Regulation of ITAM Signaling by Specific Sequences in Ig-β B Cell Antigen Receptor Subunit*

(Received for publication, May 3, 1996)

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B cell antigen receptors (BCR) are composed of an antigen binding subunit, the membrane Ig, and Ig-α/Ig-β heterodimers, that contain a transducing motif named ITAM for “immuno-receptor tyrosine-based activation motif.” Ig-α and Ig-β ITAMs only differ by four amino acids located before the second conserved tyrosine (DCSM in Ig-α and QTAT in Ig-β), which determine the in vitro association of Ig-α with the src kinase fyn. We have previously shown that Ig-α and Ig-β BCR subunits activate different signaling pathways by expressing, in B cells, FcγRII chimeras containing the cytoplasmic tails of Ig-α or Ig-β. We report here that the signaling capacity of Ig-β ITAM is regulated by peptide sequences located inside (QTAT region) or outside the ITAM (flanking sequences). Furthermore, when isolated, Ig-α and Ig-β ITAM have similar abilities as the entire Ig-α tail and the whole BCR in triggering tyrosine kinase activation, an increase of intracellular calcium concentration as well as late events of cell activation as assessed by cytokine secretion. These data show that alterations that modify the ability of Ig-α and Ig-β to interact in vitro with the src kinase fyn (switch between QTAT and DCSM) also determine signal transduction capabilities of these molecules expressed in B cells.

The transducing capacities of BCR1 are based on its multi-molecular structure. BCRs are composed of antigen binding units, the membrane immunoglobulins (mIg), noncovalently associated with transducing subunits, the Ig-α/Ig-β heterodimers. The cytoplasmic tails of these associated chains become phosphorylated after cross-linking of mIg (1) and associate with intracellular effectors (2) including the src kinases lyn, fyn, bkl, lck (3–6), and the src-related kinase syk (7, 8), as well as other kinases such as PI-3 kinase and unidentified phosphoproteins (9). By adsorbing B cell lysates on fusion proteins containing the cytoplasmic domains of Ig-α or Ig-β, the unphosphorylated cytoplasmic domains were shown to bind to Lyn and p56lck (3–6), and the src-related kinase syk (7, 8), as well as other kinases such as PI-3 kinase and unidentified phosphoproteins (9). The activation of tyrosine kinase is followed by an increase of intracellular calcium concentration (10). Typical cytoplasmic calcium increase includes an initial release of calcium from intracellular stores followed by an influx of extracellular calcium, which is involved in lymphocytes activation (11, 12). However, both cytoplasmic domains of Ig-α and Ig-β, like associated subunits of T cell antigen receptors or Fc receptors, contain an ITAM (immunoreceptor tyrosine-based activation motif), which contains conserved tyrosine- and leucine or isoleucine amino acids (YXX(L/I)X(XX)(L/I)) (13). One particularity of Ig-α and Ig-β ITAMs is their high homology because they mostly differ by the four amino acids located before the second conserved tyrosine, the same four residues determining the in vitro association of Ig-α with fyn (14).

Functional analysis of Ig-α and Ig-β cytoplasmic domains in B cells established that both are able to induce an increase of intracellular calcium concentration (15–17) with qualitative differences (18). Although Ig-β was as efficient as Ig-α in triggering protein-tyrosine kinase activation, only Ig-α-containing chimeras were able to trigger an efficient signal transduction leading to an extracellular calcium influx and interleukin-2 (IL-2) production in the IIA1.6 B cell line. Ig-β triggered an oscillatory release from intracellular calcium stores and no IL-2 secretion (18). Ig-α and Ig-β cytoplasmic domains therefore possess their own distinct signaling capabilities. In this study, the molecular basis of the different signaling capacities of Ig-α and Ig-β cytoplasmic tails were analyzed. We showed that BCR subunits transducing activities are based on the ITAM conserved sequence, but they may be regulated by unconserved sequences located inside or outside the motif.

MATERIALS AND METHODS

Plasmid Construction—Ig-α and Ig-β chimeras were constructed by adding the sequences encoding the putative cytoplasmic domains of Ig-α and Ig-β to the extracellular and transmembrane domains of cDNA coding mouse FcγRII (FcR) by recombinant polymerase chain reaction as described previously (18). Site-directed mutagenesis of the Ig-α and Ig-β chimeras was also performed using polymerase chain reaction. The resulting constructions were inserted in SHO-driven expression vector and then were sequenced.

