ELECTRON MICROSCOPIC LOCALIZATION OF IMMUNOGLOBULIN E ON THE SURFACE MEMBRANE OF HUMAN BASOPHILS

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Leukocytes from allergic individuals release histamine upon incubation with the antigen to which the individual is sensitive (1). Similarly, if antigen-unresponsive leukocytes from certain nonallergic individuals are incubated with serum from an allergic donor and challenged with the appropriate antigen, histamine is released (2). These observations indicated that both a humoral and a cellular factor were required for the antigen-induced release of histamine.

Recently, the humoral factor has been identified as immunoglobulin E (IgE) and Ishizaka and his colleagues have provided extensive evidence for the primary role of IgE in allergic reactions (3). Antiserum to IgE can mimic the effect of antigen in releasing histamine (4, 5), and leukocytes from nonallergic individuals can be sensitized by IgE for anti-IgE–induced histamine release (6).

Evidence has accumulated that the basophil is the cell responsible for histamine release: (*a*) Most, if not all, of the histamine in human peripheral blood leukocytes is found in the basophils (7–9). (*b*) There is a marked decrease in the percentage of recognizable basophils on incubation of leukocytes from allergic individuals with the appropriate antigen (10). This is apparently because of degranulation (11). (*c*) Radioactively labeled IgE appears to bind preferentially to human basophils (12).

In the present study, the binding of IgE to human peripheral leukocytes was explored at the resolution afforded by electron microscopy. For this purpose we used hybrid antibody molecules which were directed against both anti-IgE and ferritin. Basophil-enriched leukocyte preparations were incubated with or without IgE and then successively reacted with anti-IgE, the hybrid antibody, and ferritin. Ferritin thus served as a marker for IgE when the cells were examined by electron microscopy.

Materials and Methods

Preparation of Immunoglobulins.—The IgE myeloma protein (IgE_{PS}) was isolated from serum by diethylaminoethyl (DEAE)-cellulose chromatography and gel filtration (13). The final IgE preparation was uncontaminated with other serum proteins as judged by immunoelectrophoresis (14) and Ouchterlony analysis (15). $IgGw_{ar}$, a human IgG_1 myeloma protein, was

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isolated by precipitating it from serum with 0.7 M Na_2SO_4 and then eluting it from DEAE-cellulose with a 0.05 M pH 8 tris (hydroxymethyl) aminomethane (Tris) phosphate buffer. This eluted material was chromatographed on Sephadex G200 (Pharmacia Fine Chemicals Inc., Piscataway, N. J.) in order to isolate the monomeric (7S) form and to eliminate small amounts of remaining contaminants. Human Cohn fraction II (FrII)[1] was purchased from Pentex Biochemical, Kankakee, Ill. Aggregates were removed by chromatography on Sepharose 6B (Pharmacia Fine Chemicals Inc.) in a pH 8 borate-NaCl buffer. Light chains were isolated from reduced, alkylated human Cohn FrII. The polypeptide chains were separated on a Sephadex G100 column equilibrated with 1 N propionic acid.

Antisera to human IgG and IgE (provided by Dr. William Terry, National Cancer Institute) were raised in burros with two subcutaneous injections, 3 wk apart, of 1 mg of antigen emulsified in complete Freund's adjuvant. The animals were bled several times 1–3 wk after the second injection.

Burro IgG was isolated from these antisera by precipitating it with 40% saturated $(NH_4)_2SO_4$ and eluting it from DEAE-cellulose with a pH 8.0 0.01 M Tris phosphate buffer. At 20 absorbancy units (280 nm)/ml this preparation gave a single arc on immunoelectrophoresis vs. a polyvalent rabbit antiserum to burro serum.

Antibodies to horse ferritin (Pentex Biochemical, 6 × recrystallized) and to burro IgG were raised in rabbits. Two injections of 0.5 mg of antigen in complete Freund's adjuvant were given 3 wk apart and an additional dose of antigen without adjuvant was injected intravenously 2 wk later. Bleedings were taken 1–3 wk later. The IgG was precipitated with 40% saturated $(NH_4)_2SO_4$ and eluted from DEAE-cellulose equilibrated with a pH 6.5 0.0175 M sodium phosphate buffer. The IgG was free of other serum proteins as judged by immunoelectrophoresis using a polyvalent goat anti-rabbit serum (Hyland Laboratories, Los Angeles, Calif.).

Antibodies were purified by passing burro or rabbit IgG over columns containing antigen covalently coupled to Sepharose. The proteins were coupled by a method described previously (16): Settled Sepharose 2B (Pharmacia Fine Chemicals Inc.) and an equal volume of distilled water were adjusted to pH 11 with NaOH. 1 g of cyanogen bromide dissolved in 15 ml of water was added for every 10 ml of settled Sepharose, and the pH maintained at 11 for 3–4 min with constant stirring. The Sepharose was washed rapidly with large volumes of ice-cold 0.1 N $NaHCO_3$ on a sintered glass funnel. It was then resuspended in 0.1 N $NaHCO_3$ and adjusted to pH 8.6. Antigen was added and the mixture stirred for 16 hr at 4°C. The coupled Sepharose was washed, resuspended in an appropriate buffer, and stored at 4°C.

