Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 7015–7019, September 1990
Immunology

CD3ζ subunit can substitute for the γ subunit of Fc_ε receptor type I in assembly and functional expression of the high-affinity IgE receptor: Evidence for interreceptor complementation

(T-cell antigen receptor/*Xenopus* oocytes/ELISA/immunoprecipitation)

FRANK D. HOWARD*†, HANS-REIMER RODEWALD*‡, JEAN-PIERRE KINET§, AND ELLIS L. REINHERZ*†

*Laboratory of Immunobiology, Dana–Farber Cancer Institute and Departments of †Medicine and ‡Pathology, Harvard Medical School, Boston, MA 02115; and §Molecular Allergy and Immunology Section, National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, MD 20892

Communicated by Stanley G. Nathenson, July 2, 1990

ABSTRACT **The high-affinity receptor for IgE ($Fc_\varepsilon RI$) is a four-subunit structure consisting of three distinct polypeptides: the IgE-binding α chain, the four-fold membrane-spanning β chain, and the disulfide-linked γ–γ homodimer. cDNAs encoding each subunit have previously been isolated. Here we show that microinjection of *Xenopus* oocytes with a mixture of *in vitro* transcribed RNAs encoding each subunit results in expression of IgE receptors at the oocyte surface as detected by binding of IgE or anti-$Fc_\varepsilon RI\alpha$ subunit monoclonal antibody to intact oocytes. Surface expression of $Fc_\varepsilon RI$ requires injection of all three subunit (α, β, and γ) RNAs. In particular, omission of $Fc_\varepsilon RI\gamma$ RNA from the mixtures abolishes surface binding of either IgE or anti-$Fc_\varepsilon RI\alpha$ monoclonal antibody to microinjected oocytes. However, addition of CD3ζ RNA to $Fc_\varepsilon RI\alpha$ and $Fc_\varepsilon RI\beta$ RNAs restores IgE receptor surface expression when this combination is microinjected into oocytes. Metabolic labeling and immunoprecipitation of oocytes microinjected with a mixture of CD3ζ plus $Fc_\varepsilon RI\alpha$ and $Fc_\varepsilon RI\beta$ RNAs reveals a noncovalent association between the CD3ζ–ζ disulfide-linked homodimer and $Fc_\varepsilon RI\alpha$–β. These results provide direct evidence for the functional relatedness of CD3ζ and $Fc_\varepsilon RI\gamma$.**

Certain cell surface receptors consist of multiple components with specialized ligand-binding and signal-transduction functions. Such is the case for the high-affinity, tetrameric IgE receptor (Fc_ε receptor type I, $Fc_\varepsilon RI$) found on mast cells and basophils. The $Fc_\varepsilon RI\alpha$ subunit binds to the Fc region of IgE antibodies. In contrast, both the $Fc_\varepsilon RI\beta$ subunit, which spans the membrane four times, and the homodimeric $Fc_\varepsilon RI\gamma$ subunit are presumed to be involved in signal transduction (1–4). Likewise, the T-cell receptor for antigen/MHC (products of the major histocompatibility complex) is a complex formed from seven distinct transmembrane proteins. The disulfide-linked Tiα and Tiβ molecules form the idiotypic antigen/MHC-binding structure, whereas the monomeric CD3γ, CD3δ, and CD3ε subunits and the dimeric CD3ζ–ζ (or CD3ζ–η) subunits participate in an as yet unknown way in signal transduction (5–8).

$Fc_\varepsilon RI\gamma$ and CD3ζ share sequence homology in their respective transmembrane and carboxyl-terminal regions and derive from genes with a similar exon–intron organization on murine chromosome 1 (9–12). Furthermore, both CD3ζ and $Fc_\varepsilon RI\gamma$ exist on the cell surface as disulfide-linked homodimers. In addition, CD3ζ and $Fc_\varepsilon RI\gamma$ are required for cell surface targeting of their respective mature receptor complexes (4, 9–11, 13). To investigate the functional significance of these similarities we employed a *Xenopus laevis* oocyte expression system to test whether CD3ζ could substitute for $Fc_\varepsilon RI\gamma$ in mediating Fc_ε receptor assembly, surface expression, and IgE binding. Here we show that *Xenopus* oocytes microinjected with a mixture of synthetic RNAs encoding each of the $Fc_\varepsilon RI$ subunit polypeptides (α, β, and γ) express ligand-binding receptors for the Fc portion of IgE. Further, substitution of CD3ζ RNA for $Fc_\varepsilon RI\gamma$ RNA allows surface expression of an IgE-binding structure. Immunoprecipitation of metabolically labeled oocytes injected with $Fc_\varepsilon RI\alpha$ and -β RNAs and CD3ζ RNA demonstrates a noncovalent association between CD3ζ and $Fc_\varepsilon RI\beta$.

