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Immunology

Transmembrane signaling by the high-affinity IgE receptor on membrane preparations

(tyrosine phosphorylation/FcεRI/mast cells)

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ABSTRACT **Aggregating the receptor with high affinity for IgE (FcεRI) stimulates a variety of phenomena in mast cells. Previous efforts to reproduce some of these events in broken-cell preparations such as isolated membranes have had limited success, possibly because the phenomena being monitored were too distal from the initial events. One of the earliest responses is now known to be the phosphorylation of tyrosine residues on several proteins, including the β and γ subunits of FcεRI. We show that in cell sonicates or on partially purified membranes derived from tumor mast cells, aggregating FcεRI stimulates phosphorylation of receptor tyrosine residues. As in the intact cells, receptor-mediated phosphorylation occurs only on receptors that are themselves aggregated. Because even in the unfractionated sonicates the phosphorylation of other cellular components was not detectably enhanced, and because the evidence is against the receptor itself being a kinase, our results suggest that phosphorylation of FcεRI is one of the earliest events stimulated by the receptor—an event that can now be investigated on simpler biological preparations than previously available.**

Aggregation of the mast cell receptor with high affinity for immunoglobulin E (IgE) stimulates a variety of early and late cellular phenomena (1, 2). To define the mechanism of action of this receptor (FcεRI) at the molecular level, we previously attempted to develop relatively simple broken-cell preparations. Although we could prepare partially active cytoplasts and later lysed cytoplasts ("ghosts"), attempts to isolate functional membranes led to a complete loss of activity (3, 4).

Those earlier studies examined the receptor activation of phospholipase C, because that reaction was thought to be perhaps the earliest event initiated by the aggregated receptors (5). However, there is now increasing evidence that phosphorylation of tyrosines on one or more cellular proteins (6)—including the receptor itself (7–9)—might constitute even earlier events. We have, therefore, reexamined broken-cell preparations to see whether they would manifest tyrosine phosphorylation reactions that are stimulated by the receptors on intact cells.

MATERIALS AND METHODS

Reagents. The principal reagents used in these studies have been described (10). Hyperfilm-ECL and reagents for chemiluminescence assays were from Amersham. Tricine running buffer and 10% Tricine gels were obtained from Novex (Encinitas, CA).

Cells and Cell Fractions. Rat basophilic leukemia (RBL) 2H3 cells were grown as described (10); where appropriate, they were incubated with IgE at 5 μg/ml overnight. Cells were washed and resuspended in cell buffer [119 mM NaCl/5 mM KCl/1 mM $CaCl_2$/0.4 mM $MgSO_4$/25 mM Pipes/0.1% bovine serum albumin (BSA)/5.4 mM glucose, pH 7.2]. For [^{32}P]phosphate incorporation, cells were preincubated for 2–3 hr in Eagle's minimal essential medium without phosphate, supplemented with 20% dialyzed fetal calf serum (GIBCO/BRL) and containing ^{32}P (200–500 μCi/ml; 1 Ci = 37 GBq) before trypsin treatment.

To prepare cell-free fractions, cells isolated in buffer containing no added Ca^{2+} or Mg^{2+} (assay buffer) were suspended at 8–12 × 10^6 cells per ml. Just before sonication, protease inhibitors were added to yield final concentrations of aprotinin (200 kallikrein inhibitor units/ml), leupeptin (10 μg/ml), and pepstatin A (10 μg/ml). The cell disrupter (model W-225R; Heat Systems/Ultrasonic) was used at maximal output, approximately three times for 30 sec each. The sonicate was centrifuged at 14,000 × *g* for 3 min. Membranes were obtained by recentrifuging the sonicate at 45,000 × *g* for 30–60 min or at 140,000 × *g* for 50 min. When supernatants (S1) were used for complementation studies with membranes, centrifugation was performed under the latter conditions. Such S1 revealed no receptors when either the total S1 or presumptively immunoprecipitated material was blotted with anti-β antibody.

