



HIV-1 induced AIDS is an Allergy and the Allergen is the Shed gp120 – a Review, Hypothesis, and Implications

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Abstract. The possibility that the induction of T helper 2 (Th2) cytokine synthesis and the gradual increase in interleukin 4 (IL-4) and IgE levels during HIV-1 infection are an allergic response to HIV-1 proteins was raised in the author's previous article [Becker, *Virus Genes* 28, 1–4, 2004]. The present review extends this hypothesis by citing experimental reports which indicate that HIV-1 shed gp120 virions share a striking resemblance with the allergens that bind to IgE molecules bound to Fc epsilon receptor I-positive (FcεRI) cells (mast cells, basophils, monocytes, and dendritic cells, DC) and then induce them to release and synthesize the IL-4 cytokine. In the earlier review, it was established that the IL-4 cytokine is responsible for the following processes: IgE synthesis by B cells and the inhibition of antiviral IgG synthesis; the inactivation of T helper 1 (Th1) cells; and the inhibition of the antiviral cytotoxic T cell (CTL) response. The binding of the shed gp120 to B cell-bound immunoglobulin (Ig) depletes these cells. Moreover, it was reported that the viral regulatory proteins Tat and Vpr also stimulate IL-4 release from basophils. The mode of action used by HIV-1 gp120 in the induction of IL-4 [Becker, *Virus Genes* 28, 1–14, 2004] revealed that the viral protein contains a superantigen (SAg) domain that is capable of binding to the V_H3 domain of IgE and Ig; namely, the shed gp120 protein act as endogenous and environmental allergens that bind to IgE molecules, which are bound to the FcεRI receptors on hematopoietic cells. Based on these findings, it is hypothesized that gp120 is an allergen. Consequently, it can be inferred that the active replication of HIV-1 in infected individuals constantly exposes the immune system to an increase in the allergen content until the host immunity is eventually compromised. These finding suggest that HIV-1 engage in a two-pronged attack of the human immune system: it infects Th2 cells, macrophages, and DCs and then replicates at these venues; and the shed gp120 cells cause an allergy. This allergic condition consequently prevents the induction of Th1 cells, cytokine synthesis, and antiviral CTLs that are needed to clear the infecting virus and thus devitalizes the antivirals that are used to treat the virus infection. It was hypothesized [Becker, *Virus Genus* 28, 1–14, 2004] that the HIV-1 induced allergy can be treated with IL-4 inhibitors or immune response modifiers. A treatment that employs both antivirals and anti-allergen drugs may very well defeat the AIDS syndrome. Another implication of the present hypothesis is the need to improve the viral antigen used for vaccinating healthy individuals against HIV-1 infection. It is thus suggested harmful domains be eliminated from the gp120.

Key words: AIDS syndrome, endogenous allergens, hematopoietic cells, HIV-1, HIV-1 gp120, IgE/ FcεRI complex, IgE V_H3 domain, IL-4 cytokine, gp120, superantigen domain

Introduction

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HIV-1 infection in humans is a retrovirus infection
for which antiviral drugs have been developed to

inhibit the virus replication and gradually deplete the CD4⁺ T cells. Despite these efforts, the virus infection continues to engender a life threatening immune deficiency. Researchers have yet to discover how HIV-1 infection propagates AIDS? Since the appearance of patients infected with the acquired immune deficiency syndrome (AIDS) in Paris, Brussels, East Africa, and San Francisco some 20 years ago [1], this question has haunted retrovirologists, immunologists, and vaccinologists for the last 20 years. The isolation of HIV-1 and HIV-2 in 1983 stimulated molecular biological research on the virus as well as subsequent efforts to develop anti-HIV-1 vaccines. To date, the vaccination efforts using the HIV-1 gp120 envelope subunit have failed to protect humans against the virus infection. After two decades of robust research on HIV-1 and AIDS, including the publication of over 40,000 articles, the number of HIV-1/AIDS victims continues to rise [2].

The proliferation of international terrorism has heightened concerns that the smallpox virus might be used as a weapon against civilian populations. These fears have renewed the need for the mass manufacture of smallpox vaccines. However, health care officials raised the issue of the dangers involved in vaccinating individuals with impaired immune systems, such as HIV-1 patients and individuals undergoing cancer treatments [3]. While the vaccinia virus causes a mild skin infection in healthy individuals, it can cause lethal infections in people with allergies [4].

To solve the vaccinia virus enigma, it was necessary to understand the role of allergens in the pathogenesis of allergy. Advances in the fields of allergy [5,6] and cytokine control of the human immune system [7] promise to eventually uncover the reasons behind vaccinia virus pathogenicity. Recent studies have shown that the induction of an allergy by environmental and endogenous allergens depends on the particular superantigen (SAg) domain in the protein that is defined as a superallergen. Allergens that enter the human body bind to IgE molecules, which are produced in minute quantities by healthy individuals. These IgE molecules are bound to Fc epsilon receptor I (FcεRI) on the plasma membrane of hematopoietic cells (basophils, mast cells, monocytes, and dendritic cells, DCs) [8]. These cells respond to the allergen by synthesizing and releasing the cytokine inter-

leukin 4 (IL-4), which is the major factor employed by allergens to skew the host immune response [9,10]. As the levels of IL-4 gradually rise, the following changes occur: (1) IL-4 polarizes T helper 2 (Th2) cells to produce IL-4, IL-5, and IL-13 pro-inflammatory cytokines; (2) B cells synthesize IgE molecules; (3) IL-4 inhibits cytokine synthesis by T helper 1 (Th1) cells; and (4) IL-4 inhibits the precursors of cytotoxic T cells (CTLs). Thus in allergic individuals, the vaccinia virus finds a host that lacks its humoral and cellular defenses and the virus can spread to vital organs. In fact, the inclusion of HIV-1 infected people in the list of populations prohibited from receiving the smallpox vaccination alluded to the fact that HIV-1 induced AIDS might resemble an allergy.

