SURFACE IgE ON HUMAN BASOPHILS DURING HISTAMINE RELEASE*

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Human basophils bind IgE to their surface membranes (1, 2) and release histamine when incubated in vitro with anti-IgE antibodies or with antigen(s) to which the surface IgE is directed (3, 4).

In a previous study it was demonstrated that the surface IgE could be distributed either in a uniform diffuse pattern or in discrete patches and polar "caps" (2). IgE, detected with an immunoferritin stain, was also observed in pinocytotic vesicles under conditions where cap formation was prominent.

We now report experiments in which the relationship between shifts in the distribution of the surface IgE and release of histamine induced by anti-IgE or by ragweed antigen was explored. The distribution of cell surface IgE was assessed by fluorescence microscopy using fluorescein conjugated anti-IgE and by immunoferritin electronmicroscopy using anti-IgG, antiferritin hybrid antibodies. Histamine release was modulated by varying the concentration of anti-IgE or antigen and by varying the conditions of incubation (time, temperature, presence, or absence of Ca++ and Mg++); and the effect of these factors on redistribution of surface IgE was studied.

Materials and Methods

Cell Preparations.--50-60 ml blood was drawn from subjects and diluted two- to fourfold with physiological saline and anticoagulated with 0.01 M EDTA. 8–10 ml portions were layered over 3 ml Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, N. J. and Winthrop Laboratories, New York) (density 1.077) in a 13 × 125 mm tube and centrifuged at room temperature for 40 min at an average of 310-390 g at the interface (5). The interfacial cell band (mononuclear cell fraction) was aspirated and washed three times with "Tris A" buffer

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Basophils and total nucleated cells were counted in a Spire-Levy counting chamber by a method described in a previous article (6). In most experiments, $10^7$ cells were then suspended in 5 ml Tris A buffer containing either 0.01 M EDTA or 0.0006 M Ca$^{++}$ and 0.001 M Mg$^{++}$. The desired antibody preparation was added and after suitable incubation periods the cells were centrifuged at 230 g and the supernatants saved for histamine analysis.

Basophils were obtained from both atopic and nonatopic individuals. Leukocytes from most individuals released over 50% of total histamine upon exposure to an optimal concentration of anti-IgE, but the cells from some donors did not release a significant amount of histamine.

For the electronmicroscope studies, the cells were incubated with rabbit IgG anti-IgE antibody and were then similarly washed two times and incubated at 0°C for 20-30 min with 20-50 $\mu$g of hybrid (anti-rabbit IgG-antiferritin) antibody. The cells were centrifuged and suspened in 1 ml buffer containing 0.5 mg ferritin. After 20-30 min at 0°C the cells were washed two times and fixed with 3% glutaraldehyde in Sorenson's phosphate buffer pH 7.4.

**Fluorescence Microscopy.**—Cell suspensions were dropped on cold slides and a coverslip applied. The slides were then sealed with nail polish. The cells were observed either with a Leitz Orthoplan microscope (E. Leitz, Inc., Rockleigh, N. J.) equipped with an Osram HB 200 mercury lamp, BG38 and KP490 exciting filters, darkground condenser and K530 suppression filters or with a Leitz Orthoplan microscope equipped with Phloem incident light transmission and the same exciting and suppression filters. Photomicrography was performed using Kodak Tri-X (Eastman Kodak Co., Rochester, N. Y.) and exposures of 30-60 s.

**Electronmicroscopy.**—After fixation in glutaraldehyde, the cell pellets were washed with 0.25 M sucrose phosphate buffer, fixed in 1% OsO$_4$, dehydrated in ethanol, and embedded in Luft’s Epon (Luft Instruments, Inc., Lincoln, Mass.). 60-100 nm sections were cut on an LKB “Ultratome” (LKB Instruments, Inc., Rockville, Md.) and the grids stained with 10% uranyl acetate in methanol, saturated lead citrate, and then carbon coated. Grids were examined on a Hitachi HU11E electron microscope (Hitachi American Ltd., Indianapolis, Ind.).

**Histamine Assays.**—The amount of histamine released was measured by the spectrophotofluorometric technique of Shore (7) as modified by Lichtenstein and Osler (8).

**Sheep Anti-IgE Antibody.**—Adult sheep were immunized sequentially at 3-wk intervals. The first two injections consisted of 1 mg purified IgE myeloma protein (PS) (9) in complete Freund’s adjuvant injected intramuscularly. The third injection consisted of $3^1_2$ mg IgE in phosphate-buffered saline injected intravenously. Blood was collected 7–10 days after the last injection and the serum was stored at $-70^\circ$C.

