Cytoplasmic Domain Heterogeneity and Functions of IgG Fc Receptors in B Lymphocytes

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B lymphocytes and macrophages express closely related immunoglobulin G (IgG) Fc receptors (FcγRII) that differ only in the structures of their cytoplasmic domains. Because of cell type–specific alternative messenger RNA splicing, B-cell FcγRII contains an insertion of 47 amino acids that participates in determining receptor function in these cells. Transfection of an FcγRII-negative B-cell line with complementary DNA's encoding the two splice products and various receptor mutants indicated that the insertion was responsible for preventing both FcγRII-mediated endocytosis and FcγRII-mediated antigen presentation. The insertion was not required for FcγRII to modulate surface immunoglobulin-triggered B-cell activation. Instead, regulation of activation involved a region of the cytoplasmic domain common to both the lymphocyte and macrophage receptor isoforms. In contrast, the insertion did contribute to the formation of caps in response to receptor cross-linking, consistent with suggestions that the lymphocyte but not macrophage form of the receptor can associate with the detergent-insoluble cytoskeleton.

The Fcγ receptors (FcγR) are a closely related family of membrane glycoproteins comprising three classes (FcγRI, FcγRII, FcγRIII) that are distinguished by their affinities for immunoglobulin G (IgG), structures, and cellular distributions (*1, 2*). While all FcγR are characterized by homologous Ig-like extracellular regions, the structures of their membrane anchoring and cytoplasmic domains vary considerably (*1–3*). In the case of murine FcγRII, alternative mRNA splicing produces two isoforms in macrophages or B lymphocytes (*4–7*). The macrophage isoform (FcγRII-B2) has a cytoplasmic domain of 47 amino acids while the B-cell isoform (FcγRII-B1) contains a 47-residue insertion at a site proximal to the membrane. This heterogeneity appears to play a role in regulating receptor function in the two cell types since at least in fibroblasts, only FcγRII-B2 accumulated at clathrin-coated pits and permitted the endocytosis of receptor-bound antibody-antigen immune complexes (ICs) (*8*).

While FcγR have been extensively studied in macrophages, little is known about their activities on B cells. It is not clear whether they exhibit macrophage-associated functions such as endocytosis, phagocytosis, or signal transduction. This is important since even inefficient endocytosis by FcγRII in B cells would have significant consequences for the antigen-presenting functions of these cells. Thus far, however, the only well-described activity for B-cell FcγRII is in the regulation of surface Ig (sIg)–induced B-cell activation. Typically, cross-linking of sIg by antigen or antibody to sIg $F(ab')_2$ fragments causes a transient increase in cytosolic free Ca^{2+} leading to B-cell proliferation, differentiation, and Ig secretion (*9*). This sequence of events is aborted, however, if sIg is cross-linked with intact antibodies to IgG that permit cross-linking of sIg with surface FcγR (*10, 11*). Since this activation-deactivation process is associated with the phosphorylation of serine residues within the FcγRII-B1 insertion, it is conceivable that the insert plays a role in regulating the activation process (*12*). The mechanism of this regulation is unknown but may be related to the fact that FcγRII, like sIg, can form a polar cap on the B-cell plasma membrane; such caps can be associated with cytosolic molecules such as H-*ras* that may be involved in B-cell activation (*13*). We have analyzed the functions exhibited by FcγRII in B cells and the dependence of its activities on regions of the FcγRII cytoplasmic tail that were specifically characteristic of any or common to all macrophage and lymphocyte receptor isoforms. These studies were facilitated by transfection of a receptor-negative mutant of the A20 B-cell lymphoma, IIA1.6, which bears a deletion in the FcγRII gene (*14*).

We first analyzed the ability of the different FcγRII to mediate the endocytosis of ICs of HRP antigen and anti-HRP antibody in the A20 parent cells as well as in IIA.1.6 cells expressing FcγRII-B1, -B2, or deletion mutant receptors that affect coated pit localization and endocytosis in fibroblasts (*15*). Little if any internalization was detected in the FcγRII-B1 expressing A20 cells or in IIA1.6 cells transfected with the FcγRII-B1 cDNA. In contrast, IIA.1.6 cells expressing the FcγRII-B2 isoform exhibited efficient endocytosis of bound HRP-IC's (Fig. 1B). Both the rate and extent of internalization were comparable to that observed in macrophages and transfected fibroblasts (*8, 16*). This observation not only confirmed our results in transfected fibroblasts, but established their biological relevance by showing that the expression of the internalization-competent FcγRII-B2 isoform was sufficient to confer the ability to mediate IC endocytosis.

