ T cells with Der f 2 antigen^{25,26} in the presence of irradiated autologous feeder cells and elucidated the antigen-specific proliferation by [³H]thymidine uptake. In brief, NML and PBL were isolated as previously described,²² and $\gamma\delta$ T cells were purified by posi-

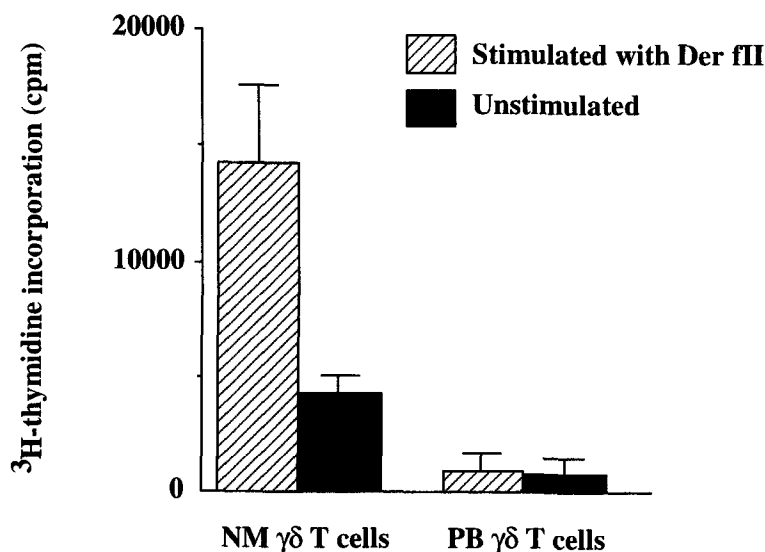


FIG. 4. Induction of cell proliferation by Der f 2 (purified mite allergen) in nasal mucosal (NM) and peripheral blood (PB) γδ T cells. Highly purified (MACS-purified) NM and PB γδ T cells were cultured in a 96-well flat bottom microplate at a density of 1×10^5 /well with irradiated autologous feeder cells (5×10^4 /well) in the presence or absence of $10 \mu\text{g/ml}$ recombinant Der f 2^{25, 26} for 5 days. [³H]Thymidine was added 16 hours before end of culture, and cell proliferation was estimated by assaying incorporation of [³H]thymidine. Although nasal mucosal γδ T cells proliferated well to Der f 2 antigen, those of peripheral blood did not.

tive selection with the use of magnetic activated cell sorting after staining with the anti-γδ TCR mAb (TCR γδ-1, Becton Dickinson, Mountain View, Calif.) and the magnetic-coated anti-mouse IgG₁ (Miltenvy, Gladbach, Germany). Subsequently, the purified nasal mucosal and peripheral blood γδ T cells were cultured in Rosewell Park Memorial Institute 1640 medium with 10% fetal calf serum and 1% penicillin-streptomycin, with or without $10 \mu\text{g/ml}$ recombinant Der f 2 (Asahi Breweries, Tokyo, Japan)^{25, 26} in the presence of autologous feeder cells for 5 days. [³H]Thymidine ($1 \mu\text{Ci/well}$) was added to the cultures 16 hours before the end of the culture, and the incorporated radioactivity was measured by liquid scintillation counting after harvesting. Although nasal mucosal γδ T cells proliferated well in response to Der f 2 antigen, peripheral blood γδ T cells did not respond well to stimulation with Der f 2 (Fig. 4). Moreover, when we examined the cytokine profile of nasal mucosal and peripheral blood γδ T cells in PAR and CIR patients by flow cytometry, we found dominant expression of T_{H2}-type cytokines in nasal mucosal γδ T cells whereas cytokines in CIR patients and those in the peripheral blood of PAR patients dominantly expressed IFN-γ.^{24, 27} These findings suggest that the expansion of γδ T cells in the nasal mucosa of PAR patients is antigen specific and that nasal mucosal γδ T cells

in PAR patients exhibit a T_{H2}-type cytokine profile independent of γδ T cells in the peripheral blood.

In contrast to the obvious heterogeneity observed in NML of PAR and CIR patients, the proportions, phenotypes, and stages of differentiation of PBL in PAR and CIR patients, showed no marked differences and were unrelated to alterations in the nasal mucosa (Figs. 1, 3, and 4). Together, these findings strongly suggest that the characteristics of lymphocytes in the peripheral blood do not precisely reflect the changes at the site of inflammation and that studies on the cellular elements in the target organ are essential to clarify the pathogenesis of these inflammatory diseases.

ROLE OF γδ T CELLS IN PAR

The precise role of nasal γδ T cells remains to be defined. There are several postulations regarding the function of γδ T cells including immune surveillance, rejection of stressed cells, regeneration of epithelial cells, antigen presentation, regulation of the differentiation of αβ T cells in the primary immune response, as contra suppressor cells, provision of help for αβ T cells in contact sensitivity, and regulation of IgE synthesis.²⁸⁻³⁴ It also has been reported that γδ T cells can recognize antigens directly. In light of our recent observations, we propose that one of the potential roles of nasal

$\gamma\delta$ T cells may be regulation of differentiation of $\alpha\beta$ T cells into T_{H1} - or T_{H2} -type cells early in the primary immune response. Yet the selective expansion of nasal mucosal $\gamma\delta$ T cells in PAR patients cannot be explained by this hypothesis alone, and further studies are underway to elucidate precisely the function of nasal $\gamma\delta$ T cells. Furthermore, like $\alpha\beta$ T cells, $\gamma\delta$ T cells may exhibit heterogeneity of function on the basis of their phenotypes.³³

