

T lymphocytes that infiltrate nasal polyps have a specialized phenotype and produce a mixed T_{H1}/T_{H2} pattern of cytokines

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Background: Nasal polyps (NPs) are inflammatory reactions in the nasal mucosa the etiology and pathogenesis of which remains unknown.

Objective: The purpose of this study was to study in detail the phenotype and function of T lymphocytes infiltrating NPs by analyzing the expression of surface markers and cytokine secretion.

Methods: NP tissue samples and peripheral blood were obtained from 18 patients. Mononuclear cells were purified from these samples, and their phenotype was investigated by triple-color immunofluorescence and flow cytometric analysis. Cytokine production was determined in cultures by using an ELISA technique.

Results: NP lymphoid cells mainly consisted of T lymphocytes. These T lymphocytes showed a CD45RO+CD45RA- phenotype and expressed pan-T cell molecules; the CD8+ subset was predominant. NP T cells showed a lower density of CD28, CD3, and TCR- $\alpha\beta$ compared with T cells from peripheral blood. NP T lymphocytes expressed the activation markers DR and CD69 and exhibited the adhesion molecule profile CD54+, CD62L-, and CD103+ CD49d^{low}. Virtually all NP T cells bore CD95 (FAS), but they did not undergo apoptosis, either spontaneously or induced by CD95 cross-linking with the mAb CH11. The pattern of cytokines secreted by NP T lymphocytes was characterized by the spontaneous and simultaneous production of IFN- γ and IL-5. Neither IL-2 nor IL-4 were detectable in nonstimulated cultures.

Conclusion: This study defines the T lymphocytes that infiltrate NPs as memory T cells in an activated status, with homing properties related to the mucosal immune system. They are resistant to anti-CD95-mediated apoptosis and produced a

mixed T_{H1}/T_{H2} cytokine pattern as defined by the simultaneous production of IFN- γ and IL-5. (*J Allergy Clin Immunol* 1998;102:953-60.)

Key words: Nasal Polyps, lymphocytes, T lymphocytes, human, phenotype, cytokines, IFN- γ , IL-5, inflammation

Nasal polyps (NPs) are edematous projections that originate from the nasal ethmoid mucosa in the vicinity of the middle turbinate. Inflammation is a prominent feature of NPs as reflected by the presence of a variety of infiltrating cells, such as eosinophils, lymphocytes, and plasma cells.^{1,2} Although NPs were recognized centuries ago, their etiology and pathogenesis remains unclear. The recurrent nature of the disease is a constant characteristic, and immune mechanisms have been implicated as contributing factors in NP formation.³⁻⁵ Lymphocytes represent a major component of the NP cellular infiltrate, and therefore they have a potential involvement in the development of the chronic inflammation occurring in this disorder. Immunohistochemical studies of NP-inflammatory populations have been previously conducted,⁶⁻⁹ although a more complete analysis of the infiltrating lymphocytes has not as yet been accomplished. In this study NP lymphocytes were purified and were phenotypically and functionally characterized. The results show that infiltrating T cells, which were the predominant lymphocytic type in NPs, exhibit a specialized phenotype and produce a mixed T_{H1}/T_{H2} pattern of cytokines.

METHODS

Patients

NP samples were obtained during routine endonasal surgery from 18 subjects (4 women and 14 men; mean age, 51.1 years [range, 20 to 73 years]). The patients were seen with a history of more than 2 years of chronic hyperplastic sinusitis with 1 or more of the following symptoms: chronic nasal congestion, chronic rhinorrhea, anosmia, or discomfort in the regions of the sinuses. All patients had undergone a sinus computed tomographic scan performed during their evaluation. Two patients had allergic rhinitis as documented by clinical history and a positive skin prick test response, and 1 patient had intolerance to aspirin. No patient had

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Abbreviations used

NP:	Nasal polyp
PB:	Peripheral blood
PE:	Phycoerythrin
PerCP:	Peridinin chlorophyll protein
7 AAD	7-amino-actinomycin-D

bronchial asthma or mucociliary disorders. Surgery was only used when medical management had failed. All patients had suspended the use of corticosteroids at least 2 months before the surgery. As a control, peripheral blood (PB) from the same patients was obtained at the time of surgery. All patients were informed about the objective of the study and gave their consent.

