

Studies of the cellular infiltrate of chronic idiopathic urticaria: Prominence of T-lymphocytes, monocytes, and mast cells

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We have used a panel of monoclonal antibodies and enzyme histochemistry in order to characterize further the perivascular mononuclear cell infiltrate found in chronic idiopathic urticaria. Biotinylated anti-mouse immunoglobulin was exposed to avidin-biotin-peroxidase-labeled complex followed by peroxidase development in order to detect binding of monoclonal antibodies. The mean percent staining obtained for 12 patients with chronic urticaria was 47% T-lymphocytes, 22% monocytes (14% by α -naphthyl acid esterase), and 11% mast cells. B-lymphocytes were not detectable, and approximately 20% of cells could not be identified. Although patients varied greatly in the ratio of Leu 3a positive helper-inducer T cells to T8 positive cytotoxic-suppressor cells, the average of all patients was not significantly different from the T4/T8 ratio in plasma. Our results suggest that the infiltrate resembles that observed in cellular immune reactions (although no antigen has been identified) and that interaction of T-lymphocytes and/or monocytes with mast cells to cause mediator release appears likely. (J ALLERGY CLIN IMMUNOL 78:914-8, 1986.)

The cause and pathogenesis of chronic idiopathic urticaria remain an enigma. However, in recent years, studies have focused on the nature of the vasculopathy and cellular infiltrate that characterizes this disorder.¹⁻⁶ We have previously reported that >90% of our patients have a nonnecrotizing perivascular infiltrate without evidence of immunoglobulin or complement deposition.⁵ The cellular infiltrate in this group consisted of a prominent increase in mononuclear cells accompanied by a ninefold increase in mast cells compared with control biopsy specimens obtained from normals. In this study we have further characterized the mononuclear cell infiltrate by use of monoclonal antibodies and selected enzymatic stains. The results confirm an increased number of mast cells in chronic urticaria (11% to 14% of the total cells) and further demonstrate that there are 22% monocytes, 47% T-lymphocytes (with highly variable helper/suppressor subpopulations), and essentially no B-lymphocytes, whereas approximately 20% of the cells are

Abbreviation used

PBS: Phosphate-buffered saline

not identifiable. Possible pathogenic mechanisms by which hive formation might occur are discussed.

MATERIAL AND METHODS

Biopsies were done with a 4 mm punch (Baker Cummins, Miami Fla.). Specimens were bisected for routine processing and quick frozen in liquid nitrogen-chilled isopentane. Staining with hematoxylin and eosin or chloroacetate esterase (for mast cell detection)⁷ was performed on paraffin sections. Fresh-cut 5 μ m serial cryostat sections were obtained by use of a motorized cryostat (Slee International, London, England). Sections were fixed in Baker's calcium-formal for 30 minutes before staining for α -naphthyl acetate esterase for monocyte macrophage detection.⁸ Sections for surface-marker studies were air-dried overnight at ambient temperature, fixed for 3 to 5 seconds in dry acetone, air-dried again, and used immediately or stored wrapped in foil at -40° C for future studies. The stored sections were left at room temperature for 1 hour before use to avoid condensation. All sections were fixed for 10 minutes in dry acetone, air-dried, and rinsed in PBS before application of monoclonal antibodies and avidin-biotin reagents.

Immunoperoxidases and enzyme-stained cells were enumerated by use of a 10 by 10 mm reticule that fits into the eyepiece of the microscope. Five grids of adjacent areas of the specimen were counted, and the total counts per five

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reticules were determined. The percentage of each cell type was then calculated.

Monoclonal antibodies specific for human thymocyte and monocyte membrane differentiation antigens included the following: T1, pan-specific for T cells; T8, specific for cytotoxic/suppressor T cells (Dako, Santa Barbara, Calif.); Leu 3a, specific for helper/inducer T cells; Leu M3, found on 70% to 93% blood monocytes and 77% to 90% pleural/peritoneal macrophages; and Leu M5, specific for monocyte/macrophage antigen, peripheral blood monocytes, large granular lymphocytes, and tissue macrophages (Beckton Dickinson, Mountain View, Calif.). Each monoclonal antibody was diluted in PBS containing 0.5% bovine serum albumin to yield a final concentration of 1 to 2 $\mu\text{g}/\text{ml}$ of antibody. Slides were incubated overnight at 4° C with a humidity chamber (Accurate Chemical and Scientific, Westbury, N. Y.) and washed with three changes of PBS (5 minutes each). Biotinylated antimouse IgG/IgM was applied for 30 minutes, and PBS was rinsed three times and exposed to avidin-biotin-peroxidase-labeled complex (Vectastain, Vector Laboratories, Burlingame, Calif.). Slides were washed as above, and the peroxidase catalyzed color reaction was carried out with preweighed 3,3'-diaminobenzidine tetra-HCl (Polysciences, Warrington, Pa.) and hydrogen peroxide, as previously described.⁹ The reaction was stopped after 10 to 15 minutes by rinsing in distilled water, and then sections were fixed overnight in 10% neutral-buffered formaldehyde, rinsed in running tap water, and counterstained with a progressive hematoxylin (Lillie-Mayer's stain) for 1 to 2 minutes. After bluing in tap water, sections were dehydrated and permanently mounted with synthetic resin.

