

IMMUNOPATHOLOGIC STUDY OF CHRONIC SINUSITIS: A PROPOSAL FOR ATOPIC AND NON-ATOPIC IGE-ACTIVATED MAST CELL ALLERGIC INFLAMMATION

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Paranasal sinus mucosa was examined in this study for mast cell membrane-bound IgE. The study material was from 54 patients identified in hospital records as having one of the following diagnoses: chronic sinusitis, chronic sinusitis with asthma, or chronic sinusitis with associated nasal polyps. Formalin-fixed tissue samples taken during endoscopic sinus surgery were routinely processed and examined with hematoxylin and eosin stains. Additional sectioned tissue was analyzed by fluorescence microscopy for mast cells after the use of anti-IgE and anti-tryptase antisera; 45% of the cases of chronic sinusitis, 50% of the cases of chronic sinusitis with polyposis, and 69% of the cases of chronic sinusitis with asthma exhibited 10 or more IgE-positive mast cells in the sinus mucosa. Significant numbers of individuals with chronic sinusitis have coexistent allergic disorders. Some individuals in this study who were not allergic by hospital record review also manifested IgE-positive mast cells in the sinus mucosa; a basis for this finding is proposed.

KEY WORDS — allergy, chromosome 5Q31.1, chronic sinusitis, IgE, interleukin-4, mast cell.

INTRODUCTION

My interest in upper airway allergy was initiated in 1980, with the immunofluorescence study of IgE-positive mast and plasma cells in adenoid tissue removed from 606 children.¹ That study and a subsequent analysis of additional cases for a total of 1,242 children revealed that 55% of the 259 children whose adenoid tissue demonstrated numerous fluorescent membrane-positive IgE mast cells had no evidence of clinical allergy.² In 1987, I proposed that some of the "false-positive," non-allergic children who manifested positive fluorescent IgE mast cells in their adenoid tissue might have been showing immune responses to viral or bacterial infections.³

A chance to examine sinus mucosa retrieved by endoscopic surgery afforded the opportunity to study upper respiratory tract mucosa in which multiple episodes of infection and/or inflammation had been clinically determined. Notably, functional endoscopic sinus surgery has been reported to ameliorate asthma in 90% of patients in long-term follow-up.⁴ Newman et al⁵ documented the surgically obtained culture results of 104 endoscopic sinus surgery patients with regard to aerobic and anaerobic bacteria and fungi; all cultures grew at least 1 aerobic organism, with coagulase-negative staphylococci being the most frequent.

MATERIALS

The cases were drawn from Munson Medical Center medical records and surgical pathology files for

endoscopic sinus surgery procedures conducted in the years 1994 through 1996 inclusive. The clinical diagnoses and historical data for these cases were drawn solely from the medical records. Fifty-four cases were studied: 22 with chronic sinusitis alone, 16 with chronic sinusitis and asthma, and 16 with chronic sinusitis with associated nasal polyposis. The definition of chronic sinusitis used herein meets the working definition proposed by Lanza and Kennedy.⁶ The site of the paranasal sinus is not further defined, because endoscopic surgical material from the ethmoid and maxillary sinuses was often jointly submitted in a single container for pathological examination.

The hospital records related to sinus surgery admission were inspected for specific mention of allergic rhinitis or hay fever and/or asthma. Asthma was well documented by physician admission history and the nurse's and anesthesiologist's preoperative notes. Allergic rhinitis, however, was not recorded in any of the 54 patients' records. The hospital records included a history of drug allergy or adverse drug reaction to 1 or more agents in 14 individuals: 6 men and 8 women. Eight cases had chronic sinusitis and asthma, 4 had chronic sinusitis alone, and 2 had chronic sinusitis and nasal polyposis. The majority of patients, 9, had had reactions to antibiotics, with penicillins and sulfonamides cited most frequently. One patient with chronic sinusitis, asthma, and nasal polyposis had asthma as a reaction to aspirin. This individual (case 35), later identified as hav-

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ing Samter triad-type asthma, also had skin test-defined atopy that resulted in subsequent desensitization therapy.