Using ^{125}I-labeled Cohn FrII (17) it was found that at 5 mg protein/ml settled Sepharose, maximal coupling (94–97%) of the added protein occurred. Ferritin was only incompletely coupled at this concentration and we therefore used only 1 mg protein/ml Sepharose to prepare the ferritin adsorbents. Sepharose-coupled ferritin released considerable brown color when eluted with acid or urea solutions. This could have been because of breakdown of the ferritin or noncovalent interaction between the ferritin and the Sepharose. For this reason the ferritin-Sepharose columns were washed extensively with acid or urea solutions just before using the adsorbents for antibody purification.

We determined the optimal conditions for eluting specific antibody from the antigen columns using ^{125}I-labeled proteins. The amount of active antibody in the starting and purified preparations was assessed by precipitin analysis. The unfractionated IgG preparation of burro anti-human IgG contained about 17% precipitable antibody. When such an IgG preparation was applied to human IgG-Sepharose columns, 25% of the protein was retained. After washing

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[1] *Abbreviations used in this paper;* Cohn FrII, Cohn fraction II; PBS, phosphate-buffered saline.

the adsorbent with buffer, various dissociating solvents were tried in separate tests. With pH 2.7–2.8, 0.05 M glycine-HCl buffer or neutral 4 M urea about 20% of the adsorbed protein was released. 85% of this eluted protein was precipitable by antigen. 8 M urea removed 40% and 6 M guanidine-HCl, 68% of the adsorbed protein, but in the former case only 60% of the material was active and in the latter instance none of it was. Therefore, for burro IgG either 4 M urea or pH 2.7, 0.05 M glycine-HCl buffer was used to release bound antibody.

To further restrict the specificity of the affinity column-purified burro anti-IgG, the latter was passed successively over columns of Sepharose-coupled light chain and IgE. Similarly anti-IgE preparations were passed over light chain and IgG columns.

Rabbit ^{125}I-labeled IgG, 25% of which was anti-ferritin antibody by precipitin analysis, was applied to Sepharose-ferritin affinity columns and 33% of the protein was retained. This could not be eluted from the ferritin-coupled Sepharose with 0.05 M glycine buffers at pH 2.1–2.8. Increasing the ionic strength of the buffer to 0.3 M at pH 2.5 had no effect. 4 M urea removed 30% of the adsorbed IgG, 6 M urea, 35%, and 8 M urea, 44%. 80% of the protein removed by 8 M urea was specifically precipitable. We decided to use 4 M urea for all elutions of rabbit antibodies since this was the mildest treatment which still gave reasonable yields.

Preparation of Hybrid Antibody.—The procedure used for the production and isolation of antibody hybrids was based on the reports of Nisonoff and Rivers (18) and Hammerling et al. (19). Purified antibody was dialyzed against pH 4.5, 0.07 M sodium acetate, 0.05 M NaCl. 2% (w/w) pepsin was added, and after 16 hr at 37°C digestion was stopped by raising the pH to 8 with 1 N NaOH. The digestion mixture was chromatographed on Sephadex G200. The initial peak represented $F(ab')_2$ fragments as judged by Ouchterlony analysis with an antiserum which detected Fab and Fc determinants. From the 280 nm absorbancy we calculated that a yield of about 50% of theoretical yield was obtained. The $F(ab')_2$ preparations of rabbit anti-burro IgG and anti-ferritin were mixed and dialyzed against a pH 5, 0.07 M sodium acetate, 0.05 M NaCl buffer. The protein concentration at this point was about 7 mg/ml. 2-Mercaptoethylamine was added to a final concentration of 0.015 M and the mixture incubated under N_2 for 1 hr at 37°C. The solution was applied to a column of Dowex AG50Wx2 (Dow Chemical Co., Midland, Mich.) to remove the reducing agent and eluted with the pH 5, 0.07 M sodium acetate, 0.05 M NaCl buffer. The protein solution (2.5 mg/ml) was brought to pH 7.0 with 1 N NaOH and stirred under 1 atmosphere of O_2 for 3 hr at 25°C. After concentrating the solution it was chromatographed on Sephadex G100. The major peak at about 40% bed volume contained the reconstituted $F(ab')_2$ fragments. The yield was 16% of the original undigested antibodies, i.e., about 25% of the theoretical yield.

Antigen-Sepharose columns were employed to isolate those $F(ab')_2$ fragments with hybrid specificity. The mixture was first passed over a burro IgG column and the specifically adsorbed fragments eluted with 4 M urea. After dialysis against buffer the partially purified preparation was passed over a ferritin column and the bound fragments again released with 4 M urea. Ouchterlony analysis performed after each step confirmed that the adsorptions were effective. The final product did contain some anti-ferritin antibodies. These may have resulted from our use, in the last step, of a ferritin-Sepharose column which contained intact rabbit anti-ferritin antibodies from a previous purification procedure. The undesired antibody was removed by reapplying the entire solution on a burro IgG column and eluting the bound antibody with 4 M urea.

