Igα and Igβ Are Functionally Homologous to the Signaling Proteins of the T-Cell Receptor

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Signal transduction by antigen receptors and some Fc receptors requires the activation of a family of receptor-associated transmembrane accessory proteins. One common feature of the cytoplasmic domains of these accessory molecules is the presence of at least two YXXA repeats that are potential sites for interaction with Src homology 2 domain-containing proteins. However, the degree of similarity between the different receptor-associated proteins varies from that of T-cell receptor (TCR) ζ and Fc receptor RIIIA γ chains, which are homologous, to the distinctly related Igα and Igβ proteins of the B-cell antigen receptor. To determine whether T- and B-cell antigen receptors are in fact functionally homologous, we have studied signal transduction by chimeric immunoglobulins bearing the Igα or Igβ cytoplasmic domain. We found that Igα and Igβ cytoplasmic domains were able to activate Ca2+ flux, interleukin-2 secretion, and phosphorylation of the same group of cellular substrates as the TCR in transfected T cells. Chimeric proteins were then used to examine the minimal requirements for activation of the Fyn, Lck, and ZAP kinases in T cells. Both Igα and Igβ were able to trigger Fyn, Lck, and ZAP directly without involvement of TCR components. Cytoplasmic tyrosine residues in Igα were required for recruitment and activation of ZAP-70, but these amino acids were not essential for the activation of Fyn and Lck. We conclude that Fyn and Lck are able to recognize a clustered nonphosphorylated immunoglobulin receptor, but activation of these kinases is not sufficient to induce cellular responses such as Ca2+ flux and interleukin-2 secretion. In the molecular structures involved in antigen receptor signaling pathways are conserved between T and B cells.

B-lymphocyte antigen receptors have two primary functions: first, to trigger secretion of specific antibodies in response to antigen, and second, to capture antigen for presentation to T cells. This antigen receptor is composed of four polypeptides, immunoglobulin heavy and light chains, Igα, and Igβ (17, 18). Igα and Igβ are transmembrane proteins that form a disulfide-linked heterodimer which in turn noncovalently linked to immunoglobulin heavy chains through polar residues in the transmembrane domain of immunoglobulin (20, 44). Igα and Igβ are essential for two aspects of B-cell antigen receptor function. They are required for cell surface transport of monomeric immunoglobulin, and the cytoplasmic domains of Igα and Igβ function as the signal transducers for the receptor (20, 26, 44, 52).

There is extensive experimental evidence that the signaling functions of Igα and Igβ are mediated by activation of Src family kinases. Cross-linking of the B-cell antigen receptor complex results in the activation of Lyn, Fyn, Blk, and Syk, all of which become receptor associated (3, 6, 58, 59). In addition, activation of the B-cell antigen receptor requires coexpression of the CD45 phosphatase (24, 27). Finally, the conserved cytoplasmic tyrosine residues in the accessory proteins are phosphorylated when the receptor is cross-linked, and these amino acids are essential for receptor function (4, 5, 15, 16, 44).

The antigen receptor on B cells shares a number of important features with the T-cell receptor (TCR) and Fc receptor RIIIA (25). All of these receptors have similar multisubunit structures, in which the signal-transducing components contain a cytoplasmic sequence motif that includes a tyrosine separated from an aliphatic residue by two amino acids (YXXA) (39). An important emerging concept is that all of these accessory proteins function by activating similar biochemical pathways (39). This idea has been substantiated for the TCR ζ chain and Fc receptor γ chain, which are functionally homologous proteins (28, 31, 33, 41, 57). On the basis of these experiments, it has been proposed that Igα and Igβ are functionally homologous to the T-cell and Fc receptor signaling proteins. However, Igα and Igβ are at best distantly related to these other antigen receptor proteins (17–19, 39, 42).

