

Identification and Partial Characterization of a Unique Marker for Human Basophils¹

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A unique marker for human basophils is needed to precisely determine the involvement of this cell type in clinical disease. To search for a marker of the basophil secretory granule, mouse hybridomas were generated against purified human basophils and screened for basophil-selective Ab. One hybridoma (2D7) produced an IgG1 κ Ab that labeled basophils, but not lymphocytes, monocytes, eosinophils, neutrophils, and mast cells by an indirect immunoperoxidase procedure. The pattern of basophil staining was cytoplasmic and granular by light microscopy. By immunogold electron microscopy, the 2D7 ligand was localized to secretory granules. Activated basophils showed reduced 2D7-dependent staining intensity, consistent with a secretory granule localization. Tissue sections of normal skin, lung, and bowel showed no reactivity with 2D7, consistent with the anticipated absence of basophils in these tissues. 2D7 staining of basophils was clearly distinct from metachromatic staining, which was presumably dependent on proteoglycan. Extracts of normal human basophils subjected to Western blotting with 2D7 exhibited two predominant bands at apparent molecular masses of 76,150 and 72,260 Da. In summary, the 2D7 ligand appears to be a specific marker for human basophils and may facilitate the assessment of basophil involvement in diseases such as asthma, anaphylaxis, and atopic dermatitis. *The Journal of Immunology*, 1995, 154: 6548–6555.

A precise determination of the relative contributions of mast cells and basophils to immediate-type hypersensitivity events and related inflammation has been difficult to achieve. These two cell types are the principal ones with abundant, constitutively expressed high affinity receptors for IgE (Fc ϵ RI) (1). Other features shared by these two cell types include metachromatic staining with basic dyes, comparable amounts of histamine stored in their secretory granules, generation of leukotriene C₄ when activated, and development from hematopoietic progenitors (2–4). Normally, mast cells reside in tissues, and basophils in the circulation. However, basophils can enter tissues at sites of inflammation, particularly during the late phase of allergic reactions (5–7) and delayed-type hypersensitivity reactions (8).

Basophils can be distinguished from mast cells by morphologic criteria and their relative lack of production of prostaglandin D₂ and tryptase. Morphologic criteria are often difficult to apply to metachromatic cells in clinical

tissue specimens. Immunohistochemical techniques to identify IgE-positive, tryptase-negative cells have been applied to pulmonary tissue obtained from asthmatic subjects and used to suggest that the number of basophils was elevated (9). However, other cell types at these sites of inflammation, such as eosinophils (10) and monocytes (11), which can express Fc ϵ RI, and B lymphocytes, which express the low affinity receptor for IgE called CD23 (12), also might appear to be IgE positive and tryptase negative. Because CD23 expression on monocytes appears to be up-regulated in atopic asthmatics (13), attempting to identify basophils by surface IgE may be particularly problematic in subjects with this disease. Detection in biologic fluids of histamine in the absence of either tryptase or prostaglandin D₂ provides indirect evidence for basophil activation (5, 6, 14, 15). These criteria have been applied to cutaneous (5, 6) and nasal (7) allergic reactions to suggest basophil involvement during the late phase of the response. The possibility of basophil-mediated anaphylaxis has been raised to explain cases of food-induced anaphylactic reactions in which no elevations of serum tryptase were detected (16).

Basophils also are closely related to eosinophils (17–21). Basophils reportedly contain small amounts (2.4×10^3 ng/10⁶ cells) of the eosinophil major basic protein and eosinophil-derived neurotoxin (214 ng/10⁶ cells). Both cell types complete their maturation in the bone marrow and are recruited into tissue sites during various inflammatory reactions, such as in the late phase of the allergic response.

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Charcot leyden crystal protein, a lysophospholipase, is abundant in membranes of eosinophils (22) and membranes and granules of basophils (23), but not in mast cells (24). IL-3 is a major growth factor for human basophils and eosinophils, but not for human mast cells (25–27). Integrins of the β_2 class also are present on eosinophils and basophils (28, 29), but not on tissue-derived or fetal liver-derived mast cells (30–32).

There is no direct marker for basophils with adequate specificity and sensitivity to distinguish them from mast cells, eosinophils, or other cell types. To identify and characterize a unique marker of human basophils, mAb were generated from splenic B cells taken from mice immunized with purified basophils. This study describes one such Ab that appears to recognize a basophil-specific protein localized in secretory granules.