Activation and Solubilization. Activation of cells and cell fractions was performed at 37°C in cell buffer and assay buffer, respectively. Assay mixtures contained 3–6 × 10^6 cells or cell equivalents per ml. Just before beginning the incubation of the cell fractions at 37°C, the mixtures were made 2 mM in Na_2ATP and 0.5 mM in Na_3VO_4, and appropriate amounts of Mn^{2+} were added. At the desired time after incubation, the cells or cell fractions were solubilized with the detergent 3-[(3-cholamidopropyl)dimethylammonio]-2-hydroxyl-1-propanesulfonic acid (CHAPS), rabbit liver lipids, and inhibitors. The final concentrations were 10 mM CHAPS, 2 mM lipids, 0.5 mM Na_3VO_4, 5 mM $Na_4P_2O_7$, 5 mM EDTA, and the same protein inhibitors as noted above. The samples were left on ice for a minimum of 30 min (10). The cell specimens and the first sonicate were centrifuged for 1 min at 14,000 × *g* at 4°C and 0.5 ml of supernatant was withdrawn for analysis; for the centrifuged sonicate and subsequent membrane fractions, this centrifugation step was omitted.

Analysis. For immunoprecipitations, the samples were reacted with 4–5 μg of the appropriate antibody for not less than 1 hr and then incubated with protein A-Sepharose for at least another hour. The pellet was washed twice with a solution of 10 mM CHAPS/2 mM lipids (11) containing the same inhibitors as the solubilizing buffer.

Samples from cell extracts or immunoprecipitates were resolved by SDS/PAGE on 10% Tricine gels and then

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Abbreviations: RBL, rat basophilic leukemia; DNP, 2,4-dinitrophenyl; CHAPS, 3-[(3 cholamidopropyl)dimethylammonio]-2-hydroxyl-1-propanesulfonic acid; PAO, phenylarsine oxide; BSA, bovine serum albumin.

transferred to a nitrocellulose membrane in a Milliblot-SDE system apparatus (Millipore). The tyrosine-phosphorylated bands were detected by enhanced chemiluminescence after blotting with a peroxidase-conjugated anti-phosphotyrosine antibody (PY-20; ICN). When blotting with the anti-β antibody JRK, conjugated sheep anti-mouse IgG (ICN) was used as the secondary antibody. Autophotographs (and autoradiographs) were quantitatively analyzed with a computing densitometer (Molecular Dynamics, Sunnyvale, CA).

The autophotographs of the blots consistently showed a band in the 52- to 55-kDa range. This band was also observed with samples of solvent exposed only to protein A-Sepharose. It probably results from release of protein A by SDS. Its binding of the conjugated antibody was avoided at later stages of these studies by addition of nonspecific mouse or human IgG (10 μg/ml) to the 4% BSA blocking solution.

RESULTS

Intact Cells. RBL cells primed with mouse 2,4-dinitrophenyl (DNP)-specific IgE were reacted with DNP-BSA and phosphorylation of tyrosine in the total cellular proteins was analyzed (Fig. 1*A*). It is apparent that even though in unstimulated cells multiple components contain phosphotyrosine, the phosphorylation of these, as well as of other components, increases when the receptors are aggregated, as described (6).

When such cells were solubilized under conditions that stabilize FcεRI (11), and the receptors were immunoprecipitated via the bound IgE, the results were as shown in Fig. 1*A* (lanes 3 and 4). In addition to an artifactual component at 52- to 55-kDa (see *Materials and Methods*), components with apparent molecular masses appropriate for the phosphorylated β (33 kDa) and dimer of the γ subunits (25 kDa) (12, 13) were seen. Their phosphorylation increased when the FcεRI were aggregated.