In this communication, we report on experiments that test the idea that T- and B-cell antigen receptors function through the activation of a set of closely related amplification cascades. Chimeric receptors that contained Igα and Igβ cytoplasmic sequences induced specific cellular responses in transfected T cells and activated both Src and Syk family kinases. Igβ, which contains only two cytoplasmic tyrosine residues, was then used as a model to explore the role of receptor tyrosine phosphorylation in the activation of Fyn, Lck, and ZAP-70.

MATERIALS AND METHODS

DNA constructs. Immunoglobulin (p459), Igα (p467), Igβ (p466), immunoglobulin M (IgM):Igα (p520), and IgM:Igβ (p523) have been described elsewhere (12, 44). IgM:Igβ-2Y/2F was obtained by site-directed mutagenesis of tyrosines 195 and 206 in Igβ in an IgM:Igβ membrane exon cassette (44).

Cell lines. Jurkat cells were grown in RPMI 1640 supple-
mented with 10% bovine calf serum, 50 U of penicillin per ml, 50 μg of streptomycin per ml, and 2 mM L-glutamine (R10). Cells were transfected with linear plasmid DNA by electroporation (37), and selection was carried out as previously described (12). Resistant cell lines were stained with 10 μg of fluorescein-labeled goat anti-human IgM (Southern Biotechnology) per ml, and positive cells were sorted on a FACStar Plus (Becton Dickinson). Analysis of surface staining was performed with the same antibody, using a FACSscan (Becton Dickinson).

**Antibodies and PC-OVA.** The anti-human CD-3 antibody OKT3 (30) was purified from either ascites fluid or tissue culture medium by precipitation with ammonium sulfate and chromatography on protein A-Sepharose (Pierce). Goat anti-human IgM Fab(ab')2 for cross-linking the B-cell receptor was from Jackson Immunoresearch; goat anti-human IgM antibody and anti-IgG1 monoclonal isotype control antibody were from Southern Biotechnology. Rabbit anti-src family kinase and anti-phosphotyrosine antibodies have been described elsewhere (60). Anti-ZAP-70 and anti-ζ antibodies were produced in rabbits by immunization with a chimeric protein composed of glutathione S-transferase fused to the either the first 300 amino-terminal amino acids of human ZAP-70 or the cytoplasmic domain of human ζ (7, 46, 55). Murine antiphosphotyrosine monoclonal antibody SH1 was produced by immunizing mice with a copolymer of phosphotyrosine, glycine, and alanine that was coupled to keyhole limpet hemocyanin (13). To modify ovalbumin with phosphocholine (Sigma), the protein was dissolved in phosphate-buffered saline and incubated overnight with para-amino-octylphosphocholine (DPPC) synthetized as described previously (8). Phosphocholine-modified ovalbumin (PC-OVA) was separated from unreacted DPPC by column chromatography on Sephadex G-25 in phosphate-buffered saline.

**Calcium flux and IL-2 measurements.** Jurkat cells were loaded with Fura-2 (Molecular Probes Inc., Eugene, Ore.) and assayed spectrofluorimetrically for calcium mobilization (12) in response to 60 μg of polyclonal anti-human IgM per ml as previously described (12). However, the unit standard was calculated according to the biological response modifiers unit standard established by the National Institutes of Health; there is a 20-fold differential between this new standard and the previous standard (12). Each experiment was done in triplicate, and the difference between cultures was less than 10%.

**Cellular activation.** Tissue culture cells were resuspended in serum-free RPMI at 107 cells per ml and equilibrated at 37°C for 15 min. Anti-CD3(1 μg/ml) or F(ab')2 anti-IgM (10 μg/ml) antibodies were added, and aliquots were removed at the indicated time points. Cells were lysed in 1% Nonident P-40–50 mM Tris-HCl (pH 8.0)–150 mM NaCl–2 mM EDTA–10 mg of aprotinin per ml–10 mg of leupeptin per ml–0.5 mM phenylmethylsulfonyl fluoride–1 mM sodium orthovanadate. Insoluble material was removed by centrifugation in a microcentrifuge for 3 min at 4°C, and the supernatants were processed for immunoprecipitation.

