Very Low Affinity B Cells Form Germinal Centers, Become Memory B Cells, and Participate in Secondary Immune Responses When Higher Affinity Competition Is Reduced

Joseph M. Dal Porto, Ann M. Haberman, Garnett Kelsoe, and Mark J. Shlomchik

Abstract

To understand the relationship between the affinity of the B cell antigen receptor (BCR) and the immune response to antigen, two lines of immunoglobulin H chain transgenic (Tg) mice were created. H50Gα and T1(V23)α mice express α H chain transgenes that associate with the λ1 L chains to bind the (4-hydroxy-3-nitrophenyl)acetyl hapten with association constants (Kₐ) of only 1.2 × 10⁵ M⁻¹ and 3 × 10⁴ M⁻¹, respectively. Both lines mounted substantial antibody-forming cell (AFC) and germinal center (GC) responses. H50Gα Tg mice also generated memory B cells. T1(V23)α B cells formed AFC and GCs, but were largely replaced in late GCs by antigen-specific cells that express endogenous BCRs. Thus, B lymphocytes carrying BCRs with affinities previously thought to be irrelevant in specific immune responses are in fact capable of complete T cell–dependent immune responses when relieved of substantial competition from other B cells. The failure to observe such B cells normally in late primary responses and in memory B cell populations is the result of competition, rather than an intrinsic inability of low affinity B cells.

Key words: mutation • antibody affinity • plasma cell • variable region gene • λ1 immunoglobulin

Introduction

Early humoral responses are heterogeneous and mainly low affinity (1–4). As the response progresses, Ab diversity is reduced and affinity increases (5–7). It is possible that early low affinity Ab is biologically irrelevant and the result of nonspecific or bystander stimulation (8, 9). Alternatively, very low affinity B cells may respond specifically but are lost as the response progresses. For example, intrinsic affinity requirements could limit cell participation in humoral responses. Survival in germinal centers (GCs) or memory differentiation may require signaling above a threshold (10–12). Alternatively, the loss of low affinity B cells from immune responses could reflect competition among antigen-reactive B cells (13). To distinguish these models for the antigen-driven selection of B cells, we generated the following two lines of transgenic (Tg) mice expressing Ig H chain Tgs that encode extremely low affinity anti-(4-hydroxy-3-nitrophenyl)acetyl antibodies when associated with the λ1 L chain: 1.2 × 10⁵ M⁻¹ for H50Gα and ~3.0 × 10⁴ M⁻¹ for T1(V23)α.

Materials and Methods

Mice. 6–8-wk-old C.B-17 and C.B-17-SCID mice were purchased from Taconic Farms, Inc. All mice were maintained in sterile microisolator cages.

Creation of H50Gα and T1(V23)α Tg Mice. The VH186.2/DFL16.1 rearrangement, containing a single mutation at codon 50, designated H50G and V23 analogue VH rearrangement from an unmutated VDJ rearrangement of the V23 gene taken from a C57BL/6 mouse 10 d after immunization with NP, were subcloned into a vector that contains the 11.6-kb Cµ fragment that

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1215–1221
includes the membrane and secretory exons (14). The H50Gμκ
lineage had two copies of the Tg, whereas two separate
T1(V23)μκ lines with similar phenotypes were used to have
three copies or a single copy of the Tg. Tg mice were back-
crossed with C.B–17 mice for five or more generations.

Flow Cytometry. The following antibodies were used: phyco-
erthrin-conjugated anti-B220 (CD45RA-PE; BD PharMingen);
fluorescein-conjugated anti–mouse IgMκ (RS.3.1-FITC); biotiny-
lated AF6–7.8 (anti-IgMκ); fluorescein-conjugated or biotinylated
Li136 (anti-λ1); and biotinylated goat anti–mouse κ L chain–
specific and biotinylated goat anti–mouse Ig (Southern Biotechnol-
ogy Associates, Inc.). Red 670–conjugated streptavidin (GIBCO
BRL) was used to reveal biotin-coupled Ab staining.

Antigens and Immunizations. Mice were immunized intraperi-
tonally with 50 μg of NP16-chicken γ-globulin (CG), (4-hydroxy-
5-iodo-3-nitrophenyl)acetyl (NIP)12-CG, or CG precipitated in
alum. For secondary/memory responses and challenge of repopu-
lated SCID mice, 20 μg of soluble NP16-CG in PBS was admin-
istered intravenously on the indicated days.

