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

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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. 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 pSV2gt and pSV2neo. The k 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 fragment containing D1.3 Vκ/κ double-transgenic mouse revealed some-what 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.

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Figure 1. Antigen induces IL-2 secretion from the A20 transfectedants. (A) Schematic representation of the transgene constructs and predicted structures of the receptors (R, EcoRI; C, Ccl; P, PstI; B, BstEI; K, KpnI; X, Xhol; S, SacI). (B) Flow cytometric analysis of transfectedants in A20 by staining for surface IgM; unfilled histograms depict the unstained controls. (C) Mean levels of IL-2 secreted by A20 transfectedants after incubation with hen egg lysozyme (HEL) for 24 h. Standard errors were below 0.5 U/ml except where indicated.

Results

The IgM/α and IgM/β but not IgM/β’−κ Chimeras Signal in A20. 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’−κ; Fig. 1 A). The transmembrane segment (which derives from the H-2Kb gene) confers sheath-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 transfectedants all stained for IgM although there were some differences in the brightness with the IgM/α chimera 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. 1C). 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. 2A) 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. 2B), 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/β$^\gamma$-light 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...
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–anti-IgM, PE–anti-B220, and biotinylated anti-CD21/35 (revealed by streptavidin). For CD21/35 expression, splenocytes were stained with FITC–anti-B220 and PE–anti-IgM and RED670–streptavidin. After fixation and permeabilization, cells were stained with a FITC-conjugated anti-IgM antibody. The analyses (10,000 gated cells) are presented for cells that have been gated as B220+.

Figure 4. Phenotypic analysis of the transgenic B cells. (A) The transgenic B cells proliferate poorly. After 48-h culture in the presence of LPS, the splenocytes from wild-type, nontransgenic mice (WT, Non-tg), or transgenic mice crossed into a transgenic background overlaid on nontransgenic controls (MT, MT). The double-transgenic mice exhibited greatly decreased proliferation of B cells. (B) Turnover of transgenic B cells. After uptake of BrdU (5-bromo-2-deoxyuridine) for 72 h, spleen cells were stained with PE-conjugated anti-B220 and biotinylated anti-D1.3 idiotype or anti-IgM (nontransgenic only) and RED670–streptavidin. After fixation and permeabilization, cells were stained with a FITC-conjugated anti-IgM antibody. The analyses (10,000 gated cells) are presented for cells that have been gated as B220+. Whereas 10–20% of the IgM+ cells in nontransgenic mice had incorporated BrdU, the figure increased to 40–50% of the D1.3 idiotype+ cells in the transgenic lines. (C) Splenic B cells from IgM and IgM/β express lower levels of mature B cell markers CD21/35 and CD23. Histograms show splenic B cells from transgenic mice crossed into a transgenic B cell population, we found that the reconstituted B cells proliferated extremely poorly on in vitro culture with LPS (Fig. 4 A). Indeed, compared with normal controls, the transgenic B cells exhibited a two- to fivefold increase in cell death during a 24-h in vitro culture. The diminished proliferative response is not unique to the IgM chimeras; it is also evident in mice transgenic for the wild-type anti-HEL IgM (Fig. 4 A). An altered turnover of the transgenic B cells is also apparent in vivo: BrdU labeling revealed that animals carrying the transgenic receptors had an increased proportion of rapidly turning over splenic B cells (Fig. 4 B). With regard to surface markers, splenic B cells from mice transgenic for both the wild-type IgM and the IgM chimeras revealed a shift to lower expression of the mature B cell markers CD21/35 and CD23 (Fig. 4 C) as compared with normal mice, though CD22 expression was unaffected. Thus, in view of their diminished proliferative response, increased in vivo turnover and decreased expression of CD21/35 and CD23, it is evident that the splenic B cells whose development is driven by the transgenic anti-HEL receptors do not show the same distribution of maturation development as splenic B cells in normal mice. However, this is an effect of all the anti-HEL transgenes and does not indicate compromised signaling by either of the individual IgM chimeras.

Upregulation of B7 by the IgM/α and IgM/β C chimeras. To monitor signaling by the IgM chimeras in the transgenic B cells, we sought to measure the proliferative 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 staining with a CTLA4–Ig fusion protein (Fig. 5 A). Triggering the receptors with HEL or anti-D1.3 idiotype antibody gave analogous results but additionally revealed that the IgM/β receptor was signaling defective (data not shown).

The IgM/α and IgM/β C 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 monitored by determining the proportion of D1.3 idiotype+ B cells that coexpress IgD and gave similar conclusions to staining with anti-μ allotype antibodies. It will be seen (Fig. 5 B) that the exclusion mediated by the wild-type HEL receptor is significantly greater than that mediated by the IgM/α and IgM/β chimeras. The IgM/β receptor is ineffective in exclusion with all D1.3 idiotype+ B cells coexpressing an endogenous rearrangement. We crossed the IgM/α and IgM/β chimeras to see whether coexpression of the two receptors yielded more complete exclusion. The double-transgenic mice exhibited greatly decreased expression of endogenous Ig gene rearrangements (Fig. 5 B) despite the fact that there was no significant increase in the abundance of transgenic IgM on the B cell surface (see Fig. 2 A).
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.

**Discussion**

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