Immunohistochemical detection of human basophils in late-phase skin reactions

By: Anne-Marie A. Irani, Candice Huang, Han-Zhang Xia, Christopher Kepley, Ala' Nafie, El Desouki Fouda, Shirley Craig, Burton Zweiman, and Lawrence Schwartz

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Abstract:

Background: Human basophils are difficult to detect with classic histochemical stains at sites of allergic inflammation. The 2D7+ anti-basophil monoclonal antibody was used to identify basophils in skin during the late-phase response to a cutaneous allergen challenge. **Methods:** The 2D7+ monoclonal antibody was used on protease-digested sections of skin biopsy specimens obtained 6 and 24 hours after an allergen or buffer challenge. The skin chamber technique was used to compare buffer- and allergen-challenged sites at 6 hours, and intradermal injection of allergen was used to compare allergen-challenged sites at 6 and 24 hours. **Results:** Dramatic increases in the numbers of 2D7+ cells and in tissue staining by 2D7+ were observed 6 hours after allergen challenge compared with buffer challenge. Histamine levels in skin chamber fluid varied with 2D7+ cell concentrations. By 24 hours, 2D7+ cells and tissue staining appeared to diminish but were still detectable in the allergen-challenged sites. Basophils localized primarily in and around blood vessels, whereas mast cells remained mostly in the superficial dermis. Mast cells were 2D7− in both the allergen- and buffer-challenged skin. Metachromatic staining of 2D7 basophils with toluidine blue was absent in these tissue sections. **Conclusions:** The 2D7 monoclonal antibody provides a more sensitive and precise marker than histochemical staining for human basophil involvement during the late-phase response to an allergen challenge. Basophil infiltration was observed at 6 hours only after allergen challenge and persisted at similar levels by 24 hours.

Keywords: Basophil | mast cell | tryptase | Factor VIII | endothelial cell

Article:

Abbreviations used
mAb: Monoclonal antibody
NBF: Neutral-buffered formalin

TTBS: Tris buffer containing saline and Tween 20

Basophils and mast cells are the major effector cells in immediate hypersensitivity reactions by virtue of the presence of high affinity receptors for IgE (FcεRI) on their cell surface. The relative contributions of basophils and mast cells to the pathogenesis of various clinical conditions is not well understood. Basophils normally reside in the peripheral blood and can enter tissues at sites of inflammation, particularly during the late phase of allergic reactions, the early phase of delayed hypersensitivity reactions, and chronic allergic inflammation. Mast cells are positioned in connective tissue and at mucosal surfaces where contact with environmental pathogens is likely to occur. Precise and sensitive methods to identify mast cells and basophils in tissue sections are needed to further advance our knowledge of their involvement in human disease.

Mast cells and basophils have traditionally been recognized by histochemical techniques in which metachromasia develops after the cells are stained with basic dyes such as toluidine blue or alcian blue. Metachromasia most likely reflects the presence of highly sulfated proteoglycans in their secretory granules. Metachromatic basophils have been distinguished from mast cells by morphologic criteria such as a nucleus with deeply divided lobes. By using such criteria, basophils have been identified in the airways[1] and induced sputum[2] of atopic asthmatic subjects, the nasal mucosa of atopic subjects,[3] and both the skin and skin chamber fluid of subjects during the late-phase response to allergen challenge.[4, 5, 6, 7] However, such criteria are often difficult to apply to clinical tissue specimens in which these cell types assume various shapes and in which only sections of the cell are examined. Furthermore, basophils lose their metachromatic staining properties when sectioned, fixed, and incubated in aqueous media or when cytocentrifuge preparations are exposed to aqueous media.[8] Immunohistochemical detection of human tryptase, a protease that is selectively concentrated in mast cells, has provided a sensitive and specific tool for identification of mast cells in tissues and dispersed cell preparations.[9] Although human basophils have been shown to contain a very small amount of tryptase in their cytoplasm,[10] and to express primarily α-tryptase mRNA,[11] they are not stained with the current immunohistochemical techniques with alkaline phosphatase–conjugated G3, a murine monoclonal antibody (mAb) against tryptase.[12] Cell-surface IgE+, tryptase cells have been identified in the airways of atopic asthmatic subjects,[13] in the lungs of patients who died of asthma,[14] in myeloproliferative and myelodysplastic syndromes,[15] and in various tissue preparations.[16] Such cells were interpreted as representing either basophils or, when metachromatic staining was absent, “phantom mast cells” that had lost cytoplasmic granules upon degranulation. However, it is now well established that other cell types (e.g., eosinophils,[17] Langerhans cells,[18, 19] dendritic cells,[20] and monocytes[21]) can express FcεRI at sites of inflammation, obscuring the utility of this receptor as a specific marker for mast cells and basophils. B lymphocytes and activated monocytes express the low affinity receptor for IgE (CD23) and may also appear to be surface IgE+ and tryptase−. Another confounding factor for basophil (and eosinophil) identification is the presence in basophils, although at lower levels than in eosinophils, of the predominant eosinophil basic proteins (e.g., major basic protein, eosinophil-derived neurotoxin, eosinophil cationic protein, and eosinophil peroxidase.)[22, 23] When such proteins are used to assess eosinophil involvement, the results may be obscured by the occurrence of concomitant basophil involvement.

