Role of Natural IgM Autoantibodies (IgM-NAA) and IgM Anti-Leukocyte Antibodies (IgM-ALA) in Regulating Inflammation

Peter I. Lobo

Abstract Natural IgM autoantibodies (IgM-NAA) are rapidly produced to inhibit pathogens and abrogate inflammation mediated by invading microorganisms and host neoantigens. IgM-NAA achieve this difficult task by being polyreactive with low binding affinity but with high avidity, characteristics that allow these antibodies to bind antigenic determinants shared by pathogens and neoantigens. Hence the same clones of natural IgM can bind and mask host neoantigens as well as inhibit microorganisms. In addition, IgM-NAA regulate the inflammatory response via mechanisms involving binding of IgM to apoptotic cells to enhance their removal and binding of IgM to live leukocytes to regulate their function. Secondly, we review how natural IgM prevents autoimmune disorders arising from pathogenic IgG autoantibodies as well as by autoreactive B and T cells that have escaped tolerance mechanisms. Thirdly, using IgM knockout mice, we show that regulatory B and T cells require IgM to effectively regulate inflammation mediated by innate, adaptive and autoimmune mechanisms. It is therefore not surprising why the host positively selects such autoreactive B1 cells that generate protective IgM-NAA, which are also evolutionarily conserved. Fourthly, we show that IgM anti-leukocyte autoantibodies (IgM-ALA) levels and their repertoire can vary in normal humans and disease states and this variation may partly explain the observed differences in the inflammatory response after infection, ischemic injury or after a transplant. Finally we also show how protective IgM-NAA can be rendered pathogenic under non-physiological conditions. IgM-NAA have therapeutic potential. Polyclonal IgM infusions can be used to abrogate ongoing inflammation. Additionally, inflammation arising after ischemic kidney injury, e.g., during high-risk elective cardiac surgery or after allograft transplantation, can be prevented by pre-emptively infusing polyclonal IgM, or DC pretreated ex vivo with IgM, or by increasing in vivo IgM with a vaccine approach. Cell therapy with IgM pretreated cells, is appealing as less IgM will be required.

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1 Introduction

Nature, by creating polyreactive pentavalent natural IgM autoantibodies (IgM-NAA), has accomplished a difficult task of protecting the host from both diverse foreign pathogens and from diverse self-neoantigens that are constantly being generated. As a result, the adaptive immune system has time to mount a highly specific immune response to foreign antigens and, in addition, such a mechanism lessens the burden on the host to maintain diverse B cell clones producing highly specific IgG autoantibodies which have the potential of causing autoimmune disease owing to their high affinity binding. Secondly, these IgM-NAA have taken over another task of subduing an excessive inflammatory response induced by both foreign and self-neoantigens. Again, we will show how the low binding affinity of IgM-NAA to live leukocytes, together with their inability to fully activate complement at body temperature (37 °C), has helped these antibodies to regulate these inflammatory cells without causing cell damage within the host. It is therefore not surprising why these IgM-NAA antibodies, which first arose in cartilaginous fish, have been conserved during evolution (reviewed in Dooley and Flajnik 2006) and why IgM-NAA make up about 70–80% of circulating IgM (Baumgarth et al. 1999; Thurnheer et al. 2003). Additionally, natural IgM is also evolutionarily functionally conserved among mammalian species, as human IgM has the same effect as murine IgM on murine cells in vitro or when used in vivo in

mice (Robey et al. 2002; Zhang et al. 2008; Lobo et al. 2015). The presence of polyreactive IgM BCR on these IgM-NAA producing B cell clones has enabled them to be rapidly activated by a foreign or an auto-neoantigen for deploying protective IgM antibodies.

Recently, B1 cells have also been shown to exist in humans. Human B1 cells, unlike murine B1 cells, are CD20+ CD43+ and CD27+. Like murine B1 cells, human B1 cells can spontaneously secrete antibody and such cells represent about 50% of umbilical cord B cells and 15–20% of circulating adult B cells, and these cells are the predominant source of human IgM-NAA (Griffin et al. 2011). CD5 is not a specific marker of human B1 cells as both B1 and B2 cells express this marker. Similarly, about 20% of CD43+ CD27+ B cells have characteristics of B2 derived pre-plasmablasts and hence CD43 and CD27 are also not reliable markers of human B1 cells (Covens et al. 2013; Tangye 2013). Human IgM-NAA are also polyreactive and bind similar autoantigens as in mice, including oxidized neodeterminants and leukocyte receptors (Chen et al. 1998; Lobo et al. 2008a; Chou et al. 2009; Lobo et al. 2015).

An important characteristic of IgM-NAA is their low binding affinity (Zhou et al. 2007). The IgM BCR expressed on B1 cells may also have a low binding affinity and this latter characteristic may be involved in preventing autoreactive B1 cells from being deleted or undergoing negative selection. In fact, several studies would indicate that autoreactive B1 cells are positively selected and this process requires both the autoantigen and the relevant BCR (Hayakawa et al. 1999; Martin and Kearney 2000; Cancro and Kearney 2004; Tian et al. 2006). The need to positively select B1 cells secreting IgM-NAA would indicate that these antibodies have an important physiological role. During life, the repertoire of IgM-NAA is shaped by T-independent antigen activation (Martin and Kearney 2002; Kretschmer et al. 2003).

IgM-NAA have been shown to have specificity for certain common epitopes present on phylogenetically conserved self-antigens. As a result, diverse IgM secreting clones with different specificities have been identified. These include IgM secreting clones with specificity for leukocyte receptors (IgM-ALA) (Lobo et al. 2008a, 2015), the Fc domain of IgG (rheumatoid factor) (Casali et al. 1987; Hardy et al. 1987), complement components (Rieben et al. 1999), collagen, thyroglobulin, intracellular constituents such as cytoskeletal proteins, cytosolic enzymes, dsDNA or nucleosomes, neutrophil cytoplasmic enzymes (ANCA) (Avrameas 1991; Vittecoq et al. 1999) as well as oxidized neodeterminants (e.g., phosphorylcholine (PC)) that are exposed when lipids are oxidized or cells undergo apoptosis (Baumgarth 2011; Gronwall et al. 2012). While some IgM-ALA have monoreactivity e.g., to some cytokines, most are polyreactive with each polyreactive IgM-NAA clone having a selective binding profile. For example, IgM anti-PC NAA will bind to ABO blood type antigens, endotoxins and oxidized neodeterminants on apoptotic cells but this autoantibody has no binding reactivity to nuclear antigens or to IgG (Baxendale et al. 2008). Conversely, IgM anti-dsDNA will bind to cytoskeletal proteins but will not bind to PC. Additionally, these IgM-NAA, by virtue of being polyreactive, also cross-react with pathogen-expressed molecules,

for example phosphorylcholine (PC) on *Streptococcus pneumoniae* and other antigens expressed by various viruses and parasites (Baxendale et al. 2008; Baumgarth 2011; Gronwall et al. 2012) Hence, it has been suggested that these natural IgM antibodies are protective, serving as a first line of defense against infections and protecting the host from pathogen-mediated apoptotic cells and oxidized neodeterminants which can induce pathogenic IgG autoantibodies (Baumgarth 2011; Gronwall et al. 2012). Additionally, polyreactive IgM-NAA have been shown to bind to idiotypic determinants on self-reactive IgG, thus providing another mechanism to protect the host from high affinity binding IgG autoantibodies that are potentially pathogenic (Adib et al. 1990; Avrameas 1991).