NML IN PAR BUT NOT CIR EXHIBIT A DISTINCT POLARIZATION TO A T_{H2} -TYPE CYTOKINE PROFILE

By reverse-transcription PCR, flow cytometry, and ELISA we examined the cytokine profile of freshly isolated NML and autologous PBL in patients with PAR and CIR. We detected increased IL-4 and IL-5 gene expression and relatively low interferon- γ (IFN- γ) gene expression in NML of PAR patients compared with those in CIR patients (Fig. 5). On the other hand, in CIR patients we demonstrated lower levels of IL-4 gene expression and enhanced IFN- γ gene expression. Of note, when we compared the IL-13 gene expression in NML from PAR and CIR patients, we found that NML in PAR but not in CIR expressed the IL-13 gene (Fig. 5), and a strong correlation existed between the levels of IL-13 gene expression and the levels of serum total and specific IgE antibodies,¹³ suggesting that IL-13 may play an important role in the maintenance of IgE synthesis within the local microenvironment. Semiquantitative analysis of the PCR products of cytokine genes in NML by densitometry relative to that of β -actin expression of each sample demonstrated a predominant T_{H2} -type cytokine profile in PAR patients and a T_{H1} -type cytokine profile in CIR patients. Analysis of the expression of cytokine genes in freshly isolated PBL from PAR patients demonstrated expression of the IL-4, IL-5, and IFN- γ genes but not of the IL-13 gene (Fig. 5).

We next examined the cytokine production by NML and PBL from PAR and CIR patients by flow cytometry and ELISA. We detected greater levels of IL-4, IL-5, and IL-13 expression in NML from PAR patients than in those from CIR patients, and NML from CIR patients demonstrated increased IFN- γ expression. When we stimulated freshly isolated NML and PBL from PAR and CIR patients with phorbol myristyl acetate (10 ng/ml) and immobilized anti-CD3 (5 μ g/ml), and the supernatants were stored at -80°C until further

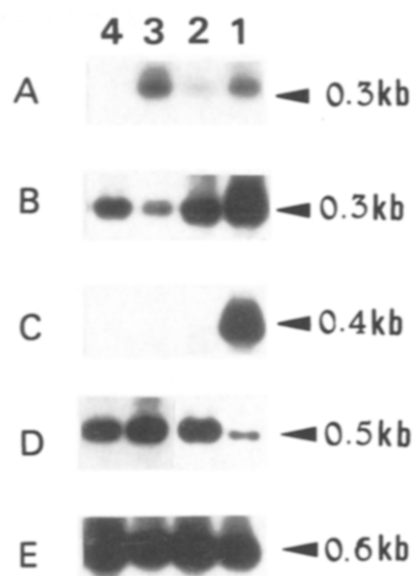


FIG. 5. Cytokine gene expression in NML from PAR and CIR patients. Poly-A RNA was extracted from isolated NML and PBL from PAR and CIR patients, and messenger RNA was selectively converted to complementary DNA (cDNA) by reverse transcriptase. Indicated cytokine cDNAs were amplified by using appropriate sense and anti-sense primers, as previously described.¹³ PCR products were electrophoresed, transferred onto nylon membranes, and visualized by chemiluminescence. β -Actin was used as the internal control for each sample. Lanes 1 and 3, Cytokine gene expression in NML and PBL, respectively, of representative PAR patient; lanes 2 and 4, cytokine gene expression in NML and PBL, respectively, of representative CIR patient. mRNAs for each cytokine are depicted as follows: A, IL-4; B, IL-5; C, IL-13; D, IFN- γ ; E, β -actin (internal control).

assay. The levels of secreted cytokines were measured using relevant cytokine assay kits (Quantikine, R & D Systems, Minneapolis, Minn., and Chemicon, Temecula, Calif.). We found that NML from PAR patients produced significant amounts of IL-13, IL-4, and IL-5 but no IFN- γ (Fig. 6, A). In contrast, NML from CIR patients did not produce detectable levels of IL-4 or IL-13 but secreted moderate levels of IL-5 and significantly greater levels of IFN- γ . As compared with NML, PBL from PAR patients secreted relatively lower levels of IL-4, IL-5, and IL-13 but greater levels of IFN- γ (Fig. 6, B). Moreover, previous work has shown that freshly isolated NML from PAR patients but not PBL expressed the IL-13 gene.¹³ Together, these findings suggest that NML in PAR patients express and produce T_{H2} -type cytokines unrelated to those in CIR and in autologous PBL.

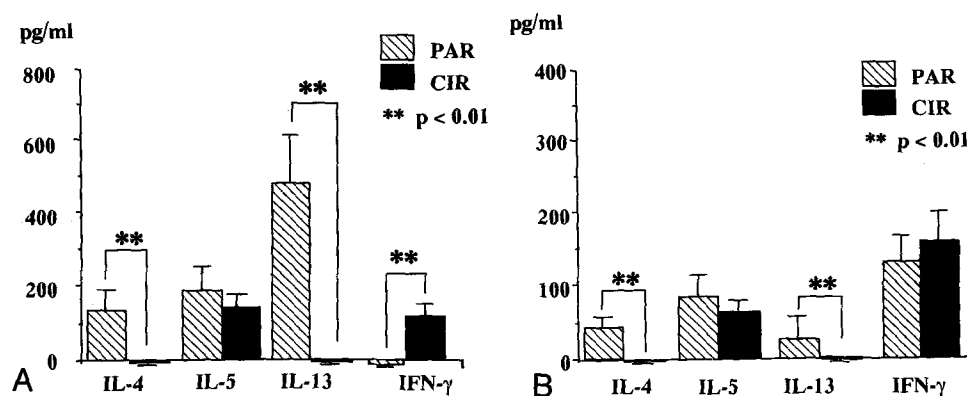


FIG. 6. Induction of cytokine secretion by NML and PBL from PAR and CIR patients. Isolated NML and PBL from PAR and CIR patients were stimulated with phorbol myristyl acetate (10 ng/ml) and immobilized anti-CD3 (5 μ g/ml) for 48 hours, and levels of secreted cytokines were measured by relevant cytokine assay kits. **A**, levels of secreted IL-4, IL-5, IFN- γ , and IL-13 by NML from PAR and CIR patients. **B**, levels of secreted IL-4, IL-5, IFN- γ , and IL-13 by PBL from PAR and CIR patients. $n = 10$; CIR, $n = 10$. * $p < 0.05$; PAR, ** $p < 0.01$.