This last step markedly decreased our final yield which over-all was only 0.25%; however, the desired preparation appeared to be highly specific, precipitating only with a mixture of burro IgG and ferritin but not with either antigen alone. The hybrid antibody isolated was sufficient for about 15 separate experiments. Considerably better yields might have resulted had we eliminated the step at which bifunctional anti-ferritin antibodies were removed. Presumably the latter antibodies would not interfere with the reaction of hybrid antibody at the cell surface or the subsequent reaction of ferritin with hybrid antibody.

Preparation of Cells.—Basophils represent at most about 1% of the cells in preparations of leukocytes from normal individuals. By combining several known techniques (20–22), Pruzansky and Patterson (10) were able to enrich such preparations so that they contained 5–20% basophils. We had similar success with their method and were able to obtain preparations sufficiently concentrated in basophils to make the electron microscopic studies practical.

Blood was drawn from one of the three normal adult male donors (A.S., H.M., M.J.) just before cell preparation. 60–80 ml containing 10 units heparin/ml were mixed with an equal amount of 6% clinical dextran (w/v) in saline (Abbott Laboratories, North Chicago, Ill.). Erythrocytes were allowed to settle for approximately 60 min and the supernatant containing leukocytes and platelets was removed and centrifuged at 1000 *g* for 10 min. The supernatant dextran-plasma was removed and the cell pellet resuspended in 2 ml of dextran-plasma. After adding 20 μl 0.12 M pH 7.4 sodium ethylenediaminetetraacetic acid (EDTA), the cell suspension was layered on 2 ml of a mixture consisting of 10 volumes of 34% Hypaque (sodium diatrizoate, Winthrop Laboratories, New York) and 24 volumes of 9% Ficoll (polysucrose, Pharmacia Fine Chemicals Inc.). The sample was centrifuged at 400 *g* for 40 min. The layer of cells at the interface was removed, suspended, and centrifuged twice in pH 7.4 phosphate-buffered saline (PBS). The cells were resuspended in 2 ml of dextran-plasma. After 10 min, elution with 40–60 ml of dextran-plasma removed erythrocytes and lymphocytes. Elution was then carried out with 100 ml of 8×10^{-4} M sodium EDTA in PBS to remove the basophil-enriched fraction

Sensitization of Cells.—For sensitization with IgE, the basophil-enriched cells were washed twice and suspended in 0.5 ml PBS containing 50 μg IgE. This and subsequent incubations were carried out for 10 min at room temperature or 0°C as noted. The cells were washed twice in 10 ml of PBS and resuspended in 0.5 ml PBS containing 100 μg specific burro anti-IgE. After incubation the cells were washed twice and resuspended in 0.5 ml PBS containing 50 μg hybrid anti-burro IgG anti-ferritin. After 10 min incubation with the hybrid antibody, the cells were suspended for a further 10 min in 2 ml PBS containing 1.0 mg ferritin. They were then washed twice in PBS before preparation for electron microscopy. Basophil-enriched preparations were similarly sensitized without prior addition of IgE, with ferritin alone, and with human IgG (370 μg IgG_{War} or 200 μg Cohn FrII), 200 μg specific burro anti-IgG, 50 μg hybrid antibody, and 1 mg ferritin.

Preparation of Cells for Electron Microscopy.—The sensitized cells in 1 ml of PBS were centrifuged in pyramidal tipped plastic capsules, and the fixation and embedding was done directly in these capsules to conserve the small pellets. The cells were fixed in 3% glutaraldehyde buffered with 0.1 M phosphate buffer at pH 7.4 for 2 hr at 4°C, washed in 7% sucrose buffer for several hours and treated with 1% osmium tetroxide for 1 hr before dehydration in graded ethanol solutions and embedding in Luft's Epon formula. 600–1000 A sections were obtained with an automatic ultramicrotome, collected on copper mesh grids, and stained with 10% uranyl acetate in methanol and with saturated lead citrate. Sections were coated with carbon before examination in a Hitachi HU 11 E electron microscope (Hitachi Ltd., Tokyo, Japan) at original magnifications of × 4500–28,000.

Quantitation of Serum IgE.—This was performed by Doctors Stephen Polmar and Thomas Waldmann of the National Cancer Institute.

RESULTS

In ultrathin sections examined by electron microscopy, sections of basophils may be distinguished from sections of other leukocytes on the basis of their distinctive cytoplasmic granules. These granules are heterogeneous in size and electron opacity, and they contain distinct punctate particles (23). By com-

parison, neutrophil granules are relatively small and uniform in electron opacity, while eosinophil granules are generally larger and more electron-opaque and are characterized by a crystalline core (24).

Despite relatively long processing of cell preparations in our experiments, the internal structure of cells was generally maintained and basophils were readily identified by scanning sections at × 4500. Each basophil section and 5–10 randomly selected control cell sections in the same grid square were then examined at higher magnifications to search for ferritin particles. As shown previously, ferritin particles used as markers in the hybrid antibody technique can be identified by their uniform size, electron opacity, and even distribution along cell surface membranes (19).