**Biochemical assays.** Immunoprecipitation, immunoblotting, and immune complex kinase assays were conducted exactly as previously described (9). Quantitation of phosphorylated reaction products of the immune complex protein kinase assays was performed either by scanning multiple exposures of relevant autoradiograms with a Bio-Rad model 620 video densitometer, with data computation via 1D Analyst software, or by analysis with a Molecular Dynamics PhosphorImager and ImageQuant software.

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**FIG. 1.** Cell surface expression of human immunoglobulin and calcium flux assays in transfected Jurkat cells. (A) Flow cytometric analysis of surface expression of human IgM on transfected Jurkat cells. Relative cell number is plotted against fluorescence intensity on a logarithmic scale. The constructs transfected are indicated at the top. Cells were assayed fluorimetrically for calcium mobilization in response to either anti-CD3 (1.5 μg/ml), anti-human IgM (10 μg/ml), or an isotype control monoclonal antibody (10 μg/ml), as indicated. (B) Calcium mobilization responses of cell lines transfected with IgM:Igα (p520) in response to 2.5 mM phosphocholine, 500 ng of PC-OVA per ml, or 10 μg of anti-human IgM per ml, as indicated by arrows. IgG, isotype control IgG1 monoclonal antibody; anti-CD3, anti-CD3 antibody; anti-IgM, anti-IgM antibody; PC, phosphocholine; JURKAT, untransfected Jurkat cells; IgM:Igα, Jurkat cells transfected with IgM:Igα (p520); IgM:Igβ, Jurkat cells transfected with IgM:Igβ (p523); IgM:Igβ-2Y/2F, Jurkat cells transfected with IgM:Igβ-2Y/2F (p517).

**RESULTS**

**Igα and Igβ induce Ca²⁺ responses in T cells.** To isolate the signaling domains of Igα and Igβ for biochemical and functional analysis, we constructed chimeric proteins composed of the external and transmembrane domains of immunoglobulins and the cytoplasmic tails of Igα and Igβ (IgM:Igα and IgM:Igβ, respectively) (44). To ensure efficient surface transport of the chimeric immunoglobulins, tyrosine 587 and serine 588 in the transmembrane domain of IgM were replaced with two valine residues (34, 35, 44, 45, 56). The Jurkat T-cell line, which has been used extensively to study TCR function, was transfected with the chimeric receptors, and T cells expressing high levels of transfected immunoglobulin were enriched by cell sorting (Fig. 1). In all cases, the transfected Jurkat cells continued to express endogenous TCR, which was used as a positive control.

In the absence of Igα and Igβ, membrane immunoglobulin has no signaling activity in transfected T cells (12). Addition of the Igα or Igβ cytoplasmic domain to immunoglobulin results in a chimeric receptor that can trigger a Ca²⁺ flux in response to either antireceptor antibodies or the ligand.
PC-OVA (Fig. 1). Monovalent phosphorylcholine was a specific antagonist in that it blocked the response to the polyvalent ligand PC-OVA, but it did not interfere with the response to antireceptor antibody (Fig. 1B). Furthermore, the response elicited by the chimeric antigen receptors was comparable to that elicited by the cross-linking the endogenous TCR. To determine whether the Ca\(^{2+}\) response induced by IgM: Ig\(\beta\) was mediated through cytoplasmic tyrosine residues, we constructed an IgM:Ig\(\beta\) mutant in which phenylalanine was substituted for tyrosines 206 and 195 (IgM:Ig\(\beta\)-2Y/2F). We found that IgM:Ig\(\beta\)-2Y/2F was completely inactive in Ca\(^{2+}\) flux assays. We concluded that the cytoplasmic domains of both Ig\(\alpha\) and Ig\(\beta\) carry all of the sequence information necessary to convert immunoglobulin into an active receptor in T cells. In addition, tyrosines 195 and 206 in Ig\(\beta\) were essential to trigger a Ca\(^{2+}\) flux.

