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Immunology

Dynamics of signal transduction after aggregation of cell-surface receptors: Studies on the type I receptor for IgE

(Fc receptor/mast cell/phosphotyrosine)

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ABSTRACT **Many ligands stimulate cellular responses by aggregating the cell-surface receptors to which they are bound. We investigated several mechanistic questions related to aggregation of receptors by using the high-affinity receptor for IgE ($Fc_\varepsilon RI$) on mast cells as a model system. We briefly exposed cells to covalently cross-linked oligomers of IgE and then added excess monomeric IgE to prevent further aggregation. Early events were examined by monitoring the phosphorylation of protein tyrosines; later events were examined by monitoring secretion. We found that aggregated receptors continue to signal both late and early events in the absence of formation of new aggregates. Additional experiments suggested that the clustered receptors undergo a dynamic process of phosphorylation and dephosphorylation. Our findings suggest that for these and related receptors that function by aggregation, the persistence of signal transduction is directly related to the intrinsic affinity of the ligand for the individual receptor.**

Many plasma membrane receptors require aggregation—often dimerization—in order to initiate signal transduction (1, 2). The mechanism by which such clustering initiates a signal is still uncertain even for the most extensively studied receptors, but activation of tyrosine kinase(s) as one of the earliest consequences has been well documented in many systems (3, 4). We are investigating the high-affinity receptor for IgE ($Fc_\varepsilon RI$) on rat mast cells—a system closely related to the one for which the importance of aggregation was first recognized (5) and to cells bearing related "multisubunit immune response receptors" (6). Like the latter but unlike the receptors for growth factors, $Fc_\varepsilon RI$ has no known intrinsic kinase activity. However, phosphorylation of tyrosines on the receptor's subunits and on several other cellular proteins has been demonstrated as a proximate consequence of aggregating the receptor (4, 7–9).

In this study, we tested to see how long clustered receptors can remain active and whether this active state is itself stable or dynamic. Our approach involved exposing cells to covalently cross-linked oligomers of IgE. Because monomeric IgE has such a high affinity for $Fc_\varepsilon RI$ (10), the receptors that bound the oligomers remain stably aggregated. Shortly after the addition of oligomers, monomeric IgE was added in great excess to prevent further clusters from forming. Whereas previous authors have concluded that aggregated $Fc_\varepsilon RI$ are only transiently active, our results indicate that stably aggregated $Fc_\varepsilon RI$ are capable of sustained signaling.

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MATERIALS AND METHODS

Reagents and Cells. All the reagents we used have been described (11–15). Rat basophilic leukemia 2H3 (RBL) cells were cultured as described (16).

Generation and Purification of Oligomeric IgE. IgE oligomers were prepared essentially as described (17). Monomeric, dimeric, and trimeric IgE were separated on a Superose 6 column (Pharmacia) by repetitive chromatography in borate-buffered saline or for use with permeabilized cells, in 119 mM NaCl/5 mM KCl/5.4 mM glucose/25 mM Pipes, pH 7.2 (assay buffer).

Activation of Cells. Cells from 3-day cultures were detached with trypsin. The cells were washed once with medium and twice with warm assay buffer containing 1 mM $CaCl_2$, 0.4 mM $MgCl_2$, and 0.1% bovine serum albumin (BSA). Routinely the cells were suspended in the wash buffer at 5×10^6 cells per ml and stimulated at 37°C with periodic mixing.

Preparation and Analysis of Samples. The total cell proteins or immunoprecipitates of the receptor were analyzed for phosphotyrosines as described (15). A standard sample was used to normalize the densitometric scans (Imagequant; Molecular Dynamics) from separate gels.

Permeabilized Cells. Trypsin-treated washed cells suspended at 5×10^6 cells per ml in warm assay buffer containing 0.1% BSA, 1 mM ATP, and 5 mM $MgCl_2$ (for cell extracts) or 1 mM $MgCl_2$ (for immunoprecipitates) were permeabilized with *Staphylococcus aureus* α-toxin for 10 min at 37°C (18).