The first concrete indication that HIV-1 infection resembles an allergy was the discovery [11,12] of increased IgE levels in the sera of HIV-1 patients. In 1990, Wright et al. [13] reported that "HIV-1 infection is characterized by a progressive depletion of helper T lymphocytes and like allergic diseases is associated with altered T cell regulation." Three years later, Clerici and Shearer [14] hypothesized that AIDS is caused by a switch from Th1 to Th2 cytokine activities. The analysis of the additional publications on the skewing of the immune responses in HIV-1 infected individuals enhances the argument that the AIDS stage of HIV-1 infection resembles the symptoms of an allergy [15,16]. On the basis of these studies, Becker [1] hypothesized that HIV-1 induced AIDS can be effectively treated with IL-4 inhibitors and/or immune response modifiers (Th2 → Th1 reversion) in addition to treatment with antivirals.

The present review cites studies on the shed viral gp120 molecules from virions and their impact on the patient's immune system, which facilitates a lucid comparison between the modes of action of HIV-1 gp120 and allergens in the induction of allergy. It was reported that the shed viral gp120 as well as the regulatory Tat and Vpr proteins induce IL-4 synthesis by binding to FcεRI⁺ hematopoietic cells. Consequently, it is hypothesized that the shed viral proteins act as an allergen that causes AIDS in infected people. The shed viral proteins skew the infected person's immunity by the same mechanism with which environmental allergens cause immune deficiency in allergy. The implications of these findings will be discussed below.

Additional Reports on Enhanced IgE Synthesis in HIV-1 Infected Individuals and its Resemblance to Allergen-induced IgE Synthesis in Allergic People

Paganelli et al. [15] studied nine HIV-1 infected patients who had hypereosinophilia and hyperimmunoglobulinemia E (hyper IgE) with chronic dermatitis and recurrent staphylococcal infections. Their immunological characterizations were compared to 51 HIV-1 positive patients without hyper-IgE, both atopic and nonatopic, and three patients who were affected by primary hyper-IgE syndrome and exhibited an increase in IgA levels and a severe decrease in B and CD4⁺ lymphocytes. The authors concluded that the hyper-IgE syndrome is a clinical manifestation of HIV-1 infection and the presence of hypereosinophilia suggests that hyper-IgE patients may have elevated levels of IL-5 cytokine.

de Martino et al. [16] investigated the relationship between IgE levels, IL-4 synthesis, and IgG, IgA, and IgM levels in children with prenatal HIV-1 infections. Their results suggest that distinct mechanisms may underlie the overproduction of IgE and IgG antibodies, while the contribution of the IgG-3 and IgG-4 subclasses to the IgG pool is marginal. In a subsequent paper, de Martino et al. [17] studied the effect of elevated IgE levels that result from the Th1 to Th2 switch and its association with an advanced stage of the disease. The study group included 54 children – 29 males and 25 females – between the ages of 13–142 months. The 15 children (27.7%) with elevated IgE levels had lower IgG-3 levels and higher IgG-4 levels than the other children. The authors did not associate the elevated IgE levels with allergy.

The IgG-3 immunoglobulins (Ig) are highly efficient, complement-fixing, and virus-neutralizing antibodies. HIV-1 patients with elevated IgE, such as prenatally infected children, had low IgG-3 and high IgG-4 counts. As the disease progressed, HIV-1 patients with elevated IgE failed to produce IgG-3 antibodies, but IgG-4 antibodies, which are specific to HIV-1 antigens, have no known role in the defense against HIV-1 infection and may increase during disease progression [18].

Pellegrino et al. [19] identified a group of long-term pediatric survivors who acquired HIV-1 via maternal transmission and received antiretroviral therapy. As of 2002, they were in good health, free

of opportunistic infections, and have thrived despite the decreased number of CD4⁺ T cells (<500/mm³). Moreover, all the children had elevated levels of anti-HIV-1 IgE serum: 20% (n = 16) of the 75 children exhibited elevated IgE in serum levels; 11 of the children had elevated anti-gp160 and anti-p24 IgE antibody levels; five children generated anti-gp120 IgE; and another five children generated IgE that was directed at non-HIV-1 specificities. Only one child exhibited increases in all the serum Ig.

Kelleher et al. [20] reported that in infected mice, the Rauscher leukemia virus (RLV) induces DCs to produce Th2 cytokine IL-4 instead of Th1 cytokine IL-12. This study also revealed that infecting mice with a pathogenic retrovirus similarly alters the Th1–Th2 cytokine balance.

The Shed HIV-1 gp120 Damage the Host Immune System

The Cleavage of HIV-1 gp160 by Cellular Precursor Convertases Enables the Shedding of gp120 from Virions

The HIV-1 env gene codes for the glycoprotein gp160 that is comprised of two parts: gp120 molecules that have specific sequences for the attachment of virions to cellular receptors; and the membrane anchored gp41, which is responsible for the fusion of the virion's membrane to the infected cell membrane. To enable the gp41 fusion sequence to fuse with the cell membrane, the gp160 is cleaved during the assembly of the newly formed virions in infected cells in order to facilitate the release of gp120.