MATERIALS AND METHODS

Materials. All restriction endonucleases were from New England Biolabs (Beverly, MA). SP6 and T7 RNA polymerases, RNasin, rNTPs, RQ1 DNase, and wheat germ extract were from Promega. G(5′)ppp(5′)G and protein A-Sepharose was from Pharmacia. Translation-grade [^{35}S]methionine (>1000 Ci/mmol; 1 Ci = 37 GBq) and [^{35}S]cysteine (>600 Ci/mmol) were from DuPont/NEN. Protein molecular weight markers were from Bio-Rad. RNA size standards were from BRL. Oocyte-positive *X. laevis* females were from Nasco (Fort Atkison, WI) or Xenopus I (Ann Arbor, MI). Type I collagenase, digitonin, protease inhibitors, and avidin-conjugated horseradish peroxidase were from Sigma. Monoclonal antibodies (mAbs) used were AR 40 (IgG1) and AR 57 (IgG2b), both directed against $Fc_\varepsilon RI\alpha$ (14); monoclonal IgE (15); and, as negative control mAbs, F23.1, an anti-murine Tiβ specific for the $V_{\beta 8.1.2.3}$ variable region (IgG2a) (16); 21Thy2D3, an anti-CD8 (IgG1); and 1HT4-4E5, an anti-idiotype of the 20a3 human T-cell line (IgG2b). The anti-$Fc_\varepsilon RI\beta$ mAb (IgG1) has been described (17). A rabbit anti-CD3ζ peptide antiserum was provided by A. M. Weissman (National Institutes of Health; ref. 18). Biotin-conjugated goat anti-mouse immunoglobulin was from Fisher Scientific (Springfield, NJ). Affinity-purified rabbit antibody to mouse immunoglobulin was from Pierce. Ninety-six-well round-bottom plates were from Nunc. *o*-Phenylenediamine was from Aldrich.

***In Vitro* Transcription of Sense RNA for Rat $Fc_\varepsilon RI\alpha$, -β, and -γ and Human CD3ζ.** Templates for RNA transcription were constructed by ligating the relevant cDNA fragment into plasmids containing SP6 and T7 RNA polymerase promoters flanking a multiple cloning segment. *Eco*RI cDNA inserts coding for rat $Fc_\varepsilon RI\alpha$ and $Fc_\varepsilon RI\beta$ were excised and ligated into the *Eco*RI site in plasmids pGEM-3Zf(−) and pGEM-4 (Promega), respectively. The *Eco*RI–*Dde* I fragment from the $Fc_\varepsilon RI\gamma$ cDNA insert was blunt-end-ligated into the *Sma* I site of pGEM-3Zf(+) after treatment with the Klenow fragment of DNA polymerase. A full-length human CD3ζ cDNA clone was a gift from A. M. Weissman and R. D. Klausner

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: mAb, monoclonal antibody; MBS, modified Barth's saline; $Fc_\varepsilon RI$, Fc_ε receptor type I.

(National Institutes of Health; ref. 9). Human CD3ζ was employed because its *in vitro* and *in vivo* translation efficiency exceeded that of murine CD3ζ by an order of magnitude (data not shown). The orientation of all cDNA inserts was verified by restriction mapping. Sense RNA, capped with GpppG, was transcribed from the promoter adjacent to the 5′ end of the cDNA insert after linearization of the template at the unique restriction site shown downstream of the 3′ end, by using commercial reagents as described (19). Approximately 1 μg of the resulting RNA was analyzed by formaldehyde/agarose (1.2%) gel electrophoresis and compared with known RNA size standards. Approximately 1 μg of each *in vitro* transcribed RNA species was translated *in vitro* with wheat germ lysate according to the manufacturer's protocol using [^{35}S]methionine. Products were analyzed by SDS/12.5% PAGE. Protein bands were visualized by autoradiography using Kodak X-Omat AR film for 12–14 hr at −70°C.

Microinjection and Surface ELISA of *X. laevis* Oocytes. Stage V and VI oocytes were harvested from oocyte-positive *X. laevis* females and manually defolliculated after treatment with collagenase (2 mg/ml) for 1 hr at room temperature in modified Barth's saline (MBS; ref. 20). After overnight incubation at 18–19°C, viable oocytes (usually >90%) were microinjected with 40–50 nl of RNA at a concentration of 1 mg/ml. Following further incubation for 24–48 hr at 19°C with daily changes of MBS, oocytes were analyzed for surface expression of receptors by ELISA. For this, oocytes were washed three times with a large excess of MBS and incubated in groups of 10–25 in 0.5 ml with first-step antibody as indicated in the figures at a final concentration of 5 μg/ml in MBS containing 10% fetal bovine serum (MBS/FBS). All incubations were for 45 min at room temperature. After each ELISA step, oocytes were extensively washed with MBS/FBS. The second-step antibody was 0.5 ml of biotin-conjugated goat anti-mouse immunoglobulin, diluted 1:100 in MBS/FBS. The third-step reagent was 0.5 ml of avidin-conjugated horseradish peroxidase (final concentration, 4 μg/ml). Before addition of the substrate, oocytes were washed five times in 50 ml of MBS and individually transferred into single wells of 96-well round-bottom plates. All liquid was removed and 100 μl of substrate (85 mM *o*-phenylenediamine in 100 mM sodium citrate buffer, pH 4.5/0.015% H_2O_2) was added to each oocyte. After appropriate incubation times (10–30 min), 90 μl of substrate from each oocyte was transferred into a flat-bottom microtiter well, the enzyme reaction was stopped by addition of 20 μl of 1 M H_2SO_4, and the OD was read at 492 nm by an ELISA reader (Titertek). The Wilcoxon rank-sum test (21) was performed to compare experimental and control groups.