Cell Culture and Transfection—The B lymphoma IIA1.6 is a FcR receptor-defective variant of A20 B cells that grows in RPMI 1640 containing 10% fetal calf serum, 10 mM glutamine, 100 units/ml penicillin, 100 µg/ml streptomycin, 50 µg/ml 2-mercaptoethanol, and 5 µM sodium pyruvate (Life Technologies, Inc.). These cells express endogenous mIgG2a. The chimera constructs were linearized with ScaI 72 h after transfection, the cells were transfected to Geneticin-containing medium (G418, 1 mg/ml; Life Technologies, Inc.). The geneticin-resistant cells were checked by fluorescence-activated cell sorter analysis for FcγR expression using the monoclonal antibody 2.4G2 (31), and cells were cloned. Detection of Tyrosine Phosphorylated Proteins—Cells were preincubated at 4 °C with or without 10 µg/ml of 2.4G2 for 15 min and then washed twice with RPMI 3.5 × 105 cells were then stimulated by F(ab′)2 fragments of mouse anti-rat IgG antiserum (50 µg/ml) at 37 °C for the indicated times. As a positive control, 2.4G2 untreated cells were stimulated by F(ab′)2 fragments of anti-mouse IgG antibodies (15 µg/
ml). At different times, the stimulated cells were lysed with 2% SDS and immediately boiled for 5 min. Proteins were precipitated by acetic acid for 30 min on ice and pelleted by centrifugation for 5 min at 10,000 × g. The samples were then analyzed on 8% polyacrylamide-SDS gels and transferred on nitrocellulose filters (Schleicher & Schuell). Red Ponceau staining of the proteins on the filters allowed verification of the transfer efficiency and the homogeneity between the different lanes. The filters were incubated for 2 h at room temperature in Tris-buffered saline containing 5% bovine serum albumin and then incubated overnight at 4 °C in Tris-buffered saline/6% bovine serum albumin with the antiphosphotyrosine monoclonal antibody Py20 coupled to HRP (ICN Flow). After washing with Tris-buffered saline containing 0.05% Triton X-100, the filters were incubated for 1 min with the Western blotting Reagent ECL (Amersham Corp.), and chimaeromunescence was detected by exposure of the filters to X-Omat films (Kodak) for 30 s to 15 min.

**Measurement of IL-2 Secretion**—IL-2 release by transfected IIA1.6 B cells (10⁶ cells/well), stimulated for 16 h under the same conditions as for the induction of tyrosine phosphorylations, was determined by monitoring the growth of the IL-2-dependent cell line CTL.L2. For the IL-2 measurement after stimulation without extracellular calcium, either 1 mM of EGTA was added in the standard medium (the viability of the cells was verified with trypan blue) or a calcium-free medium was used (Life Technologies, Inc.). 10⁴ CTL.L2 cells (supernatant free) were cultured for 24 h at 37 °C with supernatants from the activation assays. The cultures were pulse-labeled with 0.5 μCi of [³²P]thymidine (25 mCi/mmol, CEA, Gil-surt-Yvette, France) for the last 6 h of the culture period before harvesting the cells using a multiple cell harvester (Osi, Paris, France). The incorporated thymidine was detected by scintillation counting. The IL-2 secreted by mutant chimeras was compared with the IL-2 secreted, during the same experiment, by the same cells after stimulation of endogenous membrane IgG. The values obtained with supernatants of anti-IgG-stimulated cells were ranging from 2 × 10⁶ to 8 × 10⁵ cpm depending of the clones. To compare all the clones, the values were therefore normalized on mlgG stimulation, which represents the maximum of stimulation.

**Measurement of Intracellular Calcium Concentrations**—Digital calcium imaging experiments were performed on fura-2 (Molecular Probes Eugene, OR) loaded cells (0.25 μg 15 min at 37 °C in culture medium, 10⁶ cells/ml) as previously described (18). The averages of intracellular calcium changes were calculated with 50–100 single cell measurements.

**RESULTS**

The intracellular signaling activity of mlg-associated subunits, Iγ-α and Iγ-β, was analyzed by expressing, in the B cell line IIA1.6, chimeras fusing the cytoplasmic domain of either Iγ-α or Iγ-β to the extracellular and transmembrane domains of FcγRII. Both chimeras activated tyrosine kinases (Fig. 1a), but only Iγ-α chimeras (c.Iγ-α) stimulation induced intracellular calcium modifications composed of an initial release from intracellular stores followed by an extracellular calcium influx, whereas Iγ-β chimeras (c.Iγ-β) stimulation triggered an oscillatory release of calcium from intracellular stores (Fig. 1b) (18). Interestingly, only Iγ-α chimeras, as well as endogenous mlgG, were efficient to induce the secretion of cytokines.

