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BRIEF REVIEWS

Human IgM$^+$CD27$^+$ B Cells: Memory B Cells or "Memory" B Cells?[1]

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***Memory B cells are generated in germinal centers (GC) and contribute to serological immunity by rapidly differentiating into plasma cells. Human memory B cells can be identified by the expression of CD27. These cells exhibit more rapid responses than naive (CD27$^-$) B cells following stimulation in vitro, consistent with the heightened kinetics of secondary responses in vivo. CD27$^+$ B cells express mutated Ig V region genes; however a significant proportion continue to express IgM, suggesting the existence of IgM$^+$ memory B cells. The observation that mutated IgM$^+$CD27$^+$ B cells are generated in humans who cannot form GC led to the conclusions that these cells are generated independently of GC and thus are not memory cells and that they mediate responses to T cell-independent Ag. Although some studies support the idea that IgM$^+$CD27$^+$ B cells participate in T cell-independent responses, many others do not. In this review we will provide alternate interpretations of the biology of IgM$^+$CD27$^+$ B cells and propose that they are indeed memory cells. The Journal of Immunology,* 2007, 179: 13–19.**

Memory B cells are generated in germinal centers (GC)[4] in response to T cell-dependent (TD) Ag. It is within a GC that Ag-specific B cells are selected and undergo somatic hypermutation (SHM) of Ig V genes, yielding cells with increased affinity for Ag. As a result, memory B cells rapidly differentiate into high-affinity plasma cells following a re-encounter with the immunizing Ag (1–5). Early studies demonstrated that most memory B cells generated in a primary response were IgG$^+$ (6–8). Accordingly, a lineage relationship was proposed with IgM$^+$IgD$^+$ naive B cells generating IgG$^+$ memory B cells. Indeed, murine memory B cells are often distinguished from naive B cells by their ability to bind Ag with a higher affinity and express IgG (3). Subsequent studies demonstrated that memory B cells required lower concentrations of Ag and T cell help for their activation than naive B cells (9). These studies established the foundations for identifying mature B cell subsets and allowed investigations of naive and memory cells.

Similar to murine studies, many reports of human B cells relied on the differential expression of IgD to identify memory (IgD$^-$) from IgD$^+$ naive B cells (10, 11). However, this has the disadvantage of engaging the BCR during purification. After the discovery that CD27 is a marker of human memory B cells (12, 13) it became possible to isolate human naive and memory B cells independently of the BCR. Although analyses of human lymphocytes are largely restricted to in vitro experiments, studies that used differential expression of IgD or CD27 to isolate naive and memory B cells yielded important findings regarding the biology and function of human B cell subsets (10, 11, 13–22). However, the use of CD27 as a general marker of human memory B cells, especially with respect to IgM$^+$CD27$^+$"memory" B cells, has been recently questioned (23, 24). In this article, we will review the controversies generated regarding the origins and function of IgM$^+$CD27$^+$ B cells.

Identification of human memory B cells, part I: the use of IgD

Activated naive B cells that seed a GC and undergo SHM, Ig isotype switching, and selection by a specific Ag can differentiate into memory B cells or plasma cells. It is generally accepted that the processes of SHM and isotype switching are markers of memory B cells. In human tonsils, memory B cells were historically identified by the loss of IgD together with other markers such as CD38 (5, 10, 11, 17). The case for using IgD and CD38 to separate memory (IgD$^-$CD38$^-$) from naive (IgD$^+$CD38$^-$) and GC (IgD$^-$CD38$^+$) B cells was supported by the finding that the majority of tonsil IgD$^+$ cells expressed unmutated IgV region genes, while those expressed by IgD$^-$ cells were mutated (5, 11, 25, 26). Studies using these markers demonstrated that although both naive and memory B cells were in a quiescent state, memory cells exhibited enhanced responses compared to

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[4] Abbreviations used in this paper: GC, germinal center; AID, activation-induced cytidine deaminase; CVID, common variable immunodeficiency; HIGM, hyper-IgM; MZ, marginal zone; PB, peripheral blood; SHM, somatic hypermutation; TD, T dependent; TI, T independent; XLP, X-linked lymphoproliferative disease.

naive B cells in vitro (10, 17–20). Together, these articles established a scheme to identify human memory B cells.