Decroly et al. [21] reported that the cleavage of gp160 is an intracellular proteolytic process that is an essential step for virus infectivity. Furin and PC1 are expressed in the CD4⁺ human lymphoblastoid H9 cell line. Purified PC1 and furin cleave the viral gp160 into gp120 and gp41 under *in vitro* conditions. The cleavage occurs within the sequence Arg-Glu-Lys-Arg ↓ Ala-Val-Gly-Ile. Similar results were reported by Moulard [22]. Oliva et al. [23] reported that furin and other precursor convertases cleave the gp160 at the carboxyl side of the sequence Arg 508-Glu-Lys-Arg 511 (site 1), despite the presence of another consensus

sequence, Lys 500-Ala-Arg 503 (site 2). The authors analyzed a 19-residue synthetic peptide, p498 – spanning gp160-processing sites 1 and 2 – that is digested by furin at site 1. A molecular model for p498 was obtained by molecular dynamic simulations that show the peptide N-terminal side, which represents a 9-residue helical segment that encloses site 2. The C-terminal segment, a loop that exposes processing site 1, has easy access to the furin catalytic site 2.

Staropoli et al. [24] expressed trimeric envelope glycoproteins from three primary macrophage-tropic HIV-1 isolates grown in BHK cells and analyzed the furin cleavage. As detected on polyacrylamide gels, the envelope glycoprotein was secreted in a soluble form and deleted both its transmembrane anchor and the intracytoplasmic domain. Sucrose gradient centrifugation revealed that the trimers and dimers were uncleaved gp140 molecules, while gp120 were monomeric. To analyze the effect of the cleavage of gp140 into gp120 and gp41 on trimerization, the authors co-expressed furin and gp140 genes in transfected cells. It was reported that furin expression changed the subcellular localization of the glycoprotein, which was sequestered in the major furin compartment at the trans-Golgi network, and that the secreted gp120 molecules were monomeric. The binding of gp140 trimers to CD4 was reduced in comparison to the gp120 monomers that did not bind to the co-receptor CCR5.

These studies revealed that viral gp160 is synthesized and assembled into trimers during the intracellular transport of these proteins to the outer cell membrane. At the post-Golgi network, furin and coxetases cleave gp160 into gp120 monomers, which remain in a noncovalent association with the virion membrane-anchored gp41. The study by Staropoli et al. [24] seems to indicate that gp120 monomers are shed from virions and may find target cells in the serum and lymph nodes. It is possible that the damage to the host immune system and the AIDS begins with the shedding of gp120.

The Mechanisms by which HIV-1 gp120 Evades the Host Cellular and Humoral Immune Responses

It was hypothesized that HIV-1 uses the “Th1 → Th2 switch” [14] as a mechanism by

which to evade the infected host immune response. Numerous studies [review in reference 1] revealed that the CTL response to HIV-1 is initiated after infection, but an increased level of the Th2 cytokine IL-4 aborts this antiviral response. In addition to its ability to inhibit CTLs and their precursors, IL-4 abrogates the synthesis of IgG-3 – the anti-HIV-1 neutralizing antibodies – and induces B cells to synthesize antiviral IgE molecules. HIV-1 inhibits the patient’s innate, cellular, and humoral immune defenses and thus renders the infected individual defenseless against viral and microbial infections and AIDS. The ability of HIV-1 to evade the host’s protective immunity by inducing the synthesis of IL-4 also disarms the host’s ability to generate the endogenous antiviral polypeptide defensins that inhibit HIV-1 replication [25].

Hart et al. [26] reported that the binding of soluble CD4 protein to HIV-1 virions that bud from infected cells stimulated the release of gp120 molecules from the virions. This finding suggests that following infection and during the interaction of CD4⁺ T cells with the donor’s infected cells, gp120 molecules are shed from the virions. During infection of CD4⁺ T cells, the virions’ gp120 attach to both CD4 receptor molecules on T cells and the co-receptor CCR5 by its specific binding domains. After the virus is anchored to the receptor and co-receptor, the virions’ gp41 fusion sequence is introduced into the outer membrane of the infected cell, and the fusion of the virion membrane with the infected cell membrane introduces the virion core, containing two viral genomes and the attached reverse transcriptase molecules, into cellular vesicles that transport them into the cytoplasm. Consequently, gp120 molecules may be released from virions during the receptor binding process. It may be inferred that as the virus content in the serum and lymph nodes gradually rise during infection, shed HIV-1 gp120 molecules damage the innate, cellular, and humoral immune defenses of the infected host.

The induction of Th2 cytokine synthesis during an HIV-1 infection resembles the human response to environmental allergens, except that proteins with “allergenic domains” in the polypeptide sequences – deriving from manifold sources – induce the synthesis of IL-4, IL-5, and IL-13 cytokines. In comparison to allergens, it has been hypothesized

[1] that gp120 (and additional viral proteins) have “allergen-like domains” in the polypeptide sequence, which are capable of binding to different cell types in HIV-1 patients, and stimulate the expression and release of IL-4. Recent experimental evidence clearly shows that the HIV-1 viral gp120 that inhibit the immune system utilize the same mode of IL-4 induction that is employed by allergens.

The Mode of Action of gp120 used to Induce Th2 Cytokine Synthesis

Shed gp120 Molecules Induce Human FcεRI⁺ Cells to Produce IL-4 and IL-13 through Interaction with the IgE V_H3 Sequence

Patella et al. [27] reported that HIV-1 gp120 molecules, which derive from different clades, constitute potent stimuli for the release of cytokines IL-4 and IL-13 from purified basophils in healthy seronegative individuals. IL-4 mRNA levels, which are constitutively present in basophils, were increased after stimulation by IL-4. The synthesis of IL-4 mRNA was inhibited by cyclosporin A and Tacrolimus. The authors demonstrated that HIV-1 gp120 interacts with the V_H3 domain of IgE that is bound to the IgE receptor FcεRI on the surface of basophils, and that the stripping of IgE from the cell surface by low pH prevented gp120 from executing its IL-4 stimulatory effect.