In the first group of experiments, carried out at room temperature, basophil-enriched cell fractions were treated directly with burro anti-IgE, the hybrid antibody (anti-burro IgG and anti-ferritin), and ferritin. Under these conditions, no ferritin was observed on platelets or leukocytes other than basophils. The binding to basophils of the three different subjects appeared to differ in extent. Approximately one-third of the basophil sections from individual A. S. had patches of linearly distributed surface-bound ferritin. Surface-bound ferritin was observed only rarely in sections of the basophils from M. J. and typical linear distribution was restricted to less than $\frac{1}{20}$ of the cell circumference. The cells of H. M. showed an intermediate amount of surface-bound ferritin. Serum IgE was 0.08 μg/ml in A. S., 0.15 μg/ml in M. J., and 0.88 μg/ml in H. M.

In the second group of experiments carried out at room temperature, cell preparations from the three normal donors were preincubated with myeloma IgE before successive incubations with anti-IgE, hybrid antibody, and ferritin. In this system, ferritin was localized on 80–90% of basophil sections from all three donors. A trilaminar "unit membrane" was resolved with ferritin bound in a linear fashion (Fig. 1). 20–50 neutrophil sections, one or two eosinophil sections, and numerous monocyte or lymphocyte sections from each preparation were also examined, but surface-bound ferritin was never observed on these cells. In every case, however, about 10% of the platelet surface profiles had one or more small patches of ferritin. In contrast to the appearance of ferritin on basophil surfaces, platelet-bound ferritin was not evenly distributed and appeared to be "piled up" in small patches.

As a further test for specificity of the reaction with IgE, basophil preparations from A. S. and H. M. were incubated with monomeric Cohn FrII and myeloma IgG, respectively. They were then reacted successively with burro anti-IgG, hybrid antibody, and ferritin. In these experiments, no ferritin fixation was observed on basophils, other polymorphonuclear leukocytes, monocytes, or lymphocytes. A basophil preparation incubated with ferritin alone also showed no fixation of ferritin.

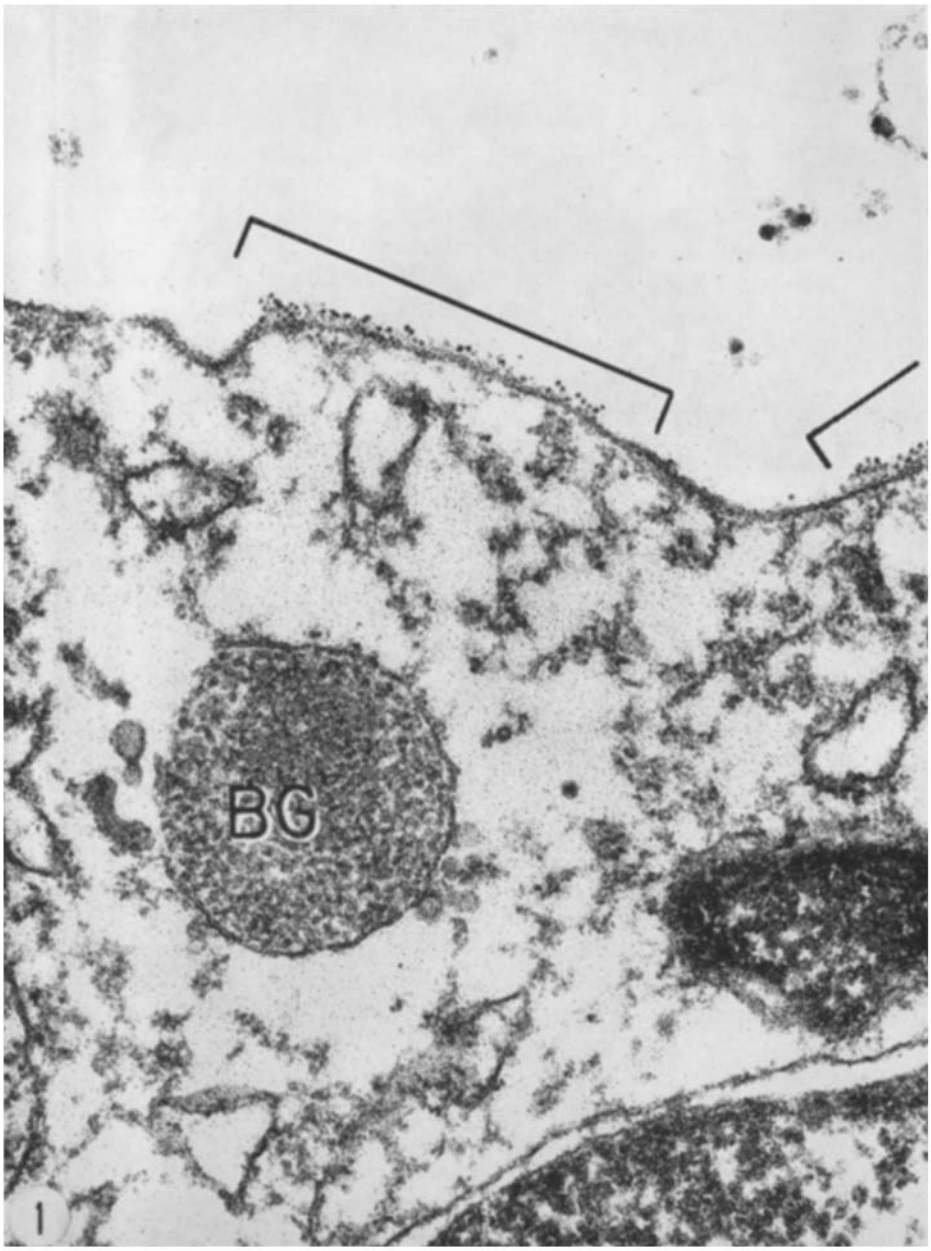

FIG. 1. Patches of ferritin (bracketed) bound to surface of a human basophil after reaction with IgE, anti-IgE, and hybrid antibody (anti-burro IgG and anti-ferritin). Ferritin particles are evenly distributed at a distance of about 200–300 A from the basophil surface membrane. Note the typical basophil granule (BG). × 77,500.