**IL-2 is induced by Ig\(\alpha\) and Ig\(\beta\).** Although Ca\(^{2+}\) flux is a measure of activation, it is a membrane-proximal event. To determine whether the B-cell receptor components could trigger complex T-cell responses, we measured IL-2 secretion in the transfected T cells. We chose IL-2 secretion because this response requires induction of nuclear events specific for T lymphocytes (47). Cross-linking either the IgM:Ig\(\alpha\) or IgM:Ig\(\beta\) chimera led to IL-2 secretion in the transfected T cells (Fig. 2). In addition, the IL-2 responses were abrogated in the IgM:Ig\(\beta\)-2Y/2F mutants (Fig. 2). Thus, B-cell antigen receptor cytoplasmic domains can trigger the entire signaling cascade for IL-2 production in T cells, and this response is dependent on the presence of tyrosine residues in Ig\(\beta\).

**Tyrosine phosphorylation of cellular proteins.** Cross-linking the TCR leads to the activation of a number of Src family kinases and the T-cell specific tyrosine kinase ZAP-70. These kinases then act on a large number of characteristic cellular substrates. As an initial measure of the proteins involved in the signaling pathways triggered by chimeric immunoglobulins in T cells, we compared the substrates that were tyrosine phosphorylated by cross-linking transfected immunoglobulin and TCR. Cross-linking IgM when it is expressed alone produces no measurable response (Fig. 3). However, addition of intact Ig\(\alpha\) and Ig\(\beta\) to IgM by transfection results in the assembly of a receptor that triggers the phosphorylation of many of the same substrates as the TCR with similar kinetics (Fig. 3). The triggers for the phosphorylation response were localized to the cytoplasmic domains of both Ig\(\alpha\) and Ig\(\beta\), since the addition of either of these sequences to membrane immunoglobulin in chimeric proteins converted immunoglobulin to an active receptor (Fig. 3). The ability to induce tyrosine phosphorylation was dependent on the presence of tyrosines 195 and 206 in Ig\(\beta\) cytoplasmic domain, since changing these residues to phenylalanine inactivated the IgM:Ig\(\beta\) chimera (IgM:Ig\(\beta\)-2Y/2F; Fig. 3).
Although both Igα and Igβ cytoplasmic domains contained sufficient information to produce chimeric immunoglobulins that triggered phosphorylation responses, IgM:Igα and IgM:Igβ were not identical. IgM:Igβ produced responses that were slightly delayed and of lower magnitude than those produced by IgM:Igα. Nevertheless, the phosphorylated substrates were qualitatively similar for all of these receptors. The only clear exceptions to this rule appeared to be proteins that were part of the specific receptor cross-linked in any given experiment. For example, additional 85-kDa tyrosine-phosphorylated proteins consistent with the chimeras appear after cross-linking with anti-IgM, but these proteins are not phosphorylated when the TCR is stimulated in the same cells (Fig. 3). Conversely, phosphorylation of a low-molecular-weight substrate with the same molecular weight as ζ was induced by cross-linking TCR but not by cross-linking IgM:Igα or IgM:Igβ (not shown). To verify that TCR proteins were not involved in signaling by the chimeric immunoglobulin receptors, we assayed for tyrosine phosphorylation of ζ by immunoprecipitation and Western blotting (immunoblotting). Cross-linking the TCR leads to rapid-onset tyrosine phosphorylation of ζ, but signaling through IgM:Igα or IgM:Igβ in the same cells has no effect on the level of ζ phosphorylation (Fig. 4). In contrast, immunoprecipitation and antiphosphotyrosine immunoblotting of the chimeric immunoglobulins after cross-linking with anti-IgM confirms that IgM:Igα and IgM:Igβ are phosphorylated only after cross-linking with anti-IgM (Fig. 4). The kinetics of Igα and Igβ tyrosine phosphorylation mirrors the kinetics of cellular substrate phosphorylation induced by the chimeric receptors in that Igβ phosphorylation is delayed in comparison with Igα phosphorylation. We conclude that many of the substrates phosphorylated in response to cross-linking the chimeric B-cell receptors in T cells are similar to those induced with TCR engagement; however, there is no detectable cross-talk between the receptors.