The authors concluded that the interaction of gp120 with the V_H3 domain of IgE that is bound to the IgE receptor FcεRI on the surface of human basophils and mast cells is responsible for the synthesis and release of IL-4 and IL-13, two cytokines that are critical to the polarization of Th2 cells. The authors stated that, “Because HIV-1 enters the body predominantly through mucosal surfaces and because the early phases of infection are associated with high levels of viremia, mast cells, and basophils can be exposed to shed or virus-bound gp120. This suggests that FcεRI⁺ cells might be a novel source of Th2 cytokines, thus contributing to the dysregulation of the immune system in HIV-1 infection ... In fact, it highlights the importance of a specific viral SA in

gp120, acting on cell types other than lymphocytes in the production of Th2-like cytokines.”

Using purified human basophils, Florio et al. [28] reported that the shed gp120 molecules bind to the V_H3 domain of Ig. The human V_H3 gene that encodes Ig is one of seven members (V_H1–V_H7) of a human gene family that predominates (>50%) the human repertoire. The authors suggested that virion-associated or shed gp120 [29] constitute the initial inducer of IL-4 favoring the Th1 → Th2 switch [14]. Shin et al. [30] reported that the gp120 binding to the V_H3 domain of IgE is identical to the binding of allergens to FcεRI-bound IgE [30].

FcεRI on DCs Delivers IgE-bound Multivalent Antigens to the Cathepsin S-dependent Pathway of MHC Class II Presentation

Maurer et al. [31] elucidated the knowledge on the FcεRI-mediated uptake and the presentation of antigens by DCs. The FcεRI-bound IgE (together with a bound antigen) is efficiently internalized into acidic proteolytic compartments. The antigen is degraded and delivered into organelles containing MHC class II, HLA DM, and lysosomal proteins. In order to keep track of the fragmented antigenic ligand, the authors interfaced with the degradation of the invariant chain (Ii), which is essential to the folding of the MHC class II molecules and the loading of peptides into the molecules' peptide-binding groove. The DCs were found to express cathepsin S, a cysteine protease involved in Ii degradation. Instead of being delivered into a recycling MHC class II pathway, the multimeric ligands of FcεRI are channeled into MHC class II compartment-like organelles of DC in which both cathepsin S-dependent Ii processing and peptide loading of newly assembled MHC class II take place.

The findings of Maurer et al. [31] explain the ability of DCs to present MHC class II molecules that carry peptides from HIV-1 gp120, p24, and p17 to Th2 cells that present antigens to B cells. In the presence of IL-4, the induced B cells synthesize IgE molecules against HIV-1 antigens.

Liu et al. [32] attempted to determine the initial source of IL-4 during the early immune response and reported that IL-10 induced the DC-primed naïve T cells to secrete IL-4.

Increased Expression of FcεRI by Peripheral Blood Monocytes in AIDS Patients and the Effect of gp120 on IgE Synthesis

Miller et al. [33] focused their study on the expression of FcεRI II (CD23) in the blood monocytes of AIDS patients, since this IgE receptor is expressed by B cells, T cells, natural killer cells (NK), follicular DCs, Langerhans cells (LC), eosinophils, keratinocytes, and platelets. Monocytes express increased levels of CD23 when encountering inflammatory and allergic diseases such as acute infectious mononucleosis, atopic dermatitis, asthma, allergic rhinitis, progressive systemic sclerosis, rheumatoid arthritis, systemic lupus erythematosus, and Kawasaki disease. The cross-linking of monocyte CD23 increases the production of Th2 cytokine IL-10, which contributes to the switch in the cytokine profile from protective Th1 cytokines (IL-2, IL-12, and IFN-γ) to Th2 cytokines (IL-4, IL-10). The authors reported that blood monocytes from AIDS patients express higher CD23 levels than those found in healthy HIV-1 seronegative individuals. Comparison of monocyte CD23 with the expression of monocyte Fcγ (CD16, CD32, CD64), and the plasma levels of IgE, IgM, IgG, IgA, Th1 (IFN-γ), and Th2 (IL-4, IL-10) cytokines revealed that CD23 expression can be correlated with monocyte CD-16 expression. This phenomenon was prominent in AIDS patients and not in other tested parameters. In fact, the serum from AIDS patients contained three times the amount of IL-10 than the controls.

Dugas et al. [34] studied the mechanisms implicated in the increased production of IgE in HIV-1 patients and examined the involvement of viral gp160, gp120, and gp41 in the increase of IgE. The study's objective was to evaluate the regulatory effects of HIV-1 gp120 on IgE production by normal human peripheral blood mononuclear cells and B-lymphocytes, as well as the role of nitric oxide (NO) in this process. Their experiments showed that gp120 specifically potentiated in a dose-dependent manner IL-4 induced IgE production. It was discovered that in the presence of Th2 cytokine, which is upregulated during HIV-1 infection, only gp120 stimulated the production of IgE. However, in the absence of IL-4, gp120 induces IgG synthesis and to a lesser extent IgA and

IgM synthesis. IgE synthesis is primarily controlled by IL-4 and by the interaction of the CD40 ligand (CD40L) with CD40 on T and B lymphocytes and is modulated by the expression of FcεRI by IL-4 stimulated B lymphocytes. The authors suggested that gp120 molecules are capable of stimulating B lymphocytes with their SAg domain after binding to their membrane IgM (see below). To test this possibility, the authors used purified B lymphocytes, some of which were stimulated with IL-4 and an anti-CD40 monoclonal antibody to induce isotype switching. It was found that gp120 did not modify the amount of IgE produced by these cells, which suggests that gp120 had an indirect effect. Furthermore, the authors reported that in addition to gp120, gp160, but not gp41, also potentiated B cells to produce IgE. HIV-1 gp160 was similarly found to stimulate B cell differentiation as reported by Chirmule et al. [35]. The authors showed that after stimulation by gp160, the differentiation of B lymphocytes into Ig secreting cells was T cell dependent. Dugas et al. [34] discovered that the interaction of gp120 with IgM on the surface of B cells did not modify IgE production. Consequently, it can be inferred that the binding of the gp120 SAg domain to the V_H3 domain of Ig is not connected to the induction of IgE synthesis. Since the switch from IgG synthesis to IgE synthesis is induced by IL-4, NO protects B lymphocytes against cell death.