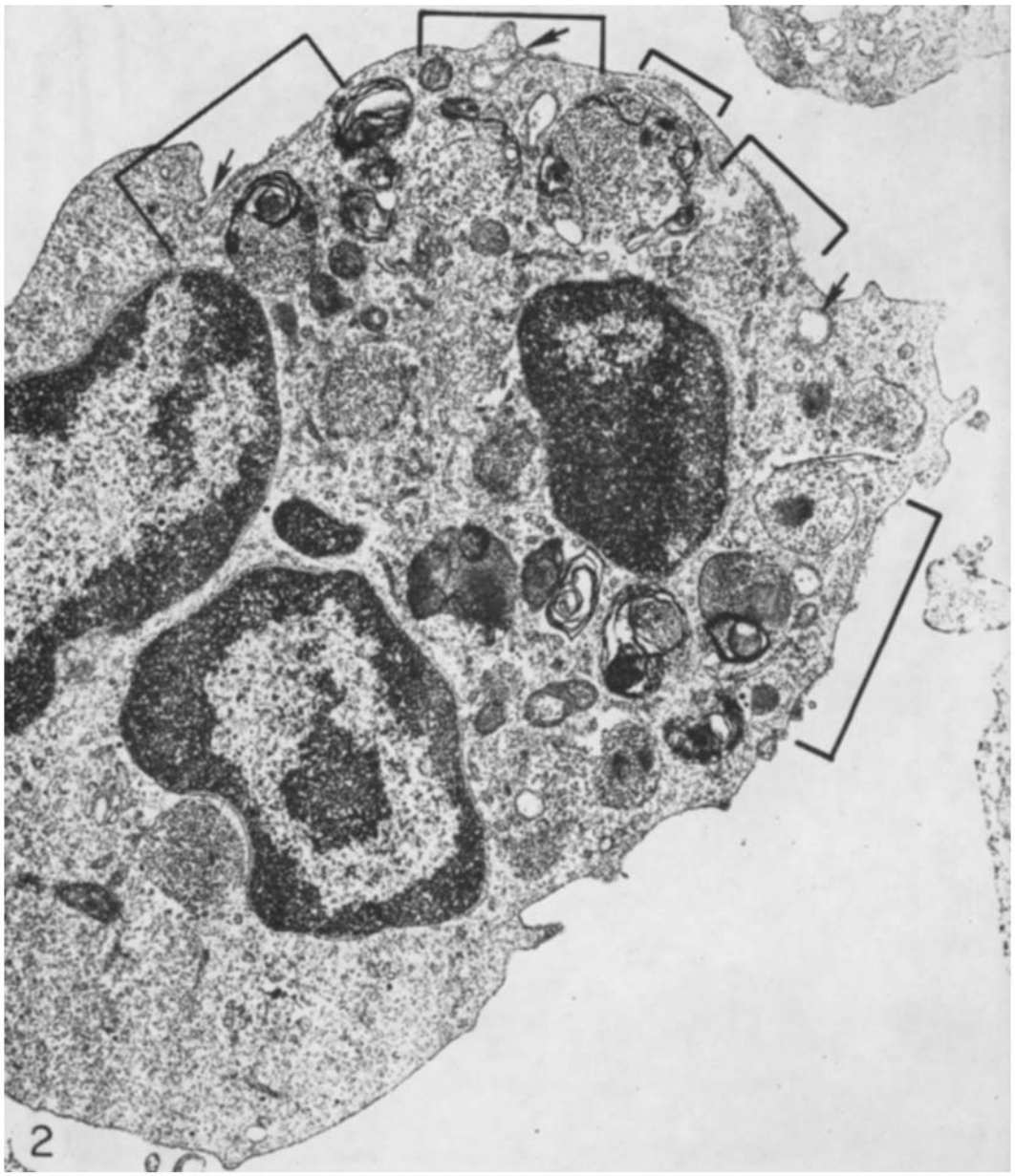

FIG. 2. Section of human basophil reacted with IgE, anti-IgE, hybrid antibody, and ferritin at room temperature. Bound ferritin is distributed in patches (bracketed) which appear restricted to a part of the cell circumference. Note that the membrane-bound ferritin is also present along surface invaginations (arrows) which are seen in longitudinal or cross-sections. × 20,700.

