Natural IgM in immune equilibrium and harnessing their therapeutic potential

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Abstract

Natural IgM antibodies are the constitutively secreted products of B1 cells (CD5$^+$ in mice and CD20$^+$CD27$^+$CD43$^+$ CD70$^-$ in humans) that have important and diverse roles in health and disease. Whereas the role of natural IgM as first line of defense for protection against invading microbes has been extensively investigated, more recent reports have highlighted their potential roles in the maintenance of tissue homeostasis via clearance of apoptotic and altered cells through complement-dependent mechanisms, inhibition of inflammation, removal of misfolded proteins, and regulation of pathogenic auto-reactive IgG antibodies and autoantibody-producing B cells. These observations have provided the theoretical underpinnings for efforts that currently seek to harness the untapped therapeutic potential of natural IgM either by boosting *in vivo* natural IgM production or via therapeutic infusions of monoclonal and polyclonal IgM preparations.

Cooperation between innate and adaptive immune compartments reinforces host protection from invading pathogens while maintaining an immunologic tolerance towards self. Immunoglobulins (Igs) or antibodies, the product of B cells, are one of the essential components of the immune system and are divided into five isotypes namely IgG, IgM, IgA, IgE and IgD. Well established as effector molecules of the adaptive compartment, Igs also participate as links or organizing factors for certain functions of the innate immune system by identifying and neutralizing the pathogens in part through the triggering of Fc receptors and activation of the complement system (1, 2).

Properties of IgM

IgM isotype represents one of the major Ig classes in the body. IgM is also the earliest type of membrane-associated Ig expressed during B-cell ontogeny, and as secreted antibodies

during antigen-specific immune responses. While the molecular weight of a monomeric IgM is 190 kDa, circulating IgM exists more frequently as a pentamer or sometimes even as a hexamer, which can convey increased avidity for the binding of an antigen. Pentameric IgM is generally associated with a J chain to form a macromolecule of about 970 kDa. In healthy adults, circulating human polyclonal IgM is generally present at about 1–2 mg/ml of blood, with a half-life of about five days. Although IgM are often potent activators of classical pathway of complement, there are great variations in the properties of different IgM-secreting B-cell clones, even when they share the same fine binding specificity.

IgM is known to bind to two receptors: Fcα/μR and the polymeric Ig receptor (3, 4). However, these receptors are not solely specific for IgM and can also recognize IgA. CD22, an inhibitory co-receptor on B cells, can also act as receptor for the glycoconjugates on soluble IgM through its sialoprotein-binding domain (5). In addition, TOSO, regulator of Fas-induced apoptosis/FAIM3, apoptotic inhibitory molecule 3 was identified as high affinity Fc receptor specific for IgM (6, 7).

TOSO is a transmembrane protein of ~60 kD expressed predominantly by lymphocytes and in contrast to its name it has no anti-apoptotic functions. However, the binding of IgM, in particular multimeric form of IgM to TOSO would facilitate B and T cell cooperation, complement activation and enhanced antibody–dependent cell-mediated cytotoxicity. TOSO recognizes Fc portion of pentameric IgM with high affinity (~ 10 nM). Multimeric form of IgM seems to be critical for this binding as higher concentrations (>100-fold) are required for binding of IgM monomers to TOSO (6, 7). Furthermore, TOSO may play a role in immune surveillance through internalization of IgM-bound immune complexes that contribute to B cell activation. As leukemic B cells generally express high levels of TOSO, this receptor represents an attractive therapeutic target for the delivery of IgM-conjugated drugs into these cancer cells (8).

Natural IgM

In health, the circulating IgM that arise without known immune exposure or vaccination, are referred to as 'natural', while 'immune' IgM are generated in response to defined antigenic stimuli. In the mouse, natural IgM (nIgM) are often without N-region additions and are germline-encoded or with minimal somatic hypermutations, although less is known about the human counterparts. nIgM can display polyreactivity, while some IgM clones have highly refined antigen-binding specificities. A major set of nIgM has been reported to recognize self-antigens (9).