2 Natural Autoantibodies and B1 Cells

In the last 40 years, much has been learned about natural autoantibodies (NAA) of different isotypes (Steele and Cunningham 1978; Dighiero et al. 1983; Hardy and Hayakawa 1986; Casali et al. 1987; Hardy et al. 1987; Nakamura et al. 1988; Kantor and Herzenberg 1993; Kasaian and Casali 1993; Mouthon et al. 1996; Clarke and Arnold 1998; Rieben et al. 1999). The term "natural antibodies" has been used to describe these Igs, as high levels of these autoantibodies are present in the umbilical cord, i.e., before foreign antigen exposure, and secondly because such antibodies can be produced under germfree conditions and in the absence of the thymus (Avrameas 1991). The full repertoire of IgM-NAA develops by early childhood. In mice, NAA are predominantly produced by the CD5+ B1 cells while marginal zone splenic B (MZB) cells contribute the remainder. These B1 cells differ from B2 cells in that they spontaneously produce IgM, IgA and IgG3 autoantibodies (Sidman et al. 1986; Solvason et al. 1991; Griffin et al. 2011) independently of T helper cells, and exhibit an enhanced response to innate immune signals such as TLR agonist (Murakami et al. 1994; Nisitani et al. 1995; Ha et al. 2006; Yang et al. 2007). Additionally, autoantibody producing B1 cells are positively selected for their self-reactivity thus implying that NAA are conserved by design (Hayakawa et al. 1999; Martin and Kearney 2000; Cancro and Kearney 2004; Tian et al. 2006). Furthermore, the finding that IgM-NAA comprise the majority of circulating IgM underscores their importance (Baumgarth et al. 1999; Thurnheer et al. 2003). Cross-sectional studies in humans and rodents would indicate that IgM-NAA decrease with age (Adib et al. 1990; Love et al. 2000; Simell et al. 2008; Griffin et al. 2011) or lose their effectiveness with age (Nicoletti et al. 1993), except for one report where follow-up of 5 healthy individuals for 25 years revealed no change in IgM-NAA levels (Lacroix-Desmazes et al. 1999). However, IgG NAA can increase (Nagele et al. 2013) but do not decrease with age (Lacroix-Desmazes et al. 1995; Bachi et al. 2013).

Natural IgM-NAA should not be confused with immune IgM that is produced several days after exposure to foreign antigens or pathogens and, in general, is antigen-specific and produced by B2 cells that require antigen binding to BCR and

additional T helper cells to generate antigen-specific antibodies. Production of immune IgM is limited as antigen-activated B2 cells migrate to B cell follicles, where with help from follicular T helper cells, these cells undergo class switch recombination (CSR) and somatic hypermutation (SHM). B2 cells differentiate into long-lived memory B cells and plasma cells that generate IgG antibodies with high binding affinity. B2 cells are distinct from B1 cells in many respects and they are derived from different progenitors (Sidman et al. 1986; Solvason et al. 1991; Montecino-Rodriguez et al. 2006). Furthermore, during an immune response, B1 cells have intrinsic mechanisms to actively inhibit CSR and SHM. In this regard, there are mechanisms to actively prevent B1 cells from entering B cell lymphoid follicles and B1 cells actively maintain low levels of activation induced deaminase (AID), which induces SHM and CSR, and through this mechanism B1 cells inhibit production of high affinity, IgG anti-self Ab, which may be pathogenic (Ishida et al. 2006; Matejuk et al. 2009).

3 Physiological Role of IgM-NAA

There are several in-depth reviews on the physiological functions of IgM-NAA (Ehrenstein and Notley 2010; Baumgarth 2011; Gronwall et al. 2012; Kaveri et al. 2012; Lobo 2016). Briefly, functions that have been attributed to IgM-NAA have included the following: (i) Providing the first line of defense against pathogens while the adaptive immune system, i.e., B2 and T cells, is being deployed to mediate a more specific and effective immune response that is longlasting and has memory. (ii) Inhibiting IgG autoantibody production and inflammatory responses by clearing apoptotic cells and binding to oxidized neodeterminants as well as by blocking pathogenic IgG autoantibodies via anti-idiotypic mechanisms (iii) Inhibiting inflammation by binding of IgM-NAA to receptors on live leukocytes, i.e., via IgM anti-leukocyte autoantibodies (IgM-ALA) and by binding of IgM anti-PC to phosphorylcholine (PC) expressed by apoptotic cells and (iv) Inhibiting expansion of B1 cells and enhancing antigen presentation to B2 and helper T cells in splenic lymphoid follicles.

The above observations indicate that IgM-NAA protect the host from invading organisms and more importantly maintain several homeostatic mechanisms primarily aimed at preventing autoimmunity and over exuberant inflammation, which can have detrimental effects on the host. Table 1 summarizes some of the physiological and pathological concepts outlined above. Several observations indicate that infective and other inflammatory states increase all IgM-NAA subsets, especially IgM anti-PC to clear the increased production of apoptotic cells that could trigger autoimmunity and secondly increase IgM-ALA to subdue excess inflammation that can be detrimental to the host (reviewed in Gronwall et al. 2012 and next section). Based on the preceding observations, one could predict that a

Table 1 Physiological function of non-pathogenic natural IgM autoantibodies

Protection from microorganisms	– Binds to bacteria and enhances phagocytosis (req. C1q, Fcα/μR) – Inhibits HIV by blocking entry and inactivating cells
Prevent autoimmunity	– Blocks anti-self IgG Ab (anti-idiotypic) IgM masks neoantigen – Binds to apoptotic cells (PC), nuclear and cytoplasmic debris, and enhances phagocytosis (req. C1q, MBP)
IgM-ALA abrogates inflammation	– Inhibits serum complement – Decreases production of TNF-α, IL-17, IFNγ – Regulates DC, T cells, and enhances T regs – Binds to CD40, CD86, CD4, CD3, and TcR – Downregulates NFκB and ZAP-70 phosphorylation – Blocks chemokine receptors
B cell homeostasis	IgM regulates B1 cell expansion via FcμR

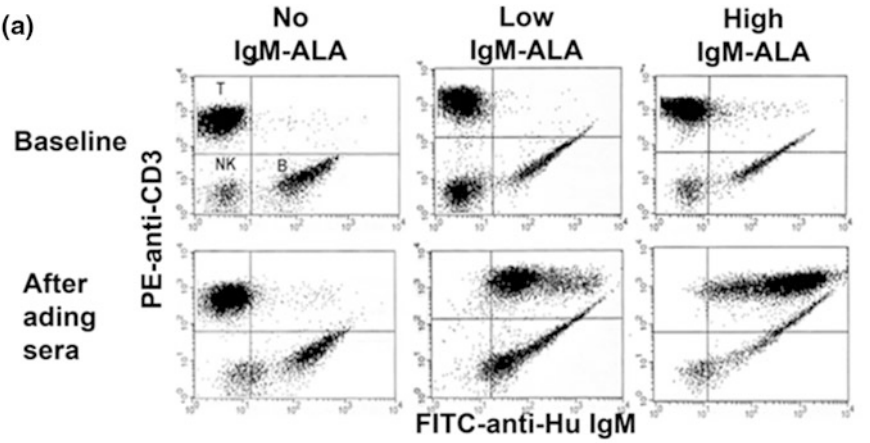
decrease in IgM-NAA, as can occur in aging (Nicoletti et al. 1993; Love et al. 2000; Simell et al. 2008; Griffin et al. 2011), could predispose to increased autoimmunity and increased morbidity and mortality from an excess inflammatory response. In this review, we will focus on the role of IgM-ALA in inhibiting inflammation.

4 Physiologic Role of IgM-ALA in Regulating Inflammation

Initial observations demonstrating that IgM can bind to receptors on live autologous and allogeneic leukocytes were made in 1970 (Terasaki et al. 1970). The role of IgM-ALA in inflammation was recognized when several investigators showed that the level of these antibodies, e.g., IgM anti-PC and IgM-ALA, increased with diverse infections and inflammatory states (reviewed in Lobo et al. 2008a). However, it was unclear whether high levels of IgM-ALA were pathogenic or not. The idea that IgM-ALA may have anti-inflammatory function and may be protective came from observations in allograft recipients, where patients with high levels of IgM-ALA were found to have significantly less rejections after kidney

Fig. 1 *High levels of serum IgM-ALA in transplant recipients are associated with better kidney allograft survival and decreased alloantibody levels*. **a** Dot plots labeled "baseline" depict IgM staining on B lymphocytes, but not on T cells, in the absence of sera. The *lower panels* (after adding sera) depict differences in the level of IgM bound to donor B and T lymphocytes (IgM-ALA) after addition of pre-transplant serum from different ESRD patients. **b** Alloantibody titer in ESRD pre-transplant sera measured by cytotoxicity is correlated to presence or absence of IgM-ALA present in the same pre-transplant sera. **c** Data depicting the difference in percentage of acute rejections and graft loss comparing high IgM-ALA versus the no and low IgM-ALA groups. *MCF* indicates increase in mean channel fluorescence of anti-IgM staining to T cells after addition of serum. Figure and legend reproduced with permission from Transplantation. 1981;32(3):233–7 copyright 1981 Wolters Kluwer Health Inc. (panel **b**) and J Clin Immunol. 2010;30(1):31–6. Copyright 2010 Springer Science + Business Media LLC. (panels **a**, **c**) ▶

(a)