The role of calcium influx in B cell signaling was analyzed by measuring IL-2 secretion after stimulation of Iγ-α chimeras or mlgG in conditions that prevented the extracellular calcium influx. Cells expressing c.Iγ-α were incubated with EGTA to chelate the extracellular calcium or in a calcium free medium. Cross-linking of c.Iγ-α or of mlgG in the 1 mM EGTA-containing medium (or in calcium-free medium) prevented calcium influx (data not shown) and IL-2 secretion by transfected cells (Fig. 1c). Both signaling events were restored by adding 1 mM CaCl₂.

Extracellular calcium influx is therefore required for the triggering of cytokine secretion after cross-linking of Iγ-α chimeras or the whole BCR. Moreover, although the cytoplasmic tail of Iγ-α and Iγ-β both contain an ITAM, only Iγ-α induced efficient signal transduction. Iγ-α therefore accounts for the ability of BCR to induce calcium influx and subsequent cell activation events. Peptide sequence of Iγ-α and Iγ-β ITAM must determine their interactions with specific cytoplasmic effectors that induce either calcium influx and cytokine secretion or calcium oscillatory release.

**FIG. 1. Iγ-α and Iγ-β cytoplasmic tails activate distinct signaling pathways.** a, chimeras were preincubated with the rat anti-FcR antibody 2.4G2 (10 μg/ml), and the stimulation was triggered by the F(ab')₂ antiseraum mouse anti-rat (50 μg/ml) for the indicated times. After separation on SDS-8% polyacrylamide gels, the proteins were immunblotted with the rat anti-FcR antibody 2.4G2 or with the anti-FcR monoclonal antibody Py20. The arrow indicates the phosphoproteins induced by the cross-linking of mlg, which peak at 2 min. b, arithmetic average of intracellular calcium concentration measurement at the single cell level in a standard medium after cross-linking of the Iγ-α or the Iγ-β chimeras. The arrow indicates the triggering of stimulation. The boxed curve represents an example of a single cell response. c, measurement of IL-2 production after cross-linking of Iγ-α and Iγ-β chimeras. Cross-linking of Iγ-α chimeras without extracellular Ca²⁺ inhibits IL-2 secretion. Chimeras were cross-linked in the same conditions than above overnight at 37 °C in a standard medium containing or not 1 mM EGTA or in a Ca²⁺ free medium. The IL-2 secretion induced by Iγ-α chimeras was restored by the addition of 1 mM of Ca²⁺ in the media. The results were normalized on the mlgG responses, which represent the maximum of IL-2 secretion.

**Requirements of the Conserved ITAM Tyrosine Residues for the Signaling Capacities of Iγ-α and Iγ-β Tails**—To identify the peptide sequences of Iγ-α and Iγ-β cytoplasmic tails involved in the induction of calcium influx, a mutational analysis was performed by using FcΓR-based chimeras (see Table I). The cytoplasmic tails of Iγ-α and Iγ-β were shown to interact with different tyrosine kinases, and the phosphorylation of conserved tyrosine residues constituting their ITAM enhanced these interactions. To evaluate in our model the role of the tyrosine residues present in the ITAM, they were individually mutated to alanine in the cytoplasmic domain of Iγ-α (c.Iγ-α-A23 and c.Iγ-α-A34) or mutated together in the Iγ-β tail (c.Iγ-β-A15,A26). These constructions were stably expressed in IIA1.6 cells, and surface expressions were evaluated by flow cytometry using indirect immunofluorescence (Fig. 2).

