The Journal of Immunology

RESEARCH ARTICLE | FEBRUARY 01 1991

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S Y Mao; ... et. al

J Immunol (1991) 146 (3): 958–966.
https://doi.org/10.4049/jimmunol.146.3.958

0022-1767/91/1463-0958$02.00/0
THE JOURNAL OF IMMUNOLOGY

Vol. 146, 958–966, No. 3, February 1, 1991
Printed in U.S.A.

IMMOBILIZATION AND INTERNALIZATION OF MUTATED IgE RECEPTORS IN TRANSFECTED CELLS[1]

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Earlier studies have shown that the mast cell receptor IgE (FcεRI) for is expressed on COS-7 cells transfected with the cDNA for each of the three types of subunits that form the tetrameric, $\alpha\beta\gamma_2$, receptor. Although such transfected COS cells fail to exhibit some of the early biochemical perturbations initiated by aggregation of the receptor on normal mast cells and related tumor lines, we show here that other characteristics of the endogenous FcεRI are retained. Thus, the unaggregated transfected wild-type receptors were found to have a restricted translational diffusion similar to that observed for endogenous receptors on mast cells as assessed by fluorescence photobleaching and recovery. Similarly, as with endogenous receptors, the mobility of transfected receptors was sharply reduced when the receptors were aggregated by reaction with small oligomers of IgE. In addition, aggregation of the transfected FcεRI caused them to be internalized by the COS cells by a cytochalasin-sensitive mechanism, albeit at a considerably slower rate than was seen with endogenous receptors on mast cells or with transfected receptors in a line of receptor-deficient mast cells. We also examined the mobility and internalization before and after aggregation, of some 13 different combinations of receptor subunit mutants in which one or more of the five cytoplasmic domains of the receptor had been truncated. Our results show that whatever interactions between the receptor and cellular components may account for the phenomena we studied, such interactions do not critically depend upon the bulk of the cytoplasmic domains of the receptor.

A large number of cell-mediated immune responses are triggered by the aggregation of surface molecules that are members of the Ig super family. In addition to sharing some structural features, these surface proteins share certain functional properties. For example, like numerous other membrane proteins, they show a retarded translational mobility even in the unaggregated state, and this is accentuated upon aggregation. After such aggregation, many are internalized, sometimes via coated pits, and become associated with the detergent-insoluble "cytoskeleton" pellet. As a group they appear to utilize the hydrolysis of phospholipids and a rise in cytoplasmic Ca^{2+}, rather than other classes of "second messengers".

With respect to these phenomena, it is natural to assume that the cytoplasmic extensions would serve as the direct link between the surface receptors and the cytoskeletal and other cellular components with which the receptors interact, and in several instances evidence for such a mechanism has recently been obtained (1–3).

This paper deals with our initial exploration to study the role of the cytoplasmic domains of the mast cell receptor with high affinity for IgE (FcεRI)[3] (4, 5). FcεRI is a tetramer consisting of an α-chain, a β-chain, and a disulfide-linked dimer of γ-chains. The α-chain is a member of the Ig super family (6, 7), and the γ-chains are highly homologous to the ζ-chains associated with the clonotypic T lymphocyte receptor (8, 9). On the functional side, the changes in receptor mobility and distribution, as well as the biochemical events that result from aggregation of the receptor, have been well documented (4, 10, 11), and follow the pattern for members of the Ig super family described above.

In order to define the role of the cytoplasmic domains of the FcεRI, we engineered mutants of each of the subunits of the receptor to truncate all, or virtually all, of their cytoplasmic domains; we found that when such mutant cDNA were transfected into COS cells in different combinations, even receptors expressing none of the five cytoplasmic domains were expressed on the surface of these cells (12).

In this paper we describe some of the properties of such mutant receptors. The surprising result is how little the functions we assessed were influenced by drastic structural changes.

MATERIALS AND METHODS

Materials. FITC isomer I was from U. S. Biochemical Corporation (Cleveland, OH). Cytochalasin D, and 2-deoxyglucose were purchased from Sigma (St. Louis, MO). The bivalent affinity cross-linker BDPE (13) was a gift from Dr. Paul Plotz (National Institutes of Health, Bethesda, MD). Dinitrophenylated-BSA (25 mol/mol) was prepared as previously described (14). Anti-DNP monoclonal mouse IgE was obtained and purified as previously described (15, 16). [^{125}I]IgE was prepared by the chloramine T method (17).

Cells. The 2H3 subline of RBL cells was grown adherent in stationary culture (18). COS-7 cells (#CRL 1651, American Type Culture

Received for publication August 20, 1990.
Accepted for publication October 29, 1990.
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[1] This work was supported in part by National Institutes of Health Grant AI14584 to M.E. N.V.-B. was supported by funds from a grant to H.M. by Hoffmann LaRoche.
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[3] The abbreviations used are: FcεRI, high affinity receptor for IgE; BDPE, bis-2,4 dinitrophenyl pimelic ester; CD, cytoplasmic domain; D_{lat}, translational diffusion coefficient; $Fc_\gamma RII$, one of the isoforms of FcR that binds IgG with low affinity; FITC-IgE, fluoresceinated IgE; FPR, fluorescence photobleaching and recovery; PCR, polymerase chain reaction; R, mobile fraction; RBL, rat basophilic leukemia.

Collection, Rockville, MD) were cultured in Dulbecco's modified Eagle's medium with 2 mM glutamine, 16% heat-inactivated fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin (Biofluids, Rockville, MD), at 37°C in a humidified atmosphere containing 5% CO_2. The CSF-independent mouse mast cell line PT18 (19) was cultured in RPMI 1640 supplemented with 16% serum, 50 μM 2-ME, 0.1 mM MEM nonessential amino acids, 1 mM sodium pyruvate, 10 mM HEPES buffer (GIBCO, Grand Island, NY), and antibiotics, at 37°C with 5% CO_2. P815 (clone 6), a mouse mastocytoma cell line stably transfected with wild-type rat IgE receptors, has been described (20).

IgE oligomer. Mouse anti-DNP IgE was cross-linked with BDPE as previously described (21). In brief, a freshly prepared solution of BDPE in dimethyl formamide was added to 4 mg IgE/ml borate-buffered saline (0.16 M NaCl, 0.2 M borate, pH 8.0) such that the BDPE:IgE molar ratio was 7:1 and the final concentration of the formamide was less than 5% by volume. The reaction was carried out for 2 h in the dark at room temperature and was quenched with 0.2 volumes of 0.02 M lysine. Under these conditions, BDPE has been shown to preferentially cross-link anti-DNP antibodies via residues near the combining sites (13). The oligomers were separated according to size by gel filtration chromatography on 2 $cm^2 \times 100$ cm columns of Sephadex G-200 and AcA22 Ultragel in tandem (22) with borate-buffered saline at a flow rate of 5 ml/h. Three-milliliter fractions were collected and analyzed by electrophoresis on 5% (w/v) polyacrylamide gels in SDS (23). The fractions containing trimeric up to hexameric IgE, were pooled and concentrated to 3 mg/ml protein. Monomers and oligomers of IgE were fluoresceinated by the method of Schreiber and Haimovich (24). The solution was adjusted to pH 9.0 by adding 0.2 volume of 1 M $NaCO_3$ buffer (pH 9.5) and reacted with 25 μg FITC/mg protein for 30 min in the dark at room temperature. The reaction mixture was purified by passage over Sephadex G-25. All conjugates had one to two fluorescein molecules coupled per IgE (FITC-IgE).

Partial degradation of cross-linked oligomers into dimers and monomers occurred during storage at 4°C (0.75 mg/ml in borate-buffered saline). Therefore, both monomer and oligomer samples were periodically further purified by HPLC. IgE monomers were additionally airfuged ($100,000 \times g$) for 30 min immediately before use.

