Is Autoimmunity a Component of Natural Immunity to HIV?

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Abstract: Antibody neutralization would be a major way to prevent HIV infection and disease progression, but the complex relationship between host and pathogen makes tough to achieve this target through immunogens based on viral envelope proteins. Autoimmunity has been associated to bacterial and viral diseases, as a consequence of inflammatory response to pathogens; it may eventually lead to harm host cells and organs. However, autoimmune-like responses have also been observed in HIV-infected patients, raising many questions about their clinical significance. Recent studies have elucidated both similarities and differences between anti-self responses in HIV infection and autoimmune diseases, identifying new molecular players that might enhance immune protection to HIV and/or modulate the clinical progression of the established infection. This paper will present the current knowledge on auto-antibodies observed in HIV infection, their putative mechanisms of generation and their possible implications for immune therapy.

Keywords: HIV, antibody, autoimmunity, neutralization, natural immunity, cross-reactive antibody.

INTRODUCTION: HUMORAL RESPONSE, HIV AND AUTOIMMUNITY

Humoral immunity is the arm of the immune response that protects host and prevents pathogen infection and spread through the production of antibodies that specifically recognize, bind and neutralize pathogens and foreign proteins. Antibodies are assisted by soluble effector molecules, such as the complement proteins. Neutralizing antibodies are usually observed only in advanced phases of HIV infection; not all infected people develop such responses to the virus [80, 113, 135]. Immunization is an effective way to increase spontaneous humoral immunity in subjects exposed to or infected by pathogens, but experimental vaccines to HIV antigens are seldom able to induce strong and long-lasting humoral responses [18].

Early studies and more recent findings have reported allo- and autoimmune responses in HIV infected patients [66, 183], and have also supported a non-pathologic role for autoimmunity in infected and exposed people [15, 37, 21]. Recent studies even lead to hypothesize a protective role for autoimmunity in preventing HIV infection [7, 17, 61]. Understanding the role of such peculiar humoral responses could reveal novel therapeutic targets and immunization strategies useful to block infection or to limit its progression.

This paper will critically review the state-of-the-art on auto-antibodies and will discuss allo- and autoimmune responses in HIV infection, with the aim to define whether such responses merely represent immune dysregulation due to the viral infection or suggest unprecedented modes of immune protection.

Keywords: HIV, antibody, autoimmunity, neutralization, natural immunity, cross-reactive antibody.
plasma cells [55]. CD40 receptor delivers intracellular signals through a series of protein kinases belonging to different pathways, such as the ERK and the NFkB cascades [169, 147], some of which, such as the p38 kinase one, are also involved in mitogen-mediated stimulation [87].

Another member of TNF receptor family, Fas (CD95), and its ligand FasL, control B cell apoptosis through the caspase cascade [148]. CD40 and Fas pathways interact in triggering apoptosis, both in pre-B cell and in B cells that have successfully completed B cell receptor rearrangement [3, 103, 82]. Cytokines and soluble factors also control B cell survival. During immature B cell selection, IL-7 and SDF-1 promote chemotaxis and interaction with stromal cells, required to ensure the effective selection of mature B cells [33, 52]. IL4, IL13 and other Th2-specific cytokines provide trophic signalling to pre- and to mature B cells displaying a functional BCR complex [88, 180, 112]. Two recently discovered B cell factors, BAFF (B cell activating factor belonging to the TNF family) and APRIL (a proliferation-inducing ligand), are also involved in B cell proliferation and survival [181]. Their effects, mediated through three surface receptors, BCMA, BAFF and TACI, are still largely unknown. BAFF has been shown to trigger both the BCR receptor complex and its pathway, through binding to BCMA receptor, interaction with CD19 co-receptor [181], and the CD40 transduction cascade. This latter pathway, resulting in NFkβ-mediated gene regulation, can be similarly stimulated by interaction with CD19 as well as by TRAF-binding motifs, recently discovered in BAFF sequence [60, 115].

**Regulatory Mechanisms Underlying Humoral Autoimmunity**

Many aspects of autoimmunity still need to be understood, but recent progresses in immunogenetics have shown that autoimmune responses may have a familiar-linked pattern of transmission, i.e. some inherited haplotypes of histocompatibility and immune-related genes confer to the carriers a higher susceptibility to develop autoimmune responses [42]. Breaking immune tolerance may have a positive significance in co-evolution of hosts and pathogens, because an awkward subset of immune specificities might prove helpful to counteract novel and uncommon pathogens.

