

Naturally occurring auto-antibodies in homeostasis and disease

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Antibodies with germline or close to germline configuration exist in vertebrates, and these so-called ‘naturally occurring auto-antibodies’ (NAb) are directed to self and altered self components. Such NAb have been attracting increasing interest because several of them, including some in their recombinant forms, have therapeutic potential. Whereas a large number of IgM and IgG NAb have tissue homeostatic roles, others modulate and regulate cellular and enzyme properties. This review describes some of these NAb and emphasizes how these low-titer, low-affinity NAb interact with self and altered self and show functional potency in homeostasis and regulation, in addition to in diseases such as infarction and systemic inflammatory response syndrome.

Introduction

Naturally occurring auto-antibodies (NAb) utilizing germline-encoded genes in the variable region (germline origin) directed against self, as well as altered self (for a review, see Ref. [1]), are generated in newborns in the absence of external stimulation [2] but the presence of the corresponding self structures [3] (Box 1). NAb first emerged in jawless fish, long before an adaptive immune response became possible [4]. Some have conjectured that NAb appeared in vertebrates to provide defense against pathogens [5]. An alternative proposal is that NAb might have emerged in now-extinct vertebrates that had acquired three germ layers and faced the difficulty of eliminating mesodermal and mesenchymal waste [6].

Soon after the existence of NAb was established by Avrameas in France and Notkins in the US (for reviews, see Refs [1,7]), steady progress has been made in characterizing NAb in terms of specificity, affinity and genetic configuration. Many IgM and IgG NAb are directed to intracellular constituents (for a review, see Ref. [6]). These have homeostatic roles in mediating clearance of such proteins upon their leakage from necrotic cells. Their functional roles have not been studied in great detail, presumably because their existence is compatible with the traditional understanding of self-tolerance. In contrast to this, NAb against plasma proteins and cell-surface-exposed structures have attracted more interest. One

reason is the increasing number of beneficial effects induced by pooled whole human IgG for intravenous application (IVIG) in treating patients with autoimmune diseases (Box 2). The occurrence of these NAb requires subtlety on the part of the immune system to prevent auto-aggression and the inappropriate recognition and clearance of self structures. Whereas some NAb bind to neoantigens (which occur, for example, on fibrillar forms of fragments from proteins [8]), others bind to self proteins and lipids upon structural changes induced by oxidative damage, enzymatic alterations and/or covalent interactions with altered lipids. Figure 1 illustrates three types of NAb involved in homeostatic processes, which will be discussed hereafter.

Homeostatic NAb to altered self

Clearance of body waste can be induced by a whole variety of eat-me tags, such as phosphatidylserine exposure, C-reactive protein (CRP)-dependent and direct complement binding (C1q), but NAb provide an additional, non-redundant means of clearance. In promoting waste removal, NAb also prevent adverse effects arising from accumulating waste that triggers adaptive immune responses culminating in autoimmune diseases, as is best known for systemic lupus erythematosus in animals lacking IgM NAb [9]. Likewise, effective NAb-dependent binding of oxidatively damaged structures prevents these structures from mediating pro-inflammatory effects (e.g. by activating endothelial cells and macrophages) [10].

IgM NAb in clearance of oxidatively damaged structures

Given the oxygen-rich environment in which we live, it is not surprising that lipid peroxidation products are ubiquitously present and emerge in physiological and pathological settings. The antigenic properties of these ‘oxidation-specific’ epitopes were first demonstrated in oxidized low-density lipoproteins (OxLDL), which are present in atherosclerotic lesions [11]. When the abundant phospholipid phosphatidylcholine (PC), which contains an oxidation-prone *sn*-2 polyunsaturated fatty acid, undergoes oxidation, highly reactive breakdown products such as malondialdehyde, 4-hydroxynonenal and the remaining ‘core-aldehyde’, 1-palmitoyl-2-(5-oxovaleroyl)-*sn*-glycero-3-phosphatidylcholine (POVPC), are generated [12]. These

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Box 1. Characteristics and proposed functions of natural autoantibodies

- Natural autoantibodies (NAb) are encoded by germline V genes. They are of the IgM isotype in lower vertebrates with restricted epitope specificity and functions, whereas in higher vertebrates, NABs are of IgM, IgG and IgA isotypes with broader functions.
- NABs recognize evolutionarily conserved molecules with an affinity for self antigen that ranges from 10^{-5} to 10^{-8} M. Many NABs are polyreactive [7], which does not mean non-specific [74]. NABs are interconnected and are complexed with anti-idiotypic IgM and IgG NABs [75]. By virtue of their capacity to recognize a wide range of antigens, NABs provide the first line of defense against microbial infections.
- NABs participate in the clearance of aging cells, cellular debris, and altered self on cells and plasma components by opsonization and recruitment of complement and probably by proteolytic activity. NABs play an important part in anti-tumor surveillance, probably through binding to repetitive motifs of carbohydrate epitopes.
- Under physiological conditions, NABs exert an anti-inflammatory activity via binding to oxidatively damaged structures or neutralizing proinflammatory cytokines, as is best illustrated by the beneficial effects of IVIG in several autoimmune and inflammatory diseases. In higher vertebrates, NABs exist that modulate and regulate cellular and enzyme functions by binding as ligands. NABs participate in the selection of immune repertoires and maintenance of immune homeostasis. For example, NABs are believed to facilitate the function of antigen-presenting cells.

aldehydes form covalent adducts with amino groups of proteins and lipids and are recognized by specific NABs [13] (Figure 1a).