B1 cells

The B-lineage compartment includes at least three distinct mature B cell subsets: B-1 that constitutively produce nIg which is most often IgM, but can be IgG and IgA isotypes; marginal zone B cells that are responsible for responses to encapsulated organisms and their non-protein antigens; and B-2 cells (also termed follicular B cells) that are recruited into T-cell dependent germinal centers upon protein antigen exposure (10, 11). In mice, B-1 cells that express CD5 have long been considered as the major source of nIgM (12, 13). While identification of their human cellular counterpart has been controversial, in part because CD5 can be an activation marker on human B cells, a recent report identified $CD20^+CD27^+CD43^+$ memory B cells as the human B-1 cell equivalent. These cells are distinct from $CD20^+CD27^+CD43^-$ activated memory B and $CD20^+CD27^-CD43^-$ naïve B cells, as B-1 cells do not display the activation markers, CD69 and CD70 (14). B1 cells are also characterized by their ability to efficiently present antigens and can provide potent signaling to T cells in the absence of specific antigenic stimulus (14–16). Compared to B-2 cells that are susceptible to BCR-mediated negative selection due to activation-induced

apoptotic death, B-1 cells are resistant to strong BCR-mediated signalling (14, 17). B-1 cells also have a special ability for self-renewal, and there is also constant addition to the peripheral B-1 repertoire from newly generated cells from the bone marrow that ensures the continuous production of nIgM throughout life (18–21).

The functional contributions of B-1 cells are intertwined in the tight regulation of their trafficking between different lymphoid compartments. B-1 cells migrate from the bone marrow and into the peritoneal cavity as they follow gradients of CXCL13, and CXCL13-deficient mice have reduced levels of peritoneal B-1 cells (22, 23). Stimulation with certain cytokines, or by infectious agent-derived ligands that bear the pathogen-associated molecular patterns (PAMPs), recognized by innate immune receptors, such as TLRs, can activate peritoneal B1 cells. This process can also induce expression of the chemokine receptor, CCR7 that can mediate their relocalization to other lymphoid organs and differentiation into Ig-producing cells. However, the homing of B1 cells into the peritoneal cavity is not an absolute requirement for mounting T-independent antibody responses as most of the production of IgM by murine B-1 cells occurs in the spleen (24).

Natural IgM in immune equilibrium

nIgM can play diverse roles in health and disease. Whereas extensive studies have documented the contribution of nIgM as a first line of defense from infection, more recent reports have highlighted their potential roles in the maintenance of immune homeostasis, the prevention from over-exuberant inflammatory responses and the developement of autoimmune disease. Taken together, these observations have provided the theoretical underpinnings to justify efforts to exploit the untapped therapeutic potential of IgM.

Natural IgM mediate protection against infection

The first indication of the crucial role of nIgs in controlling infections came from the evidence that primary Ig deficient patients display high susceptibility for recurrent infections from bacteria, virus, fungi and parasites. Indeed, reconstitution of immunodeficient animals with nIgM may itself restore the capacity of these animals to control certain infections. This may be explained by virtue of the ability of polyclonal nIgM to directly recognize a wide range of PAMPs that leads to inhibition of the growth of microbial pathogens through their direct neutralization or by activation of the classical complement pathway and amplification of humoral immune responses, leading to the enhanced phagocytosis of these opsonized pathogens (25–31) (Figure 1).

Indeed, the roles of nIgM in preventing infections likely extend beyond simple neutralization and opsonization. Immediately after the entry of pathogens into the host, interactions with nIgM also sets the stage for the shaping of the subsequent immune response. nIgM promote the recognition of pathogens by professional antigen-presenting cells, such as dendritic cells (DCs), and may guide the ensuing functional polarization of T-cell responses, as well the isotype class-switch recombination of the induced B-cell response and the induction long-term immune memory (32, 33). For example, following vaccination with *Leishmania* protein, nIgM can promote IL-4 secretion by CD11b$^+$CD11c^{lo} phagocytes (29, 34). These results indicate that formation of complexes of antigenic ligands with nIgM could be one of the mechanisms responsible for the enhanced humoral immune responses induced by alum adjuvant-containing vaccines (29, 34).