Further evidence indicates that these components are the β and γ subunits of FcεRI. (*i*) Upon reduction, the 33-kDa band was unchanged (as expected for β) but most of the 25-kDa component shifted to a molecular mass consistent with the disulfide cleavage of the cystine-linked dimer of γ chains (Fig. 1*B*, lanes 1 and 2). The residual unreduced 25-kDa band could either represent unreduced dimers or, conceivably, a proteolytic fragment of β (14, 15). That the reduced γ subunits were less intensely stained than the nonreduced dimers probably results from their weaker interaction with the anti-phosphotyrosine antibody. When cells that had incorporated [^{32}P]phosphate were monitored for phosphorylation by autoradiography, a much more stoichiometric shift in the radioactivity was seen (lanes 3 and 4). Canals (16) has observed an analogous phenomenon with the epidermal growth factor receptor. (*ii*) Immunoprecipitation with the anti-β antibody JRK yielded the same components (lane 5). (*iii*) When blotted with JRK, the immunoprecipitated receptors showed only one band at the same position as the upper band recognized by the anti-phosphotyrosine antibody (data not shown).

Fig. 2 shows the variation in phosphorylation as a function of the dose of antigen and the time after its addition. A slight decrease in the level of phosphorylation of γ and β was

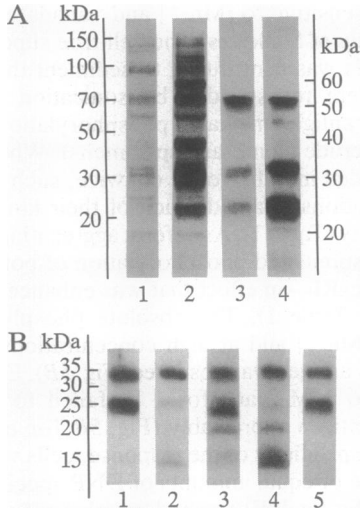

FIG. 1. Phosphorylation of protein tyrosine residues in RBL cells. Except where specifically noted, this and all of the subsequent figures are derived from autophotographs of nitrocellulose membranes to which proteins separated by SDS/PAGE had been transferred and that were then reacted with anti-phosphotyrosine antibody. Cells used for the experiments in *B* had been incubated with ^{32}P prior to stimulation with antigen. Cells were activated with DNP-BSA (100 ng/ml) for 2 min. The whole cell extract (4×10^4 cell equivalents) and the immunoprecipitated receptor (8×10^5 cell equivalents) are from different experiments. (*A*) Lanes: 1, SDS extract of unstimulated cells; 2, SDS extract of stimulated cells; 3 and 4, anti-IgE immune precipitates from CHAPS extracts of cell samples treated exactly like those shown in lanes 1 and 2. (*B*) Lanes: 1, anti-IgE immune precipitate from a CHAPS extract of stimulated intact cells; 2, same material as in lane 1 except the specimen was reduced prior to electrophoresis; 3 and 4, autoradiographs of the same blots whose autophotographs are shown in lanes 1 and 2; 5, anti-JRK immune precipitate of the same CHAPS extract used in lanes 1 and 2.

