THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 274, No. 3, Issue of January 15, pp. 1753–1758, 1999

Printed in U.S.A.

Structural Aspects of the Association of FcεRI with Detergent-resistant Membranes*

(Received for publication, August 18, 1998, and in revised form, October 1, 1998)

Kenneth A. Field‡, David Holowka§, and Barbara Baird

From the Department of Chemistry and Chemical Biology, Cornell University, Ithaca, New York 14853

We recently showed that aggregation of the high affinity IgE receptor on mast cells, FcεRI, causes this immunoreceptor to associate rapidly with specialized regions of the plasma membrane, where it is phosphorylated by the tyrosine kinase Lyn. In this study, we further characterize the detergent sensitivity of this association on rat basophilic leukemia-2H3 mast cells, and we compare the capacity of structural variants of FcεRI and other receptors to undergo this association. We show that this interaction is not mediated by the β subunit of the receptor or the cytoplasmic tail of the γ subunit, both of which are involved in signaling. Using chimeric receptor constructs, we found that the extracellular segment of the FcεRI α subunit was not sufficient to mediate this association, implicating FcεRI α and/or γ transmembrane segments. To determine the specificity of this interaction, we compared the association of several other receptors. Interleukin-1 type I receptors on Chinese hamster ovary cells and α_4 integrins on rat basophilic leukemia cells showed little or no association with isolated membrane domains, both before and after aggregation on the cells. In contrast, interleukin-2 receptor α (Tac) on Chinese hamster ovary cells exhibited aggregation-dependent membrane domain association similar to FcεRI. These results provide insights into the structural basis and selectivity of lipid-mediated interactions between certain transmembrane receptors and detergent-resistant membranes.

Multichain immune recognition receptors present on hematopoietic cells interact with Src family protein tyrosine kinases (PTKs)[1] as an early signaling step (1). Aggregation of the high affinity receptor for IgE, FcεRI, results in phosphorylation of the β and γ_2 subunits of this receptor by the Src family PTK, Lyn (2, 3). Lyn phosphorylation of the immunoreceptor tyrosine-based activation motif (ITAM) within FcεRI γ subunits allows the ZAP-70-related PTK, Syk, to associate with the receptor via its two Src homology 2 domains (1, 2). This recruitment and consequent activation of Syk leads to further downstream signaling, including phosphorylation and activation of phospholipase Cγ, mobilization of intracellular calcium, and activation of protein kinase C (4). In mast cells and basophils, which both express FcεRI, these cascades result in the exocytosis of preformed granules containing histamine and other vasoactive compounds, as well as other cellular responses.

The phosphorylation of FcεRI by Lyn is a critical event in receptor activation, but the mechanism by which receptor aggregation stimulates this event is not well understood. We have proposed a novel model for this process in which specialized membrane domains enriched in Lyn mediate this phosphorylation that occurs after the aggregation-dependent association of FcεRI with these domains (5). This model is consistent with our findings that the rapid association of FcεRI with these domains does not depend on receptor phosphorylation (6). It is strongly supported by preferential tyrosine phosphorylation of those receptors associated with membrane domains. Moreover, *in vitro* tyrosine kinase assays reproduce this activation step within these membrane domains that can be isolated because of their resistance to detergent solubilization (6). Other models that require a direct interaction between Lyn and the β subunit of monomeric FcεRI (7, 8) do not explain some observations, including the capacity of mutant and chimeric receptors lacking the β subunit to activate cells (9–14) or the difficulty in identifying the molecular basis of this interaction (15).

Lyn association with the detergent-resistant membranes (DRMs) isolated from the RBL-2H3 mucosal mast cell line (16) occurs as in other cells for Src family PTKs that contain a consensus site for dual fatty acid modifications (17, 18). These low density membranes are isolated by sucrose gradient ultracentrifugation of Triton X-100 (TX-100)-lysed cells. In other cell types, these preparations have been shown to be enriched in cholesterol, sphingomyelin, and gangliosides (19), as well as a subset of membrane-associated proteins, including certain Src family PTKs, heterotrimeric GTP-binding proteins, and glycosylphosphatidylinositol (GPI)-linked proteins (18–21). Preparations similar to DRMs are also referred to as detergent-insoluble glycolipid-enriched domains (DIGs), glycolipid-enriched membranes (GEMs), or sphingolipid-cholesterol rafts (22, 23). Caveolae, which are flask-shaped invaginations on the plasma membrane that contain the marker protein caveolin, can also be isolated using similar methods and appear to contain many of the same components (24, 25). RBL cells appear to be like other hematopoietic cells, including T- and B-cell lines (26–29), that do not contain caveolae but do exhibit DRMs that are enriched in signaling molecules (16).

FcεRI associates with isolated DRMs when the receptor is aggregated at the cell surface (6). Preservation of this association after cell lysis depends on the stability of the receptor aggregate and on using a low concentration of TX-100 in the lysate (6, 16). These interactions can be detected at the intact cell surface, as observed with fluorescence microscopy. For example, aggregation of FcεRI into patches causes co-redistribution of DiI-C_{16}, a fluorescent lipid probe with saturated acyl chains, and this lipid analog has reduced lateral mobility in these patches (30). Co-redistribution with aggregated FcεRI on

* This work was supported by Grant AI22449 from the National Institutes of Health. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Present address: G. W. Hooper Foundation, Box 0552, University of California, San Francisco, CA 94143.

§ To whom correspondence should be addressed. Tel.: 607-255-4095; Fax: 607-255-4137; E-mail: dah24@cornell.edu.