TABLE 1. Numbers of cytokine-expressing cells in the nasal mucosa

Cytokine	Epithelium		Lamina propria	
	PAR	CIR	PAR	CIR
IL-2	ND	ND	3.8 $\pm$ 2.4	6.6 $\pm$ 4.4
IL-3	7.8 $\pm$ 5.4	1.0 $\pm$ 0.8	14.5 $\pm$ 9.5	6.0 $\pm$ 2.3
IL-4	7.9 $\pm$ 4.8	0.4 $\pm$ 0.4	16.8 $\pm$ 6.2	2.0 $\pm$ 0.9
IL-5	10.5 $\pm$ 3.2	1.5 $\pm$ 0.5	16.4 $\pm$ 2.6	6.1 $\pm$ 1.7
IL-6	9.6 $\pm$ 9.5	2.5 $\pm$ 0.7	15.9 $\pm$ 4.7	6.6 $\pm$ 1.1
IL-7	5.3 $\pm$ 2.7	3.9 $\pm$ 1.7	10.9 $\pm$ 2.3	8.1 $\pm$ 3.2
IL-8	5.8 $\pm$ 3.8	7.2 $\pm$ 2.8	9.2 $\pm$ 2.2	16.8 $\pm$ 6.2
IL-10	5.9 $\pm$ 3.3	1.2 $\pm$ 0.3	13.2 $\pm$ 5.3	2.2 $\pm$ 0.8
IL-13	8.0 $\pm$ 3.3	0	16.2 $\pm$ 5.3	2.0 $\pm$ 0.2
GM-CSF	4.9 $\pm$ 1.7	1.0 $\pm$ 1.1	14.7 $\pm$ 5.7	3.6 $\pm$ 1.0
TNF- α	6.5 $\pm$ 0.8	6.8 $\pm$ 1.3	14.4 $\pm$ 6.8	19.8 $\pm$ 6.3
IFN- γ	0.2 $\pm$ 0.3	0.6 $\pm$ 0.6	2.0 $\pm$ 1.9	8.7 $\pm$ 6.2

Results are expressed as number of cells in area of 0.202 mm² at magnification $\times 200$.

ND, Not done.

* $p < 0.01$.

† $p < 0.05$.

MAST CELLS AND LYMPHOCYTES CONSTITUTE THE PREDOMINANT CELLULAR SOURCE OF T_H2-TYPE CYTOKINES IN THE NASAL MUCOSA IN PAR

In both PAR and CIR patients, cells expressing IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-13, granulocyte-macrophage colony-stimulating factor (GM-CSF), IFN- γ and tumor necrosis factor- α (TNF- α) were detected by immunohistochemistry (Table 1). When we compared the cytokine expression in the nasal mucosa between PAR and CIR, we found up-regulation of the expression

of IL-4 gene cluster (IL-4, IL-5, IL-6, IL-13, and GM-CSF) in PAR patients. The close proximity of the genes of these cytokines, within 110 to 180 kb on chromosome 5, suggest that the regulation of these cytokines may be under the same genomic control. By contrast, an increased number of cells expressing IFN- γ , IL-8, and TNF- α were noted in the nasal mucosa of CIR patients.

We investigated the cellular source of these cytokines by double immunohistochemistry with the use of relevant mAbs that can identify mast cells (anti-c-kit/anti-tryptase), T cells (anti-CD3 mAb), eosinophils (anti-EG2 mAb), and macro-

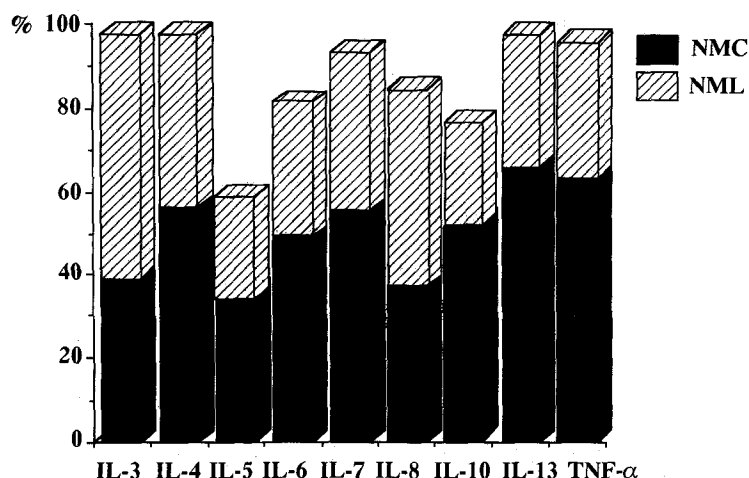


FIG. 7. Comparison of the proportion of cytokine expressing NMC and NML in total cytokine-expressing cells. Immunohistochemical analysis of cytokine expression in nasal mucosa of PAR patients was performed by double staining (alkaline phosphatase anti-alkaline phosphatase and FITC or avidin-biotin complex peroxidase and FITC) with anticytokine mAbs and relevant cell-surface antigens. Relative contribution of NMC and NML as sources of these cytokines was compared.

