The Immunoglobulin (Ig)α and Igβ Cytoplasmic Domains Are Independently Sufficient to Signal B Cell Maturation and Activation in Transgenic Mice

By Yih-Miin Teh and Michael S. Neuberger

From the Medical Research Council Laboratory of Molecular Biology, Cambridge CB2 2QH, United Kingdom

Summary

The B cell antigen receptor, composed of membrane immunoglobulin (Ig) sheathed by the Igα/Igβ heterodimer (CD79α/CD79β), mediates the response of the B cell to antigen by initiating transmembrane signaling and driving the internalization of antigen for presentation. Transfection experiments have revealed that, while these tails share an essential tyrosine-based activation motif (ITAM), they perform differently in some but not all assays and have been proposed to recruit distinct downstream effectors. We have created transgenic mouse lines expressing chimeric receptors comprising an IgM fused to the cytoplasmic domain of each of the sheath polypeptides. IgM/α and IgM/β chimeras (but not an IgM/β with mutant ITAM) are each independently sufficient to mediate allelic exclusion, rescue B cell development in gene-targeted Igα mice that lack endogenous antigen receptors, as well as signal for B7 upregulation. While the IgM/α × (IgM/β) double-transgenic mouse revealed somewhat more efficient allelic exclusion, our data indicate that each of the sheath polypeptides is sufficient to mediate many of the essential functions of the B cell antigen receptor, even if the combination gives optimal activity.

The B cell antigen receptor (BCR) [1], which is composed of membrane immunoglobulin sheathed by the Igα/Igβ heterodimer (CD79α/CD79β), mediates the response of the B cell to antigen by initiating transmembrane signaling and driving the internalization of antigen for presentation. The Igα/Igβ sheath is not only necessary for allowing the surface transport of membrane IgM but is also critical for mediating the signaling of the BCR and endocytic activities (1, 2).

Various chimeric receptors have been used to dissect the relative contributions of the Igα and Igβ cytoplasmic domains to BCR function. Because immunoreceptor tyrosine-based activation motifs (ITAMs) (3) present in both sheath polypeptides are central to their signaling activity (4–8), Igα and Igβ may be functionally redundant. Indeed, several transfection studies have failed to reveal significant differences in the signaling activities of Igα and Igβ (6, 7, 9). However, others have found that whereas chimeras containing just the Igα tail could mediate transmembrane signaling in B cell transfectants, analogous Igβ chimeras were impaired to variable extents (4, 10, 11, 12). This, in addition to the suggestion that the Igα and Igβ cytoplasmic domains bind different downstream effectors (13) and exhibit differences in antigen-presenting activity (14), is consistent with the two chains fulfilling distinct functions.

Experiments to ascertain whether there is a division of function between the Igα and Igβ cytoplasmic domains reveal that in vivo both IgM/α and IgM/β chimeras are able to induce the pro-B to pre-B transition and mediate allelic exclusion (8, 15). Here, we extend the transgenic analysis to the later stages of B cell development, to ask about the triggering of B cell maturation and activation.

Materials and Methods

DNA Constructs, Transfectants and Transgenic Mice. Plasmids driving the expression of the hen egg lysozyme (HEL)-specific receptors are based on pSV2gpt and pSV2neo. The κ transcription unit, in which the Vκ segment of the mouse D1.3 mAb (16, 17) is linked to rat Cκ, was assembled by exchanging the SacI–XhoI β-globin insert of Lk·βG (18) for an analogous PCR-generated SacI–XhoI fragment containing D1.3 Vκ. The heavy (H) chain vectors derive from pSV·Vκ1 (19) but with the VH/Vκ segment replaced by a PCR-generated PstI·BstEII D1.3 VH segment. For the chimeric λ chains, the Cλ region was replaced by Cμ/β or Cμ/β‘–14 Cμ regions (20) or Cμ/α Cμ regions (20a) (Fig. 1 A).

DNA was introduced into the mouse A20 B cell lymphoma by electroporation and stably transfected clones selected in DMEM, 10% FCS, 50 μM 2-ME containing G418 and mycophenolic acid.
Flow Cytometry. Analysis was performed on Becton Dickinson FACScan® or FACScalibur® using LYSYS II or CELLQuest software. FITC-conjugated and biotinylated goat anti-IgM, and PE-conjugated rat anti-IgD were from Southern Biotechnology (Birmingham, AL); FITC-R A3-6B2 (rat anti-B220[C57R]), PE-conjugated B3B4 (rat anti-CD23), biotinylated 53-2.1 (rat anti-Thy1.2[CD90]) and purified 7G6 (rat anti-CD21/35[CR 2/1]) were from Pharmingen (San Diego, CA); PE-conjugated R A3-6B2 and PE-CD670-streptavidin were from Gibco BRL (Paisley, UK); FITC-streptavidin was from Amersham (Amersham, UK); and PE-streptavidin was from Jackson Immunoresearch (West Grove, PA). Cells making the ES.2 monoclonal anti-D1.3 idiotype antibody (22) were a gift from R. Poljak. The flow cytometric analyses illustrated are representative of multiple individual animals aged 2–4 mo old.

