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Tolerance Induction of IgG+ Memory B Cells by T Cell-Independent Type II Antigens

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Memory B cells generated during a T cell-dependent immune response rapidly respond to a secondary immunization by producing abundant IgG Abs that bind cognate Ag with high affinity. It is currently unclear whether this heightened recall response by memory B cells is due to augmented IgG-BCR signaling, which has only been demonstrated in the context of naive transgenic B cells. To address this question, we examined whether memory B cells can respond in vivo to Ags that stimulate only through BCR, namely T cell-independent type II (TI-II) Ags. In this study, we show that the TI-II Ag (4-hydroxy-3-nitrophenyl) acetyl (NP)-Ficoll cannot elicit the recall response in mice first immunized with the T cell-dependent Ag NP-chicken γ-globulin. Moreover, the NP-Ficoll challenge in vivo as well as in vitro significantly inhibits a subsequent recall response to NP-chicken γ-globulin in a B cell-intrinsic manner. This NP-Ficoll–mediated tolerance is caused by the preferential elimination of IgG+ memory B cells binding to NP with high affinity. These data indicate that BCR cross-linking with a TI-II Ag does not activate IgG+ memory B cells, but rather tolerizes them, identifying a terminal checkpoint of memory B cell differentiation that may prevent autoimmunity. The Journal of Immunology, 2011, 186: 5620–5628.

The T cell-dependent (TD) immune response is characterized by the formation of germinal centers (GC) in the lymphoid follicles of secondary lymphoid organs where Ag-specific B cells proliferate vigorously and can undergo class switch recombination and somatic hypermutation (SHM) at their Ig gene loci (1–3). As a result, the Ag receptor (BCR) on the responding B cells changes from IgM to a switched isotype, mostly IgG in spleen and lymph node, and its affinity for Ag is diversified. Among such GC B cells, those expressing an IgG BCR with high affinity for Ag differentiate into memory B or long-lived plasma cells, both of which are responsible for immunological memory, a hallmark of adoptive immunity (4, 5). Memory B cells have a long lifespan in the spleen and possibly in other lymphoid organs without appreciable proliferation and are responsible for the recall response to the same Ag as encountered in the primary response (6–8). In the presence of Th cells, the memory recall response can be elicited by soluble TD Ags without any adjuvants and is characterized by the more rapid production of a greater amount of IgG Ab with higher affinity than observed in the primary response (9).

The heightened memory recall response is believed to be attributable to both an increase in the frequency of Ag-specific memory B and T cells and the enhanced capacity of the B cells for expansion and facilitated differentiation into plasma cells when compared with naive B cells. These latter features can be ascribed in part to enhanced engagement of Th cells by the memory B cells due to their expression of higher levels of MHC class II, CD80, and other T cell-interacting molecules (10–13). Alternatively or additionally, the phenotype of memory B cells can be ascribed to augmented signaling through the IgG BCR that is expressed by most of these cells. Indeed, increased B cell clonal expansion, plasma cell production, and Ab production upon primary immunization were observed in mice transgenic for an IgG BCR or a chimeric BCR consisting of the extracellular portion of IgM and the transmembrane/cytoplasmic domains of IgG (IgM/G) compared with mice expressing an IgM BCR (14). Furthermore, the IgG BCR, or its cytoplasmic domain, has been shown to mediate augmented calcium response, BCR oligomerization, microcluster growth, and proliferation upon BCR ligation (15–19). However, these results have been obtained from studies using transgenic naive B cells or B cell lines and not naturally derived memory B cells expressing an IgG BCR. Thus, there is no formal evidence demonstrating that the enhanced response of memory B cells is due to enhanced signaling through IgG BCR.

This issue would be best addressed by stimulating memory B cells in vivo only through the BCR without engaging T cell help. T cell-independent (TI) Ags are known to induce a primary immune response in the absence of T cell help, characterized by the rapid production of low-affinity Abs of IgM as well as particular IgG subclasses, mainly IgG3 in mice (20). Among the TI Ags, T cell-independent type I (TI-I) Ags contain mitogenic moieties such as LPS and activate B cells through the BCR and TLRs simultaneously. By contrast, TI type II (TI-II) Ags are large molecules that display highly repetitive epitopes on their surface and can directly stimulate B cells by cross-linking the BCR without engagement of other known costimulatory receptors. If the heightened memory B cell response is due to heightened IgG-BCR signaling, then TI-II Ags carrying the same epitope as the priming TD Ag should elicit a recall response, inducing much stronger...
activation and more Ab production by IgG+ memory B cells than by naive IgM+ B cells. Surprisingly, this seemingly old and important question has remained unsolved. Experimental data that can be found only in the classical literature had drawn two opposite conclusions: TI-II Ags can, or cannot, elicit the recall response (21-24).

