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IgM in Microbial Infections: Taken for Granted?

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Abstract

Much has been learned about the structure, function, and production of IgM, since the antibody's initial characterization. It is widely accepted that IgM provides a first line of defense during microbial infections, prior to the generation of adaptive, high affinity IgG responses that are important for long-lived immunity and immunological memory. Although IgM responses are commonly used as a measure of exposure to infectious diseases, it is perhaps surprising that the role of and requirement for IgM in many microbial infections has not been well explored *in vivo*. This is in part due to the lack of capabilities, until relatively recently, to evaluate the requirement for IgM in the absence of coincident IgG responses. Such evaluations are now possible, using gene-targeted mouse strains that produce only IgM, or isotype-switched IgG. A number of studies have revealed that IgM, produced either innately, or in response to antigen challenge, plays an important and perhaps underappreciated role in many microbial infections. Moreover, the characterization of the roles of various B cell subsets, in the production of IgM, and in host defense, has revealed important and divergent roles for B-1a and B-1b cells. This review will highlight studies in which IgM, in its own right, has been found to play an important role, not only in early immunity, but also in long-term protection, against a variety of microbial pathogens. Observations that long-lived IgM responses can be generated *in vivo* suggest that it may be feasible to target IgM production as part of vaccination strategies.

Keywords

IgM; microbial infection; B-1 cell

Properties of IgM important for microbial immunity

The characteristics and functions of IgM have been reviewed widely, and have been well documented (for review, see [1]). Many properties of IgM make this immunoglobulin particularly well-suited to its role in microbial immunity. It is present in high concentrations in blood (in the range of 1.5mg/ml), and is the first antibody elicited in an immune response following immunization or infection. IgM has a relatively short half-life in the serum, approximately 28 hours, in normal mice in the absence of antigen [2]. Thus, it is generally assumed that the production of IgM wanes, once B cell responses mature. However, some recent evidence, including our own, suggests that this is not always be the case, and

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demonstrates that IgM responses can be maintained for long periods following infection or immunization, perhaps due to long-lived B cells (discussed below).

Monomeric IgM (180,000 kDa) is expressed as membrane-bound antibody on all naive B cells, but it is secreted from B cells as a pentamer (five monomeric units held together by disulfide bonds that link the carboxy-terminal heavy chains and the J chain). The pentameric structure generates 10 linked antigen binding sites, affording IgM a higher valency than other immunoglobulins. IgM is generated from germline configured transcripts in B cells, prior to the onset of class switch recombination (CSR) and somatic hypermutation (SHM), and is typically of low affinity. However, its high valency can allow it bind to antigens with a wide range of avidities (10^{-3} to 10^{-11} M⁻¹; average values range between 10^{-6} and 10^{-7} M⁻¹) [3,4], and to cause agglutination or clumping, a process which facilitates the removal of foreign pathogens or antigens (e.g., viral particles). Indeed, IgM is 100 to 10,000 times more effective than IgG in mediating agglutination. Agglutination is considered to be a key component of the process of IgM-mediated virus neutralization, given that a single bound IgM can activate complement and lyse an erythrocyte, while a thousand or more IgG molecules are required [5].

High valency also allows IgM to be more efficient than other immunoglobulins at binding antigens with repeating epitopes, such as viral particles, DNA, red blood cells, and carbohydrates on pathogens or cell surfaces. Due to the low affinity and high valency of IgM, the majority of IgM are polyreactive; this latter property allows IgM to bind to a range of unrelated antigens [6-8]. Polyreactive antibodies recognize phylogenetically conserved structures, including nucleic acids, proteins, carbohydrates, and phospholipids [9,10]. Since these structures can occur in repetitive forms on some bacteria and viruses, polyreactive IgM on B cells can bind to antigenic determinants and induce antibody production independent of T cell help.