[1] The abbreviations used are: PTK, protein tyrosine kinase; ITAM, immunoreceptor tyrosine-based activation motif; RBL, rat basophilic leukemia; DRM, detergent-resistant membrane; GPI, glycosylphosphatidylinositol; CHO, Chinese hamster ovary; IL, interleukin; TX-100, Triton X-100.

intact cells is also observed for three other membrane components isolated with DRMs (16), a GD_{1b} ganglioside derivative (31), the GPI-linked protein Thy-1,[2] and Lyn.[2]

In the present study, we investigated the structural basis for the interaction of FcεRI, a multisubunit transmembrane receptor, with isolated DRMs. These membranes were visualized by whole-mount electron microscopy to compare them with similar preparations from other cells. We also compared the association of wild-type and mutant FcεRI with DRMs isolated from hematopoietic and nonhematopoietic cell lines. Finally, we investigated the specificity of this interaction by measuring the aggregation-dependent association of other transmembrane receptors with DRMs. These results provide evidence that the structural features of FcεRI that mediate the detergent-sensitive interaction with membrane domains occur selectively but not uniquely with this receptor.

EXPERIMENTAL PROCEDURES

Cell Lines and Antibodies—RBL-2H3 cells, mouse monoclonal anti-dinitrophenyl IgE, and biotinylated ^{125}I-IgE were previously described (6, 16). P815 mouse mastocytoma cells and Chinese hamster ovary (CHO) cells stably transfected with wild-type or mutant FcεRI were generously provided by Dr. H. Metzger (National Institutes of Health) and were maintained as described (32). Prior to harvesting, cells were sensitized with biotinylated ^{125}I-IgE for 4–24 h. All stable FcεRI transfectants expressed at least 50,000 IgE receptors per cell at the time of the experiments except the $\alpha\gamma_2$ P815 cell line, which expressed approximately 18,000 receptors per cell. CHO cells stably transfected with type I IL-1 receptors were from Dr. S. Dower (University of Sheffield, United Kingdom) and were maintained as described (33).

The IL-2 receptor α (Tac) and Chimera-1 receptor were transiently expressed on CHO cells for 48 h prior to performing experiments. The IL-2 receptor α DNA was provided in a pCMV mammalian expression vector by Dr. B. Howard (National Institutes of Health). This plasmid was transfected using LipofectAMINE (Life Technologies, Inc.) at 1 μg of DNA per ml and 10 μl of liposomes per ml in Opti-MEM (Life Technologies, Inc.) for 5 h in the absence of serum. The Chimera-1 DNA was constructed by ligating the DNA encoding the extracellular portion of the FcεRI α subunit, the DNA encoding the transmembrane and intracellular portions of the type I IL-1 receptor, and the pcDNA3.1 mammalian expression vector (Invitrogen, Carlsbad, CA). The rat FcεRIα cDNA used to make this construct was provided by Dr. H. Metzger (National Institutes of Health). The type I IL-1 receptor DNA was cloned using reverse transcription PCR from murine adult brain total RNA (provided by Dr. A. Trumpp, University of California, San Francisco). Sequencing confirmed that the junction of this chimeric receptor corresponds to base 669 of the FcεRI α sequence (GenBank™ accession number M17153) and base 1015 of the IL-1 receptor sequence (GenBank™ accession number M20658). The Chimera-1 plasmid was transfected using LipofectAMINE Plus (Life Technologies) at 2 μg of DNA per ml and 3 μl of liposomes per ml in Opti-MEM for 3 h.

Primary and secondary antibodies used to form receptor complexes at the cell surface were as follows. For IgE, affinity-purified rabbit anti-mouse IgE (34); for α_4 integrin, TA-2 mouse monoclonal antibody (35) provided by Dr. T. Issekutz (Toronto Hospital, Toronto, Ontario, Canada) and rabbit anti-mouse IgG Fc secondary antibody (Jackson ImmunoResearch, West Grove, PA); for IL-1 receptors, M5 rat monoclonal antibody (36), provided by Dr. S. Dower, and goat anti-rat IgG secondary antibody (Fisher); for IL-2 receptor α, 3G10 mouse monoclonal antibody (Boehringer Mannheim) and rabbit anti-mouse IgG secondary antibody (Cappel, West Chester, PA). All primary antibodies were iodinated with chloramine T as described previously (37). After cell harvest, the receptors were labeled with the appropriate primary antibody for at least 30 min at 20 °C, followed by two washes in buffered salt solution (20 mM Hepes, pH 7.4, 135 mM NaCl, 5 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 5.6 mM glucose). For α_4 integrin experiments, RBL cells were presaturated with IgE, and IgE was present during TA-2 binding to prevent any interaction of FcεRI with the Fc portion of TA-2.

Sucrose Gradient Ultracentrifugation—Confluent cells were harvested using EDTA and suspended at 8×10^6/ml in buffered saline solution with 1 mg/ml bovine serum albumin. Cells labeled with the appropriate antibody were stimulated with streptavidin (Sigma) or secondary antibodies as indicated under "Results," followed by the addition of an equal volume of ice cold 2× lysis buffer (final concentration, 25 mM Hepes, pH 7.5, 50 mM NaCl, 10 mM EDTA, 1 mM Na_3VO_4, 30 mM pyrophosphate, 10 mM glycerophosphate, 1 mM 4-(2-aminoethyl)benzenesulfonyl fluoride (Calbiochem), 0.02 units/ml aprotinin, and 0.01% (w/v) NaN_3) with the indicated concentration of Surfact-Amps TX-100 (Pierce). For aggregating FcεRI after cell lysis, we used rabbit anti-IgE because lysed RBL cells appear to contain sufficient free biotin to interfere with streptavidin aggregation.[3] After incubation on ice for at least 10 min, the lysates were then diluted with an equal volume of 80% (w/v) sucrose in 25 mM Hepes, pH 7.5, and 150 mM NaCl. Step gradients of sucrose were formed by layering 0.25 ml of 80%, 0.5 ml of 60%, 1.5 ml of 40% (containing the cell lysate), 0.75 ml of 30%, 0.5 ml of 20%, and 0.5 ml of 10% (w/v) sucrose in Beckman Ultra-Clear centrifuge tubes (11 × 60 mm). Centrifugation and the analysis 0.2-ml aliquots of the gradients were performed as described (6, 16).