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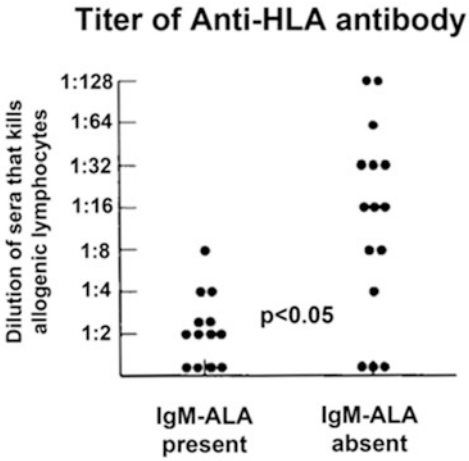


(c)

	No IgM-ALA (MCF<20)	Low IgM-ALA (MCF 21-200)	High IgM-ALA (MCF>200)
# of Patients	65	22	21
%Acute Rejections Requiring treatment	32	32	*9.5
%Graft loss at 1 yr	20	9.1	*0

*p<0.05 comparing High IgM vs. Low and No IgM

transplantation and developed less alloantibodies after a sensitizing event with alloantigens (Fig. 1) (Lobo 1981; Lobo et al. 2008a). We therefore hypothesized that the increase in natural IgM-ALA during inflammatory states provided a mechanism to regulate leukocyte function and prevent excess inflammation that may be detrimental to the host. Two characteristics, i.e., their low affinity binding to different leukocyte receptors including co-stimulatory molecules and chemokine receptors (Lobo et al. 2008a, 2015) and secondly, their inability to lyse leukocytes at body temperature, despite the presence of complement, allowed us to develop this hypothesis (Winfield et al. 1975; Lobo 1981; Lobo et al. 2008a). We postulated that the low affinity binding to leukocyte receptors modulated their function without causing cell lysis or apoptosis.

4.1 B Cell Clones Obtained from Human Umbilical Cord Produce IgM-ALA that Exhibit Leukocyte Receptor Specificity—Binding of IgM to Leukocytes Was not Mediated by FcμR

It became necessary to determine if IgM-ALA exhibited leukocyte receptor specificity, especially since these antibodies are polyreactive and could non-specifically bind to carbohydrate or other moieties and hence cross-react with several leukocyte receptors. This issue was evaluated by isolating B cell clones from human umbilical cord blood. We observed that >90% of umbilical cord B cells were IgM secreting but, surprisingly, only 10% of these IgM clones had IgM-ALA binding activity when examined by flow cytometry on a cell mixture of B (Daudi), T (Jurkat, Sup T1) and macrophage (U937) human cell lines. These observations therefore indicated that binding of IgM to receptors on human B cells and macrophages occurred independently of FcμR, which is not expressed by human macrophages (Kubagawa et al. 2009). Secondly, we observed that IgM-ALA had receptor specificity, especially since some of the IgM-ALA monoclonal antibodies only bound to receptors expressed by all leukocytes while other IgM monoclonals bound to receptors expressed by either T cells (SupT1, Jurkat) or macrophages (U937) or B cells (Daudi) (Fig. 2a) (Lobo et al. 2008a). The latter is exemplified by a T cell-specific human monoclonal IgM, which immunoprecipitated CD4 from cell lysates and bound to recombinant soluble CD4 (Figs. 2b, 3a).

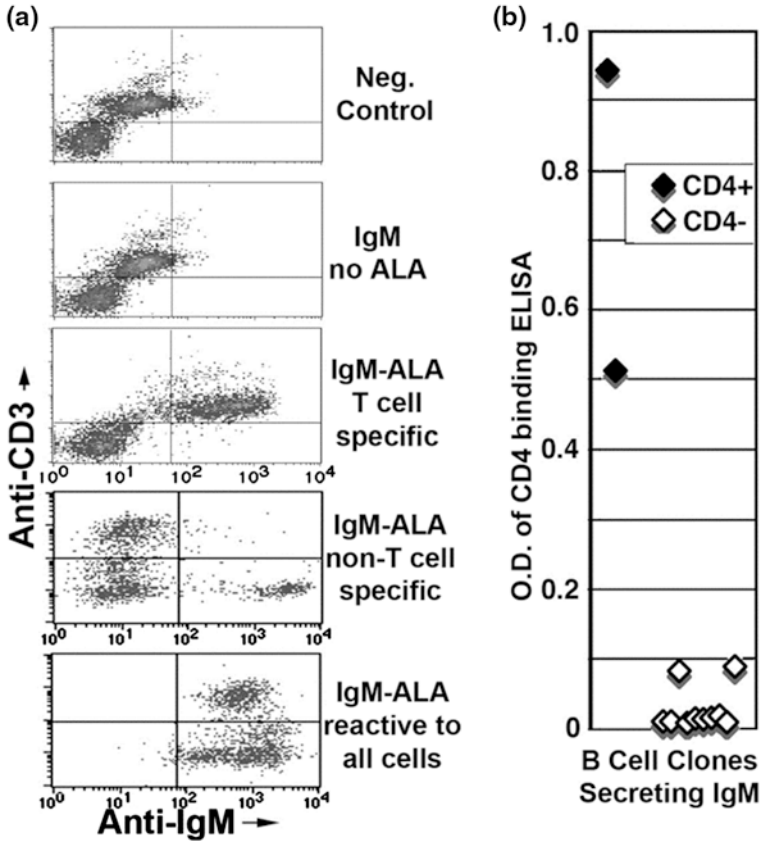


Fig. 2 *Presence of IgM-ALA in supernatants from umbilical cord B cells.* **a** and **b**, IgM-ALA reactivity in IgM-containing supernatants from B cell clones activated with EBV. IgM-ALA reactivity was detectable in 8 of 79 supernatants. **a** Supernatants were interacted with cells containing a mixture of human cancer cell lines, i.e., Jurkat (T cells), SupT-1(T cells), Daudi (B cells), and U937 (monocyte). Daudi B cells in the cell mixture were initially pre-treated with un-labelled anti-IgM and washed to block intrinsically expressed IgM. Note that the supernatants have IgM-ALA that is specific for either T or non-T cells or all four cell lines. Subsequent studies revealed that IgM anti-non-T cell had binding reactivity to only U937 cells and not to Daudi cells. **b** Data on IgM anti-CD4 reactivity using an ELISA technique. Note that only 2/79 IgM-containing supernatants had IgM anti-CD4 reactivity and both these supernatants also had IgM that bound to leukocytes. Figure and legend reproduced with permission from J Immunol. 2008 Feb 1;180 (3):1780–91. Copyright 2008. The American Association of Immunologists, Inc