Patient populations

Cases were prescreened and consisted of 12 patients, eight female and four male, age range 19 to 62 years, with chronic idiopathic urticaria with hives for >3 months in whom no evident cause could be ascertained. There was no improvement in any patient on a 5-day diet of rice, water, and lamb. None of the patients had a cutaneous vasculitis, i.e., absence of vessel-wall necrosis, neutrophilic infiltrate, or significant cutaneous deposition of immunoglobulins or complement. All laboratory data were within normal limits, including complete blood count, differential, sedimentation rate, antinuclear antibody, cryoglobulin, C4, C3, and urinalysis.

RESULTS

Fig. 1 is a representative photograph of a biopsy specimen stained with a pan-T cell monoclonal antibody demonstrating that most cells stained are T-lymphocytes. When the cells in several sections of this specimen were counted by two independent observers, 72% of the cells were found to be stained. The range for 12 patients varied from 30% to 85%, and the mean was 47%. Fig. 2 depicts the stain for monocytes with the M3 monoclonal antibody; 17% of the



FIG. 1. Skin biopsy specimen of chronic urticaria stained with a pan-T cell monoclonal antibody and by immunoperoxidase.

cells were stained. The range in 15 specimens was from 7% to 35%, and the mean was 22%. The M5 monoclonal antibody that stains for monocytes as well as large granular lymphocytes and the M3 monoclonal antibodies for monocytes revealed similar results. Thus, we have no evidence of accumulation of large granular lymphocytes; however, no marker specific for the latter cells was used. Staining for monocytes with α -naphthyl acid esterase elicited a value of 14%. Fig. 3 depicts staining for mast cells with chloroacetate esterase. A mean of 11% of cells was stained. Staining for surface immunoglobulin was uniformly negative, indicating absence of detectable numbers of B-lymphocytes. Our data for the 12 patients are summarized in Table I. Approximately 19% of cells could not be identified.

We next used monoclonal antibodies to characterize further the T-lymphocytes observed into either Leu 3a positive helper-inducer type T cells or T8 positive cytotoxic/suppressor cells. As can be observed in Table II, there was considerable patient-to-patient variability in the percentage of each T subset observed. Some, such as patient No. 5, 9, or 11 had a marked preponderance of Leu 3a positive cells; however, other patients did not. For example, patient No. 2 had a striking increase in T8 positive cells, and

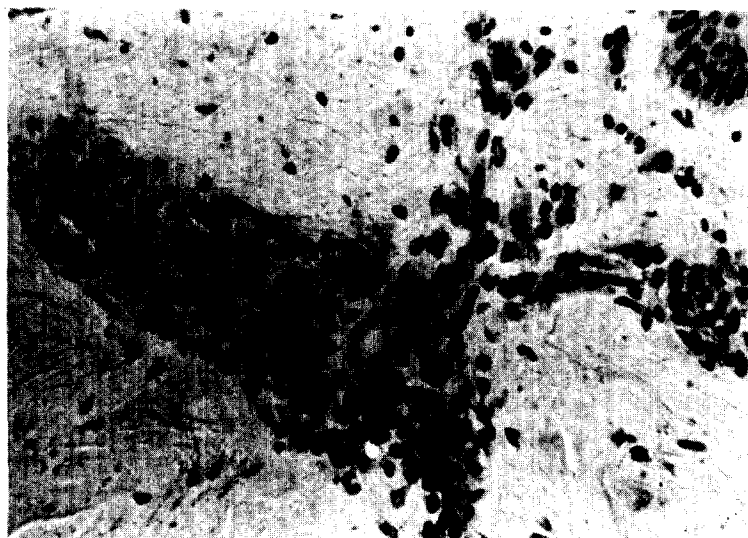


FIG. 2. Staining of a chronic urticaria lesion with M3 monoclonal antibody demonstrating monocytes within the infiltrate. Identified as the darker, rimmed cells (brown in color).