Internalization of FcγRII-B2 in B cells also required the same cytoplasmic domain determinants as transfected fibroblasts (*15*). As shown in Fig. 1B, B cells expressing B2-CT31 in which amino acids 32 to 47 of the FcγRII-B2 receptor were deleted (Fig. 1A), a mutation that only slightly reduced endocytosis in fibroblasts (*15*), were also able to mediate rapid internalization of HRP-ICs. In contrast, elimination of sequence (residues 19 to 31) that contain the presumptive coated pit localization domain (B2-CT18) or deletion of the entire cytoplasmic domain (B2-CT1) did not internalize HRP-ICs (Fig. 1B). Similarly negative was a FcγRII-B1 mutant (B1-CT53) whose cytoplasmic domain was truncated directly after the 47–amino acid insert, the region corresponding to that required for endocytosis of FcγRII-B2.

To determine whether internalization of HRP-IC's in the FcγRII-B2–expressing B cells reflected the ability of receptor-bound ligand to accumulate in plasma membrane coated pits, we examined coated pit localization of colloidal gold–conjugated ICs by electron microscopy. Colloidal gold–labeled ICs (*8*) were bound to transfected B cells and, after brief incubation at 37°C, became localized on plasma membrane torn from the cell surface (*17*). This procedure was used because B cells had too few plasma membrane coated pits to be visualized by thin section analysis. In the whole mount preparations, clathrin coated pits and planar coated regions were easily recognized by their characteristic hexagonal lattice structure (Fig. 2). Gold-labeled ICs could be localized at coated pits only in cells transfected with FcγRII-B2 (Fig. 2A). Even after scoring several hundred particles, we did not observe any gold at coated pits in FcγRII-B1 transfectants or in A20 cells

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expressing endogenous FcγRII-B1 (Fig. 2B). Although gold was occasionally found in small aggregates associated with an electron opaque material, the aggregates exhibited no similarity to clathrin lattices. Thus, the inability of B cells that express FcγRII-B1 to mediate endocytosis of HRP-IC's complexes was due to their inability to localize receptor-bound ligand at coated pits and not to a general defect in the endocytic pathway in B cells.

There is considerable indirect evidence that antigen processing may occur intracellularly. The basic pathway may include the endocytosis of antigen, delivery to and proteolytic cleavage in one or more endosomal compartments, association of the resultant peptides with class II molecules, and transport of the immunogenic complexes to the plasma membrane for recognition by antigen-specific T cells (*18, 19*). Targeting of antigens to specific cell surface receptors, such as sIg on B cells or FcR or macrophages, enhances the efficiency of presentation relative to that possible with soluble antigen by more than 1000 times (*20, 21*). However, if, in fact, B cells are incapable of FcγRII-B1–mediated endocytosis, binding of antigen to B-cell FcγRII should not lead to efficient presentation (*22, 23*). This defect in IC endocytosis and processing should be overcome in cells expressing internalization-competent FcγRII.

To determine whether the ability of B cells to present FcγRII-bound antigen correlated with the type of FcγRII expressed, the B-cell transfectants were examined for their ability to present lambda (λ) repressor to the 24.4 T cell hybridoma cell line. Transfectants were cultured with the 24.4 cells in the presence of various concentrations of soluble λ repressor or repressor complexed with two monoclonal IgG's (22D and 51F) that recognize distinct epitopes (*24*).

Untransfected, FcRγII-negative IIA1.6 cells presented soluble and IgG-complexed antigen at comparably high concentrations of λ repressor (~1 μg/ml) (Fig. 3A, upper left). Thus, the binding of the two antibodies to λ repressor (anti-λ) neither inhibited nor enhanced presentation in the absence of FcγR on the presenting cell. Cells transfected with FcγRII-B1 exhibited only a slight (two to four times) increase in presentation of the antibody-antigen complexes, showing that binding to a receptor incapable of internalization did not itself enhance presentation efficiency (Fig. 3A, upper right). In contrast, when cells expressing FcγRII-B2 were incubated with IgG-complexed λ repressor, presentation was observed at antigen concentrations that were two to three orders of magnitude lower than that obtained with soluble λ repressor alone (Fig. 3A, lower left). At low doses, this enhanced activity was completely blocked by saturating concentrations of antibody to FcγRII (anti-FcγRII) (2.4G2, monoclonal) demonstrating its dependence on FcγR. The activity was also dependent on the viability of the presenting cells because fixing the FcγRII-B2–expressing B cells (in 0.05 percent paraformaldehyde) prior to incubation with antigen-blocked presentation of immune complexed repressor or soluble repressor.