**Activation of Src kinases and ZAP-70.** Our IL-2 and tyrosine phosphorylation experiments suggested that B-cell receptor components can activate the same set of biochemical pathways as the TCR. To test this idea directly, we monitored the activation of ZAP-70, Fyn, and Lck following receptor cross-linking (Fig. 5, 6, and 7, respectively). Activation of ZAP-70 was measured by immunoprecipitation with specific anti-ZAP-70 antibodies followed by immunoblotting with antiphosphotyrosine antibodies. Western blots with anti-ZAP-70 antibodies are shown as a control for the total amount of ZAP-70 protein in each of the samples. We found that ZAP-70 was phosphorylated upon cross-linking either the Igα or Igβ chimera. The kinetics of phosphorylation were slower for Igα than for Igβ or TCR, and the level of ZAP phosphorylation achieved by cross-linking the chimeras was somewhat lower than that observed by activating ZAP through the TCR. However, activation of ZAP-70 by Igβ is analogous to activation by the TCR, since tyrosine residues in the cytoplasmic domain of Igβ were required for ZAP-70 induction (Fig. 5, IgM:Igβ/2Y-2F). Following receptor cross-linking, ZAP-70 was associated with the activated receptor, as detected by coimmunoprecipitation of ZAP-70 with phosphorylated ζ (Fig. 5). The association was specific, since ζ was associated with ZAP-70 only after TCR cross-linking.

To evaluate the activities of Lck and Fyn, the enzymes were immunoprecipitated from cell lysates by using antisera specific for Lck or Fyn, and immune complex protein kinase assays were conducted. Parallel immunoblots were also performed to determine the relative abundance of each
enzyme over the time course of the experiments. The results of these experiments demonstrate that both Fyn (Fig. 6) and Lck (Fig. 7) protein kinases activities were transiently stimulated following CD3 cross-linking. The level of peak activation for both enzymes (as determined by quantitation of autophosphorylation) was in most cases between two- to threefold. The degree of stimulation of these protein kinases as well as the transient nature of their response to CD3 cross-linking is consistent with previous observations concerning the effect of immune recognition receptor surface engagement on Src family protein tyrosine kinases in different cell types (1, 3, 6, 14, 51, 60). An additional independent measure of Fyn activation is the increased tyrosine phosphorylation of p116, which is a T-cell protein that specifically associates with activated Fyn through Fyn Src homology 2 domain (SH2)-p116 phosphotyrosine interactions (51, 23). Also phosphorylated in the Fyn and Lck immune complexes following CD3 cross-linking were the Igα and Igβ chimeras, which coprecipitate as a result of their binding to Staphylococcus aureus protein A used in the immunoprecipitation reactions. Interestingly, the chimeras with wild-type Igα and Igβ cytoplasmic sequences appeared to serve as excellent exogenous substrates in the immune complex kinase assays, and their phosphorylation paralleled the level of Fyn and Lck autophosphorylation. For Fyn, the wild-type Igα and Igβ chimeras appeared to compete with phosphorylation of
and Fyn or Lck immune complex protein kinase assays were performed. The results of these experiments show that the protein kinase activities of Lck (Fig. 7) and to a lesser extent Fyn (Fig. 6) could be stimulated by IgM:Igβ-2Y/2F. In the case of Fyn, changes in autophosphorylation were less apparent than the phosphorylation of the Fyn-associated p116 (Fig. 6). As expected, in these experiments the chimera harboring the double tyrosine mutant was not phosphorylated in the immune complex kinase assays (Fig. 6 and 7).