Immunohistology. Immunohistology was performed as previ-
ously described (3, 15). GCs were enumerated as peanut aggluti-
ees areas within lymphoid follicles (2–3 SD from three separate
sections). Endogenous Ig–expressing B cells in the GC of
H50Gμκ or T1(V23)μκ mice were enumerated using both AF6–
7.8–biotin and biotinylated goat anti–mouse IgG1, followed by
streptavidin–alkaline phosphatase. To detect apoptotic cells,
TUNEL assays were performed using in situ apoptosis detection
kits (Oncor).

Microdissection of Cells, DNA Amplification, and Sequencing. Microdissection of cells, DNA amplification, and sequencing was
performed as previously described (14, 16). Cellular material
(constituting 10–20 cells) was microdissected from individual
GCs (3, 15, 17). In H50Gμκ mice, GCs sampled were also IgMκ+
and devoid of endogenous Ig–expressing (IgMκ+ or IgGκ+) cells.
DNA was amplified by Pfu polymerase (Stratagene) using nested
primers specific for Vκ1 and Jκ1 elements (14).

Quantitation of Serum Ab. Ab titers were determined by end-
point dilution in ELISA using plates coated with NIP, BSA, NIP-BSA, or CG (7). The endpoint was the last dilution that
demonstrated a signal greater than twofold above the back-
ground. NP-specific ELISAs using differentially haptenated sub-
strates were used to quantitate high and low affinity Ab (3).

Reduction of Serum Ab by 2-Mercaptoethanol (2-ME). Serum
was assessed for NP reactivity by ELISA after treatment with
2-ME (3). For calculations of the percent of the IgMκ titer resis-
tant to 2-ME treatment, mock and 2-ME ELISA results were
converted to relative Ab concentrations by comparison to a stan-
dard purified H50Gμκ/λ1 transfectoma Ab.

Adaptive Transfers of Splenocytes. 5 × 106 splenocytes recov-
ered from H50Gμκ and C.B–17 mice 30 d after immunization
with 50 μg of NP16-CG precipitated in alum (18) were injected
intravenously into recipient SCID mice. Recipients were chal-
genled with 20 μg of NP16-CG in PBS or PBS alone 24 h later. 9 d
after immunization the recipient sera were collected.

Online Supplemental Material. The supplementary material
shows original VA sequence data as well as summary tables that
analyze the distribution of mutations in the sequences. Fig. S1
shows all codons that contained mutations in VA1 sequences de-
vised from either C.B–17 or H50Gμκ mice, as described in the
Materials and Methods and Results sections. Table S1 is a com-
parison of the two datasets, showing the frequency of overall mu-
tations as well as replacement and silent mutations. Table S2
shows the ratios of replacement and silent ratios in each subregion
of VA. Online supplemental material available at http://
www.jem.org/cgi/content/full/jem.20011550/CD1.

Results

Characterization of H50Gμκ and T1(V23)μκ Tg Mice. Splenic B
cells from naive H50Gμκ and T1(V23)μκ mice demonstrated efficient allelic exclusion, with ≤2% expressing
endogenous IgMκ protein (unpublished data). The ratio of
A1:κ L chain–bearing B cells were comparable in both Tg and control mice, and all Tg lines displayed total
surface Ig densities comparable to normal, naive B cells (unpublished data).

Humoral Immune Responses in H50Gμκ Tg Mice. Naive
H50Gμκ Tg mice had low titers (≤1:160) of NP-specific serum
Ab that sharply increased after immunization with
NP–CG (Fig. 1A). At day 12, the titers of NP-binding Tg
(IgMκ) Ab increased 1,000-fold in H50Gμκ mice. Only in
3 out of 31 H50Gμκ mice were substantial levels of IgG1
anti–NP detected (Fig. 1A), indicating that typically most
anti-NP was Tg derived. Levels for anti–NP A1 and total Ig
at day four were similar for Tg and control animals. How-
ever, C.B–17 mice later achieved 5–10-fold higher titers.
To confirm that H50Gμκ mice expressed only low affinity
Tg-encoded antibodies in response to immunization, sera of
H50Gμκ and C.B–17 mice were mildly reduced with
2-ME (3). The binding of very low affinity IgM Ab is avidity
dependent and lost upon 2-ME reduction (5), whereas
higher affinity IgM or IgG Abs are unaffected. The reduc-
tion of Tg sera led to a near complete loss (≥97%) of NP
reactivity (Fig. 1B). This loss was comparable to that ob-
served for purified H50Gμκ/λ1 transfectoma Ab (3).
In contrast, C.B–17 serum titers were unaffected.

