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REVIEW

Polyreactive antibodies in adaptive immune responses to viruses

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Abstract B cells express immunoglobulins on their surface where they serve as antigen receptors. When secreted as antibodies, the same molecules are key elements of the humoral immune response against pathogens such as viruses. Although most antibodies are restricted to binding a specific antigen, some are polyreactive and have the ability to bind to several different ligands, usually with low affinity. Highly polyreactive antibodies are removed from the repertoire during B-cell development by physiologic tolerance mechanisms including deletion and receptor editing. However, a low level of antibody polyreactivity is tolerated and can confer additional binding properties to pathogen-specific antibodies. For example, high-affinity human antibodies to HIV are frequently polyreactive. Here we review the evidence suggesting that in the case of some pathogens like HIV, polyreactivity may confer a selective advantage to pathogen-specific antibodies.

Keywords Polyreactivity · Antibodies · B cells · Tolerance · Viruses · HIV

Abbreviations

BCR	B-cell receptor
CD4i	CD4 induced co-receptor binding site
CDR3	Complementary determining region 3
HIV	Human immunodeficiency virus
Ig	Immunoglobulin
IgH	Ig-heavy chain
IgV_H	Ig-heavy chain variable region
MPER	Membrane-proximal external region
SLE	Systemic lupus erythematotus

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Introduction

Antibodies are essential to the humoral immune response against pathogens and constitute one of the first lines of defense against re-infection. The idea that antibodies are highly specific was suggested by the "lock and key" model proposed by Emil Fisher and strongly reinforced by pioneering observations on haptens made by Karl Landsteiner [1]. However, we now know that many antibodies and T-cell antigen receptors are also polyspecific or polyreactive as defined by the ability to bind to several different ligands [2–6]. Polyreactive B cells, especially those with low levels of reactivity, are part of the physiologic repertoire and produce "natural" antibodies [7–10], which are important contributors to the innate immune responses against pathogens. In addition, recent work has shown that class-switched high-affinity antibodies that react "specifically" with infectious agents such as human immunodeficiency virus (HIV) can also be polyreactive. In this review, we discuss these recent discoveries focusing primarily on the adaptive antibody response to HIV and other viruses.

Signatures of polyreactive antibodies

David Talmage first advanced the concept of antigen receptor "multiplicity" in 1959, but this was only proven 10 years later by Herman Eisen and his colleagues who first demonstrated antibody polyspecificity in a series of

experiments testing the reactivity of anti-hapten myeloma proteins [11, 12]. They showed that a myeloma protein reactive to the 2,4-dinitrophenyl (DNP) group could bind both to structurally similar (e.g., menadione) and unrelated (e.g., caffeine) molecules [12]. This "degenerate" type of binding was later documented for conventional monoclonal antibodies, for example, anti-DNA, anti-HIV-1 p24 protein and anti-DNP antibodies [13–16].

Polyreactivity is a conserved feature of antibodies among species [17] and can be found in different immunoglobulin (Ig) isotypes (IgM, IgG and IgA) [18, 19]. The affinity of polyreactive antibodies for their different ligands is generally low (K_d ranging from 10^{-3} to 10^{-7} M) compared to the high affinity of monoreactive antibodies for their specific ligand ($K_d = 10^{-7}$–10^{-11} M) [2, 19, 20]. It is generally assumed that the Ig-heavy chain variable region (IgV_H) accounts for most polyreactive binding [21]. Indeed, a number of molecular features of IgV_H have been associated with polyreactivity, including long and hydrophobic IgH complementary determining region 3 (CDR3), but none of these are predictive [21–23]. Although the precise molecular mechanism by which polyreactive antibodies bind to multiple ligands is not known, it has been proposed that the antigen-binding site of such antibodies is more flexible than monoreactive antibodies [24–28]. Consistent with this idea, antibody molecules can adopt distinct conformations in equilibrium, which allow recognition of various antigens using different interacting residues [29, 30]. An alternative, but not necessarily mutually exclusive possibility, is that antibodies undergo conformational reconfiguration upon antigen binding, allowing antigen accommodation into the binding pocket ("induced fit" mechanism) [31]. Irrespective of the mechanism, the binding of multiple antigens by a single polyreactive antibody molecule implies that the antibody is capable of several distinct physical interactions [32, 33]. These interactions confer "promiscuous," but usually low-affinity binding, but do not exclude high-affinity interactions as described for monoclonal antibodies specific to HIV [34] and human epidermal growth factor receptor 2 [35].