HIV gp120 also Damage B and T Cells

The Binding of the HIV-1 gp120 "SAg" Domain to the V_H3 Sequence of Nonimmune Ig on B Cells Causes a Precipitous Decline in B Cells

Goodglick et al. [36] studied the SAg domain in gp120 that binds to nonimmune Ig on B cells. In addition, Berberian et al. [37] reported that HIV-1 gp SAg domain binds to the V_H3 domain of Ig that are present on 4–6% of the endogenous B cells that are activated under *in vitro* conditions, which resemble the activation and depletion of B cells. The authors [36] used a panel of gp160 peptides and examined which peptides inhibit the SAg binding domain to six different Igs. Only one peptide epitope from the sequence of gp120 (aa 231–250) was found to bind to all the Igs that were

studied, and a second C2 peptide (aa 241–260) bound to five Igs.

Juompan et al. [38] and Zouali [39] studied the selective deficit in antibodies specific to the SAg-binding site of gp120. The SAg-like properties of HIV-1 gp120 for B cells allows for an unconventional mode of interaction that differs from the binding to immunization-induced antibodies, which requires a tertiary structure of the heavy and light chain variable regions. The authors examined the temporal development of V_H3^+ antibodies specific to gp120. They identified the interaction of the gp120 SAg sequence with amino acid sequences present in the first and third domains of the Ig heavy chain, namely the V_H3 site. The gp120 residues partly overlap with the *Staphylococcus aureus* protein A (SpA) binding site for V_H3^+ Igs [39]. Chirmule et al. [35] suggested that the binding of gp120 to B cell Ig molecules via the SAg domain causes humoral deficiency and B cell death by apoptosis. The authors stated that the, “finding suggest that epitopes within the gp120 SAg binding site to Ig should be excluded from candidate vaccine preparations.”

Silverman [40] reviewed the properties of B cell SAg proteins that are endowed with unconventional Ig-binding capacities similar to T cell SAg: namely, *Staphylococcal* protein A (SpA), HIV-1 gp120, and protein Fv (pFv), a human gut-associated sialoprotein. The binding of a SAg is dependent on the variable (v)-gene segment contribution from a single chain of the heteromeric antigen receptor. The SpA SAg interacts with most heavy chains from the human V_H3 gene family and contains a unique binding site that is distinct from the Fc-binding site. The authors quoted studies that have demonstrated the existence of progressive V_H3 deficits in HIV-1 patients at the mRNA and Ig levels. Characterization of the V_H3 family specificity of gp120 from many HIV-1 isolates [37] seems to indicate that retrovirally encoded B-cell SAg has a role in the pathogenesis of the infection-associated immune defect.

Karry and Zouali [41] discovered that, upon binding to the V_H3 domain, HIV-1 gp120 envelope glycoprotein is capable of cross-linking the IgM to the membrane on normal B cells and inducing their activation. Consequently, the authors identified the sequence motifs on gp120 that are involved in the binding of SAg to IgM. They showed

that the SAg-binding activity occurs in the gp120 of highly divergent HIV-1 isolated clades from various geographical regions and that carbohydrate residues are impertinent to the binding process. The authors identified a discontinuous epitope that spans the gp120 V4 variable domain and the amino-terminal region that flanks the V4 constant domain. aa 392–434 are essential to the binding with the most critical being Leu³⁹⁵, Asp³⁹⁷, and Ile⁴²⁵–Gln⁴²⁷. Residues from C2 constant domain aa 252–272 also appear to play an accessory role in the binding of gp120 SAg to normal human Igs.

The authors suggested that the ability of gp120 to bind to IgM on B cells and diminish the amount of B cells gradually strengthens as the disease progresses. In a subsequent publication, Karry et al. [42] reported that the gp120 SAg can only interact with a subset of the human V_H3^+ Ig that can convey binding to the prototypic bacterial B cell SAg protein A from the *Staphylococcus aureus*.

T cell Activation by SAg

Silverman [40] indicated that proteins with SAg domains bind to T cell receptors (TCRs), without processing into peptides, at a sequence of the major histocompatibility complex (MHC) outside the peptide-binding grooves.

Li et al. [43] reviewed the process in which T cells are activated via the simultaneous interaction of the SAg with the $V\beta$ domain of the TCR and MHC class II molecules on the surface of an antigen-presenting cell. The available knowledge on the three-dimensional structure of bacterial SAg, their complexes with MHC class II molecules, and the TCR β chain, provide a framework for understanding the molecular basis of polyclonal T cell activation.

Ghering et al. [44] developed a new Th1/Th2 *in vitro* model with which to study SAg activation of T cell clones that express TCR. The presence of IFN- γ and IL-4 positive cells with $V\beta$ -chains helped determine that these are the only cell clones that can be shifted towards Th1 or Th2 differentiation. Th1 shifts were achieved in the presence of IL-12 and anti-IL-4 antibodies, while Th2 shifts were executed at a slower pace in the presence of IL-4, IL-6, and anti-IL-12 antibodies. The authors

concluded that their model could be useful for the investigation of early and late events in normal and abnormal T cell differentiation.