Immune Response of Very Low Affinity B Cells. To di-
rectly test whether B cells with very low affinities were ca-
pable of initiating and sustaining specific humoral re-
sponses, H50Gμκ Tg mice were immunized with NIP-CG
and T1(V23)μκ Tg mice were challenged with NIP–CG and
NIP–CG. H50Gμκ/λ1 Ab has a lower affinity for NIP
(6.3 × 104 M−1) than for NP (3). T1(V23)μκ/λ1 Ab has a
Kd = 5.0 × 104 M−1 for NIP and an affinity for NP that is
too low for reliable measurement by fluorescence quench-
ing (5).

T1(V23)μκ mice had 8–10-fold increases in hapten-spe-
cific IgMκ Ab between 4 and 16 d after immunization
with either immunogen, with some mice showing 20–30-fold
increases in Ab titer (Fig. 1, C and D; ▲, Δ). IgMκ
responses by T1(V23)μκ mice to NP–CG or NIP–CG were
lower (5–10-fold) than those observed in H50Gμκ mice for
the same immunogen (Fig. 1, C and D; ▲, Δ vs. ○). IgG1
responses, presumably encoded by the endogenous Igκ loci,
were higher in T1(V23)μκ mice compared with H50Gμκ
mice. The higher levels of endogenously derived anti–NP
antibodies in T1(V23)μκ mice may reflect the extraordinar-
ily poor affinity of these Tg B cells, which confer little ad-
vantage in competition with rare B cells that express endo-
genous VH genes. B cells expressing endogenously
encoded VH genes comprise 1–3% of the initial repertoire,
of which only a small fraction of B cells would have NP/ 
NIP specificity. Thus, the emergence of these B cells in re-
sponse to NP/NIP reflects the competition between rare,
presumably higher affinity B cells and the much more 
common low affinity Tg B cells.

At the height of the response, H50G<sub>a</sub> mice challenged 
with NIP had a 100-fold increase in hapten-binding IgM<sub>a</sub>
Ab titers compared with controls (Fig. 1 D), which is 
2–10-fold lower than those after challenge with NP-CG 
(Fig. 1, C and D). This observation establishes a relation-
ship between B cell antigen receptor (BCR) affinity/avid-
ity and the magnitude of the Ab response in a single Tg 
mouse line.

**Low Affinity Tg B Cells Can Initiate Primary GCs.** PNA<sub>a</sub>
GCs in H50G<sub>a</sub> mice 12 d after immunization (Fig. 
2 A) were observed in numbers (~70–80 per section) 
equivalent to normal mice (6, 15). 65 ± 5.8% of the GCs 
in H50G<sub>a</sub> mice were stained with both anti-IgM<sub>a</sub> 
and anti-λ1 Ab, indicating that they contained NP-specific 
B cells expressing the H50G<sub>a</sub> Tg (Fig. 2, A and C). The 
percentage of λ1<sup>+</sup> GCs was only slightly higher than that 
seen in non-Tg C.B-17 mice for the same day, which was 
54 ± 3.4%. Many B cells in the splenic red pulp exhibited 
strong cytoplasmic staining with λ1- and IgM<sub>a</sub>-specific Ab 
(unpublished data), the characteristic phenotype of plasma-
blasts and antibody-forming cell (AFC) (18).

H50G<sub>a</sub> Tg B cells also formed Tg<sup>a</sup> GCs when their 
BCR affinity was less than 10<sup>-5</sup> M<sup>-1</sup>, i.e., after immuniza-
tion with NIP. Such mice had PNA<sub>a</sub> and IgM<sub>a</sub> GCs in 
numbers equivalent to C.B-17 controls, although many of 
these GCs were smaller than those in control animals (un-
published data). We also investigated the capacity of very 
low affinity B cells to form GC in T1(V23)<sub>a</sub> mice. Sur-
prisingly, there was no difference in GC number and size 
in T1(V23)<sub>a</sub> mice challenged with NP or NIP compared 
with C.B-17 controls. However, ~80% of GCs in 
T1(V23)<sub>a</sub> mice contained both Tg IgM<sub>a</sub>– and endoge-
nous Ig–expressing B cells (unpublished data). When 
present, endogenous Ig–expressing B cells (demonstrated 
with anti-IgM<sub>a</sub> Ab and anti-IgG<sub>a</sub> Ab) constituted as much 
as 50% of the PNA<sub>a</sub> cells in each GC. Presumably these 
endogenously derived B cells reflect the competitive suc-
cess of rare, higher affinity B cells. To confirm this, we mi-
crodissected and sequenced several clusters of λ1<sup>+</sup> B cells

