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Tracking Immunoglobulin Variable-Gene Expression in HIV Infection

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Abstract

B-cell SAgs interact with normal human nonimmune immunoglobulins (Igs) independently of the light-chain isotype, and activate a large proportion of the B-cell repertoire. Recently, the major envelope protein of human immunodeficiency virus 1 (HIV-1), gp120, was found to exhibit SAg-like properties for B cells with potential pathological consequences for the infected host, including accelerated apoptosis and progressive loss of B cells. This unconventional mode of interaction contrasts with its binding to immunization-induced antibodies, which requires the tertiary structure of the heavy- and light-chain variable regions. Examining the temporal development of V_H3^+ antibodies in HIV-1-infected subjects over a 7-yr period showed that V_H3^+ antibodies specific for the gp120 SAg-binding site are deficient. Quantification of V_H3^+ antibodies, which impart protective responses to infectious agents, in serum samples from HIV-seropositive slow progressors and from patients who progressed to AIDS-related manifestations reveals that paucity in V_H3^+ antibodies is a marker of rapid clinical decline. Remarkably, anti-gp160 V_H3^+ antibodies show a gradual decrease in progressors and vary with time, depending on the viral load. Thus, V_H3^+ antibodies could play an important role in protection, and their underexpression may accelerate disease progression. Investigation of the structural basis of the interaction between human Igs and gp120 shows that the viral gp120 SAg can interact only with a subset of human V_H3^+ Igs. A number of amino acid-positions present primarily in the first and third framework regions of the Ig heavy-chain variable regions correlate with gp120 binding. These residues partially overlap with the *Staphylococcus aureus* protein A-binding site for V_H3^+ Igs. Overall, these interactions could represent a novel mechanism of humoral deficiency resulting from the capacity of a viral SAg to impact an important subset of the B-cell repertoire and to induce B-cell death by apoptosis.

Index Entries: Superantigen; gp120; HIV; B cell; human antibodies; immunoglobulin variable genes.

Introduction

HIV shows remarkable genetic and biologic diversity, and certain virus isolates are believed to be more pathogenic than others. How HIV destroys the immune system and causes disease remains an intriguing question. Studies of the dynamic nature of HIV replication in vivo reveal that the virus is turning over very rapidly and in very large amounts. Billions of virus particles are continuously produced by newly infected cells, and then rapidly cleared *(1)*. This rapid turnover, which makes lentiviruses such as HIV, unique among infectious agents, results in the daily production and removal of millions of HIVs from the blood of infected people, and is very active in the lymph nodes of HIV-infected subjects. As might be expected, this infectious disease is associated with humoral and cellular immune responses against viral components in almost all infected subjects. However, the role of these responses in the control of HIV infection is not clearly understood, and the immune response developed is not efficient in protecting patients from ultimate progression to fatal disease *(2)*. Elucidation of the complex processes responsible for the spectrum of clinical manifestations is therefore currently the focus of much investigation. In this retroviral infection, the central event is the high-affinity binding of the HIV envelope glycoprotein gp120 to its receptors, which are present on the surface of positive cells of the immune system. The high-sequence heterogeneity of gp120 and its central role in anti-HIV-1 immunity raise the question of the extent of the diversity of the B-cell repertoire developed by the invaded host to cope with this varying pathogen. Even though B cells are not directly killed *(3)*, functional abnormalities of B cells and humoral immune responses are common in HIV-infected patients. They include hypergammaglobulinemia; enhanced serum levels of IgG, IgA, IgE, and IgM in children; the presence of circulating immune complexes and autoantibodies; and in vitro spontaneous IgG production by circulating lymphocytes *(4,5)*. In addition, B-cell proliferation and immunoglobulin (Ig) production induced in vitro by polyclonal activators, as well as primary responses in vivo, are impaired soon after HIV infection in most patients *(6)*. The mechanisms causing hyperactivation of the B-cell compartment in HIV-1-infected patients have not been yet clarified. Initially, B-cell hyperactivation was attributed to frequent coinfection with Epstein–Barr virus (EBV) and cytomegalovirus, two well-known B-cell activators *(5)*. Then, the direct B-cell-activating role of HIV components was adduced *(5)*. In principle, several viral proteins as well as the inactivated HIV-1 virus itself can directly stimulate B cells to proliferate and differentiate into Ig-producing cells *(7)*. The occurrence of oligoclonal Igs ranging from 10- to 45% of those examined has been reported in HIV-1^+ subjects *(8)*, and some specific HIV-1 glycoproteins may decrease responsiveness of B cells to polyclonal stimulators *(9)*. In an attempt to understand the mechanisms responsible for these alterations, several laboratories analyzed the antibody repertoire expressed in HIV-infected subjects and