The first potential specific marker of human basophils was reported in 1987, a surface antigen designated Bsp-1.[24, 25, 26] However, the IgM mAb produced against this antigen is somewhat unstable, and further molecular information on the antigen has been limited. A potential marker of human basophil secretory granules was described in 1995.[8] The murine mAb termed 2D7 was shown to recognize a specific determinant in cytoplasmic granules of human basophils by immunohistochemistry and immunogold electron microscopy.[8] Basophils activated with anti-FcεRIα mAb showed a markedly reduced staining with 2D7 mAb. Tissue sections from normal skin, lungs, and bowels appeared to show no reactivity with 2D7, which was consistent with the expected absence of basophils in these tissues. In this study immunohistochemistry with the 2D7 mAb was optimized for sections of paraffin-embedded tissue that had been fixed in either neutral-buffered formalin (NBF) or Carnoy's fluid and was used to demonstrate recruitment of basophils into the skin during the late phase of an IgE-mediated cutaneous response.

Methods

Materials

The murine anti-basophil mAb (termed 2D7) was produced as previously described.[8] Nonspecific mouse myeloma IgG1 (MOPC31-C), peroxidase-conjugated horse anti-mouse IgG, rabbit anti-human Von Willebrand factor, alkaline phosphatase–conjugated goat anti-rabbit IgG, protease type I, peroxidase-conjugated streptavidin, normal horse serum, normal goat serum, naphthol AS-MX phosphate, Fast Blue RR, 3-amino-9-ethylcarbazole, levamisole, 30% hydrogen peroxide, Percoll, and 10% neutral-buffered formalin (Sigma Chemical Co., St Louis, Mo.); vectastain elite ABC kit (Vector Laboratories, Burlingham, Calif.); and alkaline phosphatase–conjugated murine anti-tryptase mAb (G3) (Chemicon International Inc., Temecula, Calif.) were obtained as indicated.

Tissue specimens

Punch skin biopsy specimens (3 mm) were obtained from subjects undergoing local allergen challenge with either the skin chamber model[4] or intradermal injection after informed consent was obtained as approved by the Human Studies Committee at the University of Pennsylvania. Depending on subject sensitivity, either grass or ragweed allergen was used. When the skin chamber system was used, 0.3 ml of 100 PNU allergen/ml or buffer was placed in separate chambers for 5 hours and removed at the time biopsy of the blister base was done. Histamine levels in skin chamber fluids were measured in triplicate by a radioenzymatic technique as described previously.[27] More prolonged incubations in the skin chamber model were not performed because nonspecific inflammation accelerates after this time span. To compare 6- and 24-hour time points, 30 μl of allergen (100 PNU/ml) or buffer were injected intradermally into separate sites at 0 hours, and biopsies of the sites were performed at 6 hours and, in replicate sites, at 24 hours. Skin biopsy specimens were immediately frozen and then stored in liquid nitrogen. Frozen tissues were sectioned as needed.

Basophil purification

Venous blood was collected from normal volunteers after informed consent was obtained as approved by the Human Studies Committee at Virginia Commonwealth University. Peripheral blood containing 0.5% to 2% basophils was anticoagulated with 0.01 mol/L ethylenediamine tetraacetic acid diluted 1:1 with calcium-free Hank's balanced salt solution and subjected to sedimentation through 45% (density, 1.070 gm/ml), 58% (density, 1.080 gm/ml), and 65% (density, 1.090 gm/ml) Percoll.[28] Percoll densities were confirmed by measurement of the refractive index by using an American Optical Refractometer (Scientific Instruments, Keene, N.H.). After centrifugation at 700× g for 15 minutes at room temperature, basophils were collected from the 45% to 58.5% interface at purities ranging from 15% to 40%. Contaminating cells consisted predominantly of lymphocytes and monocytes. Neutrophils and eosinophils were collected from the 58.5% to 65% interface at purities of 70% to 80% and 2% to 5%, respectively. Percoll-enriched cells were then washed three times with Hanks' balanced salt solution containing 1% bovine serum albumin and 0.01 mol/L ethylenediamine tetraacetic acid at 4° C to prevent basophil activation. Cytocentrifuge preparations were made with a portion of the cells, and were fixed in either 10% NBF or Carnoy's fluid (60% ethanol, 30% chloroform, and 10% glacial acetic acid, vol/vol/vol) for 15 minutes at room temperature. The remaining cells were suspended in 0.5 ml of freshly obtained plasma, which was allowed to clot spontaneously at room temperature. Clots were immediately fixed in either 10% NBF or Carnoy's fluid for 15 hours at room temperature. Fixed clots were embedded in parraffin, and 2 μm thick sections were prepared with a Zeiss HM 325 microtome (Walldorf, Germany).

