Lateral Electromigration and Diffusion of Fcε Receptors on Rat Basophilic Leukemia Cells: Effects of IgE Binding

MICHAEL A. McCLOSKEY, ZI-YAO LIU, and MU-MING POO
Department of Physiology and Biophysics, University of California, Irvine, California 92717. Dr. Liu's present address is Department of Biological Chemistry, Division of Biology and Biomedical Sciences, Washington University School of Medicine, St. Louis, Missouri 63110.

ABSTRACT We have used in situ electromigration and post-field relaxation (Poo, M.-m., 1981, *Annu. Rev. Biophys. Bioeng.*, 10:245–276) to assess the effect of immunoglobulin E (IgE) binding on the lateral mobility of IgE-Fc receptors in the plasmalemma of rat basophilic leukemia (RBL) cells. Bound IgE sharply increased the receptor's electrokinetic mobility, whereas removal of cell surface neuraminic acids cut it to near zero. In contrast, we found only a small difference between the lateral diffusion coefficients (*D*) of vacant and IgE-occupied Fc receptors (*D*: 4 vs. 3×10^{-10} cm^2/s at 24°C). This is true for monomeric rat IgE; with mouse IgE, the difference in apparent diffusion rates was slightly greater (*D*: 4.5 vs. 2.3×10^{-10} cm^2/s at 24°C). This range of *D* values is close to that found in previous photobleaching studies of the IgE-Fcε receptor complex in RBL cells and rat mast cells. Moreover, enzymatic depletion of cell coat components did not measurably alter the diffusion rate of IgE-occupied receptors. Thus, binding of fluorescent macromolecular probes to cell surface proteins need not severely impede lateral diffusion of the probed species. If the glycocalyx of RBL cells does limit lateral diffusion of the Fcε receptor, it must act primarily on the receptor itself, rather than on receptor-bound IgE.

Integral proteins diffuse 10–10^3 times more slowly in plasma membranes than in reconstructed lipid bilayers. Hydrodynamic theory also predicts that integral proteins should diffuse much faster than they do in vivo, provided the major drag that they experience is that due to lipid viscosity (1). At least this is the indication from a host of diffusion studies employing the method of fluorescence recovery after photobleaching (FRAP).[1] Hence, the search is on for extramembranous structures that impede diffusion of membrane components, with much attention being focused on the cytoskeletal matrix. In this paper, we ask whether or not the "anomalously" slow diffusion observed in natural membranes is an artifact of the FRAP technique, rather than a true reflection of the mobilities of membrane proteins.

Several experiments (summarized in reference 2) have essentially silenced most critics who previously felt that protein cross-linkage resulting from the intense bleaching light might explain the slow diffusion reported in FRAP studies. A still untested hypothesis, however, is that high molecular weight fluorescent ligands normally used in FRAP impede the intrinsic mobility of the membrane proteins to which they are bound. This does not occur for antibodies bound to lipid haptens in pure lipid bilayers (3), but might happen if a bound ligand were to increase the drag on its receptor by interaction with polymeric constituents of the cell coat, or glycocalyx. Notably, addition of an artificial "cell coat" to the antibody-lipid hapten system markedly reduces the observed lateral diffusion coefficient (*D*) value of lipid-bound antibodies (4).

A few previous studies lend support to the suggestion that nonligated membrane receptors may diffuse much faster than ligand–receptor complexes. Using the techniques of post-field relaxation (PFR) (5) and post-inactivation recovery (6), the *D* values of ligand-free concanavalin A (Con A) and acetylcholine (ACh) receptors in embryonic *Xenopus* muscle cells were found to lie in the range of 1–4 × 10^{-9} cm^2/s (at room temperature), much faster than the FRAP-determined values for acetylcholine receptors in embryonic rat muscle (7) or Con A receptors in the same *Xenopus* muscle (Liu, Z.-y., and

---

[1] *Abbreviations used in this paper:* CBA, cell buffer with azide; Con A, concanavalin A: *D*, diffusion coefficient; FRAP, fluorescence recovery after photobleaching; PFR, post-field relaxation; RBL, rat basophilic leukemia; TRITC, tetramethylrhodamine isothiocyanate; TRr-IgE, Texas Red-labeled rat IgE.

THE JOURNAL OF CELL BIOLOGY · VOLUME 99 SEPTEMBER 1984 778–787

M-m. Poo, unpublished results). Using PFR, Tank (8) found that uncomplexed low-density lipoprotein receptors on an internalization-defective human fibroblast cell line diffuse at 1.1×10^{-9} cm^2/s; but using FRAP a D value of 1.4×10^{-11} cm^2/s was observed for the low-density lipoprotein receptor complex (9).

The goal of our present work was to use one technique, namely, PFR, to compare the diffusion coefficients of one receptor in ligand-free and ligand-bound states. The choice of IgE-Fc receptors on rat basophilic leukemia (RBL) cells offers two advantages in this regard. First, the lateral diffusion rates of IgE-Fc receptor complexes on RBL cells (and rat mast cells) have already been measured with FRAP (2, 10), so one can compare the results from two different methods for one receptor on one cell type. Second, the interaction of IgE with its receptor is strong yet univalent. By using multimer-free IgE one can therefore maintain a high molecular weight (185,000) ligand for long periods on the cell surface without inducing receptor cross-linkage or the active cellular responses of anchorage modulation and internalization that can attend the binding of lectins, F(ab′)$_2$ fragments, or other multivalent ligands. The present study therefore provides a direct test of the idea that nonspecific interaction of a macromolecular label (IgE) with the cell coat retards lateral diffusion of the IgE-bound Fc receptor.

MATERIALS AND METHODS

Cells: The 2H3 subline of RBL-IV cells (11) was provided by Dr. Henry Metzger, National Institutes of Health. Cell monolayers were grown and passaged as described in (12). Stock cultures were passaged weekly into 75-cm^2 flasks (Corning Glass Works, Corning, NY), and at this time aliquots of 1.5×10^6 cells were seeded into 6-cm-diam tissue culture dishes (Corning), using the same medium as for the stock culture. In pilot experiments, we observed a systematic increase in the starting asymmetry index (see below) with culture age; in the diffusion studies we therefore only used cultures 3–4 d old. The medium was decanted, and the monolayer was rinsed twice in liquid policeman (0.6 mM Na_2 EDTA, 0.14 M NaCl, 2.7 mM KCl, 9.5 mM $NaKP_i$, pH 7.2), and incubated for 12 min at 37°C in 4 ml of the same buffer. Cells were dislodged with a gentle stream from a pasteur pipette, diluted to 25–50 ml with Tyrode's buffer (0.137 M NaCl, 2.7 mM KCl, 1.5 mM $CaCl_2$, 0.4 mM P_i, 1 mM $MgCl_2$, 5.6 mM glucose, 0.2% BSA, 0.02 M HEPES, pH 7.4) at room temperature, and harvested by centrifugation for 4–5 min at 200 g in polypropylene tubes.

For pre-field labeling experiments cell pellets were resuspended in Tyrode's plus 0.2% BSA (Sigma Chemical Co., St. Louis, MO), and for post-field labeling in cell buffer with azide (CBA) (10 mM HEPES, 28 mM NaN_3, 1 mM NaP_i, 109 mM NaCl, 2 mM $CaCl_2$, 1.5 mM $MgCl_2$, 5.4 mM KCl, 0.1% glucose, pH 7.3). Cell viability was checked with trypan blue, and cells were diluted to 1–2 $\times 10^6$/ml and then either plated directly in electromigration chambers (post-field labeling) or labeled in suspension with fluorescent IgE (pre-field labeling). In the former case cells were used within 2 h of isolation, and most data is for those used within 30 min. In pre-field labeling, cells were kept on ice for up to 5 h before use.

Fluorescent Antibodies: Purified rat IgE from the IR162 immunocytoma (13) was supplied by Dr. Henry Metzger. For later experiments we purified IR162 IgE from ascites fluid also supplied to us by Dr. Metzger. Affinity-purified mouse monoclonal anti-2,4-dinitrophenyl IgE from the H1 dinitrophenyl-ε-26.82 hybridoma (14) was provided by Dr. Fu-tong Liu of the Medical Biology Research Institute, La Jolla, CA. IgE was conjugated with either tetramethylrhodamine isothiocyanate (TRITC, isomer R, lot A1GCZK; Baltimore Biological Laboratories, Cockeysville, MD) or Texas Red (Molecular Probes, Junction City, OR). Fluorescein isothiocyanate-labeled rabbit anti-mouse IgG was obtained from Miles Laboratories (Elkhart, IN).