IgM antibodies (Abs) against oxidation epitopes are found in normal mice, but their levels are many-fold higher in cholesterol-fed LDLR^{-/-} mice and apoE^{-/-} mice with extensive atherosclerosis. This enabled the generation and characterization of a panel of IgM mAbs specific for oxidation epitopes, cloned from the spleens of these non-immunized mice. One example is the prototypic anti-

Box 2. Intravenous immunoglobulin (IVIG)

Commercial preparations of pooled human immunoglobulin for intravenous application (IVIG) contain primarily IgG and traces of IgA and IgM from plasma of tens of thousands of healthy blood donors. Thus, IVIG contains a broad range of immune antibodies and the whole set of IgG NABs. Nevertheless, IVIG does not just represent the sum of IgG NABs and Abs from these donors; it is enriched in IgG dimers, and multimers (which can have adverse effects) are largely eliminated. The dimeric fraction consists primarily of idiotype-anti-idiotypic pairs [75] that presumably form more efficiently between antibodies from distant donors than with autologous ones in purified IgG and plasma of individuals. IVIG originally was developed to treat primary immune deficiencies. Nowadays, it is primarily used to treat a variety of autoimmune diseases. IVIG exerts long-lasting beneficial effects in many of these diseases by several mechanisms [70,71], including:

- Blockage of Fc receptors on macrophages;
- Induction of inhibitory Fc gamma receptor IIB;
- Neutralization of pathologic autoantibodies by anti-idiotypic NABs;
- Attenuation of complement overreaction by NABs interacting with C3 convertase;
- Activation of NK cells via Fc gamma receptor III, which, in turn, mediate an antibody-dependent killing of IVIG-carrying dendritic cells, whereby T-cell priming is suppressed and auto-immunity inhibited [76];
- Expansion of certain regulatory T cells [77].

OxLDL IgM mAb EO6, which specifically binds the phosphorylcholine head group of oxidized phospholipids, such as POVPC, but not of native PC [14] (Figure 1a). The sequence of EO6 mAb is of germline origin and is identical to that of the previously described T15 mAb, which binds to phosphorylcholine covalently linked to the pneumococcal cell-wall polysaccharide (for a review, see Ref. [13]). Immunization of LDLR^{-/-} mice with heat-killed phosphorylcholine-containing pneumococci led to high titers of anti-OxLDL IgM Abs that were predominantly of the T15 clonotype and reduced atherosclerotic lesion formation significantly [15]. Likewise, passive infusion of the T15 IgM NAb significantly reduced lesion formation in transplanted vein grafts of apoE^{-/-} mice [16]. Thus, T15 or EO6 IgM NABs seem to mediate this atheroprotective effect by their capacity to bind OxLDL, thereby neutralizing its pro-inflammatory effects and inhibiting the scavenger-receptor-mediated uptake of OxLDL by macrophages [14], which otherwise results in detrimental foam-cell formation in atherosclerotic lesions.

Many epidemiological studies assessed the association of IgM and IgG Ab titers against OxLDL with surrogate markers of cardiovascular disease or clinical endpoints, but there is still some controversy regarding their exact role (for a review, see Ref. [13]). However, increasing evidence points to the notion that IgG isotypes against OxLDL are associated with pro-atherogenic properties, whereas IgM isotypes against OxLDL are associated with atheroprotective properties. For example, the levels of IgM anti-OxLDL Abs inversely correlated to the extent of coronary stenosis (narrowing of a blood vessel) [17]. Likewise, high levels of IgM Abs to phosphorylcholine were found to predict decreased carotid atherosclerosis in hypertensive patients [18].

IgM NABs in clearance of dying cells

Clearance of apoptotic cells is impaired in mice lacking IgM. In fact, clearance of apoptotic thymocytes requires classical complement pathway activation by IgM [19]. The same was found for apoptotic Jurkat cells *in vitro* [20]. These authors demonstrated that human IgM binds to apoptotic cells by interacting with the phosphorylcholine group on exposed lysophosphatidylcholine. Thus, exposure of altered self on apoptotic cells differs from that observed on oxidatively damaged LDL and is dependent on intracellular activation of a phospholipase A2 [20] (Figure 1b). The generated lysophosphatidylcholine diffuses to the extracellular side and evidently exposes the phosphorylcholine group in a conformationally different arrangement to that of intact PC. Though the phosphorylcholine groups of lysophosphatidylcholine and that of protein-bound POVPC are in different contexts, the phosphorylcholine-specific T15 IgM mAb was able to reconstitute complement-dependent clearance of apoptotic cells in IgM-deficient mice when apoptotic cells were pre-incubated with 20 µg/ml as compared to 200 µg/ml murine IgM [21]. This is in line with the fact that apoptotic cells also contain oxidation-specific epitopes, as suggested earlier on the basis of phagocytosis inhibition studies, by 50 times higher concentrations of such mAbs in the absence of complement [22]. The finding, however, does not exclude that yet other IgM NAB(s) exist