Immunohistochemistry

Staining with 2D7 mAb. Frozen tissue sections were thawed and fixed in either 10% NBF or Carnoy's fluid for 15 minutes at room temperature. NBF-fixed tissue sections were subjected to digestion with 0.1% protease in 0.01 mol/L Tris buffer (pH 7.4) containing saline and 0.05% Tween 20 (TTBS) for 20 minutes at room temperature. Endogenous peroxidase activity was inhibited by incubation in methanol with 0.6% H_2O_2 for 30 minutes at room temperature. Nonspecific staining was blocked by incubation in normal horse serum at a 1:20 dilution in TTBS for 1 hour at room temperature. Tissue sections or cytospins were then incubated with 2D7 ascites fluid at a 1:300 dilution in TTBS overnight at 4° C or with a murine mAb of undetermined specificity (MOPC31-C) as a negative control. After washing in TTBS, sections were incubated with biotin-conjugated horse anti-mouse IgG followed by peroxidase-conjugated streptavidin, each for 1 hour at room temperature as recommended by the manufacturer. A freshly prepared solution of 3-amino-9-ethyl carbazole (0.2 mg/ml of 0.1 mol/L acetate buffer [pH 5.2]) containing 0.01% H_2O_2 was applied for 7 minutes at room temperature. After a final rinse in distilled water, the slides were mounted in a solution of 90% glycerol in phosphate-buffered saline. Positively stained cells developed a reddish brown color.

Staining of endothelial cells. Adjacent tissue sections were stained with polyclonal rabbit IgG anti-human Von Willebrand factor to identify endothelial cells and thereby locate blood vessels. After fixation in 10% NBF and digestion with 0.1% protease, sections were incubated with normal goat serum at a 1:10 dilution for 1 hour at room temperature to inhibit nonspecific staining. Polyclonal rabbit IgG anti-human Von Willebrand factor was applied overnight at 4° C. Alkaline phosphatase-conjugated goat anti-rabbit IgG was then applied for 1 hour at room temperature, and positively stained cells were visualized in blue after the addition of fast blue RR

(1 mg/ml) in 0.1 mol/L Tris buffer (pH 8.2) containing naphthol AS-MX phosphate (0.2 mg/ml) for up to 20 minutes in the dark.

Staining of mast cells. Alternatively, adjacent tissue sections were stained with alkaline phosphatase–conjugated anti-tryptase (G3) for demonstration of mast cells.[12] Protease digestion was omitted for sections undergoing staining with the G3 conjugate because it interfered with binding to the G3 epitope. Although basophils have been shown to contain a very small amount of tryptase in their cytoplasm[10] and to express primarily α-tryptase mRNA,[11] they are not stained with the G3 conjugate.[12]

Stained specimens were analyzed with the Image Pro image analysis program (Media Cybernetics, Silver Spring, Md.). Photomicroscopy was performed with an Olympus BX50F microscope associated with a PM-30 Exposure Control Unit (Olympus Optical Company, Ltd., Japan).

Cytochemistry

Cytocentrifuge preparations of peripheral blood basophils or neutrophils and eosinophils were stained with modified Giemsa stain (Sigma Diagnostics, St. Louis, Mo.) according to manufacturer's instructions. Frozen tissue sections were fixed in 10% NBF, and incubated with 0.5% toluidine blue in 0.5 mol/L HCl for 30 minutes at room temperature. Slides were rinsed with distilled water and mounted in Permount.

Statistical analysis

Median experimental values were analyzed with a rank sum test for nonparametric data.

Results

Detection of peripheral blood basophils in sections of paraffin-embedded clots with the 2D7 mAb

A previous study showing specific staining of peripheral blood basophils with the 2D7 mAb[8] used cytocentrifuge preparations. The purpose of the current experiments was to examine basophils in sections of paraffin-embedded tissues, in which the 2D7 antigen may be less available than in cytocentrifuge preparations. As a positive control for experiments involving tissue sections, freshly isolated peripheral blood basophils were resuspended in plasma that was then clotted, fixed in 10% NBF or Carnoy's fluid, embedded in paraffin, sectioned, and stained with 2D7 mAb. Although clots fixed in Carnoy's fluid stained well, those fixed in 10% NBF did not stain unless first digested with 0.1% protease (Fig. 1, A).