METHODS

The examination of sinus tissue for mast cells and IgE was done in the context of an ongoing study of adenoid and lymphoid tissue that exceeded 3,000 cases during a 17-year period. Sensitivity, specificity, and sequential staining studies with anti-IgE and toluidine blue were carried out in initial studies.¹ The sensitivity of 200 IU/mL of IgE, a level that reflects appreciable elevation of that immunoglobulin, has been previously described in our work.⁷ Addition of anti-tryptase antisera as a mast cell identifier was carried out in an investigation of mast cell participation in Hodgkin's disease.⁷ The above antisera, and those described below, were used against positive and negative adenoid control tissue that manifested either numerous or rare mast cells. Specifically for this study, polyclonal rabbit anti-IgE was diluted 1:100 and followed by application of a 1:200 dilution of fluoresceinated goat anti-rabbit antiserum. Monoclonal mouse anti-tryptase was diluted 1:50 and followed by fluoresceinated goat anti-mouse diluted 1:20. Thirty-minute incubation periods at room temperature were carried out for both first- and second-set antibody applications. All dilutions and interval washes were carried out with phosphate-buffered saline solution, pH 7.2 (antisera from Dako Laboratories, Carpinteria, Calif). Microscopic examination was conducted with a Zeiss halogen epifluorescence microscope with excitation filters B-490 and EY-455 and barrier filters DM-500 and O-515. Photomicrographs were taken with a Nikon EFM system with Ilford 400 ASA film.

Immunofluorescence studies of sinus mucosa and stroma by the above technique revealed twofold variation in stromal mast cell counts in various areas of 14 cases, and threefold variation in 1 case. To moderate these variances in stromal sites, the 2 highest counts per high-power field (400 $\times$) were averaged. The single field of mucosal epithelium and subtenant stroma exhibiting the greatest visible number of mast cells per high-power field was similarly recorded.

Formalin-fixed, wax-embedded, and conventionally stained hematoxylin and eosin surgical pathology-accessioned slides were microscopically examined. An analysis of the dominant inflammatory cell type present was made. That is, if inspection of the glass slide revealed 75% or greater numbers of a particular cell type, it was so characterized, ie, eosinophilic, lymphocytic, or plasma cellular. If no cell type

dominated and the inflammatory infiltrate was an admixture of variable proportions of eosinophils, lymphocytes, plasma cells, and neutrophils, the infiltrate was designated as mixed. For each case, the estimated area of inflammatory infiltrate on the hematoxylin and eosin-stained slide was microscopically determined and semiquantified as less than 10%, 10% to 40%, or greater than 40%. After examination of that single hematoxylin and eosin-stained slide that was most demonstrative of the above characterizations, the paraffin block from which the slide was prepared was further sectioned at 4 μ m for immunofluorescence staining techniques to enumerate and localize mast cells.

RESULTS

Microscopic examination of the sinus mucosa and stroma by the immunofluorescence technique revealed readily identifiable fluorescent cells in the stroma and mucosa. By their larger size and convoluted appearance, mast cells seemed to be the most numerous, but in mucosal epithelium particularly, the distinction between mast cells and basophils is difficult. For reporting purposes and discussion in this study, the cells identified are tabulated as mast cells. Notably absent from the sinus tissue were immunofluorescent IgE plasma cells, except in 2 cases (cases 17 and 54), in which 1 plasma cell was identified in each.

Elevated numbers of IgE fluorescence-positive mast cells (10 or more per high-power field) in the sinus mucosa were noted in 29 of the 54 cases (54%). By disease category, 45% of the chronic sinusitis cases bore elevated numbers of IgE-positive cells (Table 1), as did 69% of the chronic sinusitis cases with asthma (Table 2) and 50% of the cases with chronic sinusitis with polyposis (Table 3). Anti-tryptase antisera detected significant numbers of mast cells in all 54 cases except 2 (cases 49 and 51), in which negligible numbers of mast cells were seen. Photomicrographs of IgE-positive mast cells and mast cells labeled with anti-tryptase are shown in Figs 1 and 2, respectively.