Electron Microscopy—Sucrose gradient fractions containing DRMs from either anti-IgE-stimulated or unstimulated cells lysed in 0.05% TX-100 were pooled according to the distribution of ^{125}I-IgE bound to FcεRI in a parallel stimulated sample. Subsequent steps were performed with the technical assistance of Shannon Caldwell at the Cornell Integrated Microscopy Center. Formvar carbon-coated grids (300 mesh) were suspended on the top of drops of the sucrose fractions for 30 min to allow the adherence of DRMs. The grids were then extensively washed, fixed with 1% gluteraldehyde for 3 min, washed, and stained for 30 s with 2% uranyl acetate. The negatively stained DRMs were visualized with a Philips EM-201 transmission electron microscope.

RESULTS

We previously demonstrated that the PTK Lyn associates with DRMs isolated from RBL cells, and we found that this association is enhanced upon FcεRI stimulation (16). Under these standard cell lysis conditions with high concentrations of TX-100, FcεRI did not co-isolate with DRMs. However, with lower detergent lysis conditions, similar to those identified by Pribluda *et al*. (7) for enhancing the association of kinase activity with immunoprecipitated FcεRI, we found that substantial amounts of the aggregated receptor co-purified with the low density DRMs following sucrose gradient ultracentrifugation (6).

As part of our investigation of the structural basis for this receptor association with DRMs, we examined the detergent sensitivity. For low detergent conditions, we use 0.05% TX-100 to lyse RBL cells at 4×10^6/ml and then dilute this lysate 1:1 with 80% sucrose prior to sucrose gradient ultracentrifugation. Under these conditions, the micellar detergent to cell lipid ratio during the ultracentrifugation is approximately 3 (38). To exclude some artifactual possibilities, we added 0.025% TX-100 to all of the sucrose gradient steps, as shown in Fig. 1 (○ and ●). Under these conditions, as when no TX-100 is added to the gradient (6), less than 5% of the unaggregated FcεRI associates with the DRM fractions (○, fractions 3–7) and greater than 50% of streptavidin-aggregated biotin-IgE-FcεRI complexes associate with these (●). From this result, it appears unlikely that the plasma membrane is incompletely solubilized or that association of aggregated receptors with DRMs depends on the separation of DRM components from TX-100 during the ultracentrifugation process.

To investigate the requirements for DRM association of aggregated FcεRI, we lysed cells with 0.05% TX-100 prior to adding anti-IgE to form FcεRI aggregates. When this lysate was fractionated (Fig. 1, □), these aggregates migrated at a higher density in the sucrose gradient, similar to aggregates formed on cells that are subsequently lysed in 0.2% or higher TX-100 (16). In contrast, addition of anti-IgE *prior* to cell lysis with 0.05% TX-100 causes greater than 50% of FcεRI to associate with low density membranes (*e.g.* see Fig. 6*A*), similar to results using streptavidin cross-linking (Fig. 1 (●) and Ref. 6).

[2] D. Holowka, unpublished observations.

[3] K. A. Field, D. Holowka, and B. Baird, unpublished observations.

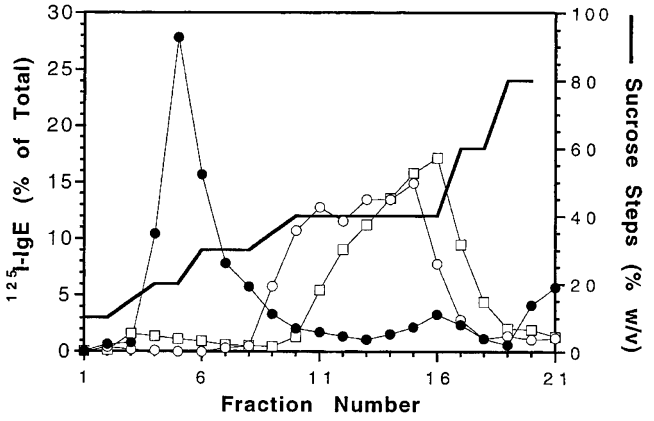

FIG. 1. **Sucrose gradient ultracentrifugation of RBL cell lysates.** RBL cells sensitized with biotinylated ^{125}I-IgE and suspended at 8×10^6/ml in buffered saline solution were stimulated for 5 min at 37 °C with 10 nM streptavidin (●) or left unstimulated (○ and □) and then lysed with an equal volume of 2× lysis buffer containing 0.1% TX-100. After 10 min on ice, the lysates were either loaded onto sucrose gradients as described under "Experimental Procedures" (○ and ●) or else first treated with 2.5 μg/ml rabbit anti-IgE for an additional 10 min prior to loading onto the gradient (□). For the samples represented by ○ and ●, 0.025% TX-100 was added to the entire sucrose gradient. The ^{125}I present in each 0.2-ml fraction is shown as a percentage of the total ^{125}I-IgE in the entire gradient including the pellet (fraction 21). The *right axis* indicates the percentage of sucrose used for each step in forming the gradient (*heavy line*).

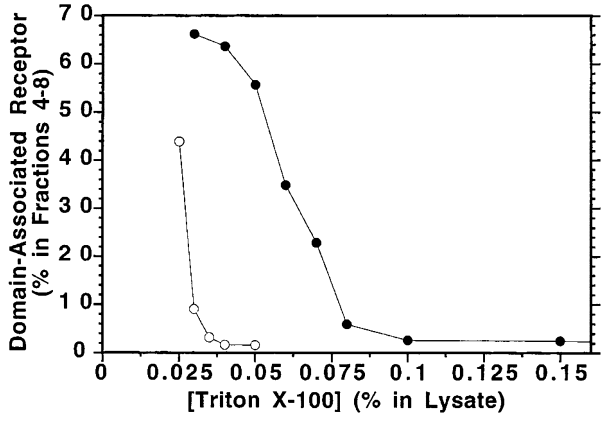

FIG. 2. **Detergent-sensitive association of FcεRI with membrane domains.** Sucrose gradient ultracentrifugation was performed on RBL cell lysates as for Fig. 1, except that the final concentration of TX-100 in the lysate was varied as indicated, and TX-100 was not included in the gradient steps. The amount of ^{125}I-IgE in the sucrose fractions containing detergent-resistant membrane domains (fractions 4–8) is compared for cells that were unstimulated (○) or that had been stimulated with 10 nM streptavidin for 5 min at 37 °C prior to lysis (●).

