tions that cause cell size to increase than those that cause it to decrease.

These questions notwithstanding, our data clearly demonstrate a pattern of punctuated evolution for a quantitative morphological character. Moreover, this pattern arose in a simple experimental system without any population subdivision (which promotes cladogenesis), and the punctuated changes were largely (if not entirely) caused by the successive fixation of several beneficial mutations. Millions of mutations occurred during these thousands of generations (9), but evidently beneficial mutations of large effect were quite rare (16). The experimental population was strictly asexual, which may have increased our ability to resolve punctuated changes. However, any difference between sexual and asexual populations with respect to the dynamics of adaptive evolution (17) breaks down when two conditions are met: (i) standing genetic variation for fitness is exhausted, as will eventually happen in any constant environment (18), and (ii) beneficial mutations are so rare that they occur as isolated events (11, 17). To the extent that these conditions are fulfilled in nature, then the selective sweep of beneficial alleles through a population might explain some cases of punctuated evolution in the fossil record. In any case, our experiment shows that punctuated evolution can occur in bacterial populations as a consequence of the two most elementary population genetic processes: mutation and natural selection.

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9. The total mutation rate equals the product of the number of cell replications per day (~5×10^8) and the genomic mutation rate [which is ~0.0025 for *E. coli* (19)].
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12. Let $R(0)$ be the initial ratio of the population of a mutant genotype to that of its progenitor and let $R(t)$ be that ratio at time t. Then $\ln R(t) = \ln R(0) + st$, where s is the selection rate constant (20). For example, if $s = 0.46$ per day, which corresponds to a relative fitness of 1.1 (= 1 + s/ln 100) in our experiment (6), then it takes 30 days (200 generations) for a single mutant to increase to a frequency of 10% in a population of 10^7, but only 5 more days are needed to reach 50%.
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25. We thank J. A. Mongold for help with the experiments; A. F. Bennett, J. E. Cohen, D. J. Hall, J. A. Mongold, and P. D. Sniegowski for discussion and comments; and A. G. Clark for suggesting that we obtain these data. This work was supported by a fellowship from the Spanish Ministerio de Educación y Ciencia to S.F.E., a fellowship from Michigan State University to V.S.C., and a grant from NSF (DEB-9421237) to R.E.L.

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Aberrant B Cell Development and Immune Response in Mice with a Compromised BCR Complex

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The immunoglobulin α (Ig-α)–Ig-β heterodimer is the signaling component of the antigen receptor complex on B cells (BCR) and B cell progenitors (pre-BCR). A mouse mutant that lacks most of the Ig-α cytoplasmic tail exhibits only a small impairment in early B cell development but a severe block in the generation of the peripheral B cell pool, revealing a checkpoint in B cell maturation that ensures the expression of a functional BCR on mature B cells. B cells that do develop demonstrate a differential dependence on Ig-α signaling in antibody responses such that a signaling-competent Ig-α appears to be critical for the response to T-independent, but not T-dependent, antigens.

Surface expression of, and signaling by, Ig on B lymphocytes is dependent on both Ig-α and Ig-β (1–5). The signaling capacity of these two molecules, which are expressed as heterodimers in the pre-BCR and BCR (2–5), has been attributed to the presence of an immunoreceptor tyrosine-based activation motif (ITAM) within both intracellular domains (6). Indeed, BCR signaling is severely compromised when the Ig-α ITAM is mutated or deleted in a myeloma cell line (3). In the presence of wild-type Ig-β, the extracellular and transmembrane domains of Ig-α are sufficient for surface Ig expression (3). Thus, by truncation of the Ig-α cytoplasmic tail, a mouse mutant can be generated that retains B cell surface Ig expression (Fig. 1) but is compromised in Ig-α signaling (3, 7), allowing the identification of a point or points at which a pre-BCR or BCR signal is critical for B cell development or function (or both).