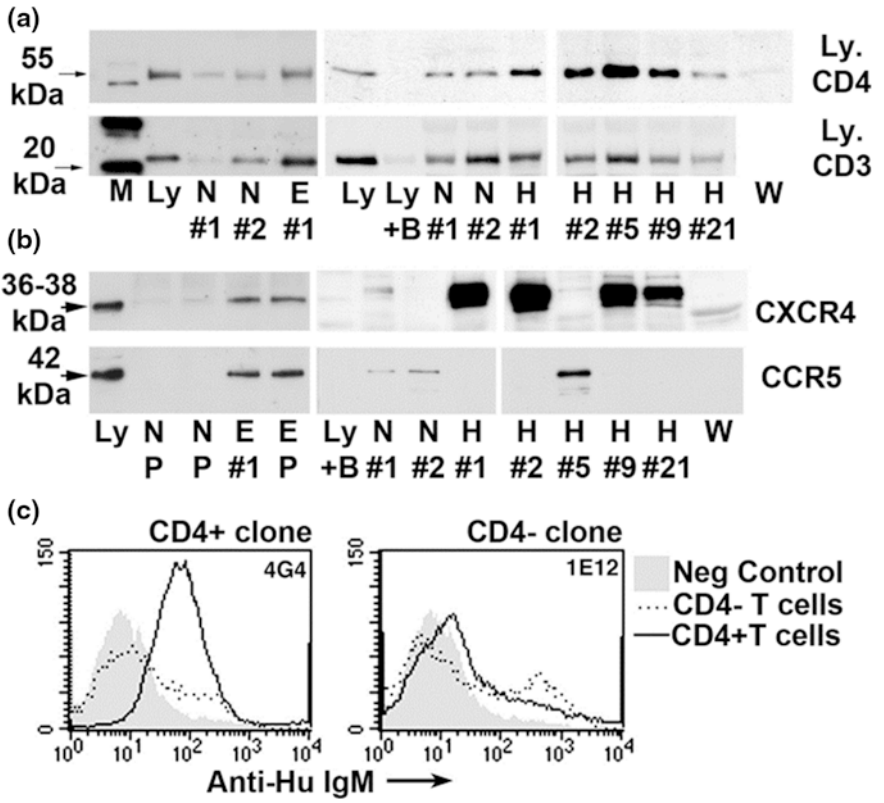
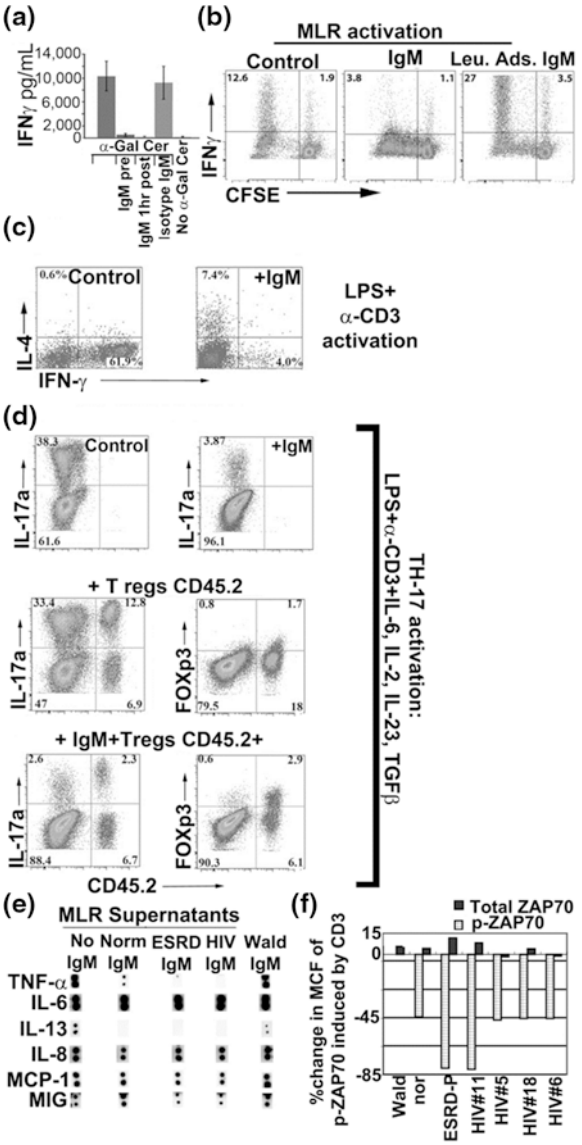


Fig. 3 *Immunoprecipitation experiments to show binding of human polyclonal IgM to CD3*, *CD4*, *CCR5*, *and CXCR4*. **a**, **b** Identical quantities of individual (labeled no. 1, 2, etc.) or pooled (labeled P) IgM from normal (labeled N), HIV (labeled H), ESRD (labeled E), or Waldenstrom (labeled W) were used to immunoprecipitate leukocyte receptors from equal amounts of whole cell lysates or recombinant soluble CD4. As controls, Western blots were performed with cell lysates in the absence of agarose beads (to control for binding of primary Ab to leukocyte receptor and to determine receptor size (labeled Ly). In another control, agarose beads without IgM were added to lysate to determine whether the leukocyte receptor nonspecifically bound to the bead (B plus Ly). Note that severalfold more receptors were immunoprecipitated by ESRD and HIV IgM when compared with normal IgM. **c** Significantly increased binding of IgM from B cell clone 4G4 to CD4+ T cells when compared with CD4− T cells (MCF 71.6 vs. 16.6). Clone 4G4 secreted IgM with anti-CD4 reactivity. Note that no increased binding was observed on CD4+ T cells using a B cell clone (IE12) secreting IgM without anti-CD4 reactivity (MCF 22.5 vs. 22.3). Figure and legend reproduced with permission from J Immunol. 2008 Feb 1;180(3):1780–91. Copyright 2008. The American Association of Immunologists, Inc

4.2 *IgM-ALA from Different Human Sera Differ in Their Repertoire for Receptor Binding. IgM Regulates Human T Effector Cells and DC Without Affecting Tregs or Chemokine Production*

This was studied by using polyclonal IgM purified from sera of normal controls, HIV patients and patients with end stage renal disease (ESRD) (Lobo et al. 2008a). IgM in these studies was purified by size exclusion chromatography, as ammonium chloride precipitation affected IgM-ALA binding [see method details in Lobo et al. (2008a, 2015)]. In these studies we used purified polyclonal IgM to immunoprecipitate CD3, CD4, CCR5 and CXCR4 from lysates of cell lines and showed that the repertoire of IgM-ALA was different among individuals, especially patients, as exemplified with HIV patients (see Fig. 3). This finding demonstrating differences in the repertoires of IgM-ALA may in part explain the different clinical manifestations of inflammation among different individuals. Prior exposure to different infective agents or foreign antigens may provide a possible explanation for the observed differences in the repertoire of IgM-ALA among different individuals (Adib et al. 1990).

Addition of polyclonal human IgM to cultures of human peripheral blood mononuclear cells (PBMC) differentially inhibited co-stimulatory receptor upregulation, cytokine production, and proliferation of T cells (Lobo et al. 2008a). This was not observed with Waldenstrom's IgM lacking IgM-ALA. IgM obtained from both normal individuals and patients downregulated expression of CD4, CD2 and CD86 but not CD8 and CD28 on blood PBMC activated with alloantigens (MLR) (Lobo et al. 2008a). Additionally, physiological doses of polyclonal IgM obtained from either normal individuals or patients inhibited production of the same set of cytokines, i.e., TNF-α, IL13, and IL-2 but not IL-6 and chemokines when human PBMC were activated by alloantigens (Fig. 4e and Lobo et al. 2008a). Other investigators using a monoclonal IgM-ALA with TCR reactivity have shown that natural IgM can inhibit IL-2 production and T cell proliferation by binding to the TCR (Marchalonis et al. 1994; Robey et al. 2002). Similarly, we have shown that IgM can inhibit Zap-70 phosphorylation and T cell proliferation induced by CD3 ligation as well as T cell proliferation induced by alloantigens (Fig. 4f and Lobo et al. 2008a). Importantly, IgM inhibited T cell proliferation in the mixed leukocyte reaction (MLR) without altering Treg levels. Finally, we show that IgM can inhibit chemokine-induced chemotaxis of activated PBMC by binding to the receptor and blocking chemokine binding. However, we also show that IgM does not inhibit chemokine production (Fig. 4e) (Lobo et al. 2008a). In these studies, patient IgM had a more inhibitory effect when compared to normal IgM and these functional differences between normal and patient IgM may be explained by differences in their IgM-NAA levels and repertoire, especially since we used identical quantities of purified IgM in these in vitro studies (Fig. 3) (Lobo et al. 2008a). Other investigators have also shown that polyclonal human IgM can inhibit proliferation of human T cells (Robey et al. 2002; Vassilev et al. 2006).



In summary, we show that the quantity and repertoire of IgM-ALA varies in different individuals especially in disease. Additionally, we show that polyclonal IgM, in physiological doses, inhibits human T effector cell activation and proliferation and that, in addition, IgM regulates production of certain cytokines by binding to co-stimulatory molecules (CD4, CD3, TCR) and inhibiting Zap-70 phosphorylation. We also show that IgM does not inhibit Tregs. IgM-ALA do not

◂**Fig. 4** *Polyclonal IgM inhibits IFN-γ production and T cell proliferation and differentiation into TH1 and TH17 cells of murine splenic cells and specific pro-inflammatory cytokines from human leukocytes activated with alloantigens.* **a** Supernatant IFN-γ in 48 h culture media of splenic cells activated with a-gal-ceramide which specifically activates NKT-1 cells. IgM was added either 0.5 h before activation (IgM pre) or one hour post activation. **b**, **c**, **d** CFSE labeled WT-B6 splenocytes (2.5×10^5 in 0.5 ml media) were activated either in a one way MLR (using 7.5×0^5 BALB/c irradiated splenocytes) or LPS (350 ng) and soluble anti-CD3. Cells were cultured for 4 to 5 days. IgM (10–15 μg) was added at the initiation of culture unless otherwise indicated. In (**d**), the effect of Tregs was evaluated by co-culturing 2.5×10^5 CD45.1 WT-B6 splenic leukocytes, containing 1.8% CD4+ Foxp3+ cells, with 0.5×10^5 sorted CD45.2 WT-B6 Tregs (76% Foxp3+) under cytokine conditions favoring TH-17 differentiation. **e** Pooled human normal, ESRD, and HIV IgM but not Waldenstrom IgM significantly inhibit the increase in TNF-α and IL-13 but not that of IL-6, IL-8, MIG, and MCP-1 produced in response to alloantigen activation of T cells. Supernatants were obtained from day 5 MLR cultures stimulated in the presence or absence of pooled IgM (15 μg/ml), added on day 0. **f** Pooled human IgM inhibits anti-CD3 mediated Zap-70 phosphorylation of human T cells. IgM was added 30 min before anti-CD3/28 and cells were cultured overnight before quantitation by flow cytometry. Figures and legend reproduced with permission from J Immunol. 2012 Feb 15;188(4):1675–85 (Panels A to D) and J Immunol 2008 Feb1; 180(3):1780–91 (panel **e**, **f**). Copyright 2008 and 2012. The American Association of Immunologists, Inc

appear to affect the production of chemokines by leukocytes, but interferes with their action by binding to chemokine receptors.