Secretion Assays. Attached cells grown in 24-well plates were washed twice with 0.5 ml of warm assay buffer/0.1% BSA/0.4 mM $MgSO_4$. Additions were made as indicated in the text and figure legends. Cell supernatants were assayed for released hexosaminidase in triplicate as described (15).

RESULTS

Phosphorylation Initiated by Oligomers of IgE. When RBL cells were reacted with trimeric IgE, phosphorylation of tyrosines on several cellular proteins (Fig. 1*A*) and on the γ and β chains of $Fc_\varepsilon RI$ (Fig. 1*B*) was stimulated. The pattern of phosphorylation was similar to that seen when cells sensitized with antigen-specific IgE are reacted with multivalent antigen (7–9, 19, 20). In the total cell extracts, three species with apparent molecular masses of 72, 40, and 33 kDa (p72, p40, and p33) were most prominently phosphorylated; the anti-receptor immune precipitates revealed phosphorylation of tyrosine(s) on the receptor's γ chains, less so on the β chains, and, with the procedures we used, no additional phosphorylated components (Fig. 1*B*; data not shown). For

Abbreviations: DNP, 2,4-dinitrophenyl; BSA, bovine serum albumin; $Fc_\varepsilon RI$, high-affinity receptor for IgE.

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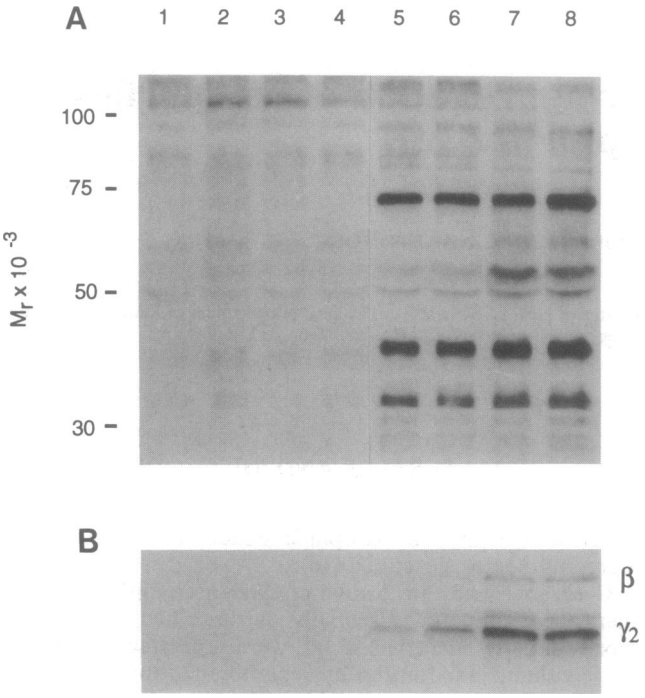

FIG. 1. Autophotograph of a Western blot using anti-phosphotyrosine antibodies. Protein-bound phosphotyrosines in RBL cells stimulated with oligomeric IgE. Cells were incubated with 0.1, 0.3, 1, or 3 μg of monomeric (lanes 1–4) or trimeric (lanes 5–8) IgE per ml for 2 min. When all of the cellular proteins were examined (*A*), the cells were disrupted with SDS, and 1×10^5 cell equivalents per lane were resolved on a SDS/12% polyacrylamide gel; when immunoprecipitated receptor was analyzed (*B*), nonionic detergent was used and 5×10^6 cell equivalents per lane were analyzed on a 14% gel.

5×10^6 cells per ml, the times we wished to study, and the conditions of our assay, we determined that 0.1 μg/ml for the trimers and 0.3 μg/ml for dimeric IgE were useful concentrations with which to stimulate the cells. This amount of IgE is less than the total IgE binding capacity of the cells (≈0.5 μg/ml; data not shown).