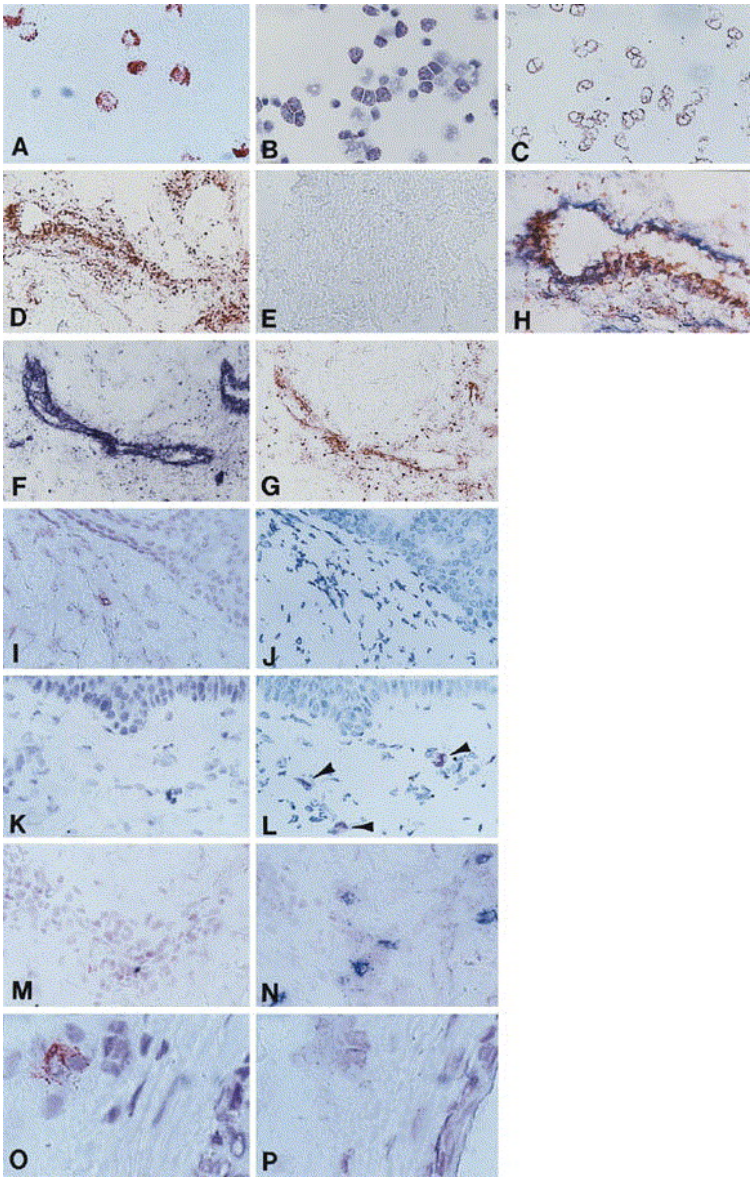

Figure 1. Staining of peripheral blood and tissue basophils with 2D7. **A** through **C,**Peripheral blood basophils of 27% purity, isolated by Percoll density-dependent sedimentation as described,[28] were placed in plasma clots **(A)** or centrifuged onto slides (**B** and **C**). Basophil-containing clot was fixed in 10% NBF, embedded in paraffin, sectioned, digested with 0.1% protease for 20 minutes at room temperature, and stained with 2D7 (10 μg/ml) **(A)**. Basophil cytocentrifuge preparations were air-dried and stained with Giemsa **(B)** or fixed in 10% NBF and stained with 2D7 **(C)**. 2D7 labeling was developed by indirect immunoperoxidase and 3 amino-9-ethyl carbazole, resulting in reddish brown stain. **D** and **E**, Staining with 2D7 on protease-digested sections of 10% NBF-fixed skin obtained 6 hours after challenge either with allergen **(D)** or buffer **I** in skin chambers. **F** through **H,** Staining with anti-Factor VIII **(F)** and 2D7 **(G)** on protease-digested sections of 10% NBF-fixed skin obtained 6 hours after challenge with allergen in skin chambers. Double labeling with 2D7 and anti-Factor VIII is shown in **H**. **I** through **L,** Staining with 2D7 mAb (**I** and **K**) and toluidine blue (**J**and **L**) was performed on adjacent sections of 10% NBF-fixed skin 6 hours after allergen challenge in skin chambers. Sections are counterstained with Gill's hematoxylin. One intact 2D7 cell in **I** appears to be toluidine blue–negative in **J**, whereas three toluidine blue–positive cells in **L** (arrows) appear to be 2D7 in **K**. **M**through **P,** Staining with 2D7 mAb (**M** and **O**) and alkaline phosphatase–conjugated G3 (**N** and **P**) was performed on adjacent sections (protease-digested before 2D7 staining) of 10% NBF-fixed skin 6 hours after allergen challenge in skin chambers. Six tryptase-positive mast cells shown in **N** appear to be 2D7, whereas one 2D7 cell in **O** is tryptase-negative. The dense blue particles seen in **K** and **M** represent precipitated hematoxylin dye and should be ignored. Magnification: **A** through **N,** 368×; **D** and **P,**920×.