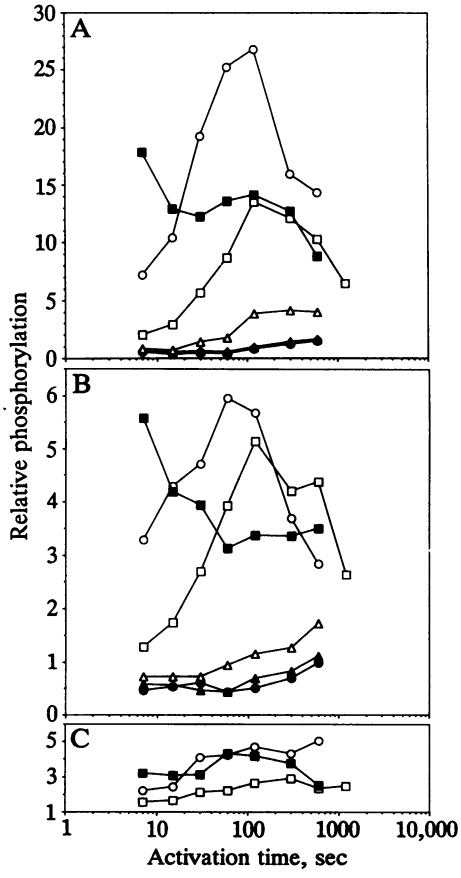

FIG. 2. Phosphorylation of tyrosines on the β and γ subunits of FcεRI in intact cells after aggregation of FcεRI. Doses of DNP-BSA used were as follows: ●, 0.1 ng; ▲, 1 ng; △, 10 ng; □, 100 ng; ○, 1 μg; ■, 10 μg. Values shown are ratios of stimulated/nonstimulated cells and are average values from two to five experiments. (*A*) Relative phosphotyrosine on γ subunits. (*B*) Relative phosphotyrosine on β subunits. (*C*) Ratio of phosphotyrosine on γ subunits/phosphotyrosine on β subunits.

consistently observed at early times with low doses. (This is represented by ordinate values of <1.) The onset of enhanced phosphorylation after addition of antigen was the same for both subunits and although the stimulated phosphorylation of the γ chains (Fig. 2*A*) was greater than that of the β chains (Fig. 2*B*), the ratio of enhanced phosphotyrosine on γ vs. β remained more or less constant regardless of the absolute level of phosphorylation (Fig. 2*C*). Similar ratios were observed whether the receptor was immunoprecipitated with anti-IgE or with anti-β antibody, so these results were not significantly influenced by partial dissociation of the receptor after solubilization.

Higher doses of antigen led to higher peak levels of phosphotyrosine, which occurred at earlier times. Immunoblots with anti-β antibodies showed a consistent yield of β chains (data not shown), confirming that the apparent dephosphorylation with time was not simply due to a decreased recovery of receptors. Furthermore, addition of the phosphatase inhibitor phenylarsine oxide (PAO) inhibited the time-dependent decrease in phosphorylation (Fig. 3).

Maintenance of the enhanced phosphorylation required a continuous aggregating stimulus. Thus, within a minute after the addition of hapten, the phosphotyrosine returned to baseline levels (Fig. 3, triangles). When the cells were incubated with PAO, the antigen-stimulated phosphorylation was severalfold higher than in the controls (Fig. 3, solid symbols). Notably, PAO did not inhibit hapten-induced dephosphorylation (Fig. 3, solid triangles).

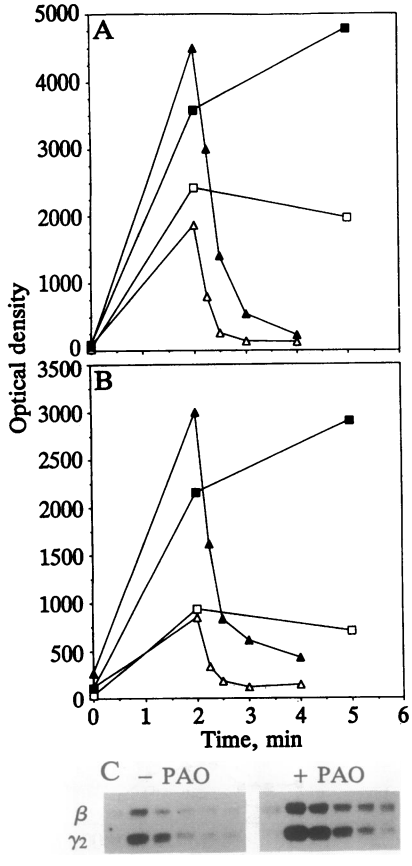

FIG. 3. Effect of PAO and hapten on phosphorylation of tyrosine residues on β and γ subunits of FcεRI after aggregation of FcεRI with antigen on intact cells. Open symbols, no PAO; solid symbols, 5 μM PAO; squares, no hapten; triangles, 50 μM hapten (added at 2 min). Data are from one of two experiments, which gave similar results. (*A*) Phosphotyrosine on γ subunits. (*B*) Phosphotyrosine on β subunits. (*C*) Autophotographs from which data for specimens to which hapten had been added were derived; left to right, samples taken at 0, 2, 2.25, 2.5, 3, and 4 min.