10 ml of the whole serum were applied to a 10 ml Sepharose-IgE column containing approximately 50 mg of IgE. After incubation for 1 h at room temperature the column was washed extensively with borate buffer. The bound antibodies were then eluted with 0.1 N acetic acid and the protein eluate neutralized with Tris and concentrated. Approximately 100 mg of protein was recovered. This preparation was then applied to a 10 ml Sepharose-IgG column equilibrated with borate buffer and containing approximately 50 mg of purified human IgG. The protein containing effluent was pooled and reapplied to the same column. The final effluent contained about 70 mg of protein. The specificity was checked by using a tanned red cell microhemagglutination technique (10). At a concentration of 8 mg protein/cubic centimeter, the purified anti-IgE gave a hemagglutination titer of 1:8 against human IgG and 1:50,000 against human IgE. 85% of this purified protein was specifically bound when re-applied to the IgE immunoadsorbent.

**Rabbit Anti-IgE Antibody.**—Rabbit anti-IgE antiserum was obtained by immunization with the Fc fragments of the IgE myeloma protein (PS) included in complete Freund’s adjuvant (9). The antiserum was absorbed with Bence Jones protein obtained from the myeloma patient and with the Fab fragment of the PS protein. The absorbed antiserum did not give a precipitin band with human immunoglobulins of the four other heavy chain classes. The anti-
serum was then applied to a Sepharose immunoadsorbent column coated with the PS myeloma protein and the bound antibodies were eluted with glycine-HCl buffer, pH 3.0. After concentration, precipitating antibody in the purified antibody preparation was quantitated by quantitative precipitin analysis using the Fe fragment of the PS myeloma protein as antigen. The antibody comprised 70% of total protein in the preparation.

The specifically purified antibody was digested with crystalline papain by the method of Porter (11). The antibody containing 4.2 mg protein in 2 ml of 0.1 M phosphate buffer, pH 7.0, was incubated with 0.04 mg of crystalline papain in the presence of 0.01 cysteine and 2 mM EDTA. After digestion at 37°C for 6 h, the digestion mixture was applied to a Sephadex G100 column (2 X 30 cm) to remove undigested protein. Fractions corresponding to the major 3.5 S peak were pooled and concentrated. The fragment of anti-IgE and undigested antibody were examined for their ability to induce reversed PCA reactions in the monkey (12). Normal *macaca irus* received intracutaneous injections of 0.05 ml of the undigested antibody and of the 3.5 S fragment followed by an intravenous injection of Evans blue. 0.02 μg/ml of undigested antibody gave definite skin blueing, whereas even 20 μg N/ml of the 3.5 S fraction failed to do so.

**Sheep Hybrid Antirabbit IgG-Antihorse Ferritin Antibody.—** Separate sheep were immunized with purified rabbit IgG and horse ferritin according to the same schedule used for preparing anti-IgE sera. The sera were stored at -70°C.

Antirabbit-IgG was adsorbed onto a Sepharose rabbit-IgG column containing approximately 5 mg IgG per milliliter of Sepharose. After extensive washing with borate buffer the bound antibody was eluted with 0.1 N acetic acid and the eluate neutralized with Tris. Approximately 7-8 mg of antibody were recovered per milliliter of sheep serum.

Antiferritin antibodies were purified in the same way except that the Sepharose-ferritin adsorbent contained only 1 mg ferritin per milliliter of Sepharose. Again 7-8 mg of antiferritin antibody were recovered from each milliliter of sheep serum. In both cases 93-99% of the protein was specifically bound when small portions were reapplied to the appropriate immunoadsorbent.