Materials and Methods

Materials

Hydrogen peroxide (30%), peroxidase-conjugated goat IgG anti-mouse IgG and IgM, alkaline phosphatase-conjugated streptavidin, 3-amino-9-ethyl carbazole, nonspecific mouse myeloma IgG1 (MOPC31C), Coomassie brilliant blue, bromophenol blue, DTT, glycerol, bromochlorodolyl phosphate/nitro blue tetrazolium, Freund's complete adjuvant, polyethylene glycol, DEAE-Sephacel, Percoll, normal goat serum, collagenase, DNase (Sigma Chemical Co., St. Louis, MO), and biotin-amidocaproate *N*-hydroxysuccinimide ester (Pierce Chemical Co., Rockford, IL) were obtained as indicated. Alkaline phosphatase-conjugated G3, a mouse mAb (IgG1 κ) against tryptase that selectively recognizes mast cells (but not basophils), was prepared and used as described previously (33). The IgG mouse mAb 29C6, against Fc ϵ RI- α (34), was a gift from Dr. R. Chizzonite (Hoffmann-La Roche, Nutley, NJ). All procedures used to obtain human specimens were approved by the committee on human studies at Virginia Commonwealth University.

Preparation of anti-basophil mAb

Basophils were isolated from whole blood essentially as described previously and purified to $\geq 95\%$ (35). Purified basophils (2.0×10^6 cell/ml) were lysed and blended with Freund's complete adjuvant (1:1) by Dounce homogenization (Kontes Co., Vineland, NJ) and used to immunize BALB/c mice by i.p. injection. Two more i.p. injections were given at least 10 days apart using the same procedure with Freund's incomplete adjuvant. Serum samples from immunized mice were tested for the presence of antibasophil Ab using the immunohistochemistry procedure described below. Three days after the last injection, 8.1×10^7 spleen cells harvested from the immunized mouse were fused with 2×10^7 P3X63Ag-653 (American Type Culture Collection, Rockville, MD) murine myeloma cells using polyethylene glycol, as described previously (36), and plated in 96-well microtiter plates at 10^5 myeloma cells/well. Hybridomas selected with medium containing hypoxanthine, aminopterin, and thymidine were grown in Dulbecco's modified Eagle's medium supplemented with 4 mmol of L-glutamine per liter and 20% (v/v) FCS. Cells were grown in a Lunaire model B106 10H incubator (Lunaire Environmental, Williamsport, PA) at 37°C in 6% CO₂. Hybridomas secreting Ab reactive with basophils, but not with mononuclear cells (see below), were selected and cloned by limiting dilution into 96-well microtiter plates containing irradiated spleen cells (1×10^5 /well) as feeder cells. One stable clone, termed 2D7, was selected for further study and subcloned. Large scale production of 2D7 was performed in a Life-cell Batch Cell Culturing System (Baxter, Fenwal Division, Deerfield, IL). The 2D7 Ab was purified from the tissue culture medium by precipitation with 45% ammonium sulfate at 4°C and ion exchange chromatography on DEAE-Sephacel essentially, as described previously (37). Purified 2D7 was dialyzed against PBS, pH 7.4, at 4°C overnight and stored at –70°C. The 2D7 mAb was characterized as an IgG1 κ Ab using an ELISA subtyping kit (Boehringer-Mannheim Biochemicals, Indianapolis,

IN). Biotinylation of purified mAb 2D7 was performed by incubating biotin-amidocaproate *N*-hydroxysuccinimide ester with mAb (2 mg/ml) in a 5:1 molar ratio, at pH 9.2 in 0.1 M sodium bicarbonate buffer for 4 h at room temperature, followed by dialysis against PBS, pH 7.4, at 4°C overnight.

Mast cell dispersion and purification

Human lung tissue was dissected free of major bronchi and blood vessels, and digested with proteases (20 μ g of elastase, 200 μ g of DNase, and 2 mg of collagenase per ml HBSS three times), essentially as described previously, to disperse lung cells (38). Dispersed cells were then filtered over a 75- μ m wire mesh screen and washed three times in Tyrode's buffer. Viable cells (determined by trypan blue exclusion) were counted by hemocytometry.

Immunohistochemistry

Percoll-enriched preparations of neutrophils, eosinophils, basophils, lymphocytes, and monocytes were obtained as previously described (35). Cytospin preparations of cells were fixed in methanol for 15 min, incubated in methanol containing 0.6% H₂O₂ for 30 min to block endogenous peroxidase, washed in dH₂O, and incubated with normal goat serum (1/500 dilution) for 2 h to block nonspecific staining. Blocked slides were washed with Tris-buffered saline containing 0.05% (v/v) Tween 20, pH 7.4 (TTBS). For screening of hybridomas, 25 μ l of hybridoma medium was applied overnight at 4°C. For testing purified 2D7 mAb, slides were incubated with varying concentrations of the Ab (diluted in PBS containing 1% (w/v) BSA) overnight at 4°C. Samples were then washed three times in TTBS and incubated with a 1/50 dilution of peroxidase-conjugated goat anti-mouse IgG/IgM Ab (Sigma Chemical Co.) for 1 h. After washing the slides again three times with TTBS, a freshly prepared solution of 0.2 mg of 3-amino-9-ethyl carbazole (AEC)/ml of 0.1 M acetate buffer, pH 5.2, containing 0.01% H₂O₂ was applied for 5 min at room temperature. After a final wash in distilled water, the slides were mounted in 90% glycerol-PBS. Positively stained cells developed a reddish brown color. Basophils fixed in Carnoy's fluid (60% (v/v) ethanol, 30% (v/v) chloroform, and 10% (v/v) glacial acetic acid) were not noticeably different in staining intensity than basophils fixed with methanol (not shown).