The Binding of Shed gp120 Molecules to FcεRI-bound IgE on DCs Resembles the Binding of Allergens to FcεRI-bound IgE

Mode of Action of gp120 in the Evasion of Human Immunity

Secord et al. [45] studied 26 HIV-1 positive children (with intrapartum or transplacenta infections) between the ages of eight and 11, who were in good health, despite diminished CD4⁺ T cell counts. The authors detected elevated serum IgE levels in seven of the children. The IgE antibodies were anti-HIV-1 p24, anti-gp160, and anti-p17 suggest that these viral proteins formed complexes with IgE bound to FcεRI on DCs were processed and presented to Th2 cells and B cells as antigenic peptides. Removal of IgE from the children's serum prevented the synthesis of anti-HIV-1 IgE molecules. The authors indicated that, "the role of IgE molecules that are produced by the long-term pediatric survivors . . . remains to be defined."

IgE molecules that are prevalent in uninfected people at a very low level, bind to FcεRI molecules on DCs. After HIV-1 infection and the shedding of gp120 DCs may serve as receptors for the shed viral molecules during the earliest stages of a primary HIV-1 infection. The binding of gp120 to IgE triggers the following events:

1. DCs respond to the binding of the shed gp120 by inducing the synthesis and release of IL-4.
2. DCs deliver IgE-bound viral antigens (gp120, p24, and p17) into a cathepsin S-dependent MHC class II pathway [31]. Instead of being delivered into a recycling MHC class II pathway, the viral ligands are efficiently channeled into MHC class II compartment-like organelles in which cathepsin S-dependent Ii processing and viral peptide loading to the newly assembled MHC class II molecules takes place. Antigenic sequences of gp120 as well as additional viral antigens are then presented by DCs to Th2 and B cells in the presence of IL-4.
3. The binding of gp120 to IgE bound to FcεRI on mast cells, basophils, and monocytes induces

the synthesis and release of IL-4 and IL-13 cytokines and the polarization of Th2 cells.

4. The finding by Secord et al. [45] that IgE with anti-gp160, anti-p24 or anti-p17 specificities existed in the tested children's sera suggests that these viral structural proteins have antigenic domains for binding to IgE.

Mode of Action of Allergens in the Inhibition of the Human Immune Response

The ability of the endogenous superallergen pFv to interact with the V_H3 region of IgE and to induce IL-4 secretion from human FcεRI⁺ cells was reported by Patella et al. [10]. pFv – a potent stimulus for the release of histamine and IL-4 from basophils – is a human sialoprotein that is found in the livers of healthy people and is released into the intestinal tract of viral hepatitis patients. Brief exposure to lactic acid to dissociate IgE from the FcεRI on basophils prevents the induction of IL-4 synthesis by the treated basophils by pFv. The authors used three human V_H3⁺ monoclonal IgM antibodies that inhibited pFv-induced secretion of IL-4 and histamine from basophils. It was concluded that the endogenous superallergen domain of pFv interacts with the V_H3 domain of the FcεRI bound IgE and induces the synthesis and release of IL-4 from human FcεRI⁺ cells. "This finding," according to Patella et al., "supports the hypothesis that the *in vivo* release of pFv, previously recognized as a B cell SAg [10, 40] and identified as a superallergen, might be pathophysiologically relevant." Silverman et al. [46] reported that an endogenous sialoprotein and a bacterial B cell SAg compete for binding to the V_H family binding interaction with human Ig.

It thus seems logical to assume that the SAg domains of HIV-1 gp120 are indeed superallergens and that HIV-1 infection of humans culminates in the development of an allergy. Therefore, HIV-1 infections should be treated with anti-allergy drugs (e.g. IL-4 inhibitors and immune response modifiers) that are capable of inhibiting IL-4 synthesis by FcεRI⁺ hematopoietic cells and the reactivation of Th1 cells to synthesize cytokines that will induce an active antiviral CTLs response as well as synthesize antiviral IgG-3 virus neutralizing antibodies by B cells. Florio et al. [28] reported that the drugs cyclosporin A [47] and Tacrolimus

(FK506) [48] inhibit IL-4 synthesis by basophils and human FcεRI⁺ cells. Tacrolimus, which is used to treat the human allergic condition atopic dermatitis [49], inhibits the release of histamine from allergen treated basophils.

The FcεRI-bound anti-allergen IgE molecules interact with additional allergen protein molecules, which bind to the anti-allergen domain of IgE by their antigenic domains. This process ensures the continued presentation of allergens to Th2 cells and the continued synthesis of anti-allergen IgE molecules by B cells in the presence of IL-4 [50]. It is suggested that, similar to allergens, HIV-1 gp120 and other shed proteins bind by their antigenic domains to antiviral IgE bound to DC FcεRI, ensuring the continuous synthesis of antiviral IgE.

By using the same mechanism that allergens utilize to induce Th2 cytokine synthesis, HIV-1 infection – mediated by the shed gp120 – subdues the host protective immune system. In other words, it prevents the synthesis of antiviral, neutralizing IgG-3 antibodies by potentiating the IL-4 induction of IgE synthesis by B cells. In addition, the induction of IL-4 cytokine synthesis by FcεRI⁺ hematopoietic cells inhibits cytokine synthesis by Th1 cells and the antiviral CTL response.