Natural IgM in immune tolerance and tissue homeostasis

nIgM may have first arisen to reinforce fundamental mechanisms for maintaining homeostasis. Apoptosis is an obligatory outcome of development, proliferation and cell differentiation that continues throughout life. Every day, $>10^{11}$ cells in our body die by

apoptosis and therefore apoptotic cell (AC) clearance is essential for tissue homeostasis. The innate immune system recognizes ACs, when they become spontaneously decorated with soluble innate immune molecules, such as complement C1Q and mannose binding lectin (MBL) that can recognize ligands specifically expressed on the AC membranes (ACMs). In fact, this specialized process of phagocytic clearance of ACs, termed efferocytosis, is one of the most fundamental functions of the innate immune system. In health, ACs do not pose an immediate threat to the host as there are redundant means to ensure rapid and efficient cell corpse clearance by macrophages and DCs. To rationalize how defects in efferocytosis may be linked to autoimmune pathogenesis, Walport and colleagues developed the "waste disposal" hypothesis (35). If efficiency of AC clearance is limited, there can be progression to secondary necrosis and the ensuing release of nuclear antigens such as high-mobility group box 1 protein, heat shock proteins and other components of dying cells (DAMPs, danger-associated molecular patterns), which are believed to activate pattern-recognition receptors of innate immune cells that include TLR, leading to inflammatory responses. Necrotic cells can also release autoantigens that can select pathogenic B- and T-cell clones, which together can lead to the development of autoimmune disease in predisposed individuals.

Naïve humans and mice have substantial levels of nIgM that recognize ACMs, while even higher levels can be induced by intravenous infusions of large numbers of ACs (36). The IgM-dependent deposition of C1Q of the classical complement pathway has a major role in determining the efficiency the clearance of ACs by macrophages (37). Furthermore, (38). Experimental models have shown that suppression of inflammatory arthritis mediated by ACs may require nIgM that can directly inhibit macrophage and DC activation and may also promote IL-10-secreting B and T cells (45, (39).

Despite the complexity of molecules displayed on ACs, studies in healthy mice have shown that humoral immune responses to AC infusions are dominated by antibodies to the oxidation-associated phosphorylcholine (PC) and malondialdehyde (MDA) neodeterminants on ACMs (36). In explanation, PC is a phospholipid head group that is an immunodominant epitope on microbial pathogens, such as *Streptococcus pneumoniae*, but the PC head group is also a component of neutral phospholipids (e.g. phosphatidyl choline, PtC) in the outer leaflet of cell membranes. In healthy cells, PC is sequestered and not available for immune recognition. Yet, during apoptotic death, the PC head group becomes exposed due to oxidative damage to the polyunsaturated fatty acid side chains of phospholipids, to generate reactive aldehydes such as 1-palmitoyl-2-(5-oxovaleroyl)-sn-glycero-3-phosphocholine (reviewed in (40)). Thereby, damaged cells are flagged via antibody recognition of PC neo-epitopes. A second major set of anti-ACM IgM recognizes MDA, which is an important degradation product of unsaturated lipids reacting with reactive oxidation species (41). nIgM recognition of ACs via the oxidation-associated phospholipid determinants, PC and MDA, enables the discrimination of ACs from healthy cells (36, 38, 42–47) (Figure 1).