DNA constructs. Mutant cDNA of the individual subunits (α,β,γ) were constructed with truncations in the presumptive cytoplasmic domains (Fig. 1). The nomenclature of these mutants is indicated in Table I. The procedures used for mutagenesis, transfections, and assessment of expression have been described (12). The chimeric mutant, $\beta\alpha_c$, was prepared by fusing the DNA sequence encoding the cytoplasmic domain of the α-chain to the sequence coding for the C-terminally-truncated β chain (β_{ct}). This modification was introduced using the PCR as described (25). The templates used were pSVL vectors containing α and β cDNA as described previously (26). These vectors were linearized at *PstI* and *NdeI* sites, respectively. Two separate reactions were carried out. One included an upstream primer preceding the insert, an antisense mutagenic oligonucleotide, and the β template; the other included a downstream primer after the insert, a sense mutagenic oligonucleotide, and the α template. The mutagenic oligonucleotide contains the last 21 bases coding for the fourth transmembrane domain of β-chain and the first 21 bases coding for the cytoplasmic domain of the α-chain. After the products were purified, a third reaction was performed in order to reconstitute the entire construct. The mutated cDNA was inserted into the pSVL expression vector and verified by sequencing the DNA. The β_{-c} mutant, which lacks all of the C-terminal cytoplasmic tail except for arginine (residue 202), was also prepared by the PCR method. The mutagenic oligonucleotide contains the last 20 bases coding for the fourth transmembrane domain of the β-chain and a stop codon following that for the arginine residue. A *Bam*HI site was also incorporated and used for inserting the cDNA into pSVL.

Transfection in COS-7 cells. After harvesting by trypsinization, cells were washed twice in ice-cold PBS (pH 7.2), and resuspended at 1×10^7 cells/ml in a phosphate-buffered sucrose solution (272 mM sucrose, 7 mM sodium phosphate, and 1 mM $MgCl_2$). A total of 60 μg of various combinations of mutagenic constructs and native cDNA was used for 8×10^6 cells. The cell suspension was incubated on ice for 10 min in Gene Pulser cuvettes (BioRad, Richmond, CA), and transfected at settings of 380 V, and 25 μF, in an electroporation apparatus (BioRad). Transfected cells were further incubated on ice for 10 min, and then cultured in complete medium. Cells were assayed about 48 h after transfection.

Flow cytometric analysis and sorting of transfectants. Transfected COS-7 cells were incubated with FITC-IgE monomers or oligomers at 4°C for 60 min, washed three times in Hepes-buffered HBSS containing 1% BSA. The percentage of cells expressing wild-type or mutant IgE receptors was assessed by using an Epics 752 flow cytometer (Epics Division of Coulter, Hialeah, FL) modified with a Cicero interface and software (Catalyss Corporation, Englewood, CO). Cell aggregates and dead cells were eliminated from the analysis by using bit maps of forward and 90° light scatter, and the cells were counted based on forward light scatter. Unlabeled transfected cells were used to correct for background- and auto-fluorescence. At least 10,000 cells were counted to obtain each fluorescence profile. The percentage of transfected cells binding IgE was then calculated by subtracting the background from the experimental fluorescence profile. The remaining cells were then sorted for the population with fluorescence intensity above background (Fig. 2), and maintained at 4°C. The absolute number of IgE bound per cell was estimated by calibrating the flow cytometer with fluoresceinated bead standards (Flow Cytometry Standards, Research Triangle Park; NC).

Measurement of translational diffusion by FPR. FPR quantifies translational diffusion by measuring the recovery of fluorescence after partial bleaching of a small area on an otherwise uniformly-labeled cell surface (27, 28). The recovery curve contains information about the D_{tat} of the labeled molecules, and about the fraction of the population of labeled molecules, R, which is free to diffuse on a time scale of minutes. Calculations and control experiments indicate that the measuring and bleaching beams do not cause detectable cross-linking of, or damage to, the low concentrations of molecules of interest in an FPR experiment (29, 30).

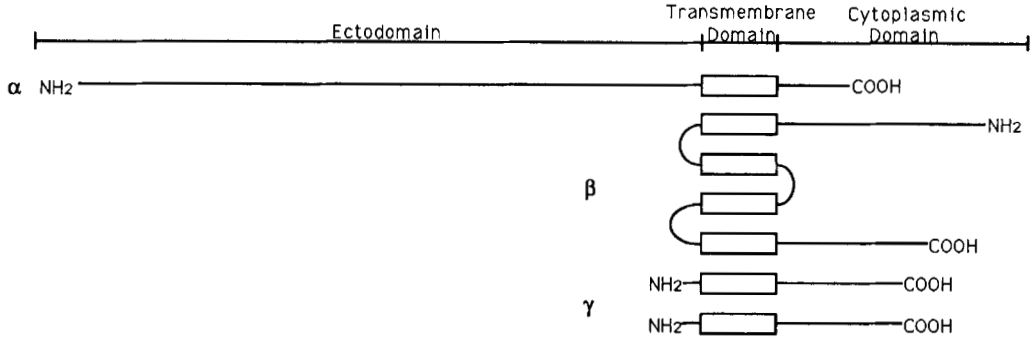
In our experiments, the FcεRI on cells were labeled with FITC-IgE monomer or oligomer for 60 min at 4°C, washed three times in HEPES-buffered HBSS containing 1% BSA. COS-7 transfectants were sorted for positive cells as described in the section on flow cytometric analysis. FPR measurements were made on cells in suspension, and at ambient temperature (19°C). To avoid internalization of receptor/IgE complexes, no sample was examined for more than 20 min. The FPR instrument used has been described elsewhere (31).

Assessment of internalization of surface-bound [^{125}I]IgE-DNP-BSA complexes. Cells in suspension (4 to 8×10^7/ml for COS-7 transfectants, and 2×10^7/ml for the other cell types) were incubated at 4°C for 1 h with 10 μg/ml of ^{125}I-labeled mouse anti-DNP IgE in the presence of 1 mg/ml normal rabbit IgG. After an additional two washes, cells were further incubated (4°C, 1 h) with DNP-BSA (5 μg/ml). The twice-washed cells were resuspended in 0.4 ml of cold medium and added to 4 ml of prewarmed medium (37°C) so that the final concentrations were 2×10^6 cells/ml for RBL, PT18, and P815 (clone 6) cells, and 4 to 8×10^6 cells/ml for COS-7 cells. One-milliliter aliquots of the cell suspension were sampled at the indicated time points and injected into ice-cold PBS to terminate internalization. The cells were centrifuged at $200 \times g$ for 5 min, washed once with cold PBS, and then incubated with 0.12 ml of 5 mM DNP-lysine (pH 8.0) for 5 min to promote dissociation of the DNP-BSA molecules from the cell-bound IgE. This was followed by the addition of 0.18 ml of 10 mM glycine-HCl buffer (pH 2.3), which reduced the pH to 2.9, for an additional 2.5-min incubation to allow dissociation of surface-bound IgE. The treatment was terminated by addition of 0.1 ml of medium containing 5 μg/ml IgE (pH adjusted to 9.0) to neutralize the pH in the presence of a large excess of unlabeled IgE. The cells were then pelleted through a phthalate oil mixture. The percentage of radioactivity that remained cell associated was considered to have been internalized (32). Cells that reacted only with [^{125}I]IgE were processed in parallel to obtain the background. The net percentage of internalization stimulated by Ag was then calculated by subtracting the background.

In the experiments in which the effect of cytochalasin D on internalization was examined, the incubation conditions were modified slightly to keep the cells at 37°C throughout. Cells were first loaded with [^{125}I]IgE by incubation at 37°C for 1 h, washed, and then incubated with cytochalasin D at 10^{-5} M for 30 min at 37°C or with carrier solvent (0.1% ethanol) alone (33). Stimulation of internalization was started by addition of 0.4 ml cell suspension to 4 ml of prewarmed medium (37°C) with DNP-BSA (1 μg/ml final concentration) in the presence of 10^{-5} M cytochalasin D. Cells were then sampled and assessed for internalization as above. Cells loaded with [^{125}I]IgE were processed in parallel in the absence of DNP-BSA to obtain the background. No difference in the net percentage of internalized ligand measured by the two different protocols was found for COS-7 cells. However, enhanced internalization was found for the other cell types by using the latter protocol.

RESULTS

Flow cytometric analysis of COS-7 cells transfected with FcεRI. It has been reported that COS-7 cells transfected with FcεRI express about 10^6 receptors/cell (26). Figure 2 shows representative flow cytometric profiles of



α WLQ-LIFPSLAVILFAVDTGLWFSTH-KQFESILKIQKTGKGKKKGCOOH

αCt WLQ-LIFPSLAVILFAVDTGLWFSTH-KQFE

βN LTS-CVVTGFCLCILGVLVQTVGLF-ELEKKLFSQWTQQPPPSAPKEPLAKAPPSAELLEIDPASPSEQPNPLALDARSKNETDMNH2

βNt LTS-CVVTGFCLCILGVLVQTVGLF-EM

βC ITE-LVLMLLFLTILAFCSAVLLI-YRIGQEFERSKVPDDRLYEELHVYSPIYSALEDTREASAPVVSCOOH

βCt ITE-LVLMLLFLTILAFCSAVLLI-YRIGQ

β-C ITE-LVLMLLFLTILAFCSAVLLI-R

βαC ITE-LVLMLLFLTILAFCSAVLLI-YRQFESILKIQKTGKGKKKGCOOH

γ NH2LGEPQ-LCYILDAILFLYGIVLTLLYC-RLKIQVRKADIASREKSDAVYTGLNTRNQETYETLKCOOH

γCt NH2LGEPQ-LCYILDAILFLYGIVLTLLYC-RLKI

Figure 1. The α, β, and γ subunits of IgE receptor consist of three topologic domains. The topographic orientation of the linear amino acid sequences are diagrammed to scale. The boxed areas indicate the hydrophobic residues predicted to span the lipid bilayer. To the right of these hydrophobic domains are residues exposed to the cytoplasm. The amino acid sequences of wild-type, truncated, and chimeric proteins are displayed below the diagram. The *arrow* indicates where the sequences of the parent molecules are joined in the chimeric protein.