We next assayed antigen presentation by IIA1.6 cells transfected with the various FcγRII deletion mutants. As expect-

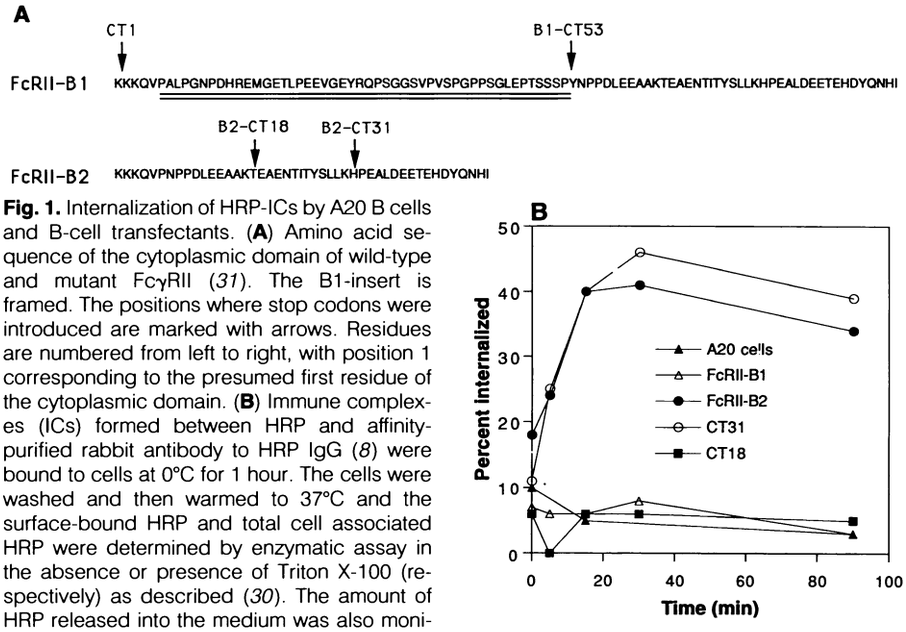

Fig. 1. Internalization of HRP-ICs by A20 B cells and B-cell transfectants. (**A**) Amino acid sequence of the cytoplasmic domain of wild-type and mutant FcγRII (*31*). The B1-insert is framed. The positions where stop codons were introduced are marked with arrows. Residues are numbered from left to right, with position 1 corresponding to the presumed first residue of the cytoplasmic domain. (**B**) Immune complexes (ICs) formed between HRP and affinity-purified rabbit antibody to HRP IgG (*8*) were bound to cells at 0°C for 1 hour. The cells were washed and then warmed to 37°C and the surface-bound HRP and total cell associated HRP were determined by enzymatic assay in the absence or presence of Triton X-100 (respectively) as described (*30*). The amount of HRP released into the medium was also monitored and was negligible. Internalized ICs were thus calculated from the difference between surface and total IC, and the results are given as a percentage of the ligand bound at 0°C in A20 cells (which express endogenous FcγRII-B1) as well as in IIA1.6 cells transfected with FcγRII-B1, FcγRII-B2, B2-CT31, and B2-CT18. Endocytosis mediated by each form of FcγRII was measured in three to five independent experiments, and the values between experiments varied by less than 10% of the total bound ligand. The results shown are from a single representative experiment. Nonspecific binding and uptake (<5%) was determined by including 2.4G2 IgG during the binding of the HRP-ICs.