TABLE II. Cytokine profile in the nasal mucosa

Type of cell	PAR	CIR
Epithelial cells	IL-α, IL-1β, IL-6, IL-8, GM-CSF, TNF-α	IL-α, IL-1β, IL-8, TNF-α
Lymphocytes	IL-α, IL-1β, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-13, GM-CSF, TNF-α	IL-α, IL-1β, IL-2, IL-3, IL-5, IL-7, IL-8, IL-10, GM-CSF, IFN-γ, TNF-α
Mast cells	IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-13, TNF-α	IL-8, TNF-α*
Eosinophils	IL-5, IL-6, GM-CSF	ND
Macrophages	IL-α, IL-1β	IL-α, IL-1β

ND, Not done.

*In CIR, few mast cells express IL-4, IL-5, IL-6, and IL-7 but none expresses IL-13 or IL-10.

phages (anti-CD68 mAb). We found that a variety of inflammatory cells such as mast cells, eosinophils, T lymphocytes, and macrophages as well as resident epithelial cells contributed as sources of these cytokines (Table II). However, mast cells and T lymphocytes constituted the predominant source of T_H2 -type cytokines, excluding IL-5, which was expressed by lymphocytes, mast cells, and eosinophils (Fig. 7). Bradding et al.³⁵ reported that mast cells accounted for >90% of IL-4- and IL-6-expressing cells and 50% of IL-5-expressing cells in PAR. In contrast, Ying et al.^{36,37} reported that $CD4^+$ T lymphocytes were the predominant source of IL-4 in allergic rhinitis, accounting for >70% of IL-4-expressing cells.^{36,37} Although it is well agreed that mast cells are an important source of IL-4, IL-5, and IL-6, this discrepancy in the

relative contribution of mast cells as cytokine-expressing cells may be the consequence of the different experimental techniques used.

It is well known that glucocorticosteroids can inhibit cytokine production in lymphocytes and eosinophils, but there is yet no evidence of the capacity of steroids to inhibit cytokine production in human mast cells.³⁸ Therefore it is important to elucidate the relative contribution of mast cells as T_H2 -type cytokine-expressing cells and their precise roles in perpetuating chronic allergic inflammation. By double immunohistochemistry, we found that NML formed the predominant source of T_H2 -type cytokines in the epithelial compartment but that mast cells accounted for about 60% of T_H2 -type cytokine-expressing cells in the lamina propria compartment. However, the numerical

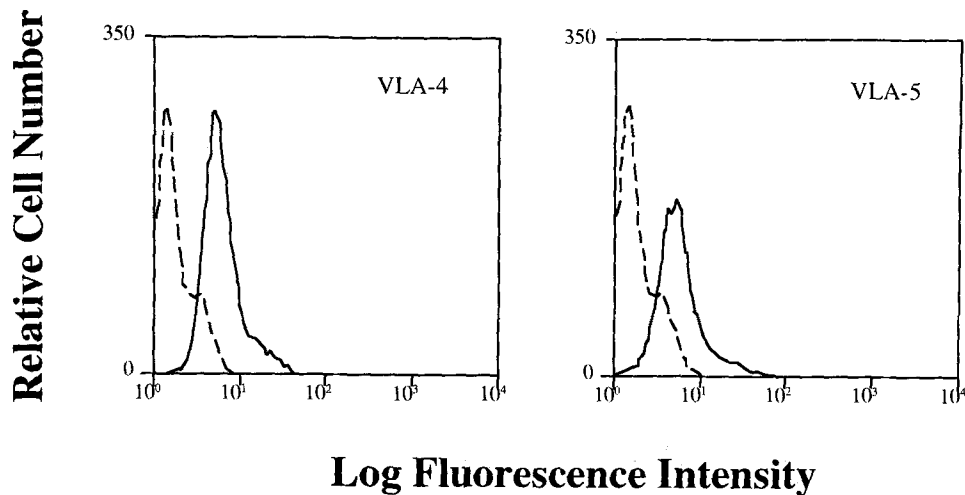


FIG. 8. Flow cytometric analysis for the expression of VLA-4 and VLA-5 in NMC from representative PAR patient. Flow cytometric analysis was performed by FACS after staining with FITC/PE-conjugated VLA-4 or VLA-5 mAbs and anti-c-kit mAb. Accurate estimation of expression of VLA-4 and VLA-5 in NMC was done by gating on c-kit⁺ (CD117) cells. *Dashed line*, Negative control; *solid line*, VLA-4⁺ or VLA-5⁺ cells.

predominance of cytokine-expressing cells cannot be equated to the amount of cytokine produced, and further studies are necessary to define the precise amounts of cytokines secreted by each cell type and the kinetics and pharmacologic modulation of cytokine production before we can conclude on the importance of each cell type.

NASAL MAST CELLS IN PAR EXPRESS VERY LATE ANTIGEN-4 AND VERY LATE ANTIGEN-5

In addition to cytokines, several types of cell-surface molecules are involved in the recruitment of inflammatory cells into specific sites of inflammation.³⁹ Lymphocytes and mast cells in the tissues are surrounded by other cells like fibroblasts and mucosal cells as well as extracellular matrix proteins (e.g., collagen, fibronectin, and laminin). Therefore the interaction of these inflammatory cells with fibroblasts and extracellular matrix may be important in the perpetuation of chronic inflammation. Recently, we demonstrated that β 1 integrins such as very late antigen-4 (VLA-4) and very late antigen-5 (VLA-5) and the vitronectin receptor integrin are involved in mast cell activation, up-regulation of cytokine expression in mast cells, and mast-cell survival.⁴⁰ We detected the expression of VLA-4 and VLA-5 in nasal mast cells (NMC) from PAR patients by staining with the relevant fluorescein isothiocyanate/PE (FITC/PE)-conjugated mAbs and the anti-c-kit mAb and then analyzing by flow cytometry (Fig. 8). Furthermore, we recently found up-regulation of the ex-

pression of VLA-4 and VLA-5 in NMC from PAR patients (manuscript in preparation).