4.3 The Function of Murine T Effector Cells, DC and NKT Cells but not Tregs Is Regulated by Binding of Polyclonal IgM to Specific Co-Stimulatory Receptors

Using murine splenic leukocytes, we observed a severalfold increased binding of IgM-ALA to live granulocytes, DC and B cells when compared to T cells and this binding occurred despite pronase digesting cells to remove FcμR. Furthermore, we showed that IgM could immunoprecipitate several different leukocyte receptors, thus indicating that the IgM bound to more than one receptor expressed on the cell membrane (Fig. 5d) (Lobo et al. 2012). However, IgM binding to all leukocytes was enhanced when cells were activated (see Fig. 5a). We therefore investigated if IgM had an inhibitory effect on the function of T cells, DC and NKT cells by binding to receptors, e.g., antigen presenting receptors and co-stimulatory receptors that are upregulated during activation.

The functional effect of physiological doses of IgM on murine T cells was examined in vitro studies using splenic cells. We showed that murine polyclonal IgM inhibited naïve T cells from differentiating into TH1 and Th17 cells (Fig. 4b, c, d), even when IgM was added 48 h after activation (Lobo et al. 2012). This inhibitory effect on T cells was independent of DC as the same inhibitory effect was noted when T cells were activated with insoluble anti-CD3/28. IgM, in addition, inhibits differentiation of Foxp3+ Tregs into TH17 cells. This is exemplified in

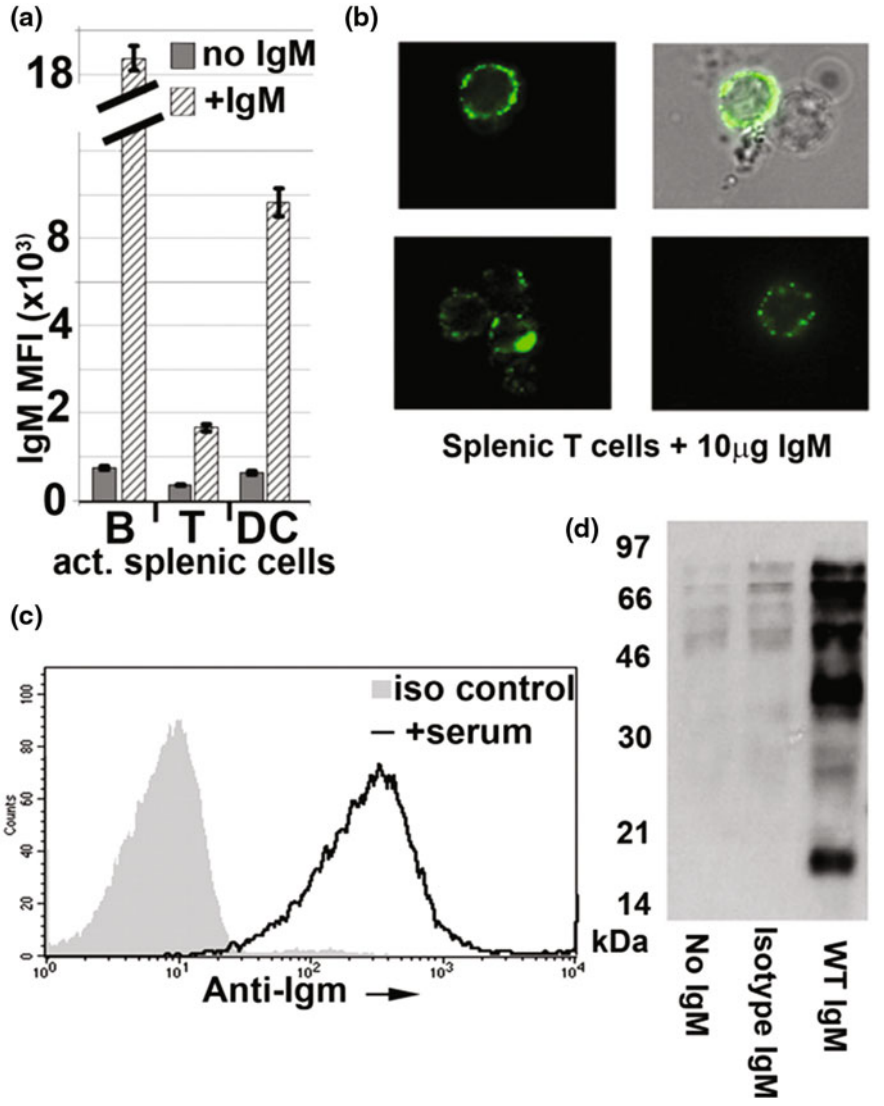


Fig. 4d where physiological doses of IgM significantly inhibited sorted Foxp3 + (CD45.2+) Tregs from losing Foxp3 expression and differentiating into TH17 effectors when co-cultured with splenic cells under cytokine conditions favoring TH17 differentiation (Lobo et al. 2012).

Since there are <1.5% DC in murine spleens, we used 7–8 day cultured murine bone marrow DC (BMDC) to investigate the functional effects of IgM on DC. We showed that polyclonal murine IgM, but not IgM pre-adsorbed with activated splenic leukocytes, bound to recombinant soluble CD40, CD86 and PD1 but not

◂**Fig. 5** *Polyclonal murine WT IgM binds to membrane receptors on leukocytes*. **a** Polyclonal IgM has severalfold increased binding to LPS-activated murine splenic B cells and dendritic cells (DC). Splenic leukocytes activated for 48 h were incubated with purified mouse IgM at 4 °C and evaluated for IgM binding using IgG anti-IgM (clone 11/41). Isotype monoclonal IgM with reactivity to KLH did not bind to activated leukocytes (data not shown). IgM binding to B cells was evaluated by blocking intrinsically expressed IgM with unlabeled IgG anti-IgM (clone 11/41). **b** depicts immunofluorescence microscopy images of IgM binding to cell membranes of splenic T lymphocytes. **c** compares binding of IgM and isotype IgM on CD3+WT B6 pronase-pretreated splenic leukocytes. Spleen cells were pronase digested to remove cell surface proteins including FcμR and to show that IgM-ALA can bind to other non-FcμR receptors on cell membranes. **d** depicts a representative example of a Western blot from two separate experiments demonstrating immunoprecipitation by WT polyclonal IgM of biotinylated membrane proteins from the murine macrophage cell line J77. In this experiment, WT polyclonal IgM is compared with an equal amount of isotype IgM that has no binding activity to leukocytes using flow cytometry. Figure and legend reproduced with permission from J Immunol 2015 Dec 1;195 (11), 5215–26 (panel **a**) J Immunol. 2012 Feb 15;188(4):1675–85 (Panels **b**–**d**). Copyright 2012 and 2015. The American Association of Immunologists, Inc

PDL-1, CD40L and CD80 indicating therefore that IgM-ALA has binding specificity to certain DC receptors (Lobo et al. 2015), just as we observed with human T cell receptors where IgM bound to CD4, CD3 and CD2 but not to CD8 (Lobo et al. 2008a). Additionally, IgM inhibited LPS-induced CD40 upregulation, but not upregulation of CD86, PDL1 and MHCII by BMDC and downregulated basal expression of PD1 on BMDC. IgM also downregulated LPS-induced p65NF-κB activation (Lobo et al. 2015) but not activation induced by LPS+ anti-CD40 (agonistic Ab), thus indicating that IgM can inhibit p65NF-kB upregulation mediated by TLR4 activation, but not when both TLR4 and CD40 are activated (Lobo et al. 2015). Interestingly, IgM inhibited TLR4 activation by a mechanism that did not involve inhibition of LPS binding to cell receptors (Lobo et al. 2015). There was however no decrease in IL12 production or increase in IL10 production when LPS-activated BMDC were pretreated with IgM, indicating therefore that LPS-induced production of IL12 and IL10 are not dependent on p65NF-κB (Lobo et al. 2015), In in vivo studies (described in the next section), we show that IgM pretreatment of LPS-activated BMDC switches these activated BMDC to a regulatory phenotype, which can inhibit innate inflammation induced by reperfusing kidneys following renal ischemia.

