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Immunological Tolerance as a Barrier to Protective HIV Humoral Immunity

Kristin M.S. Schroeder, Amanda Agazio, and Raul M. Torres

Department of Immunology & Microbiology, University of Colorado School of Medicine, Aurora, CO 80045

Abstract

HIV-1 infection typically eludes antibody control by our immune system and is not yet prevented by a vaccine. While many viral features contribute to this immune evasion, broadly neutralizing antibodies (bnAbs) against HIV-1 are often autoreactive and it has been suggested that immunological tolerance may restrict a neutralizing antibody response. Indeed, recent Ig knockin mouse studies have shown that bnAb-expressing B cells are largely censored by central tolerance in the bone marrow. However, the contribution of peripheral tolerance in limiting the HIV antibody response by anergic and potentially protective B cells is poorly understood. Studies using mouse models to elucidate how anergic B cells are regulated and can be recruited into HIV-specific neutralizing antibody responses may provide insight into the development of a protective HIV-1 vaccine.

Graphical abstract

Correspondence to: Raul M. Torres.

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Introduction

Of the more than 25 vaccines currently used in medicine, all largely depend on pathogen-specific antibodies as mediators of protection [1]. However, only two of these vaccines (HPV and Hepatitis B) were specifically designed to elicit protective antibodies against a particular viral protein presented by the vaccine. The remaining vaccines are instead administered as purified inactivated or attenuated viruses or bacteria or their products (e.g., toxoids, polysaccharide capsules) and were empirically determined to be remarkably effective. Thus, our ability to rationally design effective vaccines against clinically important pathogens has been rather limited to date, as the precise immunological cellular and molecular mechanisms by which successful vaccines provide prophylactic immunity remain ill defined. This has been especially highlighted by our unsuccessful attempts to eradicate the human immune deficiency virus (HIV-1) that continues to infect millions annually and remains a global health burden. In this review, we highlight the association that exists between autoimmunity and HIV-1 and specifically the evidence that immunological tolerance is one barrier which impedes HIV-1 protective humoral immunity both physiologically and prophylactically with a vaccine. We further argue that for pathogens such as HIV that are not easily controlled by the host adaptive immune system, either naturally or upon vaccination, the development of an effective vaccine will need to exploit our increasing understanding of B cell biology and the mechanisms that regulate whether and how these lymphocytes mount antibody responses. The recent success of checkpoint blockade immunotherapy in promoting tumor immunity documents that experimental findings in mouse models [2–4] are able to provide important scientific guiding principles for the clinic. As we discuss in this review, mouse models of antibody response can also be valuable in demonstrating proof-of-principle concepts important for HIV vaccine design.

HIV-1 was identified as the etiologic agent of acquired immune immune deficiency syndrome, or AIDS [5], over three decades ago and soon thereafter it was shown that CD4 was the high affinity HIV-1 receptor [6, 7] to which the HIV envelope gp120 protein (Env) bound to infect T cells. Despite this long-standing knowledge, an effective HIV-1 vaccine has remained elusive and presumably for the same reasons that also impede a protective host antibody response upon natural HIV-1 infection. Principal amongst these barriers is the propensity of the virus to rapidly mutate as a result of an error prone viral reverse transcriptase. Thus, although HIV-specific antibodies able to neutralize the infecting (autologous) founder virus eventually appear months after initial infection, by this time the high mutability of HIV-1 renders them ineffective [8]. HIV-1 Env is also heavily glycosylated and, again as a consequence of its genetic variability, sites of gp120 glycosylation vary with time [9]. This not only provides a dynamic physical barrier to neutralizing epitopes, but also cloaks HIV-1 with N-linked glycans, which are indistinguishable by the immune system from ‘self’ carbohydrates [8]. Further, and in contrast to most viral envelope proteins, HIV-1 virions also have a relatively low density of Env gp120/gp41 heterotrimeric spikes on the virus surface [10] with an average distance between viral proteins that exceeds the ability of a gp120-specific antibody to bind bivalently, i.e., with both antigen-binding sites [11]. Consequently, HIV-1 virions with sparsely distributed Env proteins are not expected to be able to efficiently activate potentially

protective naïve B cells expressing a weak affinity gp120-specific germline-encoded B cell antigen receptor (BCR) [12–14].