The earliest known BCR signaling event is tyrosine kinase activation leading to a cascade of intracellular protein phos-
phosphorylations. Our previous results showed that the cytoplasmic domains of Ig-α and Ig-β are able to trigger phosphorylation of similar major intracellular proteins (Fig. 1a). As expected, c.Ig-α A23 and c.Ig-α A34 or c.Ig-β A15, A26 were inefficient to trigger tyrosine phosphorylation of intracellular proteins, whereas in control experiments, stimulation of mIg triggered tyrosine phosphorylation in all the transfected cells (Fig. 3a). The stimulation of these mutated chimeras was also inefficient to induce any changes of the intracellular calcium concentration, as measured at the single cell level by video imaging (Fig. 3b), and they did not trigger IL-2 secretion (Fig. 3c). All the transfected cells were responsive to stimulation via mIg. Ig-α and Ig-β cytoplasmic tails trigger two different signaling pathways, which are therefore both dependent on ITAM tyrosine residues. As shown by others (15, 19–21), the conserved tyrosines residues are required for the induction of transmembrane signaling through Ig-α and Ig-β. Because both chains activate different signaling pathways, some Ig-α or Ig-β specific amino acids must modulate their signaling activities.

**Four Amino Acids in the ITAM Determine the Signaling Capabilities of Ig-α and Ig-β Cytoplasmic Domains**—One major difference between Ig-α and Ig-β ITAM consists of four amino acids preceding the second tyrosine of the motifs. The peptide sequence DCSM is present in Ig-α, whereas Ig-β contains the sequence QTAT. This difference has been related to the specific binding of src family kinases to the cytoplasmic tails of Ig-α or Ig-β. In a nonphosphorylated status, only the molecules bearing the four amino acids DCSM bind the tyrosine kinase fyn (14). The role of these four amino acids in the signaling activity of Ig-α and Ig-β was investigated by expressing two chimeras containing a switch of the sequences QTAT versus DCSM in Ig-α and Ig-β cytoplasmic tails (c.Ig-α QTAT and c.Ig-β DCSM) (see Table I and Fig. 2). The cross-linking of c.Ig-β DCSM triggered the phosphorylation of numerous intracellular proteins and an extracellular calcium influx leading to IL-2 secretion (Fig. 4, a, b, and c). The exchange of QTAT by DCSM inside the entire Ig-β cytoplasmic domain was therefore sufficient to convert the transducing phenotype of Ig-β in that of Ig-α tail. Because the cross-linking of c.Ig-β cytoplasmic tail was not able to trigger a calcium influx, the capacity of c.Ig-α and c.Ig-β DCSM to trigger a calcium influx is probably determined by the presence of the DCSM polypeptide sequence in their cytoplasmic tail. In contrast, the conversion of the DCSM sequence into QTAT in the Ig-α tail did not prevent the triggering of calcium influx and tyrosine kinase activation (Fig. 4, a, b, and c). In all these experiments, the transfected cells were responsive to anti-IgG. The results obtained with the switching mutants showed that the ability of Ig-β to trigger different intracellular events may be modulated by amino acids located between the conserved ITAM residues, whereas the similar switch (QTAT) in the entire Ig-α cytoplasmic tail does not affect its signaling capacity. The conformation induced by the peptide sequences surrounding Ig-β ITAM must be important to induce its interactions with specific intracellular effectors.

**The Activity of Ig-β ITAM Is Regulated by Flanking Sequences**—The sequences flanking the two motifs are different between Ig-α and Ig-β cytoplasmic tails. To test whether flanking sequences affect the signaling capacities of ITAMs, chimeras containing isolated Ig-α or Ig-β motifs (c.Ig-α m and c.Ig-β m; Table I) were expressed in IIA1.6 cells (Fig. 2). The stimulation of c.Ig-α m induced tyrosine phosphorylation of several intracellular substrates similar to those induced by the cross-linking of c.Ig-α, with a maximum of phosphorylation intensity after 1 min of stimulation (Fig. 5a). The pattern of calcium signaling obtained after cross-linking of c.Ig-α m was similar to the pattern obtained after stimulation of c.Ig-α, comprising a release of calcium from the intracellular stores and an influx of extracellular calcium (Fig. 5b). This calcium response triggered by the cross-linking of c.Ig-α m was followed by later events of signals transduction, as measured by IL-2 secretion (Fig. 5c). Thus, when isolated, the Ig-α ITAM was as efficient as the entire Ig-α cytoplasmic tail and as the whole BCR in triggering cytokine production. The ITAM of Ig-α is therefore fully functional inside the entire Ig-α tail environment, and it is not regulated directly by its flanking sequences. Surprisingly, the isolated Ig-β ITAM had different signaling abilities than the entire cytoplasmic tail of Ig-β. In contrast to intracellular calcium oscillations induced by Ig-β chimeras, the cross-linking of c.Ig-β m triggered a complete calcium response composed of an initial calcium release from intracellular stores followed by an extracellular calcium influx (Fig. 5b), like the cross-linking of mIg, c.Ig-α, or c.Ig-α m. Moreover, the stimulation of c.Ig-β m was very efficient in inducing IL-2 secretion (Fig. 5c). In contrast, no clear differences of phosphoproteins were detected after cross-linking of c.Ig-β m or c.Ig-β, whereas
the maximum intensities of the phosphorylations were respectively observed after 2 and 1 min of cross-linking (Figs. 1a and 5a). Thus, the isolated Igβ ITAM and the entire cytoplasmic tail of Igβ are both able to activate tyrosine kinases but differ in terms of calcium signaling and triggering of IL-2 secretion.