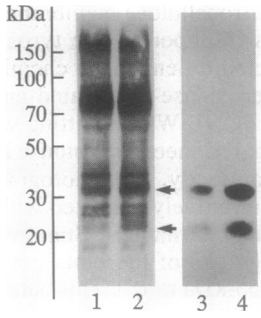

FIG. 4. Phosphorylation of protein tyrosine residues in sonicates of RBL cells stimulated after sonication by aggregation of FcεRI with DNP-BSA (100 ng/ml) for 2 min in the presence of 0.5 mM vanadate and 0.05 mM Mn^{2+}. Lanes: 1, SDS extract of unstimulated sonicate; 2, SDS extract of stimulated sonicate; 3 and 4, anti-IgE immune precipitates from CHAPS extracts of the same sonicates shown in lanes 1 and 2.

Stimulation of Sonicated Cells. Unlike the patterns observed with intact cells, the addition of antigen to centrifuged sonicates free of intact cells or large cell fragments led to only subtle changes in the pattern of protein phosphotyrosines (Fig. 4, lanes 1 and 2). However, close inspection revealed that the components at 33 and 25 kDa (arrows) showed a modest increase in phosphotyrosine. Immunoprecipitation with anti-IgE antibody of extracts from sonicates solubilized with CHAPS confirmed this increase on the β and γ subunits of the receptor (lanes 3 and 4).

Contrary to what was observed in the intact cells, both the basal and antigen-stimulated phosphorylation in the sonicates were inhibited by EDTA (data not shown, but see ref. 7) and were sensitive to [Mn^{2+}] and vanadate.

Fractionation of Sonicates. The cell-free supernatant of the sonicated cells was centrifuged to sediment the membranes. The latter were resuspended by sonication in buffer and reassayed for antigen-mediated phosphorylation of the receptor (Table 1, crude membrane specimens). When sedimented and resuspended in buffer once or twice, such washed membrane preparations retained much of their kinase and phosphatase activity (Table 1). As before, aggregating the receptors with antigen stimulated phosphorylation of both the γ and β subunits of FcεRI—an effect that was enhanced by vanadate (Fig. 5*A* and Table 1). The absolute phosphorylation was sensitive to [Mn^{2+}] and at high concentrations of Mn^{2+} the stimulation by antigen was obscured (Fig. 5*B*). Exposure of the membranes to 1 M NaCl for 1 hr failed to eliminate the response to antigen appreciably (Fig. 5*B Top* and *Middle*).

To test the specificity of the response, cells were incubated with a mixture of equal amounts of DNP-specific mouse IgE and nonspecific rat IgE; washed membranes were prepared

Table 1. Phosphorylation of tyrosines on FcεRI

Specimen	No. of exps.	Mn^{2+}, μM	VO_4^{3-} (0.5 mM)	γ*	β*	γ/β*
Intact cells	7	NA	NA	16.5	11.1	1.49
Crude sonicate	7	1, 10	–	3.9	2.6	1.50
	4	1, 10	+	12.2	7.2	1.69
Centrifuged	6	10, 50	–	1.7	1.6	1.06
sonicate	8	10, 50	+	4.8	3.2	1.50
Crude membranes	4	50, 100	–	1.6	2.1	0.76
	6	50, 100	+	3.4	4.7	0.72
Washed	2	50, 100	–	2.8	2.3	1.22
membranes	4	50, 100	+	3.2	3.3	0.97

NA, not applicable.
*Values are ratios of phosphotyrosine in the specimens stimulated with DNP-BSA (100 ng/ml) for 2 min compared to unstimulated samples.

