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Immunocompetent cells in human nasal polyps and normal mucosa*

Arne Linder[1], Alex Karlsson-Parra[2], Carina Hirvela,[1], Lars Jonsson[1] Anders Koling[1], Olof Sjoberg[2]

1 Department ofOtorhinolaryngology and Head & Neck Surgery, Uppsala University, Uppsala, Sweden
2 Department oflmmunology, UppsalaUniversity, Uppsala, Sweden

SUMMARY

The distribution ofT- and B-lymphocytes, HLA-DR- expressing cells, and macrophages was determined using monoclonal antibodies in frozen biopsy sections of nasal polyps from 12 patients, and of nasal mucosa from five disease-free controls. The relative proportion and spatial distribution of different lymphoid cells was similar with regards to both nasal polyps and normal mucosa. Numerous scattered T lymphocytes (Leu4-positive) and HLA-DR-expressing macrophage/dendritic-like cells were shown and tended to accumulate in the subepithelial areas. Aggregates of T lymphocytes and HLA-DR-positive cells were also found close to deeper glands. In the submucosal clusters, the Leu3a-positive ("helper/inducer") cells were more common than the Leu2a-positive ("suppressor/cytotoxic") cells. Furthermore, a number of epithelial, non-lymphoid cells were found to express the HLA-DR antigen, which suggests an active role for the epithelium in the immunological response of the normal mucosa as well as that of the nasal polyp.

Key words: epithelial cells, HLA-DR, nasal polyps, lymphocytes, T-cell subsets

INTRODUCTION

Nasal polyposis is a common condition, affecting about 4% of the population (Settipane and Chafee, 1977). The polyps obstruct nasal breathing and impair the sense of smell as well as the clearance and ventilation of the paranasal sinuses. Nasal polyposis may be associated with local disease, such as chronic sinusitis, but also with systemic disorders including asthma, intolerance to non-steroid anti-inflammatory drugs **(NSAID)** and immotile cilia syndrome (Ogino et al., 1986).

The sequence of events leading up to polyp formation is still controversial (Sasaki and Nomura, 1984). The nasal polyp is a mucosa! projection showing oedema and signs of inflammation (Drake-Lee et al., 1984; Frenkiel et al., 1985). Inflammatory responses may take different forms to combat various threats to the tissue. The spatial distribution of immunocompetent cells may give clues to the localization and nature of factors triggering the response. In the present study, monoclonal antibodies were used to compare the distribution of immunocompetent cells in nasal polyp tissue and normal mucosa in order to determine whether the nasal polyps show signs of an immunological response.

MATERIAL AND METHODS

Patients and controls

Nasal polyps were obtained from 12 patients undergoing polypectomy under local anaesthesia. Demographic data are given in Table 1. Internal comparisons were made in the polyposis group in order to find out whether the histological appearance differed between those treated with topical corticosteroids during the month preceding surgery and those which were not; or between first-time polypectomees and those with a recurrence. A control group was included, consisting of five patients undergoing surgery, where disease-free nasal mucosa had to be removed to obtain surgical access. The control specimens were taken from the middle turbinate and middle meatus. The study was approved by the Local Ethics Committee, Uppsala University.

Tissue processing

Immediately after removal, the polyp material was placed in Histicon medium (Histolab, Bethlehem Trading Ltd, Gothenburg, Sweden) at 4°C until snap-frozen in isopentane and then stored at -70 °C. Sections of 6 µm were cut in