the potential role of a new type of lymphocyte ligands, referred to as superantigens (SAgs). Initially described for T cells, SAgs are a group of microbial proteins known for their potent ability to activate a large number of T cells expressing a common immunoreceptor-variable-region family. A counterpart of T-cell superantigens has been described for B cells wherein the heavy-chain variable region determines the specificity of the immunoglobulin for the superantigen. While conventional antigens stimulate a small proportion of B cells, the proportion of B cells responsive to SAgs can be orders of magnitude higher. For example, *Staphylococcus aureus* protein A (SpA) activates approx 40% of human polyclonal IgMs. SAg binding is mediated by regions of the heavy chain outside the classical antigen binding site of antibodies.

Results and Discussion

Abnormal Repertoire Expression in HIV Infection

Initial studies of the paratopic expression of human antibodies to HIV-1 led to the conclusion that the humoral response to HIV epitopes is less heterogeneous than initially thought *(10,11)*. An alternative means to evaluate the diversity of the antibody repertoire to a given pathogen consists of characterizing the idiotypic determinants present on the variable regions *(12–15)*. This strategy was also applied to analyze the heterogeneity of the antibody response to gp120. Overall, the results suggest that the extent of the idiotypic repertoire towards the HIV virus is high *(10,16)*. However, these serological studies did not lead to elucidation of the molecular mechanisms underlying the generation of the functional activities of anti-HIV antibodies in the infected host. In an effort to understand these processes, several laboratories conducted studies of the genetic composition of human antibodies to HIV *(14,17–29)*. This approach requires generation of secreting B-cell clones, which can be obtained by EBV-transformation or by somatic fusion with lymphoblastoid or human–mouse heteromyeloma cells and, then, by selection of B-cell clones secreting antibodies with in vitro neutralizing or enhancing activity. To clone the variable genes expressed by these antibodies, mRNA is extracted, transcribed into cDNA, molecularly amplified by PCR, and cloned in a phagemid vector, and the nucleotide sequence is determined *(30)*. The sequences determined allowed identification of the gene elements expressed. These studies provide clues to the genetics of the humoral response to HIV. The V_H genes used exhibit significant sequence divergence from their germline counterparts. This was an indication of the high somatic mutation rate. The frequency of the mutations in the complementarity-determining regions (CDRs) was higher than in the frameworks (FRs). Most important, anti-HIV V_H genes were drawn from various V_H gene families, but anti-gp120 antibodies rarely used V_H3 genes *(14,29)*. Further studies are required to understand the mechanisms of this abnormal repertoire expression in HIV infection.