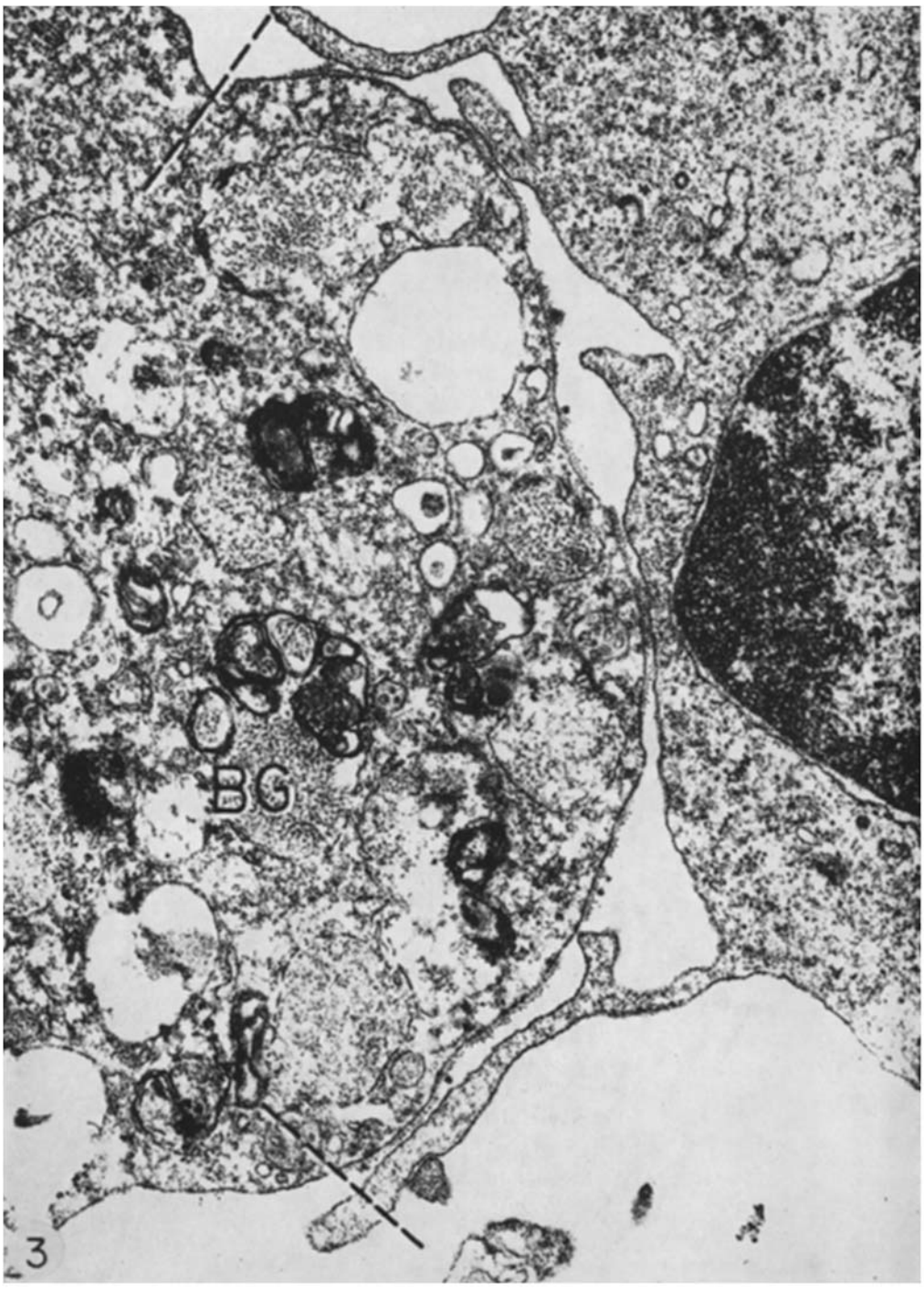

FIG. 3. Enlarged portion of another basophil reacted with IgE, anti-IgE hybrid antibody, and ferritin at room temperature. Note that evenly distributed ferritin is almost continuous between the dashed lines. × 32,500.