Immunoprecipitation of Lysates of Metabolically Labeled Microinjected Oocytes. *Xenopus* oocytes were harvested and microinjected as described above. Groups of five microinjected oocytes were metabolically labeled for 60 hr at 19°C with a mixture of [^{35}S]methionine and [^{35}S]cysteine (final concentrations, 5 mCi/ml and 1 mCi/ml, respectively, in 50 μl of MBS) beginning 6 hr after microinjection. After extensive washing with MBS, labeled oocytes were solubilized at 50 μl per oocyte in 1% digitonin/25 mM Tris, pH 7.5/0.8% NaCl/0.2% KCl containing iodoacetamide (10 mM), leupeptin (10 μg/ml), antipain (50 μg/ml), phenylmethylsulfonyl fluoride (1 mM), soybean trypsin inhibitor (10 μg/ml), aprotinin (10 μg/ml), and pepstatin (1 μg/ml). Following centrifugation for 15 min in an Eppendorf microcentrifuge, the soluble fraction was incubated with 2 μl of first-step antibody [either anti-Fc$_\varepsilon$RIβ mouse mAb (1.69 mg/ml), anti-CD8 (3.7 mg/ml), or undiluted rabbit anti-CD3ζ peptide serum] by rotating for 1 hr at 4°C with 25 μl of protein A-Sepharose. Four microliters of rabbit anti-mouse immunoglobulin affinity-purified antibody (1.5 mg/ml) was then added for an additional hour at 4°C. The bead–antibody–antigen complexes were pelleted by centrifugation, the supernatant was removed, and the beads were washed with 1 ml of lysis buffer followed by 1 ml of 0.05% digitonin/10 mM Tris, pH 7.5. Antigen–antibody complexes were solubilized in 50 μl of nonreducing Laemmli sample buffer at 100°C for 3 min. Aliquots (25 μl) were subjected to two-dimensional nonreducing–reducing SDS/PAGE using 12.5% acrylamide in both dimensions (22). Autoradiography was for 12 hr (complete gels) or 4 hr (insets) at −70°C on Kodak X-Omat AR film after treatment with 1 M sodium salicylate.

RESULTS AND DISCUSSION

***In Vitro* Transcription and Translation of Fc$_\varepsilon$RI and CD3ζ Subunit RNAs.** To generate templates for *in vitro* synthesis of RNA, cDNA inserts containing full-length coding regions for

