Membrane-bound IgE Receptor Complexes Fused with Rat Basophilic Leukemia Cells Mediate Degranulation

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Abstract. The high affinity receptor for IgE on rat basophilic leukemia (RBL) cells mediates antigen-triggered cellular degranulation. Polyethylene glycol-induced membrane fusion methods were used to introduce exogenous IgE receptors into living RBL cells, and these were tested for normal activities. In cell-cell fusion experiments, RBL cells with fluorescein-labeled rat IgE bound to receptors and containing [5-1,2-3H(N)]hydroxytryptamine binoxalate ([3H]5HT) in their secretory granules were fused to cells with receptors occupied by rhodamine-labeled anti-dinitrophenyl mouse IgE. The fused cells showed a uniform surface distribution of both types of IgE, which could be patched independently by anti-IgE or dinitrophenylated bovine gamma globulin (DNP6BGG). [3H]5HT release could be triggered specifically by DNP6BGG. In vesicle-cell fusion experiments, plasma membrane vesicles, with receptors occupied by fluorescein- and [125I]-labeled anti-DNP mouse IgE, were fused to RBL cells containing [3H]5HT. The cells showed substantial associated fluorescein fluorescence and [125I] counts, and [3H]5HT release could be triggered specifically by DNP6BGG. These experiments indicate that IgE receptors can be dissociated from their natural cellular interactions and retain the ability to reassociate with another cell's components to deliver the transmembrane signal for degranulation.

The receptor for IgE in the plasma membrane of mast cells and basophils mediates antigen triggering of degranulation in the allergic response. The crosslinking of IgE receptor complexes initiates the transmembrane signal, but the mechanism by which this signal is propagated and leads to the release of components from secretory granules has not been elucidated (see reference 25 for review). One powerful approach to understanding structure-function relationships in macromolecular complexes is purification of essential components and reconstitution into an operational system. Methods have been developed to purify the receptor for IgE from the rat basophilic leukemia (RBL) cells (18) and for reincorporation of the purified receptor into phospholipid vesicles (27). However, functional competence of these receptors is not readily evaluated because the expression of activity such as degranulation requires other parts of the cell.

Membrane fusion methods can be used to reconstitute complex cellular systems. For example, Schramm first used polyethylene glycol (PEG) to hybridize the plasma membranes from two different cells and thereby reconstitute the components necessary for receptor-mediated adenylate cyclase activity (28). Similar PEG fusion methods are now being applied with purified components of this receptor-coupled system to ask detailed questions of structure and function (7). In the IgE receptor system, Mazurek et al. (21) recently used Sendai virus-mediated fusion to introduce cromolyn binding proteins into the membranes of deficient RBL cells to reconstitute degranulation activity. We have now used PEG-induced membrane fusion methods to reconstitute IgE receptor complexes into RBL cells. We have been able to fuse RBL cells with each other (cell-cell fusion) and to fuse RBL cells with plasma membrane vesicles derived from RBL cells (vesicle-cell fusion). In both types of experiments exogenous IgE receptors fused into RBL cells were able to interact functionally with native components and cause release from endogenous secretory granules. This is the first step toward developing a fusion reconstitution system with more highly purified preparations for studying in detail the structural requirements of IgE receptors in cell triggering.

Materials and Methods

IgE and Triggering Reagents

Mouse monoclonal anti-2,4-dinitrophenyl (anti-DNP) IgE (IgEa) was
purified from ascitic fluid of CAF1 mice bearing the tumor H 1 DNP-e-26-82 (20) by a previously described procedure that uses affinity chromatography (16). Purified rat myeloma IgE from tumor IR 162 (IgEa,5), anti-fluorescein (FITC) and rhodamine (R110) monoclonal antibodies (mAbs) that were gifts from Dr. Henry Metzger (National Institutes of Health). TRITC (Research Organics, Inc., Cleveland, OH) and FITC (Molecular Probes, Inc., Eugene, OR) were used to prepare fluorescent derivatives of IgE as previously described (9, 23). [125I]-labeled IgE was prepared by the chloramine T method (19). Rabbit anti-IgEa (6) and anti-IgE~ (22) were produced and purified as previously described, and AffiniPure goat anti-rat IgG (H+L) was purchased from Jackson ImmunoResearch Laboratories, Inc., Avondale, PA.