However, concordant studies investigating the SHM of peripheral blood (PB) B cells found that cells expressing mutated IgV region genes did not strictly correlate with the loss of IgD (26, 27). PB B cells were divided into three subsets: IgM^+IgD^+, IgM^+IgD^-, and IgM^-IgD^-. Ig V region genes expressed by IgD^+ PB B cells were mostly unmutated (mutation frequency: 0.3%), and IgM^-IgD^- cells had the highest mutation frequency (3.9%). Nevertheless, the mutation frequency of the IgM^+IgD^- subset (2%) was substantially greater than that of the IgM^+D^+ subset (26–28) (Fig. 1*a*). It was speculated that mutated IgM^+IgD^- B cells may have begun to participate in a GC but exited early, before isotype switching occurred, or that they formed outside of a GC (26, 27). Evidence supporting these conclusions was provided by studies that detected mutated Ig μ transcripts in human spleen (29) (Fig. 1*a*), PB (30), and tonsil (11), albeit to a lower extent than IgG^+ B cells. Collectively, these studies were consistent with the finding that SHM can precede isotype switching in a GC (5). They provide the first evidence of somatically mutated B cells expressing IgM, thereby suggesting 1) the existence of IgM memory B cells; and 2) that memory to TD Ag may not be restricted to isotype-switched B cells (11, 26–29). These conclusions were also supported by in vivo studies of murine memory B cells, which found that IgM-memory B cells comprise a substantial proportion of secondary responses to TD Ag (6, 9, 31–34).

Identification of human memory B cells, part II: the use of CD27

Investigations performed in the early 1990s examined the expression of CD27 on human B cells. $CD27^+$ B cells represented ~25% of all PB and tonsil B cells yet were absent from cord blood (14, 16). $CD27^-$ and $CD27^+$ B cells were found to have distinct responses to stimulation, with $CD27^+$ B cells secreting 5- to 100-fold more Ig in vitro that $CD27^-$ B cells (15, 16). Human B cells could be further fractionated into three populations according to expression of IgD and CD27: $CD27^-IgD^{high}$, $CD27^+IgD^{low}$, and $CD27^+IgD^-$ (15, 16). These papers were the first to introduce the classification of B cells into naive, IgM memory, and switched memory B cell subsets.

The next chapter of human memory B cell characterization occurred in 1998 when it was reported that $CD27^+$ B cells in PB and spleen expressed somatically mutated Ig V region genes, while those expressed by $CD27^-$ B cells were unmutated (12, 13) (Fig. 1*b*). This led to the conclusion that CD27 expression identifies all human memory B cells. Consistent with earlier studies (15, 16), the human memory B cell population was heterogeneous, comprising cells that were either $IgM^{high}IgD^{low}$ (~40–50%) or IgM^-IgD^- (~30–40%; corresponding to the classically defined isotype-switched IgG^+ or IgA^+ memory cells) (12, 13, 35, 36). The phenotype of $CD27^+$ B cells (13) resembled that of tonsil IgD^-CD38^- memory B cells (18). $CD27^+$ B cells localized to the marginal zone (MZ) of human spleens (13, 37), consistent with previous findings that: 1) human B cells in the splenic MZ expressed somatically mutated IgV region genes while those in the follicle were unmutated (38) (Fig. 1*a*); and 2) TD Ag-specific memory B cells in rats localize to the splenic MZ (39). Finally, human $CD27^+$ B cells exhibited functional features of memory B cells such as undergoing greater proliferation and generating more Ig-secreting cells than $CD27^-$ B cells in vitro (13, 16, 21, 22, 35, 36), reminiscent of studies examining the responses of murine naive and memory B cells (8, 9). These studies established that $CD27^+$ human B cells correspond to functional memory B cells, while $CD27^-$ B cells are naive. This was supported by the finding that Ag-specific B cells in the PB of immunized individuals resided in the $CD27^+$ subset (40).