Autoimmunity may depend on the “original sin” occurred in the ontogeny of the immune system, i.e. the escape and the survival of some auto-reactive T and B cell clones in the course of the negative selection of immune repertoire [117, 30]. When low-affinity, auto-reactive T and B cell clones bind self-antigens with high affinity, they should enter anergy or apoptosis pathways [30]. Some trophic factors, such as BAFF, have been recently shown to promote survival of memory auto-reactive T and B cells [23, 71].

Several studies, carried out in transgenic mice, have shown that B tolerance is controlled by T helper lymphocytes. T-dependent antigens require the parallel recognition of both B and T lymphocytes to induce a specific humoral response and therefore their recognition relies on the coordinate function of T and B cells. T-independent antigens, also called B cell mitogens, are instead able to trigger B cell activation directly, through multiple molecular recognition and cross-linking on B cell surface [55, 86]. Host antigens, presented to T and B cells in the thymus during positive and negative selection, constitute the “true” self repertoire, while host antigens that can be “seen” by immune cells at periphery are likely to induce tolerance but may also give rise to autoimmune responses [184]. T helper cells also appear to play a role in the pathogenesis of hypergammaglobulinemia, i.e. polyclonal activation of B cells, a phenomenon often associated to low cytopathic viruses, like HIV and hepatitis C virus (HCV). Antigen-independent B polyclonal activation may also occur in presence of microbial antigens inducing chronic inflammation [67] and by intervention of the so-called superantigens, both acting on p38 and mitogen-activated kinase cascade [107, 164]. Neutralizing antibodies to low-cytopathic viruses usually increase when CD8+ T cell function is absent. Nevertheless, it has been demonstrated that partial removal of T helper cells may improve the generation of specific, neutralizing antibodies without affecting CTL response to virus, al least in LCMV model [139].

**AUTOIMMUNE PHENOMENA IN HIV INFECTION**

Since the Eighties, when the Acquired Immunodeficiency Syndrome was first described, many signs of B cells dysfunction have been observed in HIV-positive subjects [47, 35], such as [80], the marked over-secretion of antibodies (hypergammaglobulinemia), the presence of circulating immune complexes, and the abnormally high number of spontaneously proliferating B cells. Moreover, HIV infected subjects were shown unable to mount a proper humoral response upon vaccination [2, 102].

Impairment of B cell is not only attributable to defects in number and function of CD4+ T helper cells, because B cells do not respond to B specific mitogens and T-independent antigens, such as lipopolysaccharide (LPS), keyhole limpet haemocyanin (KLH) and *S. aureus* wall polysaccharides; addition of allogenic T helper lymphocytes also fails in restoring normal responsiveness [81, 73]. Conversely, the administration of antiretroviral drugs to acutely infected patients was shown to regulate B cells activity and survival and to restore their response to T cell-dependent antigens [162].

**B Cell Dysfunction in HIV Infection**

Mechanisms underlying B cell dysfunction are still subject of intense investigation. It is known that HIV exerts a direct effect on B cells, through its binding to B cell surface molecules [101]. Stimulation of CD40 pathway affects B phenotype and enhances B cell susceptibility to T-tropic and dual-tropic HIV strains [108]. Altered B cells display different co-receptor molecules and responsiveness to CD4+ T cells [109, 100, 111]. Fas-mediated apoptotic pathway is upregulated in B cells subsets expressing low levels of CD21 membrane receptor (Complement Receptor type 2, CR2) [110]. Due to the fact that CR2 is the cell receptor for Epstein-Barr virus [121, 157], this finding confirms the early observation of a deleterious role of EBV co-infection, that was supposed to explain the immunoglobulin hypersecretion observed in cohorts of AIDS patients [182]. Conversely, recent studies have shown that naive B cells from HIV infected patients display an activated phenotype and are able to secrete IgG spontaneously [36]. Fig. 1 summarizes
molecular mechanisms, triggered by HIV, that are likely to generate allo- and autoimmunity; all of them will be described in detail in the next paragraphs.

**Anti-Cell Antibodies**

The induction of anti-cell immune responses is relatively uncommon, albeit auto-reactive antibodies can be usually found in a small number of human sera from healthy subjects free from autoimmune diseases as well as in commercially available pooled blood serum [96, 161, 64].