PC and MDA are also dominant phospholipid-related epitopes on oxidatively modified (but not native) low-density lipoproteins (OxLDL) in atherosclerosis. The development of atherosclerosis is also associated with B-cell activation, particularly manifested by enhanced production of natural anti-OxLDL IgM and IgG autoantibodies (42). Levels of circulating anti-OxLDL IgM antibodies have been linked with reduced vascular risk in humans (48). Yet anti-OxLDL IgG antibodies show variable association with vascular risk (49). In fact, in earlier studies it was shown that bacterial vaccine that induced high levels of IgM anti-PC antibodies arrested the progression of atherosclerosis in atherosclerosis-prone mice with cholesterol levels greater than 1000 mg/dL (43). However, the underlying mechanisms were undefined as infusions of such antibodies did not affect the *in vivo* clearance of OxLDL (50).

It was nonetheless postulated that the balance of inflammation and accumulation of ACs in atherosclerosis is a direct reflection of the potential influences of specific types of atherosclerosis-associated autoantibodies. In an earlier report, it was shown that anti-PC IgM antibodies blocked phagocytic clearance of ACs (44). However, as described below, subsequent studies showed that this T15 clonotypic PC-specific IgM enhances AC clearance *in vivo* and *in vitro*, in a complement-dependent fashion (36, 38) that may suggest that there was an insufficient level of early complement factors, such as C1q and MBL, in the assays described in this earlier *in vitro* report (44).

Recent studies in murine models demonstrated that anti-ACM IgM directed against PC and MDA can have two major regulatory functions: i) enhance clearance of ACs by phagocytes, (as termed efferocytosis) (36, 38) and ii) direct suppression of pro-inflammatory responses induced by agonists for TLR3, TLR4, TLR7 and TLR9, and likely many other innate pathways (38). However, it is not clear whether these two immunomodulatory functions are mediated through the same signaling pathway, as inhibition of AC phagocytosis does not necessarily inhibit the anti-inflammatory influences of AC interactions with DCs (51). Investigations of the inhibitory properties of the prototypic anti-PC IgM, T15, which also recognizes ACs, did not decrease TLR transcript expression or induce TGF-β or IL-10 in DCs (38).

In well-characterized inducible murine models of inflammatory autoimmune diseases, collagen-induced arthritis and autoantibody-mediated arthritis, anti-ACM nIgM infusions (i.e., T15 IgM), but not isotype control or saline, protected from inflammatory arthritis (38). Also, recent observations in patients with the autoimmune disease, systemic lupus erythematosus, highlight the association of nIgM levels in protection against autoimmunity (52–54). For example, decreased levels of anti-PC IgM antibodies are characteristic features of active lupus compared to disease in remission (52, 53). These studies suggest that some nIgM may serve as regulators of the innate immune system to help maintain homeostasis and in certain cases suppress the development of inflammatory and autoimmune diseases.

Another important role of nIgM in tissue homeostasis implicates clearance of altered or malignant cells via complement-dependent cell lysis and induction of apoptosis (55, 56). Many tumor-related epitopes are carbohydrate in nature and by themselves are poorly immunogenic, thereby rendering the formulation of effective active immunization approach a daunting task. Owing to their capacity in some cases to recognize the repetitive motifs of some carbohydrate antigens and with an increased avidity due to their polymeric structure, nIgM may provide an 'endogenous' therapeutic tool for enhancing certain types of immune responses (57, 58).

Deficiency in serum IgM may predispose to the development of IgG autoantibodies suggesting that nIgM contribute to the suppression of disease-associated IgG autoantibody production (59) (Figure 1). It has also been argued that the protective effect of IgM may at times involve anti-idiotypic (i.e., targeting of the antigen receptors of some clonally related lymphocytes) down-regulation of some autoimmune responses, or result from the induction by some IgM anti-idiotypic antibodies of apoptotic death of pathogenic B-cell clones or the selection of other protective B-cell subsets (60–62). The maintenance of tissue homeostasis by nIgM may also involve the enhanced clearance of misfolded proteins, which could have clinical implications for conditions like Alzheimer's disease in which pathogenesis results from the deposition of misfolded proteins such as β-amyloid plaques in the brain. Relevant to this hypothesis, IgM antibodies have been shown to recognize and hydrolyze β-amyloid proteins (63).