Identification of human memory B cells, part III: $CD27^-$ memory B cells

Several studies identified a small population of human $CD27^-IgG^+$ B cells that express mutated IgV region genes, have increased levels of CD80 and CD86, and greater cell size and Ig secretion in vitro than IgG^- naive B cells (35, 41–43). These studies confirmed that all $CD27^+$ B cells are memory B cells but revealed a minor population of memory B cells within the $CD27^-$ fraction.

IgM^+ memory B cells—do they exist?

The finding that a substantial proportion of human $CD27^+$ B cells had undergone SHM but not Ig isotype switching (12–14, 16) provided support for the existence of IgM^+ memory B cells (26–28). However several recent studies have brought the classification of IgM^+CD27^+ B cells as memory B cells into question (23, 24, 44). The major conclusions of these studies were that IgM^+CD27^+ B cells: 1) are generated independently of GC; 2) undergo SHM during generation of the preimmune repertoire; and 3) are exclusively involved in responses to T cell-independent (TI) Ag.

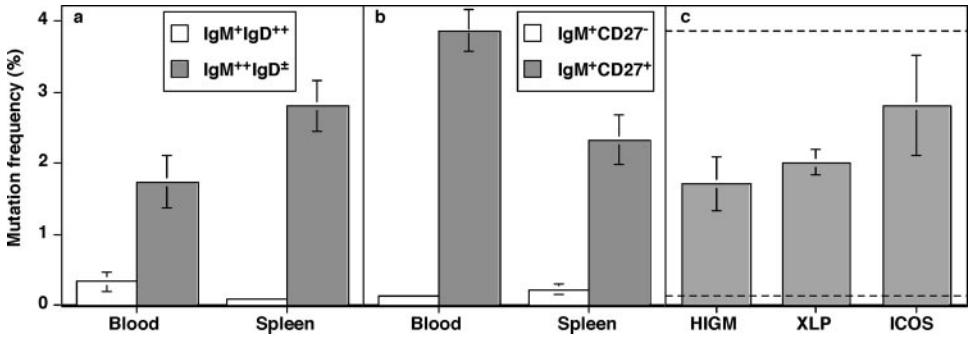

FIGURE 1. Somatic hypermutation in human B cell subsets. The frequency of SHM was determined in (*a*) IgM^+IgD^{high} (naive) and $IgM^{++}IgD^{\pm}$ (IgM memory) B cells, (*b*) $IgM^+IgD^{high}CD27^-$ (naive) and $IgM^{++}IgD^{\pm}CD27^+$ (memory) B cells present in the PB and spleen of normal donors, and (*c*) $IgM^{++}CD27^+$ B cells detected in the PB of patients with HIGM, XLP, or ICOS deficiency. The data for the generation of this graph were derived from Refs. 12, 23, 24, 38, 42, and 52). The values represent the mean ± SEM. The upper and lower dashed lines in *c* represent the frequency of SHM in naive and IgM^+CD27^+ B cells isolated from normal donors (illustrated in *b*), indicating the near-normal level of SHM in B cells from immunodeficient patients.

Consequently, it was proposed that IgM^+CD27^+ B cells do not represent bona fide memory B cells (24, 45). These studies have impacted on humoral immunology by questioning the use of SHM as a marker of memory and the dogma of how memory B cells are generated. Furthermore, these conclusions have been rapidly embraced by sections of the immunological community (45, 46) without considering alternative and equally valid interpretations of the function and origin of IgM^+CD27^+ B cells and the contribution of switched memory B cells to TI immune responses. Next, we will outline the data supporting the conclusions from these studies (23, 24, 44) and then provide alternative explanations favoring the possibility that IgM^+CD27^+ B cells are memory cells.