In this study, we have used current technology to address whether TD-induced IgG+ memory B cells respond to epitope-matched TI Ags. Our data clearly indicate that IgG+ memory B cells are activated by a TI-I Ag containing LPS, but not by a TI-II Ag. Moreover, we found that memory B cells with a BCR of high affinity for the antigenic epitope are deleted upon challenge with the TI-II Ag by B cell-intrinsic mechanisms. As a result, a subsequent recall response to the TD version of the Ag was significantly reduced, consisting only of low-affinity Abs. These data indicate that BCR cross-linking with a TI-II Ag does not activate IgG+ memory B cells, but rather tolerizes them, suggesting the presence of a terminal checkpoint in B cell differentiation that may help prevent autoimmunity.

Materials and Methods

Mice and immunizations

C57BL/6 mice were purchased from Japan SLC. B1-8 IgH knockin (B1-8 ki) mice (25) backcrossed to the congenic C57BL/6-CD45.1 strains were used where indicated. Previous studies have demonstrated that the B1-8 ki allele effectively excludes the endogenous IgH allele (26). Therefore, we used heterozygous mice in all experiments. All mice were maintained in a mouse facility under specific pathogen-free conditions. Mouse procedures were performed under the protocols approved by the Animal Care and Use Committee of the Tokyo University of Science. Mice were immunized i.p. with 100 μg (4-hydroxy-3-nitrophenyl) acetyl (NP)35-chicken γ-globulin (CGG) precipitated in aluminium hydroxide (alum) and boosted i.v. with 23 μg soluble NP35-CGG, NP35-LPS (Biosearch Technologies), NP10.9-Ficol (Biosearch Technologies), and NP15-mouse serum albumin (MSA) in PBS, unless otherwise noted.

Immunofluorescence microscopy

Freshly isolated spleens were snap-frozen in OCT compound (Sakura Finetek) and 5 to 6-μm-thick sections were prepared using a cryostat. After drying at room temperature, sections were fixed for 10 min in acetone at −20˚C and allowed to dry. Nonspecific binding was blocked using a 3% BSA/PBS at room temperature, and then the slides were washed and stained for 1 h at room temperature with different combinations of the following Abs and reagents: PE-conjugated anti-IgG1 (Southern Bio-technology Associates), biotinylated anti-CD45R/CD19, CD38, CD45R/B220, CD80, CD138, GL7 (e Bioscience; M3A) in PBS, unless otherwise noted.

RT–PCR and nucleotide sequence analysis

To analyze whether IgG+ memory B cells can respond to TI Ags and differentiate to AFCs, we first immunized C57BL/6 mice with a commonly used TD Ag, NP-CGG precipitated in alum and, 16 wk later, challenged the mice with TD, TI-I, or TI-II Ags carrying the same epitopes, NP-CGG, NP-LPS, or NP-Ficoll, respectively. The mice rechallenged with NP-CGG rapidly produced a robust NP-specific IgG1+ AFCs, as early as 5 d postimmunization, whereas those challenged with NP-LPS had a rapid but more modest response. In striking contrast, mice secondarily challenged with NP-Ficoll had no significant NP-specific IgG1 response (Fig. 1A, 1B).

The response of mice primed with NP-CGG and then challenged with NP-LPS is an authentic memory recall response because: 1) primary immunization with NP-LPS did not result in production of NP-specific IgG+ AFCs in 5 d (Fig. 1B); and 2) the ratio of the number of high-affinity NP-AFCs to total NP-AFCs was equally high after the secondary challenge with NP-LPS or NP-CGG (Fig. 1C). Challenge with LPS alone did not elicit the IgG1 recall response (Fig. 1D), in agreement with recent reports using similar systems (28, 29).
It was previously reported that pre-existing Ag-specific IgG Abs strongly suppress Ag-specific IgM production from TI memory B cells upon a secondary challenge with a TI-II Ag (30). To exclude the possibility that the inability of NP-Ficoll to elicit a specific IgG1 recall response is due to pre-existing NP-specific Abs, we transferred splenocytes from mice primed with NP-CGG 112 d earlier into naive recipients, which were then challenged with NP-Ficoll or NP-CGG (Fig. 1E). Even in this condition, NP-Ficoll did not induce a specific IgG1 recall response (Fig. 1F). These data indicate that the inability of the TI-II Ag to recall the memory response is not due to pre-existing Ag-specific Abs, but is attributable to postimmune cellular components.