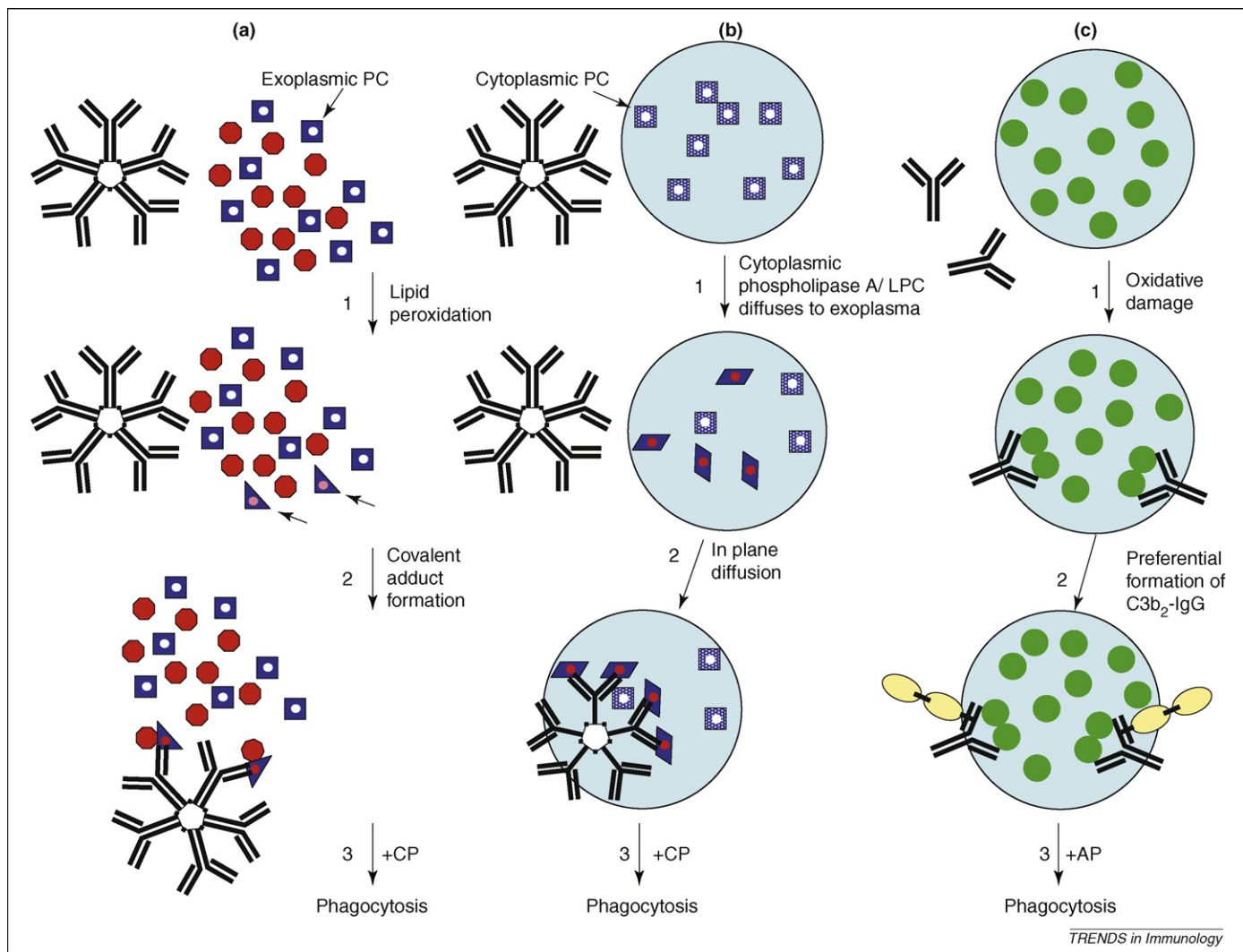


Figure 1. Possible modes of how homeostatic NAb recognize altered self and mediate clearance of waste products. **(a)** Interaction of IgM NAb with oxidation-specific epitopes on cells and particles. Red symbols represent a protein (e.g. LDL), blue symbols represent exoplasmic phosphatidylcholine (PC) associated with an unsaturated fatty acid, and the white circle within PC illustrates the 'hidden' phosphorylcholine headgroup. (1) A few PC molecules undergo lipid peroxidation and expose reactive aldehydes (blue rectangles become triangles with the phosphorylcholine head partially altered). (2) Some of the proteins react covalently with aldehydic groups of oxidized phospholipids and thereby generate neoantigens in which the phosphorylcholine head group is exposed (red in bound triangle). (3) Multivalent binding by IgM NAb to phosphorylcholine residues in the context of the bound protein activate classical complement pathway (+CP) sufficiently to opsonize the particles for efficient clearance by phagocytes. **(b)** Recognition of apoptotic cells by IgM NAb (blue patterned squares represent cytoplasmic PC): (1) some PC molecules are cleaved by phospholipase A2 to lysophosphatidylcholines that diffuse rapidly to the exoplasmic side and expose their phosphorylcholine headgroups (blue patterned rectangles become blue rhomboids exposing the red phosphorylcholine exoplasmically); (2), phosphorylcholine-specific IgM NAb bind multivalently to rearranged lysophosphatidylcholine; and (3) classical complement pathway activation by bound IgM (+CP) is sufficient to opsonize the cells for efficient clearance by phagocytes [(see part 3 of (a))]. **(c)** Opsonization of senescent red blood cells (RBC) by IgG anti-band-3 NAb (green circles represent band 3 protein that is abundant on RBC surface and is mostly anchored): (1) oxidative damage combined with alterations in anchorage induces oligomerization of some of these antigens, enabling IgG NAb to bind firmly by bivalent binding to oligomers of band 3; (2) IgG anti-band 3 NAb that have an affinity for C3 within their framework enable nascent C3b to deposit in dimeric form (yellow symbols); and (3) dimeric C3bs on bound NAb act as potent precursors of amplifying C3 convertases (+AP), whereby C3b deposition is greatly enhanced mediating an IgG- and complement-dependent clearance.

that specifically recognize lysophosphatidylcholine in its context at physiological concentration in the ng to μ g per ml range and might even differ in the ability to recruit complement, an aspect that has not been studied yet.