Optimal staining occurred at 2D7 mAb concentrations from 2 to 10 μg/ml. Under these conditions, no staining was detected with the negative control mAb MOPC31-C. Staining with 2D7 mAb appeared to be granular, which was consistent with its localization to secretory granules by immunogold electron microscopy.[8] Sections of clots containing neutrophils and eosinophils that had been fixed in 10% NBF, embedded in paraffin, and digested with protease did not react with the 2D7 mAb. A cytocentrifuge preparation of peripheral blood basophils fixed in NBF and stained with Giemsa Figure 1, Figure 1 exhibits metachromatic and immunospecific staining patterns, respectively. Thus basophils in paraffin-embedded tissue sections, like those in cytospins, can be precisely stained with the 2D7 mAb.

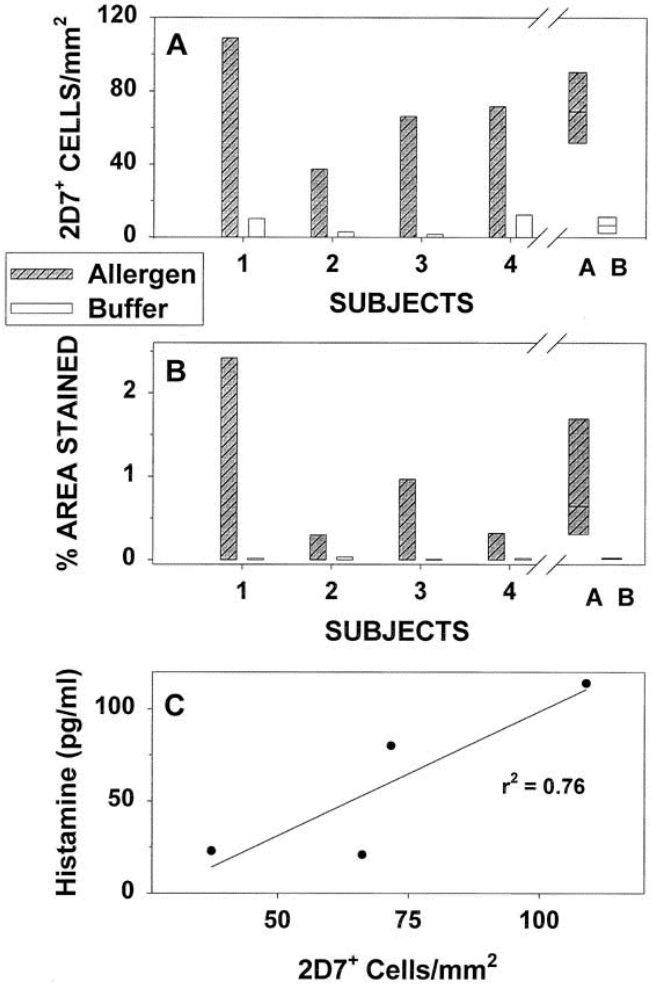

Figure 2. Quantification of 2D7 staining in skin 6 hours after challenge with allergen or buffer. 2D7 cells **(A)** and percentage of tissue section that was stained **(B)** were measured in four different subjects as described in the Methods section. Data from individual subjects are shown in bars above numbered tick marks. Group data from allergen-challenged sites (A) and buffer-challenged sites (B) are shown above corresponding letters as box plots with median (hatch mark) and 25th and 75th percentiles (rectangle). Histamine concentrations were measured in skin chamber fluids and correlated to number of 2D7+ cells in corresponding skin biopsy specimens (C).

Identification of basophils in skin biopsies 6 hours after allergen challenge with the 2D7 mAb

Paired skin biopsy specimens were obtained 6 hours after buffer challenge and allergen challenge in skin chambers of allergen-sensitive subjects. Frozen tissue sections were brought to room temperature and fixed in either 10% NBF or Carnoy's fluid for 15 minutes. As seen with the basophil clots, formalin-fixed tissue sections required digestion with 0.1% protease for 20 minutes at room temperature to permit labeling by 2D7. As shown, intense staining with 2D7 occurred in the dermis of a skin biopsy specimen from an allergen-challenged site (Fig. 1, D) but not in the buffer-challenged site from the same subject (Fig. 1, E). The staining of allergen-challenged sites appeared to be most intense in and around vascular walls and exhibited both discrete cellular localization and diffuse extracelluar staining.

Results were quantified by counting the number of positively stained, nucleated cells and by measuring the percentage of the tissue area that was stained. Fig. 2, A shows the 2D7+ basophil concentrations in allergen-challenged and buffer-challenged skin in each of four experimental subjects, with biopsy specimens from the blister base in the skin chamber.

The concentration of 2D7+ cells after 6 hours was higher in the site challenged with allergen than the site challenged with buffer in each case. In allergen-challenged dermis, basophil concentrations ranged from 37 cells/mm^2 to 109 cells/mm^2, with a median of 69 cells/mm^2. In comparison, 2 to 12 2D7+; cells/$mm^2$with a median of 7 cells/mm^2 were detected in buffer-challenged sites. These median values are significantly different from one another ($p = 0.03$).