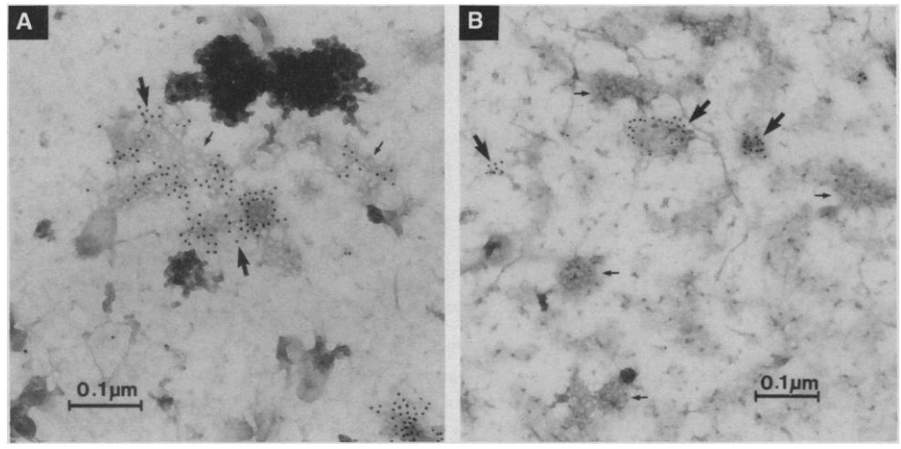

Fig. 2. EM localization of colloidal gold–labeled IC's on the surface of B cells expressing FcγRII-B2 and FcγRII-B1. Colloidal gold-DNP-BSA (dinitrophenol–bovine serum albumin) or rabbit anti-DNP ICs were formed as described (*8*). Cells (1 × 10^7/ml) were resuspended in gold-labeled ICs for 2 hours on ice and then washed by centrifugation to remove unbound gold. The cells were incubated for 3 min at 37°C, washed in cold phosphate-buffered saline (PBS), and attached to Alcian blue–coated cover slips. Plasma membrane was torn from the upper surface of the cells and prepared for electron microscopy as described (*17*) except that the specimens were not carbon-coated before examination. (**A**) FcγRII-B2 transfected B cells. (**B**) FcγRII-B1 transfected B cells. Large arrows indicate the 10-nm colloidal gold–labeled ICs and small arrows indicate the clathrin-coated areas of the membrane.

ed, mutant receptors that were unable to mediate the endocytosis of HRP-ICs were also unable to enhance complexes of the repressor and antibody to the repressor (Fig. 3B). Only the B2-CT31 mutant, a deletion mutant that exhibited both endocytosis and coated pit localization, increased presentation efficiency similar to full-length FcγRII-B2. Thus, FcγRII-B1, the receptor isoform typically expressed by B cells, is unable to mediate enhanced antigen presentation because it cannot be efficiently internalized.

Although the insertion in the FcγRII-B1 cytoplasmic domain prevented internalization and presentation in B cells, there was a possibility that the insertion might also be involved in the FcγRII-dependent modulation of B-cell activation. With Ca^{2+} as a measure of the activation response (*25*), cross-linking of sIg with anti-sIg $F(ab')_2$ elicited a rapid rise in intracellular Ca^{2+} followed by slow decay ($t_{1/2} \sim$ 250 s) (Fig. 4A, top). In contrast, adding intact IgG antibody to sIg which cross-linked surface FcγRII-B1 together with sIg, the initial Ca^{2+} transient was rapidly extinguished ($t_{1/2} < 30$ s) (Fig. 4A, middle). The rapid decay was prevented by including the anti-FcγRII 2.4G2, which prevented the binding of the intact Fc domain of the anti-sIg to FcγRII (Fig. 4A, bottom).