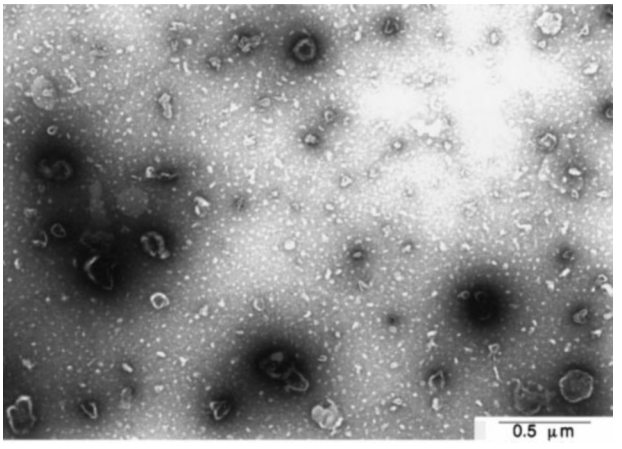

FIG. 3. **Whole mount electron microscopy of membrane domains.** Detergent-resistant membrane domains were isolated from RBL cells as in Fig. 2 using 0.05% TX-100 for lysis. The membrane domains from fractions 5 and 6 of a sucrose gradient (see Fig. 1) were adhered to Formvar carbon-coated grids, fixed, and negative-stained with uranyl acetate. Transmission electron microscopy was used to visualize the domains, and a representative field containing vesicles of various sizes is shown with a 0.5-μm *scale bar*.

These results indicate that the interactions between DRM components and aggregated FcεRI that occur on intact cells must be preserved during cell lysis and sucrose gradient fractionation, as they cannot be caused by receptor aggregation subsequent to cell lysis.

The association of FcεRI with DRMs could be mediated by protein-protein, protein-lipid, or some combination of these interactions. By varying the amount of TX-100 used to lyse RBL cells, we further investigated the detergent sensitivity of this interaction. Unaggregated FcεRI was recovered in the low density gradient fractions when insufficient TX-100 (<0.03%) was used for cell lysis (Fig. 2, ○). Under these conditions, it is possible that plasma membranes are not solubilized completely and therefore migrate at this low density because of their lipid content. With greater than 0.03% TX-100 for cell lysis, the membranes appeared effectively solubilized, and monomeric FcεRIs were found almost entirely in the 40% sucrose fraction. In addition, other membrane-bound proteins that do not associate with DRMs, including α_4 integrins (*e.g.* see Fig. 6*B*) and Src,[3] were found in the 40% sucrose fractions at 0.05% TX-100. Under these same conditions, greater than 50% of streptavidin-aggregated biotin-IgE bound to FcεRI remained associated with DRMs in the sucrose gradient (Fig. 2, ●). When concentrations of TX-100 used during cell lysis were greater than 0.05%, aggregated FcεRIs were not retained with DRMs, and complete disruption of this association occurred as low as 0.08% TX-100 (Fig. 2). As shown previously, lipid-anchored markers of these DRMs (*i.e.* Thy-1, GD_{1b} gangliosides, and Lyn) remain associated even at 0.5% TX-100 (16). This marked sensitivity for FcεRI/DRM association is consistent with a lipid-mediated interaction (see under "Discussion").

Electron microscopy of isolated, negatively stained DRMs from RBL cells lysed at 0.05% TX-100 reveals them to be vesicular structures (Fig. 3). The majority range from 50 to 200 nm in diameter; larger vesicles with diameters up to 1.4 μm were less frequently observed.[3] These vesicles are qualitatively similar in appearance and size to DRM preparations from MDCK cells (25), T- and B-cell lines (28), neuroblastoma cells (26), and RBL cells, more typically lysed in 1% TX-100.[4] Thus, DRMs isolated with lower TX-100 to cell lipid ratios are not ultrastructurally different from other DRMs that have been prepared with higher TX-100, and these preparations are not significantly contaminated with unsolubilized membrane sheets or organelles.

To investigate further the structural basis of the FcεRI/DRM interaction, we used P815 mast cells and CHO cells that were stably transfected with various FcεRI constructs. As shown in Fig. 4, P815 cells stably expressing wild-type $\alpha\beta\gamma_2$ FcεRI subunits (*wt*) show aggregation-dependent receptor association with low density DRMs that is similar to native FcεRI on RBL cells at 0.05% TX-100 (Fig. 2 and Ref. 6). Furthermore, cells expressing mutated versions of the receptor lacking either the C-terminal cytoplasmic tail of the β subunit or the cytoplasmic tail of the γ subunit show similar aggregation-dependent association (Fig. 4). Interestingly, IgE receptors entirely lacking the β subunit (FcεRI $\alpha\gamma_2$) also show aggregation-dependent DRM

[4] D. Reczeck and K. Field, unpublished observations.