IgM NAb in tumor surveillance

Malignant cells are often characterized by changes in the expression of certain proteins and their glycosylation pattern that are characteristic of their malignant transformation. Under certain circumstances, carbohydrate antigens on glycolipids and glycoproteins can constitute another 'neo-self' pattern that is recognized by NAb on malignant, but not normal, cells [23]. Most of these NAb are IgMs that are of germline origin and seemingly play a part in tumor

immunosurveillance [24]. As is the case for oxidation-specific epitopes, the repetitive character of the carbohydrate epitopes also enables a high-avidity binding of multivalent IgM NAb to their targets. This enables efficient complement activation and destruction of the target cells. However, this is often counteracted by the presence of tissue complement regulatory factors. Efficient IgM NAb share the ability to induce tumor cell apoptosis. The monoclonal IgM SAM-6, which was originally isolated from a gastric cancer patient [25], specifically recognizes an O-linked sugar on glucose-regulated protein 78 (a member of the heat-shock protein 70 family) on malignant but not normal tissue [25]. Interestingly, SAM-6 induces tumor cell apoptosis *in vitro* by inducing a toxic intracellular lipid

accumulation, in particular in the presence of oxidized lipids [26]. Other examples include PAM-1 that binds to tumor-specific N-linked carbohydrates on cysteine-rich fibroblast growth factor receptor-1 of human epithelial tumors and even pre-cancerous lesions. The mAb SC1, isolated from a patient with signet cell carcinoma, specifically binds to N-linked glycosyl residues on an 82 kDa isoform of CD55. The mAb SC1 induces engulfment of this GPI-anchored molecule and apoptosis [27]. This isoform of the decay-accelerating factor (CD55) is expressed on several tumors. All these NAbs hold promise not only for histopathological purposes but also for passive immunotherapy as adjuvant cancer treatment.

The finding that all these tumor-related epitopes are carbohydrates, which are typically poor antigens, is a major limitation for active immunization strategies. To overcome this limitation, one approach has been to use as an immunogen a paratope-specific anti-idiotypic NAb that mimics tumor-specific carbohydrate residues, such as sialyl-glycolyl gangliosides, which are expressed on several types of tumors [28]. Murine anti-idiotypic antibodies were generated against a murine mAb P3, which specifically binds sialyl-glycolyl gangliosides. One of these anti-idiotypic Abs (1E10) has germline configuration and belongs to the V(H)J558 and V κ 10 gene family. Subsequently, non-small-cell lung cancer patients were hyperimmunized with this anti-idiotypic Ab and successfully induced Abs that recognize the same gangliosides as the P3 mAb [28,29]. In fact, those patients who elicited anti-sialyl-glycolyl antibodies showed a longer survival. Thus, anti-idiotypic vaccination approaches might provide intriguing strategies to induce therapeutic, tumor-specific immune responses.

IgG and IgM NAbs in Alzheimer's diseases

Many neurodegenerative disorders are characterized by the aggregation and deposition of misfolded proteins or peptides leading to inflammation and neurological dysfunction. Generation of misfolded proteins does not just occur in neurodegenerative disorders but occurs throughout our lifetime. NAbs that target patterns generated by such misfolded fibrillar or aggregated proteins have evolved. These IgG NAbs bind up to 200 times better to the fibrillar than to the monomeric form of such peptides, as shown for light-chain-derived peptides, α -synuclein and β -amyloid [8], to which NAbs are characterized extensively [30]. They recognize both conformational and linear epitopes and have been shown to prevent *in vitro* β -amyloid oligomer-induced cell death of N2A neuroblastoma cells [31]. In fact, anti- β -amyloid NAbs are present in IVIG preparations, and pilot studies in which IVIG was infused in patients suffering from mild Alzheimer's disease have shown an improvement of the patients' mental score [30]. In addition, IgM NAbs with the ability to recognize and hydrolyze β -amyloid have been isolated from a patient with Waldenström's macroglobulinemia [32].