Reagents and antibodies

RPMI 1640 medium, L-glutamine, FCS, and HBSS were obtained from Gibco BRL (Bethesda, Md). Lymphocyte isolation medium was purchased from ICN (Costa Mesa, Calif). Mouse mAbs conjugated to FITC (anti-CD2, CD3, CD4, CD7, CD8, CD11a, CD19, CD29, CD44, CD45, CD45RA, CD62L, CD71, TCR- $\alpha\beta$, and DR), to phycoerythrin (PE) (anti-CD2, CD3, CD4, CD5, CD8, CD11b, CD11c, CD19, CD25, CD28, CD45RO, CD49d, CD54, CD56, CD58, CD69, and TCR- $\gamma\delta$), and to peridinin chlorophyll protein (PerCP) (anti-CD45, CD3, and DR); FITC-, PE-, and PerCP-conjugated mAbs of appropriate isotype used as negative controls; and FITC-conjugated goat anti-mouse IgG used in indirect immunofluorescence were provided by Becton Dickinson (San Jose, Calif). Type V collagenase, cycloheximide, and 7-amino-actinomycin-D (7 AAD) were purchased from Sigma (St. Louis, Mo). Unconjugated anti-CD103 mAb (HML-1), anti-CD95 mAb (CH11 and FITC-UB2), and anti-CD28 mAb (CD28.2) were obtained from Immunotech (Luminy, France). Immunoassays for the quantitative detection of human cytokines were obtained from Immunotech (Luminy, France) for IL-2 and IL-4 and from R&D Systems (Minneapolis, Minn) for IFN- γ and IL-5. OKT 3 anti-CD3 mAb was purchased from Ortho (Raritan, NJ). The 96-well and 24-well flat-bottomed plates used in cellular cultures were from Nunc (Roskilde, Denmark).

NP and PB cell preparation

NP samples were washed in HBSS, placed in a petri dish containing 5 mL of RPMI 1640 medium, minced into 5-mm pieces, and incubated with collagenase at a concentration of 1 mg/mL at 37°C for 1 hour. The supernatant and the digested tissue were passed through a stainless steel sieve to obtain a cellular suspension. Cells were washed twice in HBSS, and mononuclear cells were obtained by centrifugation on a Ficoll-Hypaque density gradient. With this purification protocol, more than 95% of the recovered cells were mononuclear leukocytes. The average yield was $6.5 \pm 1 \times 10^6$ mononuclear cells (range, 1 to 18). Eosinophils were not present in the cell preparations as determined either by May-Grünwald-Giemsa staining or by a technique that included immunofluorescence and flow cytometry in which the eosinophils were identified as CD45+CD16-CD49d+ cells.¹⁰ Viability was greater than 95% as determined by the trypan blue exclusion test. PB mononuclear cells were obtained from fresh heparinized venous blood by centrifugation on Ficoll-Hypaque. Viability was greater than 97%.

Immunofluorescence staining

Two- or three-color labeling experiments were performed as previously reported.¹¹ Briefly, 0.5×10^6 NP or PB mononuclear cells in 100 μ L of HBSS were incubated with saturating quantities of the appropriate FITC-, PE-, and PerCP-conjugated mAbs for 30 minutes in the dark at 4°C. After staining, cells were washed twice. CD103 antigen was detected by indirect immunofluorescence. In brief, NP and PB lymphocytes were first treated with unconjugated HML-1 mAb for 30 minutes at 4°C in the dark. After 2 washes, cells were incubated with FITC-conjugated goat anti-mouse IgG for 30 minutes in the same conditions and were washed twice. Isotype-matched FITC-, PE-, and PerCP-conjugated irrelevant mAbs were used as negative controls. Labeled cells were analyzed within 2 hours.

Flow cytometry

Cells were analyzed in a FACScalibur instrument (Becton Dickinson) equipped with an air-cooled argon ion laser, which operated at 488 nm and 15 mW of power. The instrument was equipped with 3 fluorescence detector photomultiplier tubes, with green fluorescence (FITC; FL1) being collected through a 530/30 nm bandpass, orange/red (PE; FL2) through a 585/42-nm bandpass, and red (PerCP; FL3) through a 650-nm longpass filter. Light scatter signals were recorded in linear mode, and the fluorescence signals were recorded in logarithmic mode. A minimum of 5000 cells were collected and analyzed by using the CELLQUEST software (Becton Dickinson). NP and PB lymphocytes were selected in the forward versus side scatter dot plot and additionally gated as CD45- or CD3-positive cells in the corresponding FL3 histogram. Expression of other surface molecules was explored by using the FL1 and FL2 fluorescent channels. Background fluorescence was set so that less than 1% of the cells were positively stained with the corresponding negative control. The percentage and the mean fluorescence intensity were recorded for each examined molecule.