How can we boost natural IgM *in vivo*?

In recent reports, several strategies have been described for boosting of nIgM levels. Following splenectomy or thermal injury, patients often develop a selective loss of circulating IgM and display an associated heightened susceptibility to certain types of infections. While in health, nIgM is constitutively produced, there is extensive evidence that levels can be enhanced by a number of interventions. Experimental models suggest that exogenous administration of IL-18 can restore nIgM production and also strengthen the host defenses to infection (64, 65) (Figure 2). Additional strategies to induce nIgM include: (i) parenteral administration of CpG, an unmethylated, oligodeoxynucleotide PAMP that activates B cells via TLR9; (ii) use of mimetic peptides derived from random *in-silico* screening of peptides that mimic poorly immunogenic non-protein antigens; (iii) immunization with PC-containing antigens that are also a dominant type of phospholipid-related neodeterminant on ACMs and on OxLDL; (iv) pneumococcal vaccination that exploits the molecular mimicry among the PC moieties of microbial cell-wall polysaccharide, unfractionated OxLDL and ACs; and (v) injection of internal-image bearing anti-idiotypic antibodies (43, 66–70). Indeed, idiotypic vaccination strategies that boost IgM and IgG levels against NeuGcGM3 ganglioside antigens have also shown promising results in early clinical trials (71, 72).

Harnessing therapeutic potential of natural IgM

Use of IgM-enriched therapeutic preparations

As normal human plasma contains a substantial amount of nIgM, it may be practical and economically viable to harness therapeutic potential of these IgM through the generation of therapeutic preparations in a manner analogous to intravenous IgG (IVIg) that is now extensively used for the treatment of a wide range of pathological conditions (73, 74) (Figure 2). Indeed, an IgM-enriched Ig preparation, Pentaglobin®, contains 12% IgM and this has been successfully used for treating infections associated with sepsis in patients, as well as transplant rejection, and for certain inflammatory conditions in experimental models (75–78). Such preparations may also provide benefits to combat infections that arise in patients with autoimmune disease (79). This preparation likely benefits from enrichment for a common type of VH4-34-encoded nIgM antibody that has specific high binding capacity for microbial lipopolysaccharide (80), a factor implicated in infection-induced cardiovascular collapse.

Similarly, a pilot preparation of pooled polyreactive normal IgM that contains more than 90% IgM (IVIgM), was generated from plasma of more than 2,500 healthy donors. Such IVIgM also contains anti-idiotypic antibodies that recognize disease-associated IgG-autoantibodies from patients with a diverse range of autoimmune diseases and this IVIgM can inhibit *in vitro* the activities of these IgG-autoantibodies (81). Furthermore, the *in vivo* therapeutic efficacy of IVIgM was confirmed in complement-dependent inflammatory conditions and in experimental models of uveitis, myasthenia gravis and multiple sclerosis (77, 81–84). While exploring the mechanisms for such protection, it was found that IVIgM can induce the apoptosis of mononuclear cells, suppress the functions of T cells and also prevent the activation of the complement cascade under inflammatory conditions (77, 85, 86).

Use of monoclonal IgM antibodies

Promising results obtained with polyclonal nIgM prompted the identification and use of monoclonal nIgM antibodies in experimental autoimmune diseases in mice (Figure 2). Two such monoclonal antibodies (either derived from serum or generated by recombinant approach) IgM22 and IgM46 have provided protection against Theiler's murine

encephalomyelitis virus-induced chronic progressive demyelinating disease and lysolecithin-induced demyelination in mice by promoting remyelination (83, 84, 87, 88) and are potential candidates for therapeutic trials.