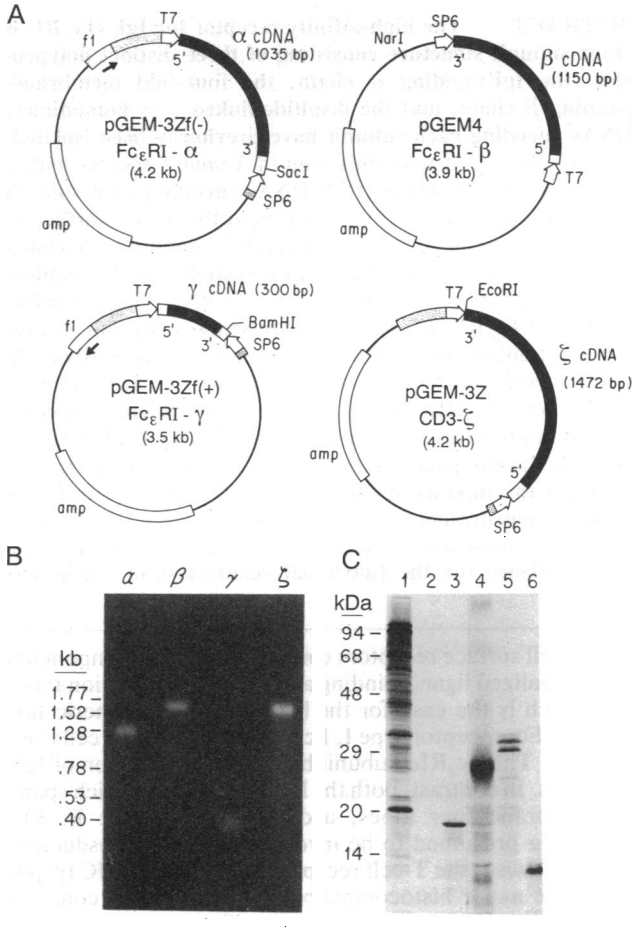


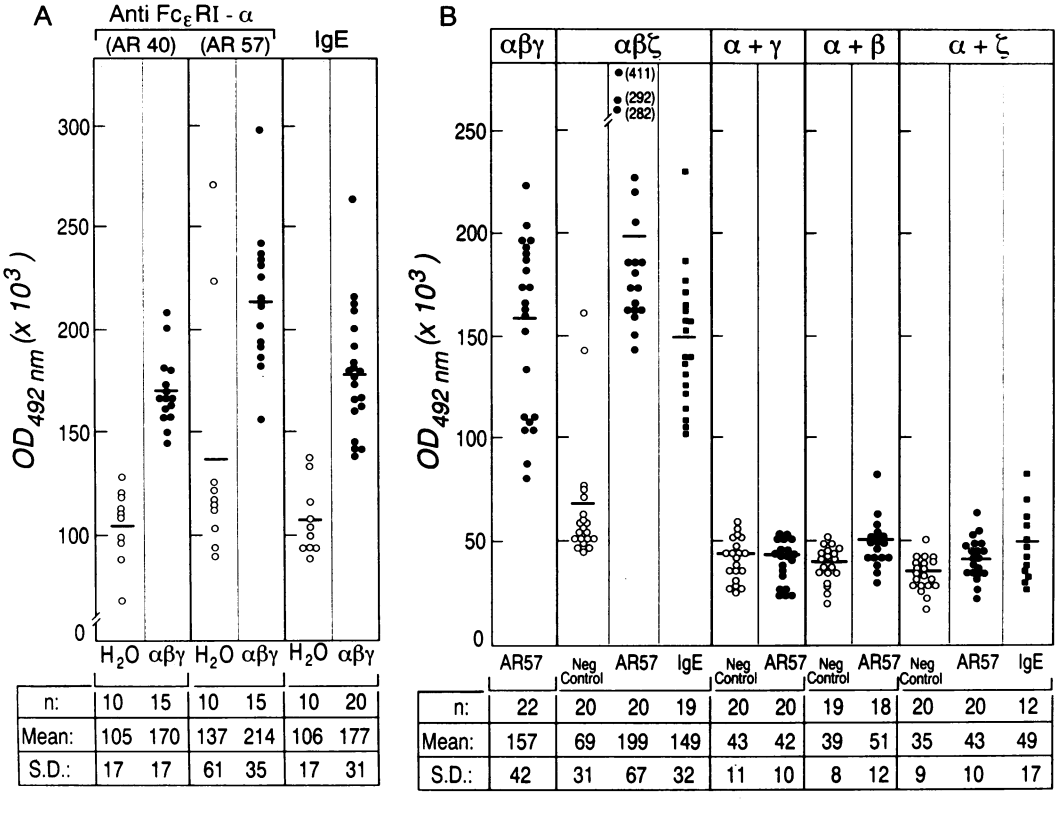
FIG. 1. Production and translation of *in vitro* transcribed sense RNA for rat Fc$_\varepsilon$RIα, -β, and -γ and human CD3ζ. (*A*) Templates for RNA transcription. The positions of SP6 and T7 RNA polymerase promoters (open arrows) are shown with respect to the cDNA insert (filled box) and the unique 3′ restriction site used for template linearization (see *Materials and Methods*). The open box represents the multiple cloning site, while the stippled box and the box with adjacent arrow denote the *lacZ* gene and f1 promoter, respectively. bp, Base pairs; kb, kilobases. (*B*) Products of *in vitro* transcription. Each linearized template was transcribed *in vitro* with the appropriate RNA polymerase (SP6 or T7). Approximately 1 μg of the resulting RNA was analyzed by formaldehyde/agarose gel electrophoresis. (*C*) *In vitro* translation of synthetic RNAs. Each *in vitro* transcribed RNA species was translated *in vitro* with wheat germ lysate. Products were visualized by autoradiography after reducing SDS/12.5% PAGE. Lanes: 1, positive control (brome mosaic virus RNA); 2, negative control (H_2O); 3, human CD3ζ; 4, rat Fc$_\varepsilon$RIα; 5, rat Fc$_\varepsilon$RIβ; 6, rat Fc$_\varepsilon$RIγ. Molecular mass standards are phosphorylase *b* (94 kDa), bovine serum albumin (68 kDa), ovalbumin (48 kDa), carbonic anhydrase (29 kDa), soybean trypsin inhibitor (20 kDa), and lysozyme (14 kDa).

$Fc_\varepsilon RI\alpha$, -β, and -γ and CD3ζ were subcloned into vectors containing SP6 and T7 RNA polymerase promoters suitable for *in vitro* RNA synthesis (Fig. 1*A*). RNA for $Fc_\varepsilon RI\alpha$, $Fc_\varepsilon RI\beta$, $Fc_\varepsilon RI\gamma$, and CD3ζ was synthesized from the corresponding linearized cDNA construct by using SP6 or T7 RNA polymerase as appropriate. This procedure yielded full-length RNA for each subunit as shown by formaldehyde/agarose electrophoresis (Fig. 1*B*). The integrity of individual subunit RNAs was verified by *in vitro* translation. SDS/PAGE analysis of translation products revealed a predominant band at the expected size for each preprotein (27 kDa for pre-α, a 31-kDa doublet for pre-β, 12 kDa for pre-γ, and 18 kDa for pre-ζ; Fig. 1*C*). Thus the synthetic RNAs were functional as substrates for protein synthesis *in vitro*.

