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

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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} cm²/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} cm²/s at 24°C). This range of D values is close to that found in previous photobleaching studies of the IgE-Fc 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). Hence, the search is on for extracellular 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} cm²/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...
The binding of lectins, F(ab')2 fragments, or other multivalent ligand for long periods on the cell surface without inducing receptor cross-linkage or the active cellular responses can compare the results from two different methods for 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 ligand for an extended period on the cell surface without inducing receptor cross-linkage or the active cellular responses that uncomplexed low-density lipoprotein receptors on an 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 systematic increase in the starting asymmetry index (see below) with culture 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 NaNO3, 1 mM Na2EDTA, 109 mM NaCl, 2 mM CaCl2, 1.5 mM MgCl2, 5.4 mM KCl, 0.1% glucose, pH 7.3). Cell viability was checked with trypan blue, and cells were diluted to 1-2 × 106/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-α26.82 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). Fluorescent isothiocyanate-labeled rabbit antimouse 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, 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 56 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-lgE) 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 concentration 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 TR-lgE, 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 undervatised mouse IgE did reveal multimeric components. Because the mouse IgE was not purified by gel filtration after its reaction with TRITC or 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 56 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).

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 cm2/s, but using FRAP a D value of 1.4 × 10–11 cm2/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 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 that uncomplexed low-density lipoprotein receptors on an 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 passed as described in (12). Stock cultures were passed weekly into 75-cm2 flasks (Corning Glass Works, Corning, NY), and at this time aliquots of 1.5 × 109 cells were seeded into 6-cm-diam tissue culture dishes (Coming) 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 polyclene (0.6 mM Na2EDTA, 0.14 M NaCl, 2.7 mM KCl, 9.5 mM Na2HP04, 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 CaCl2, 0.4 mM MgCl2, 1 mM Na2EDTA, 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.
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 × 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 × 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 × 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-aperture 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 4% H2O2 for 30-60 min and rinsed with distilled H2O 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 (Y9409, 3M, St. Paul, MN) were used as the side-walls (spacers) of the cell chamber, and a 24 × 40-mm coverslip 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), a few seconds were required to reach the desired temperature within ±0.5°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. The top of the chamber slides were insulated by laying a styrofoam wafer over each.

Using a microfluorometric technique described before (5), except that a 4-μm aperture was used to collect photons from anode- and cathode-facing poles of the cell. An asymmetry index, A(I), was calculated for each cell:

\[ A(I) = (I_+ - I_-)/(I_+ + I_-) \]

where \( I_+ \) and \( I_- \) are the fluorescence intensities at cathodal and anodal edges, after subtraction of the background. The latter was determined separately for each cell. Photon meter readings over the edge of unlabeled background were only 4% 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 remaining slides were scored single blind, with two of us using exclusive criterions for 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. 4B (qv.), the error is 33%. This is likely an upper limit to such error in the population studies, since ln(A(I)) and ln(A(I)) approach each other as the spacing between x 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 (12.3 μm) estimated from Coulter counter measurements of suspended RBL cells (18), indicating that not much swelling 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 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 x40 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 31M paper.

RESULTS

Electromigration of Vacant and IgE-occupied Fc Receptors

In the first set of experiments, RBL cells were labeled with TRG-IgE and then plated in the cell chambers. Application of an electric field caused redistribution of the labeled Fc receptors on the cell surface from a uniform to a highly asymmetric distribution. Fig. 2, a-d depicts TRG-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. 2b. This experiment was conducted with 30 mM NaN3 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 α2-macroglobulin (21), and f is...
the same cell after addition of 10 mM CuSO₄ 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),

$$m = \left(\frac{D}{3E_o r}\right) \ln\left[\frac{1 + A_s}{1 - A_s}\right]$$

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 $A_s$ begins to decrease after 15–20 min in the field, especially with post-field staining.
during the post-field staining period: as shown in Fig. 5A (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 ($A_{i20'} = 0.65$ 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 Fce 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 × 10⁻¹⁴, 3.4 × 10⁻¹⁶, and 6.6 × 10⁻¹⁷ cm²/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, CuSO₄ (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 CuSO₄, 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 NaN₃ and 0.1% glucose without noticeably different outcomes. Together, these results are consistent with passive back-diffusion of IgE-Fce 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 Aᵢ for that cell. This quantitative effect was observed directly during single cell recording as a large and sudden jump in the steady-state Aᵢ 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 (Aᵢ - Aᵢ̅), 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. 4 b we found an average D value of 4.6 × 10⁻¹⁰ cm²/s (25°C) for TRr-IgE bound to Fce receptors (range: 1.5 × 10⁻¹⁰ to 1.1 × 10⁻⁹ cm²/s). The average D value for bound mouse IgE (Texas Red- or TRITC-labeled) was 1.8 × 10⁻¹⁰ cm²/s (25°C) for the 37 cells measured (range: 5 × 10⁻¹¹ to 6 × 10⁻¹⁰ cm²/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 Fce 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 cm²/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 cm²/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 Aᵢ 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 Fce 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,
electromigration. Because this fraction may have been a select a minor fraction remained stuck to plain glass slides after the treated RBL cells, but the gestalt impression is faithfully was observed in control cells, and somewhat less in trypsin- cocalyx (27). It reportedly also binds to sialoglycoproteins, stained ceils that had or had not been trypsin-treated (as above) before staining are shown in Fig. 6. Ruthenium red is general lack of an effect of trypsin corroborates the FRAP glass are slightly lower than for untreated glass (Table II). The to be sure, substantial variation in the glycocalyx thickness provided their negative charge density is high enough (27). of acidic mucopolysaccharides like those present in the gly- component(s).

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 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 Fc 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 Fce 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 5](https://example.com/figure5.png)

**Figure 5** (A) Population recovery curves for free and TRr-lgE-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 $A$, between cells on single slides averaged 0.16 and the standard error of the mean $A$, between different slides at each time point ranged from 0.02 to 0.04. Solid triangles show $A$, 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 $A$, between slides at each time point ranged from 0.01-0.09.
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. 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 Aτ 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 Aτ 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 Aτ 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. 4B), 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.

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