TABLE I

Mutated subunits with truncated CD

Serial No.	Description	Abbreviation	Reference
EdpC 14	Terminates α after Glu 207; eliminates 15 of 20 residues of CD	α_{Ct}	12
NAD8028900	Initiates β at position equivalent to Leu-58 in wild type; eliminates 57 of 59 residues in NH_2-terminal CD	β_{Nt}	12
NAD5108901	Terminates β after Gln 205; eliminates 38 residues of 43 in COOH-terminal CD	β_{Ct}	12
NAD6048900	Initiates β at position equivalent to Leu-58 in wild type and terminates β after Gln 205; combination of defects in mutants β_{Nt} and β_{Ct}	β_{NCt}	12
SYM032790	Terminates β after Tyr 201 with Tyr → Arg substitution; eliminates 42 residues of 43 in COOH-terminal CD.	β_{-C}	This paper
SYM101989	Joins Arg 202 of β to Gln 205 of α; chimeric β with CD of α in place of COOH-terminal CD	$\beta_{\alpha C}$	This paper
NAD5108900	Terminates γ after Ile 30; eliminates 32 of 36 residues of CD	γ_{Ct}	12

populations of COS-7 cells transfected with either wild-type or mutant FcεRI molecules. In the fluorescence microscope, the positive cells appear brightly ring-stained by FITC-IgE monomers (data not shown). Different mutants were expressed to different extents both in terms of the fraction of positive cells and in the number of receptors per cell. The fractions of transfected cells binding fluorescent IgE are summarized in Table II. The mean number of receptors in the positive cells was estimated by calibrating the flow cytometer with fluorescent beads. These values differed by no more than threefold between the transfectants. The number of receptors correlated well with the percent of positive cells as judged by flow cytometry (correlation coefficient = 0.8, data not shown), and with the percent of transfectants that bound IgE-conjugated E (12).

The surface expression of the mutant receptor $\alpha\beta_{-C}\gamma$ was assessed by rosetting assay, and the fraction of positive cells was comparable to that of wild-type receptor (S.-Y. Mao, unpublished observations).

Translational diffusion of FcεRI molecules in transfectants expressing different truncation phenotypes. We measured the translational diffusion of the wild-type and 13 different mutant receptors in transfected cells. Figure 3 shows the mobile fractions for RBL and several COS-7 transfectants labeled either with FITC-IgE monomers or oligomers. The means of the D_{lat} and R measured on multiple samples in several different experiments are summarized in Table II. The values of both D_{lat} and R for monomeric wild-type FcεRI in COS-7 cells are similar to

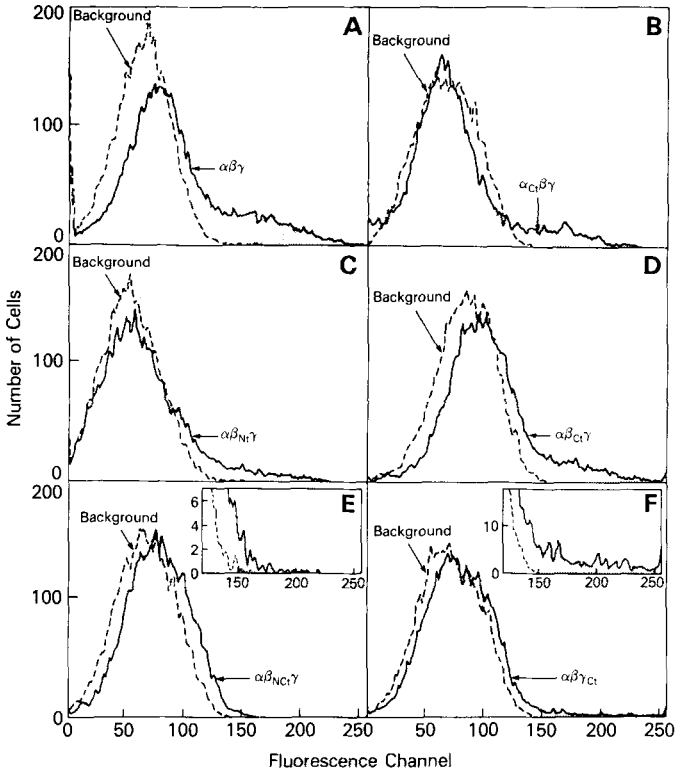


Figure 2. Flow cytometric analysis of COS-7 transfectants expressing either wild-type or mutant IgE receptor molecules. Cells were stained with FITC-IgE, and unlabeled transfected cells were used to correct for the background. A difference in 25 channels corresponds to a twofold difference in fluorescence. Cells were then sorted for the population with a fluorescence intensity above background (*shaded area*) for FPR measurements.

the values for the endogenous FcεRI in RBL cells (Table II) (34, 35). As noted previously for RBL cells (34), addition of oligomers reduced R substantially. This is thought to reflect an increase in the fraction of receptors that are immobile (34, 36). Transfected wild-type receptors were immobilized by IgE oligomers to the same extent as endogenous receptors on RBL cells. The fraction of the oligomer-bound FcεRI that remained mobile diffused freely at a rate close to that of monomeric FcεRI. It is likely that some of the cellbound IgE oligomers are not bound multivalently (37), and this could account for the fraction that remains mobile.

As can be seen from Table II, removal of the cytoplasmic domains did not affect D_{lat} of the receptors reacted either with monomeric or oligomeric IgE. Similarly, for most of the mutants, the fractions of mutant receptors containing a single truncated cytoplasmic domain that had restricted mobility before aggregation, and that became immobilized after reaction with the oligomers IgE, were the same as for the wild-type receptors. Truncation of the C-terminal domain of the β-chain, created a partial defect in immobilization. Receptors containing a truncated β C-terminal domain as well as additional truncations in the α and/or γ CD ($\alpha\beta_{NCt}\gamma$, $\alpha_{Ct}\beta_{Ct}\gamma$, $\alpha\beta_{Ct}\gamma_{Ct}$, $\alpha_{Ct}\beta_{Ct}\gamma_{Ct}$) were immobilized to the same extent as $\alpha\beta_{Ct}\gamma$ (noted in bold type in Table II).

To test the specificity of the effect seen with the truncated C-terminal domain of the β-chain, a chimeric mutant was prepared, in which the C-terminal domain of β was replaced with that of the α-chain. Absence of the latter had already been shown not to affect immobilization of the receptor (Table II: R = 33% for the transfectant containing mutant α_{Ct} and R = 34% for the wild type). The chimeric mutant, $\alpha\beta\alpha_{Ct}\gamma$, was immobilized by IgE oligomers as well as the native receptor (Table II).

We also examined the mobility of receptors containing the *human* α chain which can be expressed by co-transfection with γ-chains alone, i.e., without β-chains (8). These receptors were also immobilized normally by IgE oligomers.