HIV-1 broadly neutralizing antibodies (bnAbs) display unusual features difficult to elicit by vaccination

Despite these HIV-1 barriers that discourage the activation and recognition of neutralizing epitopes by naïve B cells, HIV-specific antibodies capable of neutralizing a wide breadth of HIV-1 genetic subtypes exist in some infected individuals; this indicates protective humoral immunity against the virus can be elicited. In the first 25 years of HIV research a handful of HIV-specific antibodies were identified that could neutralize a relatively broad range of HIV-1 genetic subtypes [15]. However, technological advancements in the isolation, culture, screening and molecular cloning of single B cells from individuals chronically infected with HIV-1 for years have facilitated the identification of hundreds of additional HIV-1 broadly neutralizing antibodies (bnAbs) [8, 16]. Considered together, these ‘second generation’ bnAbs have been instrumental in defining hallmark features of HIV-1 bnAbs and current intense effort is directed at determining how to elicit HIV-1 bnAbs with a vaccine [8].

Remarkably, these recently identified HIV-1 bnAbs are able to potently neutralize ~90% of diverse HIV-1 strains across most genetic subtypes [8, 16] and predominantly recognize epitopes within four conserved, but less immunogenic regions of the Env protein [3, 15–18]. Three of these regions are located on gp120 and include epitopes on the CD4 binding site (CD4bs), N-linked glycan-V3 loop, and the V1V2 domain, whereas the fourth region is found within the membrane proximal external region (MPER) located on gp41 [8, 19]. Features common to these bnAbs include a substantial somatic mutation load in both the complementarity determining (CDR) and framework (FWR) regions, an Ig heavy chain CDR3 (a unique sequence generated by the rearrangement of Ig gene segments during development) that is above average length and Ig light chains with shorter than usual CDR3 regions [8, 16]. Furthermore, HIV-1 bnAbs isolated from different individuals are often encoded by the same set of *IGHV* genes [8, 16].

Of particular interest, a high proportion HIV-1 bnAb directed against CD4bs and MPER epitopes also display specificity for self and thus are considered poly-/auto-reactive antibodies [17–19]. The self antigens recognized by these HIV-1 bnAbs include the enzymes kynureninase and ubiquitin ligase E3A, SF3B3 splicing factor [19, 20], phospholipids (e.g. cardiolipin) [21], dsDNA [17] and, recently, histone H2A [22]. Why some bnAbs display autoreactivity remains speculative, although evidence exists for host mimicry by HIV-1 and as a possible mechanism used by the virus to avoid adaptive immunity [20]. Additionally, poly-/auto-reactivity of bnAbs has also been shown to enhance antibody affinity for HIV-1 via heterologation defined as the simultaneous recognition of both a sparsely distributed Env spike and host derived membrane antigen [23, 24]. Based on the autoreactivity displayed by a substantial subset of HIV-1 bnAbs, Haynes and colleagues initially postulated that immunological tolerance may present a barrier to mounting a potentially protective HIV-1 antibody response [18, 25].