IgG to oxidatively stressed and senescent red blood cells

As mentioned above, NAbs of the IgG isotype occur in higher vertebrates, and this has minimized the chances for cross-reactions with unrelated antigens that can occur with IgM antibodies and cell-exposed antigens, owing to

their ten binding sites. However, bivalent binding of IgG NAbs provides only a minimal gain in avidity to overcome low affinity. Moreover, IgG antibodies are less effective activators of the classical complement pathway than IgMs and require, aside from complement receptors, Fc receptors on phagocytes. Thus, the recruitment of IgG NAbs to cell-exposed antigens for homeostatic purposes in higher vertebrates called for additional means to enhance opsonization. So far the best studied are IgG NAbs to human red blood cell (RBC) band 3 protein (an anion transport protein), which mediates clearance of oxidatively stressed and senescent RBCs (for a review, see Ref. [33]). These NAbs overcome their low affinity to native band 3 protein by bivalent binding to oligomerized band 3, as generated on oxidatively stressed and senescent RBCs, in part by intracellular SS bond formation [34,35] (Figure 1c). Bound anti-band-3 NAbs further compensate for their low numbers and low affinities by preferentially generating C3b₂-IgG complexes in the presence of active complement [36]. Because of a weak affinity for C3 within their framework (for a review, see Ref. [33]), nascent C3b binds in dimeric form, which is a very potent precursor of the amplifying C3 convertase [37]. This convertase generates C3b in amounts exceeding that which the classical complement pathway is able to form [33]. Thus, IgG NAbs such as anti-band-3 NAbs and, for example, those to rabbit RBCs, which stimulate complement amplification even when the classical complement pathway is blocked (Mg²⁺-EGTA serum), have extra potency (for a review, see Ref. [38]).

NAbs modulating cell functions

Oligodendrocytes

It has been demonstrated that immunoglobulins from mice injected with homogenized spinal cord induced remyelination in syngeneic mice treated with Theiler's encephalomyelitis virus, a model for multiple sclerosis (MS) [39]. A mAb generated from a similarly treated mouse also induced remyelination (mAb SCH94.03) and was an IgM NAb of germline origin [40]. In an attempt to obtain a human mAb with similar properties, a panel of IgM molecules was purified from sera of patients with monoclonal gammopathies, among which two induced remyelination in mice (sHlgM22 and sHlgM46) [41]. Recently, a recombinant form of HlgM22 (rHlgM22) was shown to induce myelin repair three times better than controls within five weeks after a single peritoneal injection [41]. This and previously studied IgM NAbs cross the blood-brain barrier, tag demyelinated lesions within the brain and induce retraction of >80% of lesions in the spinal cord [42]. HlgM22 exerts its effect by suppressing apoptosis via binding to lipid rafts on oligodendrocytes and cross-linking unknown surface lipids and protein molecules. The recombinant form of this IgM NAb, therefore, has therapeutic potential for treating MS patients.

Dendritic cells

Several types of NAbs modulate the functional properties of dendritic cells. One such NAb, which was isolated from the serum of a patient with Waldenström's lymphoma, was found to deviate by 14 and 6 amino acids from the closest known germline variable genes [40]. Nevertheless, this

IgM Ab (called sHlgM12) cross-links the co-stimulatory molecules B7-DC that are involved in T-cell activation on mouse and human dendritic cells. Its transient binding matures dendritic cells without them losing the ability to become primed by an antigen and to activate naïve T cells 10–100-fold more efficiently [43]. Thus, whether mice were treated with sHlgM12 before or even after B16 melanoma tumor engraftment, the treatment had a protective, T-cell-mediated effect against the tumor. Moreover, the killing of tumor cells was antigen specific and involved cytotoxic T cells. Dendritic-cell-modulating NAb have opened up a promising approach for tumor immunotherapy.

Whereas the antibodies discussed above polarize the maturation of dendritic cells towards a Th1-type immune response, other NAb participate in differentiation of dendritic cells towards a Th2-type T-cell stimulation. Because this type of immune response is impaired in patients with X-linked agammaglobulinemia (XLA), who suffer from an increased susceptibility to bacterial infections, maturation of dendritic cells from such patients (having low or no circulating immunoglobulins) has been studied [44]. Monocyte-derived dendritic cells from blood of XLA patients cultured with granulocyte monocyte colony-stimulating factor and 10% autologous plasma revealed a reduced expression of several DC-specific surface molecules (predominantly CD80 and CD86) [44]. Supplementation of the autologous plasma with physiological concentrations of IgG from IVIG normalized differentiation of these dendritic cells [44], as well as those derived from patients with common variable immunodeficiency [45]. The same potentiating effect could be achieved by physiologic concentrations of IgG NAb affinity purified from IVIG on a CD40 peptide, implying that anti-CD40 NAb are responsible for this cell maturation [44]. Dendritic cells treated with this NAb induced CD4⁺ T-cell proliferation, but not to the extent observed with CD40 ligand and via a different signaling pathway. Note, however, that IVIG added at therapeutic concentrations inhibits differentiation, maturation and function of dendritic cells [46], illustrating that IVIG is not simply the sum of all IgG NAb.

B and T cells

NAb that modulate the B- and T-cell immune responses have been known for some time in sharks, mice and humans [47]. T-cell-receptor-specific IgM NAb from sharks and humans, affinity purified on a single-chain T-cell-receptor peptide (generated from V β 8.1), bind to exactly the same overlapping 16-mers [47]. These TCR-specific IgM NAb significantly inhibit activation of antigen-primed T-cells as measured by IL-2 production, when added to *in vitro* culture in the range of 1–20 μ g/ml. Other types of IgM NAb to leukocytes (IgM-ALA) were enriched from rheumatoid-factor-depleted IgM of healthy humans and patients with end-stage renal disease by binding to CD4⁺ and CD8⁺ T cells [48]. As a typical IgM NAb, anti-leukocyte IgMs are already present in umbilical cord serum. IgM-ALA NAb inhibit T-cell activation and chemotaxis and immunoprecipitate CD3, CD4 and two chemokine receptors [48].