VCAM-1 and intercellular adhesion molecule-1 are the counterreceptors or ligands for β 1 and β 2 integrins, respectively. Studies have shown increased expression of ICAM-1 and VCAM-1 in the nasal mucosa of persons with allergic rhinitis.^{41, 42} A striking feature in allergic inflammation is the selective accumulation of activated eosinophils and basophils without increased numbers of neutrophils. Lee et al.⁴¹ demonstrated that ICAM-1 expression in the nasal mucosa of persons with allergic rhinitis and was not up-regulated after stimulation with antigen; in contrast VCAM-1 was up-regulated 24 hours after allergen challenge. Because VLA-4 is not expressed by neutrophils, the selective up-regulation of VCAM-1 in the nasal mucosa may result in the accumulation of activated eosinophils and basophils without increased numbers of neutrophils. It is known that IL-4 and IL-13 can up-regulate VCAM-1 expression in endothelial cells; thus mast cells and lymphocytes may indirectly contribute to the recruitment of eosinophils and basophils through the IL-4- and IL-13-induced up-regulation of VCAM-1 expression in the nasal mucosa.

FC ϵ RI-EXPRESSING CELLS IN THE NASAL MUCOSA IN PAR ARE MAST CELLS AND BASOPHILS AND ARE INCREASED IN PAR

Cross-linking of allergen-specific IgE bound to the high-affinity IgE receptor (Fc ϵ RI) on the surface of mast cells with multivalent allergens results in the release of inflammatory mediators, which

produces clinical symptoms. The FcεRI expression in mast cells is therefore important for the development of allergic diseases. However, the precise relation between the FcεRI and atopy is unclear. It has been reported that eosinophils and monocytes also express FcεRI.^{43,45} We therefore examined the morphologic characteristics of FcεRI-expressing cells in the nasal mucosa of PAR patients by immunoelectronmicroscopy with the anti-FcεRIα-chain mAb (CRA1), which is noncompetitive with IgE.^{46,47} Morphologically, FcεRI⁺ cells in the nasal mucosa of PAR patients were either mast cells or basophils (Table III) (Fig. 9). However, basophils constituted <6% of the total FcεRI⁺ cells. Occasionally, a few FcεRI⁺ cells with monocyte-like morphologic features were detected. Neither eosinophils nor macrophages demonstrated specific staining with the non-competitive CRA1 in the nasal mucosa of PAR patients. When we compared the number of FcεRI⁺ cells in the nasal mucosa of PAR and CIR patients after staining with CRA1, which is noncompetitive with IgE^{46,47} by using the alkaline phosphatase anti-alkaline phosphatase method (APAAP kit, Dako, Palo Alto, Calif.), we observed an increased number of FcεRI⁺ cells in the nasal mucosa of PAR patients (PAR, 18.0 ± 3.6 cells/0.202 mm²; CIR, 11.0 ± 1.2 cells/0.202 mm²; $p < 0.01$). It would therefore be of interest to investigate the FcεRI⁺ cells in the nasal mucosa of patients with seasonal allergic rhinitis, in and out of season.

HETEROGENEITY OF MAST CELLS IN PAR AND CIR

Mast cells and basophils are critical effector cells in allergic diseases but are also involved in a variety of IgE-independent biologic responses.⁷⁻¹¹ Mast cells are derived from hematopoietic precursors in the bone marrow; these precursors migrate to and mature in the peripheral tissues under the influence of local microenvironmental factors.⁴⁷ Basophils, in contrast, do not normally reside in the tissues but are usually found in the circulation. Even in a normal physiologic state, mast cells can be detected in various tissues, can increase or decrease in association with various biologic responses, and may vary in their phenotypic characteristics according to the anatomic site.⁴⁸ Human mast cells are classified into at least two phenotypically distinct subpopulations on the basis of the type of neutral proteases they contain, namely mast cells that contain only tryptase and mast cells that contain tryptase, chymase, cathepsin G, and carboxypeptidase.⁴⁸ Since Enerback's reports on the heterogeneity in rat intestinal and peritoneal mast cells, classifying them into mucosal mast cells and connective-tissue mast cells⁴⁹, much has been learned

TABLE III. Ultrafine structure of FcεRI⁺ cells in the nasal mucosa

Ultrafine characteristic	Epithelium	Lamina propria
Size	$7.8 \pm 2.4 \mu\text{m}$	$13.1 \pm 2.8 \mu\text{m}$
Nucleus		
Round or oval	$82.8\% \pm 10.8\%$	$21.7\% \pm 8.8\%$
Elongated	$11.7\% \pm 3.8\%$	$72.9\% \pm 6.8\%$
Segmented	0	$4.3\% \pm 2.7\%$
Nucleus:cytoplasm ratio	0.6 ± 1.2	0.4 ± 0.3
Microfilaments	Visible	Not prominent
Microvilli	Few or moderate	Moderate
Specific granules		
Number of granules	20.8 ± 14.6	48.4 ± 26.2
Size of granules	$0.54 \pm 0.1 \mu\text{m}$	$0.5 \pm 0.06 \mu\text{m}$

about mast-cell heterogeneity. Tissue specific heterogeneity in mast cells, heterogeneity in response to various agonists, and heterogeneity in cytokine expression are well documented.⁵⁰⁻⁵³ The distribution of the two subsets of mast cells on the basis of protease expression in the nasal mucosa and their response to various agonists have been described previously.⁵⁴⁻⁵⁶