**Cells and Plasma Membrane Vesicle Preparation**

RBL cells (subline 2H3) were maintained in stationary culture (4, 30) and used 5-6 d after passage. Plasma membrane vesicles were prepared from blebbing cells that had been treated with 50 mM (0.15% wt/vol) formaldehyde and 1 mM EDTA as described elsewhere (15). The resulting vesicles were resuspended in K buffer (135 mM NaCl, 20 mM Tris-HCl, pH 7.4) containing 10 μg/ml buparotylated hydroxytyocele and dialyzed against K buffer. Vesicles prepared in PBS (150 mM NaCl, 10 mM sodium phosphate, pH 7.4) caused massive cell death in fusion experiments. Vesicles prepared with this basic procedure are in a volume of ~1.2 ml and have a receptor concentration of ~20 pmol receptor/ml as determined by binding studies performed with [125I]-labeled IgEa (15). The use of formaldehyde apparently does not result in a large amount of cross-linking in the membranes, since most of the receptors are very mobile and readily redistribute upon bridging by antigen or anti-IgE, and since autoradiographs of SDS-polyacrylamide gels of cells from pre-bound [125I]-labeled IgE show that <10% of the label migrates as higher molecular weight products (15). For fusion experiments, the vesicles were then treated with fluorescein- and [125I]-labeled IgEa by incubating with a four- to fivefold molar excess over receptors for 12-16 h at 4°C, collected by centrifugation at 25,000 g for 45 min, and resuspended in 200-500 μl K buffer.

**Membrane Fusion**

The PEG (pharmaceutical grade, M, 1,540; Polysciences, Inc., Warrington, PA) was melted and diluted to 60% (wt/vol) with buffered medium (Earle's balanced salt solution containing 20 mM Hepes, pH 7.4), then filtered through a 0.22-μm filter, and titrated with NaOH to neutrality. Phynoehemagglutinin (PHA) type IV (Sigma Chemical Co., St. Louis, MO) was prepared in a stock solution (1 μg/ml in PBS). For cell–cell fusion experiments, cells growing adherent in culture in 75-cm² flasks were incubated with 15 μCi of [5-1,2-3H(N)]-5-hydroxytryptamine (5-HT) (Sigma Chemical Co., St. Louis, MO) to quantitate the cell equivalent (CEq) per 10⁷ total Ceq were added to the cell–cell or vesicle–cell mixture, and the samples were mixed by gently drawing in and out of plastic pipette tips for 10 min. 60% PEG was added to achieve a final concentration of 46–50%, and the cell suspension was stirred gently for 6–8 min. The samples were then diluted to 15 ml with supplemented medium and transferred into 75-cm² flasks. DNAse (type III, Sigma Chemical Co.) was added to a final concentration of 50 μg/ml. In control samples, the same amount of PEG was added after the dilution. After a recovery incubation at room temperature for 1 h and at 37°C for 1–2 h, the cells were harvested from the flasks, washed twice with buffered salt solution (BSS; 135 mM NaCl, 5 mM KCl, 1 mM MgCl₂, 1.8 mM CaCl₂, 56 mM glucose, 0.1% gelatin, 20 mM Hepes, pH 7.4), and 5 μg of rat IgEa were added to each sample. In vesicle–cell experiments, the [125I] was counted again to determine the Ceq of vesicles still associated. The cells were counted and assayed for [125I]-HT release. Samples were observed with a Leitz Ortholux II fluorescence microscope equipped with 12 (fluorescein: excitation band pass filter 450–490 nm, emission barrier filter 515 nm) and N2 (rhodamine: excitation band pass filter 530–560 nm, emission barrier filter 580 nm) cubes with a Leitz 90° water immersion fluorescence objective (NA = 1.00).