The predominant inflammatory cell infiltrate detected by hematoxylin and eosin correlated with immunofluorescence-detected mast cells is given in Table 4. In 2 of the 16 asthmatic cases, eosinophils predominated, whereas 11 cases, including the 2 that were eosinophilic-predominant, were detected by increased IgE-immunofluorescent mucosal mast cells (2/16 or 13%, versus 11/16 or 69%). Eighteen of the 38 non-asthmatic cases, or 47%, bore numerous IgE-immunofluorescent mucosal mast cells, and 11 of the 38, or 29%, showed predominant eosinophilic inflammation.

TABLE 1. CHRONIC SINUSITIS

Case	Sex	Age (y)	Cell Type and Percent	Anti-IgE MC/HPF		Anti-Tryptase MC/HPF	
				Stroma	Mucosa	Stroma	Mucosa
1	M	57	Mixed <10	0	0	17	24
2	F	30	Mixed <10	0	0	13	14
3	M	72	Mixed <10	6	0	23	16
4	M	52	Mixed <10	7	0	27	17
5	M	50	Mixed <10	25	14	44	40
6	M	31	Mixed <10	34	24	20	26
7	M	71	Mixed 10-40	0	0	15	14
8	F	40	Mixed 10-40	0	0	18	27
9	M	47	Mixed 10-40	0	0	20	42
10	F	44	Mixed 10-40	15	7	21	30
11	F	33	Mixed 10-40	15	14	11	8
12	F	24	EOS <10	0	0	18	15
13	M	40	EOS <10	13	12	21	19
14	M	69	EOS <10	23	42	9	43
15	M	33	EOS 10-40	12	23	17	45
16	M	66	EOS 10-40	21	52	18	53
17	M	42	EOS 10-40	24	21	21	29
18	F	61	EOS >40	16	0	29	26
19	F	57	LYMPH <10	0	0	23	19
20	F	31	LYMPH <10	8	12	14	20
21	F	71	LYMPH 10-40	8	2	24	22
22	M	48	PLASMA >40	4	36	15	16

MC/HPF — mast cells per high-power field, EOS — eosinophilic inflammation, LYMPH — lymphocytic inflammation, PLASMA — plasma cell inflammation.

DISCUSSION

In my original study¹ of adenoid tissue from children, the percentage of cases exhibiting fluorescence-positive IgE mast cells in stroma was 16%. Two percent of these children additionally revealed a transmucosal presence of 10 or more IgE-positive mast cells per high-power field in addition to numerous

stromal mast cells.¹ The review by Brandtzaeg et al⁸ of upper respiratory tract immunopathology noted that a shift of IgE-“armed” mast cells from the stroma into the mucosal epithelium signified allergen exposure. In this study of sinus mucosa from all clinical categories, 54% exhibited this feature of transmucosal inflammation. Among asthma cases, 69% displayed positive IgE mast cell mucosal involvement.

TABLE 2. CHRONIC SINUSITIS WITH ASTHMA

Case	Sex	Age (y)	Cell Type and Percent	Anti-IgE MC/HPF		Anti-Tryptase MC/HPF	
				Stroma	Mucosa	Stroma	Mucosa
23	F	64	Mixed <10	2	0	6	12
24	F	14	Mixed <10	9	3	12	20
25	F	29	Mixed <10	20	18	23	37
26	F	46	Mixed <10	15	22	10	20
27	F	59	Mixed 10-40	13	2	18	15
28	F	35	Mixed 10-40	10	10	33	30
29	F	45	Mixed 10-40	8	12	12	29
30	F	32	Mixed 10-40	21	18	29	22
31	F	49	Mixed 10-40	17	32	49	19
32	F	61	Mixed 10-40	32	33	30	24
33	F	63	Mixed 10-40	21	45	26	21
34	M	73	Mixed >40	5	0	18	19
35	M	44	Mixed >40	32	33	30	42
36	F	45	EOS 10-40	23	19	23	18
37	M	49	EOS >40	19	12	15	9
38	M	54	LYMPH 10-40	0	0	20	22