Both antibody preparations were digested with pepsin and the F(ab′)2 fragments recovered in the same way in separate experiments (13, 14). 250 mg of protein (10 mg/ml) was dialyzed into a 0.15 M Na acetate, 0.05 M NaCl, pH 4.5 buffer. 2% (wt/wt) pepsin was added and the solutions incubated at 37°C for 16-18 h. The digestion was stopped by bringing the pH to 8 with 1 N NaOH. The digestion mixture was next applied to a 390 ml (3.0 X 82 cm) column of G150 Sephadex equilibrated with borate buffer. The main component eluted at approximately 50% bed volume. It gave a strong precipitin line on gel diffusion against the appropriate antigen before but not after reduction and alkylation. Yields averaged 50%. The F(ab′)2 fragments of the anti-IgG and antiferritin (at 10 mg/ml) were mixed and dialyzed against 0.1 M Na acetate pH 5.0. The solution was made 0.015 M in mercaptoethylamine and incubated for 1 h at 37°C. The solution was then applied to a AG50 X 2 ion exchange column (90 ml) equilibrated with the same Na acetate buffer. Approximately 70% of the applied protein was recovered. The reduced proteins were brought to pH 7.0 with 1 N NaOH and gently stirred at room temperature for 2 h under O2. The solution was next applied to a 550 ml (3 × 80 cm) column of G100 Sephadex equilibrated with borate buffer. Approximately 130 mg of protein eluted at 50% bed volume [F(ab′)2] and 32 mg at 50% bed volume (Fab′).

One-half of the F(ab′)2 material was processed at a time. It was first applied to a Sepharose-ferritin column. After washing with borate about 57% of the applied material was recovered with the 0.1 N acetic acid elution. This should have consisted of antiferritin-antiferritin and antiferritin-anti-IgG hybrid molecules. This mixture was applied to a Sepharose-rabbit IgG column and the specifically bound protein eluted with 0.1 N acetic acid and neutralized as before. This material (9 mg) precipitated with a mixture of rabbit IgG and ferritin but with neither antigen alone. The overall yield calculated on the basis of the amount of intact antibody used was 8%. The hybrid antibody preparation was stored at 4°C in borate buffer.
Fluorescein Coupled Anti-IgE.---Because sheep IgG does not elute well from DEAE-cellulose, F(ab')2 fragments were prepared from the specifically purified anti-IgE. The procedures were identical to those described under the previous section. The F(ab')2 fragments (45 mg) were applied to a 25 ml column of DEAE-cellulose equilibrated with 0.01 M Na phosphate pH 7.5. 85% of the protein was recovered when the column was eluted with 0.01-0.03 M phosphate pH 7.5 buffer. The eluate was made 0.075 M in Na2CO3 (pH 9.3) and concentrated to 10 mg protein per milliliter. Fluorescein isothiocyanate powder was added to a final concentration of 0.05 mg/mg protein and the solution incubated at 4°C for 16-18 h. During the 1st h the pH was maintained at 9.3 by additions of small amounts of 1 N NaOH. The conjugated protein was then dialyzed against 0.01 M phosphate buffer and reapplied to a DEAE-cellulose column equilibrated with the same buffer. Fluorescein-conjugated protein was eluted at increasing ionic strengths. Generally those fractions with a 280 nm:495 nm absorbancy ratio of 0.6-1.7 (representing a fluorescein to protein molar ratio of 11-3:1) were used.

In several experiments intact sheep anti-IgE was fluoresceinated directly and the conjugated protein adsorbed onto DEAE-cellulose. To elute significant amounts of protein 0.1 M phosphate, 1 M NaCl was required. This protein had a 280 nm:495 nm absorbancy ratio of 0.71 (molar ratio 9.5:1). This material was then digested with pepsin to give the fluoresceinated F(ab')2 fragments. No obvious differences were observed when the alternatively prepared conjugates were employed. All fluoresceinated fractions retained their ability to release histamine.

For all studies in which Fab' fragments were used the latter were prepared from fluoresceinated F(ab')2 fragments. The divalent fragments were reduced with 0.005 M or 0.01 M dithiothreitol in 0.2 M, Tris-HCl, pH 8.6, incubated for 1 h at room temperature, alkylated with 0.011 M or 0.022 M iodoacetic acid and then eluted from a G100 Sephadex in order to recover the Fab' fragments. This material was then concentrated and reapplied twice to a G100 Sephadex column.

A DEAE fraction of otherwise unpurified rabbit anti-IgE was also fluoresceinated as above and subsequently purified by adsorption with an IgG-Sepharose column and elution from an IgE-Sepharose column. The specificity of this preparation for IgE on basophils was checked by electronmicroscopy using hybrid antirabbit IgG-antiferritin antibodies. Only basophils showed surface bound ferritin.