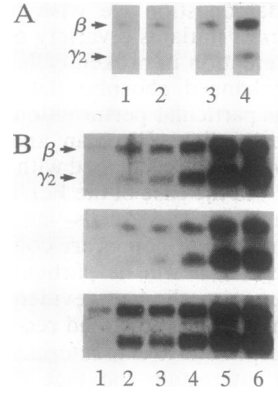

FIG. 5. Phosphorylation of protein tyrosine residues in washed membranes prepared from sonicated RBL cells and stimulated after preparation of the membranes by aggregation of FcεRI with DNP-BSA (1 μg/ml) for 2 min and in the presence of 100 μM (*A*) or variable amounts (*B*) of Mn^{2+}. Materials analyzed were anti-IgE immunoprecipitates of solubilized membranes. (*A*) Lanes: 1, unstimulated; 2, stimulated; 3 and 4, same as lanes 1 and 2, but 0.5 mM vanadate was present. (*B*) Lanes: 1, 3, and 5, unstimulated membranes; 2, 4, and 6, stimulated membranes. [Mn^{2+}] was either 50 μM (lanes 1 and 2), 100 μM (lanes 3 and 4), or 1 mM (lanes 5 and 6). (*Top*) Washed membranes. (*Middle*) Membranes washed and then incubated in 1 M NaCl for 60 min. (*Bottom*) Same as *Top* but reaction mixture contained the same number of cell equivalents of S1.

from a portion of them. The cells or membranes were stimulated via the mouse or rat IgE, and the solubilized receptors were selectively immunoprecipitated. In intact cells, enhanced phosphorylation of receptor tyrosines was observed only on those receptors that had themselves been aggregated (Fig. 6 *Left*). Paolini *et al.* (7) have reported comparable results from analogous experiments. Although all the responses were smaller, the phosphorylation of tyrosines observed upon stimulation of the washed membranes showed a similar specificity to that seen on the intact cells (Fig. 6 *Right*).

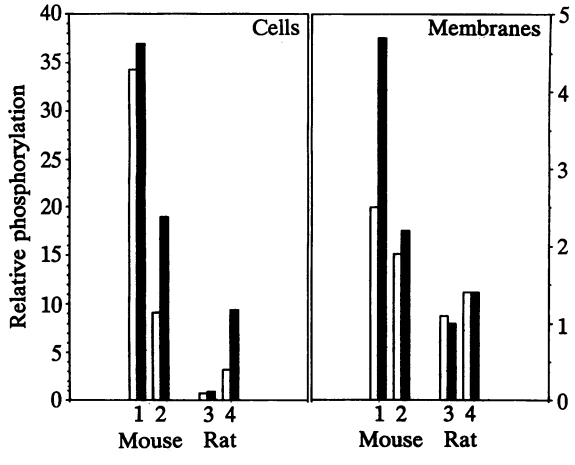

FIG. 6. Receptor-specific phosphorylation of tyrosines on γ and β subunits of aggregated FcεRI. Cells were incubated with a 1:1 mixture of DNP-specific mouse IgE and nonspecific rat IgE, and membranes were prepared from a portion of them. Cells or membranes reacted with DNP-BSA (1 μg/ml), anti-mouse IgE (12.5 μg/ml), or anti-rat IgE (12.5 μg/ml) for 2 min; the reaction was stopped; the receptors were isolated by immunoprecipitation with anti-mouse IgE or anti-rat IgE; and immunoprecipitates were analyzed in the usual manner. Open bars, β subunits; solid bars, γ subunits. Samples 1 and 3, stimulation with DNP-BSA; samples 2, stimulation with anti-mouse IgE; samples 4, stimulation with anti-rat IgE. Samples 1 and 2 were immunoprecipitated with anti-mouse IgE; samples 3 and 4 were immunoprecipitated with anti-rat IgE. (*Left*) Intact cells. (*Right*) Membranes.