**Results and Discussion**

**The Fusion Procedure**

PEG-induced membrane fusion has been widely used as a means to create hybrid cells (8) and also to introduce vesicular membranes into living cells (1, 26). The major problem in this approach is that conditions facilitating membrane fusion are severe, leaving open a narrow window between efficient fusion and cytotoxicity, and this is further complicated by variability of the cells. This is a serious limitation in reconstitution studies which depend on a measurable activity of intact cells compared to, for example, PEG-induced production of hybridomas. In the latter case, high cell viability is not necessary since a new cell line can be derived from a single living cell. Another problem facing attempts to set up an experimental system for reconstituting receptors for IgE arises from there being no known RBL cell variant that lacks these receptors but contains all of the other components necessary for receptor-mediated degranulation. We could circumvent this problem by taking advantage of the very tight binding of IgE to its receptor (Kd > 10⁹ M⁻¹) and its slow dissociation rate (19). We used two types of monoclonal IgE (IgEa and IgE~) which could be differentially labeled with fluorescent probes and [125I], to monitor the distribution of receptors from a particular source. Also, since the two types of IgE have different specificities, they could be selectively cross-linked to trigger cellular degranulation as a test of receptor function.

We carried out a large number of preliminary experiments on RBL cells to optimize conditions for causing efficient membrane fusion with the least amount of cytotoxicity. A lectin was used to cause limited membrane agglutination before fusion (24). PHA was selected because unlike some lec-
Figure 1. Fluorescence micrographs of samples containing a mixture of rhodamine-IgE-labeled cells and fluorescein-IgE-labeled cells that had been subjected to the fusion procedure. Microscope fields were viewed either with rhodamine (A, C, and E) or fluorescein (B, D, and F) optics. A and B show the same field which contains one fused cell exhibiting both fluorescent IgE markers, and two cells labeled only with fluorescein-IgE. C and D show the same field of a sample to which DNP-6BGG was added. The field contains one fused cell and one fluorescein-IgE-labeled cell. E and F show a fused cell from a sample to which anti-IgE was added. Bar, 5 µm.

tins (13) it does not by itself cause cross-linking of cell surface IgE and thereby triggering of cellular degranulation (data not shown). After PEG treatment, the cells were recultured for a few hours in order to select for healthy cells, since these adhere to plastic while the dead and dying cells do not. IgE was always bound to the exogenous source of receptors to be tested for reconstitution, and the host cells loaded with [3H]5HT always had IgE bound to receptors. To minimize any replacement of the IgE bound to receptors on the host cells by IgE, the presence of excess IgE was maintained throughout the fusion and recovery processes.

Cell–Cell Fusion

These experiments were carried out to determine in the gentlest way possible whether receptors from the plasma membrane of one cell could communicate with granules from another cell after membrane fusion. RBL cells having receptors saturated with fluorescein-labeled IgE and containing [3H]5HT in their secretory granules were combined under fusion conditions with cells having receptors saturated with rhodamine-labeled anti-DNP IgE and containing no [3H]-5HT. Fluorescence microscopy allowed visualization of the fused cells. Normal RBL cells with bound, fluorescent IgE exhibit a ring stain of fluorescence indicating a uniform distribution of receptors on the cell surface. When a reagent that cross-links IgE receptors is added to these cells, clustering occurs, and fluorescent patches are produced. As shown in Fig. 1, fused cells exhibit a ring stain and can be identified by the presence of both rhodamine (Fig. 1A) and fluorescein fluorescence (Fig. 1B). Addition of DNP-6BGG to samples caused patching of the rhodamine-IgE on fused cells (Fig. 1C), while the fluorescein IgE remained ring stained (Fig. 1D). Addition of anti-IgE which is cross-reactive for IgE caused patching of both rhodamine and fluorescein fluorescence on the fused cells (Fig. 1, E and F). Hence the IgE receptor complexes from both cells are free to diffuse over the fused cell plasma membrane and are clustered normally and independently by immunological cross-linking reagents.

Although fluorescence microscopy reveals the degree of cell–cell fusion and distributions of IgE receptor complexes on the hybridized cell, it does not allow assessment of whether those receptors become functionally reconstituted with other cellular structures along the signal transduction pathway. For this purpose IgE-mediated degranulation was tested. The release of [3H]5HT triggered by DNP-6BGG revealed the presence of IgE receptor complexes that had become functionally linked to granules containing [3H]5HT that came from cells initially labeled only with IgE. The [3H]5HT release from cells triggered by anti-IgE via their endogenous IgE receptor complexes could be used as an internal measure of the cells' capacity to undergo receptor-mediated degranulation.