TABLE 3. CHRONIC SINUSITIS WITH POLYPOSIS

Case	Sex	Age (y)	Cell Type and Percent	Anti-IgE MC/HPF		Anti-Tryptase MC/HPF	
				Stroma	Mucosa	Stroma	Mucosa
39	M	44	Mixed <10	0	0	15	30
40	M	26	Mixed <10	13	9	11	25
41	M	38	Mixed <10	14	15	13	14
42	M	70	Mixed 10-40	4	1	16	18
43	M	58	Mixed 10-40	8	5	20	10
44	F	43	Mixed 10-40	70	45	57	27
45	M	55	Mixed 10-40	38	52	19	39
46	F	48	Mixed 10-40	37	59	46	55
47	M	75	Mixed >40	0	0	14	15
48	M	59	Mixed >40	12	22	16	8
49	M	55	EOS >10	0	0	2	0
50	F	53	EOS >10	2	6	13	24
51	M	75	EOS >10	12	19	2	3
52	M	58	EOS 10-40	18	31	16	27
53	M	60	LYMPH >40	17	24	38	26
54	M	31	PLASMA >10	0	0	27	23

As noted in a previous study² from our laboratory, 55% of children who had numerous stromal fluorescent IgE-positive mast cells in adenoid tissue had no clinical evidence of allergy. A follow-up of 39 non-allergic children performed 13 years later by mailed questionnaire (20 mast cell membrane IgE-positive and 19 IgE-negative) revealed that none had

symptoms of allergy and that their personal physician had made no diagnosis of airway allergic disease (unpublished observations).

Newman et al,⁵ in their study of chronic sinusitis by computed tomography (CT), immunoassays for IgE, and peripheral tissue eosinophilia, noted that



Fig 1. (Case 36) Mast cells distributed in stroma about coiled, partly autofluorescent elastica in blood vessel (anti-IgE/anti-rabbit fluorescein, original $\times 200$).

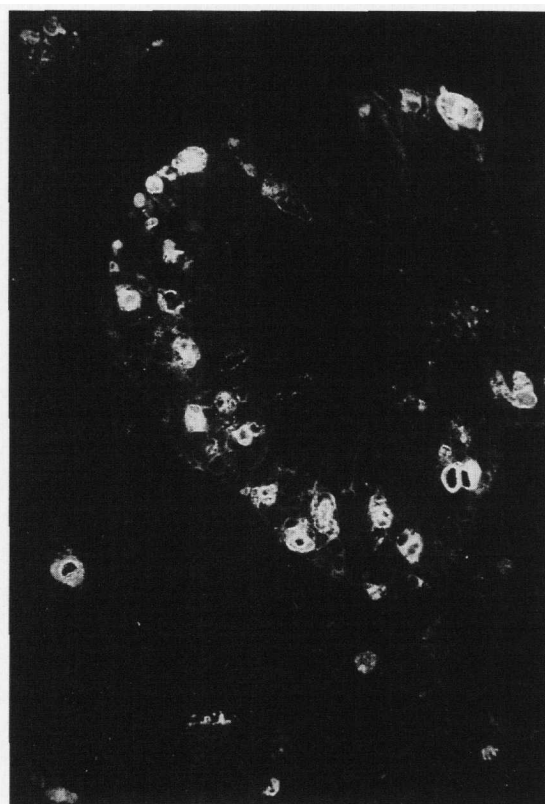


Fig 2. (Case 14) Mast cells principally resident in mucosal glandular epithelium and few in submucosal stroma (anti-tryptase/anti-mouse fluorescein; original $\times 200$).