For TRITC conjugation, to 1 mg of mouse IgE in 1 ml PBS was added 2 drops of 1 M carbonate buffer, pH 9. 10 μl of a 20 mg/ml solution of the isothiocyanate in dimethylsulfoxide was added, the mixture homogenized by inversion and left on ice for 4 h. Free dye was removed by passage through a P6 column (Bio-Rad Laboratories, Burlingame, CA) equilibrated with PBS and dialysis for 24 h (4°C) against PBS and NaN_3. Using the absorbance at 280, 515, and 555 nm (15) the molar ratio of dye/protein was estimated to be ca. 2 and 5 for two different preparations. Subsequent gel filtration produced no change in these numbers, indicating that noncovalently bound dye was not bleeding off the conjugate.

Texas Red was coupled with either rat or mouse IgE by mixing an equal volume of borate buffered saline (pH 8.0) containing 4–5 mg/ml of IgE with 0.25 M Na carbonate buffer, pH 9, and then adding powdered Texas Red (0.25 mg/mg IgE). The suspension of dye was inverted periodically during a 1-h (rat) or 2-h (mouse) incubation on ice. Free dye was removed from the blue conjugate on a P6 column as above. From absorbance at 280 and 596 nm, the estimated dye/protein ratios were 2.5 for rat and 5.5 for mouse IgE (16).

Before labeling cells, we routinely centrifuged the IgE for 30–60 min at 100,000 g (4°C) in a Beckman airfuge (Beckman Instruments, Inc., Fullerton, CA), and then used only the upper 60–70% of the supernate. In some experiments we used a Texas Red-rat IgE (TRr-IgE) conjugate which had been purified (Fig. 1) by chromatography on sequential columns of AcA 34 and AcA 22 (LKB Produkter, Bromma, Sweden) (17). The pooled monomer fractions were used directly (0.6 mg IgE/ml), but even after reconcentration to 4 mg/ml, analysis by nondenaturing PAGE (4–25%) showed no evidence of aggregated IgE. Since the D values obtained were equal to those for less rigorously purified TRr-IgE, we assume that aggregation of the latter was not a problem. Neither TRITC- nor Texas Red-mouse IgE was subjected to PAGE, but PAGE of the underivatized mouse IgE did reveal multimeric components. Because the mouse IgE was not purified by gel filtration after its reaction with TRITC or Texas

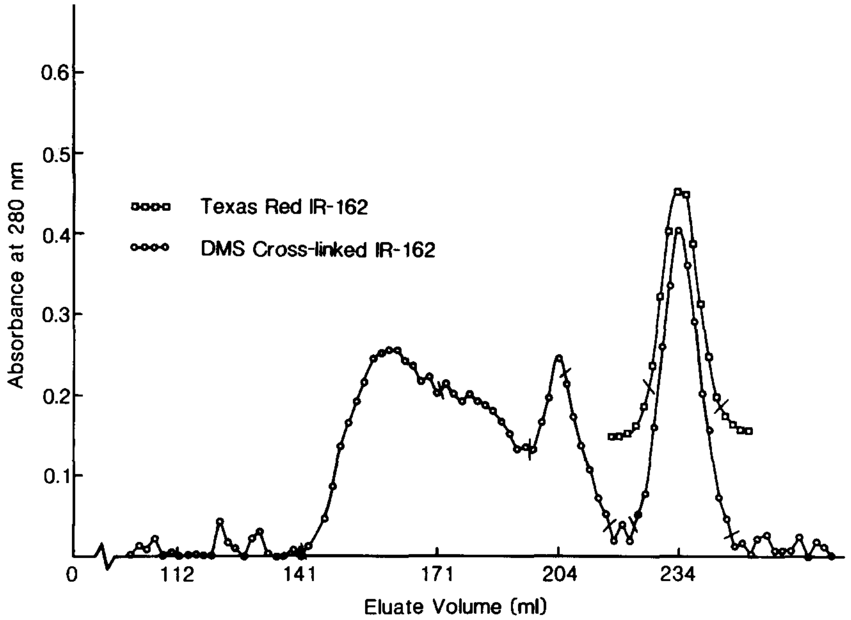


FIGURE 1 Isolation of monomeric rat IgE conjugate. Gel filtration elution profile of TRr-IgE conjugate (squares) and a mixture of monomer, dimer, and higher M_r rat IgE polymers (circles) formed in the presence of dimethylsuberimidate (*DMS*). About 10 mg of DMS-treated IR162 IgE was applied to AcA 34 column (1.6 × 95 cm), and the effluent was passed directly onto the top of another 1.6 × 95 cm column containing AcA 22. Elution was at room temperature with borate-buffered saline, pH 8, at a flow rate of 8 ml/h. Upper curve is displaced 0.15 U upward from the origin. Abscissa gives measured volumes rather than multiples of nominal fraction volume. Fractions were pooled as shown and subjected to nondenaturing electrophoresis on 4–25% polyacrylamide slab gels.

Red, if the ultracentrifugation step failed to remove this aggregated IgE, it may have been present during the diffusion experiments.

Cell Labeling: For determination of the D value of IgE-bound receptors with pre-field labeling, RBL cells suspended in Tyrode's buffer ($1-2 \times 10^6$/ml) were incubated with 10–15 μg/ml of fluorescent IgE for 30 min on ice. Cells were diluted to 25 ml with either Tyrode's or CBA, centrifuged as above, and resuspended ($1-2 \times 10^6$/ml) in the same buffer. As determined by spot photometry, binding of fluorescent rat IgE was inhibited ≥96% by prior incubation in unlabeled rat or mouse IgE (100 μg/ml) for 15–30 min at 0°C. Electromigration chambers were filled with cell suspension (60 μl) and left for 10 min at room temperature to permit cells to adhere to the substrate before application of the field.

In post-field labeling, the cells ($1-2 \times 10^6$/ml) in CBA were added to chambers and allowed 10 min at room temperature for adherence. After electromigration (and back diffusion in the case of IgE-unoccupied receptors), slides were immediately transferred to a metal block (0°C) and three aliquots (50 μl each) of IgE (10–15 μg/ml) in CBA were passed through the chamber. After 15 min at 0°C the free IgE was rinsed away with three to five aliquots (50 μl each) of CBA; for back diffusion of bound receptors, slides were brought back to 24°C for various time periods. The asymmetric receptor distribution was then quenched by a 30–60-s fixation in cold (0°C) acetone, followed by replacement with CBA. By measuring the fluorescence intensity in a 4-μm-diam spot over the edge of cells (see below) before and after acetone fixation, the loss of IgE during fixation was apparently ≤3%. By measuring the whole cell fluorescence with a larger photometer aperture the loss was apparently ≤5% ($n = 80$).

Apparatus and Procedure for Field Application: Equipment was as previously described (5), with the following modifications. The electric field was applied to a cell chamber made from a glass microscope slide. Slides were washed in concentrated HNO_3-H_2SO_4 (1:1, vol/vol) for 30–60 min and rinsed with distilled H_2O before use to minimize fluorescence background due to adsorption of IgE conjugates; without this step the Texas Red conjugates gave uniquely high background fluorescence. Two parallel strips of double-coated adhesive transfer tape (Y9469, 3M, St. Paul, MN) were used as the side-walls (spacers) of the cell chamber, and a 24 × 40-mm coverglass served as the roof. This tape gave an average chamber thickness of 0.12 mm. Buffer wells at both chamber ends were dammed with a rim of rubber cement. To maintain isothermal conditions during post-field back diffusion and to compensate for heat production during field application, the cell chamber was placed on a constant temperature aluminum block. The block was machined to hold three slides, drilled for coolant circulation, and linked to a recirculating bath set to 24°C. With a maximum current applied to the chamber, namely, 6 mA (a field of 40 V/cm when using CBA; resistivity = 80 Ω cm at 27°C), the slide temperature stabilized at 27°C.

Post-field labeling of Fcϵ receptors was performed on another metal block kept at exactly 0°C with a separate cooler. A small increase in this temperature (2–4°C) led to irreproducible and generally smaller starting asymmetries, possibly because of an abrupt increase in diffusion rates somewhere between 0–4°C. Tops of the slides were insulated by laying a styrofoam wafer over each. Using a tele-thermometer (YSI model 42SL) with a small-diameter probe (model 427, Yellow Springs Instrument Co., Yellow Springs, OH), we found that it took 45 s for the temperature of a slide to drop from 27° to 0°C, and an equal time for the reverse process. Only a few seconds were required to come within 75% of the steady value.

