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NARES: A model of inflammation caused by activated eosinophils?

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SUMMARY

Twenty patients were selected on the basis of perennial rhinitis, the absence of allergy and with an eosinophil count higher than 20% of total leucocytes in nasal secretions (NARES). Nasal endoscopy with biopsies from the middle turbinate and sinus CT were pe,formed. Biopsies were processed for histological examination and for immunofluorescence. The clinical progress during treatment was scrutinized. An acute congestive aspect of the nasal mucosa was noted in 4 cases, and micropolyposis in 9 cases. Sinus CT showed opacity of the ethmoidal cells in 87% of cases (maxillary sinuses: 75%; frontal sinus: 46%; sphenoidal sinus: 31%). An eosinophilic infiltrate of the nasal mucosa was constituted in 9 cases: In 6 cases, the cells expressed the FecRII receptor, recognized by the monoclonal antibody Bb10. Anti-H_1 drugs usually failed to result in a clinical improvement and local eosinophilia was not changed. Local corticoids were more effective but not sufficient in some cases, so that oral corticotherapy was needed. Ethmoidectomy was peiformed in three cases. NARES seems to evolve in three stages: (]) migration of eosinophils from the vessels to the secretions; (2) retention of eosinophils in the mucosa which might be linked to activation of unknown origin; (3) nasal polyposis. Numerous interactions between irritation of the epithelium, release of substance P, and eosinophils, lead to the hypothesis of a neurogenic origin of NARES.

INTRODUCTION

Non-allergic rhinitis with eosinophilia syndrome (NARES) is a condition which has been recognized since 1980 (Jacobs et al., 1981; Mullarkey et al., 1980; Mullarkey, 1988; Moneret-Vautrin et al., 1990). It is the classic syndrome of nasal hyperreactivity, but is rapidly complicated by chronic obstruction and

hyposmia with no allergy factor, and a nasal-secretion eosinophilia of over 20% of leucocytes (De Simone et al., 1987; Spector et al., 1980). The hypothesis has been postulated that NARES develops eventually into nasal polyposis. There is a lack of data concerning the state of the nasal mucosa co-existing with the high eosinophilia of the secretions. This study of 20 cases of NARES shows an eosinophilic infiltrate in only half of the cases. However, the frequency of the state of activation of the eosinophils, as demonstrated by staining with the Bb 10 antibody which recognizes the FccRII receptor, should be emphasized. The probable role of the eosinophils and their mediators is confirmed by the relative inefficiency of the anti-H_1 drugs.

MATERIAL AND METHODS

Twenty patients were selected on the basis of perennial rhinitis without allergy, and with an eosinophil count of > 20% of the total leucocytes in nasal secretions. The score of clinical symptoms (pruritis, sneezing, obstruction, rhinorrhoea, hyposmia) was calculated on the basis of a four-mark scale: 0 (symptoms absent); 1 (mild symptoms); 2 (moderate symptoms); and 3 (severe symptoms).

Methods to perform eosinophil counts in nasal secretions, skin tests and Phadiatop® are described elsewhere (Moneret-Vautrin et al., 1990). Nasal endoscopy and sinus CT-scans were also performed in each patient.

Local anaesthesia was used to obtain biopsies from the head of the middle turbinate. Biopsies were also obtained under general anaesthesia in 10 patients without nasal complaints, who were operated for head-and-neck tumours, i.e. carcinomas. All patients gave their informed consent.

Samples were snap-frozen by immersion in liquid nitrogen, forwarded to the laboratory, and kept at -80 °C until studied. Three-µm-thick serial sections were prepared at -30 °C using a cryostate microtome (Slee, London, UK), collected on clean glass slides, air dried, and processed without further fixation. The first section of each series was stained with toluidine blue for histological examination. Subsequent sections were processed for immunofluorescence.