Anti-lymphocyte antibodies have been early observed in sera from HIV infected patients [47, 35]. Due to the fact that most of these antibodies recognize HLA or CD4 molecules, their appearance has been attributed to the cytopathic effect of HIV infection or to the long-lasting exposure of haemophilic patients to blood derivatives [137, 141]. Anti-lymphocyte antibodies found in HIV-infected people reacted with T4, T8 and B lymphocytes [163, 178, 14], but also with host proteins other than immune molecules involved in HIV infection, [155, 104, 26]. Antibodies to HLA and TCR molecules have been related to repeated contacts with allo-

![Fig. (1). Molecular mechanisms underlying the HIV-autoimmunity connection.](image)
antigens, due to blood transfusions or to intravenous drug addiction, to the occurring of molecular mimicry or to the exposure of cryptic epitopes [104, 15, 43]. Anti-B cell antibodies, mostly but not exclusively found in sera from HIV-positive haemophilic patients, were associated with a significant increase in circulating IgM and IgG [31]. Other auto-antibodies target a wide number of surface and cell molecules, including proteins and cell components, such as phospholipids and dsDNA [134, 149]. Auto-antibodies, such as those targeting alpha-1 proteinase inhibitor (alpha-1-PI), an acute-phase marker synthesized by liver cells in response to various cytokines and lipopolysaccharide from Gram-negative bacteria, have been related to progression of HIV infection [19]. Similarly to what has been described for neutralizing antibodies, exacerbated autoimmune responses and even relapses have been observed in many HIV-positive patients [69, 183, 149]. However, some auto-antibodies, like those to the first external loop of CCR5 receptor, observed in HIV-positive, long term non progressing individuals (LTNP), could support a protective role in disease control [131]. Auto-antibodies seen in HIV patients may differ from those observed in autoimmune diseases: for example the former ones present a lower degree of cross-reactivity with cell molecules and a higher sensitivity to high-salt and denaturing conditions, suggestive of low avidity [134]. Fig. 1 summarizes how HIV infection can elicit anti-cell antibodies.

Lymphocytotoxic Antibodies

Some anti-lymphocyte antibodies also display cytotoxic activity, exerted through complement protein cascade, leading to antibody-mediated complement cytolysis of host lymphocytes [128]. This mechanism, observed in HIV-positive subjects in both acute and chronic stages of the disease [141, 79], was thought to contribute to lymphopenia and to immune dysfunction through massive CD4+ T-lymphocyte killing [156, 54, 165]. In Fig. 1, the generation of lymphocytotoxic antibodies is presented.

Circulating Immune Complexes (IC)

Immune complexes, i.e. circulating aggregates of antigens and antibodies, have been found in homosexual males and intravenous drug abusers but especially in HIV-positive individuals [116, 140]. Analysis of immune aggregates have revealed the presence of HIV infectious particles and HIV-related antibodies; host proteins, allo- and auto-antibodies were also found [26, 45, 183]. Immune complexes and auto-antibodies were significantly associated with late stages of the disease, suggesting the existence of a autoimmune-like alteration that might account for progressive lymphopenia, thrombocytopenia and other clinical features observed in the disease [104, 72]. The development of immune complexes following HIV infection is shown in Fig. 1.

The Anti-Idiotype Network

According to Jerne’s theory of immune network, anti-idiotypic antibodies, i.e. antibodies directed to first-wave antibodies produced in response to the pathogen, can be also generated in the course of chronic viral infections. A suggestive idiotype-based hypothesis, taking origin from HLA type II and HIV-gp120 homology, has been formulated. According to it, HIV antigens trigger auto-reactive responses and contribute to destabilize the immune network through mirror-like molecular interactions, that involve host and HIV-infected cells [62]. The idiotype-anti-idiotypic hypothesis of HIV infection, illustrated in Fig. 1, finds also support in recent observations, that showed a cross-reactivity between antibodies from HIV-infected sera and antibodies elicited to agents involved in the most common HIV co-infections, such as CMV, HBV, M.tuberculosis, S.aureus, and mycoplasmas [143]. Antibody cross-reactivity may depend on antigen complementarity; moreover, HIV envelope proteins share homology with HLA molecules, while antigens belonging to other pathogens mimic host proteins [142]. Both mechanisms may contribute to explain the presence of anti-cell and anti-lymphocyte antibodies early observed in sera from HIV-infected, ARC and AIDS patients [158, 173, 151]. Anti-idiotypic antibodies are likely to play a synergic role in immune response, contributing to amplify response to neutralizable epitopes by recognizing larger, conformational epitopes of the infectious agent or by enhancing primary antibody affinity [59, 16, 41].