Fig. 2, B shows the results of tissue staining expressed as percentages of the dermal areas stained with 2D7. These values were calculated by using image analysis. In allergen-challenged sites after 6 hours, 0.3% to 2.4% of the surface areas of the different tissue specimens displayed 2D7-specific staining. The median percentage was 0.6. These values reflect both cellular and extracellular staining patterns. In contrast, buffer-challenged specimens displayed little, if any, staining with the 2D7 antibody, with a median of 0.02% of the total surface area being stained. The median values of these paired groups are once again significantly different from one another ($p = 0.03$). Carnoy's fluid–fixed tissue sections stained best with 2D7 if sections were not digested with 0.1% protease. Tissues fixed in Carnoy's fluid compared with those fixed in NBF exhibited 2D7-specific staining patterns that were similar in distribution. Fig. 2, C shows the histamine concentrations in skin chamber fluids after 6 hours compared with the tissue basophil concentrations. Higher histamine levels corresponded to higher basophil levels.

Basophil infiltration persists 24 hours after intradermal allergen challenge

In eight allergen-sensitive subjects, skin biopsy specimens were obtained 6 and 24 hours after challenge of intact skin by intradermal injection of allergen or buffer. As shown in Fig. 3, A, 2D7+ cell counts were higher in the allergen-challenged samples after 6 hours than in the allergen-challenged sites after 24 hours in five of eight specimens, although these differences were not statistically significant as determined by a paired rank analysis.

Of note, the number of 2D7+ cells in the intradermal allergen-challenged samples after 6 hours were consistently lower than those found with biopsy specimens from the skin chamber sites shown in Fig. 2, A. In Fig. 3, B the percentages of tissue area stained by 2D7 mAb are shown. Again, most values obtained for the allergen-challenged sites after 6 hours were higher than

those of the allergen-challenged sites after 24 hours, but once again differences between median values did not reach statistical significance. On the other hand, the number of 2D7 cells or the percent area stained were not significantly different in the allergen and buffer challenge sites at 24 hours. The variations between experimental subjects probably explains the lack of statistical significance in the degree of basophil infiltration observed at 6 hours versus 24 hours after challenge. Thus by 24 hours after allergen challenge, basophils are still present in the dermis, although there appears to be a decrease in both the number of intact basophils as well as the extent of tissue staining for 2D7 antigen.

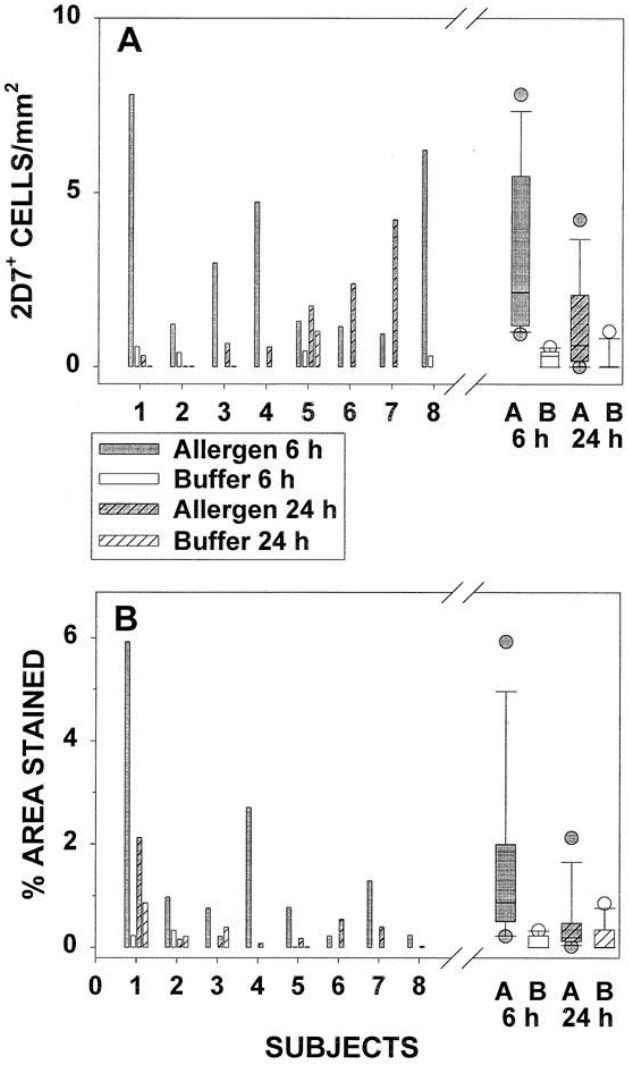

Figure 3. Comparison of 2D7 staining in skin 6 and 24 hours after intradermal injection of allergen. Samples from eight different subjects were evaluated for numbers of 2D7+ cells **(A)** and percentage of each tissue section stained **(B)** and are displayed as box and whisker plots with median (hatch mark), 25th and 75th percentiles (rectangle), 10th and 90th percentiles (whiskers), and outliers (circles).