Homologous recombination in embryonic stem (ES) cells (8) resulted in an ES cell line (EΔ6) in which a stop codon was introduced in exon four of *mb-1* (Fig. 1A). The mutated locus ($mb\text{-}1^{\Delta c/\Delta c}$) encodes a truncated Ig-α molecule whose cytoplasmic tail

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consists of 21 instead of 61 amino acids and lacks the ITAM (9). Wild-type and homozygous mutant B220$^+$ bone marrow cells express a 1-kb *mb-1* transcript (Fig. 1B). Moreover, peripheral B cells from *mb-1*$^{\Delta c/\Delta c}$ mice, although decreased in number (see below), express surface IgM at equivalent levels compared with expression in wild-type animals (Figs. 1C, 2E, and 3). These results indicate that *mb-1*$^{\Delta c/\Delta c}$ B lineage cells express a truncated Ig-α molecule that associates with Ig-β and allows Ig transport to the cell surface.

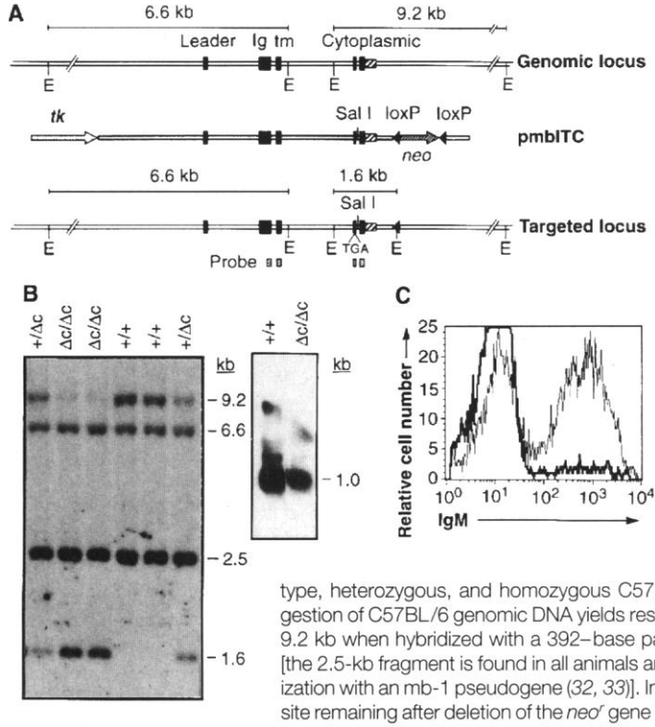


Fig. 1. Generation and identification of *mb-1*$^{\Delta c/\Delta c}$ mice. (**A**) Genomic *mb-1* locus (top) (*32*), targeting construct (middle), and targeted locus (bottom). Homologous recombination introduces a loxP-flanked *neo*^{r} gene 3′ of the *mb*-1 gene that is removed in ES cells by Cre-mediated recombination. The stop codon (TGA) introduced at amino acid 181 in the targeted allele is shown (*32*). Exons are indicated as black boxes, loxP sites as filled triangles, and selectable genes as arrows. E, Eco RI. The cDNA probe is represented as stippled boxes. (**B**) (Left) Southern (DNA) analysis of wild-type, heterozygous, and homozygous C57BL/6 littermates. Eco RI digestion of C57BL/6 genomic DNA yields restriction fragments of 6.4 and 9.2 kb when hybridized with a 392–base pair Pvu II cDNA *mb*-1 probe [the 2.5-kb fragment is found in all animals and arises from cross-hybridization with an mb-1 pseudogene (*32*, *33*)]. In the targeted locus, the loxP site remaining after deletion of the *neo*^{r} gene introduces an Eco RI restriction site, resulting in the loss of the 9.2-kb wild-type restriction fragment and the gain of a 1.6-kb fragment. (Right) Northern (RNA) analysis of bone marrow B220$^+$ cells from wild-type (+/+) and *mb*-1$^{\Delta c/\Delta c}$ animals (Δ*c*/*c*). Hybridization with *mb*-1 cDNA reveals a 1-kb transcript in both animals. (**C**) Histogram of IgM expression levels on wild-type (thin line) and *mb*-1$^{\Delta c/\Delta c}$ (bold line) splenic B cells shows equivalent levels of surface IgM expression.