Data Acquisition and Analysis: Receptor migration was measured with a microfluorimetric method as described before (5), except that a 4-μm-diam aperture was used to collect photons from anode- and cathode-facing poles of the cell. An asymmetry index, $A_i(t)$ was calculated for each cell:

$$A_i = (I_- - I_+)/(I_- + I_+), \tag{1}$$

where I_- and I_+ are the fluorescence intensities at cathodal and anodal edges, after subtraction of the nearby cell-free background. The latter was determined separately for each cell. Photometer readings over the edge of unstained cells were only 4 ± 2% above background on slides not exposed to IgE; cell "autofluorescence" was therefore neglected in both the pre- and post-field labeling experiments. For stained cells with no asymmetry, typical signal to noise ratios fell in the range of 6–8.

A_i was determined for 40 cells on each slide (10 cells in each of four parallel and widely spaced scans), and an average A_i was calculated. Roughly half of the slides were scored single blind, with two of us exchanging experimental manipulations and A_i measurements. Because of photobleaching during the measurement of A_i on single cells, the true A_i is either smaller or larger than the measured A_i, depending on which side of the cell is recorded first. To minimize bias we usually alternated the initial sampling side on sequential cells (population study). We did not try to analyze the contribution of any fluorescence quenching which may have occurred upon concentration of the fluorescent ligands on one side of the cell.

A linear least squares program was used to fit the slope of the decay of $\ln[A_i(t)]$, for both the single cell and the population experiments. To the first order, on a single spherical cell, this slope equals $-2D/r^2$, where r is the cell radius (5). For a D value of 3.8×10^{-10} cm^2/s (the empirical value) the first-, second-, and third-order curves become superposable within 6 min. Before 6′ the maximum difference between first- and third-order slopes is 8%, the first-order plot being steeper.

In the population studies it is incorrect to equate, as we did, the decay of $\ln[\overline{A(t)}]$ with $-2\bar{D}t/\bar{r}^2$, where $\bar{D}$ and $\bar{r}$ are population averages. The correct average is $\overline{\ln[A(t)]}$. However, presently available theory does not encompass the possibility that time zero asymmetries may be negative or zero, and decay to more negative values with time. Yet for the conditions of our population experiments, even the time zero distribution of A_i includes negative asymmetries, some of which are probably artifact (see Results) but many of which are real. Short of deriving more general equations, one compromises by forming the linear average and transforming it. This leads to an underestimate of the true average D value; applying both methods to the three cells in Fig. 4*B* (q.v.), the error is 33%. This is likely an upper limit to such error in the population studies, since $\ln(\bar{x}_i)$ and $\overline{\ln(x_i)}$ approach each other as the spacing between x_i decreases.

Average cell diameters were measured with a reticle calibrated with a slide micrometer. RBL cells in CBA had an average diameter of 12.1 μm (SD = 1.3 μm; $n = 330$). This is close to the average value (12.3 μm) estimated from Coulter counter measurements of suspended RBL cells (18), indicating that not much flattening of the cells occurred in CBA, and that the assumption of spherical geometry is not bad. After acetone fixation and rehydration with CBA, the diameter had shrunk to 10.7 μm (SD = 1.2 μm; $n = 120$). We made no attempt to correct for any difference between the measured A_i and the true A_i which might result from this shrinkage.

Electron Microscopy and Photography: Cells for thin sectioning were processed as follows: after elution or treatment with enzymes cells were rinsed three times in 0.1 M Na cacodylate buffer, pH 7.2, and then incubated 1 h in the following buffer: 0.2 M cacodylate, pH 7.3, 0.5% ruthenium red, and 1.3% glutaraldehyde. After washing in cacodylate buffer the cells were incubated 3 h in a solution containing 0.2 M cacodylate, pH 7.3, 0.5% ruthenium red, and 1.3% osmium tetroxide. After dehydration the cells were embedded in Epon-Araldite, sectioned, and examined at 60 kV in a JEOL JEM 100C electron microscope (JEOL USA, Electron Optics Div., Peabody, MA).

The sequence in Fig. 2 was taken with Kodak Recording film (No. 2475, Eastman Kodak Co., Rochester, NY) through a ×40 oil immersion objective on a Zeiss epifluorescence microscope (Carl Zeiss, Inc., New York). Exposure times were 15, 25, 50, and 180 s for Fig. 2, *a–d*, respectively. The film was push processed by development in DK-50 for 8 min and printed on Ilford 3.1M paper.

RESULTS

Electromigration of Vacant and IgE-occupied Fc Receptors

In the first set of experiments, RBL cells were labeled with TRr-IgE and then plated in the cell chambers. Application of an electric field caused redistribution of the labeled Fcϵ receptors on the cell surface from a uniform to a highly asymmetric distribution. Fig. 2, *a–d* depicts TRr-IgE-labeled RBL cells before and after application of an electric field of 20 V/cm for 10 min. Accumulation of the surface-bound IgE towards the cathode is obvious in Fig. 2*b*. This experiment was conducted with 30 mM NaN_3 and without glucose in the incubation medium, a condition known to block certain metabolic energy-dependent processes such as capping (19). On the majority of cells, essentially all of the fluorescence emanates from IgE exposed to the extracellular medium, because it is effectively quenched by treatment with dilute copper sulfate (Fig. 2, *g* and *h*). The paramagnetic Cu(II) ion is an effective quencher of fluorescence from rhodamine, and it enters living cells slowly enough to allow its use in distinguishing external from internal fluorophore (20). This is shown in Fig. 2, where *e* depicts a Swiss mouse 3T3 fibroblast containing rhodamine-labeled α_2-macroglobulin (21), and *f* is