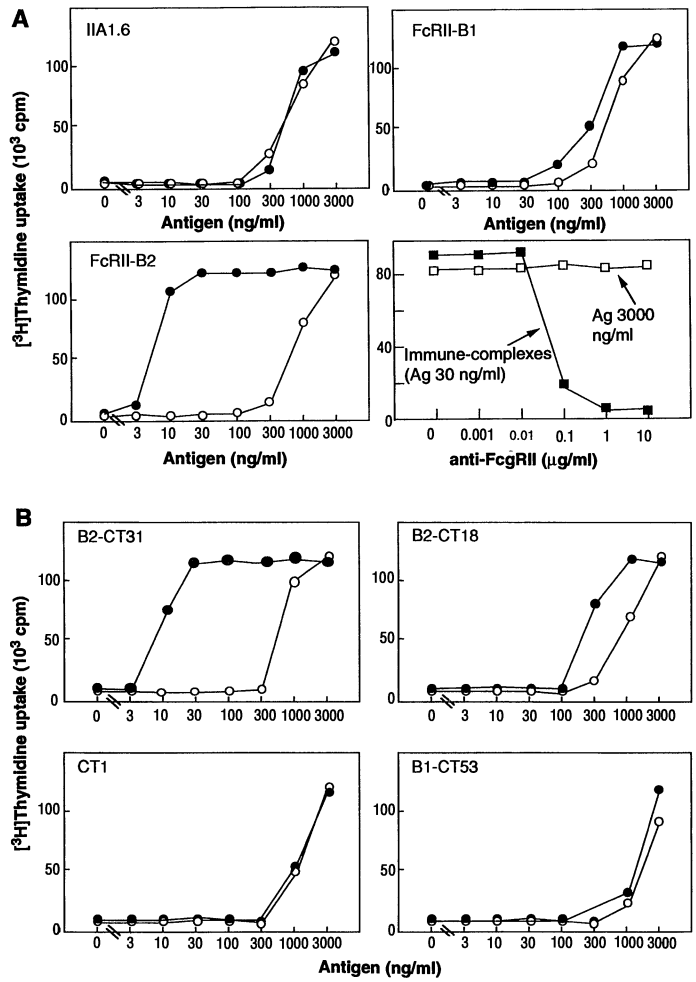

Fig. 3. Presentation of λ repressor and λ repressor immune complexes by B cells expressing wild-type and mutant FcγRII. Transfected IIA1.6 cells were cultured together with the λ repressor–specific T cell hybridoma 24.4 (*32*) for 18 to 20 hours with varying concentrations of the amino-terminal fragment 1 to 102 of λ repressor in the presence (●) or absence (○) of two IgG1 monoclonal antibodies to repressor recognizing distinct epitopes (15 μg/ml, each IgG) (*24, 32*). The presence of IL-2 released by the 24.4 cells into the culture supernatants was determined by the CTL.L2 proliferation assay (*32*) (**A**) λ repressor presentation by receptor-negative nontransfected IIA1.6 cells (upper left), FcγRII-B1-expressing IIA1.6 cells (upper right), or FcγRII-B2-expressing cells (lower left). Enhanced presentation by immune complexes in FcγRII transfectants was prevented by prior treatment with 2.4G2 (10 μg/ml) (bottom right) for 30 min in ice before the addition of λ repressor–containing immune complexes (antigen at 30 ng/ml). The 2.4G2 had no effect on the receptor-independent presentation of soluble λ repressor (3000 ng/ml) (lower left). (**B**) Presentation experiments with IIA1.6 cells expressing B2-CT31 (upper left), B2-CT18 (upper right), CT1 (lower left), or B1-CT53 (lower right). Each point represents the average of duplicate samples which varied by <10%.

Other IIA1.6 transfectants were used to determine the relative abilities of FcγRII-B1, -B2, and the deletion mutants to mod-

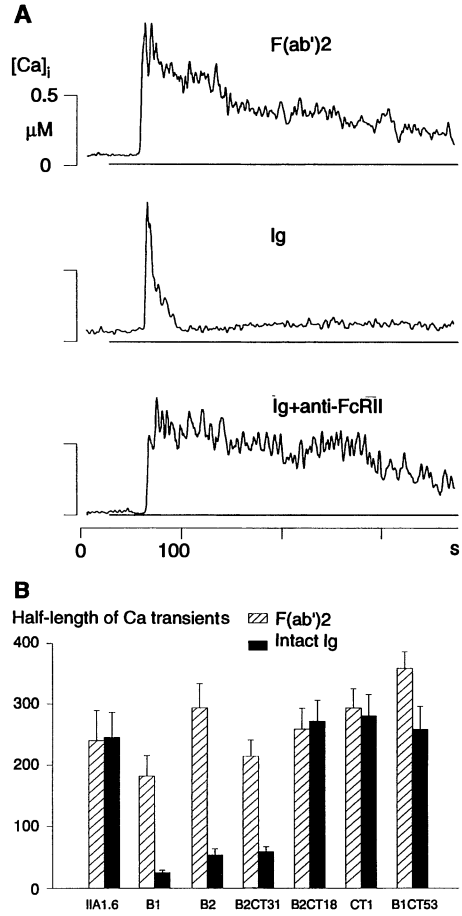

Fig. 4. Intracellular Ca^{2+} measurements in transfected IIA1.6 cells after cross-linking of sIg or co–cross-linking with wild-type or mutant FcγRII. Cells were treated with excess fura-2, and single cells were assayed fluorimetrically (*25*) for increases of intracellular Ca^{2+} in response to either $F(ab')_2$ fragments or intact rabbit antibodies to mouse IgG (Cappel, Courbevoie, France). (**A**) Recordings of changes in cytosolic free Ca^{2+} after cross-linking of sIg and FcγRII-B1 in transfected IIA1.6 cells. Intracellular Ca^{2+} was recorded in three different cells during application of different ligands for the times indicated by the bar under each trace. Top trace, $F(ab')_2$ fragments (50 μg/ml); middle trace; intact IgG antibodies to sIg (75 μg/ml). Bottom trace, the cell was first incubated with anti-FcγRII 2.4G2 (8 μg/ml) for 10 min before application of intact IgG antibodies to sIg (75 μg/ml). (**B**) Relative lengths of Ca^{2+} transients elicited by $F(ab')_2$ fragments (50 μg/ml) and intact IgG antibodies to sIg (75 μg/ml) in wild-type and transfected IIA1.6 cells. The half-time for decay of the Ca^{2+} transients was defined as the period between the onset of the ligand-induced Ca^{2+} rise and the time at which the Ca^{2+} concentration had returned to half of the maximum Ca^{2+}. Data points represent the mean (6 to 20 cells) ± SEM. For a given clone, $F(ab')_2$ and intact IgG responses were recorded on the same batch of cells.