IgM is the primary humoral component elicited by T-independent (TI) antigens [11]. Classically, TI antigens have been defined as Type 1 or Type 2; at high concentrations, the former trigger polyclonal B cell responses via non-clonally distributed co-stimulatory receptors, such as Toll-like receptors (TLRs), in the absence of a classical second signal [12]. In contrast, TI-2 antigens are classified as highly repetitive structures, such as cell membrane polysaccharides and bacterial flagella, which can crosslink B cell receptors (BCRs) to induce an IgM response [12,13]. Limited amounts of IgM are produced against T cell-dependent antigens, and high-affinity isotype-switched antibodies are the hallmark of the T cell-dependent humoral response [11,14].

Both natural and immune IgM are important in host defense

There are now considered to be two classes of IgM, natural (or innate) IgM, and immune (or adaptive) IgM. Natural IgM is produced by innate-like B-1 cells in the absence of pathogen encounter, and immune IgM is produced by both innate-like B-1 and adaptive B-2 cells following antigen or pathogen encounter. Although the cellular sources of natural and immune IgM differ, only minimal differences in the molecular properties exist between natural and immune IgM. However, the former contain more flexible intrinsic antigen-binding sites that facilitate broad interactions with a variety of antigens [15-17]. Natural IgM has been found in both humans and mice, and constitutes the majority of total circulating IgM [18-20]. Natural IgM is generated largely in the absence of exogenous antigen exposure, given that it has been found in antigen-free mice and newborn humans [21-23]. The majority of natural antibodies are polyreactive, and bind to a number of different antigens with low affinity ($K_d=10^{-4}$ to 10^{-7} M).

However, a small percentage exhibit characteristics of monoreactive antibodies that recognize fewer antigens, but with higher affinity (K_d : 10^{-7} to 10^{-11} M). The former class of IgM are germline-encoded, while somatic hypermutations can be detected in the latter [24]. Natural IgM is produced primarily by the CD5+ B-1a subset of B-1 cells that are generated during fetal and neonatal development [9,10,25], and are found primarily in the peritoneal and pleural cavities [9]. Natural antibodies are present at low titers in serum and contribute to early immunity prior to the onset of the adaptive humoral response [26,27]. Although natural antibodies were first described over 100 years ago [4], they were for a long time thought to recognize only self-antigens, and thus to be of little value for microbial immunity. However, within the past 20 years, a number of studies have documented that natural IgM can bind to a number of microbial pathogens [14,28-31].

An important advance that facilitated investigations of the role of natural IgM *in vivo* in B cell-sufficient mice was the generation of mice deficient for secretory IgM (sIgM). This mouse strain was generated by germline deletion of the μ chain secretory exon [30]. In sIgM-deficient mice, IgM is expressed on the cell membrane of B cells, but B cells are unable to secrete IgM, although they can undergo CSR and secrete IgG. Using the sIgM-deficient mice in a mouse model of acute septic peritonitis induced by cecal ligation and puncture, it was demonstrated that natural antibodies were required for protection against systemic bacterial infection [30]. Resistance to infection was restored when the sIgM-deficient mice were reconstituted with polyclonal IgM from normal mouse serum. These studies established that natural IgM can provide effective anti-microbial immunity. Examples of infections where IgM has been shown to provide a component of protective immunity *in vivo* are provided in Table 1, and several of the studies are described below.

Immune IgM, in contrast, is produced in conventional fashion primarily by spleen and lymph node B-2 cells, following antigenic exposure [32,33]. Recent studies have demonstrated that B-1b cells also contribute to the production of immune IgM in response to T-independent antigens [25,34,35]. B-1b cells are phenotypically similar to B-1a cells, although N-region diversity is often comparable to that of B-2 cells [36,37], suggesting that the properties of immune IgM produced by the two B cell subsets are similar.