Immunosorbents.---Protein Sepharose columns were prepared essentially as described previously (2, 15). In brief, washed 2B Sepharose was reacted with 300 mg of cyanogen bromide per milliliter of packed Sepharose at pH 11 and at temperatures below 30°C. Upon completion of the reaction the Sepharose was washed rapidly with 0.1N NaHCO3 (pH 8.5) and then incubated with 5 mg protein per milliliter Sepharose at 4°C for 16-18 h. When ferritin was coupled only 1 mg protein per milliliter of Sepharose was used because the coupling efficiency of this protein is low in our experience (2). Approximately 90-99% of applied protein was bound to the Sepharose. The columns were equilibrated with borate buffer and kept at 4°C when not in use.

Miscellaneous.---Rabbit IgG (Cohn Fraction II, Pentex Biochemical, Kankakee, Ill.) was purified on DEAE-cellulose. Horse ferritin (six times recrystallized, Pentex) was used as such. Fluorescein isothiocyanate was obtained from Sigma Chemical Co., St. Louis, Mo., crystalline papain from Mann Research Labs Inc., New York and pepsin from Worthington Biochemical Corp., Freehold, N. J. Purified ragweed antigen (Fr IV-C, antigen E) was kindly supplied by Dr. M. Chase, The Rockefeller University, New York.

RESULTS

Characteristics of Cell Preparations.---When reacted with Trypan Blue 95% or more of the cells excluded the dye. By toluidine blue staining (6) approximately 2-3% of the cells demonstrated typical basophilic granules. By Wright's
stain the differential count showed 2-3% basophils, 5-20% monocytes, and the rest lymphocytes.

Specificity of Reagents.—When treated with any of the fluorescent anti-IgE preparations, the percentage of stained cells agreed well with the percentage of basophils estimated by toluidine blue staining. In one experiment cells were first reacted with Fab' fragments of fluoresceinated rabbit anti-IgE. The washed cells were then sequentially reacted with sheep antirabbit IgG-antiferritin hybrid antibody and ferritin. By electronmicroscopy ferritin staining was observed only on basophils. Similarly when unfluoresceinated rabbit anti-IgE was used in the first step only basophils showed ferritin staining.

Effect of Monovalent Anti-IgE on the Distribution of Cell Surface IgE.—Cells were reacted with the Fab' fragments of fluoresceinated sheep anti-IgE. The cells exhibited diffuse staining of the surface membrane at most doses of antibody employed (0.1-12 µg/ml). With 24 µg/ml and a 90 min incubation at 37°C, about one-third of the fluorescent cells showed several more intensely stained spots which were small and discrete. Still only about 2% of the total cells were stained.

When basophils were observed in the electronmicroscope after having been incubated sequentially with Fab fragments of rabbit anti-IgE, hybrid antibody, and ferritin, the basophils had a diffuse pattern of labeling with ferritin scattered lightly in a more or less continuous pattern over the cell membrane. Capping was not seen. (See Figs. 1 and 2, top row.)

Studies with Divalent Anti-IgE.—Aliquots of the mononuclear cell fraction from each individual were incubated with various concentrations of fluorescein coupled anti-IgE for 30 min to 240 min at 37°C. These results are summarized in Table I. Basophils from two individuals shown in Table I did not release a significant amount of histamine at any of the anti-IgE concentrations tested. However, cells from all subjects exhibited the same dose and time dependence in their fluorescent staining patterns. Also, the percentage of fluorescent cells agreed well with the percentage of basophils estimated from the differential counts.

Low doses: When cells were incubated with 0.03-0.2 µg/ml of fluoresceinated divalent F(ab')2 fragments of sheep anti-IgE for 30 to 90 min, a diffuse staining pattern was observed (Fig. 1, first row). In most cases the circumference was continuously fluorescent with no evident heterogeneity. About 10% of the stained cells exhibited several areas with more intense fluorescence. The overall appearance of the cells was indistinguishable from that observed with the monovalent Fab' fragments. Photomicroscopy of cells stained diffusely with these low doses of anti-IgE was unsatisfactory due to weak fluorescence.