Tolerance checkpoints eliminate most self- and poly-reactive B-cell clones

Polyreactivity and self-reactivity can arise as a result of random rearrangement of *Ig* genes during B-cell development [36] or by somatic mutation during the germinal center reaction [37]. Indeed, the majority of nascent B cells in the bone marrow (early immature B cells) of healthy humans express self-reactive and polyreactive BCRs (75 and 55%, respectively) [23] (Fig. 1). However, the vast

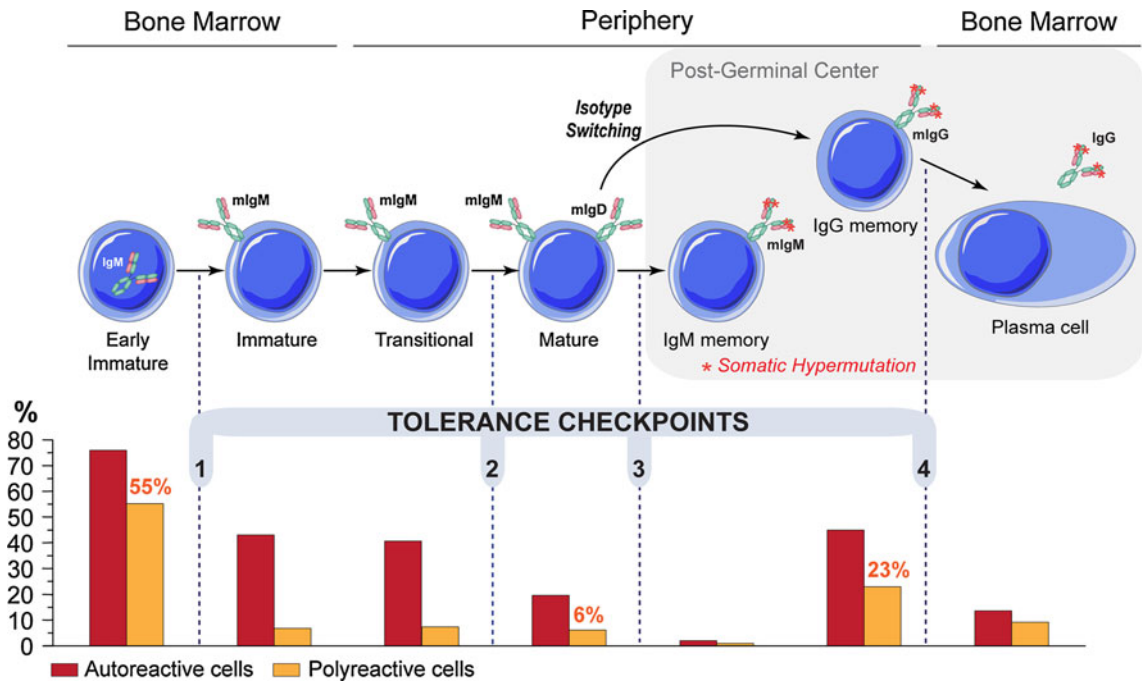

Fig. 1 Tolerance checkpoints in human B-cell development. Tolerance checkpoints between developmental stages of B-cell lymphopoiesis; a central tolerance checkpoint in the bone marrow (*1*) followed by three tolerance checkpoints in periphery (*2–4*) ensure the removal of most autoreactive and polyreactive B cells [23, 39, 63, 154]. *Bar graph* shows the frequency of autoreactive (*red bar*) and polyreactive (*orange bar*) clones determined by testing monoclonal antibodies from single B cells at the different B-cell stages [23, 39, 63, 154]. The frequency of polyreactivity in the early immature, mature naïve and IgG memory B cells is indicated above the *bars*. mIg, membrane immunoglobulin

majority of these autoreactive and polyreactive B cells are counterselected at two major B-cell tolerance checkpoints (Fig. 1) by clonal deletion, anergy or receptor editing [38]. As a result, only a small number of mature naïve B cells are self-reactive or polyreactive (20 and 6%, respectively) [23], and their average level of polyreactivity (as measured by ELISA) is far lower than that of their bone marrow progenitors. Polyreactivity is further removed from the B-cell repertoire during the transition to the IgM+ "memory" B-cell stage (2 and 1%, respectively) [39] (Fig. 1).