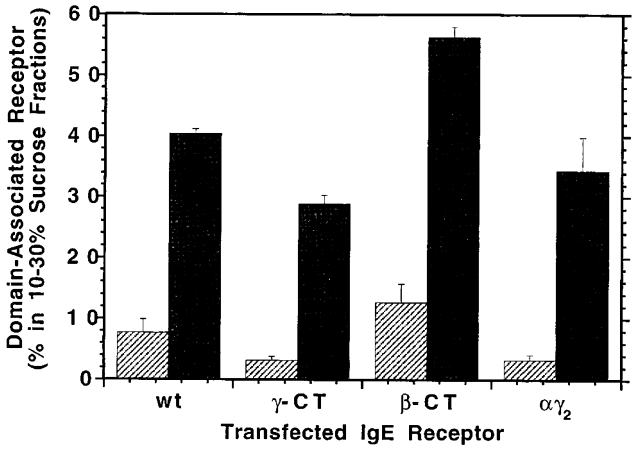

FIG. 4. **Association of transfected FcεRI with membrane domains in P815 mast cells.** The association of ^{125}I-IgE-FcεRI complexes with the isolated membrane domains is compared for various P815 cell lines stably expressing IgE receptors. The percentage of biotinylated ^{125}I-IgE found in sucrose gradient fractions 3–7 from either unstimulated (*hatched columns*) or 10 nM streptavidin-stimulated (*solid columns*) cells is shown for P815 cell lines stably transfected with either wild-type $\alpha\beta\gamma_2$ (*wt*), receptors lacking the C-terminal cytoplasmic tail of the β subunit (β-*CT*), or receptors lacking the C-terminal cytoplasmic tail of the γ subunit (γ-*CT*). For P815 cells stably transfected with human α subunits that are associated with endogenous mouse γ subunits ($\alpha\gamma_2$), the percentage of ^{125}I-IgE associated with membrane domains is shown for unstimulated (*hatched columns*) or 5 μg/ml anti-IgE-stimulated (*solid columns*) cells. Data shown are the average of two separate gradients, and the *error bars* represent the range of data.

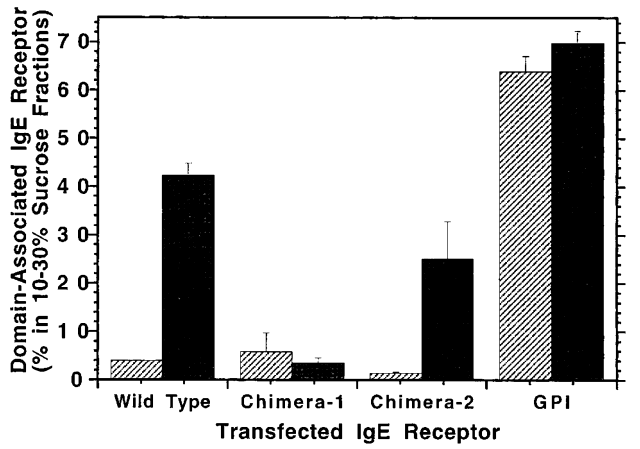

FIG. 5. **Membrane domain association of FcεRI expressed on CHO cells.** The association of various FcεRI constructs with sucrose gradient fractions 3–7 is compared for unstimulated (*hatched columns*) or 10 nM streptavidin-stimulated (*solid columns*) CHO cells. CHO cells transfected with either wild-type $\alpha\beta\gamma_2$ or chimeric receptors containing the extracellular domain of the FcεRI α subunit fused to either the transmembrane and intracellular domains of the type I IL-1 receptor (*Chimera-1*), the transmembrane and intracellular domains of the p55 IL-2 receptor (*Chimera-2*), or a GPI anchor (*GPI*) were lysed with 0.05% TX-100. Data for Chimera-1 and Chimera-2 are the average of three experiments, and the S.D. is shown; others are shown as for Fig. 4.

interactions. Some differences in the magnitude of association were observed, but the ratio of aggregated to monomeric receptors associated with the DRMs remained fairly constant. These differences could represent small contributions to the DRM association from the deleted protein segments. Alternatively, this variability could be due to differences between the transfected cell lines themselves that have been separately subcloned. Significantly, FcεRI in the β and γ subunit cytoplasmic tail mutant cells do not activate tyrosine kinases or other downstream signals (9), although they do become insoluble in high concentrations of TX-100 after extensive aggregation (32).

Nonhematopoietic CHO cells expressing wild-type FcεRI showed qualitatively similar association of this receptor with DRMs that depends on aggregation (Fig. 5, *wild-type*). However, a chimeric receptor containing the extracellular segment of the FcεRI α subunit and the transmembrane and intracellular segments of the type-1 IL-1 receptor (Fig. 5, *Chimera-1*) did not show significant DRM association in the presence or absence of receptor aggregation. This chimeric receptor demonstrates that the extracellular segment of FcεRI α is not sufficient to mediate this interaction. In contrast, a similar chimeric receptor that contains the extracellular segment of the FcεRI α subunit and the transmembrane and intracellular segments of IL-2 receptor α (p55, Tac; 39) (Fig. 5, *Chimera-2*) did show aggregation-dependent association with the DRMs. The results in Figs. 4 and 5 are consistent with the transmembrane segments of these receptors being most important for determining DRM association. We also examined the association of FcεRI α subunits linked to the plasma membrane by a GPI anchor. These receptors, which lack a transmembrane segment, associated with the DRMs even in the absence of aggregation (Fig. 5, GPI) and also remained associated to a similar extent in 0.2% TX-100,[3] conditions that would extract aggregated, wild-type FcεRI (Fig. 2). This is consistent with previous studies from our laboratory (16) and others (19, 27) on various GPI-linked proteins that show constitutive association with DRMs isolated by this method, and it supports the view that the membrane-anchoring region is most critical for these interactions.

DRM association was compared for FcεRI and other cell surface receptors. Native FcεRI on RBL cells associated with DRMs after aggregation with polyclonal anti-IgE (Fig. 6*A*). In contrast, the native α_4 integrins on RBL cells showed only low amounts of DRM association both after binding of the monoclonal antibody TA-2 (Fig. 6*B*, ○) and when further aggregated with a polyclonal antibody (●). Similarly, stably transfected type-1 IL-1 receptors on CHO cells labeled with M5 monoclonal antibody did not associate with DRMs (Fig. 6*C*, ○), even after polyclonal antibody aggregation (●) or streptavidin aggregation of biotinylated M5.[3] The possibility that these results are due to the dissociation of M5 from the receptor during lysis and gradient fractionation was excluded by labeling the IL-1 receptors with ^{125}I-IL-1. When unlabeled M5 plus secondary antibody was used to aggregate these receptors, the distribution of label was very similar to Fig. 6*C,* including the appearance of ^{125}I-IL-1-receptor complexes in the 50–60% sucrose fractions.[3]

When we examined the association of IL-2 receptor α that was transiently expressed on CHO cells, we found that these receptors labeled with a monoclonal antibody did not associate with low density DRMs (Fig. 6*D,* ○). Interestingly, when these receptors were aggregated with a polyclonal antibody, significant amounts did associate (●). In addition, some receptor aggregates also shift to the high density sucrose fractions. As with FcεRI, similar association with DRMs is seen if streptavidin is used to aggregate these receptors labeled with a biotinylated monoclonal antibody.[3] These results show that aggregation-dependent association with DRMs is restricted to certain receptors, indicating that antibody-mediated receptor aggregation is not sufficient to cause nonspecific association or entanglement with membrane domains at the cell surface.