Together, the results of these experiments indicate that Igα and Igβ cytoplasmic domains are capable of mediating the activation of Fyn and Lck in T cells. Phosphorylation of tyrosine residues in the Igβ cytoplasmic domain was found to be dispensable for Lck and Fyn stimulation. In contrast, tyrosine residues were required for the activation of ZAP-70 and for coupling surface-initiated signal transduction with T-cell activation responses.

**DISCUSSION**

There is persuasive evidence that the TCR-associated proteins ε and ζ, as well as immunoglobulin-associated Igα, Igβ, and Fc receptor γ, are signal-transducing proteins. In each case, the isolated cytoplasmic domains have been shown to function autonomously for activation of cellular signaling programs (23, 26, 31, 32, 41, 44, 54, 57). In addition, the cytoplasmic domains of TCR ζ and Fc receptor γ subunits, which are the only members of this family that are closely related, can substitute for each other in transfection assays (28, 31, 40, 41, 57). The other members of the family share YXXA SH2 target motifs spaced by seven amino acids, and in all cases tested the conserved tyrosines are essential for signal transduction (28, 32, 40, 44). There are three repeats of the target motif in ζ, and although all three are not required, an increase in the number of motifs increases the efficiency of activation, which may explain some of the redundancy in the system (22, 31, 40, 54).

Although the tyrosine residues and aliphatic amino acids appear to be critical for activation, the spacing between the YXXA motifs is not critical since some of the members of this family, including TCR ζ, have altered spacing between the SH2 target sequences (28). A complete analysis of the stringency required in the spacing between the YXXA motifs is not available, but the number of proteins with YXXA motifs spaced by 7 to 10 amino acids in a current search of the data base is very large. Thus, the known sequence requirements are at best less than rigorous and in practice fail to provide a stringent definition of the active segment of the signaling motif. We would like to suggest that sequence information alone is not sufficient to conclude that each of these proteins activates cellular responses through homologous metabolic pathways.

T-cell lines are an excellent system with which to study the signaling activity of candidate immune recognition receptors subunits, since the TCR has been the most intensively studied member of the antigen receptor family. Essential elements in the TCR signaling pathway include the Src family kinase Lck and the CD45 phosphatase (27, 29, 36). Activation of Lck leads to the tyrosine phosphorylation of TCR ζ as well as other CD3 components and the recruitment of the ZAP-70 kinase (7, 49). Fyn, another Src family kinase, is not essential for signaling in vivo, but Fyn is clearly a part of the TCR signaling pathway, since it is associated with the TCR and T cells that lack Fyn have a substantial defect in cellular activation responses (2, 11, 43, 48). Like Lck, Fyn can participate in activation of Syk
family kinases in transfection experiments with either the intact enzyme or chimeric forms of the same kinases (7, 28a). Syk family kinases are likely to be the key amplification enzymes in the signaling cascades, since chimeric receptors that contain Syk family kinases activate a number of T-cell responses (28a).

Despite our understanding of the metabolic enzymes involved in cellular activation by the intact TCR and a clear definition of the triggering proteins, there is little information on the ability of individual triggering proteins to activate the Src family kinases in vivo. For example, the cytoplasmic domains of TCR $\zeta$ and $\epsilon$ have been shown to activate complex cellular responses, but their ability to activate Lck or Fyn has not been studied. We have used chimeric immunoglobulin proteins to study the molecular mechanism of signaling by Ig$\alpha$ and Ig$\beta$. Both Src and Syk family kinases are activated by IgM:Ig$\alpha$ and IgM:Ig$\beta$, although the extent of activation was somewhat lower than that induced by the endogenous receptors. Thus, despite a high homology between Ig$\alpha$, Ig$\beta$, and TCR components, all of these proteins activate the same metabolic pathways without cross-activation of the TCR by IgM chimeras and vice versa. We conclude that activating a specific group of antigen receptors on the cell surface does not result in the recruitment of unoccupied receptors. In view of the extensive phosphorylation of cellular proteins induced by receptor cross-linking, these results suggest that there may be a specific mechanism that regulates the phosphorylation state of unoccupied receptors.