For determination of 2D7 staining after incubation of basophils in PBS, cytocentrifuge preparations of basophils, with or without methanol fixation, were incubated in PBS for 2 h. PBS-treated slides were either incubated with the 2D7 mAb and developed as described above or incubated in Wright's stain for 5 min, rinsed in distilled water, and mounted in the 90% glycerol solution.

For sequential double labeling of basophils and mast cells, cytocentrifuge preparations of dispersed human lung mast cells, Percoll-enriched basophils, or a mixture of both were prepared. Cytospins of such cells were incubated with biotinylated 2D7 (2 μ g/ml) overnight at 4°C, followed by peroxidase-conjugated streptavidin and AEC, thus staining all basophils a reddish brown. Alkaline phosphatase-conjugated antitryptase Ab (alkaline phosphatase-conjugated G3; 0.5 μ g/ml) was then added overnight at 4°C, followed by fast blue RR (1 mg/ml) and naphthol AS-MX phosphate (0.2 mg/ml of 0.1 M Tris, pH 8.2), thus staining the mast cells blue. Positive control experiments consisted of single staining of basophils with biotin-2D7 and mast cells with alkaline phosphatase-G3. Negative control experiments consisted of single staining of basophils with alkaline phosphatase-G3 and mast cell preparations with biotin-2D7.

Fresh surgical tissues were fixed in Carnoy's fluid for 24 h and transferred to absolute ethanol. Tissues were embedded in paraffin, and 4- μ m sections were prepared. Macroscopically normal-appearing areas of skin (from adult male circumcisions), lung (from lung cancer surgery), and small bowel (from bypass surgery for obesity) were used for the immunohistochemical studies described below. Tissue sections tested for staining with the 2D7 and G3 Ab, were dewaxed in xylene and rehydrated in graded ethanol solutions. Endogenous peroxidase was inhibited as described above, and slides were incubated with 2D7 (2 μ g/ml) or G3 (1.5 μ g/ml) overnight at 4°C. The immunoperoxidase reaction was visualized with AEC as described above. Photomicroscopy was performed with either a Zeiss Ultraphot (Carl Zeiss, Thornwood, NY) or Olympus BX50/PM-30 (Olympus Optical Co., Tokyo, Japan) microscope.

Electron microscopy

Percoll-enriched basophils were fixed in modified Karnovsky's (1% (v/v) glutaraldehyde, 3% (v/v) paraformaldehyde, and 4% (w/v) sucrose in 0.1 M cacodylate buffer, pH 7.4) for 1 h at 4°C. Tissues were fixed identically, except sucrose was omitted. The cells and tissues were then washed overnight in the cacodylate buffer and processed and embedded in Lowicryl K4 M (Polysciences, Warrington, PA). Thin sections were collected on Formvar-coated nickel grids and subjected to indirect immunogold staining. The sections were placed on drops of trypsin (0.3 µg/ml of 0.01 M Tris, pH 7.8, and 0.01 M calcium chloride) for 10 s at room temperature and then washed in PBS. Digested cells were incubated sequentially in 10% (v/v) normal goat serum for 30 min and in 2D7 (200 µg/ml) or MOPC31C (200 µg/ml) in Tris-saline, pH 8.2, containing 1% (w/v) BSA for 2 h. Sections were washed and placed on gold-conjugated IgG anti-mouse IgG (5-nm diameter gold particles, Ted Pella, Inc., Redding, CA) for 1.5 h. Grids were washed in Tris-saline, pH 8.2, and then in deionized water. Sections were briefly counterstained with uranyl acetate (39) and lead citrate (40), and examined with a JEM 1200EX electron microscope (JEOL USA, Peabody, MA).