DISCUSSION

We previously established that the association of FcεRI with DRMs plays an important role in the initiation of signaling by this multichain immune recognition receptor (6). Therefore, to understand fully the activation of FcεRI, it is necessary to characterize this interaction. In this study, we examined the structural basis of this aggregation-dependent association, in-

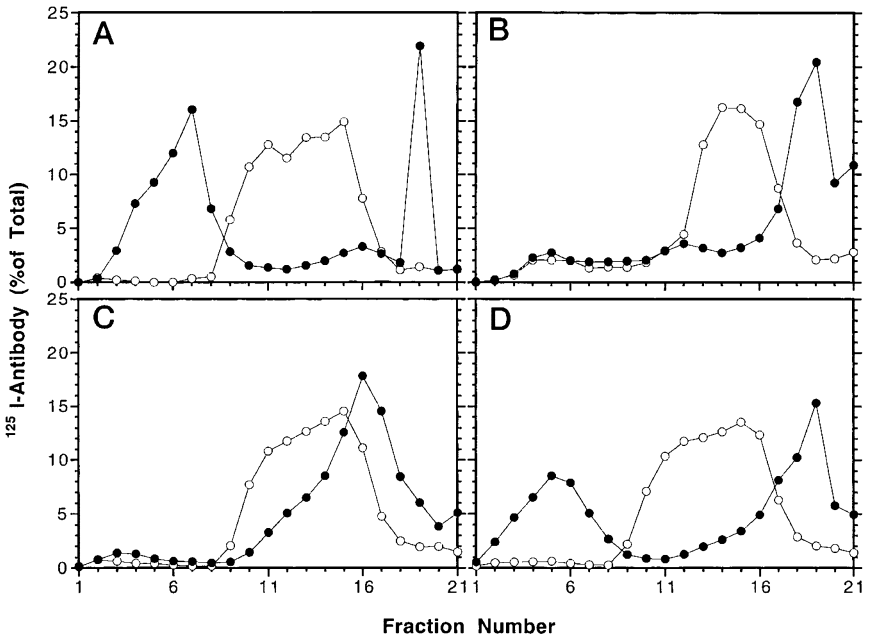

FIG. 6. **Sucrose gradient analysis of four different receptors.** The distribution of receptors in sucrose gradients is compared for FcεRI on RBL cells (*A*), α_4 integrins on RBL cells (*B*), IL-1 receptors on CHO cells (*C*), and IL-2 receptors on CHO cells (*D*). The receptors were prelabeled with an appropriate ^{125}I-modified monoclonal antibody alone (○) or followed by further aggregation with 5–10 μg/ml polyclonal antibody for 5–10 min at 37 °C (●). Cells were then lysed in 0.05% TX-100, and ultracentrifugation was performed as described under "Experimental Procedures."

cluding the contribution of different portions of the receptor.

One of the most striking characteristics of the association of aggregated FcεRI with DRMs is the detergent sensitivity of this interaction. Fig. 2 demonstrates that TX-100 concentrations as low as 0.08% nearly completely eliminated the association. The unusual requirement of low concentrations of detergent presumably reflects a weaker interaction of these membrane structures with the receptor, which is a transmembrane protein, than with lipid-modified proteins that can associate directly via their saturated acyl chains without disturbing the lipid packing of the bilayer (Fig. 5 and Ref. 16). The interaction of monomeric FcεRI with membrane domain components may also occur on the cell surface, but too weakly to be preserved during lysis at TX-100 concentrations greater than 0.03%. Thus, aggregation of FcεRI at the plasma membrane may serve to stabilize these preexisting interactions. We are currently investigating whether the appearance of unaggregated FcεRI in the low density sucrose gradient fractions at the lowest TX-100 concentrations (Fig. 2) is due to weak interactions with DRM. The selective detergent sensitivity that we observed is most readily explained by a lipid-mediated association, but we cannot rule out protein-protein interactions between FcεRI and other DRM components that are too weak to withstand higher detergent lysis conditions.

It is important to note that aggregation of FcεRI *following* detergent treatment of cells does not induce similar associations with the DRMs (Fig. 1). Thus, interactions between aggregated FcεRI and DRM components must be preserved when the cells are lysed. The ultracentrifugation of these lysates on sucrose gradients caused the low density components to rise in the gradient, where they can coalesce to form larger vesicles (Fig. 3), even in the presence of micellar TX-100 (Fig. 1).

The DRMs isolated from RBL cells lysed with 0.05% TX-100 appear similar to those from 0.2 to 1% TX-100 lysates by three criteria. First, Lyn and GPI-linked proteins are selectively enriched in both of these preparations. Greater than 50% of these lipid-linked proteins (Fig. 5) but less than 2% of total cellular protein are found in the low density sucrose fractions.[3] Second, whole-mount electron microscopy of the vesicles from 0.05 and 1% TX-100 lysates reveal similar structures (Fig. 3).[4] Third, the protein compositions of the DRMs prepared with both levels of detergent are similar as revealed by silver staining of SDS-polyacrylamide gels.[3] Thus, although it is necessary to use low detergent concentrations to preserve interactions between aggregated FcεRI and DRMs, this membrane preparation appears to be very similar to those isolated from other cells that lack caveolae (27, 28).