B-Cell Death in HIV Infection

In addition to the reduction of the proportion of mature B cells in HIV-infected subjects *(31)*, a depletion of B cells expressing V_H3^+ Ig products was described in patients with AIDS *(32)*, and people infected with HIV have aberrant and unstable expression of Ig genes, suggesting a dysregulation of the humoral immune response to HIV-associated antigens *(33)*. Since B cells are not directly infected by HIV, it is unlikely that the virus directly kills B cells. Too few cells, if any, are infectable by HIV. Therefore, HIV must be killing uninfected cells indirectly. The process of programmed cell death, normally used to clear uninfected cells and self-reactive cells, may be used. In the simian-human immunodeficiency virus (SHIV) model, intrarectal inoculation of rhesus macaques induces a sustained decrease in $CD20^+$ B cells, failure to elicit virus-binding antibodies in plasma, and high levels of antigenemia *(34)*. This B-cell loss can reach 87%, and is related to disease progression. Experiments using in situ labeling of lymph nodes from HIV-infected children and from SIV-infected macaques *(35)* revealed that apoptosis occurs predominantly in bystander cells, not in the productively infected cells themselves. Strikingly, the majority of apoptotic cells was localized in the secondary follicles, specifically in the apical light zone of the follicles—a region known to be rich in B cells, activated $CD4^+$ T cells, and follicular dendritic cells. Another mechanism might be homing of the B cells. It is also possible that the loss of B cells is linked to the fact that HIV destroys the bone marrow where naive B cells are produced. Because HIV progressively destroys the architecture of the lymph node, it might reduce the B-cell lifespan.

Superantigens in HIV Infection

Clues to understanding the altered humoral response in HIV infection came from the demonstration that HIV gp120 binds to Igs of the V_H3 gene family, suggesting that this glycoprotein is a candidate superantigen (SAg) for V_H3^+ B cells *(36)*. In another study, gp120 was found to activate the complement (C3) in nonimmune serum by a classical pathway and this C3 binding could be due to V_H3^+ anti-SAg Igs *(37)*. In Ig V_H3^+ AIDS-related Burkitt's lymphoma, a possible role for the HIV gp120 SAg was suggested *(38,39)*. Some human B-cell malignancies could also be due to a similar type of interaction. It is therefore important to emphasize the potential consequences of this interaction in disease pathogenesis. SAgs are produced by a broad range of pathogens, T-cell activation by SAgs has been observed in many species, including humans and other primates, mice, rats, cats, dogs, and rabbits. This phylogenetic diversity has supported efforts to implicate them in the pathogenesis of a variety of diseases of unknown etiology. T-cell clones triggered by SAg often disappear or become inactive after being stimulated. In vivo, SAgs can activate a large proportion of the T cells bearing the target T-cell receptor (TcR) Vβ subsets. Sub-

sequently, the number of stimulated T cells decreases through programmed cell death, and a state of anergy is seen *(40,41)*.

In HIV infection, reports that selected Vβ subsets were deleted in AIDS patients have generated much excitement *(42–45)*. However, the use of Vβ-specific monoclonal antibodies revealed no common "whole" of a specific T-cell subset in the repertoire of HIV-1$^+$ subjects *(46–48)*. It is possible that T-cell subsets triggered by SAgs may not be completely deleted, or they may be anergized for a variable time period. For example, the superantigen toxic-shock syndrome toxin-1 (TSST-1) of staphylococci is thought to be responsible for toxic-shock syndrome. In these patients, there is an acute rise in the percentage of T cells expressing the Vβ2 gene. During later stages of the disease, the percentage of Vβ2$^+$ T cells drops back to normal levels and T-cell deletion is not apparent *(49)*. It is possible that some Vβ subsets are targeted by a putative SAg without being deleted. However, during HIV infection, there is skewing of the viral burden to the Vβ12 subset without a particular correlation with a clinical stage of the disease, and this subset has been suggested to function as a viral reservoir in a majority of patients. By comparison with positively selected CD4$^+$ T cells, it was estimated that the majority of the HIV-1 viral burden in the peripheral blood of one patient was contained within the Vβ12 subset, even though this subset represented less than 2% of all T cells. It is possible that infection of a new adult host with HIV-1 initially targets Vβ12$^+$ cells, because HIV-1 is often skewed to the Vβ12 subset. These findings suggest that the Vβ selective element is a SAg that could derive from an ubiquitous integrated virus. Since HIV-1 activates transcription of herpesviruses genes, such as CMV virus genes, it could lead to expression of an otherwise silent gene, the production of which may depend on HIV-1 infection. HIV-1 appears to replicate more efficiently in T cells expressing TcR using certain Vβ genes, such as Vβ12—a Vβ specificity that is consistent with an HIV-1 SAg. It is possible that these targeted Vβ subsets are not deleted from peripheral cells, but rather represent a viral reservoir in vivo. This SAg-like activity appears to promote Vβ-slective HIV-1 replication in vitro and in vivo in patients infected with HIV-1 *(50)*. Other results obtained in humans favor a strong association between the in vivo existence of a Vβ8-specific SAg and HIV infection *(51)* and suggest that the expansion of specific-Vβ region subsets occurring in the lung might result from triggering by a SAg *(52)*. However, data obtained in HIV-1-infected implants in SCID mice are not consistent with the hypothesis that HIV-1 acts as a SAg in vivo *(53)*. Rather, they suggest that the disruption of the TCR Vβ repertoire in HIV-1 infection may be influencing T-cell development in the thymus, contributing to both the overall CD4$^+$ T-cell depletion in AIDS and limited TCR-repertoire diversity *(53)*. Enhancement of specific Ig production in SCID-hu-PBL mice after in vitro priming of human B cells with SAg was also noted *(54)*. Studies of the function of the regulatory protein Nef, which paves the way for the virus to cause disease, added another layer