PROTECTIVE AUTOIMMUNITY

Allo- and Auto-Responses in HIV-Exposed, Non-Infected Individuals

Immune responses to allo-antigens have been shown in healthy individuals exposed to HIV, such as health and sexual workers, intravenous drug users, partners and children born to HIV-positive individuals [10, 21, 95]. Some clinical evidences support a protective role of allo-responses: for instance, HLA-concordant children born to HIV-positive mothers had a higher risk of viral transmission [97]. Cohorts of exposed uninfected people, albeit repeatedly exposed to HIV, were not found infected but displayed neutralizing responses to gp120 and gp41, able to block infectivity of both HIV laboratory strains and primary isolates. Strikingly, neutralizing responses were statistically related to the presence of anti-cell antibodies to surface antigens exposed on lymphocytes, possibly involved in the process of virus infection, such as HLA class I, CD4 and CCR5. Anti-HLA and anti-CD4 antibodies, found in exposed subjects, were shown to recognize conformational epitopes, induced by the interaction with gp120, and to display HIV-neutralizing properties [9, 10, 21, 51]. Anti-CCR5 IgG and IgA antibodies, found in a subset of exposed individuals, shared the same specificity to the first external loop of the receptor and inhibited specifically R5-mediated HIV infectivity [93, 6, 91].

Anti-cell antibodies may origin from different immune mechanisms, depending on HIV exposure or HIV-independent. Anti-CD4 antibodies are triggered by the unmasking of cryptic/conformational epitopes, that become exposed after gp120-CD4 binding [51]. Anti-HLA responses depend on molecular mimicry between HIV glycoproteins and HLA domains [37]. In both cases, these anti-cell antibodies appear to be induced in response to HIV-antigenic stimuli [10]. Conversely, epitopes targeted by anti-CCR5 IgG and IgA, found in some exposed uninfected subjects and in healthy subjects, may have different mechanisms of action and different ways of generation. In fact, anti-CCR5
antibodies directed to the second external loop, i.e. the HIV binding site, are generated in response to HIV infection and block HIV entry through binding competition [17]. Anti-CCR5 antibodies recognizing the first external loop of the protein do not interfere with HIV binding directly, but induce co-receptor down-regulation, thus neutralizing virus infectivity. [93, 7]. Generation of anti-CCR5 antibodies, observed in healthy subjects not previously exposed to HIV, could be rather explained by autoimmune phenomena, triggered by membrane perturbations unrelated to HIV stimuli, such as the activity of exogenous or endogenous viruses or local inflammation [6, 91, 7].

Studies of Allo-Vaccination

Allo-immunization between sexual partners, carried out to prevent abortion, was shown to up-regulate cytokines and to down-regulate their membrane receptors, suggesting a way to achieve natural resistance to HIV [133]. In vitro experiments, carried out on CD4+ T cells from allo-immunized subjects, in fact displayed a reduced susceptibility to both laboratory and primary HIV strains [175]. Further experiments confirmed these findings, showing that antibodies generated in allo-immunized, HLA-discordant couples, were able to neutralize HIV isolates in vitro [85]. These results led the way to experimental HIV vaccinations, aimed at eliciting allo-immune responses to prevent virus spread [38, 44, 83].

Studies of allo-vaccination, carried out in monkeys, also confirmed that repeated contacts with allo-antigens from seminal fluid or fetuses increase T cell count, chemokine secretion and may induce anti-CCR5 antibodies, mechanisms resulting in natural resistance to SIV infection in vitro [11].

Monkeys immunized with human uninfected cells were protected from challenge with HIV strains grown in human cells, through the xenogenic response directed to human proteins, such as HLA molecules, exposed at surface of viral particles [40]. Anti-CCR5 monoclonal antibodies were found to neutralize HIV infection in vitro and to protect monkeys from challenge with SIV strains, once passively administered [84, 127, 166]. Other experiments, carried out on mice and monkeys, showed that anti-CCR5 antibodies could be elicited in animal models and re-boosted when required [28, 27, 7]. Anti-CCR5 antibodies from immunized animals also shared neutralizing properties with human monoclonal immunoglobulins and with natural antibodies found in exposed individuals. [93, 7].

Animal Models of Allo-Autoimmunity in HIV Infection

Animal models of HIV infection, such as naturally SIV-infected mangabeys, do not show autoimmune responses in the course of infection, similarly to a wide number of human HIV-positive patients [4]. Anti-cell antibodies were first described in the Nineties, in macaques, as spontaneously occurring responses [154]. More recently, anti-HLA and anti-lymphocytes antibodies were found in sera from SIV-infected macaques and sooty mangabey monkeys. In these experiments, neutralizing antibodies were not found effective in preventing disease progression; rather, the presence of antibodies recognizing a 18 kDa surface protein, sharing homology with H2B histone, was associated to development of disease [50]. Xenovaccination studies identified anti-HLA responses in SIV-vaccinated macaques, elicited by cell surface molecules associated to budded viral particles cultured in human cells [32]. Xenovaccination was shown to induce protective humoral responses in SIV-challenged macaques, mediated by the increased chemokine secretion [44]. Other vaccination approaches, addressed to break immune tolerance to CCR5 co-receptor protein, obtained the generation of neutralizing, high-titer auto-antibodies in experimental animal models [28, 7]. In macaques, anti-CCR5 antibodies, elicited through vaccination, were able to neutralize HIV laboratory strains and primary isolates in vitro. After challenge, vaccinated macaques succeeded in maintaining a low or undetectable viral load for a long follow-up period [27].