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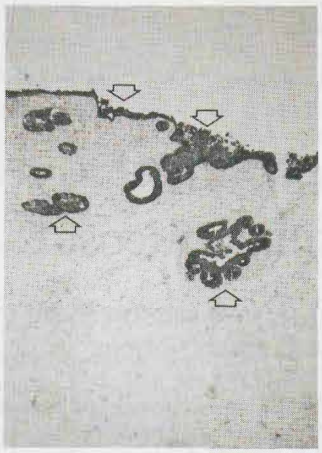
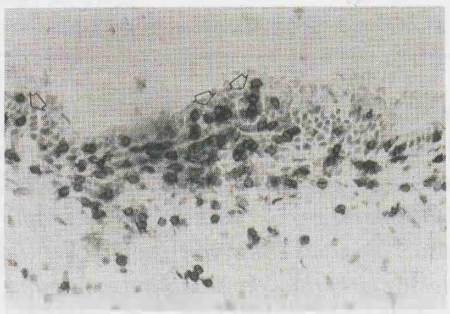
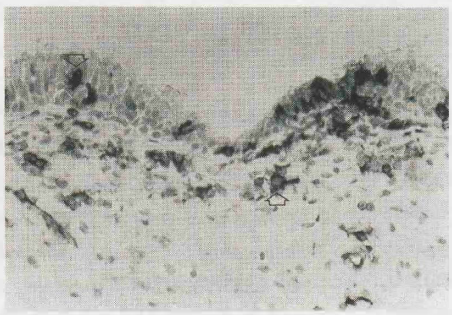
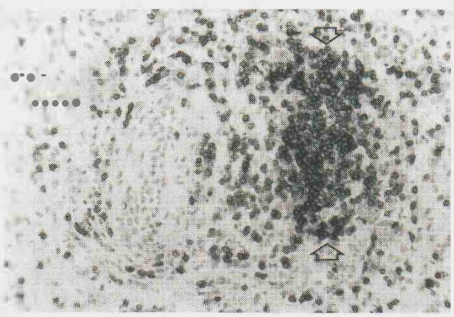


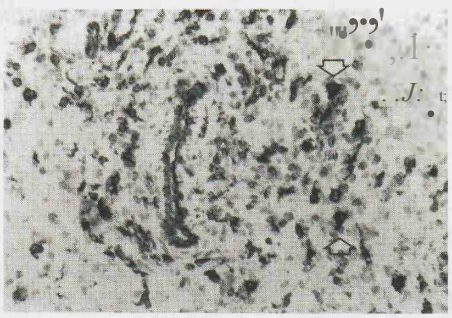
Figure 1.
Immunoperoxidase staining pattern in human nasal polyp tissue incubated with monoclonal antibody anti-cytokeratin 18. The section is counterstained with Mayer's hematoxylin. Surface and glandular epithelium cells are indicated by arrows.





Figures 2a and 2b. Immunoperoxidase staining pattern in human nasal polyp tissue incubated with monoclonal antibodies anti-Leu4 (2a) and anti-HLA-DR (2b). The sections are counterstained with Mayer's hematoxylin. Arrows indicate T lymphocytes in the epithelial layer (2a) and **HLA-DR**-expressing cells in the epithelial/subepithelial layer (2b).





Figures 3a and 3b. Immunoperoxidase staining pattern in human nasal polyp tissue incubated with monoclonal antibodies anti-Leu4 (3a) and anti-HLA-DR (3b). The sections are counterstained with Mayer's hematoxylin. The figures demonstrate a spatial correspondence between large clusters of T lymphocytes (3a) and the accumulation of HLA-DR-expressing macrophage/dendritic cells (3b).

Figure 4.
Immunoperoxidase staining pattern in human nasal polyp tissue incubated with monoclonal antibody anti-HLA-DR. The section is counterstained with Mayer's hematoxylin. Surface epithelial cells expressing the HLA-DR antigen (arrows).

Table 1. Patients and controls in the study

initials	sex	age	recurrent	steroids	asthma	allergy
nasal polyposis						
JW	m	44	+	+	+	+
EM	m	48	+	+	+	
OF	m	60	+	+		
EE	m	62	+	+		
TJ	m	79	+	+	+	
IB	m	83	+			
EL	m	58	+			
ST	m	31		+	+	+
PBE	m	52				+
SK	m	53				
AN	m	59		+		
SF	m	74		+		
controls						
POS	m	33				
LD	f	51				
ALM	f	63				
ASO	f	66			?	?
SA	m	67				+

The sex of the patient is shown as male (m) or female (f). "Recurrent" polyposis is defined as those cases previously operated on. "Steroids" means that the patient had been treated with topical nasal steroids during the month preceding surgery. "Allergy" indicates a clinical diagnosis of respiratory allergy. The record of one patient is incomplete as regards asthma and allergy ("?").

a cryostat at -20°C and then fixed in acetone at room temperature for 10 min and air-dried for 1 h. After final storage at -70°C the sections were refixed in cold acetone diluted 1:1 in distilled water for 1 min, followed by final fixation in cold acetone (100%) for 5 min. In 9 of the 12 patients separate specimens from the polyps were stained with hematoxylin-eosin in order to visualize eosinophilic granulocytes.