Anti-leukocyte IgM has also attracted interest because kidney recipients whose plasma showed high IgM binding

to T cells of the kidney donors had excellent graft survival, whereas a majority of patients having no accessible IgM-ALA NAb lost their graft within one year [48]. In addition to IgM-ALA NAb, IgG anti-F(ab')₂ antibodies, which comprise anti-idiotypic NAb and anti-hinge NAb, also exert beneficial effects in kidney transplants. Pretransplant IgG anti-F(ab')₂ NAb were significantly higher in patients with immediate kidney function and low creatinine after one year than in those who had graft failure after one year [49]. Hence, kidney graft survival is, indeed, modulated by IgM-ALA NAb that dampen T-cell activation and by anti-idiotypic NAb that keep antibodies complexed.

IgM-ALA NAb have yet another beneficial effect because their binding to CD4 prevents HIV-1 virus from attaching to leukocytes and reduces infectivity by 95% *in vitro* and in severe combined immunodeficiency mice containing human peripheral lymphocytes [50]. The IgM-ALA NAb as characterized so far are a heterogeneous group of NAb, which can vary in their receptor specificity and concentration. It remains to be investigated whether the broad and variable specificity of these IgM NAb might, in part, be due to the use of sera that were heat inactivated, a process that favors immunoglobulin complexation, as is known from ELISA studies on other targets. Humans also have IgG NAb that are protective against R5-tropic HIV-1 virus and specifically bind to the chemokine receptor 5 expressed on monocytes and T cells [51]. IgG NAb, affinity purified from IVIG on an N-terminal peptide of chemokine receptor 5, have been shown to inhibit infection of macrophages and lymphocytes with the R5-tropic strain of HIV-1.

Stimulated B cells produce Ig when the appropriate antigen is bound to the B-cell receptor. An immune response in humans peaks and is subsequently downregulated by IgG anti-hinge NAb, which increase during an immune response (for a review, see Ref. [52]). Mice, which do not produce IgG anti-hinge NAb [53], lack this type of B-cell regulation but upregulate IgM rheumatoid factors (i.e. anti-Fc antibodies) during a secondary antigen challenge [54]. In humans, IgG anti-hinge region NAb seem to bind with their Fc portion to the Fc receptor on B cells and at the same time via their Fab portion to the antigen-bound membrane Ig, whereby they exert an inhibitory effect on B-cell activation [52] (Figure 2). Thus, IgG anti-hinge NAb, known to bind to F(ab')₂ (which lack the Fc portion) but not to fluid-phase IgG, also bind to membrane Ig, when this is conformationally altered by bound antigen.

Proteolytic NAb with homeostatic roles

Certain IgA, IgM and (less so) IgG NAb can hydrolyze model tripeptide and tetrapeptide substrates [55]. Pooled human and mouse IgM and several mouse monoclonal IgMs hydrolyze peptides similar to serine proteases by nucleophilic attack of the substrate, as verified using phosphonate diesters [55]. Natural IgAs isolated from the blood and saliva of healthy humans catalyzed the cleavage of model peptide substrates with efficiency considerably superior to that of IgG NAb. This observation highlights the IgA compartment of the humoral immune response as a source of natural catalysts. In fact, IgA purified from the saliva of humans without a preceding