Flow cytometric analysis of bone marrow (*10*) revealed a small increase in the number and proportion of the *mb-1*$^{\Delta c/\Delta c}$ pro-B (B220^{lo}/CD43$^+$) cell population compared with that of controls (Fig. 2A and Table 1). However, the pro-B cell subpopulations, defined by heat-stable antigen and BP-1 expression (*11*), were present in similar proportions in *mb-1*$^{\Delta c/\Delta c}$ and control mice (*12*). In contrast, pre-B (B220^{lo}/CD43$^-$/IgM$^-$) and immature B (B220^{lo}/CD43$^-$/IgM$^+$) cells were present but diminished in *mb-1*$^{\Delta c/\Delta c}$ mice by a factor of 2 to 4 compared with the wild type (Fig. 2, A and B, and Table 1). The B220^{lo}/CD43$^-$ cells did not differ from controls in cell size distribution (determined by forward scatter) or up-regulation of CD25 expression (*12*).

Studies in previously characterized B cell–deficient mutants have demonstrated that progenitor B cells must express a functional membrane-bound pre-BCR for the pro- to pre-B cell transition to occur (*13*, *14*). The development of pre-B cells from the pro-B compartment of *mb-1*$^{\Delta c/\Delta c}$ mice implies that mutant pro-B cells assemble a pre-BCR that can signal this step, although less efficiently than the wild-type receptor.