In contrast, tolerance checkpoints are defective in patients with autoimmune diseases i.e., systemic lupus erythematotus (SLE) [40, 41], rheumatoid arthritis [42, 43] and type 1 diabetes [44], leading to the accumulation of autoreactive and polyreactive mature naïve B cells in the periphery. The mechanisms required for establishing tolerance have been investigated in patients with immunodeficiency. Tolerance checkpoints require normal B-cell receptor (BCR) signaling [44, 45], and they can be altered by mutations in toll-like receptor signaling molecules [46] and CD40/CD40L signaling [47].

In conclusion, polyreactive B cells strongly cross-reacting with self-antigens are eliminated by B-cell tolerance mechanisms. Nevertheless, low levels of antibody polyspecificity can be tolerated to constitute a reservoir of "natural" antibodies offering structural diversity that may increase the spectrum of the B-cell repertoire involved in innate immune responses against pathogens.

Polyreactive "natural" antibodies as a first line of defense against pathogens

B cells expressing low-affinity polyreactive surface immunoglobulins are present in the peripheral B-cell repertoire of adult individuals and represent half of cord-blood B cells [48–50]. They frequently co-express the CD5 surface molecule that is also a specific marker for mouse B-1 cells, a particular B-cell subset that was historically identified as a major source of self-reactive and polyreactive antibodies [2, 18, 51–53]. Nevertheless, other B-cell populations apart from $CD5^+$ B-1 lymphocytes were shown to secrete "natural" antibodies cross-reacting with self-antigens; one such population is marginal zone B cells that reside in the mouse spleen [49, 50, 54–56].

Although polyreactive IgM antibodies may circulate in complex with self-antigens [57], they bind to various invading pathogens such as viruses and bacteria, thereby facilitating their elimination [58–60]. Indeed, passive transfer of normal sera to animals, subsequently infected with bacteria or viruses, can confer protection against the infectious agent [58, 59, 61, 62]. More recently, polyreactive "natural" monoclonal antibodies were shown to possess broad antibacterial activity against gram-positive and gram-negative bacteria, and the ability to neutralize the activity of LPS endotoxin [60].

Polyreactivity in antigen-experienced post-germinal center B cells

Surprisingly, B cells can re-acquire poly- and self-reactivity during the germinal center reaction. Nearly 20% of post-germinal center IgG- and IgD-expressing memory B cells are polyreactive [63, 64], as compared to 6% on the naïve B cell compartment [23] (Fig. 1). Like the "natural" IgM antibodies, polyreactive IgG antibodies may also play an important role in the immediate response to infection as suggested by the increased susceptibility to infections in the absence of IgG in patients with hyper-IgM syndrome [65].

In addition to IgG, mouse and human IgA antibodies are also polyreactive [61, 66]; 25% of intestinal IgA-expressing plasmablasts show low levels of polyreactivity with self and foreign antigens, including commensal bacteria and rotavirus [67]. Importantly, the polyreactive IgAs, like IgGs, are somatically hypermutated indicating that the reactivity arose secondarily in the germinal center [66, 67]. IgA is the most abundant Ig isotype produced in mammals, and is believed to have a major role in the protection of mucosal surfaces against toxins, bacteria, viruses and protozoa [68].

Since the germinal center is the site of somatic hypermutation, the polyreactivity observed in the human IgG and IgD memory B cells, and IgA plasmablasts, likely represents a by-product of affinity maturation. Consistent with this idea, most of the germline precursors of polyreactive IgG memory B-cell antibodies from healthy donors or SLE patients were not poly- or self-reactive [63, 69]. In addition, germline-encoded antibodies might lose their polyreactivity when somatically mutated as a result of antigen-driven affinity maturation [70]. Although the amount of polyreactive binding by IgG memory antibodies is normally low and non-pathogenic, somatic hypermutations are responsible for creating pathogenic antibodies in patients and mice with autoimmune diseases where the checkpoints that remove autoreactive B cells are altered [71–74].