The role of mast cells in acute allergic inflammation through the release of various inflammatory mediators, including histamine, leukotrienes (LT) such as LTC₄ and LTD₄, and prostaglandins (PG) such as PGD₂, is well known. Studies suggest that mast cells may play important roles in allergic inflammation not only through the release of these inflammatory mediators but also through the release of multifunctional cytokines. Yet the precise roles of mast cells in chronic allergic inflammation is unclear. We therefore investigated the phenotypic and functional characteristics of nasal mast cells in PAR and CIR patients. NMC in both PAR and CIR patients expressed a variety of cytokines, including IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, and TNF-α, and the expression of T_{H2}-type cytokines was significantly increased in NMC from PAR patients. On the other hand, NMC from both group of patients did not express T_{H1}-type cytokines such as IL-12 or IFN-γ. Furthermore, nasal mast cells expressed CD40L (Fig. 10), the c-kit receptor, tryptase, FcεRI, β1 integrins, and intercellular adhesion molecules. Recently, we also found that nasal mast cells from PAR but not from CIR patients induced IgE synthesis in B cells (manuscript submitted). These findings strongly suggest novel and important roles for nasal mast cells in initiating and perpetuating chronic allergic inflammation.

Heterogeneity in mast cells has been well documented on the basis of protease expression and

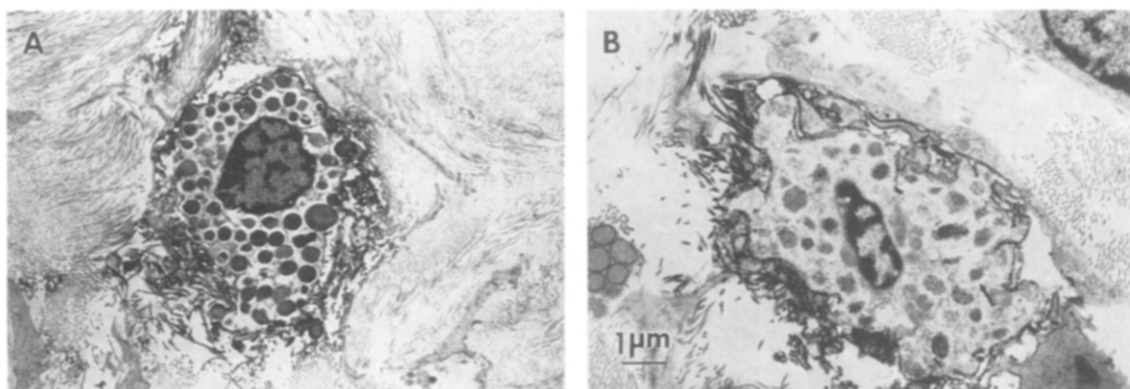


FIG. 9. Morphologic appearance of FcεRI⁺ cells in nasal mucosa of representative PAR patient. We analyzed morphologic features of FcεRI⁺ cells in nasal mucosa of PAR patients by immunoelectronmicroscopy after staining with anti-FcεRIα chain mAb, which is noncompetitive with IgE (CRA1).^{46, 47} **A**, Specific FcεRI staining on surface of unactivated mast cell. **B**, Specific FcεRI staining on surface of activated mast cell. Arrow, Partially degranulated granule.