Cells incubated for 3½ to 4 h at 37°C with sheep antibody showed some patching and capping. Those cells which still showed diffuse staining more often had discrete intensely fluorescent spots superimposed on a diffuse background.
Fig. 1. Section of human basophil reacted with Fab fragments of rabbit anti-IgE, hybrid antibody, and ferritin at 37°C. Bound ferritin is distributed diffusely, and extensively along the cell membrane. Magnification × 40,000.
| Subject | Conc. a-IgE | Time | Histamine release | Staining pattern |
|---------|------------|------|-------------------|------------------|
|         | µg/ml | min | % |                  |                  |
| P. G.   | 0.028  | 30  | 35 | Too light to determine |
|         | 0.14  | 15  | 73 | 96               | 4                | 0               |
|         | 0.14* | 30  | 74 ± 3 | 98 ± 1 | 2 ± 1 | 0 | 0 |
|         | 0.14  | 90  | 70 | 96               | 4                | 0               |
|         | 0.14  | 220 | 70 | 46               | 24               | 30              |
| 0.28    | 30    | ND  | ND | 94               | 6                | 0               |
| 0.56    | 30    | 73  | 90 | 9                | 1                | 0               |
| 1.4     | 10    | 32  | 32 | 45               | 35               | 0               |
| 1.4†    | 30    | 44 ± 8 | 8 ± 1 | 75 ± 6 | 17 ± 7 | 0 |
| 1.4     | 90    | 47  | 0  | 38               | 46               | 17              |
| 1.4     | 220   | 50  | 0  | 6                | 86               | 9               |
| 14.0.§  | 30    | 10 ± 10 | 2 ± 2 | 38 ± 11 | 59 ± 10 | 0 |
| 14.0    | 90    | 10  | 1  | 15               | 66               | 19              |
| 14.0    | 220   | 11  | 0  | 4                | 82               | 14              |
| 140     | 30    | 4   | 2  | 36               | 62               | ND              |
| B. W.   | 0.005  | 30  | 4  | Too light to determine |
| 0.010   | 30    | 10  | Too light to determine |
| 0.05    | 30    | 33  | 99 | 5                | 0                | 0               |
| 0.10    | 30    | 48  | 95 | 5                | 0                | 0               |
| 0.50    | 30    | 68  | 68 | 31               | 1                | 0               |
| 1.0     | 30    | 52  | 8  | 75               | 17               | 0               |
| 10.0    | 30    | 30  | 1  | 16               | 70               | 13              |
| 100     | 30    | 17  | 0  | 7                | 45               | 48              |
| J. A.   | 0.057  | 30  | 68 | 100              | 0                | 0               |
| 0.14    | 30    | 60  | 100 | 0                | 0                | 0               |
| 1.4     | 30    | 36  | 0  | 83               | 17               | 0               |
| 14.0    | 30    | 10  | 0  | 36               | 61               | 3               |
| A. K.   | 0.058  | 30  | <10| 100              | 0                | 0               |
| 0.11    | 30    | <10 | 100 | 0                | 0                | 0               |
| 0.11    | 90    | <10 | 97  | 3                | 0                | 0               |
| 0.11    | 240   | <10 | 50  | 38               | 12               | 0               |
| 0.14    | 30    | <10 | 95  | 5                | 0                | 0               |
| 0.58    | 30    | <10 | 73  | 19               | 2                | 1               |
| 1.1     | 30    | <10 | 30  | 45               | 18               | 7               |
| 1.1     | 90    | <10 | 0  | 29               | 49               | 22              |
| 1.1     | 240   | <10 | 2  | 15               | 53               | 31              |
| 1.4     | 30    | <10 | 1  | 67               | 28               | 2               |
| 2.8     | 30    | <10 | 0  | 59               | 38               | 3               |
| 11.0    | 30    | <10 | 0  | 38               | 40               | 22              |
| 11.0    | 90    | <10 | 0  | 9                | 80               | 11              |
| 11.0    | 240   | <10 | 0  | 14               | 50               | 36              |
| 14.0    | 30    | <10 | 0  | 42               | 58               | 0               |
| K. B.   | 0.14   | 30  | <10| 94               | 4                | 0               |
| 1.4     | 30    | <10 | 4  | 62               | 29               | 5               |
| 12.0    | 15    | <10 | 0  | 25               | 69               | 6               |
| 12.0    | 30    | <10 | 0  | 17               | 59               | 25              |
| 12.0    | 60    | <10 | 0  | 12               | 64               | 24              |
| 12.0    | 240   | <10 | 0  | 5                | 79               | 15              |
| 14.0    | 30    | <10 | 1  | 23               | 63               | 8               |

ND, Not determined.

* Average and range of values from two separate experiments.

† Average and range of values from three separate experiments.

‡ Average and range of values from four separate experiments.
Cells reacted with 0.1-0.3 µg/ml of rabbit anti-IgE and subsequently treated with hybrid antibody and ferritin showed little or no labeling when examined by electron microscopy.