Serologic polyreactivity in viral infections

Although cross-reactivity to autoantigens or polyreactivity is strongly selected against during B-cell development [75, 76], it is a common serologic feature of certain viral infections in humans, including HIV [77], Epstein–Barr virus [78–82] and hepatitis viruses [83–86]. High incidence of serologic autoreactivity was also described in patients

infected with chickenpox, measles and mumps viruses [87]. Finally, mice infected with vesicular stomatitis virus, vaccinia virus and lymphocytic choriomeningitis virus also develop polyreactive autoantibody responses [88–90].

The emergence of autoantibodies in virus-infected humans and mice, as well as the role of pathogens, such as viruses, in the development of certain autoimmune diseases has been studied for over 30 years. Several mechanisms have been suggested to explain the frequent association observed between infections and autoimmunity, including molecular mimicry between self- and foreign antigens, polyclonal T- and B-cell activation (bystander activation), and viral transformation of autoreactive B cells [89, 91–95]. However, in most cases little is known about the mechanisms that facilitate the emergence of these antibodies during infection.

The relationship between antibody polyreactivity and anti-viral immune responses is best documented for HIV. Humoral self-reactivity in humans infected with HIV was first documented 25 years ago shortly after the virus was isolated [96–98]. In addition, some of the IgG monoclonal autoantibodies isolated from HIV donors with high serum autoreactivity were shown to be somatically mutated and polyreactive, cross-reacting with multiple antigens [99]. Interestingly, another lentivirus of the *Retroviridae* family, Visna virus, which infects sheep, also triggers the development of IgM/IgG autoantibodies similar to those found in HIV patients [100].

Polyreactivity of HIV-specific antibodies

The HIV envelope protein is a trimeric glycoprotein composed of three gp120 monomers, each non-covalently bound to a transmembrane gp41 protein [101]. Two anti-HIV broad neutralizing antibodies, 2F5 and 4E10, that target a linear epitope of the membrane-proximal external region (MPER) of gp41, were found to be polyspecific and reactive against membrane phospholipids, i.e., cardiolipin [34, 102–104]. Importantly, the polyreactivity of gp160-specific IgG antibodies is a more general feature of the human memory antibody response to HIV-1. Indeed, the characterization of 134 unique anti-gp160 antibodies, isolated from IgG memory B cells in HIV clade B-infected donors, showed that the vast majority were polyreactive (75 vs. ~20% in controls), cross-reacting with both foreign and self-antigens (Fig. 2a) [105, 106]. Anti-gp41 antibodies were the most polyreactive and lipid-reactive (85–90%), as confirmed later for antibodies to the cluster II region of gp41 [107], and most recently for other human antibodies to HIV-1 gp41 [108, 109]. Although polyreactive anti-gp160 IgG antibodies isolated from clade-B infected donors have somewhat longer and more hydrophobic IgH CDR3s, the correlation was neither strong nor predictive [21–23, 106]. Similar findings were recently reported for IgG antibodies cloned from HIV clade A-infected donors (Fig. 2a) [110], strongly supporting the idea that polyreactivity is a conserved feature of HIV-specific antibodies.

Ig genes encoding high affinity antibodies to HIV-1 envelope protein are highly hypermutated [105, 110–112]. Mutation is essential for both specificity and breadth of the response as demonstrated by the finding that the germline versions of broad and potent neutralizers are generally far less active and more restricted in breadth than the mature antibody [106, 111, 113]. Surprisingly, however, the germline precursors of anti-HIV antibodies remained polyreactive (70 vs. 6% in the overall naïve repertoire) (Fig. 2b) [23, 39], indicating