Basophils recruited into allergen-challenged sites predominate in and around blood vessels

Adjacent tissue sections obtained from blister bases were stained with a polyclonal antibody against Factor VIII (Fig. 1, F) and with 2D7 (Fig. 1, G) to visualize the relationship between

basophils and blood vessels, respectively, in allergen-challenged skin. Basophils infiltrating the dermis 6 hours after allergen challenge consistently localized primarily in and around the blood vessels. In Fig. 1, H double labeling with 2D7 and anti-Factor VIII shows both stains to be extensively superposed.

2D7 mAb staining compared with staining with toluidine blue and anti-tryptase mAb

Adjacent tissue sections obtained from allergen-challenged skin chamber sites after 6 hours were stained with 2D7 mAb and toluidine blue. As shown in Figs. 1, I and J, a 2D7+ cell (I) shows no metachromatic staining (J). A total of 162 2D7+ cells were counted in three different sections, all of which showed no metachromasia. In addition, 589 2D7+ basophils identified in a section of basophil clot were also toluidine blue–negative. Metachromatic extracellular staining was not seen at all. Numerous metachromatic cells were observed in regions that appeared to contain 2D7+ mast cells as exemplified in Figs. 1, K (negative for 2D7+ cells) and L (three metachromatic cells observed).

Adjacent tissue sections obtained from allergen-challenged skin chamber sites after 6 hours were stained with 2D7 mAb and alkaline phosphatase–conjugated G3 (anti-tryptase mAb), respectively. Six G3+ mast cells are shown in Fig. 1, N, and the adjacent area shows 1 faintly stained 2D7+ cell (Fig. 1, M). In sections having both 2D7+ and G3+ cells, double labeling of the same cell was not apparent, as determined by counting a total of 114 G3+ cells and 45 2D7+ cells, respectively, in sections from two different subjects. Figs. 1, O and P show a high magnification of adjacent sections. A 2D7 cell in Fig. 1, O was G3 in the adjacent section incubated with the G3+ mAb (Fig. 1, P). Thus mast cells appear to be 2D7+ both in buffer-challenged and allergen-challenged sites.

Discussion

This study describes the immunohistochemical detection of human basophils infiltrating the skin during the late phase of an IgE-dependent immediate hypersensitivity reaction to an allergen challenge. Two challenge techniques were used, depending on the length of time required for the experiment. Biopsy specimens from the blister base in the previously described skin chamber model were used for experiments comparing allergen versus buffer challenge sites at the 6-hour time point. When comparing the 6-hour and 24-hour time points after an allergen challenge, intradermal injection of allergen or buffer was used. To perform these studies with sections from skin biopsy specimens fixed in NBF and embedded in paraffin, the 2D7 antigen had to be exposed by digestion of the sections with protease. Sections from paraffin-embedded tissue fixed in Carnoy's fluid yielded satisfactory results without protease digestion, suggesting that the 2D7 antigen was adequately exposed in Carnoy's fluid–fixed sections. Significant increases in the numbers of 2D7+ cells and the percentages of tissue stained by 2D7 were observed 6 hours after allergen challenge compared with buffer challenge. The increased numbers of 2D7+ cells correlated to the histamine concentrations in the corresponding skin chamber fluids, suggesting that basophils are an important donor of histamine during the late-phase response to a cutaneous allergen challenge. At the 6-hour time point, allergen-challenged sites showed a similar degree of 2D7 tissue staining for both the skin chamber and intradermal injection approaches, whereas numbers of 2D7+ cells were much higher in the skin chamber system than at sites of intradermal

allergen injection. Reasons for these differences in basophil accumulation may include: (1) a lower antigen concentration entering the dermis by diffusion from skin chambers compared with direct intradermal injection, which in turn may result in fewer basophils undergoing degranulation; (2) more persistent diffusion of antigen into the dermis underlying the skin chamber fluid reservoir than from a single intradermal injection of antigen; and (3) the absence of epidermal cells at the site of allergen challenge when using skin chambers, thus leading to enhanced infiltration of intact basophils. At 6 hours after an intradermal injection of allergen, clinical signs of the late-phase response (erythema and induration) were near maximal, whereas at 24 hours these signs had mostly resolved. The corresponding immunohistochemical data revealed a decrease in the numbers of intact basophils and the extent of tissue staining detected in the allergen-challenged sites at 24 hours compared with that detected at 6 hours. Indeed, at the 24 hour time point, no apparent differences in 2D7 staining were seen between the buffer and allergen-challenged sites. The 2D7 staining at 6 and 24 hours corresponds to the waxing and waning, respectively, of the erythema and induration evident clinically at these sites. Basophil infiltration into these late-phase reactions appears to be similar to the infiltration of eosinophils and neutrophils reported previously.[29]