Dose–response curves from a cell–cell fusion experiment are shown in Fig. 2. For comparison purposes Fig. 2A shows typical curves for release triggered by DNP-6BGG and anti-IgE when IgE and IgE are both present on the same cells. The DNP-6BGG curve is broad, and maximal release occurs at very low doses, whereas the anti-IgE curve has a narrower peak at higher doses. For the negative control sample (Fig. 2B), the same procedure was carried out as for the fusion sample, except that the PEG was added after dilution of the mixed cells. As was generally observed, this control sample shows some apparent DNP-6BGG-triggered release above background, which is probably due mostly to a small amount of exchange of the IgE and IgE (as shown in a vesicle–cell experiment below). Also, there may be some uptake of [3H]5HT by the IgE-labeled cells occurring during the recovery incubations after the fusion. The DNP-6BGG-triggered release of the sample containing fused cells (Fig. 2C) is considerably above that of the control, and the dose–response curve has the same shape as for normal cells (Fig. 2A).

A large number of cell–cell fusion experiments were carried out, and the various samples were evaluated by two-color fluorescence microscopy and by the degranulation assay. Occasionally the experiments did not work: most of the
cells died and/or little or no fusion was observable. There was no obvious explanation for these failures other than the variability of the cells in their reaction to the same treatment. However, we never observed false positive results; i.e., there was always the same or more [3H]5HT release stimulated by DNP16BGG in the fusion samples than in the control samples. Four representative cell–cell fusion experiments with positive results are summarized in Table I. The control samples contained either cells saturated with IgEm or IgEr that had been subjected to the fusion procedure separately before mixing the suspensions (Exp. 1) or the combined cells to which the same amount of PEG was added after dilution (Exps. 2–4). Cell viability in the final fusion and control samples was always 90% or greater by trypan blue exclusion, and recovery of cells from the initial mixtures was generally 30–50% (not corrected for fusion of cells in those samples). The percentage of fused cells was calculated by counting rhodamine-labeled cells and the number of those that also exhibited fluorescein fluorescence. This varied from 5–19% in fusion samples and was always <1% in the control samples. To put some perspective on these values the following may be considered. If all of the combined cells fused statistically in pairs (1 XX: 2 XY: 1 YY), the observed number of fluorescein- and rhodamine-labeled cells would be 66% of the rhodamine-labeled cells. Using this model, we obtained hybridization efficiencies of 7.5–29%. This may be an underestimation because large aggregates of cells (not scored) probably included a higher percentage of fused cells, and also fusion of three or more cells was occasionally observed. These fusion efficiencies compare to the <0.01% typically obtained with standard procedures for making hybridomas (14).

For a quantitative measure of the apparent functional reconstitution of receptors in the degranulation assay, the [3H]5HT release stimulated by exogenous receptors (labeled with anti-DNP IgEm) was compared to the [3H]5HT release stimulated by the endogenous receptors (labeled with IgEr). The relative DNP16BGG release was calculated for each sample: (net DNP16BGG release/net anti-IgEr release), where the net triggered release values used were obtained by subtracting the spontaneous release from the maxima of the respective dose–response curves (see Fig. 2). As shown in Table I, the relative DNP16BGG release in fusion samples was 0.2–0.4 over that in control samples in successful cell–cell fusion experiments. Considering the same model as above, if all of the combined cells fused statistically in pairs and if DNP16BGG and anti-IgEr were inherently

Table I. Cell–Cell Fusion

| Exp. | PEG | PHA | Percent fused  | Net DNP16BGG/ | Net DNP16BGG/ |
|------|-----|-----|---------------|----------------|---------------|
|      |     |     |               | DNP16BGG       | net anti-IgEr  |
| 1    | +   | −   | 0             | 3.0            | 0.12           |
|      | +   | +   | 9             | 10.3           | 0.42           |
| 2†   | a.d.| +   | 1             | 1.7            | 0.17           |
|      | +   | −   | 1             | 3.1            | 0.37           |
|      | +   | +   | 10            | 4.4            | 0.46           |
|      | +   | +   | 5             | 5.9            | 0.61           |
| 3    | a.d.| +   | 0             | 3.1            | 0.13           |
|      | +   | −   | 2             | 2.7            | 0.15           |
|      | +   | +   | 8             | 7.1            | 0.32           |
|      | +   | +   | 19            | 8.4            | 0.40           |
| 4**  | a.d.| +   | 0             | 7.2            | 0.23           |
|      | +   | +   | 10            | 9.4            | 0.53           |
|      | +   | +   | 16            | 9.4            | 0.55           |