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Figure 1. Humoral immune response in low affinity H50G<sub>a</sub> and T1(V23)<sub>a</sub> Tg mice. (A) H50G<sub>a</sub> (○) and C.B-17 controls (●) were injected intra-
peritoneally with NP-CG in alum. Serial dilutions of sera were assayed for NP binding of total Ig, Tg IgM<sub>a</sub>, anti-NP characteristic λ1, and IgG<sub>a</sub>. *, 
H50G<sub>a</sub> mice in which significant anti-NP IgG<sub>a</sub> titers may have affected the measurement of IgM<sub>a</sub> Tg levels. (B) Sera from H50G<sub>a</sub> and control C.B-17 
mice after immunization with NP-CG were assayed for their ability to bind NP-BSA after 2-ME treatment (shaded bars) or mock treatment (solid bars). 
The change in the relative NP binding of treated samples is shown as a percentage change from mock-treated samples. Values represent the mean values 
(± SD) obtained from three independent experiments. T1(V23)<sub>a</sub> Tg mice (▲) were compared with H50G<sub>a</sub> (○) and C.B-17 (●) mice in response to 
(C) NP-CG or (D) NIP-CG in alum. Sera were assayed for NP-binding components. Each symbol represents the average endpoint dilution value of se-
rum from an individual mouse measured in triplicate.
Costaining for IgMα/IgG1 and sequenced their VH regions. In fact, >75% of recovered sequences were V\textsubscript{iH}186.2, the canonical VH of anti-NP responses, which established that the endogenously derived B cells are higher affinity anti-NP B cells. It is likely that the population of B cells expressing endogenous VDJ rearrangements accounts for the normal size and frequencies of GCs in immunized T1(V23)α/β animals.

Late GCs of H50G\textsuperscript{μ} Mice Show Evidence of Increased Apoptosis. 12 d after immunization with NP-CG, B cells bearing BCRs encoded by the endogenous Igh\textsuperscript{b} loci are infrequent in the GC of H50G\textsuperscript{μ} Tg mice (Fig. 3 A). However, at day 16 of the primary response, endogenous Ig-expressing B cells (IgM\textsuperscript{b} or IgG1\textsuperscript{b}) begin to appear in the GCs, increasing to 5–10% of all PNA\textsuperscript{+} GC cells by day 20 (Fig. 3, B and C). Late GCs (16–20 d after immunization) of H50G\textsuperscript{μ} mice had two- to threefold more apoptotic B cells compared with C.B-17 controls, as indicated by TUNEL labeling (Fig. 3, D and E). GCs (n = 30) sampled from six H50G\textsuperscript{μ} mice at 16 or 20 d after immunization had an average 32% (±10%) TUNEL\textsuperscript{+}/PNA\textsuperscript{+}/IgD\textsuperscript{−} cells. This was in contrast to GCs (n = 20) from four C.B-17 mice at similar days, which contained only 12% (±7%) TUNEL\textsuperscript{+} GC cells.

Somatic Mutation in H50G\textsuperscript{μ} Mice. Somatic hypermutation was assayed in the endogenous α L chain gene of H50G\textsuperscript{μ} mice, as ectopic heavy chain Tg generally does not mutate. IgM\textsuperscript{b}/α L, GC cells from three H50G\textsuperscript{μ} mice, and three C.B-17 mice were microdissected and their V\textsubscript{α} regions amplified and sequenced (Fig. S1 and Tables S1 and S2). Of 28 H50G\textsuperscript{μ} VA1/Jα1 rearrangements examined, 22 were mutated with an average of 5.1 (±3.7) mutations per rearrangement.