At such doses, a time-dependent increase of phosphotyrosine on all five protein species was seen (Fig. 2 *A–C*, *E*, and *F*), with the maximum occurring at 8–16 min (solid squares). The leveling off or decrease in phosphotyrosine seen at later time points for the components in the whole cell extract was not due to nonspecific inactivation of cells or to a decrease in their responsiveness. Thus, cells that were stimulated for 2 min at 0, 50, and 100 min after suspension in assay buffer showed equivalent levels of phosphotyrosine (data not shown).

Little or no phosphotyrosine above that observed for unstimulated cells was seen in samples incubated only with monomeric (10 μg/ml) IgE (open squares). When the cells were incubated with a mixture of trimeric (0.1 μg/ml) and monomeric (10 μg/ml) IgE, a small increase in phosphotyrosine above that seen with monomer was observed (open circles). This was probably due to the aggregation of receptors by trimeric IgE that bound prior to complete saturation of the remaining receptors with monomer (C.W., U.M.K., S.-Y.M., H.M., and B.G., unpublished data). The critical samples are those in which a 100-fold excess of monomeric IgE (10 μg/ml) was added to cells that had been stimulated with trimer for 2 min (solid circles). From the known rate constants for the binding of IgE to $Fc_\varepsilon RI$ (10) and the conditions of the experiment, one can calculate that the monomeric IgE would saturate all unoccupied receptor sites within 3–4 min, thereby preventing new aggregates from forming. Significantly, the protein-associated phosphotyrosine in these cells remained elevated for at least 30 min.

Similar experiments were performed using dimeric IgE as an aggregating stimulus. Data from these studies have been analyzed quantitatively in order to estimate the kinetic constants for this system and will be reported elsewhere (C.W., U.M.K., S.-Y.M., H.M., and B.G., unpublished data). The phosphorylation of p72 in a representative experiment is shown in Fig. 2*D*. The results obtained after stimulation with