† Samples were treated with 3–5 μg PHA/10⁷ cells (+) or not (−) and 50% PEG (+) or the same amount of PEG was added after dilution (a.d.).
‡ Number of double-labeled cells per 100 rhodamine-labeled cells.
§ [3H]5HT release assays were performed as described in Materials and Methods. Spontaneous release values of samples with PEG added after dilution were 19.9 ± 10.9%. Spontaneous release values of fusion samples were 20.9 ± 8.45%.
¶ In this experiment fluorescein-IgEr was used and the anti-IgEr trigger was anti-fluorescein.
** In this experiment the [3H]5HT release assays were performed in 30% D₂O.

Figure 2. [3H]5HT release from cells triggered by DNP16BGG (●) or anti-IgEr (○). Dose–response curves of (A) a sample of cells labeled with both IgEr and anti-DNP IgEm; (B) the control sample from a cell–cell fusion experiment; and (C) the fusion sample of the same experiment. For cell–cell fusion experiments, IgEr-labeled cells that had been loaded with [3H]5HT were mixed with anti-DNP IgEm-labeled cells and treated as described in Materials and Methods. The error bars represent the range of duplicate samples. Dashed lines indicate spontaneous release (duplicate samples).
equally good triggers, then the relative release triggered by DNP$_{6}$BGG would be expected to be 0.5. Although this is a highly oversimplified view, it does suggest that the (net DNP$_{6}$BGG release/net anti-IgER release) shown by the fusion samples over that of the control samples reflects a significant reconstitution of exogenous receptors that are functional. In this regard it should be noted that under normal conditions \(<10\%\) of a single cell's receptors need be involved in triggering maximal degranulation (11). Consequently, the percentage of the exogenous receptors introduced by cell fusion that have been functionally integrated cannot be estimated accurately.

Other observations made in the cell-cell fusion studies are exemplified by the experiments shown in Table I. Even within the same experiment there was some variability in the success of fusion in two separate samples treated essentially the same (last two lines of Exp. 2–4). Cells subjected to 50\% PEG and no PHA sometimes showed evidence of fusion, but addition of lectin generally provided a substantial enhancement (Exp. 2). We tested the use of D$_{2}$O in the degranulation assay (in Exp. 4) since this is known to enhance the release caused by a low level of stimulation (12). In these experiments D$_{2}$O generally had no preferential effect on the fusion samples and usually caused a relative increase in release of the control sample, suggesting that release triggered by the small amount of exchanged IgE is amplified in the presence of this reagent.

In spite of the variation of fusion efficiency among experiments, our results provide strong evidence that IgE receptors from one cell can become structurally and functionally integrated into another cell after the two have been fused. (a) At the detection limit of fluorescence microscopy all receptors from each cell diffuse over the combined plasma membrane and can be cross-linked to form patches on the cell surface. (b) At least some significant percentage of these surface receptors can cross-interact with intracellular components of the mated cell to trigger release of its granular contents. An important extension of these studies will be to determine if cross-triggering can occur between heterologous cell types. For example it would be interesting to see if IgE receptors from RBL cells can cause degranulation of neutrophils after the two types of cells have been fused.

**Vesicle–Cell Fusion**

These experiments were undertaken to determine whether receptors removed from intact cells in the form of plasma membrane vesicles retain their ability to interact with other cellular components after reconstitution to trigger degranulation. Plasma membrane vesicles were prepared from RBL cells, and their IgE receptors were saturated with fluorescein- and $^{125}$I-labeled IgE. These were combined with intact cells having receptors saturated with IgE and containing [$^{3}$H]5HT in their secretory granules. In samples that had been treated with lectin and PEG under fusion conditions, easily visible fluorescein fluorescence was found to be associated with all of the RBL cells (Fig. 3 A). Much of the fluorescence appeared in clumps which probably represented vesicles that had agglutinated with the cells by means of the lectin. There were also smooth areas of uniform stain on the cell surface, and these appeared to be areas where the vesicles had become integrated with the cell by membrane fusion. Control samples included approximately the same amounts of cells and vesicles and were treated the same way, except that the PEG was added after dilution. These showed a much smaller amount of fluorescence that all appeared as clumps (Fig. 3 B).