Table 2. Effect of hapten on antigen-stimulated phosphorylation of γ and β subunits of FcεRI in washed membranes

Subunit	Time, min	Stimulation by antigen*	
		− hapten	+ hapten
γ	2	11.8*	0.52
	4	6.6	0.88
β	2	3.7	0.53
	4	2.0	0.84

*Values shown are ratios of optical densities in the respective bands for the samples to which 1 μg of antigen per ml had been added relative to the samples from which antigen had been omitted. Incubation mixtures contained 100 μM Mn^{2+} and no vanadate.

As with the intact cells, antigen-stimulated phosphorylation by the washed membranes was inhibited by hapten when the receptors were aggregated with DNP-BSA (Table 2 and Fig. 7) but not when aggregated with anti-IgE antibody (data not shown).

A summary of the results of various experiments in which the different cell fractions were activated under the diverse conditions detailed above is presented in Table 1. In it we present the maximal enhancement by antigen and the range of [Mn^{2+}] at which it was seen.

Partial Reconstitution. The soluble portion of fractionated sonicates enhanced the activity of membranes whose activity had been partially depleted by repeated sedimentation and resuspension (Fig. 5*B Top* and *Bottom*). It increased the absolute amount of phosphotyrosine associated with the FcεRI and enhanced the antigen-mediated response at the lower [Mn^{2+}] where basal phosphorylation was diminished.

Receptor-Associated Proteins. Immunoprecipitates of the receptor from stimulated cells or from cell fractions at micromolar Mn^{2+} concentrations usually revealed only the two tyrosine phosphorylated bands identified as β and γ (cf. ref. 8). However, membranes exposed to millimolar Mn^{2+} concentrations frequently revealed other weakly phosphorylated components at 70, 30, 19, and <14 kDa.

DISCUSSION

Aggregating FcεRI on intact cells enhances phosphorylation of its β and γ subunits (refs. 7–9; Fig. 1) and is receptor specific in two senses. (*i*) It is not observed when the cells undergo reaction with other reagents that lead to mast cell degranulation (ref. 7; P. Germano and J. Rivera, personal

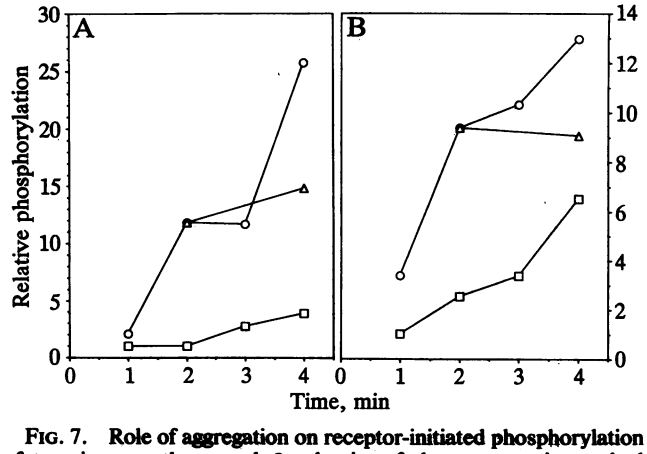

FIG. 7. Role of aggregation on receptor-initiated phosphorylation of tyrosines on the γ and β subunits of the receptor in washed membranes. Phosphorylation of tyrosine was quantitated by the procedure described in Figs. 2 and 3. □, Unstimulated preparations; ○, stimulated preparations. Where hapten was added (△), the addition was made at 2 min. Values are expressed as a ratio relative to the phosphotyrosine in the unstimulated sample after 1 min at 37°C. Data are from one of five experiments, all of which gave similar results. (*A*) Phosphorylation of γ subunits. (*B*) Phosphorylation of β subunits.

communication) and occurs only on those receptors that were themselves aggregated—i.e., there is no bystander effect (ref. 7; Fig. 6 *Left*; cf. ref. 12). Phosphorylation results from a dynamic interplay of kinases and phosphatases. Thus, addition of phosphatase inhibitors enhances phosphorylation, whereas inhibitors of receptor aggregation induce a rapid decrease in tyrosine phosphorylation (ref. 7; Fig. 3). Dephosphorylation of the receptor after addition of hapten even in the cells treated with PAO is notable; there are multiple possibilities that could explain this phenomenon, which would be interesting to explore.