We next tested the effect of polyclonal IgM on Type1 NKT cell function. For these in vitro studies, we used α-gal-ceramide, a glycolipid that is taken up by DC and presented via the CD1d MHC Class I-like molecule to Type 1 NKT cells. Only Type 1 NKT, but not T effector cells, will secrete IFN-γ after the invariant TCR on Type 1 NKT recognizes α-gal-ceramide presented by CD1d. In these studies, physiological doses of IgM inhibited α-gal-ceramide induced IFN-γ production of splenic leukocytes, even when IgM was introduced 1 h after α-gal-ceramide (Fig. 4a) (Lobo et al. 2012). We have not yet defined the mechanism for the

Table 2 In-vitro effects of IgM-ALA on human and murine leukocytes

	T cells	NKT-1 cells	BMDC
Cell receptor binding	CD4, CD3, TcR, downregulates CD4, CD2; inhibits HIV entry	TcR, CD4	Binds CD86, CD40, PD-1
Intracellular signaling	Inhibits ZAP-70 activation		Inhibits LPS-induced NFκB activation
Pro-inflammatory mediators	Inhibits production of IFNγ, IL-17, TNFα, IL-2 but not IL-6, MCP-1	Inhibits production of IFNγ, not IL-4	No effect on IL-12 or IL-10
Anti-inflammatory mediators	Enhances production of IL-4, enhances T regs		Switches BMDC to regulatory phenotype (PD-1, IL-10 dependent)
Proliferation	Inhibits T cell proliferation (alloantigen and anti-CD3/28)		

inhibitory effect of IgM on Type 1 NKT function, but it is possible that IgM directly inhibits NKT-1 cells or DC presentation of α-gal-ceramide.

In summary (see Table 2), these in vitro studies indicate that IgM-ALA regulate leukocyte function by binding and downregulating certain leukocyte receptors (e.g., CD4 and CD2 on T cells, CD40 and CD86 on DC) and inducing regulatory DC function, possibly by downregulating NFκB. Physiological doses of IgM regulate leukocyte activation, proliferation and chemotaxis to attenuate excess inflammation (Fig. 4 and Lobo et al. 2008a). There are marked individual variations in the repertoire of IgM-ALA, with specificity for different leukocyte receptors, especially in disease states, and this could potentially explain the differences in the vigor and character of inflammatory responses in different individuals exposed to the same inciting agent. Additionally, there are differences in total levels of IgM-NAA or IgM-ALA as we observed in transplant recipients (Fig. 1) and this could also influence the inflammatory response. Finally, IgM-ALA, by binding to leukocyte receptors and inhibiting cell activation, can provide another mechanism to limit viral entry into cells and replication as we have shown with the HIV-1 (Lobo et al. 2008b).

4.4 Innate Immune Inflammatory Response in Renal Ischemia Reperfusion Injury (IRI) Is Inhibited by IgM-ALA

We used an in vivo murine model of renal IRI to test the inhibitory effects of IgM-ALA on DC and NKT-1 cells (Li et al. 2007). Renal vessels to both kidneys

are completely occluded with clamps for 26 or 32 min to induce either mild or severe ischemic renal tubular injury. After unclamping the blood vessels, the extent of renal injury or decrease in renal function is evaluated at 24 h after reperfusion by quantitating plasma creatinine, which increases as this substance is normally only removed by the kidneys. The initial ischemic injury in this model is insufficient to impair renal function, but it is the innate inflammatory response to products released (after reperfusion) by ischemic renal cells (e.g., DAMPS and glycolipids) that significantly worsen kidney injury, which leads to loss of function. DAMPS and glycolipids released by ischemic renal cells are taken up by DC and in the splenic marginal zone, DC present glycolipids in the context of CD1d to activate NKT cells, which rapidly release IFN-γ to activate innate effector cells, especially granulocytes, macrophages, and NK cells (Li et al. 2007). Chemokines released by ischemic cells enhance extravasation of activated innate effectors from the blood-stream into the kidney interstitium, where these effector inflammatory cells cause further renal tubular injury with loss of kidney function and an increase in plasma creatinine. This acute loss in kidney function is referred to as acute kidney injury (AKI).

Two approaches were employed to test the protective role of IgM in suppressing this renal ischemia-induced innate inflammatory response. First, we performed renal ischemia in B6/S4-IgMko mice (referred to as IgM KO) that lack circulating IgM but have normal levels of other immunoglobulins. Their normal functioning B cells express membrane IgM BCR but are unable to secrete IgM and these mice have normal or increased levels of Tregs, Bregs and IL10. We demonstrated that these mice are very sensitive to renal ischemia and develop AKI with mild ischemia (26 min clamp time), which is insufficient to cause AKI in their WT counterparts (Lobo et al. 2012). Administering 240 μg dose of polyclonal IgM intravenously, to achieve plasma levels similar to that in their WT counterparts, protected these IgM KO mice from developing AKI with mild ischemia, thus indicating that their sensitivity to ischemia resulted from a lack of circulating IgM (Lobo et al. 2012).

A single dose (150 μg) of purified polyclonal IgM was also administered intravenously to wild type C57BL6 (WT-B6) mice to increase levels of baseline circulating IgM by about 30–50%. In this second approach, increasing plasma IgM levels protected WT-B6 from severe renal ischemia (32 min clamp time) (Lobo et al. 2012). This protection was mediated by IgM-ALA, as administering similar quantity of polyclonal IgM pre-adsorbed with activated splenic leukocytes to remove IgM-ALA, failed to protect these WT-B6 mice from severe renal IRI (Lobo et al. 2012).

In these studies, physiological doses of polyclonal IgM mediated protection by decreasing the ischemia-induced innate inflammatory response as we observed a very minimal inflammatory response with no or minimal tubular injury in the protected kidneys. This protective effect of IgM-ALA could be mediated through several mechanisms including regulation of NKT and DC and maintaining or enhancing Tregs, which also mediates protection in this model of innate inflam-mation (Kinsey et al. 2009).

4.5 *Ex Vivo Induced Regulatory DC Are Protective in Renal Ischemia. Regulatory DC Require Tregs, B Cells, Circulating IgM and IL10 to Mediate in vivo Protection*

Since IgM-ALA bound to co-stimulatory receptors and downregulated CD40 and NFκB and had severalfold increased binding to splenic DC, when compared to T cells, we investigated the role of IgM-ALA in regulating DC in this model. In these studies, we used 7–8-day cultures of BMDC, which were activated ex vivo for 48 h with LPS with or without polyclonal IgM. After washing these activated BMDC, 0.5×10^6 BMDC were intravenously infused into mice 24 h before performing renal ischemia. We showed that IgM + LPS pretreated BMDC, but not LPS pretreated BMDC, protected mice from AKI by inhibiting the ischemia-induced inflammatory response that worsens kidney injury (Lobo et al. 2015). Importantly, IgM + LPS-activated BMDC protected kidneys only when IgM was present during the 48 h BMDC culture and not when IgM was added to BMDC at the end of the 48 h LPS activation, indicating therefore that regulation of BMDC by IgM is an active process requiring both NF-κB and CD40 downregulation induced by IgM (Lobo et al. 2015). Preventing downregulation of NFκB and CD40 by adding the agonistic anti-CD40 antibody to LPS + IgM during the 48 h BMDC activation negated the protective effect. These studies would indicate that NF-κB and CD40 downregulation are required to switch activated BMDC to a regulatory phenotype (Lobo et al. 2015). It is possible that binding of IgM to CD40 induces this regulatory phenotype.