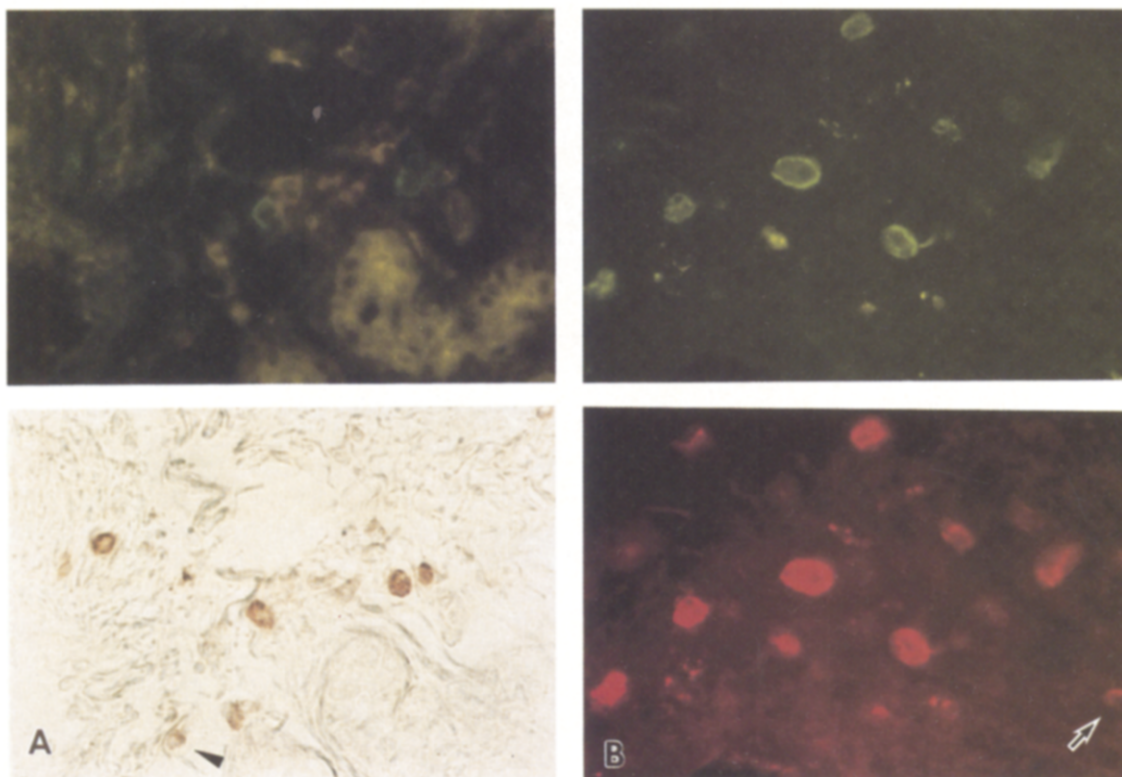


FIG. 10. Expression of IL-13 and CD40L in nasal mast cells of representative PAR patient. Immunohistochemical analysis for IL-13 and CD40L expression in nasal mast cells was assessed by double staining with anti-IL-13 and CD40L mAbs and anti-c-kit mAb (avidin-biotin complex peroxidase and FITC and FITC/PE methods, respectively) as previously described.^{13, 22} *Top* (two panels), c-kit⁺ Cells stained by FITC. **A**, Immunoreactivity for IL-13 (avidin-biotin complex peroxidase) in nasal mucosa of representative PAR patient (×400). **B**, CD40L⁺ cells (×525). Although immunoreactivity for IL-13 and CD40L was clearly localized to c-kit⁺ cells, some c-kit⁻ cells also exhibited immunoreactivity for IL-13 (arrowhead) and CD40L (arrow).

response to pharmacologic agonists.⁵⁷⁻⁵⁹ Although Kitamura⁶⁰ demonstrated tissue specific heterogeneity in mice and switching from one subtype to another, there is no such clear evidence in human

beings. Recently, Bradding et al.³⁵ reported heterogeneity in mast cells based on the cytokine expression. When we compared the expression of cytokines in nasal intraepithelial (IEMC) and lamina propria

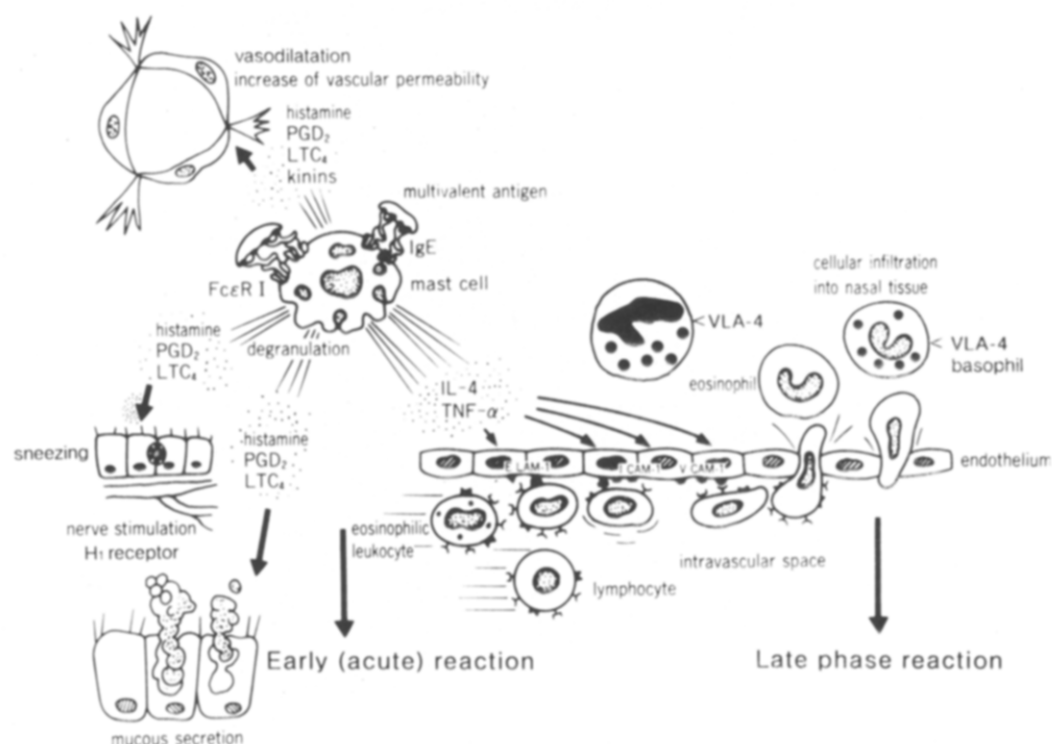


FIG. 11. Roles of NMCs in early-phase (acute) and late-phase allergic reactions.

mast cells (LPMC), we also found heterogeneity in the extent of cytokine expression between IEMC and LPMC (manuscript in preparation). We also observed differential expression of FcεRI, in that nasal mast cells from PAR patients expressed increased levels of the FcεRI as compared to nasal mast cells from CIR patients, and the FcεRI expression in mast cells was upregulated by IL-4 (manuscript submitted). This finding indicates that even within the same tissue, local microenvironmental factors may play a crucial role in modulating the immunophenotypic characteristics of mast cells.

EOSINOPHILS IN RHINITIS

Like mast cells, eosinophils are implicated in allergic diseases. However, their role in the pathogenesis of asthma and rhinitis exhibits certain differences. Eosinophil mediators such as major basic protein, eosinophil peroxidase, eosinophil-derived neurotoxin, and eosinophil cationic protein are known to be toxic to the lower- but not to the upper-airway epithelium. Moreover, in asthma there is good correlation between the degree of infiltration of activated eosinophils and the bronchial hyperresponsiveness,⁶¹ but such a relation is not so clear in the case of atopic rhinitis.^{62, 63} In the nasal mucosa of PAR patients we found that

eosinophils express GM-CSF and IL-5. We could not detect eosinophils in the nasal mucosa of CIR patients, but eosinophils may be detected in other conditions, including the nonallergic rhinitis eosinophilic syndrome and nasal polyposis. In light of our observations and those of others on the heterogeneity of mast cells and lymphocytes in various immunopathologic conditions, it would be relevant to know whether such a heterogeneity exists with regard to eosinophils and, if so, whether there is a concomitant heterogeneity in the response of eosinophils to pharmacologic agents.