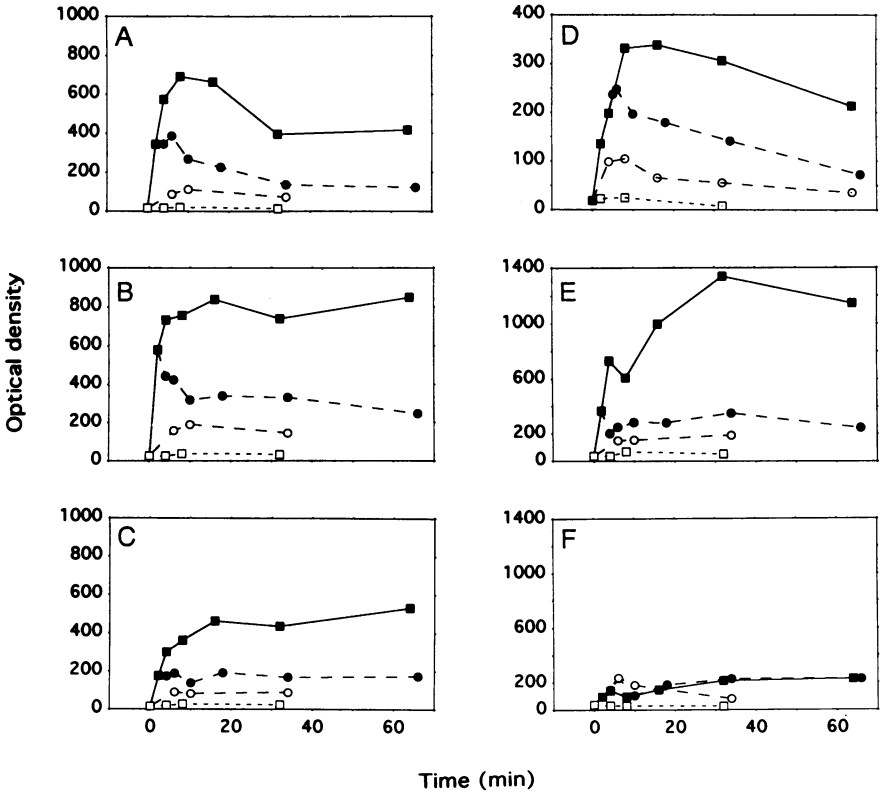

FIG. 2. Time dependence of phosphorylation of protein-bound tyrosine in RBL cells stimulated with oligomeric IgE. (*A–C*, *E*, and *F*) Average of duplicate samples from one of two separate experiments on cells reacted with trimeric IgE. (*D*) Representative result obtained from seven separate experiments on cells stimulated with dimeric IgE (0.3 μg/ml). At the indicated times, samples were removed, and whole cell extracts or immunoprecipitates of $Fc_\varepsilon RI$ were prepared, resolved on SDS/polyacrylamide gels, and analyzed for phosphotyrosine as in Fig. 1. Intensity values were obtained by densitometric scanning of autophotographs similar to those illustrated in Fig. 1 for p72 (*A* and *D*), p40 (*B*), p33 (*C*), $Fc_\varepsilon RI\gamma$ (*E*), and $Fc_\varepsilon RI\beta$ (*F*). Cells were stimulated with 0.1 μg of trimeric IgE per ml (■), 0.1 μg of trimeric IgE per ml for 2 min and then 10 μg of monomeric IgE per ml (●), a mixture of 0.1 μg of trimer and 10 μg of monomer per ml (○), and 10 μg of monomeric IgE per ml (□).

dimer alone or with dimer followed by excess monomer were qualitatively similar to our observations with trimers. Thus, in the absence of newly forming aggregates, phosphorylation of tyrosines on p72 (and on p40, p33, and the receptor's γ subunit; data not shown) declines only slowly. Cells stimulated with dimer consistently showed weaker signals than those stimulated with trimers (note ordinate in Fig. 2*D*). This result parallels previous findings that trimeric IgE is a more potent stimulus than dimeric IgE (17).

Phosphorylation of Tyrosine in Permeabilized Cells. The phosphorylation of protein tyrosines under conditions in which no new aggregates were being formed could persist either because the phosphorylated state is stable or because the proteins are subject to a dynamic process of phosphorylation and dephosphorylation. To distinguish between these alternatives, we tested whether cells would incorporate ^{32}P into the relevant proteins if exposed to [^{32}P]ATP after new aggregate formation had been halted. In a limited number of experiments, we permeabilized cells with tetanolysin (21) or with the α-toxin of *S. aureus* (18) and incubated them with [^{32}P]ATP under conditions in which incorporation of ^{32}P into the subunits of the receptor as well as into receptor-associated proteins has been observed with high doses of antigen (S.-Y.M., T. Yamashita, and H.M., unpublished data). However, with the much weaker signals generated by trimeric IgE, even the samples reacted only with trimer failed to show incorporation of ^{32}P sufficiently above background to measure accurately.

A second approach was to inhibit the action of tyrosine phosphatases to determine whether the level of phosphotyrosine increased. Treatment of RBL cells with a combination of H_2O_2 and Na_3VO_4 or phenylarsine oxide causes an antigen-dependent increase in phosphorylation of tyrosines on the subunits of the receptor and on other cellular proteins (9, 22, 23). Again, the increase in basal phosphorylation in the presence of either of these phosphatase inhibitors prevented us from detecting the modest increases in phosphorylation initiated by low doses of trimeric IgE.

A third approach—to study the effect of inhibitors of tyrosine kinases—yielded interpretable results. We used EDTA because it is a relatively mild but effective inhibitor of kinases. RBL cells permeabilized with the α-toxin of *S. aureus* responded less vigorously and more briefly than nonpermeabilized cells. Therefore, we were able to analyze only the two most prominently phosphorylated species, p72 and the γ subunits of $Fc_\varepsilon RI$. As with the intact cells, aggregating the receptors on permeabilized cells with trimers of IgE led to an increase in phosphotyrosine (Fig. 3, solid circles). Monomeric IgE alone (open triangles) failed to stimulate significant increases in phosphotyrosine compared to unstimulated cells, whereas simultaneous addition of trimeric (0.1 μg/ml) and monomeric (10 μg/ml) IgE led to a low level of phosphorylation (plus signs). Cells reacted with excess monomer 2 min after the addition of trimer again exhibited a decreased but persistent level of phosphotyrosine (open circles), indicating that receptor aggregates remain active in permeabilized cells as well. Notably, when EDTA was added to the latter samples 6 min after the addition of monomer—i.e., well after formation of new clusters of receptors had ceased—a decrease of protein-bound phosphotyrosine to basal levels was observed within 2 min (solid triangles). These results suggest that previously formed aggregates of $Fc_\varepsilon RI$ as well as other cellular components undergo continuous phosphorylation and dephosphorylation.