IgM and immunity to viral infections

Although the presence of IgM has long been used as an important diagnostic tool for identifying individuals who have been exposed to viral pathogens, and although neutralizing IgM is generated against most viruses [38], the relevance of IgM for host defense in many viral infections has not been clearly defined *in vivo*. Consequently, several studies have begun to investigate roles for natural and immune IgM in viral immunity. Ochsenbein and colleagues demonstrated that natural antibodies specific for several viruses, including vesicular stomatitis virus (VSV), lymphocytic choriomeningitis virus (LCMV), and vaccinia virus, were present, albeit at low titers, in normal mouse serum [39]. Moreover, B cell-deficient mice lacking natural antibodies were more susceptible to VSV infection, and transfer of normal serum to B cell-deficient mice restored protection, revealing that natural antibodies can play an significant role in host defense against viral infections.

To begin to address the roles of various B cell subsets in the production of IgM during viral infection, Baumgarth and colleagues demonstrated that natural and immune IgM are produced by different subsets of B cells. Natural IgM production was found to be a principal function of B-1 cells, given that these cells maintained their function, and did not differentiate into immune IgM-producing B cells *in vivo* [33]. This finding was demonstrated using antibody-mediated cell ablation to generate chimeric mice containing B-1 and B-2 cells of different BALB/c-derived IgM allotypes. Although allotype-specific immune IgM was elicited from B-2 cells

during infection, natural IgM was produced only by B-1 cells, and antibody production by the B-1 cells did not increase during infection. These initial studies of B-1 cells were extended by an investigation of the respective contributions of natural B-1-derived IgM and immune B-2 cell-derived IgM to host defense during influenza infection [31]. Natural antibody was found to be protective during mouse influenza infection, given that sIgM-deficient mice exhibited increased mortality following infection with influenza strain Mem71. To distinguish between possible roles for B-1- and B-2-derived IgM in immunity, the authors generated radiation-induced fetal liver and bone marrow chimeric mice. The experimental strategy was predicated on the observation that most B-1 cells are derived from fetal liver, and B-2 cells are derived from adult bone marrow. Using the chimeric mice, it was demonstrated that B-1 cells produced polyreactive IgM that bound to at least four different influenza strains, while B-2 cells secreted strain-specific immune IgM that exhibited minimal cross-reactivity [31]. Mice lacking either natural B-1-derived or immune B-2-derived IgM were as susceptible as mice lacking both sources of IgM, indicating that both natural and immune IgM contributed to immunity. Collectively, the studies demonstrated that B-1- and B-2 cell-derived IgM can provide independent components of the protective humoral immune response during influenza infection. Other related studies of influenza infection demonstrated that complement-dependent neutralizing natural IgM reactive to influenza virus PR8 was present in C57BL/6 mice, in the absence of infection, and could induce a modest degree of protection when administered to RAG1-deficient mice [40].

A role for IgM in host defense during influenza infection was also addressed using activation-induced cytidine deaminase (AID)-deficient mice. AID is essential for both CSR and SHM [41], so mice lacking AID produce only IgM, and thus are a useful tool for investigation of the role of IgM in microbial immunity, without the confounding effect of coincident IgG. Harada and colleagues, using AID-deficient mice in studies of influenza infection, demonstrated that IgM was sufficient for protection against both primary and secondary infections with strain PR8 [42]. Unmutated IgM was sufficient to control viral replication, although efficient virus elimination ultimately required isotype-switched IgG. Although similar mortality rates were observed in wild-type and AID-deficient mice, the latter strain exhibited greater morbidity. Moreover, virus elimination was delayed during primary infection, and viral titers were higher during secondary infection in AID-deficient mice, likely due to the absence of IgG. Although high-affinity IgG clearly plays an important role in influenza immunity, the level of protection provided by IgM it was remarkable. In those studies, the authors were unable distinguish between natural and immune IgM, so both forms of IgM could have contributed to the protection.