Internalization of IgE/receptor complexes triggered by DNP-BSA. To determine whether the COS-7 cells transfected with FcεRI can internalize these receptors after aggregation, we examined cells whose receptors had been saturated with [^{125}I]IgE before incubation with DNP_{25}-BSA. As shown in Figure 4, IgE/FcεRI complexes were internalized when aggregated by the multivalent Ag. After 60 min at 37°C, 36% of the cell-associated IgE could no longer be removed by exposure to acid pH. However, the internalization by the COS-7 transfectants was relatively inefficient when compared with RBL cells,

TABLE II

Surface expression and translational diffusion of wild-type and mutant IgE receptors

FcR Phenotype	% Positive[a] Cells	D_{lat} (10^{-10} cm^2/sec)		Mobile Fraction (×100)[b]	
		Monomer	Oligomer	Monomer	Oligomer
RBL	100	2.7	4.8	59 ± 14 (118)	33 ± 11 (99)
COS($\alpha\beta\gamma$)	20.9 ± 6.9 (7)	1.2	1.6	64 ± 14 (75)	34 ± 10 (78)
COS($\alpha_{Ct}\beta\gamma$)	14.5 (2)	1.0	2.0	62 ± 13 (42)	33 ± 11 (49)
COS($\alpha\beta_{Nt}\gamma$)	12.7 (2)	1.0	1.8	70 ± 14 (44)	35 ± 11 (63)
COS($\alpha\beta_{Ct}\gamma$)	18.5 (2)	1.3	1.7	65 ± 17 (57)	**46 ± 15** (100)[c]
COS($\alpha\beta_{NCt}\gamma$)	11.4 ± 3.4 (6)	2.2	2.4	60 ± 14 (54)	**51 ± 14** (73)
COS($\alpha\beta\gamma_{Ct}$)	9.7 (2)	1.5	2.3	56 ± 12 (24)	34 ± 10 (49)
COS($\alpha_{Ct}\beta_{Ct}\gamma$)	8.5	1.7	1.7	59 ± 10 (18)	**41 ± 13** (33)
COS($\alpha_{Ct}\beta\gamma_{Ct}$)	8.7 (2)	1.2	1.5	60 ± 10 (35)	34 ± 9 (45)
COS($\alpha\beta_{Ct}\gamma_{Ct}$)	8.1 (2)	1.3	1.5	57 ± 14 (52)	**45 ± 16** (58)
COS($\alpha_{Ct}\beta_{Nt}\gamma_{Ct}$)	12.8 (3)	2.7	4.2	58 ± 13 (25)	36 ± 9 (22)
COS($\alpha_{Ct}\beta_{Ct}\gamma_{Ct}$)	8.2	1.0	1.2	64 ± 13 (15)	**42 ± 16** (35)
COS($\alpha\beta_{ac}\gamma$)	18.5 ± 3.5 (6)	1.1	1.5	58 ± 15 (53)	33 ± 13 (75)
COS($\alpha_H\gamma_R$)	13.7	1.0	2.4	64 ± 13 (33)	34 ± 12 (49)
COS($\alpha_H(\beta\gamma)_R$)	9.9	1.5	2.5	59 ± 12 (27)	30 ± 9 (30)
COS($\alpha_H(\beta_{NCt}\gamma)_R$)	10.0	1.1	1.2	61 ± 9 (13)	40 ± 19 (6)

[a] The percentage of cells expressing wild-type or mutant IgE receptors was assessed as described in *Materials and Methods*. The number of experiments are indicated in parentheses.

[b] Cells were labeled with either FITC-IgE monomers or oligomers, sorted (COS-7 cells), and maintained at 4°C. FPR measurements were made at ambient temperature (19°C). Data are reported as mean ± SD. The total number of cells measured from multiple samples in several different experiments is indicated in parentheses.

[c] The highlighted values indicate defects in immobilization created by mutations in the receptors.

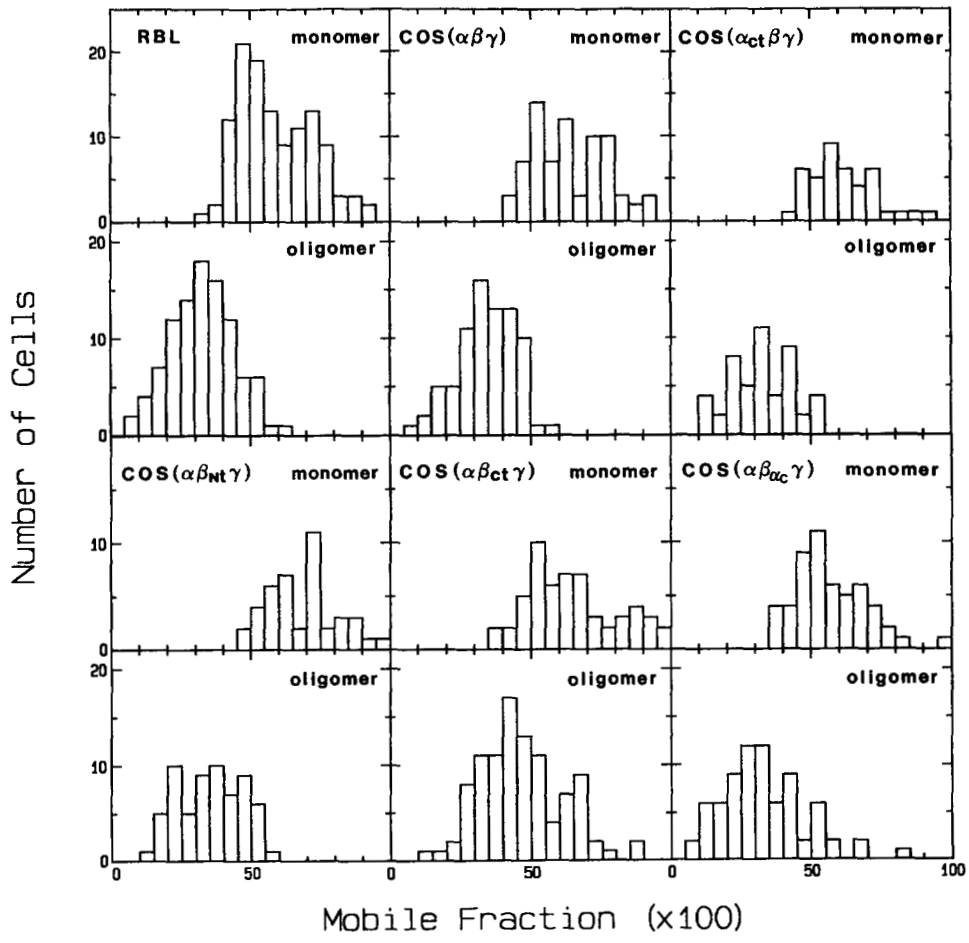


Figure 3. Distribution of values for the R from FPR measurements on RBL and COS-7 transfectants expressing either wild-type or mutant IgE receptors. Cells were labeled either with FITC-IgE monomers or FITC-IgE oligomers as indicated, and COS-7 transfectants were sorted for positive cells. FPR measurements were made at ambient temperature (19°C).

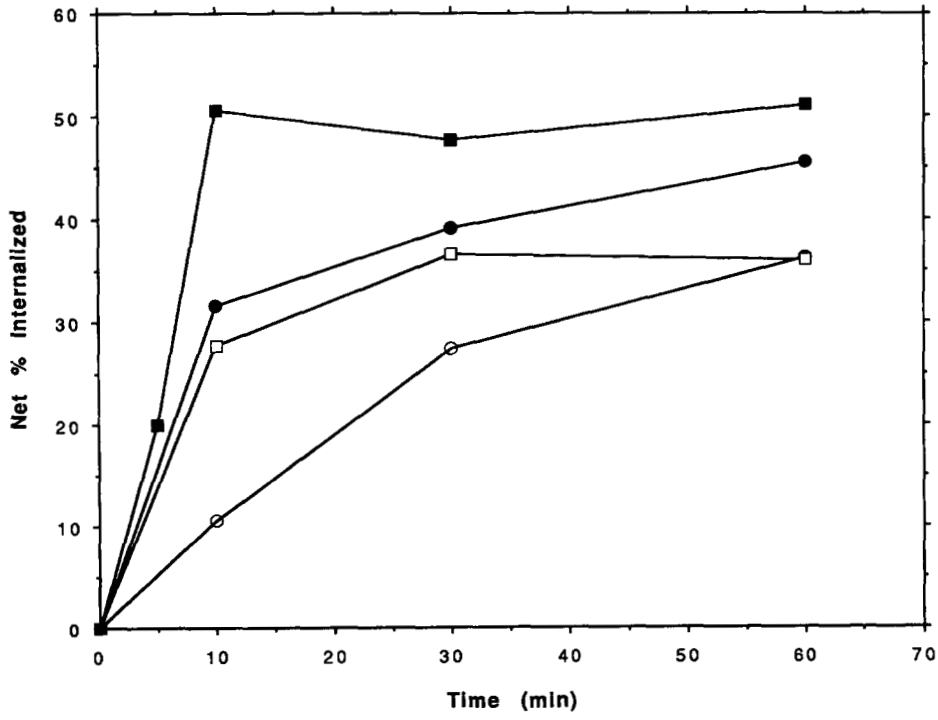


Figure 4. Internalization of ^{125}I-labeled mouse anti-DNP IgE/DNP-BSA complexes in RBL (■), PT18 (●) containing endogenous FcεRI, and in P815 (clone 6) (□), COS($\alpha\beta\gamma$) (○) containing transfected wild-type FcεRI. Cells (2×10^7 cells/ml for RBL and PT18, 4 to 8×10^7 cells/ml for COS-7 cells) were incubated (4°C, 1 hr) with 10 μg/ml of [^{125}I]IgE anti-DNP in the presence of 1 mg/ml normal rabbit IgG. The twice-washed cells were further incubated (4°C, 1 hr) with DNP-BSA (5 μg/ml). The cells were washed two more times and then resuspended in warm (37°C) medium to yield final concentrations of 2×10^6 cells/ml for RBL and PT18, and of 4 to 8×10^6 cells/ml for COS-7 cells. They were sampled at the indicated time points to assess internalization. P815 (clone 6) was labeled with [^{125}I]IgE at 37°C for 60 min, and the internalization was stimulated by adding twice-washed cells into warm medium (37°C) containing 1 μg/ml DNP-BSA. The concentrations of [^{125}I]IgE and cells were the same as those used for the RBL cells.