ulate the activation signal (Fig. 4B). As expected, the receptor-negative IIA1.6 parental cell line was activated and maintained a prolonged Ca^{2+} response ($t_{1/2}$ ~ 240 s) with either anti-sIg $F(ab')_2$ or anti-sIg. In contrast, either the FcγRII-B1 isoform or the macrophage-specific FcγRII-B2 isoform were equally effective at attenuating the Ca^{2+} signal ($t_{1/2}$ 20 to 40 s). Thus, it appeared that the presence of the cytoplasmic domain insert was not required for regulating the early activation response. Analysis of the various deletion mutants suggested that the region of FcγRII-B2 that was needed for rapid endocytosis (residues 18 to 31) was also needed to attenuate the Ca^{2+} response, whether or not the insert was present (Fig. 4B). Essentially the same pattern was observed if a late marker of sIg-mediated activation in A20 or IIA1.6 cells, secretion of interleukin-2 (IL-2), was monitored. Although the region involved in regulation of activation overlapped with a region needed for endocytosis, endocytosis itself did not correlate with regulating the activation response. Both FcγRII-B1 and -B2 were equally active at regulating activation although only FcγRII-B2 was internalized. Interestingly, the tyrosine within this region is contained within a potential consensus sequence for phosphorylation by *src*-family kinases (*26*).