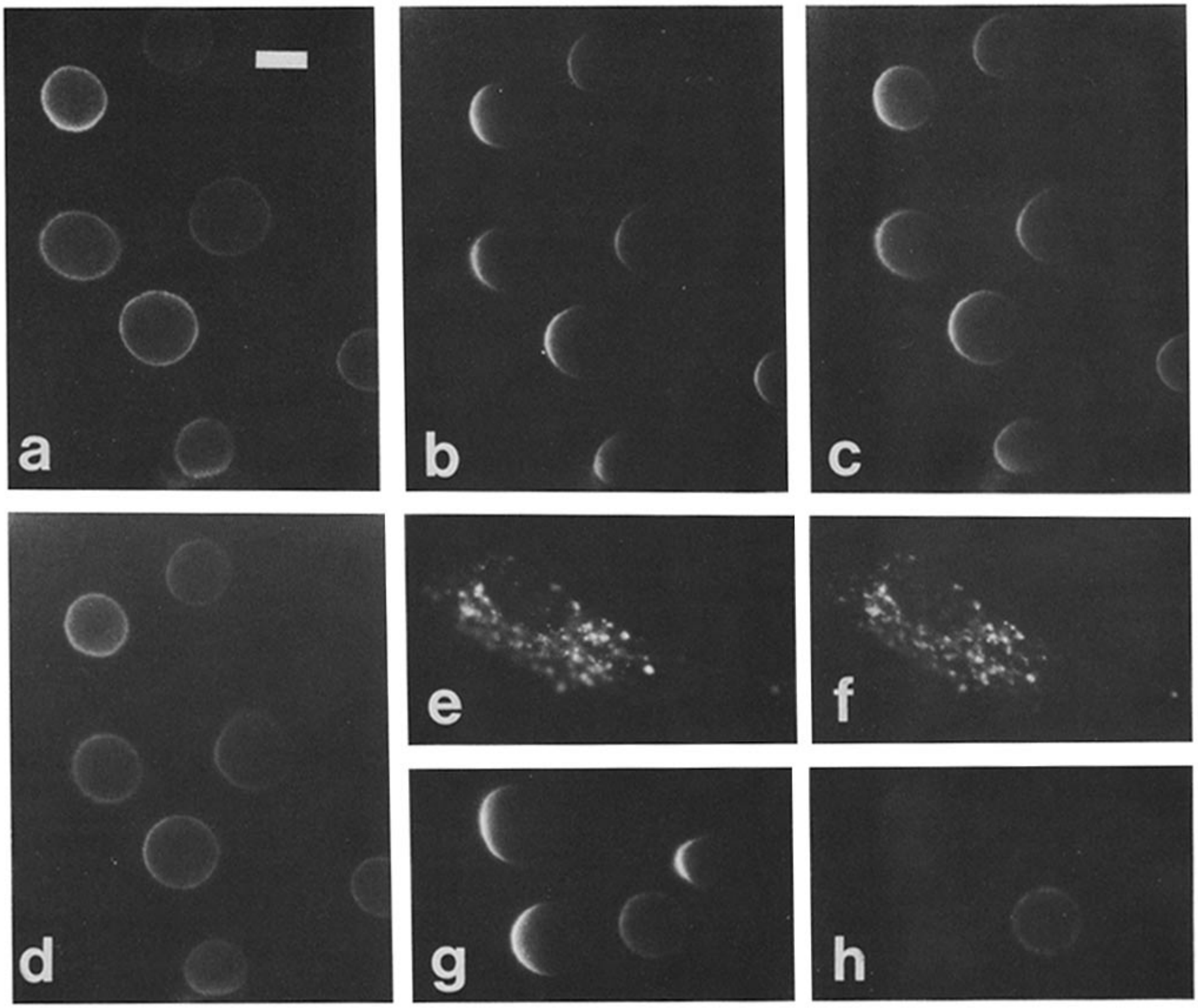


FIGURE 2 Rise and decay of asymmetric IgE distribution on RBL cells. (*a*) Before field application; (*b*) after 10 min at 20 V/cm. Bright side of crescents face cathode. Field was terminated immediately after the photograph was shot. (*c*) 4 min after field termination; (*d*) 22 min after field termination. Note back diffusion of accumulated receptors in *c* and *d*. (*e*) Swiss mouse 3T3 cells containing internalized and some surface-bound rhodamine-labeled α_2-macroglobulin; (*f*) same cell after replacement of medium with 10 mM $CuSO_4$ in 0.14 M NaCl, pH 4.5. Little quenching is detectable, and what is present is mostly due to photobleaching during first exposure; (*g*) four RBL cells in field of 20 V/cm. With field on, slide was scanned to find a "ring-stained" cell showing only weak asymmetry. Such cells were of low frequency (<2%) with rat IgE (in a 20 V/cm field). (*h*) Same cells after treatment with $CuSO_4$ as above. Note that only externally exposed IgE is redistributed by the field. Bar, 10 μm. × 723.

the same cell after addition of 10 mM $CuSO_4$ in 0.14 M NaCl. Although we plan to quantify field-induced internalization using radiolabeled IgE, the above qualitative observations are consistent with passive lateral migration of surface receptors under the influence of an electric field.

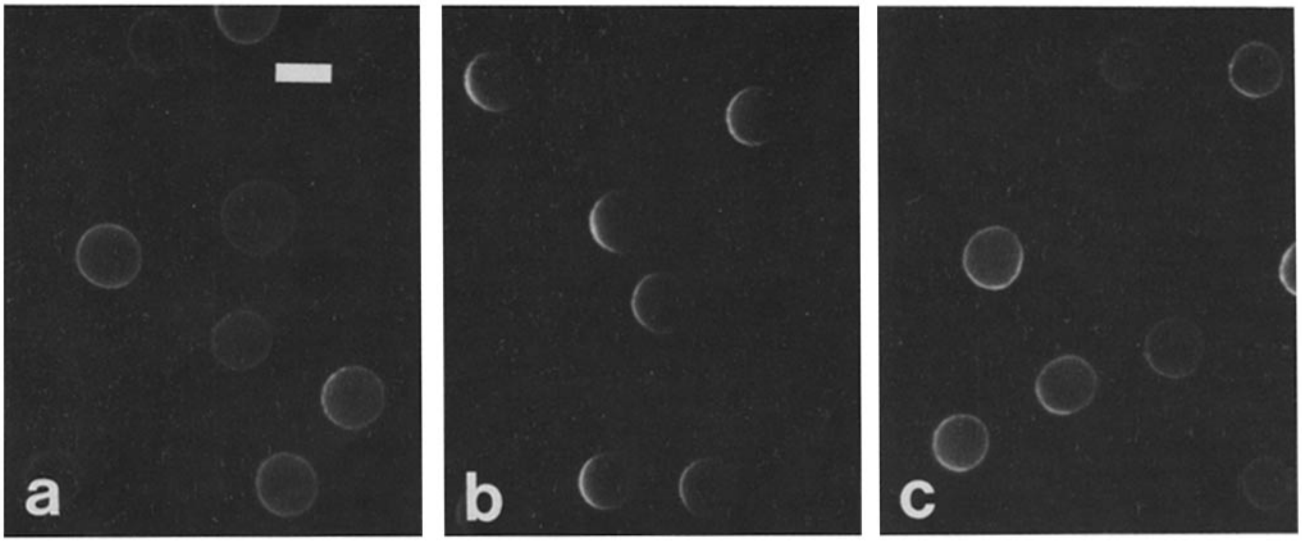
When labeling of the surface receptors for IgE was carried out after field application (post-field labeling), an asymmetric distribution of Fcϵ receptors was also observed, indicating that unoccupied receptors also migrate under the influence of the field. The graph in Fig. 3 shows the time evolution of asymmetry in the surface distribution of IgE receptors in pre- and post-field labeled cells, as determined microfluorimetrically (see Materials and Methods). Although both populations were exposed to a 10 V/cm field, the steady-state asymmetry of TRr-IgE-complexed receptors was much higher than that of the uncomplexed receptors (Fig. 3, *a* and *b*). Using a theoretical expression for *m*, the effective electrokinetic mobility (22),

$$m = (D/3E_o r)\ln[(1 + A_s)/(1 - A_s)] \qquad (2)$$

we estimate that at 10 V/cm the value of *m* for the complex is about four times greater than it is for the free receptor.[2] The simplest explanation for this observation is that bound IgE increases the electrokinetic mobility of its receptor. The difference was not due to back diffusion of free receptors

---

[2] r is the cell radius, D the lateral diffusion coefficient, E_o the electric field strength, and A_s the steady-state asymmetry index. This is an approximate calculation, complicated by uncertainty in the true A_s for $E_o > 10$ V/cm; at these field strengths A_i begins to decrease after 15–20 min in the field, especially with post-field staining.



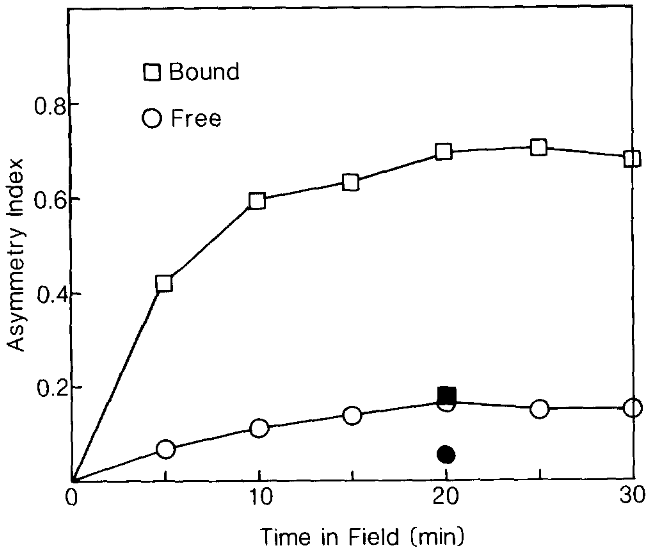


FIGURE 3 IgE binding increases the electrokinetic mobility of Fcϵ receptors. Cells in micrographs were exposed for 20 min to a steady field of 10 V/cm at 24°C, quickly cooled to 0°C, and fixed with acetone. (a) TRr-IgE bound after field application (IgE was bound in the cold before fixation); (*b*) TRr-IgE bound before field application; (c) TRr-IgE bound before field but after treatment of cells for 30 min with neuraminidase (65 U/ml). Post-field incubation at 0°C was for 15 min, irrespective of whether IgE was bound before or during this time. (a–c) Bar, 10 μm. × 723. (*graph*) Rise of asymmetry index for free and TRr-IgE complexed receptors in 10 V/cm field at 27°C. (■) *A*(20′) for bound receptors on neuraminidase-treated cells (as in c). (●) *A*(20′) for free receptors on neuraminidase-treated cells (40 cell average). Each point except the latter is an average of at least 80 cells (two slides). Standard deviation between cells on single slides averaged 0.11 for each curve (range: 0.08–0.14).

during the post-field staining period: as shown in Fig. 5*A* (solid triangles) (q.v.), no appreciable back diffusion occurred if the cells were kept at 0°C. In theory, for equal *m* values, one would also expect a higher steady-state A_i for the IgE-receptor complex if its diffusion rate were much lower than that of the unoccupied receptor (Eq. 2). However, we know from independent measurements of *D* that this is not the case (see below).