In more recent studies, Baumgarth and colleagues investigated the roles of the B-1a and B-1b cell subsets in IgM-mediated immunity to influenza [43]. Although it had been generally accepted that B-1a cell production of natural IgM is systemic, the authors demonstrated that these cells can produce local IgM in response to infection. Antibody production was not a consequence of antigen-specific B-1a cell expansion, but rather, was due to the local accumulation of IgM^{hi}/CD43⁺/CD138⁻ B-1a cells in the lung-draining lymph nodes, probably in response to infection-driven inflammatory signals. The notion that B-1a cells can migrate in response to inflammatory signals gained support from studies by Yang and colleagues, who demonstrated that peritoneal B-1a cells could migrate to the spleen following LPS administration, in some cases without undergoing cell division [44]. These studies together highlight the fact that B-1a cells are not passive IgM-producing cells, but can play an active role in microbial immunity, even though they do not undergo clonal expansion *in vivo*.

IgM has also been demonstrated to play important roles in host defense against other viral infections. Diamond and colleagues examined the role of IgM in protection against West Nile Virus (WNV) infection [32]. Here, the authors also used the sIgM-deficient mice to

demonstrate that immune IgM can protect mice against lethal WNV infection by limiting viremia and spread of infection [32]. T cell-independent immune IgM has also shown to play a role in protection against polyoma virus [45], and VSV [13,46]. Together, these studies suggest that IgM plays a larger role in immunity to viral infections than has been generally appreciated; thus, it is possible that a protective role for IgM *in vivo* has often been overlooked in a studies of other viral infections.

IgM and immunity to extracellular bacterial pathogens

An important protective role for IgM has also been demonstrated in several extracellular bacterial infections, including those caused by *Borrelia* and *Streptococcus*. Relapsing fever is caused by a number of *Borrelia* species, including *B. hermsii*, *B. recurrentis*, and *B. duttonii* [47]. A hallmark of relapsing fever is the recurrent episodes of high bacteremia in the blood ($\sim 10^8$ bacteria/mL) [48], which are rapidly cleared within 2-4 days after the emergence of novel antigenic variants [48]. IgM is a major antibody sub-class that generates immunity to *Borrelia duttonii* [49,50], and studies of patient-derived relapsing fever *Borrelia* demonstrated a protective function for both polyclonal and monoclonal IgM [51,52]. Other findings suggested that both B-1 cells and marginal zone (MZ) B cells were primary participants in the humoral immune response during *B. hermsii* infection [34,53], supporting a role for IgM during this infection [54]. Studies by Alugupalli and colleagues investigated IgM immunity using sIgM-deficient mice; they demonstrated that T cell-independent IgM was required for immunity to *B. hermsii* [34]. Evidence from studies of *xid* mice, which have a point mutation in Bruton's tyrosine kinase (Btk), and are severely deficient in B-1b cells, suggested that the B-1b cell subset is essential for host defense [34]. Accordingly, the B-1b subset was determined to be the major source of protective IgM, given that these cells underwent selective expansion during infection. Later studies from Alugupalli and colleagues demonstrated that the B-1b cell derived IgM recognized complement factor H-binding protein (FhbA), a *B. hermsii* outer surface protein and putative virulence factor that does not undergo antigen variation [55].

IgM is also crucial for host defense against a related species, *B. burgdoferi*, the etiologic agent of Lyme disease. Although *B. burgdoferi* is generally considered to be an extracellular pathogen, a number of *in vitro* studies demonstrated that the spirochete can bind and invade endothelial cells, fibroblasts, macrophages, Kupffer cells, and synovial cells [56]. Nevertheless, antibody-mediated clearance of the pathogen occurs largely in the blood and intracellular survival sequesters the spirochetes as an immune evasion strategy [56]. Initial studies demonstrated that passive immunization with IgM and IgG, independent of T cell help, conferred protection against *B. burgdoferi*; however, the contribution of the individual subclasses was not addressed [57,58]). Belperron and colleagues investigated the potential contribution of natural antibodies in preventing the dissemination of the spirochete. A five-fold increase in the pathogen DNA levels in the blood was detected in B cell-deficient mice, compared to wild-type controls, and was reduced when polyclonal IgM was transferred prior to tick feeding [59]. Later studies demonstrated that marginal zone B cells are critical for the generation of immune IgM. Antibody-depletion of marginal zone B cells lead to reduced *Borrelia*-specific IgM titers, and increased pathogen burden in the blood [60]. Collectively, these studies indicate not only that IgM, in the absence of IgG and T cells, can play a major role in controlling *Borrelia* infection, but also that B-1b cells are an important source of immune IgM during extracellular bacterial infection.