HIV-1 and autoimmunity

An association between autoimmunity and HIV has been appreciated in the literature for decades. Specifically, naïve uninfected autoimmune prone MRL/*lpr* mice were reported to harbor serum antibodies specific for HIV-1 Env [26, 27]. Similarly, individuals with the autoimmune disease systemic lupus erythematosus (SLE) have also repeatedly been found to display HIV-specific serum antibodies, again in the absence of viral infection [28–32]. Further, the incidence of HIV-1 infection in individuals with SLE is lower than anticipated [28, 33–35] and after accounting for demographic differences [30]. More recently, an association between autoimmunity and HIV-1 protective humoral immunity was documented in an individual with SLE whose plasma was found to neutralize a wide breadth of HIV-1 genetic subtypes and to control HIV-1 infection in the absence of antiretroviral therapy [17]. Env-specific antibodies derived from memory B cells from this individual identified one CD4bs-directed bnAb, CH98, which also demonstrated specificity for dsDNA [17]. Further, HIV-infected individuals who harbor HIV-1 bnAbs also display significantly higher levels of serum autoantibodies compared to infected individuals with limited HIV-1 neutralizing activity [36]. Together these findings, while largely correlative, suggest that some autoreactive antibody specificities are able to cross-react with HIV-1 Env and supports the notion that immune tolerance impedes the production of neutralizing HIV-1 antibodies.

The reason why many bnAbs with specificities to the conserved CD4 binding site on gp120 or MPER on gp41 also recognize self-antigens remains uncertain. Beyond a mechanism evolved by HIV-1 to avoid adaptive immunity, it is noteworthy that endogenous retroviruses have comprised a sizable portion of the human genome for millions of years and likely influenced the evolution of our immune system. In this regard, many HIV-encoded antigens may indeed be considered self by the vertebrate immune system and antibodies capable of recognizing HIV-1 may be released when tolerance is compromised.

Immunological tolerance as a barrier to protective HIV-1 humoral immunity

Immunological tolerance functions to reduce the potential for autoimmune disease by removing or functionally silencing autoreactive specificities from lymphocyte populations. Newly generated B cells in the bone marrow of healthy individuals display an enriched frequency of poly-/auto-reactive BCRs that are largely censored by **central** B cell tolerance mechanisms [37–39]. Although central B cell tolerance greatly culls the majority of autoreactive B cells, a small but significant population of B cells with low affinity for self-antigens continues to develop and populate secondary lymphoid compartments. Here, **peripheral** tolerance renders and/or maintains these self-reactive cells functionally anergic by extrinsic and intrinsic mechanisms that are just beginning to be defined [40]. Anergic B cells exist in both wild type mice and healthy individuals [41–45], and despite being characterized as functionally inert, these B cells can contribute to the antibody response, at least to some foreign antigens under particular circumstances [46, 47]. Whether the circumstances that promote these responses rely on a transient breach of peripheral tolerance is not currently known.

Our current understanding of the mechanisms by which B cell central tolerance is enforced on autoreactive B cells was established by mouse models and facilitated by technology permitting the precise (versus random) introduction of Ig heavy and light chain transgenes into their respective physiological loci. Taking advantage of this approach, initial autoreactive Ig knockin mice displayed specificities toward soluble and membrane-bound self antigens such as dsDNA (3H9 Ig heavy chain only) [48] and MHC class I [49], respectively. Studies with these and subsequent Ig knockin mouse models have clearly demonstrated central B cell tolerance acts on immature B cells with autoreactive specificities in the bone marrow by the following mechanisms: 1) secondary Ig light chain gene rearrangement of endogenous Ig light chain genes to edit receptor specificity (receptor editing); 2) elimination of autoreactive B cells unable to successfully eliminate their autoreactive specificity (clonal deletion); and 3) induction of a non-functional state in low affinity autoreactive B cells (anergy) [37–39, 43]. Building on these studies, investigators have more recently generated Ig knockin mice using HIV-1 bnAb with autoreactive specificities to directly determine the role that immunological tolerance plays in limiting B cells with these specificities from mounting neutralizing antibody responses (Table 1). Beyond facilitating the characterization of mechanisms of B cell central tolerance, mouse models have similarly provided much of our understanding of how B cells mount antibody responses [50] and would be expected to continue to inform on how to elicit HIV-1 neutralizing antibody responses.