A working model for cellular activation by the TCR and other members of the immune recognition receptor family is that aggregation of tyrosine-containing signaling proteins results in Src kinase activation. In this scheme, the Src kinases phosphorylate the trigger proteins and recruit and activate the Syk family kinases. One of the predictions of this model is that recognition of the clustered triggering molecules and activation of the Src kinases should be independent of the state of phosphorylation of the trigger molecules. Our experiments are the first to demonstrate that Src kinases can be activated by a trigger molecule with no cytoplasmic tyrosines. Cross-linking of IgM:Ig$\beta$2F activates Lck and Fyn, as measured increased kinase activity and phosphorylation of p116. However, the level of Fyn activation by IgM:Ig$\beta$2F is significantly lower than that observed with either the wild-type chimera or the TCR, suggesting that in addition to a recruitment function for the Syk kinases, the tyrosines in the signaling proteins may also play an important role in the activation of the Src family kinases. Despite the activation of Fyn by IgM:Ig$\beta$2F, this mutant receptor does not trigger Ca$^{2+}$ flux, IL-2 secretion, or tyrosine phosphorylation of cellular substrates. These data support the notion that although the Src kinases may be essential for activation of cellular processes by antigen receptors, they are not sufficient to trigger a biological response.

The chain of events triggered by cross-linking surface IgM in B cells is not as clearly defined as for the TCR in T cells. Several Src kinases are associated with membrane IgM in immunoprecipitation experiments (3, 6, 59), and at least three of these, Lyn, Fyn, and Btk, are activated by cross-linking immunoglobulin in B cells (3). Furthermore, Syk, which is closely related to ZAP-70, is also associated with immunoglobulin antigen receptors, and Syk is activated by receptor cross-linking (7, 21, 28a, 50, 58). An additional level of complexity is added by the different biochemical and functional characteristics of Ig$\alpha$ and Ig$\beta$. Both Ig$\alpha$ and Ig$\beta$ cytoplasmic domains activated Ca$^{2+}$ responses in transfected B-cell lines, but only Ig$\alpha$ induces high levels of tyrosine phosphorylation of cellular substrates (26, 44). In T cells, both IgM:Ig$\alpha$ and IgM:Ig$\beta$ were competent to induce phosphorylation of cellular proteins, and as in B cells, the phosphorylation responses induced by Ig$\alpha$ were greater than those induced by Ig$\beta$. In vitro, the cytoplasmic tail of Ig$\alpha$ has been shown to bind to Lyn and Fyn in B-cell extracts, but Ig$\beta$ was not (10). However, we found that both Ig$\alpha$ and Ig$\beta$ chimeras were able to activate Fyn in vivo. The difference between our results in T cells in vivo and the binding studies in cytoplasmic B-cell extracts may reflect an underlying biological difference in the function of Ig$\alpha$ and Ig$\beta$, or they could simply be result of the difference between the mono mer used in the biochemical studies and the homodimeric trigger molecule in our chimeras.

It is not yet clear which of the B-cell kinases is essential for signaling by immunoglobulin in B cells, nor is it known whether a specific set of enzymes participates in each of the many biological events induced by membrane immunoglobulins. However, our experiments in T cells allow us to make some predictions about the likely signaling functions of the immunoglobulin-associated kinases. Members of the Src family of kinases are closely related, but a careful comparison of the SH2 domains that are involved in shaping the tyrosine and aliphatic residue binding pockets shows that Lyn, Btk, and Lck are almost identical, whereas Fyn is more homologous to Src (53). On the basis of published structural data and sequence comparisons, we would expect that either Btk or Lyn, or both can function as the Lck equivalent in B cells. Fyn is present in both B cells and T cells and is likely to have a distinct role in signaling responses (2, 11, 48). In summary, our findings that immunoglobulin receptor components are functionally homologous to TCR components suggests a model for the regulatory cascade in B cells which is based on the elegant work on TCR and Fc receptors.

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The first two authors contributed equally to this work.

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