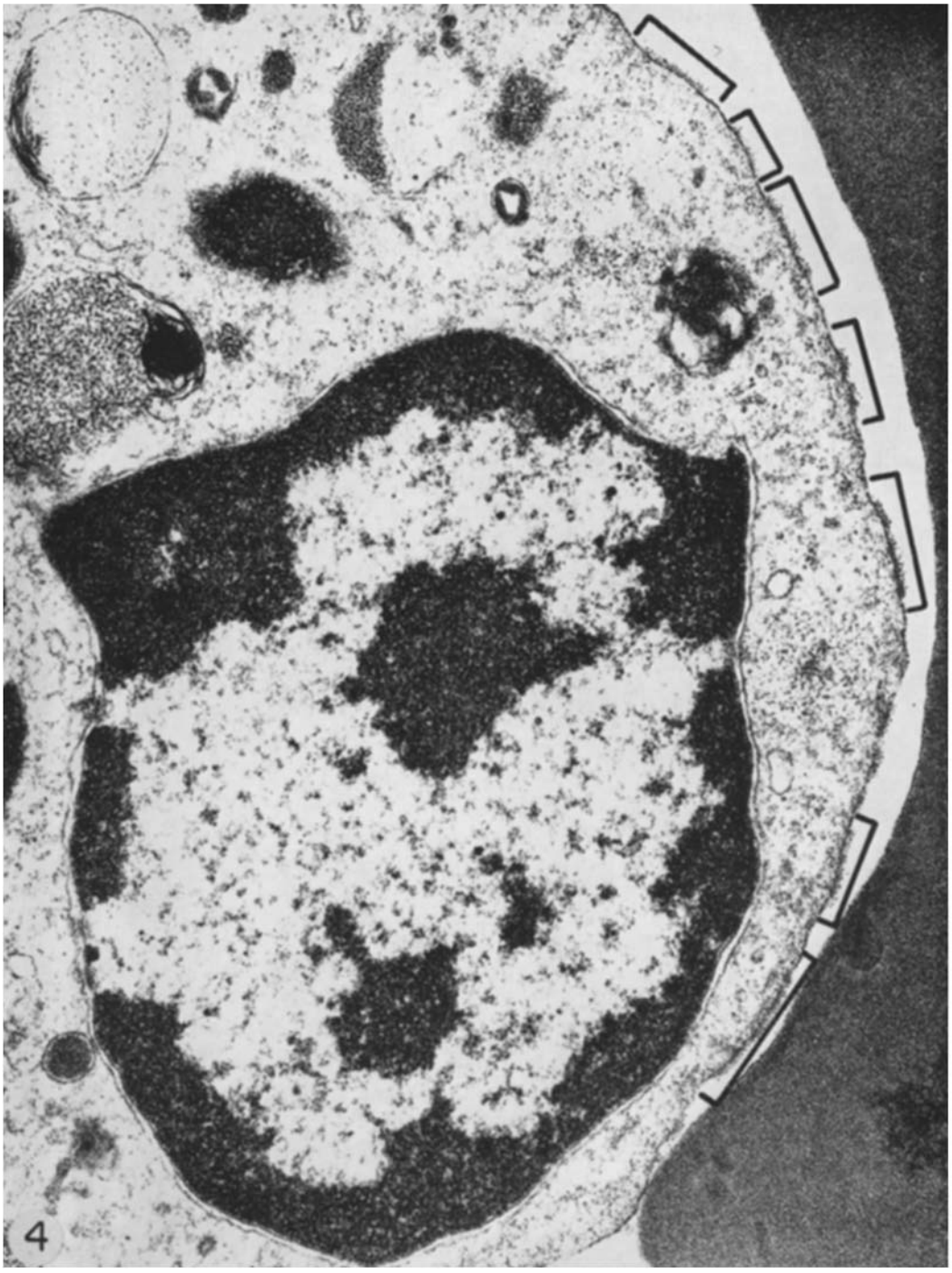

FIG. 4. Periphery of human basophil reacted with IgE, anti-IgE, hybrid antibody, and ferritin at 0°C. Ferritin label (bracketed) has a patchy distribution and was observed around the entire circumference of this cell section. × 32,500.

In the initial experiments performed at room temperature, the amount and distribution of ferritin on basophils from all three donors was similar when cells were preincubated with myeloma IgE. There was, however, a different distribution of ferritin on basophils incubated at 0°C with the same reagents and kept at this temperature until fixation. This temperature effect was observed in basophils from all three donors. As illustrated in a typical basophil section from cells prepared at room temperature (Fig. 2), all the ferritin was located toward one pole of the surface membrane profile. In most of the labeled cell profiles, ferritin covered an estimated one-fifth to one-half of the circumference. When invaginations of a portion of the cell surface were observed, as was the case in about one-fourth of the basophil sections, ferritin was invariably located over that region and in invaginations (Fig. 2). The linear pattern of bound ferritin was continuous for long stretches in some sections (Fig. 3) but was more patchy in others (Fig. 2). 10–20% of basophil sections revealed no bound ferritin and rare sections showed ferritin around the entire circumference or at opposite poles.

In contrast, most basophil sections from cells prepared at 0°C revealed the patchy pattern of ferritin binding to the surface membrane (Fig. 4), and the patches were usually distributed around the entire circumference of the cell section. In less than one-quarter of the basophil sections ferritin was restricted to a single stretch of the surface membrane as more commonly observed with basophils prepared at room temperature.

In a single experiment effects of temperature were compared. Basophil-enriched cells (M. J.) were incubated with myeloma IgE, anti-IgE, hybrid, and ferritin at 0°C. The cells were then divided; one-half were incubated at 37°C for 10 min before fixation while the other half remained at 0°C until fixation. The cells kept at 0°C until fixation revealed ferritin distributed in patches around the entire circumference of most basophil sections whereas most basophil sections from the cells warmed to 37°C revealed ferritin bound to a limited stretch of the surface membrane as noted in experiments carried out entirely at room temperature.

DISCUSSION

Our results with hybrid antibody labeling confirm and extend recent observations with radioautographic labeling that IgE associates preferentially with basophils (12). We elected to create hybrid antibody with anti-ferritin and anti-IgG specificities since Hammerling et al. have stressed that this technique provides a uniform labeling of cell surface antigens (19).