THE HIV-AUTOIMMUNE CONNECTION

Since more than two decades, basic and clinical studies on HIV biology and pathogenesis have provided a wide amount of information on its structure, its life cycle and its interactions with host cells. Nevertheless, many aspects of HIV life cycle remain still unclear and do not fit in usual models of viral infection. The high incidence of allo- and autoimmune responses, observed in cohorts of HIV-infected or even in HIV-exposed individuals all around the world, have made clear that similar responses do not occur by chance. In mice and humans, T and B cell repertoires maintain some auto-reactive cell clones life-lasting, able to trigger auto-reactive responses under exogenous or endogenous stimuli, such as viral infections, local inflammation or gene mutagenesis [42].

Receptor cross-talking, cell-to-cell trafficking, priming and memory are the combinatorial strategy of immune networking, set and conserved in vertebrate trafficking to provide a broad, prompt but specific system to discriminate between host and pathogens, species and individuals. Lipids in cell membranes also play an active role in trafficking, signalling and viral interaction [138]. Moreover, professional APC have been shown to communicate with other immune cells through exosomes, i.e. lipid vesicles exposing surface molecules and co-receptors such as CD4, HLA class I and II [160, 122].

Retroviruses possess the ability to cope with host mechanisms of self- and non-self recognition, altering them to their own sake. They can use lipid rafts and cell machinery for exocytosis to enter and to bud from infected cells. Moreover, HIV can successfully escape from adaptive immunity by using exosome pathway to spread from infected APC and target other immune cells. Natural antibodies to immune surface molecules, such as CD4, HLA class I and II, etc, suggest that HIV actually uses this treacherous way to target host cells stealthily and evade immune surveillance successfully [56].

Endogenous ERV elements similar to HIV, widespread in vertebrate and in human genome, may similarly affect immune response and induce the generation of low-affinity, allo- and auto-antibodies recognizing HLA and co-receptor molecules. ERV gene products are usually expressed in the course of ontogenesis and in some host tissues, and anti-ERV antibodies have been found both in mice and humans.
ERV also carry SAg-encoding sequences, able to induce non-specific lymphocytes activation and desensitisation [5, 106]. Various evidences indeed support the occurrence of ERV-mediated autoimmunity, at least in some animal or human populations displaying a “permissive” immune repertoire [153].

The following paragraphs examine in detail the tricky interactions between HIV and autoimmunity and the possible ways to take therapeutic advantage from the connection.

Exploiting Allo- and Auto-Immune Response to Fight HIV Infection

Addressing HIV-associated cell molecules could prove valuable to elicit anti-viral responses and to enhance virus neutralization, for example via complement-mediated cytolysis [150, 152, 179]. New and unexpected therapeutic targets could help to modulate immune responses conveniently, in order to neutralize HIV infectivity or to slow down its progression [48, 120]. Anti-cell antibodies to antigens expressed in immune cells may also improve T cell deletion and induce anergy and apoptosis of T helper lymphocyte [177]. In order to avoid harmful and undesirable immune responses, allo-immunization, evoking innate responses to highly polymorphic cell antigens, may result safer and easier to be obtained [120]. Some clinical applications of allo-immunization are already in use, to treat at risk pregnancy and to prevent abortion [175].

Redundant host antigens, such as the numerous family of chemokines and their receptors, offer suitable targets to elicit allo- and auto responses [77, 176]. The feasibility of this approach have been shown by natural genetics evidence, in subpopulations of CCR5-delta32 homozygous individuals, by clinical testing of computer-designed synthetic inhibitors of chemokine receptors, such as CCR5 and CXCR4, and, most of all, by natural occurring of anti-CCR5 auto-antibodies, in healthy blood donors, in HIV-exposed as well as in HIV-positive subjects [44, 93, 166, 17, 92, 131]. The safety of auto-immune vaccinations is warranted by the absence of immune dysfunction in subjects displaying anti-CCR5 antibodies as well as in mice and monkeys undergoing immunization [28, 27, 7, 17].