IgM has also been demonstrated to be effective during *Streptococcus pneumoniae* infection, *S. pneumoniae*, an encapsulated extracellular gram-positive bacterium, causes a number of conditions, including otitis media, pneumonia, septicemia, and meningitis [61]. During the early 1980s, it was demonstrated that natural antibodies specific for bacterial phosphocholine (of the T15-idiotype) could protect naïve hosts from *S. pneumoniae* infection [28]. Later studies

demonstrated that higher bacterial loads were higher in sIgM-deficient and C1q-deficient mice than in wild-type controls [62]. Because natural IgM is essential for immunity to *S. pneumoniae* infection, the function of B-1 cells was investigated with the use of CD19-deficient mice, which exhibit modest T cell-dependent responses, and impaired production of natural antibodies [63,64]. Impaired natural antibody production was due to the loss of the B-1a subset, which was responsible for polysaccharide-specific IgM [25]. CD19-deficient mice were more susceptible than wild-type mice to *S. pneumoniae* infection, indicating a requirement for natural IgM in host defense. In the same study, the authors also utilized a human CD19-overexpressing transgenic mouse, which, in contrast to the CD19-deficient strain, maintained B-1a cells and natural antibodies; these mice were protected from infection. However, immunization of the CD19-deficient mice generated long-lived protective IgM from B-1b cells, indicating that the B-1b subset was required for production of immune IgM. Thus, during Streptococcus infection, B-1a cells are responsible for the production of natural IgM, and B-1b cells produce immune IgM in response to pathogen encounter. The above work was important not only in that it highlighted a major role for IgM in host defense, but also in that it proposed that natural and immune IgM are produced from different B-1 cell subsets, i.e., the B-1a and B-1b cells, respectively.

IgM immunity during intracellular bacterial infections

The studies of IgM described above were performed on viruses and extracellular bacteria. Antibodies, for a long time, had not been considered to play a role in protection against intracellular bacteria, although research in the past several years has demonstrated that antibodies can provide an important component of immunity against some intracellular bacteria and other intracellular pathogens [65]. The emphasis in most studies has been on a role for IgG in host defense [66], but there is increasing evidence that IgM can also provide a component of protective immunity in such infections. For example, IgM has been documented to provide immunity against the facultative intracellular bacterium, *Nocardia brasiliensis*. The bacterium causes a chronic infectious disease known as actinomycetoma, which is characterized by swelling, abscesses and ulcers that discharge microcolonies at the site of infection. [67,68]. Serum obtained from mice immunized with *N. brasiliensis* antigens contained high titers of IgM, and conferred protection in uninfected hosts by preventing bacterial dissemination [69]. Protection was not achieved when IgG was transferred into infected mice, and isotype utilization analyses demonstrated that the protective sera primarily contained polyreactive antigen-specific IgM.

Antibodies also exhibited efficacy during infection with *Francisella tularensis*, another facultative intracellular bacterium. Several groups have supported a role for antibodies in protection against attenuated strains of *F. tularensis* [70-73], although a specific role for IgM was not addressed in detail in those studies. IgM responses were induced following *F. tularensis* infection in mice [74], however, and other studies demonstrated that T cell-independent immunity was effective [75]. Immunization with purified *F. tularensis* LPS induced predominantly IgM responses that was associated with protection against the live vaccine strain LVS [71,76,77]. In a related study, *F. tularensis* LPS-mediated protection was attributed to B-1a-derived IgM, based on the fact that wild-type, but not *xid* mice, were protected from fatal LVS infection [78]. These data, and the observation that LPS immunization was associated with an early robust B-1a response within the spleen [78], revealed that B-1-derived IgM can be highly effective against *F. tularensis*, once again underscoring the importance of this B cell subset and IgM in adaptive immunity. The latter study by Cole and colleagues, identifying B-1a cells as a source of immune IgM, stands in contrast with the studies described above that highlight a role for B-1b cells in immune IgM-mediated protection, suggesting that B-1 cell subsets can play differing roles in infections by different pathogens.