Cell culture

NP and PB lymphocytes were cultured at a concentration of 1×10^6 cells/mL in 96-well flat-bottomed plates in a volume of 250 μ L per well for cytokine production, and in 24-well flat-bottomed plates in 1 mL of volume per well for apoptosis detection. The cultures were incubated at 37°C with 5% CO₂ for 48 hours in a culture medium consisting of RPMI 1640 supplemented with 10% FCS, L-glutamine (2 mmol/L), and gentamycin (0.05 mg/mL). Cytokine production was evaluated in nonstimulated (spontaneous production) and stimulated cultures with anti-CD3 mAb (1 μ g/mL) plus anti-CD28 mAb (1 μ g/mL). After 48 hours, culture supernatants were collected and stored at -20°C until testing for IL-2, IL-4, IL-5, and IFN- γ production by using an ELISA technique. To determine apoptosis, NP cells were cultured both in the absence and in the presence of CH11 anti-CD95 mAb (200 ng/mL), which has been demonstrated to induce apoptosis in susceptible targets.¹² At the end of the culture period, cells were harvested, washed twice, and analyzed for apoptosis estimation.

Apoptosis measurement

Apoptotic T lymphocytes were detected as previously reported.¹³ Briefly, 0.5×10^6 lymphocytes were incubated with FITC-labeled anti-CD3 for 30 minutes at 4°C and washed twice. The cells were then incubated with 7 AAD (20 μ g/mL) for 30 minutes in the dark. Cells were washed twice and analyzed by flow cytometry. 7 AAD emits a red fluorescence, this being detected through 650 nm longpass filter (FL3). Apoptotic T cells were detected as CD3+ 7 AAD+ lymphocytes. Data were given as percentages of total CD3+ lymphocytes.

Statistical analysis

Values were given as mean $\pm$ SEM. Data were analyzed with the Mann-Whitney *U* Test. For qualitative data, the Fisher exact test was used. Probability (*P*) values less than .05 were considered significant.

RESULTS

Phenotypic characterization of NP T-cells

T-, B-, and NK-cell lymphoid populations present in NP samples and PB from the same patients were identified according to their simultaneous expression of CD45 and CD3 (T cells), CD19 (B cells), and CD56+ CD3- (NK cells) by using either a double- or a triple-color immunofluorescence staining and flow cytometric analysis. As can be seen in Fig 1, NP lymphoid cells mainly consisted of T lymphocytes. The T-cell percentage was higher in NPs than in PB, whereas B-cell proportions did not differ, and NK-cell figures were lower in NP samples. Because T cells represented the majority of the lymphocytes infiltrating the NPs, their phenotype was investigated to characterize this population further. To this end, the simultaneous expression of CD3 along with other molecules of interest was assessed by triple-color immunofluorescence and flow cytometry. The molecules studied were distributed in four different groups.

The first group consisted of T-cell specific markers. Table I shows that the NP T lymphocytes (defined as CD3+) expressed pan-T cell molecules, such as CD2, CD5, and CD7, and most of them expressed the $\alpha\beta$ type of TCR. These characteristics were also shared by the patients' PB T lymphocytes (Table I). In contrast, the proportion of NP CD8 T cells was higher than that found in PB, and that of NP CD4 T cells was lower than that found in PB (Table I, Fig 2). Moreover, the NP contained less T lymphocytes bearing CD28 than the PB of the same patients (Table I, Fig 2), and in addition, the intensity of expression (assessed as mean fluorescence intensity) of CD28, as well as of CD3 and $\alpha\beta$ TCR, was markedly lower in NPs than in PB T lymphocytes (Table II, Fig 2).

The second group consisted of activation markers. Among the T-cell activation markers studied (CD25, CD122, CD71, DR, and CD69), only DR and CD69 expression was found to be increased in the NP T-cell population (Table I and Fig 2). PB T cells did not express these activation markers, and a proportion of them exhibited low levels of CD25, a molecule that was absent from NP T cells (Table I, Fig 2).

The third group consisted of adhesion molecules. A variety of adhesion molecules were studied, including Ig superfamily members (CD54 and CD58); selectins (CD62L); β_1 (CD29 and CD49d), β_2 (CD11a, CD11b, and CD11c), and β_7 (CD103) integrins; and CD44. Table I shows that CD49d was extensively expressed by T lymphocytes from both the NPs and PB (Table I), although the expression level was lower in NP than in PB T cells (Table II, Fig 2). The NP and PB T lymphocytes were CD11a+, CD11b-, and CD11c-, and no significant difference was noticed between PB and NP T cells in the