Upon the assembly and expression of a

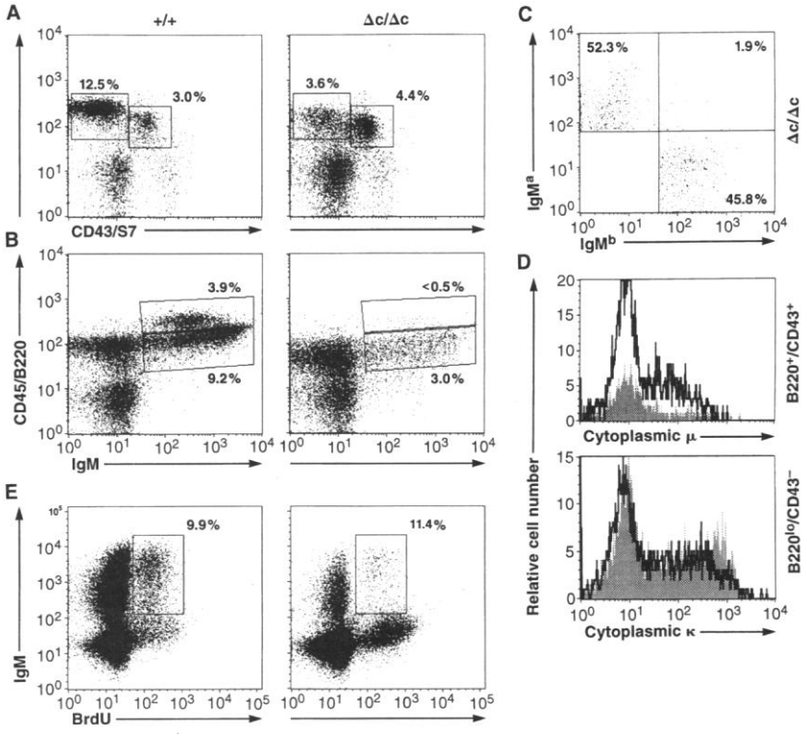


Fig. 2. Representative flow cytometric analyses of bone marrow B cell development in 9- to 13-week-old wild-type (+/+) and *mb*-1$^{\Delta c/\Delta c}$ (Δ*c*/Δ*c*) mice. Percentages of gated cells within the B220$^+$ population are indicated. (**A**) B220 versus CD43 surface expression of IgM$^-$ cells. Pro-B cells are B220^{lo}/CD43$^+$ and pre-B cells are B220^{lo}/CD43$^-$ (*11*). (**B**) B220 versus IgM expression defines newly generated (B220^{lo}/IgM$^+$) and mature recirculating (B220^{hi}/IgM$^+$) B cells. (**C**) Expression and percentages of IgM^a versus IgM^b allotypes by IgM$^+$ bone marrow cells from *mb*-1$^{\Delta c/\Delta c}$ mice. (**D**) Intracellular staining of bone marrow lymphoid cells. (Top) Cytoplasmic μ heavy chain expression within the B220$^+$/CD43$^+$ gate. (Bottom) Intracellular κ light chain expression in B220^{lo}/CD43$^-$ cells. Histograms for control animals are shaded and for mutants outlined in bold. Differences in cell number reflect the proportion of cells within a given gate (Table 1). The percentage positive cells in control and mutants are, respectively, 18 versus 23% for cytoplasmic μ in B220$^+$/CD43$^+$ cells and 47 versus 44% for cytoplasmic κ in B220^{lo}/CD43$^-$ cells. (**E**) Control and mutant splenic B cells exhibit equivalent BrdU incorporation after 3 days as determined by IgM versus BrdU staining (*19*). Percentages are for BrdU$^+$/IgM$^+$ splenocytes. Each dot plot represents 50,000 events.

functional Ig heavy chain gene, B cells arrest further rearrangement at the remaining allele in a process termed allelic exclusion. The signal that mediates allelic exclusion has also been attributed to the pre-BCR (*15*). The absence of cells coexpressing μ^a and μ^b allotypes demonstrated that allelic exclusion is intact in *mb-1*$^{\Delta c/\Delta c}$ B cells (Fig. 2C). The wild-type Ig-β chain of the mutant B cells is likely sufficient to mediate allelic exclusion, as well as pre-B cell generation, at reduced efficiency (*16*).

Ig heavy and κ light chain rearrangements were apparently undisturbed by the Ig-α mutation. We find comparable levels of cytoplasmic μ heavy chain expression in B220$^+$/CD43$^+$ cells and equivalent intracellular κ light chain expression in B220^{lo}/CD43$^-$ cells from mutant and control animals (Fig. 2D).

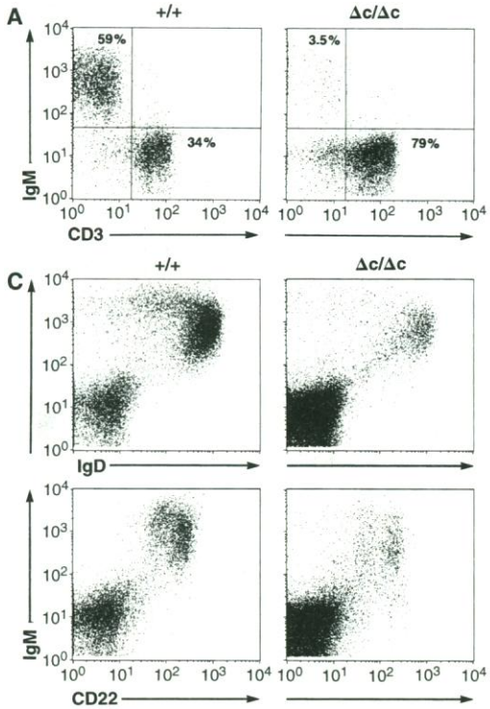
The marked reduction of a B220^{hi}/IgM$^+$ mature B cell population in *mb-1*$^{\Delta c/\Delta c}$ bone marrow and peripheral lymphoid compartments (Figs. 2B and 3 and Table 1) identified the most severe consequence of the Ig-α truncation for B cell development, namely, a decrease in the size of the long-lived mature B cell pool. *mb-1*$^{\Delta c/\Delta c}$ B cell numbers in the spleen were typically reduced to 1% of their value in controls (range, 0.3 to 2.5%; 0.2 to 1 × 10^6 versus 4 to 6 × 10^6 cells per spleen), representing 1 to 4% of splenic, lymph node, blood, and peritoneal cells (Fig. 3) (*12*). The latter finding implies that generation of the B-1 subpopulation of B cells, which express low levels of the CD5 antigen and occur at a high frequency in the peritoneum of normal mice (*17*), is also dependent on an intact Ig-α signaling chain.