In contrast, Igα ITAM activity is modulated by amino acids located between the conserved residues and by the ITAM flanking sequences, although the signaling capacities of both ITAMs require conserved tyrosine residues.

The transducing events triggered by Igα ITAM are similar to those triggered by the entire intracellular domain of Igα and by the whole BCR. In this cascade of intracellular signaling in terms of calcium signaling and triggering of IL-2 secretion.

These results show that the two isolated Igα and Igβ ITAMs are able to trigger the same intracellular events leading to cytokine production and that the unconserved environment of an ITAM can regulate the signaling activity of this kind of tyrosine-based activating motifs.

**DISCUSSION**

Stimulation of BCR triggers intracellular events such as protein kinase activation and increase of intracellular calcium concentration resulting in cell activation. In the present work, we evaluated the relative contributions of different domains of Igα and Igβ cytoplasmic tails in B cell signaling. The cytoplasmic domain of Igα, and more specifically its ITAM, reflects the transducing capacities of the whole BCR in terms of phosphoproteins induction, calcium mobilization, and IL-2 secretion, which is dependent on the triggering of a calcium influx. In contrast, Igβ ITAM activity is modulated by amino acids located between the conserved residues and by the ITAM flanking sequences, although the signaling capacities of both ITAMs require conserved tyrosine residues.

The transducing events triggered by Igα ITAM are similar to those triggered by the entire intracellular domain of Igα and by the whole BCR. In this cascade of intracellular signaling...
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Table 1

| Chimeric constructs of FcR type II and cytoplasmic domains of Ig-α and Ig-β |
|---------------------------------------------------------------------------|
| FeR-Ig-α/β chimeras and mutants     | Cytoplasmic domains |
|-----------------------------------|---------------------|
| c.Ig-α                            | RKRQNEKFVGMPODQEDL| |
| c.Ig-α A23                        | RKRQNEKFVGMPODQEDL|
| c.Ig-α A34                        | RKRQNEKFVGMPODQEDL|
| c.Ig-α QTAT                       | RKRQNEKFVGMPODQEDL|
| c.Ig-α m                          | RKRQNEKFVGMPODQEDL|
| c.Ig-β                            | RKRQNEKFVGMPODQEDL|
| c.Ig-β A15,A26                    | RKRQNEKFVGMPODQEDL|
| c.Ig-β DCSM                       | RKRQNEKFVGMPODQEDL|
| c.Ig-β m                          | RKRQNEKFVGMPODQEDL|

Amino acid residues are numbered starting at the first cytoplasmic amino acid residue proximal to the transmembrane domain. The sequences of Ig-α and Ig-β cytoplasmic tails are aligned, and mutations are indicated in bold letters. Tyrosine residues are indicated by arrows. Tyrosine residues are numbered starting at the first cytoplasmic amino acid residue proximal to the transmembrane domain. The sequences of Ig-α and Ig-β cytoplasmic tails are aligned, and mutations are indicated in bold letters. Tyrosine residues are indicated by arrows.

Fig. 5. Ig-α ITAM functions as the entire cytoplasmic tail of Ig-α, whereas the motif of Ig-β does not function as the entire intracellular domain of Ig-α. a, intracellular protein phosphorylation induced by the cross-linking of the chimeras for the indicated times. The cells were also stimulated by their endogenous mIgG (arrow). b, measurement of intracellular calcium concentration after cross-linking of chimeras containing the Ig-α or Ig-β ITAM (c.Ig-α m and c.Ig-β m, respectively). The arrows indicate the triggering of the stimulation, and the boxed curves represent an example of a single cell response. c, stimulation of both chimeras triggered IL-2 secretion. The cells all produced IL-2 after cross-linking of mIgG. This experiment was done under the same conditions as described in the legend to Figs. 3 and 4.