**TD and TI-I, but not TI-II, challenge induces a rapid extrafollicular foci response**

To confirm the above data in situ, NP-CGG–primed mice were challenged with soluble NP-CGG, NP-LPS, or NP-Ficoll 14 wk later, and then spleen sections were analyzed by immunofluorescence microscopy. In the primary response, IgG1+ cells emerged by day 5, sparsely scattered in the follicles. There were a few small clusters of GL7+ IgG1+ cells near the T cell zones, which are presumably immature GCs (Fig. 2B). By day 10, many IgG1+ cells were found in GCs, and some were seen in foci in the red pulp (Fig. 2C). By contrast, large numbers of IgG1+ cells emerged in the follicles at day 2 after the secondary immunization with NP-CGG, and they moved to the red pulp and formed large foci of AFCs by day 5, which declined in size by day 10 (Fig. 2D–F). A few GCs were detectable at day 10, but they were not NP specific (data not shown). These data indicate that IgG1+ memory B cells swiftly expand in the follicles in response to rechallenge with a TD Ag and differentiate into extrafollicular AFCs, which are much more numerous than in the primary response. In addition, the NP-specific IgG1+ memory B cells rarely generate new GCs.

Secondary challenge with NP-LPS also induced the foci of AFCs by day 5, but to a lesser extent than with NP-CGG (Fig. 2G, 2H). By contrast, essentially no IgG1+ AFCs (Fig. 2J–L) or anti-NP AFCs of any isotype (Fig. 2M) were detectable in the sections from mice secondarily challenged with NP-Ficoll, contrasting to the robust anti-NP extrafollicular foci formation after primary immunization with NP-Ficoll (Fig. 2N). Small GC-like clusters in the follicles were observed in the sections from all the mice primed with NP-CGG irrespective of the secondary challenge. These data indicate that NP-specific IgG1+ memory B cells do not differentiate into AFCs upon encounter with multivalent NP-Ags that do not elicit T cell help, except when a TLR is costimulated.

**TI-II Ag tolerizes IgG1+ memory B cells binding Ag with high affinity through a B cell-intrinsic manner**

We next asked whether the Ag-specific memory B cells simply ignore the TI-II Ags or recognize them and are somehow affected. For this, we challenged NP-CGG–primed mice with NP-Ficoll or PBS as a control at day 60, and then challenged the mice again with soluble NP-CGG 3 wk later. Upon tertiary challenge with NP-CGG, the anti-NP IgG1 response was significantly lower in mice that had been secondarily challenged with NP-Ficoll as compared with mock (PBS)-challenged mice (Fig. 3A). This effect was due mainly to the loss of IgG1 Abs that bind NP with relatively high affinity (Fig. 3B, 3C). This serological finding was...
directly confirmed by sequencing γ1 transcripts containing \( VH_{186.2} \) and related H chain V region (\( VH \)) sequences from sorted splenic plasma cells (CD4\(^{-}\)CD8\(^{-}\)k\(^{2}\)B220\(^{low}\)CD138\(^{+}\)) induced by the tertiary challenge with NP-CGG. NP responses in C57BL/6 mice are known to be dominated by Abs comprised of a \( VH_{186.2} \) H chain and \( \kappa \) L chain, and the H chains of high-affinity Abs carry a replacement mutation, tryptophan to leucine at codon 33 (W33L), which results in a 10-fold increase in the affinity of the Ab for NP (31). All 12 clones obtained from mock-challenged mice used \( VH_{186.2} \), and 66.7% of these contained the W33L replacement. By contrast, only 2 out of 12 clones obtained from the NP-Ficoll–challenged mice used \( VH_{186.2} \), and none of the sequences had the W33L replacement (Table I).

To determine whether the defect in the recall response is B cell intrinsic, purified splenic B cells from mice that had been primed with NP-CGG and challenged with various doses of NP-Ficoll...
B cell transfer system using VHB1-8 gene knockin (B1-8 ki) mice. To determine how TI-II Ags induce tolerance of the Ag-specific memory B cells do not expand or differentiate into Th cytokines such as IL-2, IL-4, and IL-5 (Fig. 3). The data shown in Figs. 3 and 4 could also be interpreted as mediated suppression was not reversed by simultaneous treatment with Th cytokines such as IL-2, IL-4, and IL-5 (Fig. 3F). These results indicate that Ag-specific high-affinity IgG+ memory B cells are tolerized by TI-II Ag through a B cell-intrinsic mechanism.