Investigation of the population dynamics in B cell development has indicated that the daily production of B cells in the bone marrow far exceeds the number of newly generated B cells that are selected into the mature peripheral B cell population (*18*). In *mb-1*$^{\Delta c/\Delta c}$ mice, the newly generated B cell compartment in the bone marrow is reduced to between one-quarter and one-third of the value in controls, whereas the mature peripheral B cell pool is diminished to ~1% of the control value (Figs. 2B and 3 and Table 1). Thus, there is a decrease in the size of the mutant peripheral B cell pool far exceeding the value expected from the reduction of the compartment of newly generated B cells. This result could be explained by a smaller fraction of newly formed B cells emigrating from the bone marrow or by a shortened half-life of mature B cells.

To discriminate between these two possibilities, we examined the in vivo incorporation of 5-bromo-2-deoxyuridine (BrdU) by B lineage cells (*19*). The percentage of bone marrow B220$^+$ cells that were labeled with BrdU was similar in control and mutant (*19*), suggesting that the kinetics of progenitor B cell maturation to the immature B cell stage was normal. Furthermore, BrdU incorporation in *mb-1*$^{\Delta c/\Delta c}$ splenic B cells and in wild-type cells occurred at equivalent frequencies (Fig. 2E) after 72 hours of continuous labeling (10.7 ± 1.4% versus 10.6 ± 2.1%, n = 6). Because there is negligible proliferation as small pre-B cells develop to mature B cells, these results suggest that the fraction of newly generated *mb-1*$^{\Delta c/\Delta c}$ B cells emigrating from the bone marrow to the periphery is reduced compared with that of wild-type animals. Although the basis for the selection of short-lived IgM$^+$ cells into the pool of long-lived peripheral B cells is not clear (*20*), these data indicate that newly formed IgM$^+$ cells require an Ig-α–mediated signal or signals to establish the pool of mature B cells.

The 3-day BrdU labeling period is insufficient to detect a reduction in the average lifespan of long-lived B cells, which would ultimately affect the accumulation of peripheral B cells in adult animals. Indeed, *mb-1*$^{\Delta c/\Delta c}$ B cell numbers do not increase with age (*12*). In contrast, the λ5T mouse mutant, despite inefficient B cell development, accumulates a sizable peripheral B cell compartment with time (*13*). This finding suggests that not only the generation, but also the maintenance, of peripheral B cells is affected by the *mb-1*$^{\Delta c/\Delta c}$ mutation, possibly as a result of increased apoptotic cell death.

Histologically, *mb-1*$^{\Delta c/\Delta c}$ B cells were detected in the spleen as small clusters adjacent to relatively large T cell zones (Fig. 3B) (*21*). These mutant B cells displayed a mature phenotype similar to that of controls as indicated by surface expression of IgM versus IgD, CD22, CD86 (B7.2), CD23, and major histocompatibility com-