Our own work has focused on humoral immunity during infection by intracellular bacteria of the genus *Ehrlichia*. The ehrlichiae are tick-born rickettsial pathogens that cause a number of serious diseases in both humans and other mammals. Our earlier studies demonstrated that outer membrane protein-specific antibodies are highly protective in both immunodeficient and immunocompetent mice [79,80]. Those studies focused largely on the role of high affinity IgG [80]. Using a heterologous ehrlichial immunization model, we also demonstrated that antibody-mediated cross-protection against fatal ehrlichial challenge infection can be achieved independent of CD4 T cells [81]. This finding led us to focus on a possible role for IgM in protective immunity. As part of these studies, we identified a major population of CD11b-positive, CD5-negative, CD11c-expressing B cells plasmablasts that were responsible for the CD4 T cell-independent production of antigen-specific IgM within the spleen, during peak ehrlichial infection [82]. The appearance of the CD11c-expressing B cell population also coincided with a robust serum IgM response. We used AID-deficient mice and sIgM-deficient mice, to address the respective contributions of IgM and IgG, to protection against challenge following heterologous infection/immunization. Both gene-targeted strains were protected against fatal challenge infection, revealing that either IgM or IgG is sufficient for immunity (RR, GW, unpublished data). We have not yet determined the subset of B cell responsible for protection, although the cell surface phenotype, as well as the presence of an IgM response in splenectomized mice, suggests that B-1b cells, but not MZ B cells, are involved (RR, GW, unpublished data; [82]). These data together indicate that IgM can also be a major component of host defense against intracellular bacteria, even though the intracellular niche occupied by intracellular pathogens is often thought to preclude protective humoral immunity. Since there is limited evidence that antibodies can access bacteria while inside host cells, an alternative explanation is that encounter occurs during transfer of the bacteria from cell to cell [83].

IgM and long-term immunity

IgM-mediated immunity is usually considered to be transient, and therefore of little value for protection against re-infection. However, several recent studies have begun to challenge this notion. In their studies of *B. hermsii* immunity, Alugupalli and colleagues demonstrated that B-1b cells can provide long-term T cell-independent immunity against re-infection. Protection was due to immune IgM, given that it was generated in AID-deficient mice, and was associated with the expansion and persistence of B-1b cells within the peritoneal cavity for at least as long as 230 days post-infection [84]. IgM production was not constitutive, however, but was induced following re-infection, indicating that the response was due to a memory cell response, not persistent antibody. This finding has provided important evidence that IgM can be produced during re-infection by memory B-1 cells, although the candidate memory cells have not yet been characterized in detail. The idea that peritoneal B-1 cells can contribute to long term protective immunity was also supported by the studies of adaptive B-1b responses elicited during *S. pneumoniae* infection by Haas and colleagues, described above [25]. In that study, B-1b-mediated adaptive IgM immunity was maintained for at least as long as 10 weeks post-infection. In our studies of ehrlichial immunity, we have also observed long-term humoral immunity: serum IgM responses and antibody-mediated protection against virulent ehrlichial challenge infection were observed for as long as 150 days post-immunization (RR, GW, unpublished data). In their study of B1 responses following *F. tularensis* LPS vaccination, Cole and colleagues [78] found that IgM responses were persistent, and also provided long-term protection against the *F. tularensis* LVS. IgM levels remained detectable for as long as 70 days post-immunization, although the candidate IgM-producing cells were no longer detected, leaving open the question as to the cellular origin of the long-term protective IgM.