in which about 50% of the cell-associated IgE was internalized in 10 min with a $t_{1/2}$ of 5 min. Similarly, PT18 mouse mast cells internalized aggregated immune complexes efficiently with a rate comparable with that of RBL (Fig. 4). The P815 cells transfected with wild-type IgE receptor (clone 6) internalized the aggregated receptor complexes almost as effectively as RBL, providing that the IgE binding step was carried out at 37°C. Monomeric IgE remained largely exposed on the cell surface of each cell type; only 3% was resistant to acid stripping at 60

min in PT18, 7% in P815 (clone 6), 10% in RBL, and 14% to 27% in COS-7 transfectants. These values were subtracted from the values obtained after addition of Ag. The net values are summarized in Table III. The relative efficiency of internalization can be estimated from the ratio of % internalized at 10 min and 60 min, and is shown in the last column of Table III.

The same assay was carried out for various mutant receptors to assess the possible role of the cytoplasmic domains on the rate and extent of internalization. The percentage of internalized IgE/FcεRI complexes after 60 min at 37°C ranged from 21% to 37% for various mutants. Truncation of the C-terminal domain of the β-chain appeared to reduce somewhat the extent of internalization; in this assay the chimeric mutant $\alpha\beta\alpha_c\gamma$ was internalized least efficiently. The differences between these mutants are more apparent when the initial rates of internalization are compared (Fig. 5). It can be seen that the double mutant $\alpha_{Ct}\beta_{Ct}\gamma$ has the lowest rate, and that the other three mutants lacking the C-terminal domain of the β-chain, $\alpha\beta_{Ct}\gamma$, $\alpha\beta_{-C}\gamma$, and $\alpha\beta\alpha_c\gamma$, also internalize with a much reduced rate. On the other hand, receptors consisting of the human α-chain and the rat γ-chain, i.e., without the β-chain, internalize as rapidly and completely as the rat wild-type receptor.

Effect of cytochalasin D on internalization. Aggregation of IgE/FcεRI complexes at the cell surface either by Ag or by anti-IgE appears to promote an association of the receptor with actin (38). In order to further characterize the internalization observed in COS-7 transfectants, cytochalasin D, an inhibitor of actin polymerization, was used to assess the effects of such inhibition on the internalization induced by Ag. For both endogenous and transfected receptors, inhibition of internalization was observed as shown in Table IV. Internalization was more effectively inhibited in RBL cells (86%) than in COS-7 cells (48% inhibition at 60 min). The inhibitory effect of cytochalasin D on the *rate* of internalization was marked in the case of RBL cells, in agreement with previous results (33), whereas little effect was observed on the *rate* of internalization in COS-7 cells. For the mutant receptors, the extent but not the rate of internalization was also greatly reduced by cytochalasin D (Table IV).

TABLE III

Internalization of wild-type and mutant receptors induced by Ag

Cell	FcR Phenotype	% Internalized[a] at 60 min	Relative[b] Internalization
RBL	$\alpha\beta\gamma$ (E)[c]	51 (3)[d]	100
RBL	$\alpha\beta\gamma$ (E)	68 (3)[e]	92
PT18	$\alpha\beta\gamma$ (E)	46 (1)[d]	69
PT18	$\alpha\beta\gamma$ (E)	41 (3)[e]	100
P815 (clone 6)	$\alpha\beta\gamma$ (T)	0.2 (1)[d]	
P815 (clone 6)	$\alpha\beta\gamma$ (T)	36 (2)[e]	77
COS	$\alpha\beta\gamma$ (T)	36 (3)[d]	29
COS	$\alpha_{Ct}\beta\gamma$ (T)	28 (5)	24
COS	$\alpha\beta_{Nt}\gamma$ (T)	32 (2)	42
COS	$\alpha\beta_{Ct}\gamma$ (T)	28 (2)	15
COS	$\alpha\beta_{-C}\gamma$ (T)	37 (1)	16
COS	$\alpha\beta\gamma_{Ct}$ (T)	30 (4)	39
COS	$\alpha_{Ct}\beta_{Ct}\gamma$ (T)	25 (3)	7
COS	$\alpha\beta_{mc}\gamma$ (T)	21 (2)	15
COS	$\alpha_H\gamma_R$ (T)	31 (3)	25

[a] Percentage of the total cellbound [^{125}I]IgE that is internalized at 60 min after stimulation.

[b] Relative efficiency of internalization was estimated from the ratio of the percentage internalized at 10 min and 60 min.

[c] E and T refer to endogenous or transfected, respectively.

[d] Cells were labeled with [^{125}I]IgE followed by binding of DNP-BSA at 4°C for 60 min in each step. Internalization was stimulated by adding cells into medium prewarmed to 37°C. Data reported are the mean, with the number of experiments indicated in parentheses. All of the COS-7 transfectants were assayed by using this protocol.

[e] Cells were labeled with [^{125}I]IgE at 37°C for 60 min and internalization was induced by subsequent addition of medium containing DNP-BSA at 37°C.

DISCUSSION

The lateral diffusion coefficients measured for membrane proteins range from 10^{-11} to 5×10^{-9} cm^2/s with values for many proteins averaging about 2×10^{-10} cm^2/s (39). This value is much lower than that expected on the basis of free diffusion in a lipid bilayer (40). It is plausible that membrane-spanning proteins interact with the cytoskeleton via their cytoplasmic domains and that this accounts for their restricted lateral mobility (41). Results of studies on class II MHC molecules transfected into B lymphoma cells are consistent with such a mechanism: they showed that when the cytoplasmic domains were truncated the lateral mobility increased (42). On the other hand, truncation mutants of class I MHC Ag, epidermal growth factor receptors, and vesicular stomatitis virus glycoprotein exhibited no such increased diffusion (43–45). Possibly these proteins interact with cytoskeletal components via their transmembrane domains (for review, see Reference 39). It is also possible that the interactions with the cytoskeleton are mediated—directly or indirectly—by the external domains. For example under-glycosylated class I MHC molecules in transfected Chinese hamster ovary cells show an enhanced lateral diffusion (46). Furthermore, such external interactions would account for the finding that steroylated dextrans incorporated into 3T3 cells show a restrained mobility much like many integral membrane proteins (47, 48). Direct evidence for interactions between the ectodomains of plasma membrane proteins has recently been demonstrated for the IL-6R: gp130 complex (49).

The lateral mobility of IgE receptors on RBL-2H3 cells has been measured with FPR by several investigators (30, 34–36, 50) and yielded values of 2 to 3×10^{-10} cm^2/sec for the lateral diffusion constant, and of 64% to 88% for the mobile fraction, for the non-cross-linked receptors. We observed similar values for D_{lat} and the R in COS-7 cells transfected with wild-type receptor cDNA derived from RBL-2H3 cells (Table II). We also studied mutant FcεRI containing one or more truncated cytoplasmic domains, and found that, in general, the absence of these domains enhanced the lateral mobility of the unaggregated monomeric receptor little, or not at all (Table II).

When the IgE receptors in RBL-2H3 cells are cross-linked, the cells undergo a series of rapid membrane and cytoskeletal changes (reviewed by Oliver et al. (10)), including stimulated assembly of F-actin, transformation of the surface from a microvillous to lamelar topography, increased cortical stiffness (51), and increased fluid pinocytosis. The aggregated IgE receptors themselves are immobilized within a few minutes upon cross-linking (35), the mobile fraction decreasing sharply to 30%. This rapid immobilization has been shown to correlate well with the subsequent secretory events (34, 35).