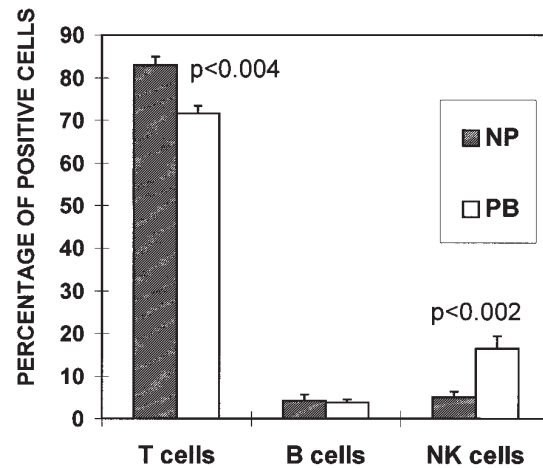


FIG 1. Classification of the lymphoid populations infiltrating NPs. Percentage of lymphocyte subtypes present in NP infiltrates and in patients' PB was evaluated by staining with appropriate fluorochrome-conjugated mAbs and flow cytometric analysis. T lymphocytes, B lymphocytes, and NK cells were identified as CD45+CD3+, CD45+CD19+, and CD45+CD3-CD56+ cells, respectively. Results represent mean $\pm$ SEM of 8 experiments.

expression of CD44 and CD58 (Table I). However, CD54

TABLE I. Phenotypic studies on NP and PB T lymphocytes

	NPT cells	PBT cells	P value*
T-cell molecules			
CD2	95 $\pm$ 2†	95 $\pm$ 3	NS
CD7	79 $\pm$ 3	89 $\pm$ 3	NS
CD5	87 $\pm$ 9	98 $\pm$ 1	NS
CD4	47 $\pm$ 3	57 $\pm$ 4	<.05
CD8	51 $\pm$ 4	35 $\pm$ 3	<.01
TCR $\alpha\beta$	87 $\pm$ 4	91 $\pm$ 2	NS
TCR $\gamma\delta$	3 $\pm$ 1	6 $\pm$ 3	NS
CD28	57 $\pm$ 9	76 $\pm$ 4	NS
Activation molecules			
CD25	5 $\pm$ 4	22 $\pm$ 14	<.03
CD71	1 $\pm$ 1	1 $\pm$ 1	NS
CD122	3 $\pm$ 1	3 $\pm$ 2	NS
DR	26 $\pm$ 8	10 $\pm$ 3	<.01
CD69	77 $\pm$ 4	5 $\pm$ 2	<.0002
Adhesion molecules			
CD11a	95 $\pm$ 2	99 $\pm$ 1	NS
CD11b	8 $\pm$ 2	10 $\pm$ 3	NS
CD11c	11 $\pm$ 3	11 $\pm$ 7	NS
CD29	89 $\pm$ 4	95 $\pm$ 2	NS
CD49d	79 $\pm$ 4	84 $\pm$ 8	NS
CD44	99 $\pm$ 1	92 $\pm$ 7	NS
CD54	61 $\pm$ 10	12 $\pm$ 4	<.001
CD58	65 $\pm$ 15	90 $\pm$ 2	NS
CD62L	6 $\pm$ 1	55 $\pm$ 10	<.008
CD103	66 $\pm$ 8	5 $\pm$ 1	<.004
Other molecules			
CD45RA	12 $\pm$ 5	58 $\pm$ 7	<.0004
CD45RO	84 $\pm$ 4	50 $\pm$ 6	<.0005
CD95	86 $\pm$ 4	52 $\pm$ 3	<.0001

NS, Not significant.

**P* values were obtained by the Mann-Whitney *U* test.

†Percentage of positive cells detected within the CD3+ subset by flow cytometric analysis. Results represent mean $\pm$ SEM of 8 experiments.