**Intermediate doses:** Cells incubated with 1–2 µg/ml of fluoresceinated sheep anti-IgE for 30 min at 37°C showed a patchy staining pattern (Fig. 2, second row). By electronmicroscopy appropriately treated cells also showed a patchy distribution of the ferritin. Pinocytotic vesicles containing ferritin were observed. Cells incubated for longer times showed a shift in the staining pattern from patch to cap formation and obvious internalization.

**High doses:** When cells were incubated at 37°C for 30 min with 10–100 µg/ml of fluoresceinated sheep anti-IgE, the labeled antibody was observed predominantly at one end of the cell covering less than 3/4 of the cell circumference (Fig. 2, third row). Increased internalization was observed with cells incubated for longer times (1½–4 h) and the total fluorescence per basophil appeared to be somewhat reduced. Such cells showed little or no surface staining but only multiple small round areas of cytoplasmic fluorescence. By electronmicroscopy capping and extensive staining of pinocytotic vesicles was observed in appropriately treated cells (Fig. 3). Even at these high doses only basophils were labeled.

In some experiments, cells were reacted with fluoresceinated anti-IgE in 0.01 M Tris-EDTA buffer, a buffer in which histamine release is inhibited. The presence or absence of divalent cations had no effect on the redistribution of the surface IgE. The effect of temperature during incubation on the distribution was also tested. Representative results are shown in Table II. Cells incubated with high doses of anti-IgE at 0°C for 30 min showed some patchy staining; at lower doses, the staining pattern was diffuse.

**Correlation of Histamine Release with Distribution of Cell Surface IgE.**--

**Monovalent anti-IgE:** Cells from sensitive individuals were incubated with varying amounts of monovalent anti-IgE (Fab or Fab' fragments) at 37°C. Histamine release was always 2% or less at anti-IgE concentrations of 2 µg/ml or less. At higher doses (10 or 24 µg/ml) some histamine release was observed. In a separate experiment, Fab' fragments which had been refractionated on G100 Sephadex several times produced progressively lower histamine release. Indeed, after three fractionations the central portion of the effluent peak gave no histamine release at 4 µg/ml, whereas bivalent F(ab')2 gave maximal release (68%) with a concentration of 0.04 µg/ml.

**Divalent anti-IgE:** Cells from sensitive individuals were incubated with varying amounts of divalent anti-IgE in the presence of Ca++ and Mg++ at 37°C for 30 min. Maximal histamine release occurred with low concentrations of anti-IgE (0.03–0.2 µg/ml). When cells were incubated with intermediate doses of fluorescent anti-IgE (1–2 µg/ml), suboptimal histamine release occurred. With high doses of anti-IgE (10–100 µg/ml) more or less complete inhibition of histamine release occurred. These results are in agreement with the
results of previous studies (16). At all doses, histamine release was essentially complete within 30 min with no additional release even after 220 min. The relationship between the dose response curve for histamine release and for redistribution of surface IgE for one subject is shown in Fig. 4.

Cell Surface IgE Distribution with Antigen Induced Histamine Release.—In these experiments, cells from subjects sensitive to ragweed antigen E were incubated with a concentration of antigen giving maximal histamine release or with 500–1,000 times excess antigen. After washing, the cells were then incubated with fluoresceinated Fab' fragments of sheep anti-IgE or with unfluoresceinated rabbit Fab fragments, hybrid antibody and ferritin. In all instances, the cells showed uniform diffuse surface staining. The presence or absence of Ca++ and Mg++ and incubations at 0°C and at 37°C produced no detectable differences in the IgE distribution. These results are summarized in Table III.

DISCUSSION

In this study we have extended previous investigations on those factors which influence the surface distribution of IgE on human basophils and have studied the relationship between changes induced in the surface distribution and histamine release.

It was previously shown that with 50 μg/ml of divalent anti-IgE, capping of the surface IgE occurred at room temperature and at 37°C but that capping was inhibited more or less completely at 0–4°C (2). In the present work we have found that the degree of patch or cap formation was highly dose, time and temperature dependent. At low doses of anti-IgE no gross redistribution was observed. At higher doses patch formation and capping were seen. At still higher doses, rapid redistribution with prominent internalization was observed. The degree of redistribution was also affected by time. Even with low doses, there was some patching and capping when incubations were prolonged to 220 min. In general, redistribution appeared to be a direct function of concentration of anti-IgE, time, and temperature.