Ag-specific memory B cells do not expand or differentiate into AFCs, but are eliminated in response to TI-II Ags

To determine how TI-II Ags induce tolerance of the Ag-specific memory B cells at a cellular level, we adopted an established system described in Fig. 4. The NP-Ficoll challenge appeared to induce selective elimination of NP-specific memory B cells and was sensitive to small amounts of the Ag (Fig. 4D). As a control, primary immunization with NP-Ficoll induced a marked expansion of donor-derived naive NP+ B cells in recipient mice by day 10 (Fig. 4E).

Next we analyzed the BrdU content of the NP+ high memory B cells on day 5 after the secondary challenge. Whereas 5.5% of such cells retained BrdU in the control mice, <1% retained BrdU in the NP-CGG–challenged mice, and essentially no cells were BrdU+ in NP-Ficoll–challenged mice (Fig. 4F). The loss of BrdU+ cells is likely due to the rapid proliferation of these cells in the NP-CGG–challenged mice, but to an elimination of such cells in the NP-Ficoll–challenged mice, considering the results shown in Fig. 4A and 4D. This interpretation is also supported by an independent experiment showing that the NP+ memory B cells began to undergo apoptosis on day 2 in NP-Ficoll–challenged mice, before the NP+ cells began to expand in the NP-CGG–challenged mice (Supplemental Fig. 2). Together, these data strongly suggest that high-affinity binding by TI-II Ags induces clonal deletion of memory B cells that have generated in the late phase of GC reaction at least partly by apoptosis.

TI-II Ag challenge eliminates IgG1+ memory B cells selected for high-affinity Ag binding

The data shown in Figs. 3 and 4 could also be interpreted as showing that NP+ memory B cells were anergized, rather than deleted, upon binding to NP-Ficoll through downmodulation of their BCR. If this were the case, such memory B cells would be hidden in the donor-derived NP-Ficoll population after the NP-Ficoll challenge in the system described in Fig. 4, but they must retain the normal frequency of VH186.2 genes encoding the H chain binding to NP with high affinity. To evaluate this possibility, we sorted all donor-derived B cells except plasma and GC B cells (CD45.1+ B220+/CD138+CD38+CD19+) from the spleens of the recipient mice 5 d after the secondary challenge with NP-Ficoll or PBS as described in Fig. 4 and sequenced the VH186.2 only. The data presented in Table I shows that the NP-Ficoll challenge affected anti-NP VH gene repertoire and SMH pattern in splenic plasma cells after tertiary NP-CGG challenge. The average number of mutations per VH sequence. The percentage of clones carrying tryptophan-to-leucine replacement at codon position 33.
replacement/silent mutations (R/S ratio) in CDR1 and CDR2 was significantly lower in the former population (Table II and data not shown). These data indicate that IgG1+ memory B cells highly selected for high-affinity binding to NP were clonally deleted upon encounter with NP-Ficoll.

Tolerance of IgG1+ memory B cells is not induced by a TD Ag that is not recognized by T cells

Finally, we asked whether the deletion of Ag-specific IgG1+ memory B cells upon binding TI-II Ags is simply due to the lack of cognate T cell help. Thus, we challenged the NP-CGG–primed

Table II. TI-II Ag eliminates the well-selected high-affinity IgG1+ memory B cells

<table>
<thead>
<tr>
<th></th>
<th>No. of Clones Sequenced</th>
<th>VHT Mutationa Average</th>
<th>R/S Ratiob</th>
<th>Position 33c W–L (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS (n = 3)</td>
<td>12</td>
<td>9.4</td>
<td>6.1 (4.1/0.7)</td>
<td>2.2 (3.1/1.4)</td>
</tr>
<tr>
<td>NP-Ficoll (n = 4)</td>
<td>15</td>
<td>7.9</td>
<td>3.0 (2.8/0.9)</td>
<td>1.6 (2.4/1.5)</td>
</tr>
</tbody>
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Adoptive transfer and primary immunization was performed as described in Supplemental Fig. 1. On day 60 immunization, recipient mice were injected with PBS or NP-Ficoll. Five days later, memory B cells (propidium iodide–CD45.1B220CD38bhighCD138d) were sorted from pooled splenocytes of these mice, from which the B1-8 ki (VHT186.2)–g1 mRNA sequences were analyzed.