We used structural variants of FcεRI to determine what portions of the α, β, and γ subunits are involved in aggregation-dependent DRM association. Previous results with RBL cells showed that tyrosine phosphorylation of the β and γ subunit ITAMs is not necessary (6). Now we find with transfected P815 mast cells that the ITAM-containing cytoplasmic segments of these subunits are not required for DRM association (Fig. 4). Thus, there appears to be no involvement of proteins with Src homology 2 domains that bind to phosphorylated ITAMs. Receptor variants lacking the entire β subunit (FcεRI $\alpha\gamma_2$) also showed DRM association (Fig. 4). This observation is consistent with the capacity of FcεRI $\alpha\gamma_2$ to activate PTK signaling in these transfected P815 cells (9), as well as in U937 monocytes and transfected NIH-3T3 fibroblasts (10). Thus, the β subunit may amplify FcεRI activation by providing a docking site for Lyn or other signaling molecules, but this subunit is not required for the initial step in receptor activation.

Aggregated FcεRI associated with DRMs on CHO cells (Fig. 5), showing that there is no requirement for hematopoietic cell-specific proteins or lipids, such as the GD_{1b} ganglioside derivatives on mast cells that are recognized by the AA4 antibody (40, 41). This observation is consistent with the capacity of FcεRI to become phosphorylated by Lyn and to activate Syk when cotransfected with these kinases in NIH-3T3 cells (2, 10). Unlike wild-type FcεRI, GPI-linked FcεRI α associates with DRMs on CHO cells in the absence of any aggregation (Fig. 5) and in both low and high levels of TX-100,[3] as seen for other GPI-linked proteins, including Thy-1 (16). Thus, the GPI lipid tail is probably critical for mediating aggregation-independent association of some proteins with DRMs.

DRM association under low TX-100 conditions does not occur for all receptors after aggregation, as shown in Fig. 6 for α_4 integrins and type-1 IL-1 receptors. This result enabled the use of chimeric receptors to assess the structural portions critical for this interaction. We found that only one (Chimera-2) of the two chimeras that contain the extracellular segment of the FcεRI α subunit showed aggregation-dependent association with DRMs, indicating that this segment is not sufficient. The association of these chimeras corresponds to that of the source

receptors: IL-2 receptor α and Chimera-2 associate with DRMs, whereas IL-1 receptor and Chimera-1 do not (Figs. 5 and 6). These results indicate that the structural basis for IL-2 receptor α and FcεRI association with DRMs primarily resides with the transmembrane and/or cytoplasmic segments of these receptors. Furthermore, because FcεRI β is not necessary for association with DRMs, and FcεRI γ is not significantly exposed at the extracellular side of the plasma membrane (42), it is likely that extracellular segments of FcεRI are not involved in this interaction.

A recent study demonstrated that the structural basis for the association of the influenza hemagglutinin protein with detergent-resistant membrane domains resides in its transmembrane segment, but the amino acid residues primarily responsible for this interaction did not suggest a predictable structural motif (43). Our results also point to the transmembrane segments of the FcεRI α and/or γ subunits being primarily responsible for aggregation-dependent association of FcεRI with DRMs, as extracellular FcεRI α, cytoplasmic FcεRI γ, and FcεRI β are not necessary or sufficient (Figs. 4 and 5). Although it remains formally possible that the 19-residue cytoplasmic segment of FcεRI α is important for these interactions, this seems unlikely in light of previous studies indicating that this short sequence is not necessary for FcεRI signaling (9) or detergent insolubility (32). Covalently attached palmitic acid has been reported for the FcεRI β and γ subunits (44), and it is possible that these lipid modifications are involved in DRM association of this receptor. More extensive mutational analyses are required to define completely the structural basis for FcεRI and IL-2 receptor α interactions with DRMs.

Our results point out that caution must be used when interpreting data from studies involving IL-2 receptor α chimeras. For example, chimeras with extracellular and transmembrane segments of IL-2 receptor α and ITAM-containing cytoplasmic segments of the FcεRI β or γ subunits (8, 12, 13, 45) or the T cell receptor ζ subunit (12, 45) have been used extensively to investigate the activation of mast cells and T cells. These studies, which showed kinase activation upon aggregation of the chimeric receptors, suggested that ITAM sequences were the only segments of these these receptors essential for signaling. However, our results with DRMs in Figs. 4 and 5 would predict that these chimeric constructs might associate with specialized domains in the plasma membrane because of structural features normally provided by other portions of multichain immune recognition receptors, and they could consequently interact with domain-associated Lyn or similar Src family PTKs. Both FcεRI and IL-2 receptors utilize Src family PTKs in early signaling events (46, 39), and DRM association may represent a common mechanism for interaction of such receptors with these kinases.

In conclusion, the present study supports a model of FcεRI activation that involves the selective interaction of this receptor with specialized membrane domains immediately following receptor aggregation on cells (for a review, see Ref. 5). These domains, as isolated, are enriched in Lyn and facilitate the coupling of the receptor with this kinase in a process that may also be utilized by other receptors. The transmembrane segments of FcεRI probably play the major role in this lipid-protein interaction, which also occurs for IL-2 receptor α after aggregation. Further study will be required to test this hypothesis more fully and to answer other important questions concerning the structural basis for this interaction, including the specific DRM components involved. Other questions about the role of compartmentalization in IgE receptor signaling also remain to be answered. These include the regulation of Lyn activity associated with membrane domains on cells and the role of tyrosine phosphatases in the regulation of signaling orchestrated by FcεRI interactions with these membrane domains.