In these studies, we needed to exclude the role of IgM anti-PC in mediating this anti-inflammatory effect, especially since there are 20–30% apoptotic cells in the ex vivo culture of IgM + LPS pretreated BMDC (Chen et al. 2009). Such a possibility seemed unlikely, as in prior studies mice were administered large numbers of apoptotic cells (2.5×10^7 thymocytes) to induce regulatory activity of in vivo antigen presenting cells (APC) (Chen et al. 2009), while in our studies, only 0.5×10^6 BMDC were used (Lobo et al. 2015). However, to exclude this possibility, we increased apoptosis in the ex vivo pretreated BMDC to >80% by subjecting activated LPS + IgM pretreated BMDC to UV irradiation. Such UV-irradiated apoptotic LPS + IgM pretreated BMDC failed to protect mice from ischemia-induced AKI, thus excluding the role of apoptotic cell/IgM complexes in inducing protection. These studies clearly demonstrated that IgM-ALA mediated protection by switching ex vivo LPS-activated BMDC to a regulatory phenotype. Regulatory BMDC required IL10 but not IDO (indoleamine 2,3-dioxygenase) as IgM + LPS pretreatment of *Il10* ko BMDC, but not *IDO* ko BMDC, failed to protect mice from developing AKI after renal ischemia (Lobo et al. 2015) In further studies, we also show that injected regulatory BMDC require the presence of other in vivo suppressive mechanisms such as circulating IgM, IL10, Tregs and B cells to mediate protection (Lobo et al. 2015).

In summary, both the in vitro and in vivo studies indicate that IgM-ALA inhibits the ischemia-induced innate inflammatory response by several mechanisms,

including switching activated DC to a regulatory phenotype, inhibiting NKT cell IFN-γ production and inhibiting chemotaxis of leukocytes by binding to chemokine receptors. However, IgM-ALA require the presence of other in vivo suppressive mechanisms such as IL10, Tregs and B cells to effectively inhibit the ischemia-induced inflammatory response. Conversely, these other in vivo suppressive mechanisms such as Tregs and Bregs also require IgM-NAA to effectively inhibit this innate inflammatory response as *Igm* ko mice that lack secretory IgM were sensitive to mild ischemia despite having normal levels of Tregs and Bregs (Lobo et al. 2015).

4.6 Inflammation Mediated by Adaptive Immune Mechanisms in Allograft Transplantation Is Inhibited by Polyclonal IgM

In these studies we used two approaches to test if IgM could inhibit allograft rejection. These studies were prompted by several prior observations including our clinical observations (Fig. 3) and the in vitro studies demonstrating that IgM (a) inhibited alloantigen-activated T cell proliferation and differentiation into Th1 and Th17 independently of DC (Fig. 4) and (b) induced regulatory function in DC (Lobo et al. 2012). First, cardiac transplants were performed intra-abdominally in *Igm* KO mice using B6-bm12 donor hearts, which are minimally incompatible at the MHC class II locus (Ia) with the recipient. In this transplant model, cardiac rejection occurs at >2 months in WT recipients as there is a mild chronic form of cellular rejection and a vasculopathy that is initiated by a T cell-mediated inflammatory process. However, in *Igm* KO recipients, graft loss occurred significantly earlier, i.e., at 2–3 weeks (Lobo et al. 2012). Additionally, there were considerably more TH17 cells infiltrating the cardiac allograft in the *Igm* KO recipients, despite no significant difference in infiltrating Tregs between the groups (Lobo et al. 2012). The observed histological findings on T cells mirror the in vitro studies, where IgM inhibited naïve T cells and Foxp3+ T cells from differentiating into TH17 cells without affecting levels of Tregs (Fig. 4d).

In the second approach, circulating levels of IgM in WT-B6 mice were increased by intravenous IgM injections to determine if higher IgM levels inhibited the severe and rapid rejection that occurs by day 5 in the setting of fully MHC-incompatible donor hearts (i.e., from BALB/c donors) (Lobo et al. 2012). 175 μg IgM was administered 24 h *after* ascertaining that cardiac surgery was successful, and the dose of IgM was repeated on days 3 and 5. Histological evaluation on day 6 clearly demonstrated that IgM markedly inhibited the severe inflammation in the cardiac allograft induced by rejection. This lack of leukocyte infiltration in the cardiac parenchyma of IgM-treated recipients was also associated with no or minimal CXCL1+ leukocytes and with no or minimal fragmentation of capillaries, as identified by the endothelial cell marker CD31 (Lobo et al. 2012).

In summary, these studies indicate that physiological doses (175 μg) of polyclonal IgM can abrogate inflammatory responses mediated by an adaptive immune mechanism. Potential mechanisms include (a) a direct inhibitory effect of IgM-ALA on T effector cells, but not Tregs. In vitro studies have shown that IgM can bind and down-modulate CD3/TCR and certain specific co-stimulatory receptors such as CD4 and CD2 but not CD8, and these mechanisms could be involved in inhibiting T effector cell proliferation and production of certain specific cytokines (e.g., TNF, IFN-γ, IL17, but not IL6, and chemokines) as well as in inhibiting their differentiation into TH1 and TH17 pro-inflammatory cells (b) by binding of IgM to CD40 and switching activated DC to a regulatory phenotype with downregulation of CD40 and p65NF-κB and (c) by inhibiting chemotaxis. It is highly unlikely that IgM anti-PC could have a significant role in inhibiting allograft rejection in our studies as we used small doses of polyclonal IgM (175 μg) and did not infuse large numbers of apoptotic cells (Chen et al. 2009).

4.7 Autoimmune-Mediated Insulitis in NOD Mice Is Inhibited by Polyclonal IgM

Insulitis in the NOD mouse is primarily mediated by autoimmune T cells but there is data to indicate that B cells are also involved (Kendall et al. 2004; Xiu et al. 2008; Ryan et al. 2010). Adoptively transferring CD3+ T cells from diabetic NOD mice can rapidly (<2 weeks) induce diabetes mellitus (DM) in young 4–5-week-old non-diabetic NOD mice thus indicating that T effectors are primarily involved in islet cell injury in this model. Because our in vitro studies demonstrated that polyclonal IgM inhibited T cell proliferation and differentiation into TH1 and TH17 cells (Fig. 4b, c, d), we performed studies to determine whether IgM could inhibit autoimmune insulitis that results in islet cell destruction and DM in NOD mice (Chhabra et al. 2012). Around 4–5 weeks after birth, these NOD mice develop a silent and non-destructive inflammatory process characterized by leukocyte infiltration of the perivascular and periductal regions in the pancreas as well as the peripheral islet regions and consisting of a heterogeneous mixture of CD4 and CD8 T cells, B cells, macrophages and DC (peri-insulitis). At 8–12 weeks of age, the immune infiltrate enters the islet areas and induces beta cell destruction (insulitis) and significant destruction first becomes evident around 12–13 weeks of age with mice exhibiting overt diabetes (DM).

In these studies, the effect of increasing IgM levels on development of DM was studied (Chhabra et al. 2012). At 5 or 11 weeks of age, NOD mice were administered bi-weekly intraperitoneal polyclonal IgM (50 μg/dose) and IgM was discontinued when mice were 18 weeks old. At 25 weeks of age, 0% of mice ($n = 30$) treated with IgM beginning at 5 weeks developed DM while 80% of control mice ($n = 30$) developed DM. Importantly, only 20% of pre-diabetic mice ($n = 20$) treated with IgM beginning at 11 weeks of age developed DM at 25 weeks of age.

At 18–25 weeks of age the pancreas revealed no or minimal insulitis in NOD mice treated with IgM beginning at 5 weeks of age. Other investigators using monoclonal polyreactive natural IgM in the neonatal period have also obtained similar results (Andersson et al. 1991, 1994). Importantly, despite discontinuing IgM at 18 weeks of age, the majority (73%) of NOD mice were protected from developing insulitis even when evaluated at 28 weeks of age, thus indicating that the anti-inflammatory effect of IgM also involves the induction of other regulatory mechanisms.

In summary, the beneficial effects of polyclonal IgM in inhibiting autoimmune insulitis could be mediated via several mechanisms including inhibition of autoimmune T effectors, blocking IgG autoantibodies via anti-idiotypic mechanisms and by inhibiting the B cells that produce them. Additionally, IgM, by switching activated DC to a regulatory phenotype and maintaining Tregs could enhance this protective effect.

5 Pathogenic Effects of IgM-NAA Under Non-physiological Conditions

In this section we will show how protective IgM-NAA can, under non-physiological conditions, become pathogenic and induce inflammation.