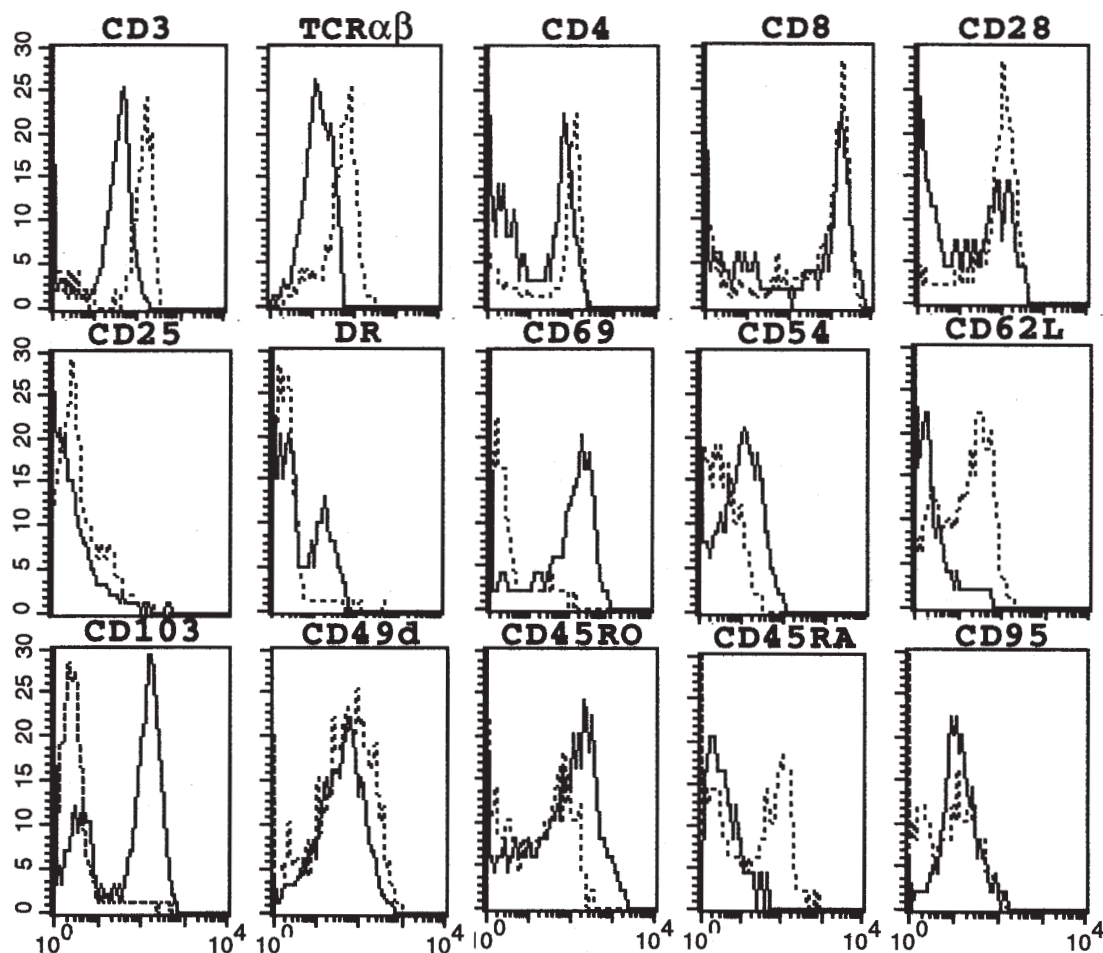


FIG 2. Phenotypic study on T lymphocytes from NPs and PB. Shown is a representative example. Two or three-color labeling experiments were performed as detailed above. A representative histogram plot of the molecules that showed differential expression is shown. Continuous lines represent NP T cells, and dotted lines represent PB T cells. Percentage of cells expressing CD8, CD28, DR, CD69, CD54, CD103, CD45RO, and CD95 was higher in NP than in PB T lymphocytes. The level of expression of CD3, TCR, CD28, and CD49d was lower on NP than on PB T lymphocytes, whereas CD54 was expressed with higher intensity on NP T cells.

TABLE II. Differences in antigen density expression by PB and NP T cells

	NP cells	PB cells	P value*
CD3	111 ± 17†	222 ± 33	<.0002
TCRαβ	22 ± 4	47 ± 5	<.0003
CD28	87 ± 17	164 ± 15	<.004
CD54	41 ± 14	17 ± 3	<.03
CD49d	61 ± 10	147 ± 18	<.02

*P values were obtained by the Mann-Whitney U test.

†Mean fluorescence intensity was calculated for every molecule within the CD3+ subset by flow cytometry analysis. Results are expressed in arbitrary units of fluorescence intensity and represent mean ± SEM of 8 experiments.

was expressed by an average of 61% of NP T cells but only by 12% of PB T cells (Table I), and the intensity of expression was also higher in NP T cells (Table II, Fig 2). CD62L expression was absent on NP T cells, whereas a

high proportion of PB T cells were CD62L+. In marked contrast, CD103 ($\alpha_E\beta_7$) was exclusively found on T lymphocytes obtained from the NP samples (Table I, Fig 2). To rule out a possible effect of collagenase on these results, experiments were conducted in which the expression of CD3, CD28, and TCR- $\alpha\beta$ was analyzed in PB cells either untreated or treated with the same purification protocol. No differences were found in either the intensity of expression or in the percentage of positive cells (data not shown).