"IgM^+ $CD27^+$ B cells are generated independently of GC"

The evidence for. Somatically mutated IgM^+CD27^+ B cells were detected in patients with hyper-IgM (HIGM) syndrome (23, 24, 47) resulting from mutations in *CD40* or *CD40L* (48) (Fig. 1*c*). B cells from HIGM patients do not undergo Ig isotype switching to produce IgG, IgA, and IgE in response to TD Ags (48), presumably due to the defective formation of GC (49) (Table I).

IgM^+CD27^+ B cells expressing mutated Ig V genes have also been detected in the immunodeficiency X-linked lymphoproliferative disease (XLP) due to mutations in *SH2D1A* (encoding SAP; (42, 50) and in common variable immunodeficiency (CVID) resulting from mutations in *ICOS* (51, 52) (see Fig. 1*c* and Table I). Humoral immunodeficiency in these patients results from decreased expression of ICOS and IL-10 production by $CD4^+$ T cells (50, 52). In XLP and ICOS-deficient CVID, patients lack isotype-switched B cells and there is a paucity of GC in spleens and lymph nodes (42, 50, 52). The level of mutation in V genes expressed by IgM^+CD27^+ B cells in patients with HIGM, XLP, and ICOS deficiency approximated that observed in phenotypically identical B cells isolated from normal donors (12, 23, 24, 42, 52) (see Fig. 1, *b* and *c*). Thus, the presence of mutated IgM^+CD27^+ B cells in individuals unable to form GC led to the conclusion that these cells are generated independently of GC and therefore are not memory B cells (23, 24, 45).

Alternative scenarios. Several pieces of evidence suggest that some GC may form in immunodeficient patients. First, abortive GC and GC-like structures have been found in lymph nodes of HIGM (49) and the lamina propria of XLP patients (42). Furthermore, GC can form in the lamina propria of some gene-targeted mice that are unable to form such structures in spleens and lymph nodes (53–56). GC can also form in response to TI Ag; however, they are smaller and shorter-lived than TD GC, as they collapse at the early centrocyte stage (57). Despite this, SHM does take place in TI GC, albeit at a lower frequency than in TD GC (57, 58). Thus, a second possibility is that mutated IgM^+CD27^+ B cells arise in the early stages of a GC. Consequently, when the ability to form a "mature" GC is defective, IgM^+ but not isotype-switched memory B cells could be generated (Fig. 2). There are a number of studies that support this hypothesis. SHM and isotype switching do not occur at the same stages of a GC reaction, with SHM preceding isotype switching (5, 11). Similarly, in the absence of signaling through ICOS and, thus, an intact GC, memory B cells could still be generated but with reduced Ag affinity (59). The finding that human IgM^+CD27^+ B cells have undergone fewer divisions in vivo than switched memory B cells (60) is consistent with the former cells exiting a GC earlier than the latter. The reduced proliferation in vivo of IgM^+CD27^+ B cells relative to switched memory B cells (60) is also consistent with the finding that Ig isotype switching by B cells increases with cell division (36). Thus, while $CD4^+$ T cells are required for the development and selection of high-affinity switched memory B cells in GC, it is plausible that optimal T cell help is dispensable for the initiation of a GC. Such a scenario would allow the generation of low-level mutated IgM^+, but not switched, memory cells in immunodeficient patients who do not develop well-formed GC (Fig. 2).

In addition to these scenarios, it is possible that IgM^+CD27^+ B cells are generated independently of GC yet still represent bona fide memory B cells. The fact that Ag-specific IgM and IgG1 memory B cells are generated from Bcl-6-deficient bone marrow, despite an inability to form GC, support this possibility (61). Similarly, mutated Ag-specific B cells could be generated in lymphotoxin-$\alpha^{-/-}$ and $CD28^{-/-}$ mice, which do not form GC, when immunized with TD Ag (53, 62, 63). However, the mutation frequency was lower in Bcl-$6^{-/-}$ and lymphotoxin-$\alpha^{-/-}$ than wild-type mice (53, 62). B cells expressing mutated Ig V region genes have also been detected in splenic extrafollicular regions in mice immunized with TD Ag (58) and murine models of autoimmunity (64). These studies demonstrate that SHM and memory B cell generation can occur in response to TD Ag but in the absence of well-formed GC.