Conclusions: Taking natural IgM to clinic

Although the therapeutic opportunities of nIgM appear vast, so far the promise of clinical utility has been evaluated in only a small range of diseases. Several different approaches to nIgM-based therapy are being considered for suitability for clinical trials. A natural human monoclonal antibody, IgM22 that binds to oligodendrocytes and promotes their remyelination will soon complete primate toxicology testing prior to submission for the FDA approval (Moses Rodriguez, personal communication). Interestingly, anti-idiotype-based approaches that boost IgM antibody directed against NeuGcGM3 ganglioside *in vivo* have also recently reached phase III clinical trials for the treatment of metastatic breast cancer (71).

One of the main reasons for the slow progress in translating the basic research findings on nIgM to clinical application is the issue inherent to the nature of the molecules themselves. The existence of IgM in either monomeric or pentameric forms presents a dilemma regarding the identification of the form of the therapeutic molecule that will achieve the most attractive profiles for efficacy, tolerability and safety. The pentameric form poses further potential challenges regarding the stability of such manufactured 'IgM-based therapeutics'. Similar to IgG, the glycosylation pattern of IgM can also play a critical role in its effector functions, and μ constant region-associated high mannose glycoconjugates can mediate the recruitment of MBL, which can enhance the clearance of ACs (36), while in other settings this may instead trigger downstream complement activation and associated undesirable complications. Hence, the specialized effector functions of IgM can provide clinical opportunities but also pose challenges for the purification of well-characterized homogeneous preparations that are required for therapeutic applications.

As for monoclonal IgM, identification of the most relevant and specific molecular targets, and then optimizing production at an industrial scale are all likely to be technically challenging and time-consuming. Further, depending on the molecular targets, each particular monoclonal IgM may have a limited range of clinical indications. For these reasons, pooled polyspecific IgM may present clear advantages, as the plasma fractionation industry is well suited for these manufacturing challenges. In fact, an Ig preparation containing 12% pooled IgM is currently used for the treatment of patients with sepsis (75, 76). Early results from pilot IVIgM preparations containing about 90% IgM have also shown immunomodulatory potential in experimental models of autoimmune and inflammatory diseases (77, 81–84). In the future, data from planned investigations should provide insights into the ideal concentration of IgM in the Ig preparations that strikes the optimal balance between therapeutic efficacy and minimal adverse reactions. IgM-containing Ig preparations may also be used as supplemental therapy for patients who are treated with agents that induce B-cell and Ig-depletion or other immunosuppressive regimens that can be associated with increased susceptibility to infection.

Acknowledgments

Abbreviations used in this paper

nIgM	natural IgM
AC	apoptotic cell
ACM	apoptotic cell membrane
OxLDL	oxidized low-density lipoproteins
PC	phosphorylcholine
MDA	malondialdehyde
IVIgM	intravenous immunoglobulin M