One referee suggested that long preincubation in the cold may have caused microtubule depolymerization and released constraints on the receptor; however, we observed marked enhancement by TRr-IgE even when cells were labeled at room temperature and never placed on ice ($A_i[20'] = 0.65$ at 10 V/cm). This referee also wondered whether noncovalently bound dye in the IgE preparation may have partitioned into the membrane during the preincubation, and in some way given an artificially high A_i. This is clearly not so, because when cells were labeled at room temperature with nonfluorescent rat IgE, exposed to a 10 V/cm field for 20 min, quenched at 0°C, and then labeled with a mouse antibody against rat IgE (23) followed by fluorescein isothiocyanate-labeled rabbit anti-mouse IgG, the asymmetry was insignificantly different ($P < 0.005$) from that shown in the graph in Fig. 3.

Rat IgE_{162} has an isoelectric point near 5.9 (24), and is thus negatively charged at the pH (7.2) of these measurements. Why then does it increase the tendency of its receptor to move toward the cathode, as if it were a cation? The most logical explanation that we can offer at this time is that drag on the receptor due to electro-osmotic flow along the membrane is the dominant force impelling it towards the cathode (25). The 185,000 M_r ligand may increase substantially the molecular area exposed to this flow. The marked diminution of free and bound *m* values by prior removal of cell surface neuraminic acids (Fig. 3, *c* and graph) is consistent with this interpretation; i.e., the resulting lower charge density should reduce the electro-osmotic flow.

Diffusive Recovery

SINGLE CELLS (PRE-FIELD LABELING): Fig. 2, *c* and *d* show two stages in the diffusive recovery of TRr-IgE occupied Fcϵ receptors on seven RBL cells after termination of the electric field. Asymmetry plots for two cells exposed to the same field are shown in Fig. 4, *A* and *B*. Although "ring staining" is often used as evidence against the internalization of externally applied fluorescent probes, as shown in Fig. 2, *g* and *h*, by itself it can be a rather poor criterion. The cell in

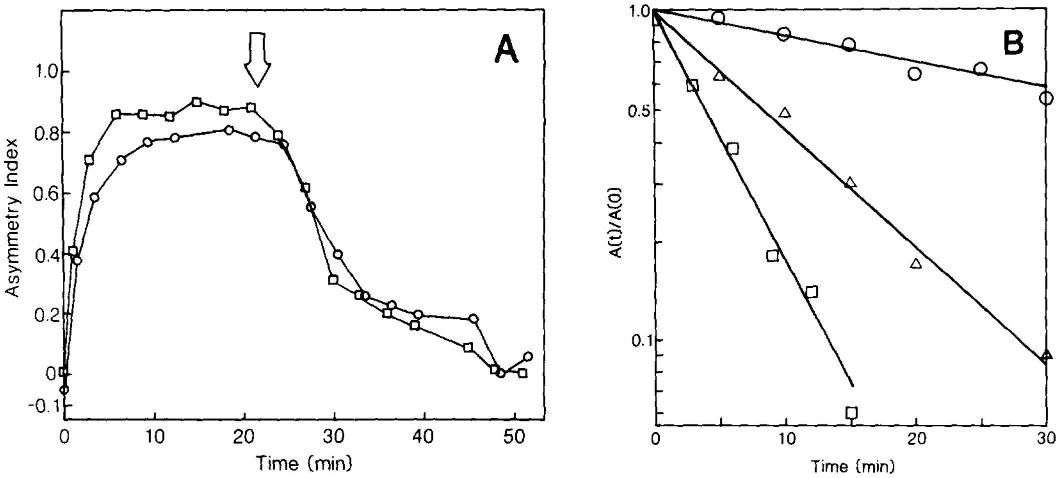


FIGURE 4 (*A*) Progression of asymmetry index on TRr-IgE-labeled RBL cells exposed at time zero to 30 V/cm field (room temperature). Arrow indicates point of field termination. Despite nearly equal diameters, the steady-state asymmetries differ by 0.05–0.10, and in the same direction as the *A*(0) values. (*B*) Post-field relaxation of receptor distribution on single cells obeys a diffusion equation. Back diffusion of TRr-IgE-labeled Fc receptors at room temperature. *A*(*t*)/*A*(0) rather than *A*(*t*) is plotted because starting asymmetries differ. Note the variation in *D* values between cells: 5.8×10^{-10}, 3.4×10^{-10}, and 6.6×10^{-11} cm^2/s. Corresponding diameters are 12.8, 14.3, and 12.6 μm.

the upper left of *g* has a crisp fluorescent ring, yet upon addition of a quenching agent (*h*), the ring remains. However, $CuSO_4$ (10 mM) did quench the fluorescence of TRr-IgE on almost all cells during the period of back diffusion (Fig. 2, *g* and *h*). Moreover, the fluorescence of those few cells (<2%) showing weak asymmetry was only partially quenched by $CuSO_4$, and the nonquenchable fraction (presumably internalized) was not formed into a crescent (Fig. 2*h*). As shown in Fig. 4*B*, relaxation of asymmetry on single cells fits fairly well the exponential decay expected from the solution to the equation for diffusion on a spherical surface (5, 22). Using TRr-IgE, recovery has been followed in the presence and absence of 30 mM NaN_3 and 0.1% glucose without noticeably different outcomes. Together, these results are consistent with passive back-diffusion of IgE-Fcε receptor complexes in the plane of the membrane.

The electric field induced some cells to form one to three large vacuoles, both during field application and shortly thereafter. The vacuoles were fluorescent, and sometimes close enough to one edge or the other of the cell to contribute to the total fluorescence intensity measured there, thus yielding an artificially high or low A_i for that cell. This quantitative effect was observed directly during single cell recording as a large and sudden jump in the steady-state A_i at the time of vacuole formation. Although cells with obvious vacuoles were studiously avoided when scoring slides for the population studies, because of the inconvenience of changing focal planes on every cell, some were inadvertently included. Despite their usually low frequency, because of the large magnitude of ($A_i - \bar{A}_i$), they probably contributed measurably to the standard deviation. Inexplicably, on a few slides vacuoles were very frequent. Vacuole induction may explain some of the unexpectedly large, negative asymmetries observed at short time points.

From a series of 19 plots like those shown in Fig. 4b we found an average *D* value of 4.6×10^{-10} cm^2/s (25°C) for TRr-IgE bound to Fcε receptors (range: 1.5×10^{-10} to 1.1×10^{-9} cm^2/s). The average *D* value for bound mouse IgE (Texas Red- or TRITC-labeled) was 1.8×10^{-10} cm^2/s (25°C) for the 37 cells measured (range: 5×10^{-11} to 6×10^{-10} cm^2/s). However, the average coefficient of variation for the mouse IgE curves (0.56) was much lower than that for rat IgE (0.92), the "long-time" tails being much flatter. Furthermore, even without field application, after 1–2 h at room temperature the mouse IgE was much more heavily internalized than the TRr-IgE, as judged by its resistance to quenching by Cu(II) and its heterogeneous distribution. Without further work we cannot say whether the apparent difference between *D* values of murine and rat IgE is real or an artifact due to aggregation of the mouse IgE. This difference also showed up with the post-field labeling method, although the absolute *D* values estimated with the separate techniques were different.

POPULATION STUDY (POST-FIELD LABELING): By labeling the Fcε receptors either immediately after field termination or after defined periods of PFR, we obtained diffusional recovery curves for IgE-bound and IgE-free receptors, respectively, as shown in Fig. 5. The initial asymmetry was created by application of a 40 V/cm field for 30 min at 27°C. For TRr-IgE the apparent *D* values of free and bound receptors were 4.0 and 3.1×10^{-10} cm^2/s. Identical experiments using mouse IgE yielded the plots shown in Fig. 5*B*. The data is a combination of points for TRITC- and Texas Red-labeled mouse IgE, mostly the former; when used on separate days there was little difference between the *D*'s obtained with the two fluorophores. The average *D*'s for free and mouse IgE-bound receptors were 4.5 and 2.3×10^{-10} cm^2/s (Table I). A Student's *t* test on all four slopes indicates they are truly different ($P < 0.05$ that they are not); but inasmuch as the distribution of A_i values on individual cells looks skewed, the validity of this *t* statistic is uncertain. Note that the mouse IgE not only yielded slower bound and faster free receptor *D* values, it also detected a lower initial asymmetry (0.31 vs. 0.44).