Another possibility is that subsets of cells exist within the IgM^+CD27^+ population that differ in their dependence on GC for development. The most compelling evidence for this is that when IgM^+CD27^+ B cells in patients with HIGM, XLP, or ICOS deficiency were quantitated, only ~20–40% of the number observed in normal individuals was generated (23, 47, 50, 52) (Table I). These results clearly suggest that the vast majority of IgM^+CD27^+ B cells are in fact dependent on GC for their development, while a subset may form via one of the mechanisms detailed above.

Table I. *Characteristics of IgM^+ $CD27^+$ B cells in immunodeficient patients*[a]

Immunodeficiency	Gene Mutation	Percentage of IgM/D^+CD27^+ B Cells (%)	SHM (%)	GC	No. IgM^+CD27^+ B Cells as Percentage of Those in Normal Donors (%)	Ref.
HIGM ($n = 7$)	*CD40L*	1.9	1.5	Aborted	~20–25%	23, 24, 49
XLP ($n = 12$)	*SH2D1A*	3.0	2.0	Absent (spleens); some GC in gut	20–40%	42, 50
CVID ($n = 9$)	*ICOS*	5.6	2.8	Absent (lymph node)	10–20%	52
Controls		~15–30	2.7–3.5	Normal	NA	13, 16, 24, 42, 50, 52

[a] One patient with HIGM (designated FF in Ref. 23) is omitted from the calculations performed, as this individual had an extraordinarily high frequency of IgM^+CD27^+ B cells (i.e., 60%). NA, Not applicable.