The immobilization occurs at 4°C and in the presence of cytochalasin, suggesting that it is not dependent on assembly of F-actin (35). Nevertheless, interaction with

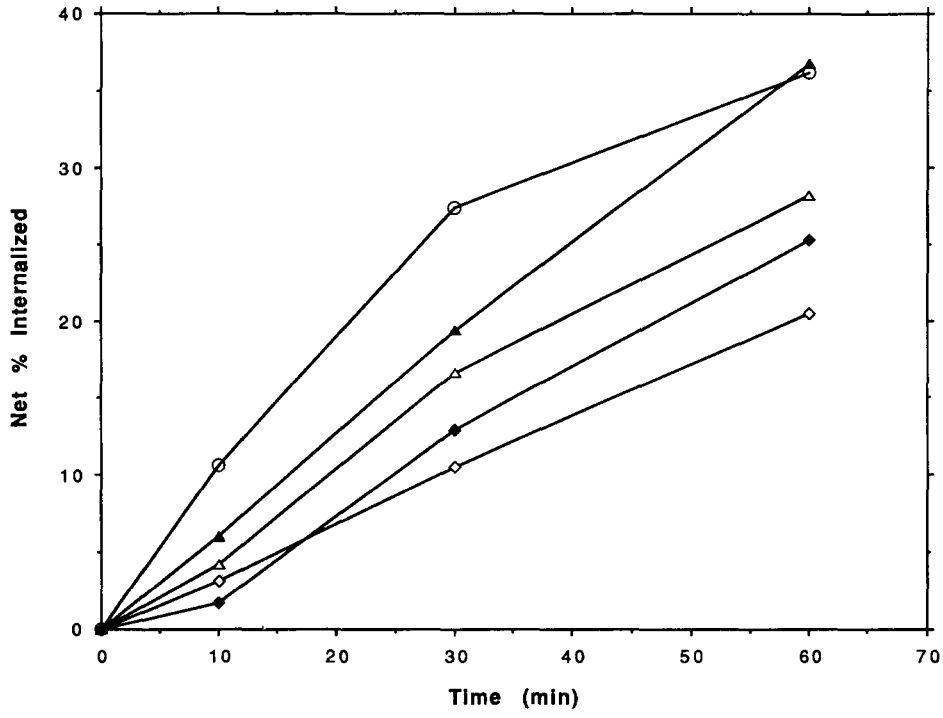


Figure 5. Effect of mutation in the β-chain C-terminal domain on the internalization of ^{125}I-labeled mouse anti-DNP IgE/DNP-BSA complexes by COS-7 transfectants. COS-7 cells transfectants expressing $\alpha\beta\gamma$ (○), $\alpha\beta_{ct}\gamma$ (△), $\alpha\beta_{-c}\gamma$ (▲), $\alpha\beta\alpha_c\gamma$ (◇), and the double mutant $\alpha_{ct}\beta_{ct}\gamma$ (◆) were assayed for internalization as described in Figure 4.

TABLE IV

Effect of cytochalasin D on internalization induced by Ag

FcR Phenotype	% Inhibition[a]		
	10 min	30 min	60 min
RBL	97	87	86
COS($\alpha\beta\gamma$)	35	35	48
COS($\alpha_{Ct}\beta\gamma$)	0	38	49
COS($\alpha\beta_{Nt}\gamma$)	0	50	36
COS($\alpha\beta_{Ct}\gamma$)	0	100	71
COS($\alpha\beta_{-C}\gamma$)	78	93	88
COS($\alpha\beta\gamma_{Ct}$)	53	73	65
COS($\alpha_{Ct}\beta_{Ct}\gamma$)	0	72	71
COS($\alpha_H\gamma_R$)	68	57	69

[a] Cells were preincubated with cytochalasin D for 30 min before stimulation. Cells were assessed for internalization of IgE as described in *Materials and Methods*. Data are calculated for each point.

cytoskeletal elements is suggested by the finding that aggregated FcεRI rapidly become nonextractable by the detergent buffers commonly used to dissolve plasma membranes, and are found associated with the remaining cytoskeletal structures (38).

Here we have shown that none of the five cytoplasmic domains of the tetrameric rat IgE receptor is required for it to become immobilized as a result of aggregation. The one possible exception was the partial defect in receptor immobilization caused by truncation of the C-terminal domain of the β-chain (Table II). The significance of this partial effect was explored by studying the mutant in which this domain was replaced with the C-terminal domain of the α-chain, whose cytoplasmic domain had already been shown to be unnecessary for the aggregation-induced receptor immobilization. The receptors with the chimeric mutant β-chain were found to be normally immobilized by IgE oligomers. It is likely that the partial defect in immobilization observed for the C-terminally truncated β-chain was caused by indirect changes, and does not imply that this region itself contains a critical site for specific receptor-cytoskeletal interactions induced by aggregation of the receptor. Furthermore, chimeric receptors consisting of the human α-chain and rodent γ-chain—lacking a β-chain altogether—were also immobilized like the wild-type receptor. Thus it appears highly unlikely that the C-terminal cytoplasmic extension of the β-chain itself contains a specific region required for an interaction that would account for the immobilization.

In most cell types, endocytosis through coated pits is the major route by which cell surface proteins are internalized and this process is selective and efficient. It has been proposed that a component of the coated pit binds a specific site on proteins destined to be internalized (3, 52); possibly this may require or be enhanced by aggregation of the surface proteins (53, 54). Recent evidence implicates the cytoplasmic domains of cell surface receptors in this rapid internalization. Mutations in the cytoplasmic domains of receptors for low density lipoprotein (52), for transferrin (55, 56), for poly-Ig (57), for mannose-6-phosphate (58), and for IgG (59) markedly inhibited the spontaneous or stimulated clustering of these receptors in coated pits, and prevented high efficiency endocytosis. Tyrosine residues in the cytoplasmic domains of the receptors for low density lipoproteins, transferrin, poly-Ig, and mannose-6-phosphate have been identified to be especially critical components of the recognition site that is required for high efficiency endocytosis. Indeed, when a particular cysteine residue in the cytoplasmic extension is substituted by tyrosine, influenza hemagglutinin is internalized via coated pits, whereas the wild-type hemagglutinin is not (60). Vega and Strominger (61) have compared the cytoplasmic regions of a panel of proteins endocytosed via coated pits, and proposed that a structural feature shared among these molecules may be involved.

The endocytosis of IgE receptor in RBL-2H3 has been extensively investigated (32, 33, 62–67). Ag cross-linked IgE receptor complexes are internalized effectively (50% to 70%), with a $t_{1/2}$ of about 5 min (cf. Fig. 4) and Oliver et al. (68) have shown by electron microscopy, that this

rapid internalization of FcεRI is also via coated pit/coated vesicles.

In this study we examined wild-type FcεRI in two cell lines containing endogenous receptors (RBL and PT 18) and in two types of transfected cells: transiently transfected COS cells and a clone of P815 cells permanently transfected with wild-type FcεRI (20). Monomeric unaggregated transfected IgE receptors were constitutively internalized only slowly, albeit on the COS cells the rate was about twofold greater than that observed with the other cells. In all cells, the endocytosis was markedly stimulated by aggregation of the IgE-FcεRI by Ag and this stimulated endocytosis was inhibited by cytochalasin D, or by a combination of the metabolic inhibitors NaN_3 and 2-deoxyglucose (S.-Y. Mao, unpublished observations). However, the COS-7 cells internalized the (transfected) receptors about five times more slowly than the other cells we examined.

We also examined mutated FcεRI although because of the difficulty in obtaining permanently transfected lines, such mutants have so far been examined only in transiently transfected COS-7 cells. We found that removal of the various cytoplasmic domains of the IgE receptor led at most to only modest decreases in either the constitutive or aggregation-induced internalization relative to the wild-type transfected receptor. The mutants containing a β-chain with a truncated C terminus again showed the most substantial functional changes (Table III). In this instance, substituting the cytoplasmic domain of the β-chain with that of the α-chain did not restore the wild-type phenotype, but the transfectant containing a β-chain-less receptor consisting of a human α-chain and a rodent γ-chain internalized as efficiently as the wild-type receptor. This again makes it doubtful that the C-terminal cytoplasmic domain of the β-chain contains a critically important site that is required for this type of stimulated endocytosis. It suggests that the functional changes induced by modification of this part of the receptor are caused by indirect perturbations.