Surface Expression of Fc_ε Receptors on *Xenopus* Oocytes. Previous studies demonstrated that injection of poly(A)$^+$ mRNA from mast cell lines conferred IgE binding activity on *Xenopus* oocytes (23, 24). In the present study, a mixture of synthetic $Fc_\varepsilon RI\alpha$, -β, and -γ RNAs was injected into *Xenopus* oocytes to test directly for $Fc_\varepsilon RI$ assembly, surface expression, and ligand binding. Individual intact oocytes were analyzed for surface binding of anti-$Fc_\varepsilon RI\alpha$ mAbs and monoclonal IgE by ELISA. As shown in Fig. 2*A*, oocytes injected with all three $Fc_\varepsilon RI$ subunit RNAs ($\alpha\beta\gamma$) displayed significant binding of anti-$Fc_\varepsilon RI\alpha$ mAb (AR57 or AR40) and IgE as compared with control oocytes injected with H_2O. Thus, in Fig. 2*A*, comparing $\alpha\beta\gamma$ oocytes with H_2O-injected oocytes, $P < 0.0001$ using mAb AR 40 (IgG1) or IgE, and $P = 0.004$ in the case of mAb AR 57 (IgG2b). Omission of the first-step mAb prior to addition of biotin-conjugated goat anti-mouse immunoglobulin or use of isotype-matched mAbs with an irrelevant specificity [mAbs 4E5 (IgG2b), anti-idiotype, and 21Thy2D3 (IgG1), anti-CD8] yielded only background-level binding equivalent to H_2O-injected controls (data not shown). These results demonstrate surface expression and ligand binding of $Fc_\varepsilon RI$ in *Xenopus* oocytes injected with a mixture of synthetic $Fc_\varepsilon RI\alpha$, -β, and -γ RNAs.

To investigate subunit requirements for surface expression and ligand binding, various receptor-subunit RNA combinations were microinjected into oocytes and tested by ELISA. As shown in Fig. 2*B*, omitting either $Fc_\varepsilon RI\beta$ RNA or $Fc_\varepsilon RI\gamma$ RNA from the injected mixture resulted in no detectable binding over background. Thus the injection of $Fc_\varepsilon RI\alpha$ and -β RNAs or $Fc_\varepsilon RI\alpha$ and -γ RNAs failed to produce significant surface expression of $Fc_\varepsilon RI$ [$P < 0.0001$ for $\alpha\beta\gamma$ comparing F23.1 negative control mAb with AR 57 (anti-$Fc_\varepsilon RI\alpha$ subunit) or IgE, and $P < 0.0001$ comparing $\alpha\beta\gamma$ with α + γ- or α + β-injected oocytes in the case of AR 57 binding]. Furthermore, no binding of anti-$Fc_\varepsilon RI\alpha$ subunit mAb to oocytes injected with RNA for $Fc_\varepsilon RI\alpha$ alone was detected (data not shown). Similar results were obtained with six batches of oocytes from four different frogs. These data are in agreement with previous experiments on the rodent $Fc_\varepsilon RI$, which demonstrated a stringent requirement for expression of all three $Fc_\varepsilon RI$ subunits to obtain surface expression in the monkey fibroblast cell line COS (4, 13). Collectively, these findings show that the assembly and expression requirements of the tetrameric $Fc_\varepsilon RI$ complex are similar in widely differing expression systems. As such, these requirements are not restricted to a single cell type nor are they peculiar to the oocyte system.

CD3ζ Can Substitute for $Fc_\varepsilon RI\gamma$ in Assembly and Surface Expression of a Functional IgE-Binding Receptor. Given the sequence homology in the transmembrane regions of $Fc_\varepsilon RI\gamma$ and CD3ζ (10, 13), we investigated whether CD3ζ could substitute for $Fc_\varepsilon RI\gamma$ in forming a ligand-binding surface receptor for IgE. To test this possibility directly, oocytes were microinjected with a mixture of synthetic RNAs coding for $Fc_\varepsilon RI\alpha$, $Fc_\varepsilon RI\beta$, and CD3ζ ($\alpha\beta\zeta$) and examined for receptor surface expression and ligand binding. As shown in



n:	10	15	10	15	10	20	22	20	20	19	20	20	19	18	20	20	12
Mean:	105	170	137	214	106	177	157	69	199	149	43	42	39	51	35	43	49
S.D.:	17	17	61	35	17	31	42	31	67	32	11	10	8	12	9	10	17

FIG. 2. Surface expression of $Fc_\varepsilon RI$ in *Xenopus* oocytes. Each dot represents a single oocyte. The number of injected oocytes (n), the mean (horizontal bar), and standard deviation (S.D.) are indicated for each group. (*A*) Monoclonal anti-$Fc_\varepsilon RI\alpha$ mAb (AR 40 or AR 57) or IgE binding to oocytes injected with $Fc_\varepsilon RI\alpha$, -β, and -γ RNAs (●) or water (○). (*B*) Binding of anti-$Fc_\varepsilon RI\alpha$ mAb (AR 57) (●) or IgE (■) or isotype control mAb F23.1 (Neg Control; ○) to oocytes injected with mixtures of synthetic $Fc_\varepsilon RI\alpha$, -β, and -γ and CD3ζ RNAs as indicated above each group.

Fig. 2*B*, $\alpha\beta\zeta$ RNA-injected oocytes were specifically reactive with anti-$Fc_\varepsilon RI\alpha$ mAb AR 57 relative to an isotype-matched control antibody [$P < 0.0001$ for $\alpha\beta\zeta$ comparing the negative control mAb F23.1 with mAb AR 57 (anti-$Fc_\varepsilon RI\alpha$ subunit)]. Furthermore, IgE binding experiments performed in parallel demonstrated that $\alpha\beta\zeta$ oocytes specifically bound monoclonal IgE antibody ligand (Fig. 2*B*, $P < 0.0001$ for $\alpha\beta\zeta$ comparing the negative control mAb F23.1 with IgE). Thus CD3ζ can complement $Fc_\varepsilon RI\alpha$ and -β subunits to form a surface receptor structure that retains the mAb- and ligand-binding properties of the natural ($\alpha\beta\gamma$) receptor.