of complexity. The findings suggest that Nef is a virally encoded T-cell SAg and, as such, may be vital in the establishment of HIV infection in a new host and in subsequent disease pathogenesis *(55)*. Thus, Nef is required for optimal HIV pathogenesis, and this may be due to its SAg properties, which transform $CD4^+$ cells to the activated state for virus replication *(56)*.

Potential Consequences of Superantigen Signaling

Despite the fact that the interaction of B cells with SAgs is not as fully investigated as that of T cells, there are similarities between the two cell types. Both cell types proliferate in response to SAg. SAg-directed responses of B and T cells are mediated by the V_H and Vβ domains, and the responses are only slightly influenced by the expressed L-chain or α-chain *V* regions. B-cell SAg recognition differs from that of conventional antigens *(57–59)*. In conventional interactions, the V_L and V_H segments provide the first two CDRs, and the CDR3 is formed by V_L-J_L joining in the L-chain, and by V_H-D_H-J_H joining in the H-chain. Variations in antigen specificity result from variations in the surface residues of the canonical structures of the CDRs and in their relative positions. In SAg interactions, only the V_H region seems to be required for recognition. As there are a limited number of V_H genes, this property results in stimulation of a large proportion of the repertoire. By exerting profound effects on the humoral arm of the immune system, B-cell SAg may have a potential pathogenic role in shaping the normal B-cell repertoire and in the pathogenesis of HIV-induced disease in humans *(57,60–64)*.

In principle, SAg interactions with the B-cell receptor may lead to activation, proliferation, differentiation, anergy, or induction of programmed cell death. For T cells, SAgs and conventional antigens trigger different pathways of cell activation. For example, while endogenous retroviral SAgs may induce T-cell proliferation without inducing phosphatidyl inositol turnover or interferon gamma secretion, conventional antigens trigger both proliferation and lymphokine gene expression *(65,66)*. Thus, while injection of some SAgs may induce clonal deletion, the same cells could be resistant to the induction of deletion by other antigens. It is possible that clonal deletion is operative at the level of SAg, but fails at the level of antigen. In the case of HIV, binding of gp120 to CD4 molecules on T cells results in dysregulation of expression of costimulatory molecules, thereby contributing to T-cell hyporesponsiveness *(67)*. gp120 can modulate lymphocyte function in vitro and interfere with T lymphocyte signal transduction in activated T lymphocytes *(68)*. Although the molecular mechanism of this anergizing effect is not completely understood, it seems that gp120 can induce uncoupling of the TcR from the earliest events in signal transduction *(69)*. The HIV-1 glycoprotein inhibits CD3-induced inositol trisphosphate production and Lck activation, calcium influx, T-cell proliferation, and tyrosine phosphorylation. It also dissociates the tyrosine kinase $p56^{lck}$