Recent clinical findings lend further support to the exploitation of auto-antibodies to target HIV. The analysis of human antibodies with neutralizing properties showed that 2F5 and 4E10, two of the most effective monoclonal antibodies, derived from HIV-positive patients, are cross-reactive antibodies recognizing cardiopentin [61]. These antibodies, administered in association with 2G12 (another HIV neutralizing monoclonal antibody) to acutely and chronically HIV-infected patients, obtained a significant delay in viral rebound after HAART interruption [167]. Epitopes bound by 2F5 and 4E10 antibodies belong to a high-conserved region of gp41 protein, common to various HIV strains, that hosts broadly neutralizable epitopes [126, 25]. In spite of the auto-reactivity of the assayed antibodies, no adverse side effects have been reported in patients undergoing the trial [70].

The high degree of similarity between HIV and human proteins explains why many attempts to elicit anti-HIV responses risk to fail or, worst, cause anti-self reactions that further endanger immune system and host tissues [144, 68, 99, 143]. Not surprisingly, the majority of clinical trials, carried out in healthy volunteers and in HIV-infected patients in the last twenty years, that were aimed at stimulating active immune responses to HIV, have not obtained yet a definitive proof of efficacy and safety [34, 146]. Conversely, strategies exploiting allo- and autoimmune response, aimed at eliciting natural resistance and tolerance to HIV, still at first assays in human patients, have shown promising results that would deserve further testing [85, 18, 167].

Inflammation Bursts Out

Mutations in local environment - such as a Th1-Th2 switch in cytokine pattern or vice versa - may contribute to auto-reactive responses to target organs. Anti-bacterial and anti-viral responses are typically Th1-biased, i.e. trigger local inflammation. Infectious stimuli, i.e. the presence of inflammatory mediators and cytokines, such as prostaglandins, IFN-gamma, TNF-alpha, IL-10, aim at recruiting effector immune cells at the site of infection. Oxidative stress exerts a synergistic effect on HIV infection, because inflammatory stimuli also promote HIV transcription, via NFkB activation [119].

Viral infections have been often invoked to explain the onset of autoimmunity, as in type-I diabetes, demyelinating diseases, keratitis and myocarditis [129]. Killing and clearance of pathogens also induces the release and the presentation of many antigens, both foreign and self ones. When T-suppressor activity is unable to down-modulate inflammation, it may proceed on and become chronic, addressing specific target cells, tissues and organs. However, natural anti-self antibodies found in healthy individuals or those observed following bacterial or viral infections are usually distinct from those produced in autoimmune diseases [145, 29]. Anti-phospholipids antibodies found in HIV-positive sera showed a different specificity and affinity in respect to antibodies isolated from SLE patients, namely a higher sensitivity to salt concentration and denaturing agents and a lower cross-reactivity with other host molecular targets [134, 138].

Oxidative Stress and its Messengers

Direct cytopathic effect of HIV-1 infection on host cells produces large amounts of cell debris. Host proteins and lipids are processed by phagocytes and dissolved into biologic fluids, undergoing further presentation to immune cells. Massive killing of CD4+ T cells makes available cell-free CD4, HLA-II and co-stimulatory molecules, promoting the occurrence of anti-CD4 and anti-HLA responses [9, 21, 95].

Local production of reactive oxygen species and lytic enzymes from activated macrophages also induces lipid peroxidation and endothelial damage. Not surprisingly, HIV infection has been shown to induce over-expression of Th1 cytokines, such as TNF-alpha and IL-1beta, ICAM-1 and VCAM-1 adhesion molecules; HIV infection also increases the expression of inducible nitric oxide synthase, probably involving the NFkB transcription pathway [125]. These factors induce a status of oxidative stress and lead the way to
metabolic alterations, targeting lipid metabolism and platelet aggregation [75]. Over time, HIV-induced endothelial and lipid damage may lead to develop lesions similar to those observed in early atherosclerosis [138].

**Mimicry: When Form Shapes Activity**

In local perturbed micro-environment, conformational changes in self proteins may easily occur, due to the generation of fragments as well as to the protein exposure to lysosomal or peroxysomal enzymes from phagocytes, and to acidic and oxidizing conditions. Cleavage and/or unmasking of hydrophobic epitopes may lead to explore new, thermodynamically-allowed conformations, able to trigger immune responses. For instance, anti-CD4 antibodies, found in HIV-infected patients or in exposed uninfected subjects, can recognize gp120 binding site as well as other cryptic epitopes, that are usually hidden from the surface of molecule [22, 21].