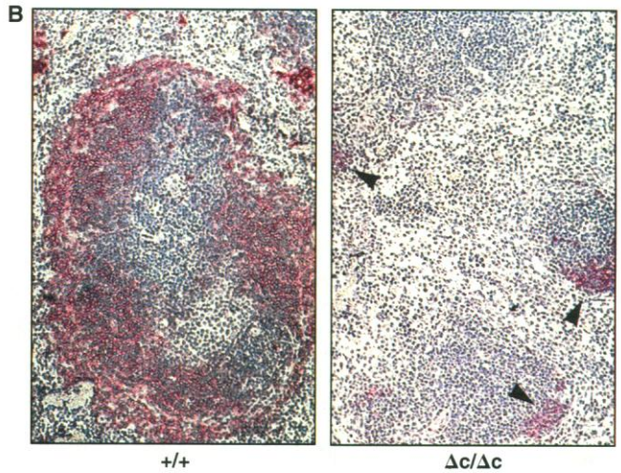


Fig. 3. Phenotype of peripheral lymphocytes from wild-type (+/+) and *mb-1*$^{\Delta c/\Delta c}$ (Δc/Δc) littermates. (**A**) Splenocyte surface expression of IgM and CD3. (**B**) Histological splenic sections from wild-type or mutant animals stained with anti-IgM and counterstained with hematoxylin. IgM-positive cells are stained red (arrow), and the dark hematoxylin staining reveals T cell zones as indicated by anti-CD4 staining of serial sections (*12*). Magnification, ×200. (**C**) Representative flow cytometric profiles of peripheral splenic wild-type and *mb-1*$^{\Delta c/\Delta c}$ B cells. IgM versus IgD (top) or CD22 (bottom) are shown. Ten times more events were collected from *mb-1*$^{\Delta c/\Delta c}$ than from wild-type splenocytes to enhance the distribution of expression.

plex (MHC) class II (Figs. 3 and 4) (*12*). The distribution of Ig isotypes in the sera of *mb-1*$^{\Delta c/\Delta c}$ mice was similar to that in wild-type mice, demonstrating that a functional Ig-α is not essential for Ig class switch recombination. However, the concentrations of serum Ig were generally reduced to between 10 and 25% of the value in control mice (*12*).

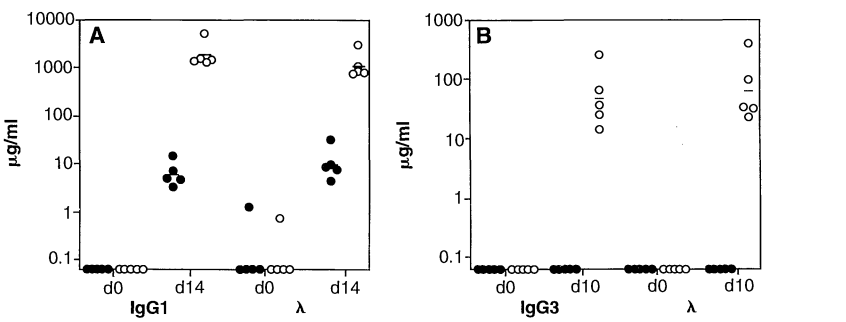
Antigens that elicit an antibody response can be classified according to their dependence or independence on MHC class II–restricted T cell help. The ability of *mb-1*$^{\Delta c/\Delta c}$ B cells to mount an antibody response to both types of antigens was investigated (*22*). *mb-1*$^{\Delta c/\Delta c}$ mice generated a hapten-specific response to the T-dependent (TD) antigen, 4-hydroxy-3-nitrophenylacetyl coupled to chicken γ-globulin (NP-CG) (Fig. 4A), that was reduced to ~1% of the value in control animals. Given the average reduction in *mb-1*$^{\Delta c/\Delta c}$ B cell numbers by a factor of 100, this level of response would be expected in the presence of saturating T cell help (*23*). That *mb-1*$^{\Delta c/\Delta c}$ B cells interact productively with T cells was supported by the finding that mutant B cells were able to up-regulate MHC class II (*12*) and CD86 in vitro upon antibody to immunoglobulin (anti-Ig) treatment (*24*) to levels similar to those seen in the controls (Fig. 4C). This result would be consistent with data showing that the cytoplasmic tails of Ig-α and Ig-β, expressed in the context of fusion proteins, can facilitate antigen presentation in vitro, even in the absence of signaling by the Ig-β ITAM (*25*).