Direct immunofluorescence was performed with monospecific fluoresceinated antisera directed to lgG, IgA, lgM, C1q, C3, and C9 (Behring, Marburg, FRG): All were diluted at 1: 20 to 1: 50 in **PBS,** and applied to the sections for 30 min at room temperature in a moist chamber.

The monoclonal antibody Bb10 was graciously provided by Dr. M Capron. This antibody has been raised to hypo-dense eosinophils and recognizes the FccRII receptor for lgE, expressed only by activated eosinophils. Detection of the antigen with the Bb 10 antibody was performed by means of an indirect immunofluorescence method. A first 60-min incubation was carried out at room temperature in a moist chamber, after covering the sections with 10 µl of the antibody diluted in PBS. This was followed by three washes in PBS at room temperature,

and a second 30-min incubation with FITC-conjugated sheep-anti-mouse Ig serum (Institut Pasteur Production, Paris, France). One section incubated with this second-step reagent alone served as a control to establish the positivity of BblO labelling in each case. Following the last incubation, all slides were washed three times, mounted in PBS/glycerol and placed at 4 °C in a moist chamber. All sections were examined within 24 hours using microscopes (Leitz Orthoplan or Olympus BH-2) equipped with Ploem systems of epi-illumination.

The presence of eosinophils was assessed by examining the slides with a phase-contrast microscope. When present, these cells were enumerated on a minimum of 5 microscopic fields; data were expressed as the number of cells per 0.05-mm^2 field. Labelling with Bbl0 was performed only in those samples in which significant numbers of eosinophils had been observed. Positivity was always controlled by consecutive examination of each field in a phase-contrast microscope and under ultraviolet light, and compared to the control slide, especially since mast cells and eosinophils are known to bind nonspecifically to fluoresceinated reagents.

RESULTS

The diagnosis of **NARES** is evident in these 20 patients whose symptoms have lasted for over a year (from 1 to 19 years) and for which several studies of nasal secretions show that local eosinophilia is stable at over 25% and even up to 95%. Prick tests were negative to all inhalants: house dust mites, birch-, ragweed- and grass pollen, cat and dog epithelia, and molds *(Penicil/ium, Aspergil!us, Alternaria)*. The absence of atopy was confirmed by a negative Phadiatop®.

The differences observed concerning clinical symptoms with allergic rhinitis and common vasomotor rhinitis have already been reported (Moneret-Vautrin et al., 1990). Briefly, all the symptoms are more intense in **NARES,** and the total score is 9.5 vs. 4.8 in vasomotor rhinitis, and 5.7 in allergic rhinitis ($p < 0{,}01$).

The intensity of the NARES is much higher than that of the other varieties; 19 subjects have a score between 6 and 14. The 20th has a score of 3. The frequency of olfactory problems should be insisted upon, as they are present 13 times out of 20 whereas they are almost always absent in patients with allergic rhinitis, and only seldomly observed in banal vasomotor rhinitis. In two cases a dry morning-cough was noted.

The progression during treatment was closely observed. After 10 days of treatment, the prescription of anti-H_1 drugs (mequitazine or loratadine) alone' allowed clear clinical improvement in only one-third of the cases. The female patient with an eosinophilic infiltrate of 55 non-activated cells per field was responding very favourably after one year of follow-up. Three times out of 4, the eosinophilia of nasal secretions was unchanged after 10 days of treatment with anti-H_1 drugs.

When eosinophilia significantly lessened, this did not always imply clinical improvement. When anti-H_1 drugs were continued during one to three months, no significant change was observed, so that local corticoids were added to anti-H_1 drugs. The association was efficient in 7 cases out of 10. However, local eosinophilia was significantly reduced only three times. A better result was obtained by systematical corticotherapy per os at the beginning of treatment. One case did not respond to high doses of corticoids and developed during a period of 18 months into severe nasal polyposis. In 3 cases a total ethmoidectomy with sphenoidectomy was performed by endoscopic surgery.