Functional reconstitution of the receptors originating in the plasma membrane vesicles was tested with the degranulation assay, and positive results were observed in many experiments. The dose–response curves of one of these is shown in Fig. 4. As with the cell-cell fusion some release is triggered by DNP$_{6}$BGG in the control sample (Fig. 4 A), but this is considerably less than in the fusion sample (Fig. 4 B). The vesicle–cell fusion experiments failed more often than the cell–cell fusion experiments, and this was attributed mostly to a higher level of cytotoxicity under the conditions required for fusion. In these experiments there was always more fluorescent and $^{125}$I-labeled vesicle material associated with the cells in the fusion sample than in the control sample, but DNP$_{6}$BGG-triggered [$^{3}$H]5HT release was not always significantly greater in the fusion sample. In no experiment did we observe significantly more DNP$_{6}$BGG-triggered release in the control sample. In the case of vesicle–cell fusion we found that degranulation assays carried out in the presence of 30\% D$_{2}$O showed positive results more often, suggesting that the low level stimulation by exogenous receptors was enhanced by this reagent.

The results of three vesicle–cell experiments with positive results are shown in Table II. Cell viability was \(>85\%\) by trypan blue exclusion for both fusion and control samples at the final stage. However, cell recovery was typically lower in the fusion samples (30–60\%) than in control samples (50–75\%). The amount of cell equivalents of vesicles associated with the cells before and after the fusion procedure was quantified by the bound $^{125}$I. The fusion samples had 0.1–0.4 Ceq of vesicles associated per cell after the final wash, which was 24–34\% of the amount added initially and 3–4 times the amount associated with cells in the control samples in the same experiment. For the fusion samples (net DNP$_{6}$BGG release/net anti-IgE release) was 0.1–0.4 over that of control samples, showing functional reconstitution of exogenous receptors. Exchange of IgE for IgE on the endogenous cellular receptors was probably the main reason for DNP$_{6}$BGG release in the control samples. This is indi-

![Figure 3. Fluorescence micrographs from a vesicle–cell fusion experiment in which fluorescein-IgE was bound to vesicles and unlabeled IgE was bound to cells. A is the fusion sample and B is the control sample. Fluorescein optics were used, and the photographic procedures were identical for the fusion and control samples. Bar, 5 µm.](image-url)
uated by Exp. 2 of Table II, in which the addition of excess IgE after the fusion procedure was omitted, resulting in high release in the control samples containing no PHA or no PEG. Although the greater number of vesicles associated with the cells in fusion samples by the combination of PHA and 50% PEG might possibly result in enhanced IgE exchange, we feel this is very unlikely on the basis of three observations (data not shown). (a) PHA-mediated agglutination of vesicles to cells persists in both fusion and control samples until trypsin treatment of cells after the recovery incubation. (b) A sample containing the same amount of vesicles but no PHA or PEG shows nearly identical degranulation in response to DNP-BGG as the control sample with PHA and 3.5% PEG, and this is less than the response of the fusion sample. (c) IgE-receptor dissociation is not enhanced under the conditions of the fusion procedure with 50% PEG.