TABLE 4. DISTRIBUTION OF IgE MAST CELLS BY INFLAMMATORY CELL TYPE

	IgE-Positive (>10 MC/HPF)	IgE-Negative (<10 MC/HPF)
Asthmatic cases (n = 16)	11	5
Mixed	9	4
EOS	2	
LYMPH		1
Non-asthmatic cases (n = 38)	18	20
Mixed	8	13
EOS	7	4
LYMPH	2	2
PLASMA	1	1

IgE antibodies were associated with severe chronic sinusitis. The majority of patients with extensive sinus disease had a negative radioallergosorbent test (RAST) result. A subsequent study of an additional 80 patients by this group revealed atopy, as defined by an elevated IgE level and/or a positive RAST score, in 40% of patients.⁹

Baroody et al¹⁰ analyzed the relationship between total IgE serum levels and sinus mucosal thickness on CT scans. Their study of 86 patients revealed a significant correlation between the extent of sinus disease as determined by CT scan and serum levels of IgE. They suggest that inflammatory responses to precipitating events such as viral or bacterial infections or IgE-mediated allergic reactions could be contributing factors. A smaller study by the same group¹¹ revealed sinus mucosal abnormalities by CT in 6 of 10 adults with ragweed allergic rhinitis. In discussion of hypotheses that may link chronic sinusitis and allergic rhinitis, they note that the character of inflammation in subjects with allergies differs from that in subjects without allergies. They cite the work of Hamilos et al,¹² which demonstrated similar eosinophilic leukocytic infiltrates in allergic and non-allergic hyperplastic sinusitis. The work of Hamilos et al would indicate that eosinophilic infiltrates cannot signify allergic inflammation unless fully characterized clinically with appropriate allergy skin testing or, as in their analysis, a broad panel of cytokine measurement.

Burrows et al,¹³ in their study of 2,657 subjects in Tucson, Arizona, for asthma and allergic rhinitis, found the prevalence of asthma to be closely related to serum IgE level. Atopy was defined in this study by skin tests employing a variety of allergens com-

mon to the Tucson area. The prevalence of asthma followed the serum IgE level more closely than the degree of atopy. Slavin¹⁴ reported, in his study of 80 adults who had sinusitis and asthma, that two thirds were non-atopic on the basis of history and skin tests.

Ponikau et al¹⁵ recently made a novel contribution to the study and understanding of chronic rhinosinusitis. They contend that allergic (eosinophilic) fungal sinusitis was present in 93% of their 101 surgical chronic rhinosinusitis cases. They believe that type 1 hypersensitivity is not the common denominator in chronic rhinosinusitis. In the absence of a study of mast cells in sinus mucosa in their work, they cite Jankowski et al,¹⁶ who state that mast cells are not increased in the nasal mucosa in chronic sinusitis.

Marsh et al^{17,18} have proposed, in their genetic research of Amish families, an explanation for elevated IgE that does not have a basis in allergen-driven IgE production. They propose that a generalized up-regulation of interleukin 4 (IL-4) resident on chromosome 5Q31.1 leads to B-cell switching to production of IgE instead of IgG. This polyclonal increase in IgE would not be detected by multiallergen testing. Furthermore, they note that mast cells and basophils may constitute a source of IL-4 throughout life. Galli¹⁹ suggests that the function of mast cells may be to initiate and orchestrate an early host response to microbial infection that in the context of immune activation equips the mast cell to function as a strategically located, highly reactive, and potent effector cell against invading pathogens. Umetsu and DeKruyff²⁰ suggest that it is likely that allergic individuals produce Th2 cytokines against many encountered antigens, including infectious agents, as well as allergens.

In summary, I identify a substantial number of individuals with chronic sinusitis who manifest numerous IgE-positive mast cells in the sinus mucosa. Many of these cases, particularly those associated with asthma, have an IgE origin through an atopic, allergen-driven, HLA-D encoded sequence of IgE formation. In non-allergic cases, significant amounts of mast cell-bound antibody identified in sinus mucosa mast cells may originate from previously committed plasma cell-generated IgG antibody against microbial organisms now re-emergent as IgE. I consider the IgE-positive mast cells in sinus mucosa to reflect a variety of antigenic experience, including allergens and infectious agents.

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