Physical Association of CD3ζ with $Fc_\varepsilon RI\alpha$–β. The above observation that a hybrid receptor ($Fc_\varepsilon RI\alpha$–$Fc_\varepsilon RI\beta$–CD3ζ) could be expressed on the surface of *Xenopus* oocytes suggested a physical association between CD3ζ and $Fc_\varepsilon RI\alpha$–β. To examine this possibility, oocytes were injected with the $Fc_\varepsilon RI\alpha/Fc_\varepsilon RI\beta$/CD3$\zeta$ RNA mixture, labeled with [^{35}S]methionine and [^{35}S]cysteine, immunoprecipitated with an anti-$Fc_\varepsilon RI\beta$ mAb, and subsequently analyzed by two-dimensional nonreducing–reducing SDS/PAGE. As shown by the representative autoradiograph in Fig. 3*A*, such analysis revealed a major off-diagonal protein migrating at ≈32 kDa in the nonreducing dimension and at ≈16 kDa in the reducing dimension, consistent with the expected mobility of a ζ–ζ homodimer. Parallel immunoprecipitation and two-dimensional gel analysis of metabolically labeled $\alpha\beta\zeta$ RNA-injected oocytes with anti-CD3ζ peptide antiserum identified an off-diagonal pattern virtually identical to the anti-β immunoprecipitate (Fig. 3*B*), confirming the identity of $Fc_\varepsilon RI\beta$-associated protein as CD3ζ. The CD3ζ homodimer migrated as a doublet possibly reflecting posttranslational modification. In contrast, no CD3ζ species were detected in $\alpha\beta\zeta$-injected oocytes immunoprecipitated with an irrelevant isotype control mAb (anti-CD8, Fig. 3*C*) or in H_2O-injected oocytes immunoprecipitated with anti-CD3ζ peptide antiserum (Fig. 3*D*). A protein complex migrating at ≈23 kDa in the nonreducing dimension and at ≈16 kDa in the reducing dimension (Fig. 3 *A* and *B*, arrow) may represent a heterodimer between CD3ζ and a fragment of $Fc_\varepsilon RI\beta$ (17) or between CD3ζ and an unidentified endogenous oocyte protein. This species had identical mobilities in anti-CD3ζ and anti-$Fc_\varepsilon RI\beta$ immunoprecipitates (compare Fig. 3*A* with Fig. 3*B*) and thus was associated with the $\alpha\beta\zeta$ complex. The ≈16-kDa protein running on the diagonal in anti-ζ immunoprecipitates represents CD3ζ monomer (Fig. 3 *A* and *B*). $Fc_\varepsilon RI\beta$ was clearly visualized as a doublet at ≈31 kDa running on the diagonal in both anti-β and anti-ζ immunoprecipitates on shorter exposure of the same autoradiograms (see *Insets* in Fig. 3 *A* and *B*). This doublet pattern was previously described for $Fc_\varepsilon RI\beta$ *in vivo* (25) and *in vitro* (Fig. 1*C* and ref. 3). The absence of detectable $Fc_\varepsilon RI\alpha$ protein in the immunoprecipitates is not surprising in light of prior data showing that the α subunit readily disassociates from the complex, even under mild detergent conditions (26). In addition, the high background in the 40- to 60-kDa range would obscure detection of the $Fc_\varepsilon RI\alpha$ glycoprotein. The coimmunoprecipitation of CD3ζ and $Fc_\varepsilon RI\beta$ demonstrates specific physical association between CD3ζ and $Fc_\varepsilon RI\alpha$-β in $\alpha\beta\zeta$-injected oocytes and provides biochemical evidence for receptor subunit complementation to form a hybrid receptor, $\alpha\beta\zeta$–ζ.

Implications. Recently it has become apparent that CD3ζ and $Fc_\varepsilon RI\gamma$ subunits participate in a variety of immune receptor structures on different cell types. Thus, in T lym-