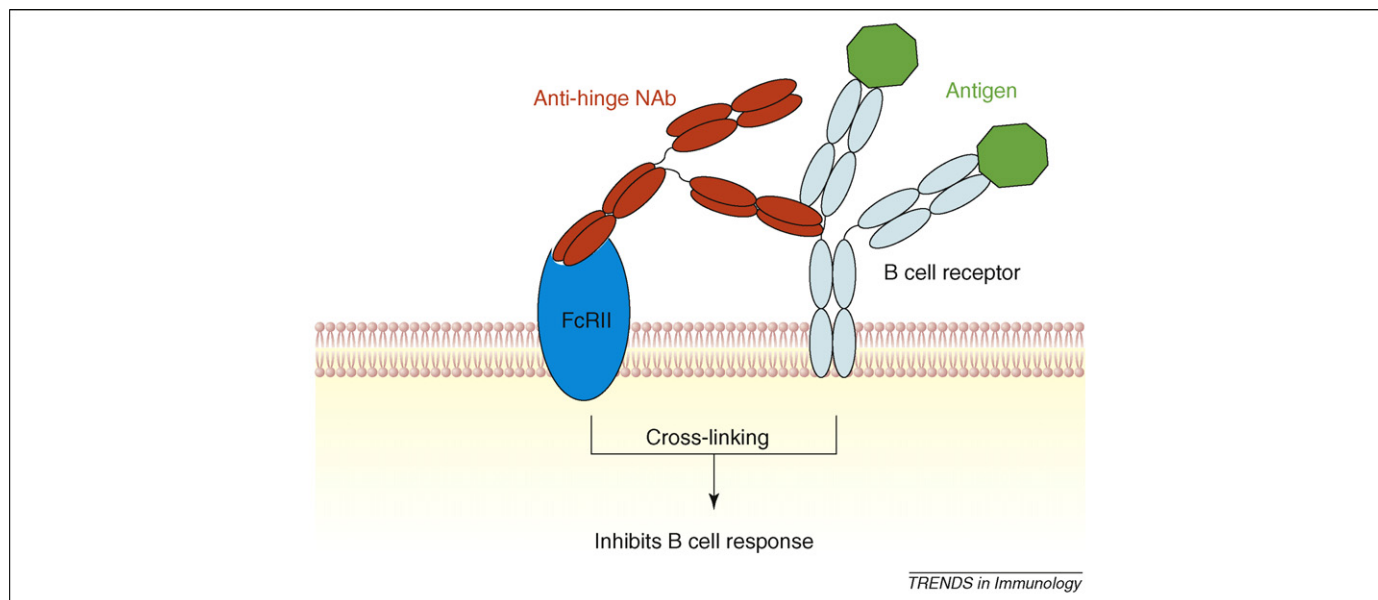


Figure 2. Putative mechanism of how IgG anti-hinge NAb inhibits the B-cell activation. Anti-hinge NAb binds via their Fab to the hinge region of antigen-complexed B-cell receptor and with their Fc portion to FcRII [52].

HIV infection contains NAb that specifically bind to a largely conserved region within gp120 of the HIV coat protein (comprising residues 421–433) and catalyze its cleavage [56]. Moreover, a peptide comprising the aforementioned amino acid residues not only blocked IgA-mediated proteolysis but also significantly reduced *in vitro* infection of peripheral blood mononuclear cells by HIV virus [56]. This indicates that these IgA NAb might have potential as a topical microbicide. Despite their lower catalytic activity [56], serum IgM and IgG NAb to these conserved epitopes in gp120 might also contribute to resistance against HIV infections [57].

Another group of catalytic antibodies is directed to the procoagulant factor VIII. About 10–30% of patients with congenital hemophilia A develop allo-antibodies against factor VIII (FVIII) after replacement therapy with exogenous FVIII [58]. Furthermore, auto-antibodies in patients with autoimmune hemophilia (acquired hemophilia) also display FVIII hydrolytic activity [59]. The two types of anti-factor-VIII antibodies share similar properties with respect to isotype, epitope specificities and mechanism of inhibition. Nevertheless, it remains to be investigated whether these IgG antibodies emerge from a true autoimmune or alloimmune reaction or are recruited from natural factor-VIII-hydrolyzing IgG-producing B cells [59]. Interestingly, high levels of IgG that present with hydrolytic activity directed against FVIII seem to correlate with survival from a systemic inflammatory response syndrome (SIRS) in severe sepsis [60]. Although the data indicate that these catalytic IgG molecules might participate in the control of thrombosis in the course of SIRS, it remains unclear whether the high titers of anti-FVIII pre-existed as NAb in some of these patients or were up-regulated during sepsis.

Catalytic antibodies have mostly been reported for pathological conditions; thus, it remains unclear whether catalytic antibodies might have been ‘induced’ by the antigen implicated in the disease or resulted from the loss of

repressive control over catalytic antibody-producing clones that were generated spontaneously under physiological conditions. The fact that injection of transition-state analogs induces catalytic antibodies in normal mice but ten times more in autoimmune-prone mice [61] indicates that the ability to generate catalytic antibodies is physiological.

Disease-promoting effects of NAb

Although NAb are *a priori* beneficial and can alleviate chronic diseases such as atherosclerosis, some NAb can promote diseases induced by a sudden impact such as in infarction and/or SIRS, two conditions that did not exert an evolutionary pressure.

Ischemia-reperfusion injury

The acute inflammatory response as it occurs in infarctions and ischemia-reperfusion injury models involves not only neutrophil infiltration and complement activation but also NAb and was first observed in ischemic skeletal muscle [62]. Hypoxia and pH changes lead to exposure and release of normally hidden autoantigens. Vertebrates possess a variety of IgM and IgG NAb against such proteins, presumably to clear them when accidentally liberated. During ischemia-reperfusion, however, a sudden release of large amounts of such autoantigens and the increased contact with NAb during reperfusion results in complement activation, involving both classical and lectin pathway initiation. However, a large portion of complement activation is apparently due to complement amplification because damage to intestinal cells was reduced significantly in factor D knockouts subjected to ischemia-reperfusion [63]. Surprisingly, from a study involving 21 hybridoma-producing NAb, a single type of IgM (specific to non-muscle myosin H chain type II) triggered intestinal injury in RAG-1^{-/-} mice, which lacked Ig and were otherwise resistant to reperfusion injury. This NAb bound to intestinal antigen and initiated complement activation in an apparently C1q-independent manner but attracted