Our results might at first appear to be in conflict with the reported role of the cytoplasmic domains in endocytosis of a variety of other receptors (above) and of the FcγR in particular. Thus, Miettinen et al. have shown that the two isoforms of the low affinity FcR for IgG (FcγRII) exhibit distinct differences in localization to coated pits as a result of cytoplasmic domain heterogeneity (59). The FcγRII-B1 isoform, which has an in-frame insertion that increases the length of the cytoplasmic domain from 47 to 94 amino acids, and a tail-less mutant, were found, respectively, to accumulate nearly 100-fold and 10-fold less in coated pits than the FcγRII-B2 isoform as revealed by electron microscopy. However, in biochemical assays, the differences in the rates of endocytosis between these FcγRII isoforms were similar to the differences we observed between the endogenous FcεRI in RBL cells (or the exogenous receptors in the transfected P815 cells) and the transfected wild-type FcεRI in COS cells. In the study by Miettinen et al. (59), the FcγRII variants that were endocytosed less efficiently appeared to be internalized in phagocytic-like vacuoles, possibly accounting for the slowed but eventual degradation of the immune complexes. The slower Ag-induced internalization of FcεRI in COS cells could also be via a route that is independent of coated pits and similar to the slow process of internalization observed with FcγRII.

Our preliminary data on the P815 mouse mastcytoma cell line stably transfected with wild-type IgE receptors (20) (Fig. 4) suggest that, on these cells, FcεRI is endocytosed as efficiently as on RBL cells, and may, therefore, involve coated pits. For this reason, and because the P815 cells exhibit some of the normal biochemical changes in response to receptor aggregation (20), whereas the COS cells do not (L. Miller, and C. Fewtrell, unpublished observations) we plan to study endocytosis of FcεRI on P815 cells transfected with mutant receptors, as such transfectants become available.

Acknowledgments. We thank Taiyin Wei and George Poy for technical assistance.

REFERENCES

1. **Nagafuchi, A., and M. Takeichi.** 1989. Transmembrane control of cadherin-mediated cell adhesion: a 94 kDa protein functionally associated with a specific region of the cytoplasmic domain of E-cadherin. *Cell Reg. 1:37.*
2. **Ozawa, M., H. Baribault, and R. Kemler.** 1989. The cytoplasmic domain of the cell adhesion molecule uvomorulin associates with three independent proteins structurally related in different species. *EMBO. J. 8:1711.*
3. **Pearse, B. M.** 1988. Receptors compete for adaptors found in plasma membrane coated pits. *EMBO. J. 7:3331.*
4. **Kinet, J.-P., and H. Metzger.** 1990. Genes, structure and actions of the high affinity Fc receptor for IgE. In *Fc receptors and the Action of Antibodies.* H. Metzger, ed. American Society of Microbiology, Washington, DC, p. 239.
5. **IUIS Nomenclature Committee** 1990. Nomenclature of Fc Receptors. *WHO Bull. 67:449.*
6. **Kinet, J.-P., H. Metzger, J. Hakimi, and J. Kochan.** 1987. A cDNA presumptively coding for the alpha subunit of the receptor with high affinity for immunoglobulin E. *Biochemistry 26:4605.*
7. **Shimizu, A., I. Tepler, P. N. Benfey, E. H. Berenstein, R. P. Siraganian, and P. Leder.** 1988. Human and rat mast cell high-affinity immunoglobulin E receptors: characterization of putative alpha-chain gene products. *Proc. Natl. Acad. Sci. USA 85:1907.*
8. **Miller, L., U. Blank, H. Metzger, and J.-P. Kinet.** 1989. Expression of high-affinity binding of human immunoglobulin E by transfected cells. *Science 244:334.*
9. **Küster, H., H. Thompson, and J.-P. Kinet.** 1990. Characterization and expression of the gene for the human Fc receptor gamma subunit: definition of a new gene family. *J. Biol. Chem. 265:6448.*
10. **Oliver, J. M., J. Seagrave, R. F. Stump, J. R. Pfeiffer, and G. G. Deanin.** 1988. Signal transduction and cellular response in RBL-2H3 mast cells. *Prog. Allergy 42:185.*
11. **Beaven, M. A., and J. R. Cunha-Melo.** 1988. Membrane phosphoinositide-activated signals in mast cells and basophils. *Prog. Allergy 42:123.*
12. **Varin-Blank, N., and H. Metzger.** 1990. Surface expression of mutated subunits of the high affinity mast cell receptor for IgE. *J. Biol. Chem. 265:15685.*
13. **Plotz, P. H., R. P. Kimberly, R. L. Guyer, and D. M. Segal.** 1979. Stable model immune complexes produced by bivalent affinity labeling haptens: in-vivo survival. *Mol. Immunol. 16:721.*
14. **Isersky, C., A. Kulczycki, Jr., and H. Metzger.** 1974. Isolation of IgE from reaginic rat serum. *J. Immunol. 112:1909.*
15. **Liu, F. T., J. W. Bohn, E. L. Ferry, H. Yamamoto, C. A. Molinaro, L. A. Sherman, N. R. Klinman, and D. H. Katz.** 1980. Monoclonal dinitrophenyl-specific murine IgE antibody: preparation, isolation, and characterization. *J. Immunol. 124:2728.*
16. **Holowka, D., and H. Metzger.** 1982. Further characterization of the beta-component of the receptor for immunoglobulin E. *Mol. Immunol. 19:219.*
17. **McConahey, P. J., and F. J. Dixon.** 1966. A method of trace iodination of proteins for immunologic studies. *Int. Arch. Allergy. Appl. Immunol. 29:185.*
18. **Barsumian, E. L., C. Isersky, M. G. Petrino, and R. P. Siraganian.** 1981. IgE-induced histamine release from rat basophilic leukemia cell lines: isolation of releasing and nonreleasing clones. *Eur. J. Immunol. 11:317.*
19. **Pluznik, D. H., N. S. Tare, M. M. Zatz, and A. L. Goldstein.** 1982. A mast/basophil cell line dependent on colony stimulating factor. *Exp. Hematol. 10(Suppl. 12):211.*
20. **Miller, L., G. Alber, N. Varin-Blank, R. Ludowyke, and H. Metzger.** 1990. Transmembrane signalling in P815 mastocytoma cells by transfected IgE receptors. *J. Biol. Chem. 265:12444.*
21. **Menon, A. K., D. Holowka, and B. Baird.** 1984. Small oligomers of