Monoclonal antibodies

The following monoclonal antibodies (Becton-Dickinson, Sunnyvale, Calif, USA) were used. Anti-Leu4 (CD3) directed to all T lymphocytes; anti-Leu2a (CD8) directed to "suppressor/cytotoxic" T cells; anti-Leu3a (CD4) against "helper/inducer" T cells and also cells of monocyte/macrophage lineage; anti-Leu12 (CD19) against B cells and, finally, anti-HLA-DR, reactive with monocytes and macrophages, B cells and activated T cells. In addition, we used the monoclonal DAKO-macrophage antibody and the anti-cytokeratin 18 antibody (Dakopatts, Glostrup, Denmark) reacting with human macrophages and simple epithelial cells, respectively.

Immunohistological staining

Subsequent incubations were carried out sequentially for 30 min at room temperature and in a humidified atmosphere. This was followed by washing for 5 min in phosphate-buffered saline **(PBS)** between each step. Endogenous peroxidase activity was blocked by incubation in 0.3% H_2O_2 for 15 min. The antibodies were diluted in PBS containing 4% bovine serum albumin (BSA) at 1:32 for anti-Leu4, anti-Leu2a, anti-Leu3a, anti-Leu12; 1:128 for anti-HLA-DR; and 1:64 for DAK.0-macrophage and anticytokeratin. Next, PAP complexes were allowed to react. The reaction was developed using buffered aminoethylcarbazole for 15 min. No staining was observed when the primary antibody was omitted. The slides were counterstained with hematoxylin and mounted in glycerin gelatin. The positive cells in a representative field (x40 objective) were identified and counted in consecutive sections. For each specimen, three such fields were assessed and the mean recorded. Macrophage- and dendritic-like cells that displayed cytoplasmic Leu3a-antigen were excluded in T-cell calculations.

RESULTS

The clinical features of the polyp patients and controls are summarized in Table 1. The polyp group contained patients with localized disease as well as those with asthma indicating a generalized disease. No patient had a documented intolerance to **NSAID.** The biopsies from the polyp patients were initially subgrouped according to recent steroid treatment and previous polypectomy, respectively. As no constant difference in the histological appearance of the polyps, between the two groups, was detected, the polyp patients are presented as one group. The nasal polyp biopsies showed different degrees of oedema. Accordingly, the absolute number of cells per visual field was generally less in the polyp tissue than in the controls. This held true for all cell types including the immunocompetent cells. Eosinophilic infiltrates were found in the polyps from 8 out of 9 patients studied.

Epithelium

The epithelium was well delineated by the anti-cytokeratin 18 antibody in the polyp mucosa and in the submucosal glands (Figure 1) and likewise in the corresponding structures of the controls. In the surface and glandular epithelium of all biopsies, a subset of the cells was found to express the HLA-DR antigen (see below).

T- and B-lymphocytes

The relative numbers of the different lymphocyte subtypes and the spatial distribution of the lymphocytes were the same both in polyps and control biopsies. Numerous scattered T lymphocytes (Leu4-positive cells) were found throughout the tissue. The cells tended to accumulate towards the subepitheliial areas, occasionally penetrating to the epithelial layer (Figure 2a). Clusters of T lymphocytes (Figure 3a) were observed in the subepithelial regions and in deeper glandular areas. There was local variation in the ratio between Leu3a-positive ("helper/inducer") and Leu2a-positive ("suppressor/cytotoxic") T cells in the stroma, but in the large cell clusters the "helper/inducer" phenotype was generally predominant. Only a small number of B cells (Leul2-positive cells) were seen scattered throughout the polyp tissue and normal mucosa.