ROLE OF NMC IN ALLERGIC RHINITIS

After cross-linking of allergen-specific IgE on the surface of mast cells with multivalent antigen, several morphologic changes occur in the mast cells, including swelling of the cytoplasmic granules and subsequent solubilization of its granule contents. Histamine, tryptase, PGD₂, and LTC₄ are among the mast-cell products that can be detected immediately after exposure to allergens.^{64, 65} Histamine induces vasodilation, increased vascular permeability, and increased glandular secretion in the ipsilateral and contralateral sides through neural reflexes. Sneezing results from the action of histamine on H₁ receptors present on the sensory

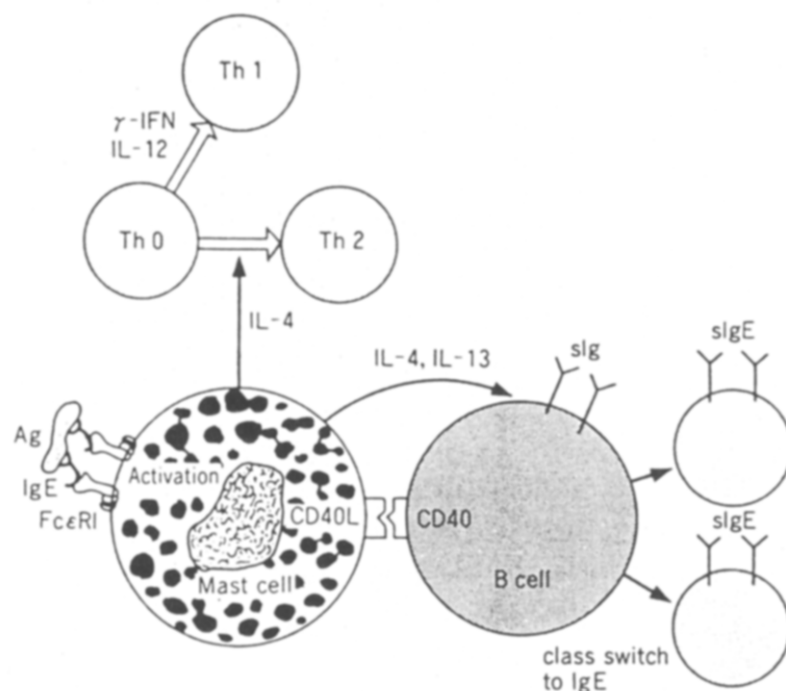


FIG. 12. Role of mast cells in IgE production. NMC may play important roles in early- and late-phase allergic reactions and in chronic allergic inflammation. Mast cells may perpetuate chronic allergic inflammation by secreting IL-4, which is necessary for differentiation and maintenance of CD4⁺ cells into T_{H2} type cells. Mast cells may also be involved in maintenance and amplification of IgE synthesis locally, in target organ. Ag, Antigen; slg, surface immunoglobulin.

nerve endings of the trigeminal nerve (Fig. 11). Of particular importance is the action of histamine on the subepithelial blood vessels, causing vasodilatation, hyperaemia, and mucosal edema. Prostaglandins such as PGD₂ also cause edema by vasodilatation and increased vascular permeability. The precise role of tryptase is not yet known. The physiologic effects of newly synthesized leukotrienes including LTC₄, LTD₄, and LTE₄ are mediated by increased vascular permeability, vasodilatation, and induction of mucous secretion.

It is well known that IEMC (mucosal mast cells) are increased in the nasal mucosa on exposure to allergens and play a crucial role in allergic inflammation through the allergen- and IgE-dependent release of these inflammatory mediators. Recent studies have shown differences in the allergen-induced histamine releasability of nasal IEMC and LPMC and heterogeneity in the response to pharmacologic agents.⁶⁶ At the same time, our studies demonstrated that LPMC are the predominant source of T_{H2}-type cytokines in PAR. Therefore, in contrast to the earlier school of thought that proposed that only surface mast cells (IEMC) play a crucial role in the pathogenesis of allergic rhinitis, we propose that both IEMC and LPMC play important but diverse roles in perpetuating allergic inflammation.

In short, IEMC may play important roles in the immediate or acute phase of allergic reaction through the release of inflammatory mediators including tryptase, histamine, PGD₂, and LTs, whereas LPMC may play important roles in the late phase of allergic reaction by up-regulating the expression of adhesion molecules including VCAM-1 and ICAM-1 (IL-4, IL-13, and TNF-α) on endothelial cells and by inducing basophil and eosinophil recruitment (IL-4 and TNF-α). LPMC may also play important roles in promoting chronic allergic inflammation by regulating the growth, differentiation, and IgE isotype switching of B cells (IL-4, IL-6, and IL-13); by maintaining the levels of VCAM-1 and ICAM-1 expression on endothelial cells (IL-4, IL-13, and TNF-α); and by promoting eosinophil development and survival (IL-3 and IL-5). In view of our findings on the presence of a substantial number of B cells in the nasal mucosa and our recent evidence that mast cells from PAR patients can induce IgE synthesis in B cells (manuscript submitted), we propose that mast cells have a novel role in promoting chronic allergic inflammation by amplifying and maintaining IgE synthesis locally in the target organ (Fig. 12).

CONCLUSION

We demonstrate that the cellular changes in the target organ more definitively reflect the underlying pathogenesis than do those in the peripheral blood compartment. Furthermore, our results suggest important roles for nasal mucosal $\gamma\delta$ T cells and mast cells in perpetuating allergic inflammation. Our observations and those of others demonstrate that mast cells have crucial roles in the early phase of the allergic reaction and in the late-phase reaction and chronic allergic inflammation. As a source of a myriad of inflammatory mediators and cytokines, mast cells may therefore be an important target for therapeutic manipulations in allergic diseases of the airways.

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