Nasal endoscopy frequently showed an abnormal aspect of the nasal mucosa, as there were only 7 normal cases. An acute congestive aspect was noted in 4 cases. Micropolyposis of the middle meatus was found in the nine remaining cases; it was unilateral in 2 cases.

Only in 3 cases, sinus CT-scans were normal. They most often showed partial or total opacity of the ethmoidal cells (87%). Added to this was hyperplasia of the mucosa of the maxillary sinuses (75%), frontal sinuses (46%) and even the sphenoidal sinus (31%). The spread of the opacities was not correlated to the severity of the local eosinophilia.

The study of the paranasal mucosa of 10 subjects with a supposedly healthy nasal mucosa showed the absence of an eosinophilic infiltrate: It was either nonexistent or demonstrating a density of less than 3 cells per field, except in one subject where it reached 10 cells per field with positive Bb 10 staining. It was over 8 cells per field in 9 patients with NARES. In two subjects with an asymmetrical affection of the nasal mucosa, the eosinophilic infiltrate was present on the same side as the micropolyposis, but not on the other. In general, a normal (Kajita et al., 1985) or simply congestive (Moneret-Vautrin et al., 1990) endoscopic aspect corresponded 8 times out of 11 to normal histology. In contrast, a micropolypoid aspect corresponded to an eosinophilic infiltrate 6 times out of 9 (Table 1).

The eosinophilic infiltrate consisted, 6 times out of nine, of cells expressing the antigen recognized by the monoclonal antibody Bb 10. Staining involved all the eosinophils in 2 cases, two-thirds in one case, half the eosinophils in 2 cases, one-third in 1 case. There was no relation between the quantity of infiltrate and the level of staining by BblO. In particular, the most dense infiltrate with 55 cells per field, was negative for Bb10. In one female patient, the eosinophilic infiltrate reacted with the Bb10 antibody on one side, and was negative on the other side. All in all, 3 out of 11 patients with a normal endoscopic aspect had activated eosinophils vs. 4 out 9 with micropolyposis.

No abnormal distribution of IgA or IgM plasma-cells was noted. There was no infiltration by metachromatic cells. A C3 deposit at the transition of the mucosa and the epithelium was observed in one patient, and on the biopsy of a control subject.

Table 1. Study of eosinophilia of secretions and tissue eosinophilic infiltrates in 20 cases of **NARES.**

	Sex	Age	eosinophils in nasal % secretions	eosinophils in nasal mucosa n°/field	% marking by Bbl0 antibody
Normal or congestive mucosa					
1	F	37	85	1	-
2	M	53	25	0	-
3	F	39	30	0	-
4	F	49	38	0	-
5	F	33	32	0	-
6	F	28	90	9	100%
7	F	41	75	<3	-
8	F	44	60	10	50%
9	M	40	90	0	-
10	F	35	85	3	not measured
11	F	40	50 to 90	10 (right); 0 (left)	+ variable (right; left)
Micropolypoid mucosa					
12	M	59	26	15	66%
13	F	44	30	15	33%
14	F	50	90	55	-
15	M	26	96	3	-
16	M	36	90	3	-
17	M	38	90	10	100%
18	F	19	80	17 (right; left)	- (right); 50% (left)
19	F	52	95	0	not measured
20	M	30	65 a 95	15 (right); 0 (left)	- (right; left)

DISCUSSION

The severity of the symptomatology, the frequent inefficiency of anti-histamine agents, and the positive effect of corticoids indicated a strong local inflammatory reaction. The fact that the secretions were rich in eosinophils suggested an eosinophilic infiltration of the paranasal mucosa. This work confirmed its existence in 9 cases out of 20, sometimes localized in one nasal fossa. The site of the biopsy was the head of the middle turbinate on grounds of easy access. As the micropolyposis seemed to be initiated near the middle meatus, one can discuss the choice of the site, and postulate that an eosinophilic infiltrate would have been more frequently detected even if the biopsy had only been performed in that place.