The distribution and type of somatic mutations observed in VA1 genes present in Tg and control GC B cells is summarized in Tables S1 and SII. 57 of 68 H50G\textsuperscript{μ} VA1 mutations encoded amino acid replacements. The 68 unique mutations were a striking contrast to the 11 unique mutations found in C.B-17 GCs. Mutations in the H50G\textsuperscript{μ} VA1/Jα1 rearrangements had an overall replacement/silent (R/S) ratio three times greater than C.B-17 rearrangements. In addition, the R/S ratio of mutations in CDR1 and CDR2 of H50G\textsuperscript{μ} VA1 genes was 10- and 6-fold higher, respectively, than the predicted ratios based on codon usage. These data demonstrate enhanced mutation and selection in the endogenous VA1 gene rearrangements of H50G\textsuperscript{μ} GC B cells.

Low Affinity Primary and Memory Responses in H50G\textsuperscript{μ} Mice. To determine the degree of affinity maturation in the primary and memory responses of H50G\textsuperscript{μ} mice, Tg and C.B-17 mice were immunized with NP-CG and 60 d later were rechallenged intravenously with soluble NP-CG. Fig. 4 shows the change in the NP-specific IgM\textsuperscript{b}/Tg Ab titers. Upon secondary immunization, a prompt and significant increase in NP-specific IgM\textsuperscript{b} Ab was observed. By day 9 of the secondary response, Tg-IgM\textsuperscript{b} endpoint titers increased from 1:4,500 to 1:72,000 (Fig. 4, C). No significant Ab response (>1 out of 200) was observed in unprimed H50G\textsuperscript{μ} mice given the same soluble antigen (Fig. 4, D).

Affinity maturation, presumably through the mutation of VA1, did enhance the avidity of H50G\textsuperscript{μ} Ab, as re-
flected by the increased portion that bound NP<sub>5</sub>-BSA (Fig. 4; Δ, bars). However, this affinity maturation was modest, as 85–90% of secondary Ab generated by H50G<sup>µ</sup> mice remained sensitive to 2-ME treatment (unpublished data). Interestingly, the rechallenge of primed H50G<sup>µ</sup> mice with soluble NP-CG elicited a rapid increase in low affinity Ab, as inferred from the precipitous decrease in the NP<sub>5</sub>/NP<sub>22</sub> binding ratio of the serum Ab (Fig. 4, bars). Only 30% of the NP-specific IgM<sup>a</sup> titer at day 9 of the secondary response showed the ability to bind NP<sub>5</sub>-BSA. This is a contrast to the 70% observed in the late primary response (Fig. 4) and consistent with the reactivation of low affinity memory B cells and their differentiation into AFC (7).

To exclude that T cell priming alone was responsible for the memory response of H50G<sup>µ</sup> mice, Tg and control mice were primed with the carrier protein CG, and 60 d later challenged intravenously with soluble NP-CG. Average NP-specific λ<sup>1+</sup> Ab titers from CG-primed H50G<sup>µ</sup> mice were 15-fold lower than cohorts primed with NP-CG (Table I; 1,220 vs. 1,33,400). On challenge with soluble antigen, NP-CG–primed H50G<sup>µ</sup> mice exhibited a 38% absolute increase in the serum Ab titer capable of binding NP<sub>5</sub>–BSA, whereas there was no appreciable increase in the NP<sub>5</sub>–BSA–binding titer in CG-primed H50G<sup>µ</sup> mice. Thus, T cell priming alone cannot account for the extent of low affinity Ab elicited in the memory responses of H50G<sup>µ</sup> mice.

To minimize the influence of preformed Ab on secondary responses, splenocytes from naive or NP-CG–primed (day 30) H50G<sup>µ</sup> and C.B–17 mice were adoptively transferred into SCID recipients, which were then challenged intravenously with soluble NP-CG. SCID mice that received primed H50G<sup>µ</sup> splenocytes generated Ab titers that were more than 100-fold higher than SCIDs that were re-populated with naive H50G<sup>µ</sup> splenocytes. The magnitude of the increase was similar in control mice receiving C.B–17 donor cells (unpublished data). These differences in serum Ab were not due to the transfer of residual AFC, as SCID mice that received primed H50G<sup>µ</sup> splenocytes but were not challenged with antigen had no appreciable anti-NP serum Ab (titer of 1:60).

Discussion

Normally, very low affinity cells are rare in late GCs, in memory cell populations, or as long-lived AFC (19, 20). By fixing the B cell repertoire at low affinity using a non-mutating ectopic Tg, we show that the virtual absence of low affinity cells in these compartments is due to competition from higher affinity cells. When clonal competition is reduced, very low affinity B cells fill GCs and contribute to memory responses.