Several microbial and viral proteins share sequence homology and/or tri-dimensional conformation with cell proteins, receptors and molecules [143, 142]. HIV antigens also present similarities to surface molecules found on immune cells. V3 loop domain from HIV-gp120 shares homology with a conserved fragment of Vbeta domain of T immune cells. V3 loop domain from HIV-gp120 shares also present similarities to surface molecules found on proteins, receptors and molecules [143, 142]. HIV antigens homology and/or tri-dimensional conformation with cell molecule [22, 21].

Epitopes, that are usually hidden from the surface of particles and become part of viral envelope. It is still unclear whether this phenomenon constitutes the molecular basis for allo- and autoreactivity seen in HIV-infected patients [66, 105, 24].

HIV particles were also shown to bud from membrane domains other than rafts, such as the exosome pathway [122, 132, 114]. Exosomes are lipid aggregates similar to liposomes, which have been observed in cultured cells belonging to immune lineages and in professional antigen presenting cells, such as macrophages and dendritic cells [160, 1, 170]. These findings lent support to a recent, provocative theory, the Trojan exosome hypothesis, suggesting that HIV also uses exosome pathway as a secondary - but not trivial - way to infect host immune cells and to escape immune surveillance successfully [56].

**Sleeping Beauty: The Role of Endogenous Retroviruses**

Many findings, included the occurrence of autoimmune-like symptoms in retroviral infection, support the putative role of endogenous retroviruses (ERV) in immune regulation and in immune-related diseases [76].

ERV mobile genetic elements, sharing similarity to transposons and to retroviruses, are widely and abundantly distributed in the genome of all vertebrates - man included - and in the genome of many invertebrates, such as Drosophila melanogaster, accounting for up to 1% of the whole genomes dimensions [118]. ERV elements usually display a LTR-gag-pol-env-LTR organization, but most of them are defective in some genes or interrupted by stop codons. Some ERV are nevertheless transcribed and express proteins or whole retroviral-like particles in a tissue-specific (e.g., placenta) or stage-specific (embryo development) manner [159]. Human ERV RNAs have been also detected in PBMC from a cohort of normal donors; moreover, one or more lymphocyte mitogen agents elicited ERV transcription in a few hours [76].

Natural, neutralizing antibodies to ERV proteins have been observed in mouse models [58] and, more strikingly, in healthy individuals [78]. Humoral responses to ERV have been also found in sera from patients suffering from autoimmune diseases, such as Sjogren’s syndrome and systemic lupus erythematosus (SLE), suggesting that ERV can be actually transcribed and expressed also in humans [13].

ERV surface glycoproteins exert a protective role to retroviral infections in mice, presumably competing with exogenous retroviral particles for receptor binding [65]. This finding might explain why ERV sequences are widespread and conserved in many vertebrates. Moreover, the presence of many ERV sequences homologue to HIV env genes in human genome also suggests that endogenous ERV gene products might protect host from retroviral infections via viral interference [20].

Mouse ERV, similar to the MMTV exogenous oncovirus, have been shown to express a superantigen, Mls-1, involved in immune (dys)-regulation [63]. Superantigens (SAg) from bacteria or viruses are known to induce rapid, polyclonal T and B activation, followed by anergy and/or lymphocyte depletion. In fact, ERV-encoded SAgS are expressed in the thymus and take part to negative selection of reactive T cell clones, through the intervention of professional APC, such as
dendritic cells and B lymphocytes [5, 106]. Priming of naïve mice with SAg-expressing cells first induces the activation of T cells expressing a specific V-beta TCR chain, followed by T cell tolerance and polyclonal B cell activation, leading to IgM and IgG production [107]. Experiments carried out in a mouse model of autoimmune disease, the pre-diabetic NOD mouse, have shown that IFN-alpha expression, following a viral infection, induces the expression of a SAg encoded by the polymorphic family of HERV-K18 endogenous retroviruses [106]. Expression of SAg can be therefore mediated by inflammatory stimuli, such as IFN-alpha, and this finding provides a link between inflammation and viral infections, suggesting a possible mechanism for autoimmunity onset [153].

ERV may also exert a mutagenetic role in immune (dys)regulation, through insertional mutagenesis or trans-activation of flanking genes. Antibodies to an ERV-encoded, nuclear protein of 28 kDa (p28), were commonly found in SLE patients. The p28 antigen is expressed by HRES-1 locus, a single copy ERV element placed on human chromosome 1. HRES-1 presents a HindIII polymorphism, inherited in a Mendelian fashion. One of the polymorphic forms of HRES-1 has been found more frequently in SLE-susceptible families and is therefore considered a candidate gene involved in SLE onset [98].