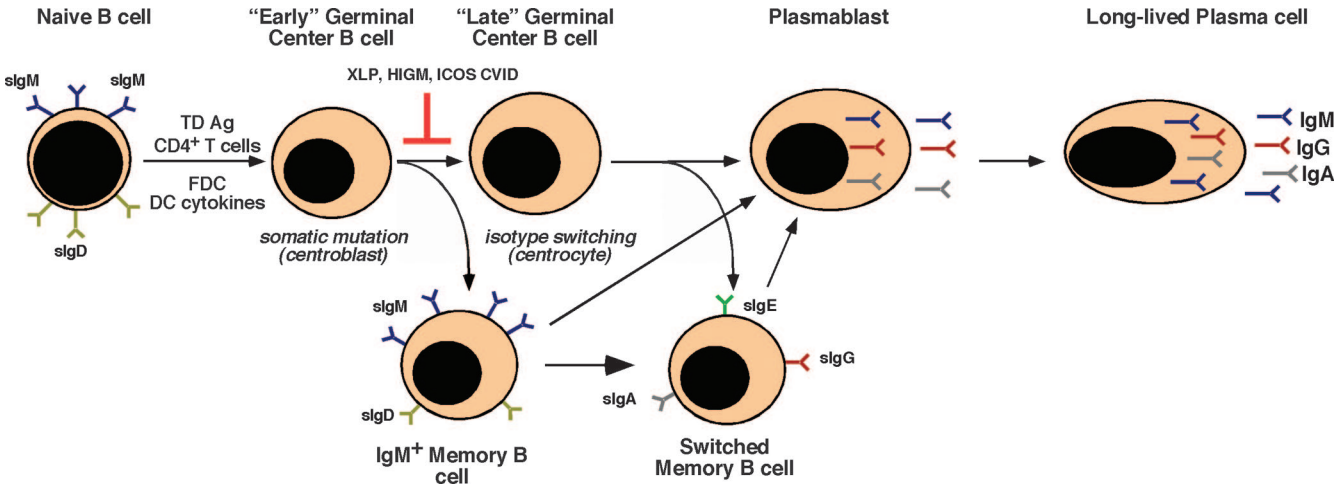

FIGURE 2. GC development in immunodeficient patients. Following interactions with Ag, naive B cells become activated and seed a GC. SHM occurs early in GC at the centroblast stage and precedes Ig isotype switching, which takes place in centrocytes (5, 11). B cells exhibiting increased affinity for the immunizing Ag are selected and differentiate into either memory B cells or Ig-secreting plasma cells. Memory B cells can subsequently differentiate into plasmablasts and plasma cells following re-exposure to specific Ag. IgM^+ memory B cells may be generated at the centroblast stage before the onset of isotype switching. Consequently, in conditions where the formation of GC is compromised, such as human immunodeficiencies caused by inactivating mutations in *CD40/CD40L* (HIGM), *SH2D1A* (XLP),or *ICOS* (CVID), abortive GC may be capable of yielding some IgM^+ but not isotype-switched memory cells.

"IgM^+ $CD27^+$ B cells undergo SHM during generation of the preimmune repertoire"

The evidence for. Based on analogies with birds, sheep, and cattle (45), it was proposed that human IgM^+CD27^+ B cells mutate their Ig V region genes during prediversification of the B cell repertoire, and this would take place within the MZ (23, 24). This proposal was supported by the finding of somatically mutated Ig V genes in murine immature B cells that expressed activation-induced cytidine deaminase (AID), an enzyme required for the induction of SHM (65).

The evidence against. If IgM^+CD27^+ B cells mutate their Ig V region genes during development independently of GC, it may be anticipated that these cells would express AID (24). However, IgM^+CD27^+ B cells in the MZ do not express AID (66). Thus, IgM^+CD27^+ B cells do not appear to undergo SHM in the MZ during ontogeny. Another study found that the exposure of preterm neonates to environmental Ag resulted in the generation of B cells expressing somatically mutated and Ag-selected Ig (67), demonstrating that the immune system of premature neonates can support the generation of productive GC, where SHM, Ag selection and Ig isotype switching can occur, within the first few weeks of extrauterine life (67). This contrasts with the supposition that children <2 years of age are unable to diversify their B cells via GC reactions due to an immature MZ (24). As highlighted recently (66), the fact that mutated IgM^+CD27^+ B cells are detected in young children cannot be considered as direct evidence supporting the idea that SHM in these cells occurred during prediversification of the B cell repertoire (24, 45).

"IgM^+ $CD27^+$ B cells are exclusively involved in humoral immune responses to TI Ags"

The evidence for. IgM^+CD27^+ B cells were reduced ~10-fold in children and adults who were either congenitally asplenic or had their spleens surgically removed (44). Asplenic individuals are highly susceptible to infection with encapsulated bacteria (68). Infants also have a poor response to polysaccharide (i.e., TI) Ag, and this has been correlated with an immature MZ (37, 68, 69). Finally, IgM^+CD27^+ B cells cannot be detected until 1–2 years of age (16, 24, 37, 68) and, once generated, they reside in the MZ (13, 37). Based on these observations and because MZ B cells mediate TI immune responses in rodents (68), it was postulated that children and asplenic patients respond poorly to polysaccharide/TI Ag because they lack IgM^+CD27^+ B cells in blood and the MZ (44). It was next concluded that IgM^+CD27^+ B cells are responsible for providing protection against pathogenic encapsulated bacteria such as *Streptococcus* and *Haemophilus* and TI Ag in general (44). These conclusions were supported by related findings such as: 1) undetectable IgM^+CD27^+ B cells in CVID patients suffering from recurrent encapsulated bacterial infections while the $CD27^+$ B cells in patients who did not have such infections were predominantly IgM^+ (44, 70); 2) a diminution in IgM^+CD27^+ B cells in the PB of individuals >65 years old, who are also susceptible to encapsulated bacterial infections (71); and 3) the detection of a clone in the IgM^+CD27^+ B cell compartment that was enriched in the spleen and PB following vaccination against *Streptococcus pneumoniae* (24).