In contrast to the TD response, *mb-1*$^{\Delta c/\Delta c}$ mice did not mount a measurable response (*26*) to an optimal dose (*27*) of the T-independent (TI) type 2 antigen, NP-Ficoll, at 7, 10, or 14 days after immunization (Fig. 4B) (*12*). Responses to TI type 2 antigens are thought to rely on extensive surface Ig cross-linking (*28*). The present data support this concept in that an uncompromised signal through the BCR (generated presumably through receptor cross-linking by a large multivalent antigen) appears to be required for the TI type 2 response.

mb-1$^{\Delta c/\Delta c}$ B cell development and function resembles that of the X-linked immunodeficiencies in humans and mice resulting from mutations of Btk tyrosine kinase, *XLA* and *xid*, respectively (*29*). This finding is in accord with the view that the Btk signal transduction pathway originates at the BCR (*30*) and suggests that the function of Btk is dependent on the Ig-α ITAM. Interestingly, ~10% of X-linked agammaglobulinemia (XLA) patients are females who exhibit a random pattern of chromosome X inactivation, suggesting that a distinct autosomal defect is responsible for the immunodeficiency in these patients (*31*). Mutations in *mb-1* that disrupt the link between the antigen receptor and Btk may be responsible for some cases of atypical XLA.

Table 1. Bone marrow B cell populations in control and *mb-1*$^{\Delta c/\Delta c}$ littermates. Total nucleated cells were counted from two femurs, and B cell numbers were calculated on the basis of flow cytometric analysis. Pro-B cells (B220$^+$/CD43$^+$), pre-B cells (B220^{lo}/CD43$^-$/sIgM$^-$), immature B cells (B220^{lo}/CD43$^-$/sIgM$^+$), and mature B (B220^{hi}/CD43$^-$/sIgM$^+$). Numbers are multiplied by 10^{-6}.

Genotype	*n*	Total cells	Lymphocytes (%)	B220$^+$	Pro-B	Pre-B	Immature B	Mature B
Δc/Δc	5	21.0 ± 5.0	25.1 ± 5.9	1.95 ± 1.1	0.75 ± 0.20	0.80 ± 0.50	0.33 ± 0.18	0.07 ± 0.07
+/+	7	25.5 ± 3.9	31.8 ± 4.5	4.81 ± 1.32	0.44 ± 0.19	1.88 ± 0.34	1.28 ± 0.57	0.91 ± 0.20



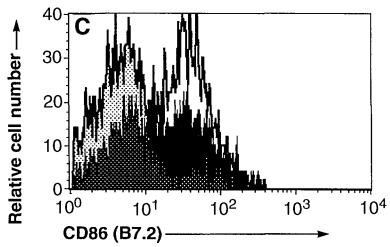


Fig. 4. Immune response of C57BL/6 wild-type (○) and C57BL/6 *mb-1*$^{\Delta c/\Delta c}$ (●) littermates to TD and TI type 2 antigens (*22*). Bars indicate geometric means. (**A**) IgG1 and λ antibody response measured 0 and 14 days after immunization with 100 μg of NP-CG. (**B**) NP-specific IgG3 and λ antibody measured 0 and 10 days after NP-Ficoll (10 μg) immunization. The hapten-specific NP primary response is dominated by λ1-bearing antibodies, and the TI type 2 response to NP-Ficoll is derived predominantly from conventional B cells (*34*). (**C**) Expression of CD86 on control (open) and mutant (shaded) B220$^+$ splenocytes (*24*) before (stippled) or after anti-IgM (1 μg/ml) overnight stimulation.

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7. C57BL/6-derived ES cells, B6III [B. Lederman and K. Bürki, *Exp. Cell. Res.* **197**, 254 (1991)], were transfected with the pmb1tc targeting construct generated from isolated C57BL/6 genomic clones. Selection for G418 resistance and gancyclovir sensitivity (300 μg/ml total G418; 2 μM gancyclovir) resulted in 31 out of 59 double-resistant colonies to be correctly targeted as described in Fig. 1 and verified by alternative probes and Southern analyses (*12*). The *neo*^{r} gene was removed by Cre-mediated deletion (*8*). ES clones heterozygous for the Ig-α mutation were injected into CB.20 blastocysts, and resulting chimeras were crossed to either CB.20 or C57BL/6 strains for germline transmission. Heterozygous offspring from these crosses were intercrossed to generate mice homozygous for the mutation in either a (C57BL/6 × CB.20)F_2 or C57BL/6 background.
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9. The targeting vector was designed to exchange the tyrosines in the Ig-α ITAM with phenylalanines. Sequencing of the targeted allele from IgM$^+$ splenic B cells verified the presence of these exchanges; however, sequencing revealed a five-nucleotide deletion [nucleotides 4506 to 4510 (*33*)] in the membrane-proximal region of the cytoplasmic tail that had occurred during homologous recombination (*12*). This