NONEFFECT OF CELL COAT DEPLETION: The native Fcε receptor of RBL cells is trypsin resistant (26). On the other hand, treatment of several cell types with trypsin will release a large fraction of their cell surface proteoglycans (27, 28). Recovery curves for bound receptor before and after treatment of RBL cells with 0.5% trypsin (in PBS-EDTA for 30 min at 37°C) were obtained by successively measuring different cells (every 30 s) on one slide during the period of back diffusion. Data from two to three slides were then averaged, the logarithms taken and fit to a straight line. *D* values for bound receptor before and after trypsinization were not significantly different ($P < 0.05$) (Table II).

Trypsinization reduced the adhesiveness of RBL cells, and

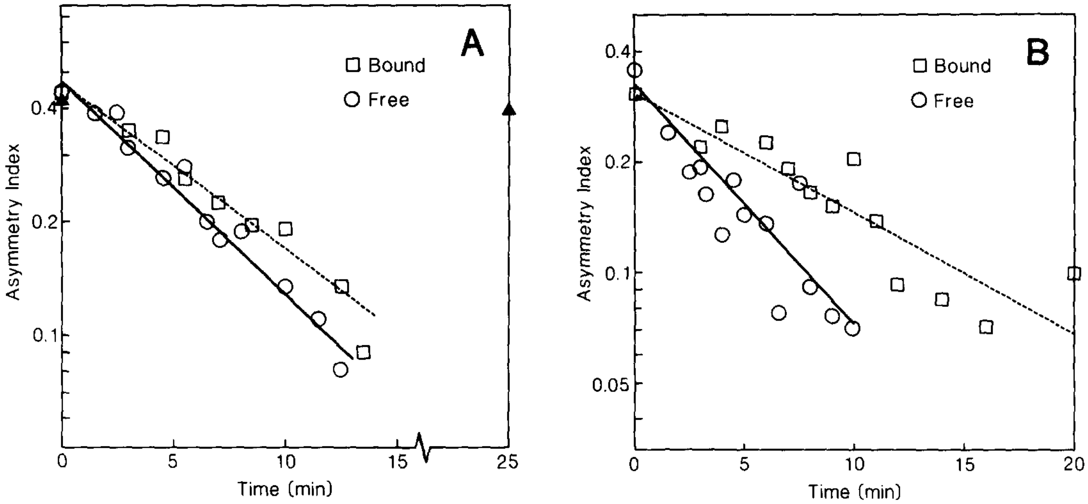


FIGURE 5 (*A*) Population recovery curves for free and TRr-IgE–complexed Fcε receptors, obtained as described in Materials and Methods. Initial asymmetry was created by application of a 40 V/cm field for 30 min at 27°C; back diffusion at 24°C. Each point is an average of 40–160 cells (one to four slides). The standard deviation in A_i between cells on single slides averaged 0.16 and the standard error of the mean A_i between different slides at each time point ranged from 0.02 to 0.04. Solid triangles show A_i at time zero and after 25 min at 0°C before staining (40 min total at 0°C). (*B*) Population recovery curves for free and bound receptors using mouse IgE as the ligand; plots are a combination of data for TRITC- and Texas Red-labeled antibody. Initial asymmetry again induced by field of 40 V/cm applied for 30 min at 27°C; back diffusion was at 24°C. Each point is an average of 40–760 cells (average = 210). Standard error of the mean A_i between slides at each time point ranged from 0.01–0.09.

TABLE I

*Diffusion Coefficients of Vacant and IgE-occupied Fcε Receptors**

	Mouse IgE		Rat IgE	
	cm^2/s	*n*	*cm^2/s*	*n*
Vacant	4.5 ± 0.2	(2,960)	4.0 ± 1.8	(1,040)
Occupied	2.3 ± 1.2	(2,720)	3.1 ± 1.5	(760)

* All values have been multiplied by 10^{10}. *D*'s were estimated from the relaxation rate of the asymmetry index by post-field labeling of the Fcε receptor either immediately (occupied) or at various times after field termination. Field of 40 V/cm was applied for 30 min at 27°C. Diffusion was at 24°C. Cells were immobilized on acid-washed glass slides. *n* refers to the number of cells examined.

TABLE II

*Effects of Trypsin Treatment on Diffusion Coefficient of TRr-IgE Occupied Fcε Receptor**

Immobilizing substrate	Control		Trypsin	
	cm^2/s	*n*	*cm^2/s*	*n*
Single cell averages				
Clean glass	4.0 ± 2.3	(8)	4.6 ± 1.8	(19)
Population averages				
Clean glass	ND		3.0	(180)
Polylysine-coated glass	2.2	(180)	2.2	(180)
LGT-Agarose	2.0	(180)	2.0	(180)

* All values have been multiplied by 10^{10}. TRr-IgE was bound to cells before field application, and PFR was used to estimate the diffusion coefficient by measuring either the decay of asymmetric receptor distribution on individual cells (single cell study) or the average decay of a population scanned during the period of back diffusion (see Results). Back diffusion was at room temperature. Initial asymmetries were created by field of 10–25 V/cm applied for 5–15 min at room temperature. *n* refers to the number of cells observed; in each poulation study three separate experiments were averaged, with 60 cells being measured in each experiment (one cell every 30 s for 30 min).

a minor fraction remained stuck to plain glass slides after the electromigration. Because this fraction may have been a select subpopulation with a lower *D* value than the average, we resorted to immobilization of trypsinized cells on polylysine-coated slides and also in ultra-low gelling temperature agarose (Sea Prep, FMC Corporation, Rockland, Maine). The Fc receptor *D* values for cells in agarose and on polylysine-coated glass are slightly lower than for untreated glass (Table II). The general lack of an effect of trypsin corroborates the FRAP results of Wolf et al. (2), who also found relatively slow diffusion of IgE-Fc receptor complexes after eluting monolayer RBL cells with trypsin. The trypsin concentration and exposure time were lower in their experiment, however.

Representative electron micrographs of ruthenium red-stained cells that had or had not been trypsin-treated (as above) before staining are shown in Fig. 6. Ruthenium red is a hexavalent cationic complex routinely used for visualization of acidic mucopolysaccharides like those present in the glycocalyx (27). It reportedly also binds to sialoglycoproteins, provided their negative charge density is high enough (27). To be sure, substantial variation in the glycocalyx thickness was observed in control cells, and somewhat less in trypsin-treated RBL cells, but the gestalt impression is faithfully depicted in Fig. 6. One sees a darker and thicker border of ruthenium red on the control cells. The above is little more than suggestive evidence that the glycocalyx of RBL cells is not a major constraint to lateral diffusion of the Fcε receptor. It is entirely possible that remnants of the glycocalyx that are left after trypsin retard diffusion of the receptor so much that no further encumbrance results from the trypsin-releasable component(s).

DISCUSSION

Our primary finding was that IgE does not cause a precipitous decline in the lateral diffusion rate of Fcε receptors on RBL cells. This result is directly relevant to numerous studies of protein mobility that have employed high-molecular-weight fluorescent reagents, and to the possibility that these reagents hinder diffusion of the species being studied. The implicit assumption has usually been that diffusion of ligand-receptor complexes accurately reflects that of unligated receptors. Our