The evidence against. There are several alternative explanations for these conclusions that are given in the following sections.

First alternative explanation: both IgM^+ $CD27^+$ and switched memory B cells are reduced in individuals susceptible to infection with encapsulated bacteria

The studies of asplenic (44) and elderly (71) individuals overlooked the fact that both IgM^+CD27^+ and switched memory B cells are equally severely diminished (i.e., 5- to 10-fold) in the PB of these individuals. It is also worth noting that CVID patients documented as suffering encapsulated bacterial infections had very few (<3.5%) $CD27^+$ B cells, whereas the CVID patients who did not experience such infections had a normal frequency (8–70%) of these cells (44). Furthermore, the reduction in the frequency of switched memory B cells was much

greater than that of IgM^+CD27^+ B cells (i.e., 40-fold vs 5-fold, respectively) in CVID patients suffering from recurrent bacterial infections compared with healthy donors (70). Finally, whereas IgM^+ and switched $CD27^+$ B cells were also reduced in asplenic adults in an independent study, congenitally asplenic children had normal development of all $CD27^+$ B cells (24). Therefore, a direct correlation between the inability of certain groups of individuals to mount efficient responses to polysaccharide Ag and the exclusive lack of IgM^+CD27^+ B cells cannot be made from the data presented in these studies. It is more likely that the inability of some individuals to respond to encapsulated bacteria results from a reduction in all memory B cells rather than reflecting a loss of only IgM^+CD27^+ B cells or is reflective of a broader immunological problem.

Second alternative explanation: the Ag-specificity of IgM^+ $CD27^+$ and IgG^+ $CD27^+$ B cells suggests that switched memory B cells are involved in TI immune responses

The proposal that IgM^+CD27^+ B cells are involved in TI immune responses would predict that they are enriched with specificities for polysaccharide Ag (24, 45). The ability of Ig expressed by IgM^+CD27^+ and IgG^+CD27^+ B cells to bind polysaccharide Ag, such as those on *S. pneumoniae* and *Haemophilus influenzae*, was recently examined (72, 73). Strikingly, <4% of Ig molecules isolated from IgM^+CD27^+ B cells recognized bacterial polysaccharide Ag (72). Thus, while some IgM^+CD27^+ B cells can produce polysaccharide-specific Ig, the vast majority do not, demonstrating that TI Ag are not the only types of Ag recognized by these cells. In contrast, ~15–20% of naive and IgG^+CD27^+ B cells expressed Ig specific for these typical TI Ag (72, 73), suggesting that B cells with anti-polysaccharide specificities are actively counterselected for entry into the IgM^+CD27^+ subset. This conclusion is supported by the finding that IgM^-CD27^+ B cells secreted higher level of anti-*S. pneumonia* Ig than IgM^+CD27^+ B cells following in vitro stimulation (74). These studies indicate that while IgM^+CD27^+ B cells may participate in TI immune responses, isotype-switched memory cells could also be involved in TI immune responses. This conclusion is not that surprising because it is known that B cells in adults responding to vaccination with *H. influenzae* or *S. pneumonia* originated from switched memory B cells inasmuch as they are IgG^+ or IgA^+ and have undergone SHM and Ag selection (75–78). For this reason, it would be of interest to know whether B cell clones exhibiting specificity for *S. pneumoniae* were detected in the switched memory in addition to the IgM^+CD27^+ B cell population (24). Therefore, the data supporting an exclusive role of IgM^+CD27^+ B cells, but not switched memory B cells, in response to encapsulated bacteria is limited.

Third alternative explanation: murine memory B cells generated in response to TI Ag are phenotypically distinct from those generated in response to TD Ag.