The final group consisted of other molecules. The human circulating T cells are heterogeneous for CD45RA and CD45RO isoform expression.¹⁴ However, virtually all NP T cells showed a CD45RO⁺ CD45RA⁻ phenotype (Table I, Fig 2). CD95 (FAS) is a surface molecule that can transmit apoptotic signals in many cell systems.¹⁵ CD95 was expressed by half of the PB T-cell fraction, whereas the majority of NP T cells were CD95⁺

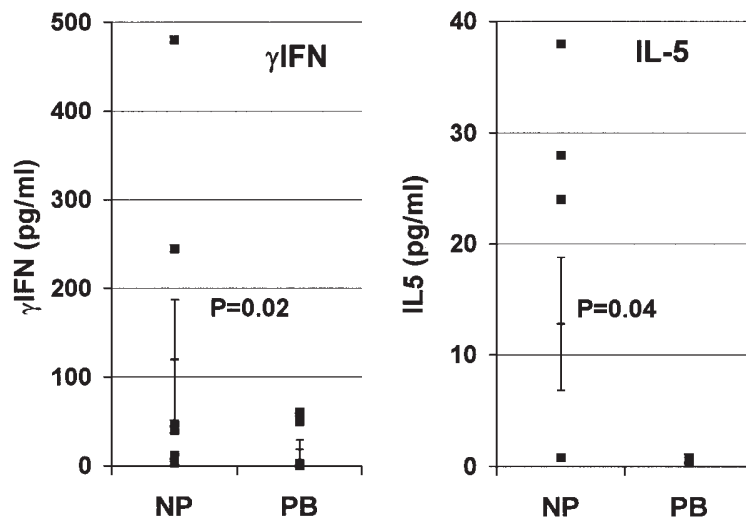


FIG 3. Spontaneous IFN- γ and IL-5 production by NP and PB lymphocytes. Lymphocytes isolated from PB and NPs (1×10^6 cells/mL) were cultured for 48 hours. At the end of the culture period, supernatants were collected, and IFN- γ and IL-5 production was determined by an ELISA technique. The sensitivity of these ELISAs was 3 pg/mL. Experimental points are shown. Horizontal and vertical lines represent mean $\pm$ SEM of 7 (IFN- γ) and 6 (IL-5) different experiments. Statistical difference was established by Fisher exact test.

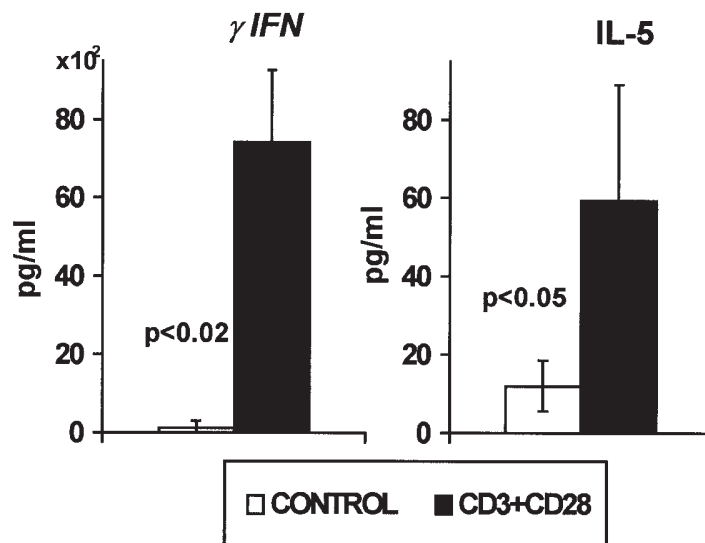


FIG 4. IFN- γ and IL-5 production by NP T lymphocytes stimulated with anti-CD3 plus anti-CD28. Lymphocytes isolated from NPs were cultured for 48 hours in absence (control) and presence of mAbs anti-CD3 (1 μ g/mL) and anti-CD28 (1 μ g/mL). At the end of the culture period, IL-5 and IFN- γ production was determined in the supernatants by ELISA. Results represent mean $\pm$ SEM of 5 experiments.

(Table I, Fig 2). The high expression of CD95 by NP T cells might indicate increased commitment of these cells to apoptosis. To test this hypothesis, apoptosis was evaluated in NP T lymphocytes freshly isolated and after a culture period of 48 hours in the presence and absence of the anti-CD95 mAb CH11. The basal percentage of

apoptotic NP T lymphocytes (determined as CD3+ 7 AAD+ cells) was low, and this figure did not increase after 48 hours in either nontreated or anti-CD95-treated cultures ($9\% \pm 3\%$, $13\% \pm 3\%$, and $14\% \pm 4\%$, respectively; mean $\pm$ SEM of 5 experiments). As a positive control of anti-CD95 induced apoptosis, Jurkat cells were

cultured (1×10^6 cells/mL) for 20 hours in the absence and presence of the anti-CD95 mAb CH11 (200 ng/mL). The percentage of apoptotic cells as determined by the same method was 15% and 82% for control and CH11-treated cells, respectively.