In an attempt to understand the biological function of capping and patching in basophils, we have studied the correlation between the redistribution of cell surface IgE and histamine release. In the anti-IgE induced histamine release, previous studies have shown that divalent cations are necessary for the biochemical processes following the antigen-antibody reaction on the cell surface to proceed (17). It has also been found that basophils from some individuals do not

Fig. 2. Photomicrographs of basophils stained with different concentrations of fluoresceinated anti-IgE. Top row, diffuse labeling with Fab' anti-IgE 10 μg/ml. Second row, patchy distribution with Fab(ab')2 anti-IgE 1–2 μg/ml. Third row, capping with (Fab')2 anti-IgE 10–14 μg/ml. Fourth row, last frame is microscopic field of the frame immediately above viewed with phase microscopy; first two frames demonstrate internalization. Magnification: first row, frames 1 and 2, × 1980, last frame × 990; second row, × 1980; third row, first frame × 990, last two frames × 1980; fourth row, first two frames × 990, last frame × 1980.
Fig. 3. Section of human basophil reacted with intact rabbit anti-IgE, hybrid antibody and ferritin at 37°C. Bound ferritin is distributed in a dense patch or cap occupying less than one-fourth of the cell membrane. × 40,000.
TABLE II
Histamine Release and Surface IgE Distribution with Divalent Anti-IgE:
Effect of Temperature and Concentration.

| Subject | Conc. a-IgE | Time | Temp | Histamine release | Staining pattern |
|---------|-------------|------|------|-------------------|-----------------|
|         | µg/ml       | min  | °C   | %                 | Diff. Patchy Caps Intern. |
| P.G.    | 0.14        | 30   | 0    | 0                 | 0 0 0             |
|         | 0.14        | 30   | 37   | 74                | 98 2 0             |
|         | 1.4         | 30   | 0    | 4                 | 96 4 0             |
|         | 1.4         | 30   | 37   | 44                | 8 75 17            |
|         | 14.0        | 30   | 0    | 0                 | 18 76 6            |
|         | 14.0        | 30   | 37   | 10                | 2 38 59            |
| H.M.    | 26.0        | 30   | 0    | 0                 | 56 40 4            |
|         | 26.0        | 30   | 37   | 0                 | 14 52 34           |

* See Table I for complete data.

![Graph showing histamine release and distribution](image)

Fig. 4. Histamine release and distribution of cell surface IgE with different doses of divalent anti-IgE and incubations of 30 min at 37°C. All data are from an experiment involving cells from the same subject (PG).

release a significant amount of histamine even when antigen-antibody reactions occur on their surfaces (1), but release histamine if they are challenged by anti-IgE in the presence of D_2O (6). The present experiments clearly showed that capping of cell-surface IgE occurs on such cells as the result of antigen-antibody reaction and that divalent cations are not required for cap formation. It appears that the biochemical processes regulating the later stages of histamine release are not involved in cap formation.

Importantly, for the purpose of this study, it appears that both histamine release and redistribution require divalent anti-IgE. Patching and capping were not observed with high doses of Fab' fragments of anti-IgE even with prolonged
## TABLE III

Histamine Release and Cell Surface IgE Distribution with Antigen E

| Subject | Conc. antigen | Time | Temp | Histamine release | Staining pattern |
|---------|---------------|------|------|-------------------|------------------|
| J. S.   | 0.0033*       | 40   | 35   | 75                | Diff.            |
|         | 3.3           | 40   | 37   | 44                | Patchy           |
|         | 0.0033*       | 120  | 37   | 51                | Caps Intern.     |
|         | 3.3           | 120  | 37   | 40                |                 |
| B. W.   | 0.0002*       | 30   | 37   | 82§               | Patchy           |
|         | 0.001         | 30   | 37   | 72§               | Caps Intern.     |
|         | 0.02          | 30   | 37   | 50§               |                 |
| J. A.   | 0.001†        | 30   | 0    | 5                 | Patchy           |
|         | 0.001†        | 30   | 37   | 90                | Caps Intern.     |

* Optimum release with this dose.
† Approximately 25 times optimum dose.
§ Results from a separate experiment.

incubations. In addition, histamine release did not occur with Fab' fragments unless very high concentrations were used, and histamine release was progressively less as Fab' fractions were successively purified by gel filtration. In a previous study (16) there was no significant histamine release with Fab fragments obtained by papain digestion of anti-IgE. That we have slight release with the Fab' fragments at high concentrations may be due to residual undigested antibody or aggregated Fab' material. It does not appear that low avidity of the monovalent anti-IgE is the reason that histamine release and capping do not occur, since by fluorescence or electronmicroscopy the monovalent antibody stained the cells equally as well as divalent antibody used in the same concentrations.