aAverage number of mutations per VHT sequence.
bAverage numbers of replacement and silent mutations per VHT sequence are in parentheses.
cPercentage of clones carrying tryptophan-to-leucine replacement at codon position 33.
mice with NP-conjugated MSA that forms a TD Ag but is not recognized by mouse T cells because they are tolerant to this self-Ag (Fig. 5A). The NP-MSA challenge did not induce an IgG1 recall response in these mice (data not shown); however, rechallenge of these mice with NP-CGG 3 wk after the secondary challenge induced the same level of anti-NP IgG1 Ab response as in control mice, indicating that NP-MSA did not tolerize memory B cells (Fig. 5B).

To examine the effect of the NP-MSA challenge on Ag-specific IgG1+ memory B cells, we immunized mice containing B1-8ki memory B cells as shown in Fig. 5C and analyzed them in the same way as described in Fig. 4D. Five days after the challenge, the donor-derived IgG1+ memory B cells (CD45.1+CD38highNP+) were present in the mice challenged with NP-MSA at a similar frequency and number to the mock-challenged mice (Fig. 5D, 5E). These data indicate that Ag-induced elimination of Ag-specific memory B cells that we observed with NP-Ficoll challenge is not due to the unavailability of cognate T cell help, but probably depends on the nature of the Ag that is intrinsic to the TI-II Ag (Fig. 6).

**Discussion**

**Unresponsiveness of IgG+ memory B cells to a TI-II Ag**

Previous studies have demonstrated that transgenic/knockin B cells expressing IgG1 or chimeric IgM/IgG1 (containing the transmembrane/cytoplasmic region of IgG1) BCRs exhibit enhanced calcium signaling upon cross-linking in vitro and heightened response to primary TD Ag immunization in vivo (14, 16, 17). In contrast, a recent report demonstrated that B cells expressing a transgenic IgG2a exhibit dampened BCR signaling, leading to poor cell activation and proliferation in vitro and poor response to TI-II Ag immunization in vivo, but nonetheless had an augmented proliferative response to CD40 ligation in vitro and augmented Ab production in TD Ag-immunized mice (33). Together with data showing relatively low expression of these transgenic IgG BCRs, these studies suggest that IgG-BCRs generate spontaneous signals that render naive B cells unresponsive to BCR stimulation alone, similar to B cells rendered anergic by chronic self-Ag stimulation (34), but are hyperresponsive to stimulation by Th cells. However, all of these studies have examined the phenotype of naive B cells expressing the transgenic IgG BCRs, which have survived early developmental perturbation induced by the transgene in the bone marrow (17, 33). Therefore, the above interpretations may not be applicable to normal IgG+ memory B cells. We have clearly shown in this study that TD-Ag (NP-CGG)-induced physiological IgG+ memory B cells do not clonally expand and develop into plasma cells upon immunization with an epitope-matched TI-II Ag, NP-Ficoll. Although other TI-II Ags would be tested in the future, the data shown in this study indicate that the unresponsiveness of IgG+ B cells to BCR stimulation may stem from the distinctive nature of IgG BCR independent of the cellular context.

**Clonal deletion of memory B cells by a TI-II Ag**

More importantly, our data demonstrated that the memory B cells expressing an IgG1 BCR with high affinity for the antigenic epitope are eliminated in vivo upon binding with epitope-matched TI-II Ag, resulting in a poor recall response to a subsequent TD Ag rechallenge. This affinity-dependent elimination of memory B cells indicates that the elimination was caused by direct interaction of the BCR with the Ag. The elimination appeared to be due to apoptosis of the memory B cells, and not to anergy, as confirmed by sequence analysis of the remaining repertoire. Memory B cell function was eliminated from splenic B cells purified from primed mice by a brief TI-II Ag treatment in vitro, indicating that the effect is BCR signal-mediated and requires no cells other than B cells. These results together suggest that high-affinity binding to a TI-II Ag by the IgG BCR on memory B cells signals apoptosis, whereas low-affinity binding to the same TI-II Ag is ignored by such cells (Fig. 6). By contrast, both high- and low-affinity binding to TI-I or TD Ags, the latter being provided with cognate T cell help, signal clonal expansion and differentiation. Noticeably, IgG+ memory
B cells are neither activated nor tolerized upon binding to TD Ags without cognate T cell help. Unlike the memory B cell response, TI-II Ag binding to an IgM BCR on naive B cells, irrespective of its affinity, signals cell activation and proliferation (35) (Fig. 6).