In the vast majority of the HIV bnAbs Ig knockin mouse strains recently generated, B cells have been found to be censored by central B cell tolerance, highlighting the role of this tolerance checkpoint in limiting the development of B cells with specificities to both autoantigens and HIV-1 Env (Table 1). Specifically, developing B cells in 2F5 Ig knock-in mice, expressing either the germline or somatically mutated IgH and/or IgL chain genes from the 2F5 bnAb reactive against MPER and kynureninase, are predominately (>95%) eliminated by clonal deletion [51, 52]. Nevertheless, receptor editing facilitates the appearance of a small number of anergic peripheral B cells expressing the 2F5 IgH chain with endogenous Ig light chains. B cells from Ig knockin mice derived from the bnAbs 4E10 and 3BNC60, which recognize the MPER and CD4bs, respectively, are similarly predominantly removed by B cell central tolerance [53, 54]. In these models, the 4E10 HIV-1 bnAb also reacts with several self antigens whereas an autoantigen recognized by 3BNC60 has not been reported (Table 1).

While the role of central B cell tolerance in limiting the development of B cells in bnAb Ig knock-in mice has been well established, the contribution of peripheral tolerance in restraining existing autoreactive peripheral B cells from mounting a protective HIV-1 antibody response has yet to be fully characterized. Studies in the autoreactive Ig models of B cell anergy have shown that anergic B cells can mount an antibody response under various conditions, such as stimulation with highly multimerized antigens and/or strong toll-like receptor stimulation [55–57]. As such, a strategy designed to activate the peripheral pool of anergic B cells in bnAb Ig knockin mice would be expected to provide insight for vaccination approaches. Indeed, in a proof-of-concept study, following serial immunizations using gp41 peptide-liposomes and toll-like receptor agonists, the few existing anergic B cells

that express germline 2F5 IgH and IgL genes produced limited amounts of isotype-switched antibodies with tier 1 HIV-1 neutralizing ability [52, 58].

As an extension of these observations, a recent study in our lab has demonstrated that autoimmune prone mice, with known defects in both central and peripheral tolerance, are able to produce neutralizing antibodies against tier 2 HIV-1 subtypes [22]. Interestingly, the lupus prone B6.Sle123 mouse strain, which harbors 3 chromosomal regions from the autoimmune NZM2410 strain on the C57BL/6 genetic background [59], produced tier 2 HIV-1 neutralizing antibodies following alum and TLR immunization alone, in the absence of Env ([22] and unpublished data). Notably, we further demonstrated that an experimental breach of immunological tolerance in naïve wild type C57BL/6 mice using the pristane hydrocarbon [60] not only leads to the production of serum autoantibodies but also, upon immunization with HIV-1 gp140, leads to an Env-specific tier 2 HIV-1 neutralizing antibody response (Figure 1). In this study, elevated serum titers of IgM autoantibodies against a lupus-associated autoantigen, histone H2A, correlated with tier 2 HIV-1 neutralization. Furthermore, IgM anti-H2A monoclonal antibodies isolated from immunized B6.Sle123 mice also demonstrated specificity for the CD4 binding site on gp120 and were able to neutralize tier 2 strains of HIV-1 [22]. Together, these findings highlight the ability of poly-/auto-reactive antibodies against SLE-associated autoantigens to neutralize HIV-1. Furthermore, these findings suggest that similar responses may be exploited in a protective HIV-1 vaccine.

How common are breaches in peripheral tolerance and is this a consideration to promote HIV-1 humoral immunity?

Under some settings (e.g., common infections) and under the influence of genetic and environmental factors, anergic B cells escape peripheral tolerance and likely contribute to autoimmunity via the production of autoantibodies [61–64]. Given this potential risk, it remains unclear why the immune system has evolved to allow these potentially dangerous B cells to populate peripheral lymphoid compartments unless conditions exist in which they may prove useful. Accordingly, Goodnow and colleagues have documented that *bona fide* peripheral anergic autoreactive B cells in both humans and mice can be recruited into an antibody response against a foreign antigen, form germinal centers and lose autoreactivity through somatic hypermutation [46, 47]. Understanding the mechanisms and conditions that allow autoreactive B cells to be transiently released from peripheral tolerance and mount humoral responses will not only provide insight into the etiology of autoimmune disease, but may also be exploited to provide HIV-1 humoral immunity. This would represent an approach with similar rationale to how checkpoint blockade immunotherapy successfully unleashes tumor immunity.