Cytokine production by NP T lymphocytes

The pattern of cytokines produced by NP T lymphocytes was also examined. To delineate between T_{H1} and T_{H2} subsets of T lymphocytes, the cytokines IL-2, IL-4, IL-5, and IFN- γ were selected for this study. First, the *in vivo* function of NP-infiltrating lymphocytes was investigated by determining the cytokine production in cultures performed under basal, nonstimulated conditions. As a control, similar experiments were performed with PB lymphocytes from the same patients. No spontaneous IL-2 or IL-4 production was detected in nonstimulated cultures of either population (data not shown). However, the NP T cells spontaneously produced appreciable quantities of IFN- γ and IL-5, whereas the PB lymphocytes showed minimal production of IFN- γ and no production of IL-5 (Fig 3). The spontaneous production of IL-5 and IFN- γ by NP T cells was actively carried out because the addition of cycloheximide (an inhibitor of protein synthesis) at 10 μ g/mL completely prevented the cytokine secretion in these cultures (data not shown). In addition, the NP-cell cytokine production was investigated in a T cell–specific stimulation system consisting of a combination of mAbs against CD3 and the costimulatory molecule CD28. As shown in Fig 4, this T-cell activation system provoked a 5- and 60-fold increase for IL-5 and IFN- γ secretion, respectively.

DISCUSSION

The majority of NP-infiltrating lymphocytes are T cells. This study analyzes in detail the phenotype and function of this population. In accordance with previous immunohistochemical studies, present results reveal that CD8 $^{+}$ T cells were more abundant than CD4 $^{+}$ T cells in NP infiltrates.^{9,16} In addition, most NP T cells expressed CD45RO, indicating that they are memory T lymphocytes.¹⁴ These primed T cells bore the $\alpha\beta$ type of TCR, and expressed a variety of pan-T cell markers (CD3, CD2, CD5, and CD7) also exhibited by the PB T cells. However, the expression of TCR, CD3, and CD28, 3 molecules necessary for T-cell activation by antigen, was markedly lower on NP T cells than on PB T cells. This characteristic might be related to the memory status of NP T cells. This is based on the finding that, whereas naive T cells, such as CD45RA $^{+}$ CD45RO $^{-}$ PB T cells, are known to express high levels of TCR/CD3 complex and to require strong costimulation through CD28 for activation, memory T cells require less antigen-recognition signals and are less dependent on costimulation for activation.^{17,18} These features may ensure an efficient response of selected cells to a specific antigen irrespective of the nature of the presenting cell. In agreement with present results, subpopulations of human PB T lymphocytes showing characteristics of memory cells presented reduced surface expression of CD28.¹⁹

NP T-cell size was similar to that of PB T-cell small resting lymphocytes, as indicated by the study of the forward scatter parameter by flow cytometry. In addition, cell cycle analysis demonstrated that only a marginal number of NP T cells were in S or G2/M phases (data not shown). These data are consistent with the characteristic small size and G0 state commonly reported for memory T lymphocytes and allowed a clear distinction of NP T cells from the activated effector T-cell stage.¹⁴ However, NP T cells were in a special activated status as demonstrated by the expression of the T-cell activation molecules CD69, DR, and CD54. A similar state of activation has also been demonstrated on memory T cells that accumulate at sites of chronic inflammation such as in rheumatoid arthritis,²⁰ chronic inflammatory liver disease,²¹ pulmonary sarcoidosis,²² Hashimoto's Thyroiditis and Graves' disease,²³ and inflammatory bowel diseases.²⁴ The activation level showed by these cells cannot be simply explained by their memory cell status because CD45RO $^{+}$ T lymphocytes isolated from PB do not usually express activation markers.²⁵ Consistent with this, PB memory (CD45RO $^{+}$) T cells from the same patients with NPs lacked those activation markers. This activation state does not seem to contribute to an important extent to the migratory capacity of memory T cells. In this respect it has been shown that CD69 expression does not distinguish migrating from nonmigrating memory T lymphocytes,²⁵ thus suggesting that the expression of activation molecules on NP T cells was locally triggered.