Kelly-Welch et al. [51] described the consequences of binding IL-4 to its receptor (IL-4R α subunit) on the hematopoietic cell lineage. Four cellular genes are actively expressed as a result of IL-4 interaction with IL-4R: I-ε (ε germline transcript from the 140-bp Iε exon [52]; CD23 coding for FcεRI; IL-4 receptor IL-4Rα; and MHC class II. Consequently, the IL-4 responsive genes activities deleteriously galvanize the damage that IL-4 incur the HIV-1 patient's immune system, which ultimately result in AIDS by a mechanism in which allergens damage the human immune system and cause allergies.

Comparison of the gp120 SAg Domains that Bind to the V_H3 Domain of IgE to the Binding Domains in Allergens

HIV-1 gp120 “SAg” domain

Florio et al. [28] studied the ability of gp120 peptides derived from HIV-1 MN gp120 domains C1, C2, V3, C4, and the C5 of gp120 by measuring their

ability to block histamine release from purified basophils by binding to the V_H3 domain of IgE [53]. HIV-1 gp120 peptides no. 1922 (aa 31–50) from the gp120 C1 domain and peptide no. 2015 (aa 501–520) from the C2 domain did not inhibit gp120 from binding to IgE. However, peptide no. 1985 (aa 301–320 and aa 241–260) from the V3 domain did inhibit the binding of gp120 to IgE. Furthermore, two overlapping peptides from the C3 domain of gp120 (aa 331–350, 341–360) were able to inhibit gp120 binding to IgE. Consequently, the gp120 sequence aa 231–360, spanning C2-V3-C3 loops in gp120 or part of this sequence, might be involved in the binding of gp120 to the V_H3 domain of IgE. The variable V3 loop of gp120 is involved in binding to the co-receptor CCR5 [53], and the amino acid sequence aa 308–331 is its principal antigenic domain [54].

The Allergens “SAg” (Superallergen) Domains

Elsayed et al. [55] studied the structural requirements for binding to IgE of the epitopes of three allergens from cod, egg, and tree pollen. The authors reported that the major allergen of cod, “Allergen M,” is a 113 aa polypeptide comprised of three domains – AB, CD, and EF – that consist of three helices interspaced by one loop. Three sites encompassed IgE binding epitopes: peptides aa 33–44, aa 65–74, and aa 88–96. A novel peptide (aa 49–64) of the CD-domain encompassed two repetitive aa sequences (D-E-D-K) and (D-E-L-K) separated by six amino acid residues spacer is essential for the binding of the superallergen domain to IgE.

Mine et al. [56] reported on the antigenicity and allergenicity of genetically modified egg white allergen from the ovomucoid third domain. The authors referred to their previous study [57] in which they identified two IgE binding sites (aa K29–S44 and aa T49–C56). Using synthetic peptides – aa Y31–N33, aa F37–C38 and aa K55–C56 – the authors demonstrated that these amino acid sequences are critical to the binding of the allergen to IgE.

Additional Viral Proteins that Compromise the Cellular Immune System

Marone et al. [58] reported that HIV-1 regulatory protein Tat, which is released from HIV-1 infected

cells induces human basophils to secrete IL-4 and IL-13 and may thus add to the dysregulation of the host immune system in mucosal surfaces.

Ayyavoo et al. [59] studied the HIV-1 regulatory protein Vpr, a virion-associated protein that influences T cell proliferation and cytokine production that induces apoptosis, and interferes with NF κ B-mediated transcription of cellular genes. The authors examined the effect of the expression of viral protein Vpr in BALB/c female mice on the induction of antigen-specific immune responses by using a Vpr DNA vaccine. In the presence of Vpr, inflammation was markedly reduced and CD8⁺ CTL activity, Th1 immune responses *in vivo*, and the level of IFN- γ were markedly inhibited as well. Ayyavoo et al. concluded that the viral Vpr protein causes a profound shift in favor of Th2 responses; however, the mode of action of Vpr still needs to be examined. Moreover, the authors asserted that, "clarifying the molecular/cellular roles of accessory genes in HIV-1 pathogenesis will likely generate novel approaches for therapy and enhance our strategies for HIV-1 vaccine development" [59].

Hypothesis and Suggestions

Hypothesis

The shed HIV-1 proteins (gp120, and possibly Tat and Vpr) act as allergens that induce Fc ϵ RI⁺ hematopoietic cells to release the Th2 cytokine IL-4 and cause the AIDS that is a form of allergy.

The studies that were presented in the previous [1] and current reviews clearly enhance the understanding of the molecular and immunological changes in HIV-1 infected people that lead to AIDS. In the present review, studies that elucidate the mechanism by which HIV-1 virions and shed viral proteins cause the acquired immune deficiency are explicated:

1. HIV-1 infects Th2 cells and causes a partial depletion of these cells in the peripheral blood.
2. HIV-1 gp120 molecules that are shed from virions bind to the V_H3 domain of IgE molecules bound to Fc ϵ RI⁺ on hematopoietic cells (basophils, mast cells, monocytes, and DC). The binding of gp120 induces these cells to release

and synthesize IL-4 and IL-13. DC process and incorporate the IgE bound gp120 and present the viral antigenic peptides via MHC class II molecules.