immunoglobulin E (IgE) cause large-scale clustering of IgE receptors on the surface of rat basophilic leukemia cells. *J. Cell Biol. 98:577.*
22. **Segal, D. M., J. D. Taurog, and H. Metzger.** 1977. Dimeric immunoglobulin E serves as a unit signal for mast cell degranulation. *Proc. Natl. Acad. Sci. USA 74:2993.*
23. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature 227:680.*
24. **Schreiber, A. B., and J. Haimovich.** 1983. Quantitative fluorometric assay for detection and characterization of Fc receptors. *Methods Enzymol. 93:147.*
25. **Higuchi, R., B. Krummel, and R. K. Saiki.** 1988. A general method of in vitro preparation and specific mutagenesis of DNA fragments: study of protein and DNA interactions. *Nucleic. Acids. Res. 16:7351.*
26. **Blank, U., C. Ra, L. Miller, H. Metzger, and J.-P. Kinet.** 1989. Complete structure and expression in transfected cells of high affinity IgE receptor. *Nature 337:187.*
27. **Cherry, R. J.** 1979. Rotational and lateral diffusion of membrane proteins. *Biochim. Biophys. Acta 559:289.*
28. **Jacobson, K., E. Elson, D. Koppel, and W. Webb.** 1983. International workshop on the application of fluorescence photobleaching techniques to problems in cell biology. *Fed. Proc. 42:72.*
29. **Edidin, M.** 1990. Molecular associations and membrane domains. *Curr. Top. Membranes Transport. 36:81.*
30. **Wolf, D. E., M. Edidin, and P. R. Dragsten.** 1980. Effect of bleaching light on measurements of lateral diffusion in cell membranes by the fluorescence photobleaching recovery method. *Proc. Natl. Acad. Sci. USA 77:2043.*
31. **Wolf, D. E., and M. Edidin.** 1981. Diffusion and mobility of molecules in surface membranes. In *Techniques in Cellular Physiology.* P. Baker, ed. Elsevier, Amsterdam, *P.1/1, part 105:1.*
32. **Furuichi, K., J. Rivera, and C. Isersky.** 1984. The fate of IgE bound to rat basophilic leukemia cells. III. Relationship between antigen-induced endocytosis and serotonin release. *J. Immunol. 133:1513.*
33. **Ra, C., K. Furuichi, J. M. Mullins, C. Isersky, and K. N. White.** 1989. Internalization of IgE receptors on rat basophilic leukemia cells by phorbol ester: comparison with endocytosis induced by receptor aggregation. *Eur. J. Immunol. 19:1771.*
34. **Menon, A. K., D. Holowka, W. W. Webb, and B. Baird.** 1986. Clustering, mobility, and triggering activity of small oligomers of immunoglobulin E on rat basophilic leukemia cells. *J. Cell Biol. 102:534.*
35. **Menon, A. K., D. Holowka, W. W. Webb, and B. Baird.** 1986. Cross-linking of receptor-bound IgE to aggregates larger than dimers leads to rapid immobilization. *J. Cell Biol. 102:541.*
36. **Schlessinger, J., W. W. Webb, E. L. Elson, and H. Metzger.** 1976. Lateral motion and valence of Fc receptors on rat peritoneal mast cells. *Nature 264:550.*
37. **Fewtrell, C., and H. Metzger.** 1980. Larger oligomers of IgE are more effective than dimers in stimulating rat basophilic leukemia cells. *J. Immunol. 125:701.*
38. **Robertson, D., D. Holowka, and B. Baird.** 1986. Cross-linking of immunoglobulin E-receptor complexes induces their interaction with the cytoskeleton of rat basophilic leukemia cells. *J. Immunol. 136:4565.*
39. **Edidin, M.** 1990. Translational diffusion of membrane proteins. In *The Structure of Biological Membranes.* P. L. Yeagle, ed. The Telford Press, *In press.*
40. **Hughes, B. D., B. A. Pailthorpe, L. R. White, and W. H. Sawyer.** 1982. Extraction of membrane microviscosity from translational and rotational diffusion coefficients. *Biophys. J. 37:673.*
41. **Jacobson, K., A. Ishihara, and R. Inman.** 1987. Lateral diffusion of proteins in membranes. *Annu. Rev. Physiol. 49:163.*
42. **Wade, W. F., J. H. Freed, and M. Edidin.** 1989. Translational diffusion of class II MHC molecules is constrained by their cytoplasmic domains. *J. Cell Biol. 109:3325.*
43. **Edidin, M., and M. Zuniga.** 1984. Lateral diffusion of wild-type and mutant Ld antigens in L cells. *J. Cell Biol. 99:2333.*
44. **Livneh, E., M. Benveniste, R. Prywes, S. Felder, Z. Kam, and J. Schlessinger.** 1986. Large deletions in the cytoplasmic kinase domain of the epidermal growth factor receptor do not affect its lateral mobility. *J. Cell Biol. 103:327.*
45. **Scullion, B. F., Y. Hou, L. Puddington, J. K. Rose, and K. Jacobson.** 1987. Effects of mutations in three domains of the vesicular stomatitis viral glycoprotein on its lateral diffusion in the plasma membrane. (Published erratum appears in *J. Cell Biol. 106:325,* 1988.) *J. Cell Biol. 105:69.*
46. **Weir, M., and M. Edidin.** 1988. Constraint of the translational diffusion of a membrane glycoprotein by its external domains. *Science 242:412.*
47. **Wolf, D. E., J. Schlessinger, E. L. Elson, W. W. Webb, R. Blumenthal, and P. Henkart.** 1977. Diffusion and patching of macromolecules on planar lipid bilayer membranes. *Biochemistry 16:3476.*
48. **Wolf, D. E., P. Henkart, and W. W. Webb.** 1980. Diffusion, patching and capping of steroylated dextrans on 3T3 cell plasma membranes. *Biochemistry 19:3893.*
49. **Taga, T., M. Hibi, Y. Hirata, K. Yamasaki, K. Yasukawa, T. Matsuda, T. Hirano, and T. Kishimoto.** 1989. Interleukin-6 triggers the association of its receptor with a possible signal transducer, gp130. *Cell 58:573.*
50. **McCloskey, M. A., Z.-Y. Liu, and M.-M. Poo.** 1984. Lateral electromigration and diffusion of Fc(epsilon) receptors on rat basophilic leukemia cells: effects of IgE binding. *J. Cell Biol. 99:778.*
51. **Liu, Z. Y., J. I. Young, and E. L. Elson.** 1987. Rat basophilic leukemia cells stiffen when they secrete. *J. Cell Biol. 105:2933.*
52. **Davis, C. G., M. A. Lehrman, D. W. Russell, R. G. Anderson, M. S. Brown, and J. L. Goldstein.** 1986. The J.D. mutation in familial hypercholesterolemia: amino acid substitution in cytoplasmic domain impedes internalization of LDL receptors. *Cell 45:15.*
53. **Marsh, M., and A. Helenius.** 1980. Adsorptive endocytosis of Semliki Forest virus. *J. Mol. Biol. 142:439.*
54. **Schlessinger, J.** 1980. The mechanism and role of hormone-induced clustering of membrane receptors. *Trends Biochem. Sci. 6:210.*
55. **Iacopetta, B. J., S. Rothenberger, and C. K. Lukas.** 1988. A role for the cytoplasmic domain in transferrin receptor sorting and coated pit formation during endocytosis. *Cell 54:485.*
56. **Jing, S. Q., T. Spencer, K. Miller, C. Hopkins, and I. S. Trowbridge.** 1990. Role of the human transferrin receptor cytoplasmic domain in endocytosis: localization of a specific signal sequence for internalization. *J. Cell Biol. 110:283.*
57. **Mostov, K. E., A. de Bruyn Kops, and D. L. Deitcher.** 1986. Deletion of the cytoplasmic domain of the polymeric immunoglobulin receptor prevents basolateral localization and endocytosis. *Cell 47:359.*
58. **Lobel, P., K. Fujimoto, R. D. Ye, G. Griffiths, and S. Kornfeld.** 1989. Mutations in the cytoplasmic domain of the 275 kd mannose 6-phosphate receptor differentially alter lysosomal enzyme sorting and endocytosis. *Cell 57:787.*
59. **Miettinen, H. M., J. K. Rose, and I. Mellman.** 1989. Fc receptor isoforms exhibit distinct abilities for coated pit localization as a result of cytoplasmic domain heterogeneity. *Cell 58:317.*
60. **Lazarovits, J., and M. Roth.** 1988. A single amino acid change in the cytoplasmic domain allows the influenza virus hemagglutinin to be endocytosed through coated pits. *Cell 53:743.*
61. **Vega, M. A., and J. L. Strominger.** 1989. Constitutive endocytosis of HLA class I antigens requires a specific portion of the intracytoplasmic tail that shares structural features with other endocytosed molecules. *Proc. Natl. Acad. Sci. USA 86:2688.*
62. **Isersky, C., J. Rivera, D. M. Segal, and T. Triche.** 1983. The fate of IgE bound to rat basophilic leukemia cells. II. Endocytosis of IgE oligomers and effect on receptor turnover. *J. Immunol. 131:388.*
63. **Furuichi, K., J. Rivera, and C. Isersky.** 1985. The receptor for immunoglobulin E on rat basophilic leukemia cells: effect of ligand binding on receptor expression. *Proc. Natl. Acad. Sci. USA 82:1522.*
64. **Furuichi, K., J. Rivera, T. Triche, and C. Isersky.** 1985. The fate of IgE bound to rat basophilic leukemia cells. IV. Functional association between the receptors for IgE. *J. Immunol. 134:1766.*
65. **Furuichi, K., C. Ra, C. Isersky, and J. Rivera.** 1986. Comparative evaluation of the effect of pharmacological agents on endocytosis and coendocytosis of IgE by rat basophilic leukaemia cells. *Immunology 58:105.*
66. **Furuichi, K., J. Rivera, L. M. Buonocore, and C. Isersky.** 1986. Recycling of receptor-bound IgE by rat basophilic leukemia cells. *J. Immunol. 136:1015.*
67. **Rivera, J., J. M. Mullins, K. Furuichi, and C. Isersky.** 1986. Endocytosis of aggregated immunoglobulin G by rat basophilic leukemia cells: rate, extent, and effects on the endocytosis of immunoglobulin E. *J. Immunol. 136:623.*
68. **Pfeiffer, J. R., J. C. Seagrave, B. H. Davis, G. G. Deanin, and J. M. Oliver.** 1985. Membrane and cytoskeletal changes associated with IgE-mediated serotonin release from rat basophilic leukemia cells. *J. Cell Biol. 101:2145.*