Memory T cells appear to recirculate to maximize potential encounters with pathogens, which preferentially infect particular sites. The migration of memory T lymphocytes through high endothelium venules to peripheral lymph nodes is greatly diminished, which seems to be related to the loss of CD62L (a high endothelium venule–specific homing receptor) by these cells.²⁶ Consistent with their memory-cell status, NP T cells lacked CD62L expression. In contrast, the memory T-cell capacity of migrating into particular tissues is mediated by other tissue-specific homing molecules.^{27,28} When we looked for homing molecules related to a specific location in the nasal-associated lymphoid tissue, we thought of CD103, the $\alpha_E\beta_7$ integrin expressed by intraepithelial lymphocytes and lamina propria T lymphocytes from gut-associated lymphoid tissue.²⁹ The high expression of CD103 showed by NP T cells suggested that this molecule could account for the specific retention of NP T cells in mucosal areas in the nasal-associated lymphoid tissue. Moreover, the expression of CD103 by mucosal T cells other than T lymphocytes from gut-associated lymphoid tissue might implicate this integrin as a common mechanism for homing to the mucosal immune system. Although CD49d is strongly expressed by isolated PB memory T cells¹⁹ and by memory T cells obtained from inflamed thyroid,²³ NP T cells showed low expression of CD49d. A similar profile of integrins (CD49d ^{low} , CD103 $^{+}$) is also expressed by normal lamina propria T lymphocytes.³⁰

CD95 (Fas) belongs to the TNF/nerve growth factor receptor family,¹⁵ and their ligation, either by the natural contrareceptor Fas-ligand or by antibodies, transduces signals of apoptosis to many cell types, including T lymphocytes.¹² However, the mere expression of CD95 is not sufficient to assure this phenomenon.³¹ Because most NP T cells expressed CD95, their susceptibility to apoptosis was assessed. Present data reveal a lack of susceptibility of NP T cells to spontaneous apoptosis in culture. This effect could mean that FasL was not present in such cultures, even though the Fas-FasL pathway was active in NP T cells. Therefore NP T-cell apoptosis induced by CD95 cross-linking with the mAb CH11 was analyzed next. Again, no cell death was observed. These results suggest that apoptosis through CD95 was inhibited in NP T cells. In contrast, CD45RO+ T lymphocytes isolated from healthy gut lamina propria, which share many phenotypic and functional similarities with NP T cells, also express CD95, and on CD95 ligation, they develop apoptosis.³² Taken together, these data may suggest that the lack of susceptibility of NP T cells to apoptosis might contribute towards maintaining the size and duration of the inflammatory response in NP.

Finally, the capacity of NP T cells to secrete several cytokines (IL-2, IL-4, IL-5, and IFN- γ) was evaluated in nonstimulated cultures in an attempt to approximate the in vivo function of the infiltrating lymphocytes. NP cells actively produced low, but detectable, quantities of IFN- γ into the supernatants of nonstimulated cultures, whereas IL-4 remained undetectable. These findings are in agreement with previous observations with mitogen-stimulated NP T cells³³ and NP tissue homogenates.^{34,35} Nevertheless, the possibility that NP T cells could produce very low quantities of IL-4 cannot be totally excluded. A large number of eosinophils is present in the NP cellular infiltrate.³⁶ IL-5 is a relevant chemotactic and survival cytokine for eosinophils.³⁷ However, the source or sources of IL-5 in NPs has not been completely clarified. This study demonstrates that, unlike PB T lymphocytes, NP cells actively produced low but detectable levels of IL-5 under nonstimulated conditions. Recently, IL-5 synthesis by NP T cells and eosinophils has also been demonstrated by an immunohistochemical technique.^{37,38} In the present system, T lymphocytes appeared to be responsible for the spontaneous IL-5 secretion because, in the NP-cell preparation, no eosinophils could be detected either by flow cytometric analysis or May-Grünwald-Giemsa staining. Furthermore, IL-5 and IFN- γ secretion was greatly increased in these cell cultures when a specific T-cell stimulus, the combination of mAbs against CD3 and CD28, was used. In addition, a similar pattern of cytokine production has been observed in cultures of T cells that infiltrate the intestinal mucosa of patients with ulcerative colitis,²⁴ a chronic mucosal intestinal inflammation in which eosinophils are also prominent. Thus the IL-5 production by NP T cells could contribute to the accumulation and survival of eosinophils in NP.

In summary, this study defines the T lymphocytes that infiltrate NP as memory T cells in an activated status, with

homing properties related to the mucosal immune system. They are resistant to anti-CD95-mediated apoptosis and secrete a mixed T_{H1}/T_{H2} cytokine pattern, as defined by the simultaneous production of IFN- γ and IL-5.

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