Secretion Due to Previously Formed Aggregates. It was of interest to determine whether all of the events required to initiate cellular secretion were similarly maintained. We took advantage of the fact that aggregation of only a small fraction of the RBL cell's receptors is sufficient to induce substantial secretion (17, 24, 25). We first reacted cells with trimeric IgE

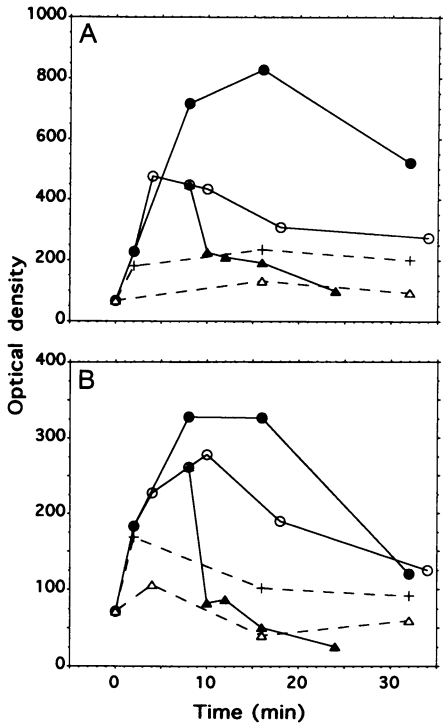

FIG. 3. Effect of EDTA on protein-bound phosphotyrosine in permeabilized cells stimulated with oligomeric IgE. Cells permeabilized with *S. aureus* α-toxin were incubated with 0.1 μg of trimeric IgE per ml (●), a mixture of 0.1 μg of trimeric and 10 μg of monomeric IgE per ml (+), or 10 μg of monomer per ml alone (△). After 2 min, 10 μg of monomer per ml was added to certain samples stimulated with trimer (○). After an additional 6 min, EDTA was added to a final concentration of 2 mM (*A*) or 7 mM (*B*) (▲)—i.e., sufficient to chelate the Mg^{2+} in the medium. Samples were removed at the times indicated and analyzed as described in Fig. 2. (*A*) Phosphotyrosine associated with $Fc_\varepsilon RI\gamma$ chain. (*B*) Phosphotyrosine associated with p72 in cell extracts. Data points represent averages of duplicate samples.

under conditions in which the secretory response of RBL cells is inhibited—namely, at low levels of extracellular Ca^{2+} (14). After 2 min, monomeric IgE was added to saturate the unoccupied receptors. Ten minutes later, 1 mM Ca^{2+} was added to the buffer, and secretion of β-hexosaminidase was monitored. Fig. 4*A* shows the average results from four independent experiments in which the samples taken at each time point were assayed in triplicate for release of enzyme. In each experiment, the "positive control" (solid squares) consisted of cells incubated with trimeric IgE for 10 min without Ca^{2+} and then incubated with Ca^{2+} in the absence of monomer. The enzyme released at 20 min was arbitrarily defined as 100%. The actual secretion for this time point in four experiments varied between 13% and 36% of the total cellular activity. Cells that were incubated in Ca^{2+}-containing buffer for 10 min and then stimulated with trimer released hexosaminidase somewhat more slowly, but by 20 min after exposure to trimer they had released similar amounts as the "positive control" cells (solid circles). The simplest explanation for the difference in the initial rate of release is that in the latter experiments there were no preformed aggregated receptors at the time the Ca^{2+} was added. Spontaneous release, which occurred over the course of the assay, was between 8% and 11% of maximal release (plus signs). These values were similar to those observed with cells reacted only with monomeric IgE (10–11%) (open circles).