In our experiments, the immunoferritin labeling appeared remarkably specific. In the absence of preincubation with IgE, only basophils gave evidence of IgE on the surface membrane. Basophils again were the only leukocytes with evidence of surface-bound IgE after preincubation with myeloma

IgE; however, piled-up patches of ferritin were noted on about 10% of platelet sections. These patches were seen only in cell preparations preincubated with myeloma IgE, and never in those preparations preincubated with myeloma IgG or ferritin alone. It is not clear whether these ferritin patches represent IgE bound to specific platelet receptors or a nonspecific interaction since the ferritin was never distributed in the linear arrangement noted with basophil sections.

We were unable to detect fixation of IgG to any leukocyte. Others have reported fixation of IgG to neutrophils and monocytes using methods different from that employed in this study (12, 25, 26). In most studies antigen-antibody aggregates have been used, a situation which appears to favor fixation to leukocytes (27). Since we used monomeric IgG, our experiments are not strictly comparable and this difference may account for our negative results.

The amount of endogenous IgE on the basophil surface appears to vary between individuals. Basophils from three nonallergic individuals showed significant differences in the amount of surface-bound IgE. This did not correlate with the serum IgE levels of the three.

After preincubation with myeloma IgE, basophils from the three subjects were remarkably similar in appearance. 80–90% of the basophil sections showed evidence of IgE on the surface. The peripheral leukocytes of only about 20% of the normal population appear to be capable of passive sensitization with IgE for anti-IgE–induced histamine release (4, 5). Although radioautographic studies with IgE-^{125}I have failed to demonstrate differences in binding between responder and nonresponder basophils (12), the ferritin antibody method should be able to detect more subtle quantitative differences. In our limited sample there was a probability of less than 1% that the cells of all three randomly selected donors would fall among the 20% responder population, $(\frac{1}{5})^3$, but a probability of 50% that all three would be in the 80% nonresponder population, $(\frac{4}{5})^3$. The similar amount of IgE bound by the basophils of the three subjects in this study would therefore most likely represent an amount insufficient to mediate histamine release if this capacity does correlate with the amount of basophil-bound IgE.

The distribution of IgE on basophils appeared to be temperature dependent. Basophil sections from preparations incubated with IgE, anti-IgE, hybrid, and ferritin at 0°C usually had surface patches of ferritin around the entire circumference of the cell. On the other hand, basophil sections from cell preparations incubated with the reagents but at room temperature usually had all ferritin localized to one segment of the basophil surface profile. In about one-fourth of the sections prepared at room temperature invaginations of the surface membrane were seen and the ferritin was invariably localized to this region. It is of interest that mouse lymphocytes treated with fluorescent anti-immunoglobulin reagents demonstrate a temperature-dependent distribution

of fluorescence and hence of the surface immunoglobulin molecules to which fluorescent antibodies are attached (28). At 4°C a diffuse speckled distribution is seen which becomes much more polar as the cells are warmed to room temperature or higher. It is also of interest that the IgE localization in that area of the cells showing invaginations may have its counterpart in these experiments on the surface immunoglobulins of mouse lymphocytes. The role of anti-IgE in this temperature-dependent localization is now under investigation.

The method used in this study is not ideally suited to determine the number of IgE molecules which can be bound to a basophil. Such an estimate could be more readily made by directly quantitating the amount of radiolabeled IgE which could be adsorbed by a cell preparation of accurately known basophil content. We can, however, make an estimate of the upper limit by assuming that each ferritin molecule is indirectly attached to a discrete IgE molecule. In those areas where the ferritin particles were evenly and linearly distributed there were about 8 ferritin molecules/1000 A. Since the depth of focus was at least 600 A there could have been as many as 8 IgE molecules/6×10^5 A^2. The diameter of a basophil is about 10μ (29). Assuming a spherical shape, the surface area should be 3×10^{10} A^2. Thus if the total surface area of the basophil were homogeneously covered with IgE receptors there could be as many as 4×10^5/basophil. It seems likely that more than one ferritin particle was bound per surface IgE, and we did not find a homogeneous distribution of ferritin over the cell surface. It is probable, therefore, that some lesser number of IgE molecules is bound by a cell under the circumstances used in these studies. While the number could easily be an order of magnitude less it is unlikely to be two orders of magnitude less.

SUMMARY

We have examined human leukocyte preparations for the presence of surface-bound IgE by electron microscopy. Basophil-enriched leukocytes were reacted with burro anti-IgE, a hybrid antibody to burro IgG and ferritin, and ferritin, with or without prior incubation of the cells with an IgE myeloma protein.

In the absence of preincubation with IgE small amounts of ferritin were fixed to the surface of basophils but on no other cells. When cells were preincubated with IgE the amount of ferritin fixation on the basophils was markedly increased and a small amount of ferritin was also bound to platelets but again to no other leukocytes. The distribution of ferritin on the basophil surface appeared dependent upon the temperature at which the cells were kept during and after reaction with the various reagents. Basophil sections from cells kept at 0°C had ferritin bound to the surface membrane in patches distributed around the entire circumference. Basophil sections from cells prepared at room temperature had ferritin distributed assymetrically covering a surface membrane segment one-fifth to one-half of the circumference.

In control studies in which monomeric IgG was substituted for the IgE and burro anti-IgG was used instead of burro anti-IgE, no cellular fixation of ferritin was observed.

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