Western blotting

Percoll-enriched preparations of basophils, neutrophils, eosinophils, and human lung-derived mast cells were obtained as described previously (35, 38) and washed three times in PBS. Cell pellets were resuspended at a concentration of 5.7×10^7 cells/ml directly in 2% SDS (w/v), 10% (v/v) glycerol, 60 mM Tris (pH 6.8), and 0.1% (w/v) bromophenol blue with or without 10 mM DTT; heated at 95°C for 5 min; and microfuged at $10,000 \times g$ for 5 min. Supernatants (1.4×10^6 cell equivalents) were applied to an 8 to 16% polyacrylamide gel in 25 mM Tris (pH 8.3), 192 mM glycine, and 0.1% (w/v) SDS (Novex System, San Diego, CA) and subjected to electrophoresis. Gels were either stained directly with 0.1% (w/v) Coomassie blue in 30% (v/v) methanol and 10% (v/v) glacial acetic acid for 2 h and destained with 30% (v/v) methanol and 10% (v/v) glacial acetic acid or subjected to Western blotting. Immunoblotting onto a nitrocellulose transfer membrane was performed for 30 min at 30 V. Membranes were blocked for 1 h with Blotto (5% nonfat milk and 0.05 M Tween-20 in PBS) and washed three times with TTBS. Membranes were then incubated overnight at 4°C with 1 µg/ml of biotinylated 2D7 or biotinylated MOPC31C diluted in PBS containing 1% (w/v) BSA and 0.05 M Tween-20. The next day, membranes were washed three times with TTBS and incubated with alkaline phosphatase-conjugated streptavidin (1/2500 dilution) for 1 h. After three washes, protein bands were visualized using bromochloroindolyl phosphate-nitro blue tetrazolium substrate in buffer containing 100 mM NaCl and 5 mM MgCl₂·100 mM Tris (pH 9.5). Reactions were stopped by rinsing with PBS containing 20 mM EDTA. For assessing molecular mass, the electrophoretic mobilities of prestained m.w. markers (lot ECJ103, GIBCO BRL, Grand Island, NY) were examined in parallel lanes.

Basophil activation

Percoll-enriched basophils (1.5×10^6) were activated with anti-FcεRIα or irrelevant control Ab, as described previously (41). Stimulated and unstimulated cell pellets were resuspended in SDS sample buffer and subjected to SDS-PAGE and Western blotting, as described above. Histamine in replicate pellets and supernatants was measured by RIA (AMAC-Immunotec, Westbrook, ME), as previously described (41). Densitometric analyses of the Western blots to estimate the amounts of Ag and of the Coomassie blue-stained gel to estimate total protein were performed using Image-Pro (Media Cybernetics, Silver Spring, MD). The net percent histamine release was calculated by the following formula: net % release = $\{[(\text{stimulated supernatant}) - (\text{spontaneous supernatant})] / [(\text{stimulated pellet}) + (\text{stimulated supernatant}) - (\text{spontaneous supernatant})]\} \times 100$.

Results

Identification of an anti-basophil mAb

In the fusion related to the current study, 130 of 200 wells showed hybridoma growth. Nine hybridomas produced anti-basophil Ab, as judged by the initial screening pro-

cedure that showed labeling of basophils but not of mononuclear cells. One hybridoma, 2D7, was chosen for further study based on its apparent specificity for basophils. As shown in panels A (light microscopy) and B (phase contrast microscopy) of Figure 1, basophils, but not monocytes or lymphocytes, were labeled by the 2D7 Ab. In panels C (light microscopy) and D (phase contrast microscopy) of Figure 1, neutrophils (78%) and eosinophils (17%) obtained from the peripheral blood of a normal donor also exhibited negative staining with the 2D7 Ab. Mast cells in sections of Carnoy's fluid-fixed skin, lung, and bowel, respectively, were evaluated for staining with the anti-tryptase Ab, G3 (panels E, F, and G of Fig. 1) and with 2D7 (panels H, I, and J of Fig. 1). Mast cells in several tissues were examined because heterogeneity of mast cells obtained from different tissue sites is known to occur for various proteases present in their secretory granules. Mast cells in sections of skin, lung, and bowel were numerous, as expected, and showed intense staining of tryptase. No staining was apparent with the 2D7 Ab, suggesting the absence of 2D7 ligand in mast cells and other cell types normally resident in these tissues. Mast cells (6%) dispersed from fresh surgical lung, Percoll-enriched basophils (32% purity), and a mixture of the two preparations were stained sequentially with biotin-2D7 (developed with streptavidin-peroxidase and AEC) and alkaline phosphatase-G3 (developed with fast blue RR and naphthol AS-MX phosphate). Among the dispersed lung cells, G3 labeled the mast cells, but 2D7 staining was negative (not shown). With the basophil preparation, 2D7 labeled the basophils, but G3 staining was negative (not shown). Mixtures of mast cells and basophils (Fig. 1K) showed that the mast cells (blue) could be clearly distinguished from the basophils (reddish brown).

Staining of basophils with the 2D7 Ab was further characterized by examination of aqueous extracted and immunologically activated basophils. To better compare the sensitivity of basophil staining with 2D7 to that of a classical histochemical stain, a methanol-fixed cytocentrifuge preparation of basophils was exposed to PBS for 2 h at room temperature and stained with either Wright's stain or the 2D7 Ab. Wright's stain failed to stain the granules of these basophils (Fig. 2A), as anticipated. However, immunostaining of the basophils with the 2D7 Ab, although producing a more diffuse pattern, was strong (Fig. 2B).