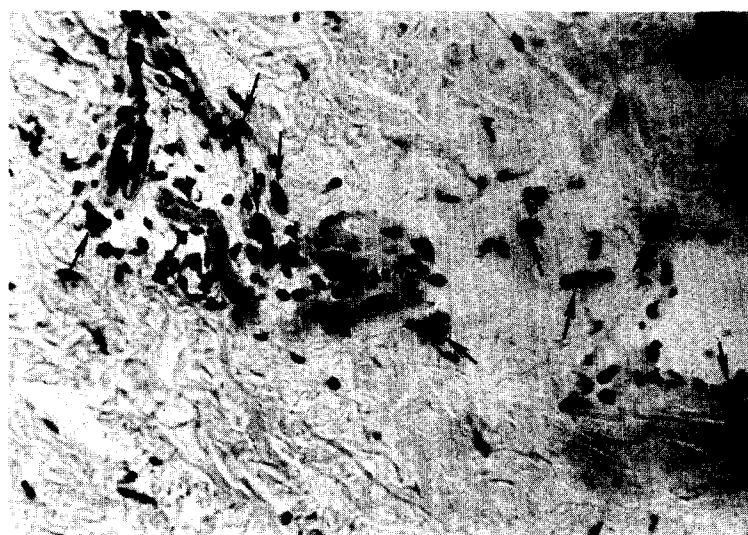


FIG. 3. Chloroacetate esterase stain of chronic urticaria lesion demonstrating mast cells in a predominantly perivascular location (see arrow heads). Although staining is red, the mast cells are the large cells scattered about the infiltrate.

the average of all the patients of 64/36 of T4/T8 closely resembles the normal T4/T8 ratio in plasma.

DISCUSSION

The infiltrative type of hive observed in chronic idiopathic urticaria is likely the result of the perivascular cellular accumulation (and their products) and the degranulation of mast cells. The wheal-and-flare reaction observed could be caused by the direct action of factors released from activated mononuclear cells or by vasoactive mediators liberated from mast cells. The latter factors may include histamine,^{10, 11} pros-

taglandin D₂,^{11, 12} leukotrienes C, D, and E,^{13, 14} platelet activating factor,^{15, 16} the kallikrein/kinin system,¹⁷⁻¹⁹ and possibly others. Since antihistamines usually relieve the pruritus of chronic urticaria but often do not eliminate the hives, it is likely that multiple mediators are of pathogenic significance.

Since chronic urticaria has not known association with atopy²⁰ and most patients lack any evidence of food-dependent reactivity,²¹ there is no reason to think the reaction is IgE mediated. By contrast, if IgE antibody to some unknown agent were responsible, one might consider the cellular reaction to be a manifes-

TABLE I. Percentage of cells counted in skin biopsy specimens

Patient No.	T cells	Monocytes*	Mast cell†	Unidentified‡
1	45	29	20	6
2	60	25	7	8
3	30	33	10	27
4	37	27	9	27
5	30	19	10	40
6	70	18	8	4
7	41	23	10	26
8	57	11	7	25
9	72	7	11	10
10	40	29	20	11
11	51	15	10	24
12	30	33	5	32
Mean	47	22	11	19

*Reported as M3 positive cells.

†By chloroacetate esterase.

‡Calculated by subtracting the sum of T cells, monocytes, and mast cells from 100.

tation of a late-phase reaction.²²⁻²⁴ Clearly, there is no evident immunoglobulin deposition, evidence of complement activation, or neutrophil accumulation in most cases; thus, mast cell activation by anaphylatoxins also appears to be unlikely. Based on the data presented herein, we would consider the possibility that a product secreted either by activated lymphocytes or monocytes may cause mast cell accumulation and activation. Thueson et al.^{25, 26} first demonstrated the existence of such a factor and demonstrated it to be capable of causing histamine release from basophils. We have recently purified such a factor from human mononuclear cells²⁷ and demonstrated it to be capable of degranulating synovial mast cells.²⁸ Other workers have described IgE binding factors from alveolar macrophages²⁹ and a B-lymphoblastoid cell line³⁰ and from late-phase reactions³¹ that are also capable of activating basophils and mast cells. The relationship of these various factors is unclear, but it is possible that they all represent a link between cellular immunity and mast cell degranulation. At this point, we can only speculate that mast cell degranulation in chronic urticaria might be caused by such factors, but it is an interesting possibility that can be tested.

Our study is not in agreement with that of Mekori et al.⁶ who found a marked accumulation of helper T cells in chronic urticaria. Although some patients did have a marked predominance of helper T cells, we found the T cell subpopulations to be highly variable from patient to patient with no overall difference from the ratio observed in plasma. The differences between these studies may relate to patient selection or staining methods, and yet patient No. 2 (Table II) in which suppressor cells predominate demonstrates that the

TABLE II. Percent T cell subclasses

Patient No.	Leu 3a cells	T8 cells
1	60	40
2	32	68
3	73	27
4	43	57
5	83	17
6	71	29
7	46	54
8	68	32
9	85	15
10	53	47
11	86	14
12	73	27
Average percent	64	36

proportion is indeed variable. Based on the results illustrated in Tables I and II, the most striking accumulation of mononuclear cells by percentage is actually the monocytes rather than the T-lymphocytes, even though the latter cells predominate. Thus, a monocyte-mast cell interaction is possible. Finally, it is possible some unknown stimulus causes mast cell degranulation and that mast cell products, including chemotactic factors,^{31, 32} cause the mononuclear cell infiltrate.

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