REFERENCES

1. Cambier, J. C. (1995) *J. Immunol.* **155,** 3281–3285
2. Scharenberg, A. M., Lin, S., Cuenod, B., Yamamura, H., and Kinet, J. P. (1995) *EMBO J.* **14,** 3385–3394
3. Zhang, J., Berenstein, E. H., Evans, R. L., and Siraganian, R. P. (1996) *J. Exp. Med.* **184,** 71–79
4. Beaven, M. A., and Baumgartner, R. A (1996) *Curr. Opin. Immunol.* **8,** 766–772
5. Field, K. A., Holowka, D., and Baird, B. (1999) in *Signal Transduction in Mast Cells and Basophils* (Razin, E., and Rivera, J., eds) pp. 102–114, Springer-Verlag, New York
6. Field, K. A., Holowka, D., and Baird, B. (1997) *J. Biol. Chem.* **272,** 4276–4280
7. Pribluda, V. S., Pribluda, C., and Metzger, H. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 11246–11250
8. Jouvin, M. H., Adamczewski, M., Numerof, R., Letourneur, O., Valle, A., and Kinet, J. P. (1994) *J. Biol. Chem.* **269,** 5918–5925
9. Alber, G., Miller, L., Jelsema, C. L., Varin-Blank, N., and Metzger, H. (1991) *J. Biol. Chem.* **266,** 22613–22620
10. Lin, S., Cicala, C., Scharenberg, A. M., and Kinet, J. P. (1996) *Cell* **85,** 985–995
11. Juergens, M., Wollenberg, A., Hanau, D., Henri-De-La-Salle, H., and Bieber, T. (1995) *J. Immunol.* **155,** 5184–5189
12. Eiseman, E., and Bolen, J. B. (1992) *J. Biol. Chem.* **267,** 21027–21032
13. Wilson, B. S., Kapp, N., Lee, R. J., Pfeiffer, J. R., Martinez, A. M., Platt, Y., Letourneur, F., and Oliver, J. M. (1995) *J. Biol. Chem.* **270,** 4013–4022
14. Repetto, B., Bandara, G., Kado-Fong, H., Larigan, J. D., Wiggan, G. A., Pocius, D., Basu, M., Gilfillan, A. M., and Kochan, J. P (1996) *J. Immunol.* **156,** 4876–4883
15. Vonakis, B. M., Chen, H., Haleem-Smith, H., and Metzger, H. (1997) *J. Biol. Chem.* **272,** 24072–24080
16. Field, K. A., Holowka, D., and Baird, B. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 9201–9205
17. Resh, M. D. (1994) *Cell* **76,** 411–413
18. Shenoy-Scaria, A. M., Dietzen, D. J., Kwong, J., Link, D. C., and Lublin, D. M. (1994) *J. Cell Biol.* **126,** 353–363
19. Brown, D. A., and Rose, J. K. (1992) *Cell* **68,** 533–544
20. Arreaza, G., Melkonian, K. A., LaFevre-Bernt, M., and Brown, D. A. (1994) *J. Biol. Chem.* **269,** 19123–19127
21. Sargiacomo, M., Sudol, M., Tang, Z., and Lisanti, M. P. (1993) *J. Cell Biol.* **122,** 789–807
22. Harder, T., and Simons, K. (1997) *Curr. Opin. Cell Biol.* **9,** 534–542
23. Brown, D. A., and London, I. (1999) *Annu. Rev. Dev. Cell Biol.* **14,** 111–136
24. Anderson, R. G. W. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90,** 10909–10913
25. Lisanti, M. P., Scherer, P. E., Vidugiriene, J., Tang, Z., Hermanowski-Vosatka, A., Tu, Y. H., Cook, R. F., and Sargiacomo, M. (1994) *J. Cell Biol.* **126,** 111–126
26. Gorodinsky, A., and Harris, D. A. (1995) *J. Cell Biol.* **129,** 619–627
27. Fra, A. M., Williamson, E., Simons, K., and Parton, R. G. (1994) *J. Biol. Chem.* **269,** 30745–30748
28. Parolini, I., Sargiacomo, M., Lisanti, M. P., and Peschle, C. (1996) *Blood* **87,** 3783–3794
29. Fra, A. M., Williamson, E., Simons, K., and Parton, R. G. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 8655–8659
30. Thomas, J. L., Holowka, D., Baird, B., and Webb, W. W. (1994) *J. Cell Biol.* **125,** 795–802
31. Pierini, L., Holowka, D., and Baird, B. (1996) *J. Cell Biol.* **134,** 1427–1439
32. Mao, S. Y., Alber, G., Rivera, J., Kochan, J., and Metzger, H. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89,** 222–226
33. Guo, C., Dower, S. K., Holowka, D., and Baird, B. (1995) *J. Biol. Chem.* **270,** 27562–27568
34. Menon, A. K., Holowka, D., and Baird, B. (1984) *J. Cell Biol.* **98,** 577–583
35. Issekutz, T. B., and Wykretowicz, A. (1991) *J. Immunol.* **147,** 109–116
36. Gallis, B., Prickett, K. S., Jackson, J., Slack, J., Schooley, K., Sims, J. E., and Dower, S. K. (1989) *J. Immunol.* **143,** 3235–3240
37. Holowka, D., and Baird, B. (1983) *Biochemistry* **22,** 3466–3474
38. Kinet, J. P., Alcaraz, G., Leonard, A., Wank, S., and Metzger, H. (1985) *Biochemistry* **24,** 4117–4124
39. Minami, Y., and Taniguchi, T. (1995) *Curr. Opin. Cell Biol.* **7,** 156–162
40. Guo, N. H., Her, G. R., Reinhold, V. N., Brennan, M. J., Siraganian, R. P., and Ginsburg, V. (1989) *J. Biol. Chem.* **264,** 13267–13272
41. Oliver, C., Sahara, N., Kitani, S., Robbins, A. R., Mertz, L. M., and Siraganian, R. P. (1992) *J. Cell Biol.* **116,** 635–646
42. Blank, U., Ra, C., Miller, L., White, K., Metzger, H., and Kinet, J.-P. (1989) *Nature* **337,** 187–189
43. Scheiffele, P., Roth, M. G., and Simons, K. (1997) *EMBO J.* **16,** 5501–5508
44. Kinet, J. P., Quarto, R., Perez, M. R., and Metzger, H. (1985) *Biochemistry* **24,** 7342–7348
45. Letourner, F., and Klausner, R. D. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88,** 8905–8909
46. Benhamou, M., and Siraganian, R. P. (1992) *Immunol. Today* **13,** 195–197