Staining of activated basophils was next evaluated. Percoll-enriched basophils (40% basophils, 45% mononuclear cells, and 15% neutrophils and eosinophils) were incubated with mouse monoclonal IgG anti-FcεRI-α (29C6), as described previously (35), for 30 min at 37°C. The net percent release of histamine was 54% (spontaneous release, <5%). Cytospins of activated and resting basophils were prepared, fixed in methanol, and stained with 2D7. The 2D7 Ab stained approximately 75% of the polymorphonuclear cells, consistent with the ability of the Ab to label resting basophils (Fig. 2C). After activation, a few of

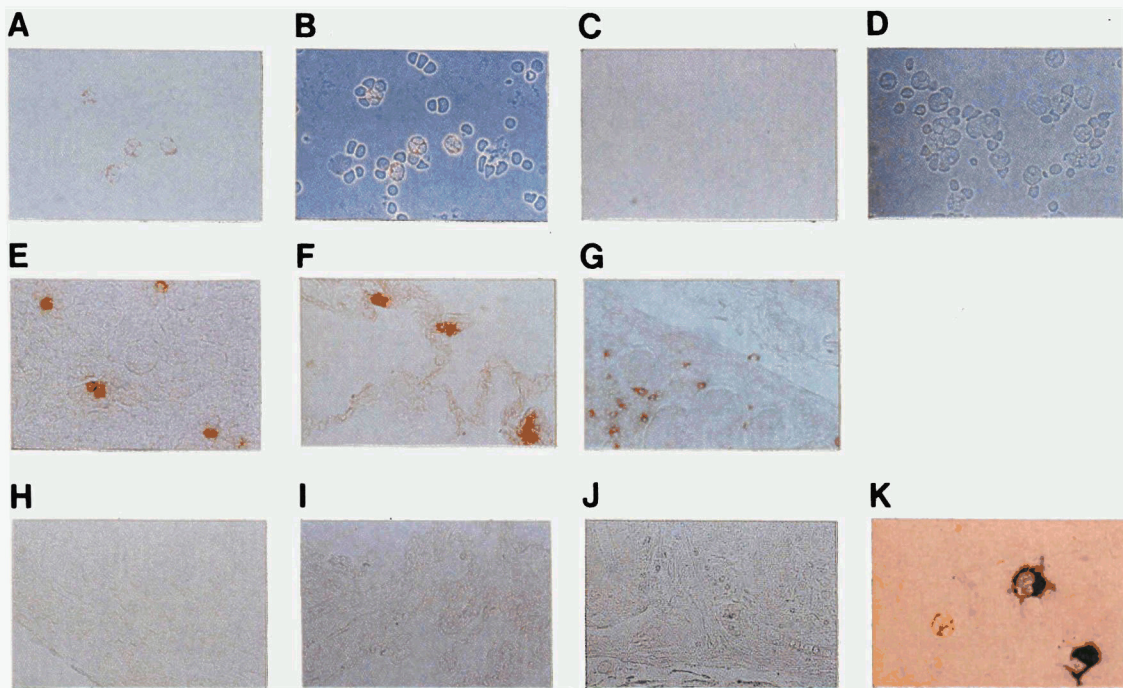


FIGURE 1. Specificity of the 2D7 mAb for human basophils. Light (A) and phase contrast (B) photomicrographs are shown of the same field of a cytocentrifuged methanol-fixed Percoll-purified preparation of peripheral blood cells containing 30% basophils, 21% monocytes, 42% lymphocytes, and 7% neutrophils and eosinophils after staining with the 2D7 mAb using the immunoperoxidase technique (magnification, $\times 160$). RBC are also present. Only the four basophils appear to be stained. Light (C) and phase contrast (D) photomicrographs are shown of the same field of a cytocentrifuged methanol-fixed preparation of peripheral blood cells containing neutrophils (78%), eosinophils (17%), monocytes and lymphocytes (3%), and approximately 2% basophils after staining with the 2D7 mAb as described in A and B. No reactivity was detected to eosinophils and neutrophils (magnification, $\times 160$). Sections of Carnoy's fluid-fixed skin (E and H), lung (F and I), and bowel (G and J) stained with the G3 mAb (1.5 $\mu\text{g}/\text{ml}$) against tryptase (E, F, and G) and the 2D7 mAb (H, I, and J) using the indirect immunoperoxidase technique (magnification: E, F, H, I, and J, $\times 250$; G, $\times 132$). Mast cells stain a reddish brown, whereas 2D7 results in no detectable stain. K shows sequential double labeling of a methanol-fixed cytocentrifuge preparation of a 1/1 mixture of dispersed human lung mast cells (6% purity) and Percoll-enriched peripheral blood basophils (16% purity) with biotin-2D7/streptavidin-peroxidase/AEC (reddish brown) and alkaline phosphatase-G3/fast blue RR and naphthol AS-MX phosphate (blue; magnification, $\times 250$). Two blue mast cells are seen, with one reddish brown basophil.