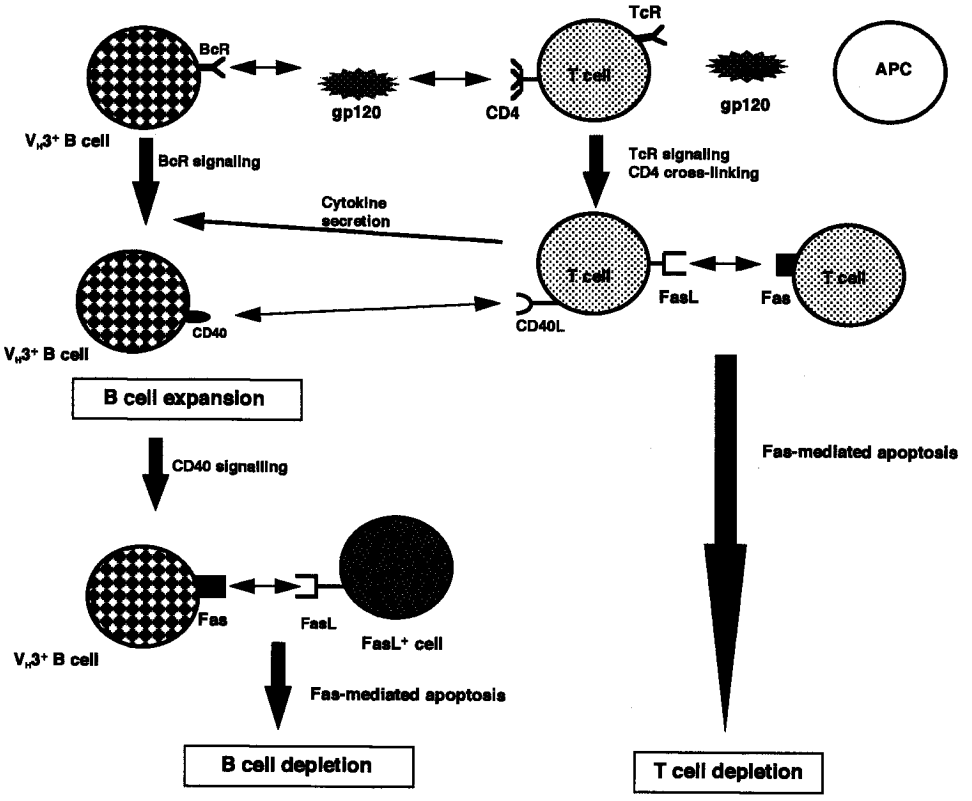

Fig. 1. Model for superantigen-mediated B-cell expansion and deletion. gp120 is envisioned as initially having an activating effect on V_H3^+ B cells, leading to upregulation of CD40 expression. Ligation of this latter marker by CD40L expressed on activated T cells will result in Fas expression on B cells *(99–102)*. Encounter of Fas^+ V_H3^+ B cells with $FasL^+$ lymphocytes will lead to Fas-mediated apoptotic death *(99–102)*, and to depletion of V_H3^+ B cells.

The gp120 Superantigen-Binding Site

In principle, definitive assignment of B-cell epitopes of foreign antigens requires information on the three-dimensional structure obtained from crystallographic data. However, determining the relationship between primary sequence and protein conformation is difficult, and most B-cell epitopes have been identified by indirect means, such as reactivity with phylogenetically related antigens, or by protein modification using either chemical procedures or site-directed mutagenesis. For infectious agents that exhibit antigenic variation and evolve at a high rate, such as the envelope glycoprotein of HIV, study of various isolates provides a convenient approach for identifying critical residues necessary in interaction with Igs. HIV-1 subtypes A through E—or clades—have been classified according to *env* and *gag* gene sequences *(79,80)*. They are characterized by their geo-

from CD4 in CEM cells, with a concomitant inhibition of CD4-linked kinase activity, suggesting that a negative signal is triggered by gp120 that results in down-modulation of CD4-$p56^{lck}$ complexes, inhibition of subsequent signal transduction, and impairment of CD3 signaling *(70)*.