Positive results from the vesicle–cell fusion experiments indicate that some portion of the receptors contained in the isolated plasma membranes retain their ability to mediate the signal for degranulation after they have been reconstituted with intact cells. We would expect (net DNP-BGG release/net anti-IgE) = 1 with successful introduction of 0.1 Ceq of competent receptors per cell (11; assuming release triggered by anti-IgE and DNP-BGG is equal). The specific relative DNP-BGG release of 0.44 seen in Table II, Exp. 1 (0.59 minus 0.15) corresponds to functional reconstitution of 0.03–0.10 Ceq of receptors per cell if the occupancy data of Fewtrell (11) are used as a reference. These rough calculations indicate that a significant fraction of the exogenous receptors associated with the cells after the fusion procedure have been functionally reconstituted. Since there is evidence that serine esterases are involved in the signal transduction process (17) we generally did not use protease inhibitors in preparing the vesicles, and proteolysis is one way in which the exogenous receptors could be rendered inactive. Polyacrylamide gels of IgE receptors immunoprecipitated from solubilized vesicles showed intact α, β, and γ receptor subunits (not shown), but we cannot rule out that some portion of the receptors or some other critical protein may have been digested. Vesicles prepared in the presence of protease inhibitors did not yield more positive results (data not shown).

The vesicles used in the vesicle–cell fusion experiments are prepared by causing the cellular plasma membrane to bleb (15), and they have been shown to lack an intact cytoskeleton (3, 29). Since there is strong evidence that clustered IgE receptors become associated with the cytoskeleton and that this event is correlated with the signal for degranulation in normal RBL cells (23), it is possible that some continuing interactions with the cytoskeleton are important for maintaining receptor activity. Therefore we also tried plasma membrane vesicles prepared by nitrogen cavitation (10) in several fusion experiments. We obtained positive results in some of these experiments (data not shown), but functional reconstitution of exogenous receptors delivered to the cell in this type of vesicle as measured by the degranulation assay was not better than that observed with the blebbed membrane vesicle.

Table II. Vesicle–Cell Fusion

| Exp. | PEG | Ceq attached/ cell | Net [3H]5HT Release | Net DNP-BGG | Net DNP-BGG/ net anti-IgE |
|------|-----|-------------------|---------------------|-------------|--------------------------|
| 1    | a.d. | 0.026 (7.6)       | 3.4                 | 0.15        |
|      | +    | 0.096 (24)        | 15.3                | 0.59        |
| 2    | a.d. | 0.0 (0)           | 1.3                 | 0.02        |
|      | +    | 0.045 (7.1)**     | 30                  | 0.46        |
|      | a.d. | 0.036 (7.5)       | 31                  | 0.47        |
|      | +    | 0.15 (33)         | 40                  | 0.61        |
| 3    | a.d. | ND§               | 3.7                 | 0.12        |
|      | +    | ND                | 9.6                 | 0.29        |

* Fusion samples were treated with 2.3–4.9 ng PHA/10⁶ cells and 46–50% PEG (+). Control samples included PHA and had the same amount of PEG added after dilution (a.d.).
| Ceq of IgE associated with the cells after the final washing. Numbers in parentheses indicate the percentage of Ceq of vesicles added initially to the cells that remained associated after the final washing.
| [3H]5HT release assays were performed as described in Materials and Methods. Spontaneous release values of samples with PEG added after dilution were 8.9 ± 4.4%. Spontaneous release values of fusion samples were 10.8 ± 0.8%.
| Excess IgE was not added to the samples in this experiment.
| No PHA was added to this sample.
| ** No PHA was added to this sample.
| In this experiment, the [3H]5HT release assays were performed in 30% D₂O.
| ND, not determined. [¹²⁵I]IgE was not used in this experiment.
vesicles (Table II). Thus it appears that if cytoskeletal associations are important they can reform.

We have shown that IgE receptors can maintain their ability to mediate signal transduction during transfer between the plasma membranes of two different RBL cells. An important use of this reconstitution approach will be to modify the receptor in a specific manner and assess the effect on function. For example, we have recently shown that the cytoplasmically exposed portions of the receptor can be cleaved proteolytically from inside-out plasma membrane vesicles or from solubilized vesicles (Hammes, S., D. Holowka, and B. Baird, manuscript submitted for publication), and a reconstitution experiment should show whether these portions are important for signal transduction. As a first step to this experiment we have had some success in solubilizing plasma membrane vesicles, reforming them by removal of detergent, and fusing them with intact cells (data not shown). Although realizing the full potential of the vesicle–cell reconstitution method will require that the fusion efficiency be improved, this approach looks promising as a way to study structure–function relationships in more detail. It should eventually be possible to reconstitute purified, modified IgE receptors in reformed vesicles or synthetic lipid vesicles (27) and then into intact cells using methods similar to those described here.

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