Mouse LTR elements have been shown to trans-activate immune-related genes, such as CD4, TCR, MHC class I [76]. Human LTR elements have been identified in HLA-DRB1 locus from patients affected by rheumatoid arthritis and in type-1 diabetes. Disease association with some homozygous HLA haplotypes supports the association of LTR integration with an increased susceptibility to autoimmune diseases [130].

A TWO-HITS MODEL TO EXPLAIN HIV-AUTOIMMUNITY CONNECTION

Similarly to the model explaining the onset of type-1 diabetes and other autoimmune diseases, a “two-hits” model for allo- and auto-immune responses to HIV can be drawn. Expression of ERV proteins or SAg and ERV-mediated membrane trafficking may induce the generation of low-affinity, allo- and/or autoimmune responses to self-proteins in a subset of genetically susceptible individuals, by a number of molecular mechanisms involving mutagenesis, molecular mimicry or immune dysfunction. Albeit anti-self responses are usually promptly quitted, primed allo- and auto- T and B cell clones are maintained lifetime. Under a subsequent immune stimulus, such as inflammation, oxidative stress or viral infection, anergic T and B cells can be reactivated, generating high-affinity antibodies to allo- and self molecules that trigger auto-immune responses or overt autoimmune diseases. The association of HLA, CD4 and co-stimulatory molecules to HIV particles, occurring in budding from lipid rafts or through exosome trafficking, may add further stimulus to autoimmune responses, providing uncommon, cryptic self-epitopes to be exposed and presented. This hypothesis designs two possible molecular scenarios, likely to steer immune response to HIV in susceptible and in resistant subjects.

The majority of at risk subjects are likely to carry an HIV-favourable haplotype or combined genetics/epigenetics factors of susceptibility; furthermore, they possibly lack a protective ERV interference. In these people, HIV infection and replication take probably place in a fast, exponential fashion, giving rise to an overwhelming immune stimulus and to immune dysfunction. Nonetheless, even a stealth entry of HIV, too weak to prime and activate APC and helper cells properly, could go unnoticed and prevent the development of a protective response.

On the other hand, naturally HIV-resistant individuals could have undergone a previous immune priming, due to ERV proteins or to other suitable stimuli; this early event generated a pool of T helper and B memory cells, able to trigger immune responses to uncommon and even allo- and anti-self antigens [136]. Once exposed to HIV, these subjects readily mount a protective response, addressed to allo- and self antigens associated with viral particles; these latter are awkward targets, usually poor or no immunogenic at all for the majority of the population, due to the negative selection of lymphocyte repertoires and/or to the lack of proper stimuli [70]. Other factors indeed, such as the local environment, the network of cytokines and soluble messengers, the presence and the extent of pro-inflammatory stimuli and, not least, timing, quality and quantity of viral antigens, are likely to play a significant role in the development of HIV-resistance [91].

As suggested in the two-hits model, natural allo- or auto-immune responses, able to neutralize HIV, have been observed in few cohorts of infected or exposed subjects [57]. The atypical occurrence of some neutralizing antibody specificities in vivo may be conveniently explained by deletion/anergy of specific auto-reactive B and T cell clones in the majority of individuals [61]. Moreover, this finding might also explain why nearly all vaccine strategies have failed in eliciting effective virus neutralization in most vaccinees [18].

Passive administration of human monoclonal antibodies has recently proven its HIV-neutralizing potential in patients, preventing the rebound of viral load after ART interruption [167]. Strikingly, some of the rare human neutralizing antibodies are poly-specific auto-antibodies to host molecules. For instance, the human monoclonal antibodies 2F5 and 4E10, that cross-react with cardioplin [61], and the anti-CCR5 antibodies, recognizing the first external loop of the co-receptor, found exclusively in highly exposed healthy individuals or in some HIV-positive patients, share a very long hydrophobic third complementarity-determining regions (CDR3 domain) [90, 25]. CDR3 structures are typical of natural auto-antibodies and have been found in self-reactive antibodies to HIV in humans and primates [8, 172, 174].

CONCLUSIONS

The occurrence of natural allo- or auto-responses in healthy individuals, without signs or symptoms of autoimmune disease, as well as the possibility of eliciting and maintaining strong and long-lasting neutralizing antibodies in animal models, suggest that some mechanisms of autoimmunity may be successfully exploited to confer a better protection or a stronger response to HIV in at risk, exposed subjects as well as in HIV-positive people. Further studies will be required to investigate the HIV-autoimmune
connection and to define safe and effective targets and successful strategies of intervention. Allo- and auto-immune responses could offer an unexplored key to understand HIV tricks in immune escape and provide unexploited strategies to fight HIV with its own weapons.

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