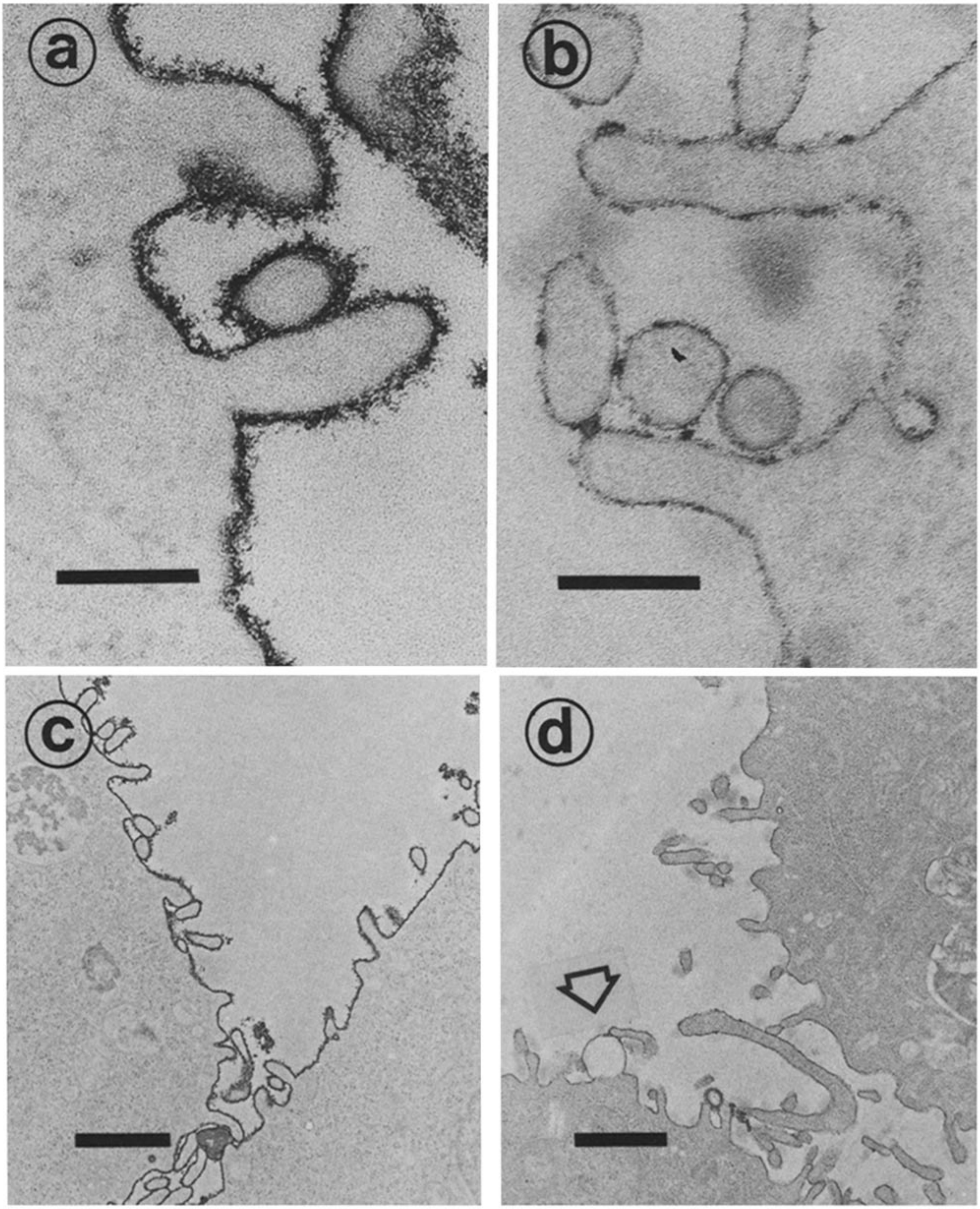


FIGURE 6 Electron micrographs of control (*a* and *c*) and trypsin-treated (*b* and *d*) RBL cells stained for mucopolysaccharides with ruthenium red. Note the thinning of glycocalyx caused by trypsin. Hyaline blebs (arrow in *d*) and a partially clumped distribution of ruthenium red were frequently observed on trypsinized cells. (*a* and *b*) Bar, 0.2 μm; × 120,000; (*c* and *d*) bar, 1.0 μm; × 15,800.

work validates this assumption for Fcε receptors on RBL cells. Whether the assumption is good for other receptors or other cells remains to be determined.

A priori, there is some reason to expect that the glycocalyx of RBL cells should limit diffusion of IgE-bound Fc receptors, because in electron micrographs (Fig. 6) it often appears to extend well beyond the top-most projection of cell bound IgE (29). Wank et al. (30) also suggest that steric constraints imposed by glycocalyx components may account for the rate constant 30-fold slower for association of IgE with cell-bound Fcϵ receptors than with Triton X-100-solubilized Fcϵ receptors. We see no obvious reason why the glycocalyx could not limit the reaction rate without affecting lateral diffusion of the receptor, but the existence of either constraint is not yet proven. One would like to extend the trypsin and neuraminidase results, and define more precisely what the contributions of different glycocalyx components are, both to the diffusional and the electrokinetic mobilities.

Because of several complications, which arose in our application of PFR to RBL cells (see Materials and Methods and Results), we emphasize the comparison between free and bound Fcϵ receptors; the values of the separate D's are no doubt inexact, though perhaps no more so than those derived from FRAP.[3] One complication is the limited field-induced internalization that we observed—even under conditions known to block internalization of other ligands on other cell types. This may be behind the previously noted biphasic time dependence of A_i at higher field strengths. Tank (8) found much more extensive field-induced internalization of low-density lipoproteins by human fibroblasts. In our case internalization was sometimes evident from the emergence of a fluorescent membrane delimiting one to three large intracellular "vacuoles." Very similar vacuoles also appear in rat peritoneal mast cells exposed to steady electric fields, and resemble the intracellular cavities seen during mast cell degranulation (33, 34).

The considerable variation in A_i that one found among different cells on the same slide was a bit exasperating. We traced some sources of this variation to the following. First, human error in placement of the photometer aperture is appreciable with current methodology. Second, there is substantial variability in the A_i values between cells before field application, due not only to the above, but probably also to morphological distinctions, e.g., a higher concentration of surface projections on one side of the cell than the other. Third, cells were infrequently rotated during or after the acetone fixation so that their crescents became randomly oriented. Fourth, the diffusion coefficients differ substantially from cell to cell (Fig. 4*B*), and possibly the electrokinetic mobilities differ as well. A wide variation in single-cell D values has been observed in previous FRAP studies of other membrane proteins (35, 36).

Whether the apparent difference between the D's of mouse and rat IgE is due to a species difference per se remains to be seen. From the relative degrees of cellular internalization and analytical PAGE we surmise that our fluorescent mouse IgE may have been more highly aggregated than the fluorescent rat IgE. Cross-linkage of IgE bound to Fcϵ receptors is a signal for receptor internalization as well as cellular degranulation (37, 38). Recent evidence also indicates that as with surface Ig on B lymphocytes (39) and C3b receptors on polymorphonuclear leukocytes (40), cross-linkage of IgE receptors on RBL cells renders them insoluble in nonionic detergents, probably due to association with the insoluble cytoskeletal matrix (41). It is intriguing to think that the slower D value we found for mouse IgE was due to signaling of cytoplasmic attachments upon binding of contaminant IgE multimers.

The enhanced cathodal electromigration of Fcϵ receptors upon binding of IgE was a surprise. Equally tenable explanations may exist, but our current hypothesis is that the occupied receptor has an increased exposure to the electro-osmotic flow. This might result from a less streamlined shape of the IgE-Fcϵ receptor complex, from an increased cross-sectional area without shape changes, or from vertical extension into a plane where the electro-osmotic flow is greater than it is at the height of vacant receptors. There is an interesting contrast between the action of IgE and that of Con A, binding of which completely prevents electromigration of Con A receptors in *Xenopus* muscle cells (22).

Unoccupied Fcϵ receptors in RBL cells have an apparent electrokinetic mobility (at 10 V/cm) about 1/27 that of unoccupied Con A receptors in *Xenopus* muscle cells (5), whereas the ratio of PFR-measured diffusion coefficients is about 1/13. After binding of IgE, m is still lower by a factor of about 7, and D by a factor of about 17. It is tempting to try to relate these differences in m to what is known regarding the molecular structures of the receptors and cell surfaces, but there are too many *unknowns* at present. Future studies of electromigration using reconstituted lipid-protein systems may help isolate the parameters most important in determining m.

We thank Dr. Henry Metzger and Dr. Fu-tong Liu for their generous gifts of purified rat and mouse IgE, and also for ascites fluid containing the antibodies. We are also indebted to Dr. Daniel Conrad for ascitic fluid containing the B5 monoclonal antibody against rat IgE.

This work was supported by a grant from the National Institutes of Health (GM 30666-02).

Received for publication 28 February 1984, and in revised form 22 May 1984.