Some cells were incubated with monomeric IgE in the absence of Ca^{2+} for 10 min, after which trimeric IgE was added together with Ca^{2+}. Their relative release of enzyme

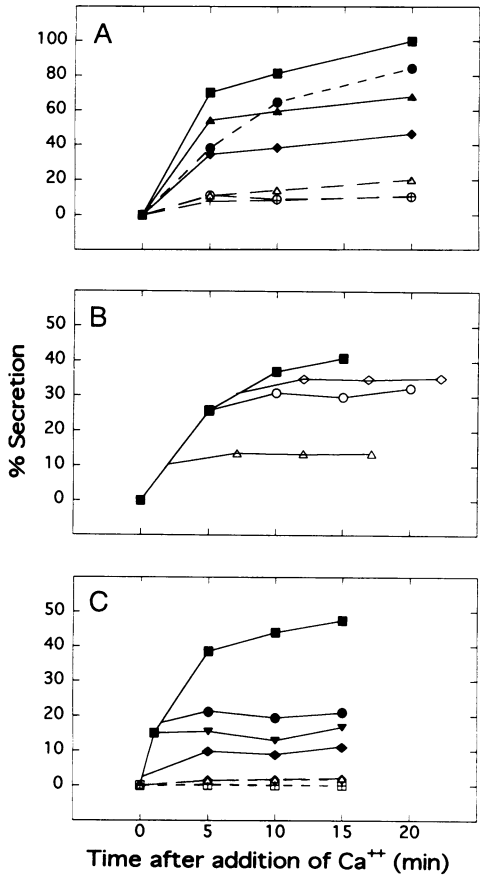

FIG. 4. Secretion of hexosaminidase from RBL cells. (*A*) Cells stimulated with trimeric IgE. Cells were incubated with or without monomeric IgE or trimeric IgE or both for 10–12 min in the absence of Ca^{2+} as follows: ■, 0.1 μg of trimeric IgE per ml; ▲, 2 min with 0.1 μg of trimeric IgE per ml and then 10 min with 10 μg of monomeric IgE per ml; ◆, a mixture of 0.1 μg of trimeric IgE per ml and 10 μg of monomeric IgE per ml; △, 10 min with 10 μg of monomeric IgE per ml; ●, 0.1 μg of trimeric IgE per ml added at the same time as Ca^{2+} (see below); +, no additions; ○, 10 μg of monomeric IgE per ml. Secretion in all samples was initiated by addition of 1 mM Ca^{2+}. Percentage of hexosaminidase released by the cells from the sample denoted by ■ at 20 min after addition of Ca^{2+} was arbitrarily set at 100%, and the release from all other samples is given relative to it. (*B*) Effect of hapten on cells stimulated with antigen in the presence of Ca^{2+}. Cells were "sensitized" with 0.5 μg of murine anti-DNP IgE per ml for 1 hr. After washing, the cells were suspended in Ca^{2+}-containing buffer and secretion was initiated by addition of 10 ng of DNP_{25} BSA per ml (■). The hapten DNP caproate (50 μM) was added after 2 min (△), 5 min (○), or 7 min (◇). Data show absolute percentage secretion and points are the averages of triplicate samples. (*C*) Effect of hapten on cells initially stimulated with antigen in the absence of Ca^{2+}. Cells sensitized with anti-DNP IgE were incubated with 10 ng of DNP_{25} BSA per ml in the absence of Ca^{2+} for 10 min. Hexosaminidase release was measured in triplicate 5, 10, and 20 min after addition of Ca^{2+}. Data show absolute percentage secretion and points are the averages of triplicate samples. A representative result from two separate experiments is shown. No hapten added (■); DNP caproate (50 μM) was added 10 s (◆), 60 s (▼), or 90 s (●) after addition of Ca^{2+}; during the Ca^{2+}-free incubation control samples contained no additions (○), hapten alone (△), or 10 ng of antigen per ml for 10 s followed by hapten for 10 min (◇); cells were reacted with antigen but no Ca^{2+} was added during the course of the experiment (□).