deletion results in a reading frame shift and the introduction of a stop codon at amino acid 181, changing the last two amino acids from Glu-Asn to Asp-Leu. This mutated locus encodes a truncated Ig-α chain harboring 21 of the 61–amino acid cytoplasmic tail.
10. Bone marrow, spleen, lymph node, and peritoneal exudate cells were isolated from *mb*-1$^{\Delta c/\Delta c}$ or control (C57BL/6 × CB.20)F_2 littermates. Cells were stained directly with fluorescein isothiocyanate (FITC)– or phycoerythrin-coupled reagents or indirectly with biotinylated antibodies followed by streptavidin-CyChrome (Pharmingen, San Diego) and analyzed with a FACScan (Becton Dickinson). Monoclonal antibodies used were anti-CD43 (S7), anti-IgM (R33-24), anti-B220/CD45R (RA3-6B2), anti-IgM^a (RS3.1), anti-IgM^b (MB86), anti-CD3 (145-2C11), anti-CD5 (53-6.7), anti-IgD (1.3-5), and anti-CD22 (Lyb 8.2). For intracellular staining, cells were fixed in paraformaldehyde and stained in 0.5% saponin (Sigma) as described [M. Assenmacher, J. Schmitz, A. Radbruch, *Eur. J. Immunol.* **24**, 1097 (1994)] with either FITC-conjugated anti-IgM heavy chain (M41) or anti-κ (R33-18).
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22. C57BL/6 *mb*-1$^{\Delta c/\Delta c}$ or control littermates (9 to 12 weeks of age) were immunized intraperitoneally with either 100 μg of alum-precipitated NP_{15}-CG or 5 to 10 μg of NP_{27}-Ficoll and serum collected 7, 10, or 14 days later (*12*). NP-specific λ1 and IgG3 (TI) or IgG1 (TD) Ig titers were assayed by enzyme-linked immunosorbent assay (ELISA) as described [J. Roes and K. Rajewsky, *J. Exp. Med.* **177**, 45 (1993)]. The limit of detection for NP-specific IgG3, IgG1, or λ1 ELISA is 0.064 μg/ml. Five mutant and control mice were used for each immunization.
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Substitution of L-Fucose by L-Galactose in Cell Walls of *Arabidopsis mur1*

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An *Arabidopsis thaliana* mutant (*mur1*) has less than 2 percent of the normal amounts of L-fucose in the primary cell walls of aerial portions of the plant. The survival of *mur1* plants challenged the hypothesis that fucose is a required component of biologically active oligosaccharides derived from cell wall xyloglucan. However, the replacement of L-fucose (that is, 6-deoxy-L-galactose) by L-galactose does not detectably alter the biological activity of the oligosaccharides derived from xyloglucan. Thus, essential structural and conformational features of xyloglucan and xyloglucan-derived oligosaccharides are retained when L-galactose replaces L-fucose.

To date only one mutant of *Arabidopsis thaliana* has been shown to affect the glycosyl compositions of cell wall polysaccharides (*1*). Plants carrying the *mur1* mutation are more brittle than wild-type plants, are slightly dwarfed, and have an apparently normal life cycle. The scarcity of cell wall mutants suggests that such mutations are lethal if they eliminate sugar residues essential for the integrity of the cell wall or the function of cell wall–derived oligosaccharins (*2*). The *mur1* mutation offers an opportunity to study the effects of the greatly reduced amounts of L-fucose (L-Fuc) on the structure and function of the

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