The above studies together suggest the existence of multiple B cell subsets and mechanisms responsible for the maintenance of long-lived protective IgM responses (Figure 1). Hsu and colleagues reported that B-1b cells elicited in response to (4-hydroxy-3-nitrophenyl)-acetyl

(NP)-Ficoll generated a population of long-lived extrafollicular IgM-secreting plasmablasts [35] such a mechanism for the maintenance of IgM could also be relevant during infections. In the study by Hsu and colleagues, long-term antibody production was not maintained by newly generated naive B cells, although such a mechanism could perhaps support long-term IgM production during chronic infections. Finally, natural antibody-producing B1 cells could also contribute to the maintenance of long-term IgM. Implicit in the notion that IgM immunity can be maintained by long-lived cells is the possibility that memory B cells and/or long-lived plasmablasts can be generated from B-1 cell subsets following vaccination. Such strategies could involve the use of co-adjuvants, allowing specific targeting of B-1 cell subsets via innate lymphocyte receptors [85].

Concluding Remarks

Although IgM responses have often been “taken for granted” as a source of transient immunity prior to onset of high-affinity IgG, the studies summarized in this review suggest that IgM plays a more important role in microbial infections than has been generally appreciated. The unique properties of IgM allow this immunoglobulin to be highly effective in the prevention and in the elimination of diverse types of microbial infections. Thus, the potential exists to better utilize IgM for prevention and treatment of infections, through immunization and/or passive antibody administration. It is now clear that a major source of both natural and immune IgM is from specialized subsets of B-1 cells, but much remains to be learned regarding the origins, development, and maintenance of B-1 responses. The knowledge gained from the study of such responses should aid in the development of vaccines designed to generate effective long-lived IgM immunity.

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Figure 1. Possible mechanisms that would support long-term maintenance of IgM

Four non-mutually exclusive sources of long-lived IgM responses during microbial infection are proposed (from top left, clockwise). 1) Newly formed naive cells, upon encounter with chronic antigen or persistent infection, may differentiate into IgM-secreting cells, 2) Natural antibody-producing cells may provide a continuous source of pathogen-specific polyreactive IgM, 3) Memory B-1b cells elicited during primary infection may serve as a source of IgM following antigen re-exposure, 4) Long-term IgM-secreting plasmablasts or plasma cells may maintain production of IgM in the spleen extrafollicular regions or the bone marrow. Ag: antigen; Ab: antibody.

Table I
Examples of pathogens where IgM has been demonstrated to play a protective role *in vivo*

Pathogen	References	Comments
Influenza	[31] [43] [33] [42] [40]	Natural and immune IgM, produced by B-1 and B-2 cells, respectively, required for protection
West Nile virus	[32]	Early production of neutralizing immune IgM limits viremia and dissemination
Lymphocytic choriomeningitis virus (LCMV)	[39] [86]	Natural IgM mediates early clearance
Vesicular stomatitis virus (VSV)	[39] [87] [29]	Natural IgM prevents dissemination and facilitates trafficking to spleen; T cell-independent/complement-dependent neutralizing IgM
<i>Francisella tularensis</i>	[78] [77] [75] [70] [71] [76] [73] [88]	B-1a secretion of IgM; long-term responses generated against LPS
<i>Ehrlichia muris</i>	[82]	Unusually large IgM plasmablast response; long-term protection
<i>Nocardia brasiliensis</i>	[69]	Immune IgM limits bacterial dissemination
<i>Borellia hermsii</i> and related	[34] [84] [51] [52] [54]	Immune IgM produced largely by B-1b cells
<i>Streptococcus pneumoniae</i>	[89] [62] [25] [90,91]	B-1a production of natural IgM that recognizes polysaccharides or phosphocholine; long-lasting immunity by B1-b derived IgM
<i>Cryptococcus neoformans</i>	[92] [93]	IgM binds to polysaccharide capsule and facilitates activation of complement pathway
<i>Brugia pahangi</i>	[94] [95]	B-1 production of IgM facilitates granuloma formation
<i>Toxoplasma gondii</i>	[96]	Direct binding of IgM to parasite; enhancement of phagocytosis
<i>Plasmodium chabaudi chabaudi</i>	[97]	Neutralization of pathogen during asexual blood stage