Although immature B cells in bone marrow and spleen, but not mature B cells, are known to undergo apoptosis upon BCR cross-linking in vitro, this susceptibility of memory B cells to BCR cross-linking was unknown until this report. To our knowledge, the only other remotely similar previous report demonstrated that human tonsillar IgD+ memory B cells are susceptible to BCR-induced apoptosis only when they are preactivated through CD40 or BCR in vitro (36).

**Mechanism for TI-II-mediated clonal deletion**

Previous reports demonstrated that large amounts of multivalent Ag can suppress an ongoing TD immune response and prevent a recall response in mice, although the mechanism for the immunosuppression was not described (37, 38). These studies differ from ours in an important way. These investigators used an extremely high dose (typically 3 mg) of a nonimmunogenic Ag (FITC-conjugated dextran) as a suppressant, whereas we used a rather low dose (25 μg) of TI-II Ag (NP-Ficol) that is immunogenic in a primary response. Thus, the mechanism for the suppression of memory recall response is likely to be totally different in the two systems.

Our study indicated that the Ag binding-induced elimination of memory B cells is not simply due to the lack of concomitant T cell help, because injection of NP-conjugated MSA did not eliminate memory B cells is not simply due to the lack of concomitant T cell help. Unlike the memory B cell response, TI-II Ag binding to an IgM BCR on naive B cells, irrespective of its affinity, signals cell activation and proliferation (35) (Fig. 6).

repetitive epitopes similar to TI-II Ags, such as dsDNA or ssDNA, in an important way. These investigators used an extremely high dose (typically 3 mg) of a nonimmunogenic Ag (FITC-conjugated dextran) as a suppressant, whereas we used a rather low dose (25 μg) of TI-II Ag (NP-Ficol) that is immunogenic in a primary response. Thus, the mechanism for the suppression of memory recall response is likely to be totally different in the two systems.

Our study indicated that the Ag binding-induced elimination of memory B cells is not simply due to the lack of concomitant T cell help, because injection of NP-conjugated MSA did not eliminate NP-specific IgG* memory B cells. This notion is also supported by the failure to rescue the same memory B cells from NP-Ficol-induced elimination in vitro by the addition of Th cytokines. Thus, the apoptotic fate of memory B cells is likely determined by the way in which the IgG-BCR binds TI-II Ags, in which each of the highly repetitive epitopes binds to the IgG-BCR with high affinity. How this particular state of binding is translated into an apoptotic signal through IgG, but not IgM, awaits further investigation.

**Implications for peripheral self-tolerance**

In mice, self-reactive B cells are known to be tolerized at their immature stages either by clonal anergy in the case of soluble self-Ag (39) or by receptor editing in the case in which self-Ags are in a multivalent membrane-bound form (40), and failure to escape self-reactivity by the latter mechanism is resolved by clonal deletion (41, 42). However, B-1 and marginal zone B cells produce IgM Abs that are polyreactive and bind weakly to self-Ag. These natural IgM Abs protect individuals from a variety of pathogens in the early immune response (43, 44). In humans, a considerable fraction of mature naive B cells express low-affinity self-reactive and polyreactive Abs (45). Moreover, >20% of circulating IgG* memory B cells in humans potentially express polyreactive and low-affinity self-reactive Abs, most of which acquired the self-reactivity by SMH (46). A similar conclusion was reached by an analysis of human anti-dsDNA Abs by site-directed reversions of the somatically derived mutations (47). Thus, self-reactive memory B cells would potentially be generated through GC reactions during TD immune responses, but those binding to self-Ags with high affinity may normally be eliminated. Accordingly, it has been proposed that GC B cells acquiring high-affinity binding to soluble self-Ags are deleted, because administration of high-dose soluble TD Ags into immunized mice in which GCs were being formed rapidly induced apoptosis of high-affinity Ag binding GC B cells (48–50). In addition, our results suggest that memory B cells binding with high avidity to self-Ags that contain highly repetitive epitopes similar to TI-II Ags, such as dsDNA or ssDNA, would be deleted. These intra-/post-GC self-tolerance mechanisms together may normally prevent the generation of pathogenic high-affinity self-reactive IgG Abs in TD immune responses while allowing generation of the low-affinity self-reactive and polyreactive repertoire that is important in innate humoral immunity.

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**Disclosures**

The authors have no financial conflicts of interest.

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