1.1 *Binding of IgM-NAA at cold temperatures can induce these antibodies to become pathogenic*: This is best exemplified in human kidney transplant recipients having high IgM-ALA and IgM anti-endothelial cell antibody (IgM-AEA) levels at the time of the kidney transplant. These recipients have a high incidence of delayed kidney graft function (DGF) (Lobo et al. 1984; Sturgill et al. 1984). DGF occurs when, after vascular anastomosis, warm blood is allowed to flow into a cold kidney. At cold kidney temperatures, binding of IgM-AEA to glomerular endothelial cells causes complement-induced glomerular endothelial cell injury. We show that this self-limiting injury can be prevented by warming the kidney prior to re-instituting blood flow. Such observations highlight the nature of IgM-NAA, i.e., their potential for complement mediated cytotoxicity under non-physiological cold conditions (Terasaki et al. 1970; Winfield et al. 1975; Lobo 1981).

1.2 *Binding of natural IgM to unmasked neoantigens can induce these antibodies to become pathogenic.* This is best exemplified by unmasking of the ubiquitous neoantigen "non-muscle myosin heavy chain type IIA and C (NMM)" after acute ischemia to the small bowel, skeletal muscle (hind limb) and heart in mice. About 1–2% of IgM-NAA B-1 cell clones in mice secrete IgM anti-NMM, probably to protect against NMM derived from infectious organisms (Zhang et al. 2004; Betapudi 2014). Injury in this murine model is predominantly mediated during

reperfusion by innate inflammation triggered by IgM binding to the unmasked NMM neoantigen and activation of complement (Austen et al. 2004; Zhang et al. 2004, 2006, 2008). Hence *Rag1* KO mice, which lack IgM-NAA, are normally protected from ischemia to the small bowel or hind limb but succumb to ischemic injury after infusion of polyclonal IgM or monoclonal IgM anti-NMM (Zhang et al. 2004, 2006). Additionally, in this murine model, one can observe binding of IgM and complement to NMM expressed by ischemic epithelial cells in the bowel or striated muscle cells in the hind limb (Austen et al. 2004; Zhang et al. 2004).

Interestingly, IgM anti-NMM mediated innate inflammation is not observed after renal ischemia, even though endothelial cells in murine glomeruli and peritubular capillaries express NMM (Arrondel et al. 2002; Renner et al. 2010). It is possible that NMM in the peritubular capillaries or in the tubules is not unmasked after ischemia. After renal IRI, one can detect increased IgM binding to glomeruli but not to the extensive network of NMM-containing capillaries that surround the outer medullary renal tubules where most of the ischemia-induced kidney injury occurs (Renner et al. 2010). Additionally, depleting B1 cells did not protect tubules from renal injury, but decreased glomerular injury, thus indicating that the tubular injury seen after renal ischemia is not mediated by natural IgM and complement (Renner et al. 2010). Other studies would indicate that the inflammatory response after renal ischemia is mediated by innate immune cellular mechanisms involving NK and NKT cells, which are activated by products (DAMPS) released by ischemic tubules (Li et al. 2007). Hence, unlike small bowel or hind limb ischemia, *Rag1* KO mice and *Igm* KO mice without secretory IgM are not protected from renal ischemia (Park et al. 2002; Burne-Taney et al. 2005; Lobo et al. 2012; Gigliotti et al. 2013; Lobo et al. 2015).

1.3 *Pathogenesis mediated by non-physiologic expansion of specific IgM-NAA clones*. This is best exemplified by hepatitis C-induced expansion of certain B1 cell clones that specifically secrete IgM-NAA that binds to self IgG, i.e., rheumatoid factor (RhF). Excess RhF production predisposes to formation of large circulating IgM/IgG complexes, referred to as cryoglobulins as these complexes precipitate ex vivo in the cold, which cause thrombosis of small blood vessels, especially in the kidney glomeruli and skin (Charles and Dustin 2009; Gorevic 2012). Patients are treated by plasmapheresis to remove cryoglobulins and agents to deplete B cells. There is no good explanation as to why expansion of RhF-secreting B1 cell clones is commonly seen after chronic hepatitis C infection and, in addition, we do not understand the normal physiological role of RhF, even though RhF was the first IgM-NAA to be discovered.

6 Conclusion

Figures 6 and 7 summarize our concepts regarding the inter-relationship between pathogens and natural antibodies. In both murine models and humans, the evidence shows that these polyreactive and low affinity binding IgM-NAA function under physiological conditions to (i) provide a first line of defense against invading microorganisms, (ii) protect the host from autoimmune inflammation mediated by autoimmune B2 and T cells that have escaped tolerance mechanisms, (iii) protect the host from endogenous oxidized neodeterminants and other neoantigens that are unmasked during tissue damage, and (iv) regulate excess inflammation mediated by both innate and adaptive immune mechanisms. The full repertoire of IgM-NAA develops during the first few years of life, but their levels and repertoire differ among healthy individuals, as well as in disease, and could contribute to the varying inflammatory response as, e.g., after an infection or alloantigen exposure. We hypothesize that infections maintain high protective levels of IgM-NAA, especially IgM-ALA and anti-PC (reviewed in Adib et al. 1990; Kearney 2000; Lobo et al. 2008a) and this could explain the significantly low incidence of autoimmune disorders such as SLE or sarcoidosis in rural parts of Africa, where malaria and other infections are endemic (Greenwood 1968; Lobo 1972; Jacyk 1984; Symmons 1995). Further support for this concept comes from a murine model of SLE, where malaria infection or purified IgM from malaria infected mice protected NZB mice from SLE-induced renal failure and death (Greenwood et al. 1970; Hentati et al. 1994). IgM-NAA have an important role in regulating inflammation even though there are other suppressive mechanisms (e.g., Tregs, Bregs, IL10, TGFβ). We show that IgM-NAA require Tregs, B cells and IL10 to effectively regulate inflammation (Lobo et al. 2015). Conversely, our studies and that of others, using mice deficient

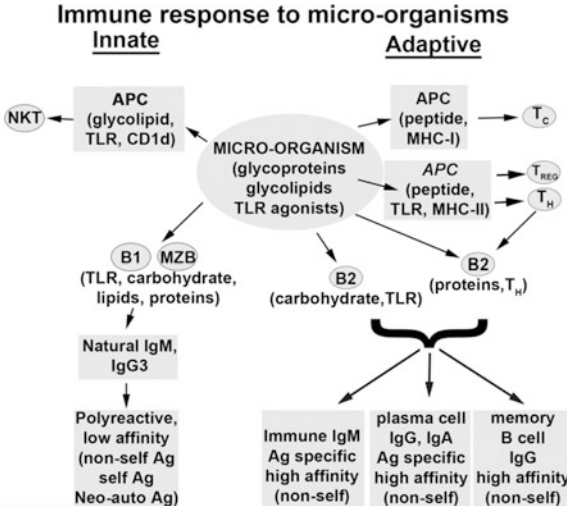


Fig. 6 Immune response to microorganisms

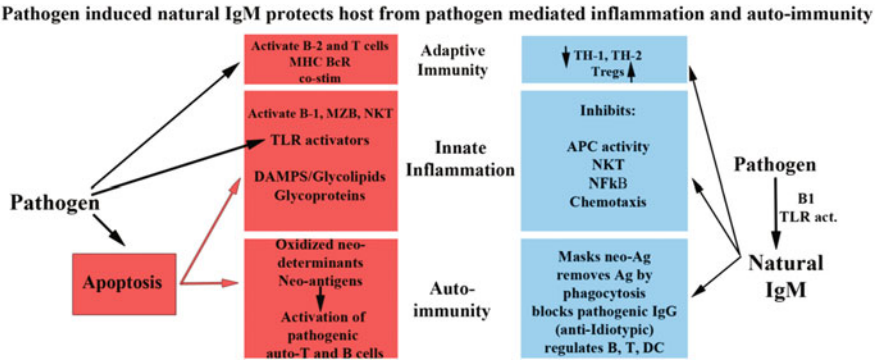


Fig. 7 Pathogen-induced natural IgM protects the host from pathogen-mediated inflammation and autoimmunity

in IgM secretion, would also indicate that Tregs and Bregs also require IgM-NAA to effectively control inflammation (Boes et al. 2000; Ehrenstein et al. 2000; Lobo et al. 2012). Understanding how diverse infectious agents increase IgM-ALA would help with development of a vaccine to increase IgM-NAA. We need to also determine if prolonged high IgM-NAA levels can induce excess immunosuppression that may be detrimental to the host. Cell therapy, especially with IgM pretreated DC, could provide an alternative approach requiring minimal quantities of IgM to prevent ischemic acute renal failure (e.g.,in high-risk patients undergoing cardiac surgery) or delayed graft function after renal transplantation (Lobo et al. 2015).

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