3. The regulatory viral proteins Tat and Vpr are released from infected cells and bind to basophils that respond with the synthesis of the Th2 cytokine IL-4.
4. HIV-1 gp120 also binds to Ig that are present on the cell membrane of B cells and precipitate their depletion.
5. The shed HIV-1 gp120 has a "SAG" sequence in the C2 and C4 constant domains [42] for binding to the V_H3 domain of IgE and Ig [28]. It is the binding of the shed viral proteins to the IgE bound to Fc ϵ RI on hematopoietic cells that triggers the synthesis and release of IL-4 and IL-13 Th2 cytokines. The gradual increase in the amount of the shed viral proteins leads to a gradual increase in the infected individual's IL-4 level. In the presence of elevated IL-4 concentrations, polarizations of Th2 cells occurs, IL-4 binds to IL-4R on T cells and induces the expression of four cellular responsive genes coding for IL-4, I ϵ , Fc ϵ RI, and MHC class II. Moreover, in the presence of IL-4, B cells synthesize IgE molecules, Th1 cells synthesis of cytokines is inhibited, and CTL precursors are inhibited. The continuous synthesis of IL-4 is the cause of AIDS.
6. A comparison of the mode of action in which HIV-1 gp120 "SAG" sequence binds to the V_H3 domain of IgE [28] to the mode of action of allergens revealed that a "superallergen" domain exists in each allergen. Both the viral SAG domain of gp120 and the superallergen domain of an allergen bind to IgE- Fc ϵ RI complex on hematopoietic cells and induce them to synthesize and release the Th2 cytokines IL-4 and IL-13.

Based on the homology between the modes of action of the viral "SAG" and the allergen superallergen domains, it is hypothesized that AIDS is an allergy-like disorder. Treatment modalities with immune response modifiers and IL-4 inhibitors that are used for the treatment of allergic people may also be useful in the treatment of HIV-1/AIDS patients.

Implications of the Hypothesis

1. Klausner et al. [2] contended that since anti-HIV-1 vaccines using HIV-1 gp120 as the major antigen failed to protect vaccinees from HIV-1 infection, it is imperative that an international cooperation be formed to develop an effective vaccine. The present review outlines studies that provide evidence for the hypothesis that during HIV-1 infection shed HIV-1 gp120 molecules induce the synthesis of IL-4 by binding to IgE that is bound to the FcεRI on mast cells, basophils, monocytes, and DC. The increased levels of IL-4 in the lymph nodes and serum of HIV-1 infected person is responsible for the allergy-like syndromes found in AIDS. The presentation of the gp120 antigens to T cells by DCs, the synthesis of antiviral IgE by B cells in the presence of IL-4, and the binding of gp120 to Ig on B cells and the pursuant depletion of these cells are responsible for the allergen-like damage that is incurred by the infected person's immune system.
2. Based on the hypothesis that the shed HIV-1 gp120 has the properties of an allergen, it is suggested that a genetically modified or engineered gp120 protein should be used for the development of a protective vaccine. Karry et al. [42] suggested that the SAg domain should be deleted from gp120 prior to its use as a vaccine. In the absence of the SAg domain, the induction of IL-4 will be prevented, B cells will synthesize neutralizing antiviral IgG-3 antibodies, and antiviral CTLs will be functional. Garrity et al. [60] suggested that since immunodominant epitopes are known to suppress the primary immune response in other antigenic determinants, dampening of the V3 loop epitope of gp120 shifted the dominant type-specific neutralizing antibody response away from the third variable domain (V3) to an epitope in the first variable domain (V1) of gp120. The authors suggested that by this approach, more broadly protective vaccines may be developed.
3. The reasons for the protection of HIV-1 patients from vaccination with the smallpox vaccine [8] can be explained. The gp120-induced allergy prevents Th1 cytokine synthesis and the induction of anti-HIV-1 CTLs in infected individuals. Therefore, additional virus proteins

that are presented by HLA class I CD8⁺ CTLs need to be used for the development of effective anti-HIV-1 vaccines. The delivery of anti HIV-1 vaccines through the skin (e.g. the Peplotion HIV-1 vaccine) [61] or the vaginal route may eliminate the need for syringes.

4. HIV-1 induced AIDS should be considered an allergy and should be treated with drugs that inhibit the synthesis of Th2 cytokines (e.g. immune response modifiers and IL-4 inhibitors). A treatment combining antivirals and anti-allergens should be pursued as part of a comprehensive treatment regime. The inhibition of Th2 cytokine activity and stimulation of Th1 cytokine synthesis will resurrect the HIV-1 patient's cellular immune response and virus-infected cells will be destroyed by an active antiviral CTL response. Consequently, the stage will be set for the clearance of the virus and the induction of antiviral neutralizing IgG-3 antibodies.

Summary and Conclusions

The transmission of HIV-1 to humans and the mega-epidemic that has developed over the last 20 years has baffled virologists and public health experts. The sexual transmission of HIV-1, the appearance of high virus titers in the patient's blood, the co-purification of HIV-1 with blood factor VIII causing infections of hemophiliacs, and the decline of CD4⁺ T cells, led to the conclusion that effective antiviral drugs must be developed for the treatment of HIV-1 patients. Concomitantly, the viral glycoprotein gp120 was used for vaccine development on the assumption that the antigenic domains in the viral glycoprotein will induce neutralizing antibodies in vaccinees and will protect them against HIV-1 infection.

The collective results of the studies on HIV-1/AIDS that were reviewed by the present author in the previous and current articles introduce a new approach to solving the HIV-1 enigma. The ability of the shed virus proteins gp120, Tat, and Vpr to induce Th2 cytokine synthesis by FcεRI⁺ hematopoietic cells, which is similar to the mode of action employed by allergens, may lead to the development of effective treatments that will correct the skewed proliferation of Th2 cytokines and

restore the Th1 cytokine balance. The restoration of the proper balance between cytokines (Th2 → Th1 reversion, [1]) together with antiviral and anti-allergy drugs that inhibit virus replication and may prevent the development of AIDS in HIV-1 patients. As suggested by Klausner et al. [2], the development of effective and protective gp120 vaccines, lacking deleterious domains, may provide anti-HIV-1 vaccines for the immunization of all members of high-risk populations that will induce protective antiviral CTLs, neutralizing antibodies, and memory T and B cells. These methods and treatments will ultimately lead to the decline of the HIV-1/AIDS pandemic.

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