B Cell Activation. Production of IL-2 from A20 transfectants (10⁴ cells in 200 μl) cultured for 24 h in medium containing various concentrations of HEL was monitored by the culture supernatant to IL-2-dependent HT2 cells (2.5 × 10⁶ cells in 100 μl medium). The viability of the HT2 cells was determined after 24 h as previously described (20) and the assay calibrated with recombinant IL-2 standards.

To monitor proliferative responses, splenocytes that had been depleted of erythrocytes by hypotonic lysis were cultured in triplicate aliquots (2 × 10⁵ in 200 μl) in RPMI, 10% FCS, 50 μM 2-ME in the presence of 1 μg/ml LPS (Sigma, Poole, UK) for 48 h before pulsing with 0.5 μCi [³H]thymidine for 15 h and scintillation counting.

To monitor CTLA4 binding, spleen cells depleted of erythrocytes were cultured (10⁶ cells/ml) in medium in the presence or absence of 10 μg/ml Fab(2) goat anti-mouse IgM (Jackson Immunoresearch) for 24 h before staining with mCTLA4-H-γ-1 fusion protein (gift from P. Lane) and FITC-conjugated goat anti-human IgG (Jackson Immunoresearch). BrdU Uptake. Mice were given two intraperitoneal injections 4 h apart of 1 mg BrdU (5-bromo-2-deoxyuridine) (Sigma) in PBS and the drinking water supplemented with 1 mg/ml BrdU for 72 h following the protocol of Torres et al. (23). Spleens were removed and, after staining for CD45R (B220) and either D1.3 or FITC-conjugated anti-BrdU antibody (Becton Dickinson, San Jose, CA) for cytofluorimetric analysis.

Results

The IgM/α and IgM/β but not IgM/β⁻⁻ C Chimeras Signal in A 20. To discriminate between the functions of Igα and Igβ cytoplasmic domains, we constructed a set of plasmids encoding either wild-type or chimeric HEL-specific receptors. The chimeras are composed of mouse μ, and rat κ, Ig chains directly linked through a hydrophobic transmembrane segment to the cytoplasmic domains of either Igα, Igβ, or a mutated Igβ whose ITAM tyrosines are substituted by leucines (IgM/α, IgM/β, or IgM/β⁻⁻; Fig. 1 A). The transmembrane segment (which derives from the H-2Kb gene) confers shear-independent surface transport (24) and the receptors do not show detectable association with endogenous Igα or Igβ chains (20).

The plasmids encoding the various receptors were transfected into the A20 B cell lymphoma; the transfectants all stained for IgM although there were some differences in the brightness with the IgM/α chimer being the least well transported to the cell surface (Fig. 1 B). The IgM/α and
IgM/β chimeras, as well as the wild-type IgM receptor, were able to initiate signaling after antigen binding as judged by the production of IL-2 from transfectants of the A20 lymphoma (Fig. 1 C). However, the signaling activity was abolished by mutation of the ITAM tyrosines in the Igβ cytoplasmic domain.

Receptor Expression in Transgenic Mice. The transcription units encoding the various receptors were introduced into the germline of transgenic mice. Cytofluorimetric analysis of spleen cells with anti-D1.3 idiotype antibody (Fig. 2 A) as well as with anti-rat κ and labeled HEL (data not shown) revealed that they were all expressed on the B cell surface, although the IgM/α staining was weaker than that of the other receptors. With the mice bearing the chimeric receptors, the receptors were also expressed on some CD45R(B220)2 cells. These correspond to a subpopulation of T cells (Fig. 2 B), probably reflecting the expression pattern of the IgH enhancer in transgenic mice (25). (The absence of surface expression of the wild-type transgenic IgM receptor in this subpopulation is consistent with the fact that wild-type IgM, but not the IgM chimeras, requires endogenous Igα/Igβ for surface transport).

IgM/α and IgM/β but not IgM/βγ/L drive B cell development. Signals transmitted through membrane Ig are required for B cell development; μMT mice that carry a targeted disruption of the μ membrane exon are B cell deficient (21). To see whether the individual Igα and Igβ cytoplasmic domains of the IgM chimeras were sufficient to signal for B cell maturation, the various transgenic lines were bred into a homozygous μMT background. It was immediately evident that the HEL-specific IgM BCR as well as the IgM/α and IgM/β chimeras all had a significant effect on B cell development; their presence led to a substantial (around two log) increase in serum IgG levels as compared with

Figure 2. Expression of transgenes. (A) Flow cytometric analysis of splenocytes of transgenic mice. Cells were stained with FITC-conjugated anti-D1.3 idiotype and PE-conjugated anti-B220 and gated for propidium iodide exclusion. Numbers indicate percentage of splenocytes in each quadrant (Non-tg, nontransgenic). (B) Transgene expression on T cells. Splenocytes were stained with FITC-conjugated anti-IgM, biotinylated anti-Thy1.2, and PE–streptavidin; the profiles present the IgM staining of Thy1.2+ gated cells.