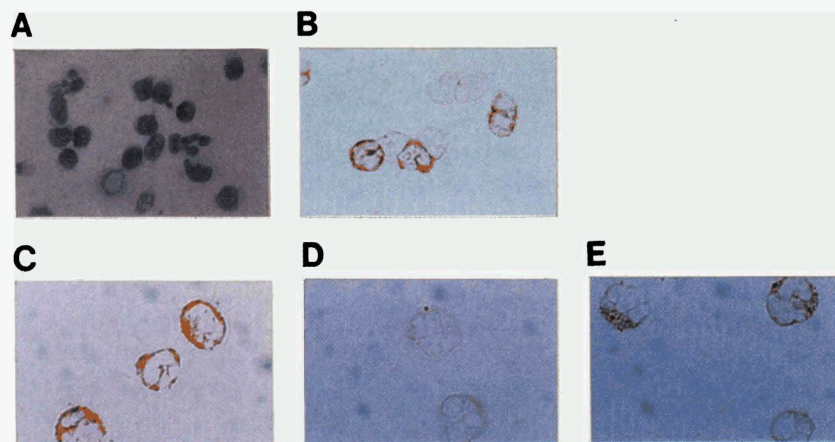


FIGURE 2. Characterization of basophil staining with the 2D7 mAb. A Percoll-enriched basophil preparation containing 20% basophils, 58% lymphocytes and monocytes, 9% neutrophils, and 2% eosinophils was cytocentrifuged, fixed in methanol, incubated in PBS for 2 h at room temperature, and stained with Wright's stain (A) or the 2D7 mAb (B) using the immunoperoxidase technique (magnification, $\times 330$). PBS-treated basophils lack cytoplasmic staining with Wright's stain, but still stain strongly with 2D7. Another preparation of Percoll-enriched basophils (40% basophils, 15% neutrophils and eosinophils, and 45% mononuclear cells), unstimulated (C) or stimulated with 3 $\mu\text{g}/\text{ml}$ of the 29C6 mAb (D and E), was stained with the 2D7 mAb using the immunoperoxidase technique (magnification, $\times 330$).

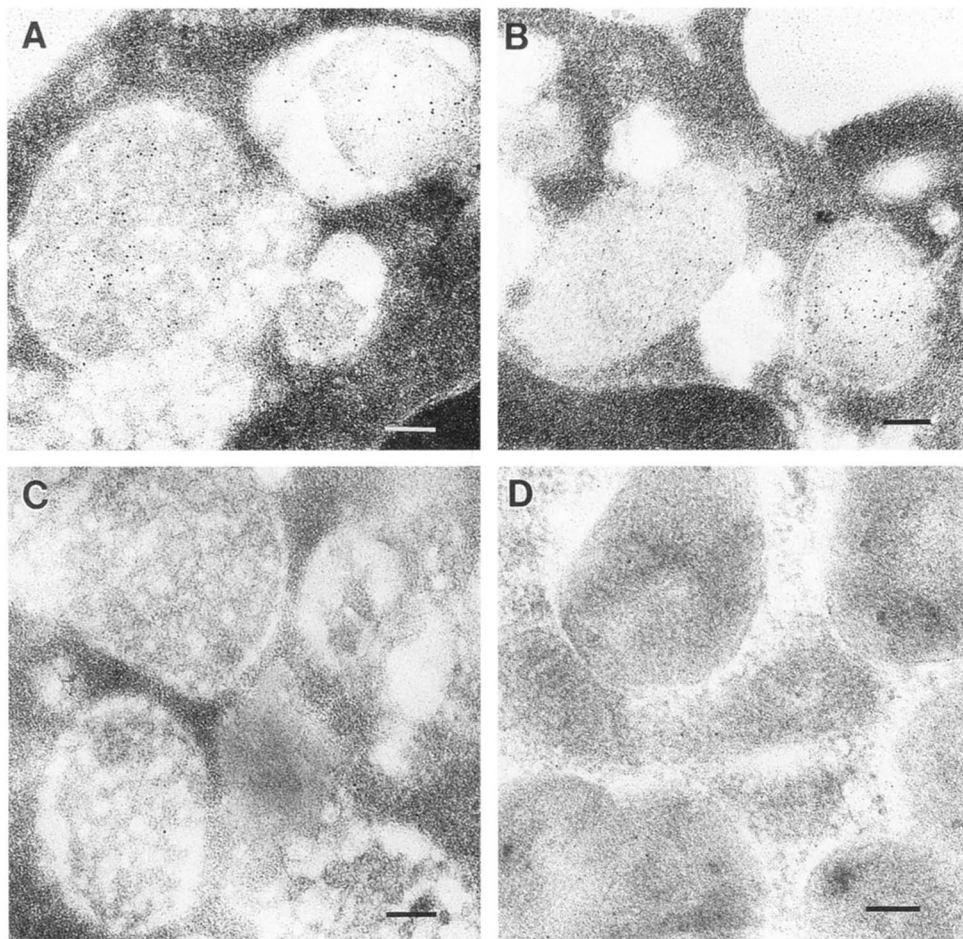


FIGURE 3. Subcellular localization of the 2D7 Ag by immunogold electron microscopy. Basophils labeled with 2D7 (A and B), a basophil labeled with MOPC31C (C), and a mast cell labeled with 2D7 in a section of skin (D) are shown. Bars = 0.1 μ m.

the polymorphonuclear cells exhibited small numbers of darkly stained granules which appeared larger than those in resting basophils; others were either unstained or showed a light homogeneous staining (Fig. 2, D and E). Mononuclear cells were not stained. Because 75% of the polymorphonuclear cells were basophils, most of the cells with this morphology should be basophils. The apparent decrease in the intensity of staining of activated basophils suggests that the 2D7 ligand was either released or destroyed.