REFERENCES

1. Saffman, P. G., and M. Delbruck. 1975. Brownian motion in biological membranes. *Proc. Natl. Acad. Sci. USA.* 72:3111–3113.
2. Wolf, D. E., M. Edidin, and P. R. Dragsten. 1980. Effect of bleaching light on measurements of lateral diffusion in cell membranes by the fluorescence photobleaching recovery method. *Proc. Natl. Acad. Sci. USA.* 77:2043–2045.
3. Smith, L. M., J. W. Parce, B. Smith, and H. M. McConnell. 1979. Antibodies bound to lipid haptens in model membranes diffuse as rapidly as the lipids themselves. *Proc. Natl. Acad. Sci. USA.* 76:4177–4179.
4. Hafemen, D. G., V. von Tscharner, and H. M. McConnell. 1981. Specific antibody dependent interactions between macrophages and lipid haptens in planar lipid monolayers. *Proc. Natl. Acad. Sci. USA.* 78:4552–4556.
5. Poo, M.-m., J. W. Lam, N. Orida, and A. W. Chao. 1979. Electrophoresis and diffusion in the plane of the cell membrane. *Biophys. J.* 26:1–22.
6. Poo, M.-m. 1982. Rapid lateral diffusion of functional ACh receptors in embryonic muscle cell membrane. *Nature (Lond.).* 295:332–334.
7. Axelrod, D., A. Wight, W. Webb, and A. Horwitz. 1978. Influence of membrane lipids on acetylcholine receptor and lipid probe diffusion in cultured myotube membrane. *Biochemistry.* 17:3604–3609.
8. Tank, D. W. 1983. Ph.D. Thesis. Cornell University, Ithaca, New York.
9. Barak, L. S., and W. W. Webb. 1982. Diffusion of low density lipoprotein receptor complex on human fibroblasts. *J. Cell Biol.* 95:846–852.
10. Schlessinger, J., W. W. Webb, E. L. Elson, and H. Metzger. 1976. Lateral motion and valence of Fc receptors on rat peritoneal mast cells. *Nature (Lond.).* 264:550–552.
11. Barsumian, E. L., C. Isersky, M. G. Petrino, and R. P. Siraganian. 1981. IgE induced histamine release from rat basophilic leukemia cell lines: isolation of releasing and nonreleasing clones. *Eur. J. Immunol.* 11:317–323.
12. Taurog, J. D., C. Fewtrell, and E. L. Becker. 1979. IgE mediated triggering of rat basophilic leukemia cells: lack of evidence for serine esterase activation. *J. Immunol.* 122:2150–2153.
13. Bazin, H., P. Querinjean, A. Beckers, J. F. Heremans, and F. Dessy. 1974. Transplantable immunoglobulin-secreting tumours in rats. IV. Sixty-three IgE secreting immunocytoma tumours. *Immunology.* 26:713–723.
14. Liu, F.-t., J. W. Bohn, E. L. Ferry, H. Yamamoto, L. A. Molinaro, L. A. Sherman, N. R. Klinman, and D. H. Katz. 1980. Monoclonal dinitrophenyl specific murine IgE antibody: preparation, isolation, and characterization. *J. Immunol.* 124:2728–2737.
15. Amante, L. A., A. Ancona, and L. Forni. 1972. Conjugation of immunoglobulins with tetramethylrhodamine isothiocyanate. *J. Immunol. Methods.* 1:289–301.
16. Titus, J. A., R. Haugland, S. O. Sharrow, and D. M. Segal. 1982. Texas red, a hydrophilic, red-emitting fluorophore for use with fluorescein in dual parameter flow microfluorometric and fluorescence microscopic studies. *J. Immunol. Methods.* 50:193–204.

---

[3] It is noteworthy that both FRAP and PFR give about the same D value for IgE-bound Fcϵ receptors. Along with other evidence presented here, this belies a suggestion (31, 32) that the divergent FRAP and PFR results obtained with some other cell types differ because PFR and FRAP measure fundamentally different processes.

17. Segal, D. M., and E. Hurwitz. 1976. Dimers and trimers of immunoglobulin G covalently cross-linked with a bivalent affinity label. *Biochemistry.* 15:5253–5258.

18. Meyer, C., L. M. Wahl, B. M. Stadler, and R. P. Siraganian. 1983. Cell cycle associated changes in histamine release from rat basophilic leukemia cells separated by counterflow centrifugal elutriation. *J. Immunol.* 131:911–914.

19. Hudson, L., and F. C. Hay. 1980. Practical Immunology. Blackwell Scientific Publications, London. 45.

20. Hafeman, D. G., L. M. Smith, D. T. Fearon, and H. M. McConnell. 1982. Lipid monolayer-coated solid surfaces do not perturb the lateral motion and distribution of C3b receptors on neutrophils. *J. Cell Biol.* 94:224–227.

21. Maxfield, F. R., J. Schlessinger, Y., Shechter, I. Pastan, and M. C. Willingham. 1978. Collection of insulin, EGF and α_2-macroglobulin in the same patches on the surface of cultured fibroblasts and common internalization. *Cell.* 14:805–810.

22. Poo, M.-m. 1981. In situ electrophoresis of membrane components. *Annu. Biophys. Bioeng.* 10:245–276.

23. Conrad, D. H., E. Studer, J. Gervasoni, and T. Mohanakumar. 1983. Properties of two monoclonal antibodies directed against the Fc and Fab' regions of rat IgE. *Int. Arch. Allergy Appl. Immunol.* 70:352–360.

24. Conrad, D. H., H. Bazin, A. H. Sehon, and A. Froese. 1975. Binding parameters of the interaction between rat IgE and rat mast cell receptors. *J. Immunol.* 114:1688–1691.

25. McLaughlin, S., and M.-m. Poo. 1981. The role of electroosmosis in the electric field-induced movement of charged macromolecules on the surfaces of cells. *Biophys. J.* 34:85–93.

26. Metzger, H., D. Budman, and P. Lucky. 1976. Interaction of IgE with rat basophilic leukemia cells. V. Binding properties of cell free particles. *Immunochemistry.* 13:417–423.

27. Luft, J. H. 1976. The structure and properties of the cell surface coat. *Int. Rev. Cytol.* 45:291–382.

28. Goldberg, R. L., and B. P. Toole. 1984. Hyaluronate coat formation and cell spreading in rat fibrosarcoma cells. *Exp. Cell Res.* 151:258–263.

29. Holowka, D., and B. Baird. 1983. Structural studies on the membrane-bound immunoglobulin E-receptor complex. 2. Mapping of distances between sites on IgE and the membrane surface. *Biochemistry.* 22:3475–3484.

30. Wank, S. A., C. DeLisi, and H. Metzger. 1983. Analysis of the rate-limiting step in a ligand-cell receptor interaction: the immunoglobulin E system. *Biochemistry.* 22:954–959.

31. Zagyansky, Y. A., and S. Jard. 1979. Does lectin-receptor complex formation produce zones of restricted mobility within the membrane? *Nature (Lond.).* 280:591–593.

32. Edidin, M. 1981. Molecular motions and membrane organization and function. *In* Membrane Structure. J. B. Finean and R. H. Michell, editors. Elsevier, Amsterdam. 37–82.

33. Horsfield, G. I. 1965. The effect of compound 48/80 on the rat mast cell. *J. Pathol. Bacteriol.* 90:599–605.

34. Lawson, D., C. Fewtrell, and M. C. Raff. 1978. Localized mast cell degranulation by concanavalin A-sepharose beads. *J. Cell Biol.* 79:394–400.

35. Smith, L. M., H. R. Petty, P. Parham, and H. M. McConnell. 1982. Cell surface properties of HLA antigens on Epstein Barr virus transformed cell lines. *Proc. Natl. Acad. Sci. USA.* 79:608–612.

36. Edidin, M., and T. Wei. 1982. Lateral diffusion of H-2 antigens on mouse fibroblasts. *J. Cell Biol.* 95:458–462.

37. Isersky, C., J. Rivera, D. M. Segal, and T. Triche. 1983. The fate of IgE bound to rat basophilic leukemia cells. II. Endocytosis of IgE oligomers and effect on receptor turnover. *J. Immunol.* 131:1–9.

38. Fewtrell, C., A. Kessler, and H. Metzger. 1979. Comparative aspects of secretion from tumor and normal mast cells. *Adv. Inflammation Res.* 1:205–221.

39. Braun, J., P. S. Hochman, and E. R. Unanue. 1982. Ligand induced association of surface immunoglobulin with the detergent-insoluble cytoskeletal matrix of the B lymphocyte. *J. Immunol.* 128:1198–1204.

40. Jack, R. M., and D. T. Fearon. 1983. Cytoskeletal attachment of C3b receptor and co-redistribution with Fc receptor on human polymorphonuclear leukocytes. *Fed. Proc.* 42:1235.

41. Baird, B., A. Menon, D. Robertson, and D. Holowka. 1984. Bridging of receptors for immunoglobulin E on the cell surface triggers their interaction with the cytoskeletal architecture. *J. Cell. Biochem.* (Suppl. 8A): 269.