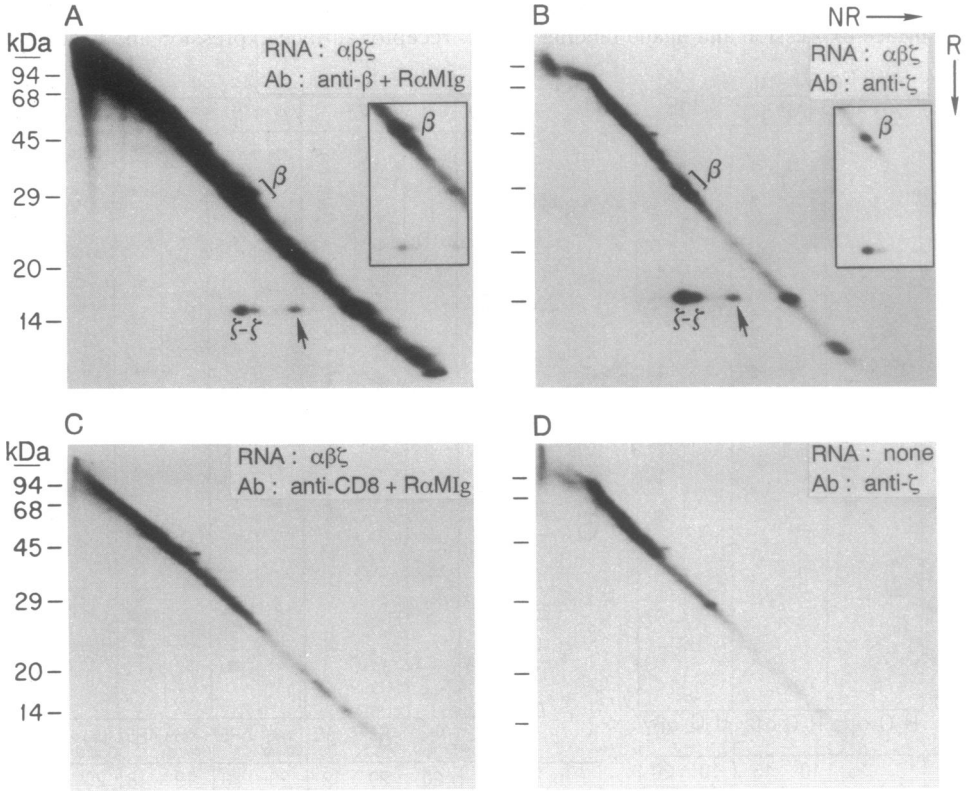


FIG. 3. Association of CD3ζ with $Fc_\varepsilon RI\beta$. Oocytes injected with $Fc_\varepsilon RI\alpha$, $Fc_\varepsilon RI\beta$, and CD3ζ RNAs were analyzed by immunoprecipitation and two-dimensional nonreducing (NR)–reducing (R) SDS/PAGE. The positions of the $Fc_\varepsilon RI\beta$ and CD3ζ products are indicated. (*A*) Oocytes injected with $Fc_\varepsilon RI\alpha$, $Fc_\varepsilon RI\beta$, and CD3ζ RNAs and immunoprecipitated with anti-$Fc_\varepsilon RI\beta$ mAb. (*Inset*) A short exposure showing area containing $Fc_\varepsilon RI\beta$ and CD3ζ. (*B*) Oocytes injected as in *A* and immunoprecipitated with anti-CD3ζ peptide antiserum. (*Inset*) A short exposure showing area containing $Fc_\varepsilon RI\beta$ and CD3ζ. (*C*) Oocytes injected as in *A* and immunoprecipitated with an isotype-matched control mAb of irrelevant specificity (21Thy2D3). (*D*) Oocytes injected with H_2O and immunoprecipitated with the anti-CD3ζ peptide antiserum used in *B*. RαMIg, rabbit anti-mouse immunoglobulin.

phocytes, CD3ζ associates with the CD3–Ti complex, forming a complete surface T-cell antigen receptor (8). In natural killer cells, however, CD3ζ has been shown to associate with Fc_γRIII-2 (CD16) (27) and certain unidentified structures (28) in the absence of other CD3 components. Fc_εRIγ associates with Fc_εRIα and -β subunits to form a surface receptor for IgE on rodent mast cells and basophils (4). In addition, the γ subunit is found in murine macrophages, where it associates with Fc_γRIIa, the murine homologue of CD16 (29), as well as in human natural killer cells, where it also associates with CD16 (30). The ability of a given subunit to function in the context of several distinct receptor types implies an important common transduction and/or assembly role for such elements in different cellular systems.

Here we have shown that CD3ζ can substitute for Fc_εRIγ in assembly and surface expression of the high-affinity IgE receptor. This substitution is reminiscent of the property of isotypy that has been well described for immunoglobulin molecules. Receptor "isotypy" in signal-transduction components might result not only from complete substitution of one subunit for another but also from formation of mixed dimers (i.e., CD3ζ–Fc_εRIγ). That a given receptor can variably associate with multiple distinct subunits to form differing receptor isoforms suggests an additional level of receptor complexity. This situation has already been documented among members of the CD3ζ/CD3η/Fc_εRIγ gene family by the finding that CD3ζ–η heterodimers and CD3ζ–ζ homodimers are expressed in the same cell type (31). Evidence suggests that such receptor types mediate distinct signaling events (32). The potential for association between a receptor's ligand-binding component and any of a number of distinct transduction subunits suggests a signaling-diversification strategy that could be operative in a single cell type. Moreover, restricted expression of particular transducing elements to a discrete stage of differentiation could provide an important regulatory mechanism in the developmental process. The present data add impetus to examine various cell types for the presence of receptor complexes diversified by subunit complementation.

F.D.H. and H.-R.R. made equivalent contributions to this work. We thank Drs. A. M. Weissman (Experimental Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD) and R. D. Klausner (Cell Biology and Metabolism Branch, National Institute of Child Health and Human Development, National Institutes of Health) for making available the CD3ζ cDNA construct and the anti-CD3ζ peptide antiserum, Dr. R. Siraganian (Clinical Immunology Section, Laboratory of Microbiology and Immunology, National Institute of Dental Research, National Institutes of Health) for mAbs AR 40 and AR 57, Dr. M. Ayane (Max-Planck Institut, Freiburg, Federal Republic of Germany) for invaluable advice on RNA work, and Dr. L. Ryan (Dana–Farber Cancer Institute, Boston, MA) for help with the statistical evaluation. H.-R.R. was supported by the Deutsche Forschungsgemeinschaft. This work was supported in part by National Institutes of Health Grants AI19807 and AI21226.