The specificity of Ig-α and Ig-β tails to trigger different signaling events seems determined by nonconserved sequences between the cytoplasmic tails of Ig-α and Ig-β. One of the differences between the two ITAM-amino acid sequences is constituted of four amino acids located between the conserved tyrosine residues (DCSM in Ig-α and QTAT in Ig-β). Our results show that this difference of four amino acids do not seem to play a role in the transduction events triggered by the isolated motifs (Fig. 5), but it is important when the motifs are examined in their entire cytoplasmic environment. Indeed, the conversion of the four amino acids QTAT into DCSM in the Ig-β cytoplasmic tail changed the signaling activity of Ig-β into those of Ig-α (Fig. 4), perhaps by enhancing the basal level of fyn associated with the chimera, because only the DCSM-bearing ITAMs were able to interact with fyn in vitro (14). In contrast, when the amino acids DCSM were changed into QTAT in Ig-α cytoplasmic tail, the transducing capacities of Ig-α were not switched, showing that QTAT is necessary but
not sufficient to inactivate the calcium influx and/or to activate calcium oscillations. These results indicate that there are other amino acids inside or outside the Ig-β ITAM that also regulate its signaling capacities.

The ITAM of Ig-β, in contrast to the entire cytoplasmic domain of Ig-α, is fully efficient to trigger intracellular events leading to lymphokine secretion. This indicates that ITAM flanking sequences regulate the activity of the Ig-β ITAM. These sequences are inefficient, however, when the four amino acids QTAT are replaced by DCSM (Fig. 4). This suggests that other amino acids that differ between the two motifs, like the four amino acids located before the first tyrosine of the two motifs (DENL in Ig-α and EDHT in Ig-β), may play a role in the regulation capacity of flanking regions of Ig-β ITAM. This sequence could regulate the Ig-β ITAM by interacting with intracellular proteins, like the two unidentified p40 and p42 phosphoproteins (9), which bind specifically to Ig-α and Ig-β intracellular signaling. First, the nonconserved sequences located inside or outside the ITAM induce conformational modifications, which modulate the affinity of cytoplasmic effectors for conserved tyrosine residues located in the ITAM. Second, each domain of the cytoplasmic tails of Ig-α and Ig-β (QTAT, DCSM, or flanking sequences) may interact with distinct intracellular effectors, which are specifically recruited by receptor aggregation and thus determine the activation of different intracellular signaling pathways. However, the fact that the deletion of one of the flanking sequences is enough to allow Ig-β to trigger IL-2 secretion after stimulation (data not shown) supports a conformational role for the flanking sequences.

The signaling activity of the BCR-associated subunits can be regulated by cross-linking of mlg with FcR (22, 26) involving the phosphatase PTP1C, which acts as a trans-regulator of the Ig-α/Ig-β ITAM activity (27). Our results show that QTAT or flanking sequences regulate Ig-β ITAM signaling activities, meaning that these activating motifs can be regulated in a cis-position. It remains to establish what is the function of these sequences in the whole BCR complex and whether cytoplasmic ligands of Ig-β cytoplasmic tail may regulate the signaling activity carried out by Ig-α cytoplasmic tail, as is suggested by results showing that mlg stimulation of spleen B cells could trigger calcium oscillations (28). During B cell activation and differentiation, BCR composition may vary because heterodimers with cytoplasmic deleted isoforms of Ig-α or Ig-β have been reported (29, 30). The heterodimers that contain one of these deleted forms therefore probably have the signaling capacities of either Ig-α or Ig-β. Variations in cytosol composition could also determine the activation of one chain more than the other or could trigger different events depending on the effectors present in the cells, like for Ig-β chimeras in T cells that are able to induce IL-2 secretion (17, 20). The analysis of relative signaling capacities of Ig-α and Ig-β and their peptide sequence requirement is a first step in understanding BCR signaling events during the different stages of B cell differentiation or activation.

Acknowledgments—We thank D. Lankar and M. A. Marloie for excellent technical participation and Dr. M. Partiseti and Dr. J. Verheugen for help in the calcium studies. We thank Dr. S. Amigorena and Dr. J. Salamero for advice during manuscript preparation.

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J. Biol. Chem. 1996, 271:23786-23791.
doi: 10.1074/jbc.271.39.23786

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