2D7+ basophils and diffuse 2D7-specific staining were observed primarily in and around blood vessels 6 hours after allergen challenge. One might anticipate such a tissue distribution for cells emigrating from peripheral blood into tissues. Furthermore, as basophils enter they are likely to encounter allergen, chemokines, and cytokines and thereby be activated to degranulate and release 2D7 antigen. This could account for the diffuse 2D7-specific staining observed in some regions of each allergen-challenged section. Whether basophils apoptose or recirculate in the blood stream as the late-phase reaction resolves is uncertain. In vitro basophils are known to survive and begin regranulation within several hours after f-met-leu-phe-induced degranulation.[30] However, the current results suggest that basophil regranulation, if it occurs in vivo after IgE-mediated degranulation, is more likely to be completed after basophils have returned to the peripheral circulation, because few 2D7+ basophils were detected at sites where biopsies were done 24 hours after allergen challenge. This conclusion presumes that the 2D7 antigen is produced by regranulating basophils and targeted to their newly forming secretory granules.

The possibility that released 2D7 antigen was taken up by other cell types, particularly mast cells, was considered. For example, major basic protein released by eosinophils is taken up by mast cells, causing mast cells to become major basic protein–positive in tissues where eosinophils had been recruited and activated.[31] However, no evidence was found in the current study for tryptase-positive/2D7+ cells. We conclude that mast cells do not take up the 2D7 antigen and do not become 2D7+ at cutaneous sites of basophil activation. Also, unlike staining with 2D7, G3 staining does not reveal tissue deposits of tryptase, even though tryptase is clearly released by mast cells at these allergen-challenged sites.[4, 32] Presumably, released tryptase has been removed from these sites by 6 hours or, alternatively, tryptase diffuses from these sites during processing of tissue.

In contrast to the findings with 2D7 immunohistochemistry, no apparent differences were found in the number of toluidine blue cells in allergen and buffer-challenged sites 6 hours after challenge. Mast cells, located mostly in the superficial dermis, stained metachromatically with

toluidine blue, whereas 2D7+ regions did not show metachromatic staining. A likely explanation is that basophils lose metachromatic staining properties when made permeable and exposed to aqueous buffers, regardless of prior fixation.[8] Alternatively, the lack of metachromatic staining with toluidine blue may be a result of extensive activation of basophils, even though IgE-mediated activation of basophils would also result in diminished 2D7 reactivity as previously demonstrated.[8] Furthermore, basophils activated in vitro by simply cross-linking IgE receptors generally retain their metachromatic staining properties. However, enhancement of IgE-mediated activation of basophils (priming) occurs when these cells are exposed to IL-3, granulocyte-macrophage colony-stimulating factor, and IL-5;[33, 34, 35] nerve growth factor;[36, 37] insulin-like growth factor II;[38] and IL-1α and IL-1β.[39] Evidence that priming occurs in atopic subjects in vivo includes increased spontaneous release of histamine by basophils isolated from the peripheral blood of children allergic to food with severe atopic dermatitis,[40, 41] of subjects with atopic respiratory disease,[42] and after airway allergen challenge of sensitive subjects.[43] IL-3–primed basophils show a marked decrease in metachromasia after activation with anti-IgE antibody.[44]

Previous studies with the skin chamber model have reported evidence of basophil, but not mast cell, activation during the late-phase response after allergen challenge.[4, 45] There are an array of cytokines and chemokines that may directly activate basophils, but not mast cells. If these are generated at sites of allergic inflammation, they could account for enhanced activation of basophils and a consequent diminution in the metachromatic staining of this cell type. Such basophil histamine–releasing factors do not depend directly on interactions between IgE and antigen. P23, for example, is a histamine-releasing factor that interacts with a particular subset of IgE molecules (IgE-plus).[46] Other histamine-releasing factors appear not to involve IgE or FcεRI. These include IL-3 and IL-5[37, 47, 48, 49, 50] and the chemokines RANTES,[51] MCP-1,[52, 53, 54, 55] MCP-2,[56] MCP-3,[57] macrophage inflammatory protein-1α (MIP-1α),[58] and connective tissue activating peptide III (CTAPIII) and neutrophil activating peptide-2 (NAP-2).[59] These interleukins and chemokines do not activate mast cells.[60, 61] The complement anaphylatoxins C5a and C3a also activate basophils, but unlike chemokines these anaphylatoxins can activate mast cells found in skin but not those found in lung tissue.[62, 63]

We conclude that basophils activated in vivo at sites of allergic inflammation might be undetectable by staining with basic dyes and that the 2D7 mAb provides a more sensitive probe to detect basophil involvement under such conditions. By using the 2D7 mAb, the data provide evidence for substantial basophil recruitment during the late phase of a cutaneous allergen challenge.

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