The ability of mice to generate memory responses to TI Ag has recently been examined. Memory B cells specific for TD or TI Ag differed with respect to life span, sensitivity to stimulation, and surface phenotype (79). If human IgM^+CD27^+ B cells represent cells generated in response to TI Ag, it may be anticipated that they would also exhibit such differences to isotype-switched memory cells. However, this is not the case. Rather, the phenotype of IgM^+CD27^+ and switched memory B cells is remarkably similar (28, 42, 80). One notable exception is the preferential expression of CD1c on IgM^+CD27^+ B cells (24); however, the physiological significance of this is unclear. Furthermore, the behavior of the IgM^+ and IgM^- subsets of human $CD27^+$ B cells in response to stimulation with TI and TD stimuli is also similar, with both populations exhibiting greater responses than naive B cells (22, 42, 80). This is quite different from murine TI Ag-specific memory B cells, which respond more like naive than TD memory cells (79). Finally, extrapolating from murine studies where some memory B cells generated during TI responses expressed IgG3 (79) and from the role of the human IgG2 subclass in clearing polysaccharide Ag in adults (81), it may be anticipated that human memory B cells involved in TI responses would express IgG2, rather than just IgM. Indeed, some of the mutated B cells noted to respond to *S. pneumoniae* and *H. influenzae* express IgG2 as well as other Ig subclasses (76).

Fourth alternative explanation: human IgM^+ $CD27^+$ and murine MZ B cells contribute to TD immune responses

Two studies have recently indicated that IgM^+CD27^+ B cells can participate in immune responses to TD Ag. It was found that memory B cells generated in normal individuals vaccinated against hepatitis B and HIV-infected patients differentiated into Ag-specific IgM-, IgG-, and IgA-secreting B cells in vitro in response to CD40L, IL-2, and IL-10. The IgM-secreting cells represented ~30% of all Ag-specific Ig-secreting cells generated (82, 83). Although it could be argued that $CD27^-$ B cells gave rise to Ag-specific IgM-secreting cells, this is unlikely because <5% of $CD27^-$ B cells differentiate into Ig-secreting cells in vitro in response to CD40L, IL-2 and IL-10; this response is dominated by $CD27^+$ B cells (19, 21, 36, 82). Thus, the most likely source of the Ag-specific IgM-secreting B cells would be IgM^+CD27^+ B cells, suggesting that they can efficiently respond to TD Ag. These findings are entirely consistent with data obtained in rodents, indicating that IgM-expressing memory B cells can be generated following immunization with TD Ag (6, 9, 31–34).

Because human IgM^+CD27^+ B cells reside in the splenic MZ (13, 37) and murine MZ B cells are vital for efficient responses to TI Ag (68), it has been inferred that human MZ (i.e., IgM^+CD27^+) B cells would also have this function (44, 68). However, murine MZ B cells are also capable of responding to TD Ag with respect to forming GC and undergoing SHM and Ig isotype-switching as efficiently as follicular B cells (84). These studies highlight the versatility of murine MZ B cells and would also be consistent with the notion that human MZ (i.e., IgM^+CD27^+ and IgM^-CD27^+) B cells could efficiently contribute to TD and TI humoral immune responses.

Conclusions

Some interesting correlative observations led to the proposal that human IgM^+CD27^+ B cells are generated independently of GC and therefore do not represent genuine memory B cells. Instead, these cells were suggested to be involved in responses to TI Ag, specifically providing protection against infection with encapsulated bacteria. Although these interpretations may be correct, when much of the available data are considered it is equally feasible that the majority of IgM^+CD27^+ B cells are in fact GC-derived memory cells that can participate in TD immune responses. For this reason, it would appear premature to

conclude that IgM^+CD27^+ B cells arise independently of GC and are restricted in their responses to TI Ag. To definitively ascribe a function to IgM^+CD27^+ B cells, it will be necessary to determine whether B cells with specificities for TD and TI Ag accumulate within this population following immunization or infection and whether they can participate in recall responses. Such studies will resolve the controversies regarding this important subset of human B cells.

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Disclosures

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