1. Kinet, J.-P. (1989) *Cell* **57**, 351–354.
2. Kinet, J.-P., Metzger, H., Hakimi, J. & Kochan, J. (1987) *Biochemistry* **26**, 4605–4610.
3. Kinet, J.-P., Blank, U., Ra, C., White, K., Metzger, H. & Kochan, J. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 6483–6487.
4. Blank, U., Ra, C., Miller, L., White, K., Metzger, H. & Kinet, J.-P. (1989) *Nature (London)* **337**, 187–189.
5. Meuer, S. C., Acuto, O., Hercend, T., Schlossman, S. F. & Reinherz, E. L. (1984) *Annu. Rev. Immunol.* **2**, 23–50.
6. Clevers, H., Alarcon, B., Wileman, T. & Terhorst, C. (1988) *Annu. Rev. Immunol.* **6**, 629–662.
7. Davis, M. M. & Bjorkman, P. J. (1988) *Nature (London)* **334**, 395–402.
8. Weissman, A. M., Bonifacino, J. S., Klausner, R. D., Samelson, L. E. & O'Shea, J. J. (1989) *Year Immunol.* **4**, 74–93.
9. Weissman, A. M., Hou, D., Orloff, D. G., Modi, W. S., Seuanez, H., O'Brien, S. J. & Klausner, R. D. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 9709–9713.
10. Kuster, H., Thompson, H. & Kinet, J.-P. (1990) *J. Biol. Chem.* **265**, 6448–6452.
11. Huppi, K., Mock, B. A., Hilgers, J., Kochan, J. & Kinet, J.-P. (1988) *J. Immunol.* **141**, 2807–2810.
12. Reth, M. (1989) *Nature (London)* **338**, 383–384.
13. Miller, L., Blank, U., Metzger, H. & Kinet, J.-P. (1988) *Science* **244**, 334–337.
14. Shimizu, A., Tepler, I., Benfey, P. N., Berenstein, E. H., Siraganian, R. P. & Leder, P. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 1907–1911.
15. Liu, F.-T., Bohn, J. W., Ferry, E. L., Yamamoto, H., Molinaro, C. A., Sherman, L. A., Klinman, N. R. & Katz, D. H. (1980) *J. Immunol.* **124**, 2728–2736.
16. Staerz, U., Rammensee, H., Benedetto, J. & Bevan, M. (1985) *J. Immunol.* **134**, 3994–4000.
17. Kinet, J.-P., Perez-Montfort, R. & Metzger, H. (1983) *Biochemistry* **22**, 5729–5732.
18. Orloff, D. G., Frank, S. J., Robey, F. A., Weissman, A. M. & Klausner, R. D. (1989) *J. Biol. Chem.* **264**, 14812–14817.
19. Krieg, P. A. & Melton, D. A. (1984) *Nucleic Acids Res.* **12**, 7057–7070.
20. Coleman, A. (1984) *Transcription and Translation*, eds. Hames, B. D. & Higgins, S. J. (IRL, Washington), pp. 271–302.
21. Rosner, B. (1982) *Fundamentals of Biostatistics* (Duxbury, Boston), pp. 150–155.
22. Godding, J. W. & Harris, A. W. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 4530–4534.
23. Pure, E., Luster, A. D. & Unkeless, J. C. (1984) *J. Exp. Med.* **160**, 606–611.
24. Liu, F.-T. & Orida, N. (1984) *J. Biol. Chem.* **259**, 10649–10652.
25. Rivera, J., Kinet, J.-P., Kim, J., Pucillo, C. & Metzger, H. (1988) *Mol. Immunol.* **25**, 647–661.
26. Kinet, J.-P., Alcaraz, G., Leonard, A., Wank, S. & Metzger, H. (1985) *Biochemistry* **24**, 4117–4124.
27. Lanier, L. L., Yu, G. & Phillips, J. H. (1989) *Nature (London)* **342**, 803–807.
28. Anderson, P., Caligiuri, M., Ritz, J. & Schlossman, S. F. (1989) *Nature (London)* **341**, 159–162.
29. Ra, C., Jouvin, M.-H. E., Blank, U. & Kinet, J.-P. (1989) *Nature (London)* **341**, 752–754.
30. Hibbs, M. L., Selvaraj, P., Carpen, O., Springer, T. A., Kuster, H., Jouvin, M.-H. E. & Kinet, J.-P. (1989) *Science* **246**, 1608–1611.
31. Baniyash, M., Garcia-Morales, P., Bonifacino, J. S., Samelson, L. E. & Klausner, R. D. (1988) *J. Biol. Chem.* **263**, 9874–9878.
32. Mercep, M., Bonifacino, J. S., Garcia-Morales, P., Samelson, L. E., Klausner, R. D. & Ashwell, J. D. (1988) *Science* **242**, 571–574.