Figure 3. The IgM, IgM/α and IgM/β, but not IgM/βγ/L, transgenes reconstitute the splenic B cell pool in μMT mice. Splenocytes were stained with FITC-conjugated anti-IgM and PE-conjugated anti-B220; the numbers denote the percentage of B220+ IgM+ cells within gated region and are representative of multiple animals (WT 57 ± 7% [n = 8]; IgM 26 ± 19% [n = 4]; IgM/β 15 ± 6% [n = 5]; IgM/α 15 ± 7% [n = 8]). WT, wild-type background; μMT, homozygous μMT background; Non-tg, nontransgenic.
50% of the D1.3 idiotype nontransgenic mice had incorporated BrdU, the figure increased to 40–

anti-CD23, gating by scatter and B220

For CD23 expression, cells were stained with FITC–anti-B220 and PE–

RED670–streptavidin), gating by lymphocyte scatter and B220

anti-IgM, PE–anti-B220, and biotinylated anti-CD21/35 (revealed by

(shaded). For CD21/35 expression, splenocytes were stained with FITC–

mice crossed into a

CD21/35 and CD23. Histograms show splenic B cells from transgenic

b

from IgM and IgM/

a

or the IgM/

b

receptor is significantly greater than that mediated by

(Fig. 5

b

coexpressing an endogenous rearrangement. We crossed

the receptors with HEL or anti-D1.3 idiotype antibody

gave analogous results but additionally revealed that the

IgM/βγ−ΔL receptor was signaling defective (data not shown).

The IgM/α and IgM/β chimeras Give Partial Allelic Exclusion but the Combination is Optimal. The ability to mediate

the feedback regulation of endogenous Ig gene rearrangement

allelic exclusion) provided another parameter for

comparing the signaling ability of the different receptors.

Allelic exclusion in the transgenic mice was readily moni-

tored by determining the proportion of D1.3 idiotype B cells

that coexpress IgD and gave similar conclusions to

staining with anti-IgM and upregulation of B7 was judged by stain-

responses to anti-IgM and IL-4. However, as with LPS, the

responses were very poor; therefore, we used upregulation

of the B7 costimulatory molecules as our readout. Splenic

B cells from the transgenic mice were cultured for 24 h

with anti-IgM and upregulation of B7 was judged by stain-

ing with a CTLA4–Ig fusion protein (Fig. 5 A). Triggering

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The results show that both the IgM/α and IgM/β chimeras can broadly perform many of the major in vivo functions of the complete BCR. Furthermore, the ability to drive the maturation and activation of peripheral B cells is dependent upon the ITAM; this parallels previous findings on pre-B cell development (8, 15).

Transfection experiments using cell lines have revealed that both Igα and Igβ are needed for surface transport of membrane Ig (26, 27). Thus, mice carrying targeted disruptions of Igβ cannot express surface Ig and are B cell deficient (28). In contrast, both the IgM/α and IgM/β chimeras described here allow extensive B cell maturation because, by virtue of their mutant transmembrane sequences, these chimeras can be transported to the B cell surface without an attendant Igα/Igβ sheath. The IgM/β chimera performs slightly better than the IgM/α chimera in several of the assays but this may simply reflect the more efficient surface transport of the IgM/β chimera.

Therefore, our results so far do not lend significant support to the idea that Igα and Igβ cytoplasmic domains perform distinct autonomous functions within the context of the intact BCR. Nevertheless, it is clear that the chimeras are not as effective as the complete BCR. Thus, the (IgM/α) × (IgM/β) double transgenic mouse is considerably more efficient than its single transgenic parents in effecting allelic exclusion of endogenous Ig gene rearrangement. This is consistent with cell line transfection experiments indicating cooperativity between the two cytoplasmic domains with the heterodimer giving a stronger signal than the component homodimers (29). Indeed, the structural conformation of the heterodimer could differ substantially from that of the homodimers and this could lead to differences in the kinetics of phosphorylation or efficacy of effector protein (e.g., Syk) recruitment, as well as in the sensitivity to antigen binding.

However, although the chimeric HEL-specific receptors do not perform as well as the wild-type IgM BCR in driving the reconstitution of a splenic B cell compartment in μMT mice, the difference is relatively small and the impaired B cell maturation is certainly not nearly as dramatic as that observed by Torres et al. (23) in mice carrying a targeted disruption of the mb-1 gene that leads to the synthesis of a BCR with a truncated Igα tail. The different performance of the various compromised BCRs in driving pre-B and B cell development could well be accounted for by a requirement for differing qualities of signal at the various maturational checkpoints. It will obviously be interesting to correlate the differentiative potential of the various mutant BCRs with their biochemical signaling activities.

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