varied between 11% and 20% (open triangles). This demonstrates that the doses of monomeric IgE we used could substantially block the clustering of receptor sites by the trimeric IgE. When trimeric and excess monomeric IgE were added simultaneously 10 min prior to addition of Ca^{2+}, the cells responded with 35–46% release (solid diamonds). This confirms that even low numbers of aggregated receptors elicit a significant response. When cells were reacted first with trimeric IgE for 2 min, and then with 100-fold excess monomeric IgE for an additional 10 min, all in the absence of Ca^{2+}, release of enzyme was 54–68% 5–20 min after Ca^{2+} was restored to the buffer (solid triangles). This result is consistent with the proposal that receptors aggregated during the first few minutes were able to generate signals sufficient to stimulate a secretory response even after a lapse of 10 min.

Our results are in apparent contrast to observations made on cells reacted with antigen-specific IgE incubated with a multivalent antigen and then with a univalent antigen (hapten). In such an experiment, if the hapten is added any time during the cellular response, it abruptly halts both the early and later events (7, 19, 26, 27). To ensure that this apparent discrepancy was not due to that aspect of our protocol in which Ca^{2+} was withheld during the initial clustering of receptors, we studied the effectiveness of hapten to inhibit continued secretion of cells initially stimulated with or without Ca^{2+}. Fig. 4*B* shows the secretory response of cells stimulated with antigen in the presence of Ca^{2+} (solid squares). Addition of hapten [50 μM 2,4-dinitrophenyl (DNP) caproate] at different times completely stopped further secretion. Fig. 4*C* shows secretory responses of cells whose sensitized receptors were aggregated with antigen for 10 min in the absence of Ca^{2+}. If hapten was added to antigen cross-linked receptors within 10 s in the absence of Ca^{2+} no release was observed after addition of Ca^{2+} (open triangles); if not, these cells released a similar amount of hexosaminidase after addition of Ca^{2+} (solid squares) as cells incubated in the presence of Ca^{2+} from the outset (Fig. 4*B*, solid squares). These results are consistent with previous studies that found that RBL cells are not readily "desensitized" by aggregating $Fc_\epsilon RI$ in the absence of Ca^{2+} (27). The continued release after addition of Ca^{2+} could again be inhibited by hapten. The latter observation is of interest. It demonstrates that the phosphorylations that occur at low levels of Ca^{2+} (7, 19) are insufficient by themselves to initiate the cascade of events leading to degranulation once Ca^{2+} is restored.

Thus, interpretation of our results with the oligomers is not compromised by the particular protocol we used. The findings clearly indicate that stable aggregates of receptors can stimulate for many minutes the full biochemical cascade that leads to secretion.

DISCUSSION

Comparison with Prior Studies. Based on experiments similar to those shown in Fig. 4*B*, in which monovalent hapten inhibited the secretion stimulated by antigen-aggregated receptors, much more abruptly and completely than it displaced the cell-bound antigen (28), Oliver *et al.* (29) and others (30) have postulated that the effect of hapten was chiefly to prevent the formation of new aggregates of receptors rather than to dissociate preexisting ones. They therefore hypothesized that aggregated receptors are able to initiate signals only briefly and are rapidly converted to an inactive species that may even inhibit further responses.

Seagrave *et al.* (28) used a highly multivalent antigen—an antigen that is much more likely to induce the well-recognized process of hyperaggregation-induced inhibition of degranulation and desensitization of receptors (31–33) than the paucivalent stimulus we used in this study. In such experiments, it may well be that the (hyper)aggregated receptors have a relatively short half-life of activity and that new receptors must be continuously recruited to generate further signals. In addition, although unlikely by itself to account for the phenomenon, the following may contribute to the difference in the kinetics of hapten-induced inhibition of secretion and release of cell-bound antigen: clearly, the

hapten will reduce the time an individual receptor is associated with the surface-bound antigen and, consequently, the time the receptor remains in a clustered state. On the other hand, with a highly multivalent antigen, it is not surprising that the latter remains bound to the cell surface for longer periods of time than any individual receptor remains bound to the antigen.