How common are breaches in peripheral tolerance that allow autoreactive B cells to differentiate into antibody secreting cells? While this is not yet clear, anti-nuclear autoantibodies (ANAs) can be detected in the sera of approximately one quarter of the population, with relatively high levels in about 10% of these individuals, and this frequency is maintained across ethnically and racially diverse populations [65]. This suggests that

peripheral tolerance may not be fully intact or is transiently relaxed in a sizeable proportion of the population. Healthy individuals that harbor elevated levels of circulating ANAs share a number of proinflammatory features with SLE individuals, including a type I interferon signature [66, 67]. Indeed, pro-inflammatory settings, such as certain bacterial [68–70] and viral [71–74] infection as well as acute tissue injury [75, 76], often lead to significant acute autoantibody production. Adjuvants, including hydrocarbons, have also been shown to promote autoantibody production in healthy individuals and wild type mice [77, 78] and over 90 drugs that are currently used in the clinic also transiently relax immunological tolerance and promote serum autoantibody production [79, 80]. Together these findings suggest that the transient relaxation of peripheral tolerance may be a more common occurrence than currently considered and may possibly be a normal feature of antibody responses under certain conditions [46, 47].

Mouse models have been used to directly demonstrate the functional restraint of anergic autoreactive B cells and the experimental breach of this restraint by genetic lesions [81, 82] or combinations of antigenic and inflammatory signaling pathways. B cell hyperactivity is closely associated with SLE and autoantibody production and consistent with this, *in vivo* stimulation of the BCR together with TLR7/9 agonists promotes autoantibody production from anergic autoreactive peripheral B cells [83]. The provision of abundant T cell help, as well as the loss of T cell suppression, has also been experimentally shown to activate anergic B cells to produce autoantibodies [84–86]. BAFF is a limiting B cell pro-survival cytokine for which non-autoreactive B cells typically outcompete autoreactive B cells [87, 88]. Thus, heightened BAFF levels relaxes this counter-selection and, together with TLR7/9 agonists, breaks peripheral tolerance and drives autoimmunity in the absence of T cells [89]. A proof-of-concept mouse study demonstrated that BAFF treatment in wild type mice prior to immunization with HIV-1 Env promotes the production of tier 1 HIV-1 neutralizing antibodies that were not elicited in the absence of BAFF pre-treatment [90]. Whether similar approaches will be useful for consideration in HIV-1 vaccine design will be informed by a better understanding of the mechanisms that not only enforce peripheral tolerance but also allow autoreactive B cells to be released from this constraint.

Concluding remarks

Despite the significant progress made in the identification and characterization of HIV-1 bnAbs and design of promising Env immunogens, HIV-1 continues to be a major global health burden and a viable vaccine is not yet on the horizon. The induction of bnAbs that are capable of neutralizing HIV-1 strains across the genetically diverse genetic subtypes is crucial to the development of a protective vaccine. Evidence is accumulating that immunological tolerance prohibits the induction of a subset of bnAbs, namely antibodies with poly-/auto-reactive specificities capable of neutralizing HIV-1. Whether transiently relaxing peripheral tolerance can be accomplished without detriment remains to be determined as well as how this might be accomplished in consideration of vaccine design. Regardless, it remains important to further understand the mechanisms that regulate the production of antibodies by poly-/auto-reactive anergic B lymphocytes in the periphery of healthy individuals, as these findings will directly inform on HIV-1 vaccine design. In this

context, mouse models of B cell autoreactivity are critical in providing insight into how to reach this goal.