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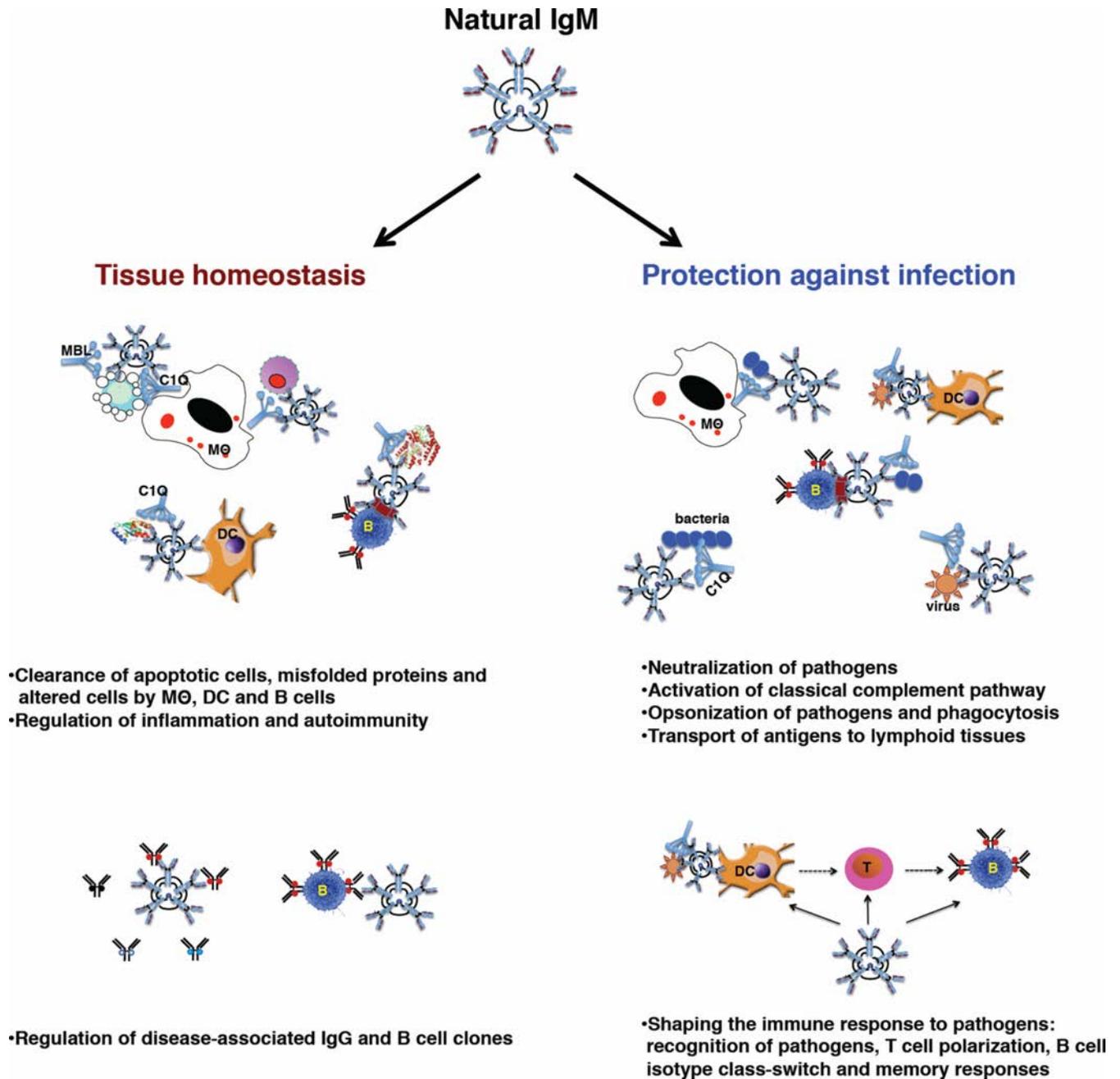
Figure 1. Natural IgM in immune equilibrium
nIgM can maintain immune equilibrium through regulating the tissue homeostasis and by acting as a first line of defense against invading microbes and shaping the subsequent immune response. The role of nIgM in tissue homeostasis implicates activation of compliment pathways and helps to prevent inflammation, autoimmunity and malignancy. These functions include recognition of apoptotic cell membranes and promoting the clearance of apoptotic cells by phagocytic cells such as DC and MΘ, clearance of altered or malignant cells or misfolded proteins, and regulation of the disease-associated IgG and B cell clones. Further, nIgM can confer protection against invading pathogens through direct neutralization of pathogens, activation of classical complement pathways, opsonization of

pathogens and phagocytosis by DC and MΘ, and transportation of antigens to lymphoid organs for initiating the immune responses by innate immune cells. In addition, nIgM can also regulate the immune response to pathogens by influencing the T cell polarization and B cell class-switch.