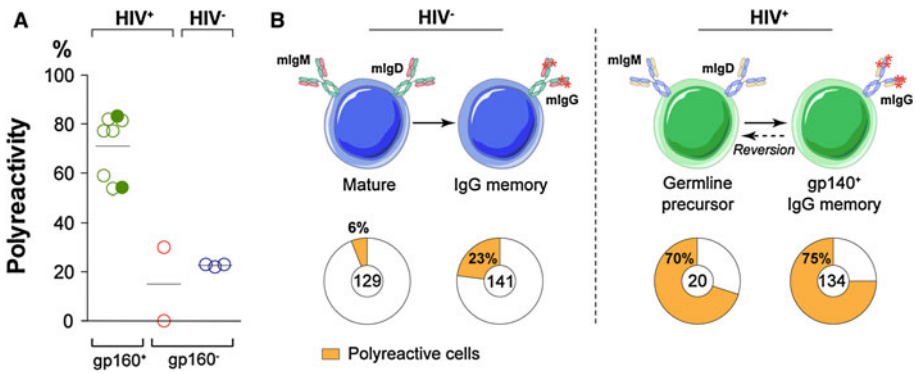

Fig. 2 Polyreactivity of anti-HIV gp160 antibodies. **a** Frequency of polyreactive anti-gp160 IgG memory B-cell antibodies isolated from HIV patients (HIV^+ $gp160^+$) infected with clade B (*open green circles*) or clade A (*filled green circles*) viruses compared to non gp160-binding B-cell antibodies from 2 of the clade B patients (*red circles*, HIV^+ $gp160^-$) [105, 106, 110], and historical control IgG memory B-cell antibodies isolated from healthy donors (HIV^- $gp160^-$) [63]. *Each symbol* represents a donor. **b** Evolution of antibody polyreactivity throughout affinity maturation (transition from mature naïve/germline precursor B cells to IgG memory B cells) for B cells in healthy donors (HIV^-) and gp160-specific B cells in HIV patients (HIV^+). *Pie charts* summarize the frequency of polyreactive (*orange*) and non-polyreactive (*white*) antibodies isolated from the B-cell compartments in HIV-infected and healthy donors indicated by the *schematic diagram*. The number of antibodies tested is indicated in the center of the *pie chart* [39, 63, 106]

that polyspecific B cells may be positively selected during the anti-HIV antibody response even before the germinal center reaction [106]. This raises the fascinating question of whether polyreactive naïve B cells are able to interact with HIV and *ipso facto*, are preferentially recruited to mount a specific adaptive B-cell response against the virus antigens [114], as recently suggested for gp160 [109]. In support of this idea, polyspecific monoclonal antibodies/sera isolated from non-infected humans or from patients with autoimmune diseases can cross-react with viral antigens (i.e., gp120, p24) and/or bind to HIV [109, 115–118].

In conclusion, although different mechanisms may account for the emergence of self-reactive/polyreactive antibodies in HIV-infected patients [119, 120], HIV gp160-specific antibodies frequently display polyreactivity, which may be functionally important.

Why are HIV-specific antibodies polyreactive?

In addition to the rapid mutation, several structural features of the HIV envelope protein make it a poor target for antibodies. These include: (1) carbohydrate shielding [121, 122], (2) conformation masking [123], (3) steric occlusion [124, 125] and (4) transient epitope exposure [126]. Besides these well-established "defense" mechanisms, the low density of functional HIV gp160 expressed at the viral surface may render the anti-HIV antibodies less efficient for viral neutralization by impeding bivalent binding to the virus.

As measured by cryoelectron tomography, mature HIV viruses express only 10–15 randomly distributed viral spikes [127], which would be spaced too far apart (145 nm) for a bivalent antibody to bridge [128, 129]. Bivalent binding is an important property of antibody binding, which increases their relative binding affinity (avidity) to their targets. We have proposed that the low spike density on HIV exerts selective pressure that favors heterotypic bivalent interactions. For example, 2F5 and 4E10 bind to their epitope in the gp41 MPER, which is buried in the HIV lipid membrane, and interact simultaneously with virion lipids [130–136]. Because these polyreactive antibodies bind to lipid alone [34, 103, 132, 137, 138], it has been proposed that lipid binding by 2F5 and 4E10 increases the concentration of antibody, allowing it to "surf" the HIV virion in the vicinity of their specific epitopes on gp41 [136]. Importantly, the interaction of lipophilic residues in the 4E10 and 2F5 IgH CDR3s with HIV membrane lipids was shown to be required for their neutralizing potency [136, 139].