Subcellular localization of the 2D7 Ag

The indirect immunogold technique was used to localize the 2D7 Ag to a subcellular compartment in basophils from peripheral blood. Gold particles were concentrated over most, although not all granules, confirming the light microscopic impression of staining of cytoplasmic granules. Gold particles were found on both moderately electron-dense and electron-lucent granules, but appeared to be more concentrated over electron-dense compartments (Fig. 3, A and B). Also, in granules containing both electron-dense and electron-lucent portions, the gold labeling was more concentrated over the electron-dense portion of

the granule. Few, if any, gold particles were present over other cytoplasmic structures, the nucleus, or extracellular regions. Other cell types present in the basophil preparations, such as lymphocytes, monocytes, and an occasional neutrophil, failed to be labeled. Substitution of 2D7 with normal mouse IgG resulted in little or no labeling of basophils (Fig. 3C). Also, mast cells in the skin were not labeled using the 2D7 Ab (Fig. 3D). Similar negative labeling results were obtained for mast cells in sections of lung and bowel (not shown).

Western blotting analysis of 2D7 ligand

Whole cell extracts from preparations of basophils of 40 to 50% purity were subjected to Western blotting under reducing conditions. An identical aliquot of the extract was subjected to SDS-PAGE and stained with Coomassie blue. Extracts of human lung-derived mast cells (15% pure) and a preparation of neutrophils (95%) and eosinophils (5%) stained with biotin-2D7 and of basophils stained with biotin-MOPC31C mAb of unknown specificity were used as negative controls. As shown in Figure 4, for basophils, the

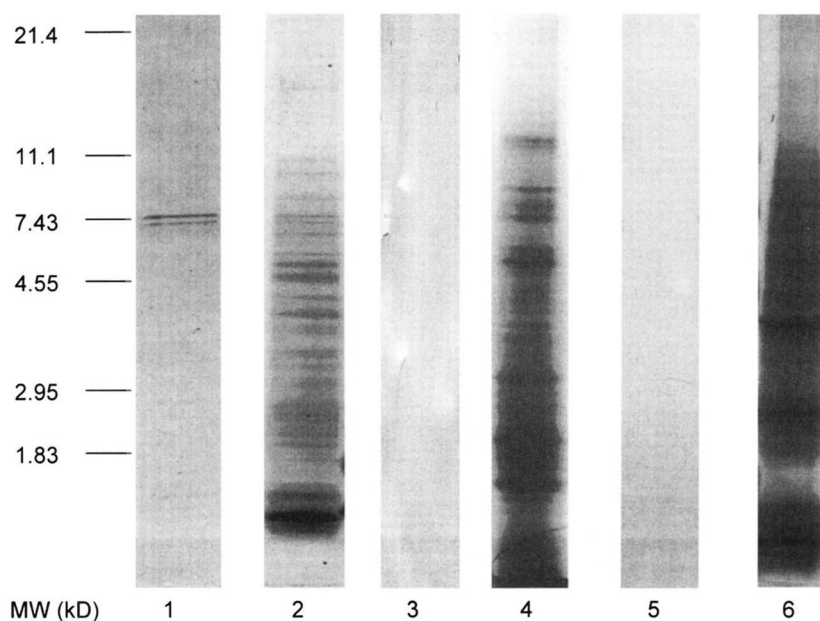


FIGURE 4. Western blotting analysis of 2D7 Ag. Equal aliquots of an extract of Percoll-enriched basophils (50% purity) obtained from peripheral blood were subjected to SDS-PAGE under reducing conditions in two 8 to 16% polyacrylamide gradient gels. In one case (*lane 1*), the sample was electrophoretically blotted onto nitrocellulose, stained with the biotin-2D7 mAb (1 μ g/ml), and developed with streptavidin-alkaline phosphatase. In the other case, the sample in the gel was stained directly with Coomassie blue (*lane 2*). As a control, equal aliquots of an extract of neutrophils (95%) and eosinophils (5%) obtained from peripheral blood (*lanes 3 and 4*) and of mast cells obtained from human lung (15% purity; *lanes 5 and 6*) were treated as described above and stained with biotin-2D7 (*lanes 3 and 5*) and Coomassie blue (*lanes 4 and 6*). The positions of prestained m.w. markers are shown to the left of the experimental lanes. The markers and their apparent molecular masses, according to the manufacturer, include myosin (H chain) at 214,200 Da, phosphorylase B at 111,400 Da, BSA at 74,250 Da, ovalbumin at 45,500 Da, carbonic anhydrase at 29,500 Da, and β -lactoglobulin at 18,300 Da.