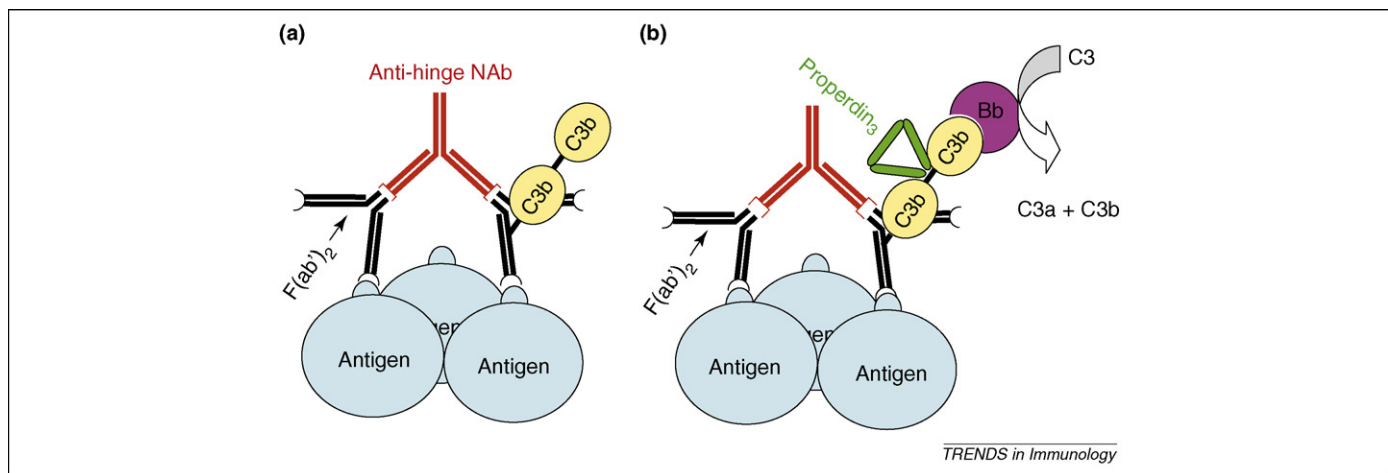


Figure 3. How secondary immune complexes comprising F(ab')₂, unknown antigens and anti-hinge NABs stimulate complement amplification. **(a)** Binding of anti-hinge NABs to F(ab')₂-containing immune complexes (ICs) generates secondary ICs in which one shortened heavy chain of F(ab')₂ is rigidified by bound anti-hinge NAB on one end and by antigen on the other end. This rigidification provides sufficient conformational stability for the short-lived, nascent C3b to bind covalently as a dimer, as shown *in vitro* [69]. **(b)** C3b₂-containing complexes have a half-life of 3–4 min in 20% plasma, as verified on artificially generated C3b₂-IgG complexes (for a review, see Ref. [38]). These complexes stimulate complement amplification seven to ten times better than bound C3b because they first bind the oligomeric properdin that enhances factor B binding during the course of C3 convertase assembly [37].

mannan-binding lectin and, hence, triggered the lectin pathway [64]. IgM complexed to non-muscle myosin probably initiated intestinal injury because peptides mimicking portions of the antigen blocked reperfusion injury in reconstituted RAG-1^{-/-} and even wild-type mice [65].

The IgM NAB described above is certainly not the only substance mediating ischemia-reperfusion injury; in analogous experiments on rats, endogenous CRP and IgM anti-phosphorylcholine NABs were shown to deposit on intestinal tissue after mesenteric ischemia-reperfusion [66]. In addition, human IgG autoantibodies to DNA, histones and phospholipids (reviewed in [67]), as they occur in patients with certain autoimmune diseases, can restore injury in the heterologous RAG-1^{-/-} mice.

SIRS

SIRS can develop from severe sepsis but can also develop in patients with trauma that have no infections. Although it is known that an excessive complement activation induces paralysis of neutrophils, the cytokine storm of macrophages and the release of tissue factor in SIRS [68], it has been unclear how excess complement activation is induced. Early in an inflammatory process, proteases are released from pathogens and activated neutrophils, which can cleave nearby endogenous IgG into F(ab')₂-like fragments. Such fragments from pre-existing and induced IgG antibodies can form immune complexes (ICs) with released autoantigens and pathogens and persist because they lack the Fc portion or parts of it. F(ab')₂-containing ICs, but not IgG-containing ICs, can form secondary ICs with IgG anti-hinge NABs that have a high affinity for the exposed hinge region of F(ab')₂ fragments, normally required in B-cell regulation [52]. Such secondary ICs – comprising F(ab')₂, unknown antigens and anti-hinge NABs – with molecular weights of up to 750 kD, were in fact detected in septic patients at the onset of a SIRS [69]. In addition, the plasma concentrations of F(ab')₂ and that of complement factor Bb, a measure of complement amplification, correlated linearly with the concentration of plasma elastase. The anti-hinge-NAB-stabilized,

F(ab')₂-containing secondary IC stimulates complement amplification, as is illustrated in Figure 3. Thus, anti-hinge NABs, which exist in old-world monkeys and humans but not mice [53], seem to contribute to excess complement activation at the onset of a SIRS by providing the biophysical prerequisites for deposition of dimeric C3b, a very potent precursor of the amplifying C3 convertase [38].

Concluding remarks

We have focused here on how some NABs recognize altered self and gain functionality despite their low affinity and low concentrations. Whereas induced IgG antibodies operate by high affinity, most NABs exert their effects by multivalent binding as IgMs to minimally altered epitopes; some bind as IgGs, of which a few act by stimulating complement amplification. Although some NABs or their recombinant forms are ready to be tested in clinical trials, many others have yet to be generated in recombinant form (e.g. against context-specific epitopes or even against idiotopes of immunoglobulins). In fact, anti-idiotypic NABs (paratope and framework-specific), which have only been touched upon here, exert beneficial effects in the treatment of many autoimmune diseases by IVIG. Anti-idiotypic NABs complex autoantibodies (for a review, see Refs [70,71]), in addition to NABs with a pathological potential [72], and even modulate enzyme activities, such as the amplifying C3 convertase [73]. Therefore, NABs should no longer be regarded as an immunological curiosity; they have important physiological roles, many of which have yet to be studied in more detail in highly purified or monoclonal form. Likewise, it seems to be a necessity to unravel how NAB production is controlled.

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