While the present studies were in progress, we were informed of the studies of Ferrarini et al. (18). These workers studied guinea pig basophil degranulation in the presence of antiguinea pig immunoglobulin. Their studies are somewhat different than our own, and though their conclusions are similar, the basis for these conclusions are somewhat different. First, it is uncertain whether their system primarily dealt with cell activation via IgG or IgE antibodies or both. More important, their conclusion that redistribution was not required for histamine release was based on the observation that monovalent Fab' fragments of the anti-Ig did not induce observable redistribution but did cause degranulation. As stated above, in previous studies (16) and in the present work, we have failed to obtain significant histamine release with monovalent fragments, the most highly purified preparations showing less than 1/10,000 the activity of the divalent antibody. It may be that the results of Ferrarini et al. in fact, are due to small amounts of aggregated Fab', and therefore correspond to those experiments in which we used low doses of divalent antibody. Thus it
appears that bridging of the cell-surface IgE by divalent anti-IgE was required to initiate both the redistribution of IgE and histamine release.

The present data clearly demonstrate that cap formation of surface IgE is not a prerequisite for histamine release. As shown in Fig. 4 and Table I, the dose and time dependence of redistribution and histamine release are quite different. Thus at lower doses of anti-IgE (0.1 μg/ml), no redistribution is observed under conditions which give maximal or near maximal histamine release. At these doses histamine release is completed long before redistribution becomes significant. Most notably, those doses which redistribute the surface IgE most effectively (10-14 μg/ml) in fact cause marked inhibition of histamine release.

One might have supposed that the failure to induce histamine release at high doses of anti-IgE was due to the formation of 1:1 anti-IgE:IgE complexes in which the requirement for bridging of surface IgE would not be met. The present experiments clearly show that this is not the case since at such high doses cap formation (which also requires bridging of surface IgE) does take place. These results appear to indicate that only certain kinds of bridging of surface IgE are effective in causing histamine release.

There are some parallels between our results on surface IgE on basophils and studies concerning immunoglobulins on lymphocytes. Taylor et al. (19) have shown that mouse spleen cells reacted with antimouse immunoglobulin demonstrated capping when incubated at 37°C for short periods. In addition they found that capping was not affected by the presence or absence of Ca++ and Mg++, and that Fab' fragments of the anti-Ig did not induce capping. Edidin and Weiss (20) using cultured fibroblasts have found that capping was induced in the presence of antibody to H2 histocompatibility antigens if the cells were incubated at 37°C. Loor et al. (21) using mouse spleen cells have also found capping in the presence of intact F(ab')2 of anti-Ig but not in the presence of Fab' fragments from such antibodies. They found a similar temperature dependence on cap formation. Finally, Unanue, Karnovsky, and coworkers (22, 23) have demonstrated that the movement of cell surface immunoglobulin is sensitive to the concentration of anti-Ig. They demonstrated cap formation within 5 min, when a high concentration of anti-Ig was used. Relatively slower rates of cap formation on basophils in the present experiments may be due to slightly smaller number of IgE molecules on basophils. Rabellino et al. (24), reported 10⁵ Ig molecules per B lymphocyte, whereas the number of IgE molecules per basophil has been estimated to be 10,000-30,000 (2, 6). Failure of visible cap formation by ragweed allergen may also be due to a smaller number of reactive IgE antibody molecules, relative to the total surface IgE. All of these results support a model of the cell membrane which permits movement of protein in the plane of the membrane (25).

SUMMARY

The distribution of surface IgE on human basophils was studied using fluorescence microscopy and immunoferritin electronmicroscopy. Redistribution of the
IgE was dose, time and temperature dependent and required divalent anti-IgE. Cells which can release histamine in vitro were indistinguishable in these respects from cells which cannot. The redistribution was unaffected by the presence or absence of Ca++. No correlation between the conditions required for optimal histamine release and for redistribution was observed. At low doses, optimal histamine release occurred in the absence of, or before, redistribution. At higher doses redistribution occurred in the absence of histamine release. Antigen-induced histamine release was unaccompanied by gross redistribution of the surface IgE.

Since both histamine release and redistribution require bridging of IgE on basophils, it is concluded that only certain kinds of cross-linking of the IgE effectively stimulates these cells.

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