As with intact cells, phosphorylation on cell-free sonicates and partially purified membranes was stimulated by aggregating the receptors, was inhibited by disaggregation (Table 2 and Fig. 7), and involved only those receptors that were themselves aggregated (Fig. 6 *Right*). Also, as in whole cells, the levels of phosphorylation reflect competing activities of kinase(s) and phosphatase(s). Dephosphorylation of the receptor induced by hapten is not evident (cf. Figs. 3 and 7), likely because of the basal kinase activity stimulated by Mn^{2+} alone (Fig. 7, squares). However, further phosphorylation induced by antigen was inhibited. A phosphatase inhibitor (vanadate) enhanced phosphorylation (Fig. 5*A*), but, unlike PAO in whole cells, it prevented or diminished the effect of the hapten on dephosphorylation of the receptor (data not shown).

The broken-cell preparations share other characteristics with intact cells. In the latter, both early and later events show maximal activity over a relatively narrow range of temperatures (17). This was also true of receptor-stimulated phosphorylation of the receptors both on intact cells and on the washed membranes (C. Pribluda and V.S.P., unpublished observations). Similarly, the broken-cell preparations worked most efficiently around neutral pH. On the other hand, the reaction in the broken cells also showed some differences with the reaction in whole cells. Thus, even in the presence of the phosphatase inhibitor vanadate, the specific enhancement of phosphorylation engendered by aggregation of FcεR on the membranes was palpably less than is obtained by using intact cells (Table 1 and Fig. 2; ref. 7). Also, on intact cells the stimulated phosphorylation of γ chains is greater than the phosphorylation of β chains, whereas, in the membranes, this preferential phosphorylation is less prominent (Table 1). However, at higher doses of antigen, the phosphorylation of γ appears to be more decisively favored (cf. Fig. 5 and Tables 1 and 2). The basis for these differences requires further analysis.

In intact cells, the tyrosine phosphorylation of other components is much more prominent than phosphorylation of the receptor itself (18–22), but, in the sonicates or on the membranes, the receptor itself is the principal and perhaps the only product of receptor-initiated phosphorylation. Thus, phosphorylation of the receptors is an early step and cannot be a consequence of the phosphorylation of tyrosines on these other components. This exclusive phosphorylation of the receptor might be expected if the receptor itself were a kinase, but several facts suggest otherwise. (*i*) It lacks the consensus sequences associated with tyrosine kinases (23, 24). (*ii*) Aggregation of FcεRI transfected into CHO cells results in only minimal phosphorylation of the receptor (U. M. Kent and H.M., unpublished observations). (*iii*) Older *in vitro* studies suggested that FcεRI and the predominant tyrosine kinase were separable components (13).

The fundamental question we wish to address is how aggregation of FcεRI initiates a variety of biochemical perturbations. The retention in broken cells of the mechanism for aggregation-enhanced phosphorylation of receptor tyrosines makes this particular perturbation especially amenable to detailed dissection. Eiseman and Bolen (25) have proposed that FcεRI is preassociated with a src-like kinase—$p56^{lyn}$ and $pp60^{c\text{-}src}$ in the case of the FcεRI in RBL cells and $p62^{c\text{-}yes}$ in the murine PT-18 cells—and that aggregation activates the system. Our findings are consistent with such a model. In this regard, it will be particularly interesting to determine whether our preliminary evidence for complementation between the membrane-bound receptor and a soluble component will permit one to characterize the latter factor(s). Finally, we find interesting the fact that enhancing the activity of one or more tyrosine kinases by the addition of Mn^{2+} appears to mimic the effect of aggregation in enhancing phosphorylation of the receptor. Exploring the relationship between these phenomena may help to clarify the mechanism of action of the receptor.

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