Although competition is reduced, low numbers of B cells expressing endogenous VH gene segments provide detectable interclonal competition. Endogenously derived cells are rare in H50G<sup>µ</sup> Tg mice, especially early in the response. However, many B cells expressing non-Tg BCR emerge in immunized T1(V23)<sup>µ</sup> mice, and to a lesser ex-

Table I. Memory Response to Soluble Antigen from NP-CG Primed and Carrier Primed Mice<sup>a</sup>

<table>
<thead>
<tr>
<th>Immunization Protocol&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Serum Response&lt;sup&gt;c&lt;/sup&gt; (end-point dilution)</th>
<th>Percent NP5/NP22 Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mice 1&lt;sup&gt;0&lt;/sup&gt;(i.p.)</td>
<td>α-NP22 λ1 titer (1/X)</td>
<td></td>
</tr>
<tr>
<td>CB-17 NP-CG NP-CG</td>
<td>135,826 ± 69,607 (53)</td>
<td></td>
</tr>
<tr>
<td>H50G&lt;sup&gt;µ&lt;/sup&gt;</td>
<td>33,417 ± 10,919</td>
<td>38</td>
</tr>
<tr>
<td>C.B-17 CG NP-CG</td>
<td>4,797 ± 2,010 (23)</td>
<td></td>
</tr>
<tr>
<td>H50G&lt;sup&gt;µ&lt;/sup&gt;</td>
<td>2,218 ± 1,581 (8)</td>
<td></td>
</tr>
<tr>
<td>C.B-17 none NP-CG</td>
<td>130 ± 60 (0)</td>
<td></td>
</tr>
<tr>
<td>H50G&lt;sup&gt;µ&lt;/sup&gt;</td>
<td>60 ± 20 (0)</td>
<td></td>
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</table>

<sup>a</sup>Serum data were obtained from mice 9 d after secondary challenge with soluble antigen.

<sup>b</sup>Primary immunizations were by intraperitoneal administration of 50 μg NP-CG or CG alone in alum. Secondary/memory responses were elicited through the intravenous injection of 20 μg soluble NP-CG 60 d after primary challenge.

<sup>c</sup>The NP-specific 1A<sup>+</sup> end-point serum titer was used as a measure of the secondary immune response to allow direct comparison between H50G<sup>µ</sup> and C.B–17 mice.
tent H50Gµκ mice, as demonstrated by the presence of non-Tg Ab (Fig. 1) and histology (Fig. 3). Presumably, the extremely low affinity of T1(V23)µκ B cells for the NP and NIP haptens allows them to be overwhelmed by rare B lymphocytes that escape allelic exclusion and respond to NP/NIP, as indicated by their frequent expression of V\(_{\text{H}186.2}\), a hallmark of high affinity NP Ab. Thus, higher affinity B cells expressing endogenous genes outcompete a numerous but very low affinity Tg population.

As V(D)J hypermutation creates L chain diversity in GCs (Figure S1), intraclonal competition also exists. R/S ratios in VA CDR1 and VA CDR2 suggest strong selection for GC B cell mutants. Although VA mutations can improve BCR affinity in H50Gµκ mice, the low affinity of secondary Ab in these mice (Fig. 4) suggests that this pathway for affinity maturation is limited. Nonetheless, the high frequency (1.4%) of point mutations in the VA1 rearrangements of H50Gµκ GC B cells is almost twice that found in a similar mouse with a germline affinity, VH 186.2 Tg (14), and much higher than wild-type mice. The high frequency and intense selection of VA1 mutations in H50Gµκ mice could reflect the difficulty in improving BCR affinity through L chain mutations alone. However, mutation rates may be intrinsically higher in low affinity B cells.

Our data do not rule out the role(s) for differential signaling governed by affinity/avidity in determining B cell fates (11, 12). Aside from memory development, other aspects of B cell differentiation like isotype switching could be governed by BCR signal strength.

The ability of very low affinity B cells to establish GCs and humoral memory has implications for understanding natural B cell responses. Low affinity BCRs can acquire higher affinities with a single point mutation (19, 21), and the many low affinity B cells that reach GCs are the potential precursors of effective memory B cells. This view is also consistent with direct estimates of clonal diversity in nascent GCs (6) and computer models, which suggest that GCs are seeded by 40–50 precursors (22). Finally, the latent genetic potential of low affinity clones could underlie “clonal shifts” in secondary responses whereby dominant, primary clonotypes are replaced by mutated, dissimilar clones (2, 23).

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