The monoclonal antibody Bbl0 identifies the eosinophils which have FciRII receptors (Capron et al., 1981; Capron et al., 1984). The expression of these receptors is a sign of eosinophil activation releasing inflammatory mediators such as MBP, ECP, EPO, EDN, PAF, leukotrienes, free radicals of oxygen, and substance P (Kauffman et al., 1987; Venge et al., 1987; Aliakbari et al., 1987; Ayars et al.,

1989). All these mediators enhance local vasodilatation and epithelial lesions (Devillier et al., 1988; Pernow, 1985). Substance P induces the eosinophils to express FceRil, and increases their cytotoxicity (De Simone et al., 1987). Seven eosinophilic infiltrates out of 9 were made up of eosinophils activated in variable proportions, from 33% to 100% of the total eosinophils. Thus NARES is indeed a model of eosinophil-caused inflammation.

The affection could develop in several stages. The first stage might be a migration of eosinophils from the blood vessels through the lamina propria and the epithelium and then out into secretions; at this stage, the mucosa is not yet infiltrated. The second stage involves retention of the eosinophils in the mucosa. These eosinophils may be in a quiescent state, or activated. The factors of attraction of the eosinophils as well as those which induce activation of eosinophils in the nasal mucosa in NARES remain unknown. Since no infiltration of metachromatic cells is observed - as opposed to allergic rhinitis - triggering factors other than the release of basophil- or mast-cell mediators must be looked for (Middleton, 1988; Togias et al., 1988).

The origin of the infiltration could be an initially neurogenic inflammation (Devillier et al., 1988; lchimura et al., 1988; Pernow, 1985) which could result from an intense epithelial irritation, since it has been shown that the epithelium is often altered in NARES and in nasal polyps (Wladislawsky, 1984). The possibility of such irritation of the epithelium might be envisaged in 3 patients. One of the patients was a chemist who had been exposed to irritating chemical vapours. The other two patients were fighter pilots having been exposed to the inhalation of oxygen several hours a day. Various gases can have a primary irritating effect, or encourage the local production of free radicals of oxygen, which can cause lesions of the nasal epithelium (Ayars et al., 1989). They might also inhibit the activity of neutral endopeptidase, which is necessary for the inactivation of substance P (Nadel, 1989).

A gradual activation of all the eosinophils may be hypothesized. We published a case of unresponsiveness to high doses of corticoids. It corresponded to a considerable eosinophilic infiltrate of the mucosa, and two-thirds of the cells were activated (Moneret-Vautrin et al., 1989). Eosinophils normally possess receptors for glucocorticoids (Altman et al., 1981; Peterson et al., 1981). It may be feared that the state of activation implies the disappearance of these receptors, making the eosinophils insensitive to corticoid action, as has been shown in malignant hyper-eosinophilia syndromes (Prin et al., 1989). From this point of view, local treatments would be inefficient, especially since the polypoid development of the mucosa causes poor spray penetration. For this reason, a sufficient, oral dose of corticosteroids with an average of 30 mg per dose, is advisable as a complement to local corticoid treatment. The corticoids would be beneficial not only in opposing adherence to the epithelial cells and chemotaxis and,

consequently, the flow of eosinophils into the mucosa, but also in acting on degranulation (Bascom et al., 1989). Thus, it is important to diagnose NARES in an early stage in order to use sufficient corticotherapy immediately.

Not only the endoscopic but also the scannographic aspects observed in these 20 patients are noticeable: Indeed, the scanner showed only 3 normal cases out of 20. The sinus mucosa thus seems to be affected earlier than the nasal mucosa. Moreover, three patients developed nasal polyposis, and ethmoidectomy was performed two years after the diagnosis of NARES. These facts lead to assume that NARES is a state of chronic inflammation mediated by eosinophils, which can legitimately be considered as the predecessor of nasal polyposis.

As NARES might be due to a neurogenic inflammation, it would seem necessary to study the immunoreactivity of the NARES nasal mucosa for neuropeptides in general, and substance P in particular.

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