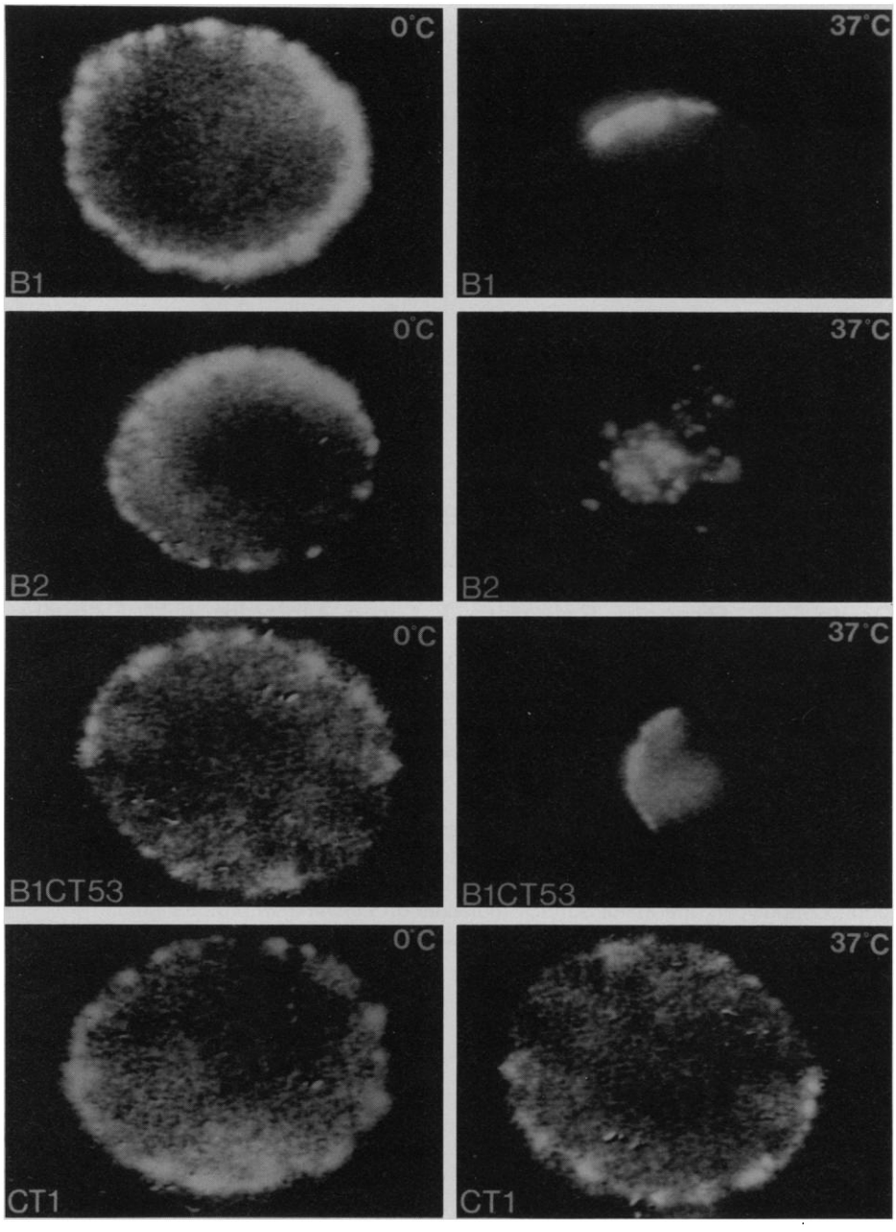

Fig. 5. Capping of FcγRII is enhanced by the FcγRII-B1 insert. IIA1.6 cells expressing FcγRII were incubated with the monoclonal anti-FcγRII, 2.4G2 IgG (30 μg/ml), for 15 minutes on ice. After being washed, the cells were treated with FITC-labeled mouse antibody to rat Ig (Immunotech, Marseille-Lumigny France) under the same conditions. After two washings, the cells were resuspended in RPMI 1640 containing 10% fetal calf serum and incubated for 1 hour at 0°C or 37°C. Cells were then fixed with methanol and analyzed by intensifying fluorescence digital-imaging microscopy (Nevicon Camera; Quantel Image Processor). Representative cells are shown expressing either B1, B2, B1-CT53, or CT1 (tail-minus) FcγRII.

Although the FcγRII-B1 insertion was not needed for the regulation of activation, we did not know whether it contributed to the ability of B-cell FcγRII to form polarized caps after cross-linking with anti-FcγRII. Capping was achieved by incubating the different FcγRII-expressing cell lines at 0°C with the anti-FcγRII 2.4G2 and then with fluoroisothiocyanein (FITC)-labeled $F(ab')_2$ fragments of a mouse antibody to rat IgG. After being washed, the cells were incubated at 0° or 37°C for 1 hour, fixed, and viewed by fluorescence microscopy. Cells expressing FcγRII-B1 exhibited typical cap structures after incubation at 37°C. In contrast, under these conditions, the cells expressing FcγRII-B2 have internalized the antibody into intracellular vesicles. No caps were detected with FcγRII-B2-expressing cells, even after shorter (5 minutes) or longer incubations at 37°C (2 hours).

Examination of the various deletion mutants showed that efficient capping correlated with the presence of the FcγRII-B1 insertion. The B1-CT53 mutant, bearing a deletion of all B2-like sequences distal to the insert, formed caps at an efficiency similar to full-length FcγRII-B1, while deletion of the insert, as in the CT1 mutant, reduced capping efficiency. CT1 was not internalized but remained diffusely distributed on the plasma membrane even after incubation at 37°C (Fig. 5), suggesting that inefficient capping did not correlate with a reduced capacity for endocytosis, but with the absence of the B1 cytoplasmic domain insert.

Our results show that the different functions associated with FcγRII in B cells and macrophages is at least partly determined by the type of receptor made in each cell type, as opposed to intrinsic differences between the cells themselves. However, the receptor's one truly B-cell–specific function (modulation of activation) could be mediated equally well by both FcγRII-B1 and -B2. Why, then, do B-cell FcγRII contain the characteristic cytoplasmic domain insertion? Perhaps the most likely possibility is to ensure that FcγRII-B1 in B cells is not capable of endocytosis and, as a result, that B cells cannot present antigen via FcγRII. Should B cells be capable of presenting antigen bound as antigen-antibody complexes, B cells would be subject to stimulation by T cells regardless of the actual specificity of the B-cell sIg. Accordingly,

expansion of B cells would not be dependent on their clonal specificity, resulting in a situation that may contribute to autoimmunity (*23*). Aside from blocking endocytosis, the only other B-cell activity specific to FcγRII-B1 was capping. This also may be an important feature of B-cell function since capping of sIg leads not only to the co-capping of FcγRII, but also includes several other molecules that may be involved in the activation process; these include MHC class II molecules, CD19, C3d receptor, and cytoplasmic H-*ras* (*13*, *27*–*29*). Thus, the increased propensity of FcγRII-B1 to cap may ensure that the receptor is distributed together with the molecules whose activities it must regulate after sIg-FcγRII cross-linking.