Heterotypic binding by anti-HIV antibodies is not limited to polyreactive lipid-binding antibodies. For example, the anti-gp120 antibody, 21c, which targets the CD4-induced co-receptor binding site of gp120 (CD4i), also binds to the CD4 molecule alone, or to both CD4i and

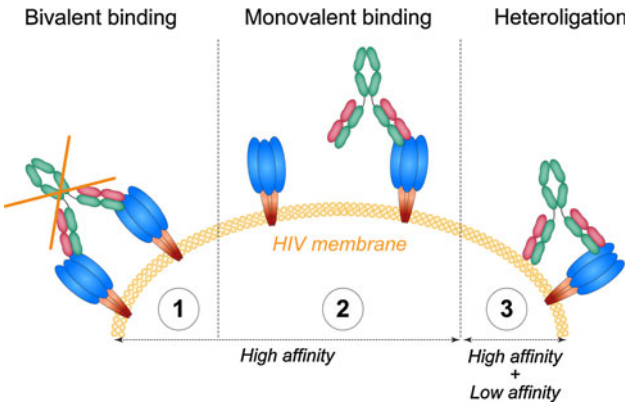

Fig. 3 Heteroligation of polyreactive HIV gp160-specific antibodies. gp160 glycoprotein is expressed at a very low density on the HIV surface (10–15 spikes) [127], indicating that the viral spikes are spaced too far apart for a bivalent antibody to bridge [128, 129] (*1*). Therefore, non-polyreactive anti-gp160 antibodies likely bind to their target with only one of their two high-affinity binding sites (monovalent binding) (*2*). In contrast, polyreactive anti-HIV gp160 antibodies are able to bind bivalently to the virus by heteroligation between one high-affinity anti-HIV-gp160 combining site and a second low-affinity site on another molecular structure on the HIV virion (*3*)

CD4 when it is complexed with gp120 [140]. Finally, polyreactive anti-gp160 antibodies are capable of bivalent heterotypic binding or heteroligation between one high-affinity anti-HIV gp160 combining site and a second low-affinity site on currently undefined HIV-1 virus surface components [106] (Fig. 3). This unusual form of bivalent binding, where one antibody arm binds to a specific ligand and the second to a hetero-ligand, increases the antibody's apparent affinity for the virus and may in part account for the positive selection of polyreactive antibodies in the anti-HIV response in humans [106]. Heteroligation may also enhance the potency of recently described broadly neutralizing glyco-peptide-specific antibodies [141]. These antibodies bind specifically to the gp120-V3 loop and to surrounding glycans, but also have high affinity for glycans alone, which would allow for heteroligation [106, 141].

Implications for vaccine development

Although not all broadly neutralizing antibodies isolated from HIV-infected humans show polyspecificity [113, 142, 143], many of them are polyreactive and cross-react with autoantigens [34, 111, 144]. Since most polyreactive B cells are removed from the repertoire early in B cell development [23, 75, 76], it has been suggested that tolerance might limit the production of broadly neutralizing HIV-1 antibodies [34, 102]. In support of this idea, knock-in B cells expressing the polyreactive IgG 2F5, a broadly neutralizing anti-HIV antibody, failed to pass central and peripheral tolerance

checkpoints [145, 146]. However, the polyreactive precursors of most HIV antibodies are far less reactive than 2F5 [106, 109, 111]. In addition, chronic infection may interfere with normal selection mechanisms. Moreover, these polyreactive cells are present in low numbers in normal healthy individuals and are not pathogenic like the autoantibodies produced in the course of systemic autoimmune diseases [147, 148]. Thus, polyreactive B cells are not completely removed from the B-cell repertoire and may even be beneficial to combat the infection as discussed above. It is therefore unlikely that polyreactivity would be a significant barrier to anti-HIV vaccine development.

Conclusion and future directions

In this review, we discussed infection-associated antibody polyreactivity focusing primarily on HIV since it is the best-studied example. Since exceptionally broad anti-HIV antibodies can effectively protect animals from infection, it has been proposed that it might be possible to vaccinate against HIV by eliciting such antibodies. Since polyreactivity is linked to the specific B-cell response to HIV, it is therefore crucial to understand how such reactivity might impact vaccine development. It may be equally important to consider and study polyreactivity in antibody-based therapies against other viral infections in humans such as influenza virus [149, 150], dengue virus [151, 152] and cytomegalovirus [153].

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