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Highlights

- bnAbs isolated from HIV infected individuals display autoreactive specificities.
- B cell development in HIV-1 bnAb Ig knockin mice is limited by central tolerance.
- Autoimmune individuals and autoimmune-prone mouse strains produce HIV-1 bnAbs.
- If a transient breach in tolerance would be helpful to develop bnAbs is not known.
- How peripheral tolerance/B cell anergy limits the HIV response is not fully understood.

Figure 1.

Polyclonal B cell activation of autoimmune strains of mice (red) leads to increased levels of serum autoantibodies of which certain IgM and IgG autoantibody specificities are able to neutralize tier 2 HIV-1 genetic subtypes. Immunization of wild type mice (white) with HIV Env elicits an IgM and IgG gp120-specific antibody response that are not able to neutralize HIV-1. HIV Env immunization of wild type mice in which peripheral tolerance has been experimentally breached (pale red) elicits autoreactive tier 2 HIV-1 neutralizing antibodies.

Table 1
Mouse models to Study Immunological Tolerance in the HIV neutralizing antibody response.

knock in mouse model	Mechanism of immunological tolerance	HIV Env Antigen recognized	Autoantigen Recognized	HIV neutralizing Abs produced	Reference
5 IgH+L mutated	Central tolerance (clonal deletion and receptor editing) in the bone marrow. Remaining peripheral B cells (5%) rendered anergic, expressing 2F5 IgH with endogenous mouse light chains.	MPER	Kynureninase (ELDKWA)	Tier 1 neutralization (monoclonal Abs derived from hybridomas) following immunization with gp41-peptide liposomes.	[51]
2F5 IgH+L germline	Central tolerance (clonal deletion and receptor editing) in the bone marrow. Less anergic, peripheral B cells than 2F5 SHM KL, expressing 2F5 IgH with endogenous mouse light chains.	MPER	Kynureninase (ELDKWA)	Neutralization not tested. Limited MPER-specific Ab production following immunization with gp41-peptide liposomes.	[52]
4E10 IgH+L mutated	Central tolerance (clonal deletion and receptor editing) and downregulation of BCR in the bone marrow. Very few peripheral B cells.	MPER	Cardiolipin, membrane lipids, splicing factor-3b subunit-3, type-1 inositol triphosphate	Neutralization not tested. Limited gp41-specific Ab produced.	[53]
3BNC60 IgH+L germline	Central tolerance (clonal deletion and receptor editing) in the bone marrow. Remaining peripheral B cells are anergic and skewed towards marginal zone phenotype.	CD4BS	Not reported	Anergic cells were activated and produced Env-specific Abs with immunization with highly multimerized HIV Env immunogens	[54]
Autoimmune Mouse model	Mechanism of immunological tolerance	Env-specific antibodies	Autoantibody specificities	HIV neutralizing Abs produced	Reference
Slc123	Impaired central and peripheral tolerance	CD4BS, gp140 (YU2)	Nuclear Antigens Histone H2A IgM	heterologous tier 2 JRFL & YU2	[22]
MRL/lpr	Impaired central and peripheral tolerance	gp140 (YU2)	Nuclear antigens Chromatin IgG	heterologous tier 2 JRFL & YU2	[22]
Wild type Mouse model	Mechanism of immunological tolerance	Env-specific antibodies	Autoantibody specificities	HIV neutralizing Abs produced	Reference
C57BL/6 BAFF treatment	Relaxation of peripheral tolerance at the transitional B cell selection checkpoint	gp140 (YU2)	phosphatidylcholine, dsDNA, and cardiolipin	Tier 1 HIV after serial Env immunizations following 10 days pre-treatment with BAFF	[86]
C57BL/6 pristane treated	Impaired peripheral and possibly central tolerance	gp140 (YU2) IgM and IgG	Nuclear Antigens Histone H2A IgM	Tier 2 HIV with pristane treatment alone; Env immunization leads to increased potency and breadth	[22]