McCloskey (34) reported that doses of the multivalent lectin wheat germ agglutinin, so high that they do not themselves stimulate secretion, immediately abrogate early signals and the secretory response generated by antigen reacting with cell-bound IgE. He also observed that the lectin rapidly immobilizes $Fc_\varepsilon RI$. His findings parallel earlier observations on mast cells, already referred to, showing that excessive cross-linking can reduce or eliminate secretory responses (see above). Although McCloskey's results would be consistent with the assumption that newly clustered receptors are only transiently active, there are other possible explanations for his data. Therefore, his results cannot be used to assess directly the active lifetime of aggregated $Fc_\varepsilon RI$. His and many previous data do show that there are cellular mechanisms—still undefined—that can reduce this lifetime, particularly if the receptors are hyperaggregated.

Our data demonstrate that small, stably aggregated receptors can elicit both early and late cellular processes for substantial lengths of time and that during that time they are dynamically phosphorylated and dephosphorylated. Recent *in vitro* studies in which the tyrosine kinase activity of receptors immunoprecipitated from antigen-activated cells was assessed also support the conclusion that aggregated receptors can remain active for a long time (V. Pribluda, C. Pribluda, and H.M., unpublished data).

Our finding that aggregated receptors undergo active phosphorylation and dephosphorylation was not necessarily predictable from previous studies in which addition of phosphatase inhibitors increased the phosphorylation of the subunits of $Fc_\varepsilon RI$ (and of other cellular proteins) in cells reacted with antigen (9, 23). In those experiments, individual receptors in the cluster would be expected to be exchanging in and out of the cluster relatively rapidly, because of the high dissociation rate constant characteristic of single antibody–antigen interactions—particularly hapten–anti-hapten interactions (also see above). Therefore, one cannot rule out that it was principally the dephosphorylation of disengaged receptors that was inhibited by the phosphatase inhibitors. Such a phenomenon cannot explain our own data because the dissociation of IgE from $Fc_\varepsilon RI$ is too slow (10). Therefore, our results imply that both monomeric and aggregated receptors are rapidly phosphorylated and dephosphorylated and that by a mechanism yet to be defined, aggregation alters the balance between these reactions.

Wider Implications. The characteristics we have described for $Fc_\varepsilon RI$ are likely not unique to this receptor and may be particularly relevant to those aspects of the immune system in which the biological response is importantly influenced by the intrinsic affinity of the receptors for the ligand. As noted in our introductory remarks, the "multisubunit immune response receptors" of which $Fc_\varepsilon RI$ is an example share many structural and functional attributes (6). In previous comments, one of us (H.M.) has attempted to explain affinity maturation of B lymphocytes on the relationship between the intrinsic affinity of the receptor for ligand, the length of time an individual receptor spends in a cluster, and, therefore, the probability it will generate a signal (35). Such a relationship would be meaningful only if the receptors would continue to be active for extended times as long as they remained aggregated, as our new data on $Fc_\varepsilon RI$ suggest.

A similar phenomenon may be relevant to recent observations showing that homologs of an antigenic peptide can inhibit activation of T lymphocytes induced by the native antigen, even though both peptides are bound with similar affinity to the same major histocompatibility complex (MHC) on the antigen presenting cell (36). If the altered antigen–MHC combination engages the same antigen-specific T-cell receptors as are engaged by the native antigen, but with a lower affinity, then the receptors bound to the former will competitively cocluster with the receptors engaging native antigen. Because of the lowered affinity of some of the receptors in the mixed clusters, the latter as a whole will have a shortened lifetime. Consequently, if the lifetime of aggregated T-cell receptors determines the persistence of the responses they trigger—as our data suggest is true for $Fc_\varepsilon RI$—then this mechanism would account for the inhibitory activity of the altered antigen–MHC complex.

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