2D7 mAb revealed two major bands with apparent molecular masses of 76,150 and 72,260 Da (*lane 1*), whereas numerous bands were apparent in the lane stained with Coomassie blue (*lane 2*). A faint 2D7-positive band also was observed at 123,700 Da. The same pattern of 2D7-positive bands was obtained under nonreducing conditions, whereas no staining with control biotin-MOPC31C was observed (not shown). When extracts from resting (2% release) and degranulated (26% net release) basophils were examined in a single experiment, a 50% decrease in the staining intensity of the 2D7-labeled band relative to the Coomassie blue-stained protein was found for the activated cells, consistent with a portion of the 2D7 ligand being released by regulated secretion (not shown). In contrast, biotin-2D7 did not stain the extracts of neutrophils and eosinophils (*lane 3*) or of mast cells (*lane 5*) that contained comparable amounts of Coomassie blue-stained protein (*lanes 4 and 6*, respectively).

Discussion

Human basophils contain a protein in their secretory granules that is recognized by a mouse IgG1 κ mAb called 2D7, which appears to be a selective marker of this cell type. The Ag appears to be selectively localized to basophils,

because mast cells, eosinophils, neutrophils, monocytes, and lymphocytes and the endogenous cell types present in skin, lung, and bowel did not react with this Ab under the conditions employed. The Ag remained available for staining with the 2D7 mAb after basophil cytoplasts were exposed to PBS. In contrast, metachromatic staining of basophils with basic dyes was lost, thereby distinguishing the 2D7 ligand from the proteoglycan or protein components of the basophil granules presumed to be required for dye binding. Localization of the 2D7 ligand to basophil secretory granules was demonstrated by immunogold electron microscopy and attenuation of the staining intensity of activated basophils that overall had released 54% of their histamine content.

Further characterization of the 2D7 ligand(s) was performed by a Western blot analysis. The 2D7 mAb recognizes identical or similar linear determinants that are present in two major protein subunits of 76,150 and 72,260 Da apparent molecular mass. Also, a protein of 123,700 Da apparent molecular mass is recognized by the 2D7 mAb, although the staining intensity of this band is quite weak. Because the same staining patterns were observed under reducing and nonreducing conditions, it is unlikely that these bands of protein were disulfide linked

to other proteins, to themselves, or to one another in their native state. However, any of these 2D7 ligands could reside in a multimeric protein with noncovalently linked subunits. Another consideration is whether each protein band recognized by 2D7 is a distinct gene product or a processed form of the larger species. Understanding the relationship among these three protein bands must await further characterization studies. The biotin-2D7 Ab did not recognize any proteins extracted from a preparation of neutrophils and eosinophils and one of the human lung-derived mast cells also subjected to Western blotting. The 2D7 Ab thus recognizes a unique basophil-specific protein constituent and also has potential clinical usefulness as a more sensitive and specific basophil marker than traditional basic dyes.

Because the basophil accounts for approximately 1% of leukocytes, it has been difficult to assess its involvement in human disease. One Ab, BSP-1, has been shown to be selectively expressed on the basophil cell surface (42). However, this Ab does not exhibit sufficient sensitivity for immunohistochemical identification of basophils, and the corresponding ligand is not likely to be released by activated basophils. Anti-IgE Ab have been used to identify basophils by immunohistochemistry in tissue sections (9). However, this strategy may lack specificity. First, a mast cell identification must be excluded by staining negatively for tryptase. Secondly, eosinophils (10), Langerhans cells (43), and activated monocytes (11), which can express FcεRI, and follicular dendritic cells, B cells, and other cell types (12), which express the low affinity receptor for IgE (CD23), also must be excluded as the cell source of the surface IgE. Detection of basophils at tissue sites of inflammation with the 2D7 mAb may now be possible with greater specificity and sensitivity than those of these double staining techniques. However, sensitivity may still be compromised in activated basophils, because the staining intensity of such cells is reduced due to the apparent release of the 2D7 ligand.

Future studies will concentrate on further characterization of the 2D7 ligand and the use of this molecule as an indicator of basophils at tissue sites of inflammation and of basophil activation. The value of this approach has been demonstrated for mast cells using the mast cell-specific marker, tryptase. Ab against this enzyme have been used to detect mast cells in tissues by immunohistochemistry and to measure tryptase in blood and other biologic fluids by immunoassay. Consequently, the involvement of mast cells in various clinical events, such as systemic anaphylaxis, can be precisely determined. However, the participation of basophils in such events, as mentioned in the introduction, remains uncertain. Because mast cells and basophils respond differently to pharmacologic agents, determining the extent that each cell type is involved in a clinical event will not only aid in the diagnosis, but also may influence treatment.

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