DC, dendritic cell; MΘ, macrophage; C1Q, complement C1Q (classical complement pathway); MBL, mannose binding lectin (lectin complement pathway); B, B cell; T, T cell. Further, recent reports have also highlighted the potential roles of nIgM in tissue homeostasis via multiple ways such as
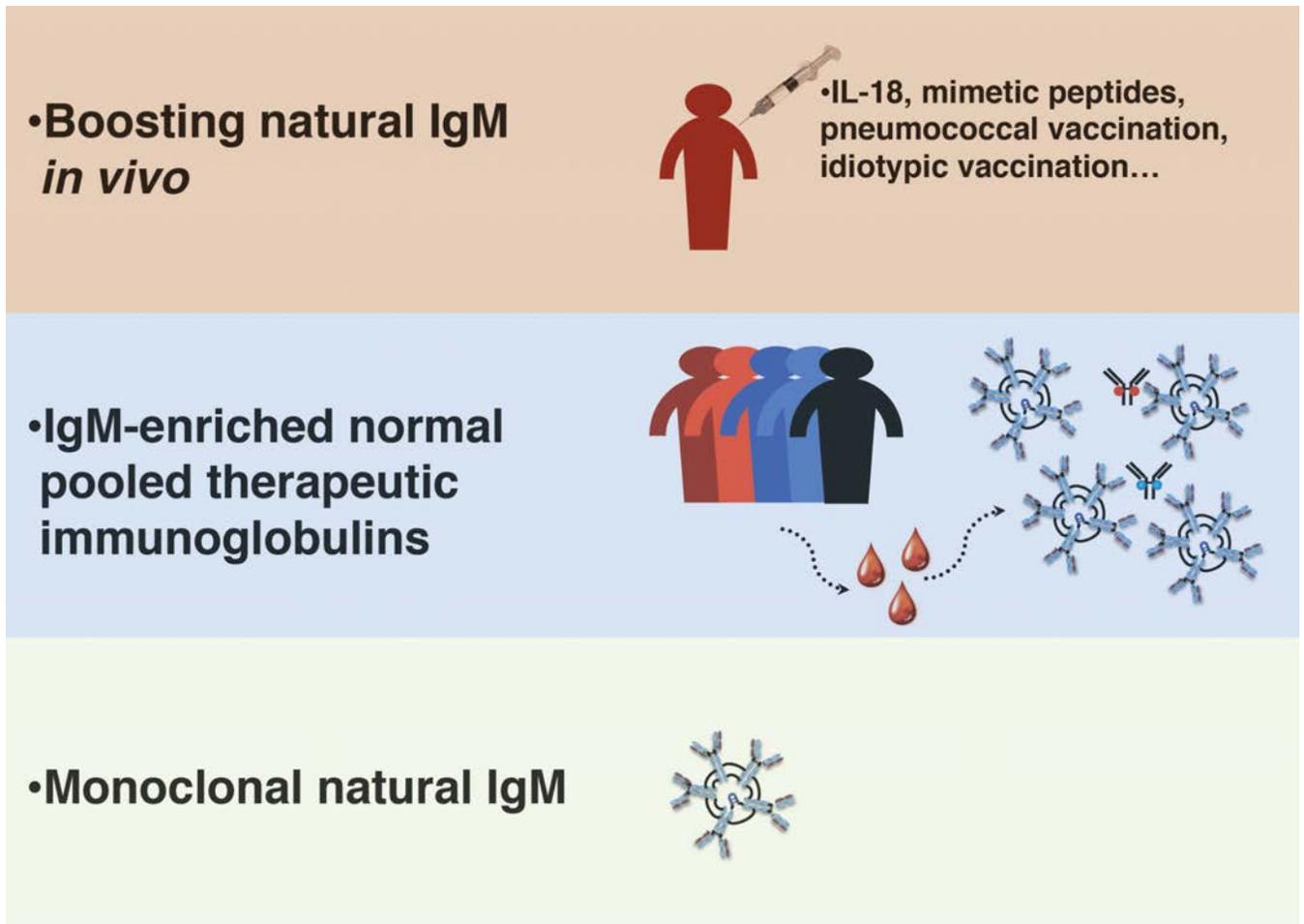
Figure 2. Means to harness therapeutic potential of natural IgM