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25. Intracellular Ca^{2+} concentrations were measured in single cells that had been exposed to fura-2 and analyzed by phocal microfluorometry (Joyce-Loeble, Cambridge, England) as described (by G. Grynkiewicz, M. Poenie, and R. Y. Tsien [*J. Biol. Chem.* **260**, 3440 (1985)]. Cells were incubated with 1 μM fura-2-acetoxy-methyl-ester (Molecular Probes) for 15 min at 37°C and then plated in culture medium on a polylysine-coated (500 μg/ml, 10 min) glass cover slip. The fluorescence on single cells was measured. Absolute intracellular calcium concentrations were calculated online by the Phocal software, on an IBM AT computer. Anti-IgG (±2.4G2 Fab) was diluted in Ringer solution at the indicated concentrations (Fig. 4) and applied by pressure ejection through a glass pipette.
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31. FcγRII-B1 and -B2 encoding cDNAs were isolated (*5*) and obtained from M. Hogarth (Parkville, Victoria, Australia) and J. Ravetch (New York). B2-CT31, B2-CT18 and CT1 encoding cDNA's were derived as described (*9*). The designation refers to the FcγRII isoform (B1 or B2) followed by the remaining length of the cytoplasmic domain (Fig. 1B). B1CT-53 was obtained by introducing a stop codon, by polymerase chain reaction, in position 54 of the B1 cytoplasmic domain. All plasmids were sequenced again after mutagenesis. cDNA's were introduced in pKC3, an SV40 early promoter-based expression vector, and co-transfected by electroporation with pSV1NEO in IIA1.6 cells, as described [K. R. Thomas and M. R. Capecchi, *Cell* **51**, 503 (1987)]. Cells resistant to G418 (1 mg/ml) were cloned in soft agar, and several individual cell lines, expressing similar numbers of wild-type or mutant FcγRII were selected. Receptor-positive cells were enriched, in some cases, by "panning" on bacteriological plates coated with IgG [B. Seed and A. Aruffo, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 3365 (1987)]. Expression was quantified by determining the binding of ^{125}I-labeled Fab fragments of the FcγRII-specific monoclonal antibody 2.4G2 [J. C. Unkeless, *J. Exp. Med.* **150**, 580 (1979)]. All cells (including the A20 parent cell line) expresses between 1×10^5 to 4×10^5 receptors per cell.
32. Culturing of the 24.4 T cell hybridomas and antigen presentation assays were performed as described [M-Z. Lai *et al.*, *J. Immunol.* **139**, 3973 (1987)]. The IC's were formed prior to use by incubating different concentrations of purified λ repressor with (at 15 μg/ml) each of two different monoclonal antibodies to λ repressor (51F and 22D) at 37°C for 15 min. Release of IL-2 was determined by monitoring the growth of the IL-2-dependent cell line CTL.L2 as described (*33*). Bacteriophage λ repressor cI protein fragment 1 to 102 was purified [R. M. Breyer and R. T. Sauer, *J. Biol. Chem.* **264**, 13348 (1989)]; it was provided by R. M. Breyer (Institut Cochin de Genetique Moleculaire, Paris). The monoclonal antibodies to λ repressor were purified from culture supernatants by chromatography (MAB-trap G-protein columns; Pharmacia, Bois d'Arcy, France). The hybridoma T cell 24.4, was isolated from BALB/c mice injected with the 12-24 peptide of λ-repressor as described (*33*).
33. M-Z. Lai *et al.*, *J. Immunol.* **139**, 3973 (1987).

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Stage-Specific Adhesion of *Leishmania* Promastigotes to the Sandfly Midgut

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Although leishmaniasis is transmitted to humans almost exclusively by the bite of infected phlebotomine sandflies, little is known about the molecules controlling the survival and development of *Leishmania* parasites in their insect vectors. Adhesion of *Leishmania* promastigotes to the midgut epithelial cells of the sandfly was found to be an inherent property of noninfective-stage promastigotes, which was lost during their transformation to metacyclic forms, thus permitting the selective release of infective-stage parasites for subsequent transmission by bite. Midgut attachment and release was found to be controlled by specific developmental modifications in terminally exposed saccharides on lipophosphoglycan, the major surface molecule on *Leishmania* promastigotes.

The preliminary part of the *Leishmania* life cycle, in which ingested amastigotes from an infected host transform into rapidly dividing extracellular promastigotes, can take place in the blood meal of many different hematophagous arthropods. Within an unsuitable host, however, the parasites are later destroyed or passed out with the feces (*1*). For most leishmanial species, as well as for most other trypanosomatids, the establishment of a true infection within an appropriate vector requires the attachment of parasites to the midgut epithelium (*2*). This attachment is thought to retain parasites in the gut during passage of the digested blood meal and may also be involved in stage differentiation (*3*). The next phase in the development of transmissible infections within the sandfly is the detachment and anterior movement of promastigotes to the cuticle-lined foregut, where some attach by

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