HLA-DR-expressing cells and macrophages

The HLA-DR staining pattern was similar in polyp tissue and in the control biopsies. A large number of scattered HLA-DR-expressing cells, the vast majority having a macrophage/dendritic appearance, were present in all specimens. Examination of serial sections from the same biopsy showed an accumulation of HLA-DR-expressing macrophage/dendritic cells in areas corresponding to the T-cell clusters (Figure 2b). Several of these HLA-DR-exptessing cells were also found in the epithelial layer (Figure 3b). Additional staining with the macrophage-reactive antibody showed a distribution similar to that of the HLA-DR-expressing macrophage/dendritic cells. A variable number of epithelial cells were found to express the **HLA-DR** antigen (Figure 4). These cells occurred both in surface and glandular epithelium, and did not stain with anti-macrophage antibodies.

DISCUSSION

The object of this study was to compare the distribution of immunocompetent cells in specimens from nasal polyps and normal nasal mucosa subjected to the same immunohistochemical staining. In order to minimize the influence of regional differences in the distribution of immunocompetent cells (Stoop et al., 1991), control biopsies were taken from the mucosa of the middle turbinate and middle meatus, corresponding to the region where the polyps were obtained. Studying the normal nasal mucosa, we demonstrated that T lymphocytes were more prevalent than B lymphocytes and commonly aggregated around macrophages in subepithelial and paraglandular clusters. We also found that the "helper/inducer" phenotype was more common than the "suppressor/cytotoxic" one among the T lymphocytes. These results agree with the observations by Winther et al. (1987), but are in contrast with the report of Hameleers et al. (1989), Fokkens et al. (1990) and Stoop et al. (1991), who found the "suppressor/cytotoxic" phenotype to be more numerous than the "helper/inducer" one. Our material contained an abundance of subepithelial and paraglandular lymphocyte clusters, which may account for the higher prevalence of the "helper/inducer" phenotype, since there is an agreement among the investigators that this phenotype dominates in the clusters.

The nasal polyp cases in this study were initially subgrouped so that those recently treated with steroids could be compared with those which were not, and the cases with a recurrence could be compared with those not previously operated upon. In the present, albeit limited, material we were unable to demonstrate a difference between the subgroups. The study included patients with asthma, indicating generalized disease, and those with local disease only. The relevance of the findings to the subgroup of polyposis associated with asthma and intolerance to analgesics ("aspirin disease") remains to be established, as none of the patients studied had a documented intolerance to NSAID. The polyps displayed a T-cell predominance and a subepithelial clustering of mainly Leu3a-positive ("helper/inducer" phenotype) cells, which was comparable with the findings in the normal mucosa. Our results as regards nasal polyps corroborated those of Larocca et al. (1989). This pattern is distinctly different from the relative decrease in "helper/inducer" cells as described in infected tissues, such as chronically inflamed maxillary sinus mucosa (Nishimoto et al. 1988).

The nasal mucosa is constantly exposed to a potentially harmful environment, and signs of immunological activity are to be expected as part of the normal state. In the present material, a response to an antigen is suggested by the association between HLA-DR-expressing cells, identified as macrophages, and clusters of T-cells dominated by the Leu3a-positive ("helper/inducer") phenotype. Such clusters were as prevalent in normal mucosa as in polyp tissue. Similar findings of activated macrophages in the superficial parts of polyps have been reported by Petruson et al. (1988a, b). Stoop et al. (1991) discuss a possible interaction between T lymphocytes and eosinophils in nasal polyps. Our data confirm a high prevalence of eosinophilic infiltrates in polyp tissue, but the methods used do not allow any conclusions concerning possible interactions between eosinophils and other immunocompetent cells.

Since the HLA-DR antigen is intimately linked to the antigen presentation in the immune response, our finding of HLA-DR expression on epithelial non-lymphoid cells is noteworthy. This finding is supported by similar observations by other authors (Fokkens et al. 1990; Hameleers et al. 1989; Winther et al. 1987), and may indicate that the epithelial cells play an active role as an immunological barrier to foreign substances in polypoid as well as normal mucosa.

To conclude, the present study indicates that the distribution and activation of immunocompetent cells does not differ between nasal polyps and normal mucosa. It would thus appear that the formation of nasal polyps is not dependent on an altered activity of those cells.

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Arne Linder, MD, PhD
Dept. of ORL and Head & Neck Surgery
University Hospital
S-751 85 Uppsala
Sweden