Although the potential of B-cell SAgs to alter signaling pathways has not been documented, it may be possible that there is an inappropriate activation via gp120 binding to V_H3^+ B cells. Such a binding event may render the B cells anergic or unable to respond to further exogenous stimuli. More dramatically, when these cells encounter antigen, they may become prone to apoptosis, thereby limiting the available repertoire of protective antibodies against HIV-1 and associated opportunistic pathogens. It has been suggested that this property is a potentially specific and significant factor in homosexual transmission of HIV infection *(71)*.

Tackling the Antibody Repertoire with the gp120 Superantigen

The observations that HIV infection is characterized by accelerated apoptosis and progressive loss of B cells raise the question of whether these abnormalities are related to the property of gp120 to act as a SAg for V_H3^+ B cells. This possibility was tested by a number of investigators using immunochemistry-based *(72,73)* and molecular biology-based approaches *(10,14,74)*. The results showed marked depletion of V_H3^+ Igs and B cells in HIV infection. Further studies revealed that people infected with HIV have aberrant and unstable expression of Ig genes *(33)*, suggestive of humoral immune dysregulation and responses to HIV-associated antigens and SAgs. In an experimental model, a study of Ig V_H usage during primary infection of rhesus monkeys with chimeric simian–human immunodeficiency viruses (SHIV) concluded that the envelope glycoprotein of HIV-1 does not selectively expand or deplete the V_H3 repertoire of primate B cells during acute AIDS virus infection *(75)*. However, it is possible that peculiarities of the experimental system could account for the inability to detect systematic alterations in the Ig repertoire of SHIV-infected monkeys, that gp120 interacts differently with human and rhesus monkey Igs, or that V_H3-specific perturbations might develop later in the clinical course of SHIV infection of monkeys. In human studies where the temporal development of V_H3^+ antibodies in HIV-1-infected subjects was examined over a 7-yr period, V_H3^+ antibodies specific for the gp120 SAg binding site were found to be deficient *(76)*. More important, paucity in V_H3^+ antibodies—which impart protective responses to infectious agents *(77)*—was a marker of rapid clinical decline. Anti-gp160 V_H3^+ antibodies showed a gradual decrease in progressors and varied with time, depending on the viral load. Thus, disease aggravation is associated with a decrease of the magnitude of the humoral response, and V_H3^+ antibodies seem to play an important role in protection *(78)*. The depletion of V_H3^+ B cells may accelerate disease progression (*see* Fig. 1).

graphical distribution, with strains A, C, and D dominating in sub-Saharan Africa, C in India; and E in Thailand. HIV-1 B is the dominant subtype in the Unites States and Western Europe; it has also been isolated occasionally in Africa, Thailand, and India, and it is the dominant subtype in the Caribbean and South America. gp120s from clades B and E are highly divergent antigenically, as judged by antibody reactivity profiles. The clade E, which seems to be of recent origin, forms a very distinctive serotype at the level of anti-gp120 antibodies. gp120 molecules from clade D isolates tend to be poorly immunoreactive compared with gp120 from other clades. This may be a consequence of the high degree of sequence divergence found among clade D isolates *(79)*.

It is obvious that certain structures in the envelope glycoprotein, such as the CD4-binding site, must be functionally conserved. However, it is unclear whether SAg epitopes on gp120 are also highly preserved within and between clades. Using gp120s from different clades, we have shown that the SAg binding activity is well-conserved *(81)*. That gp120s from different clades exhibit the SAg property is not inherently surprising. HIV-1 clades are defined on the basis of primary sequence, whereas SAg binding is a much more complex process that depends on interactions with motifs that are likely to be influenced by the tertiary and quaternary structures of the protein. Previous studies have shown that the CD4-binding site of gp120 is quite well-conserved between different clades of gp120s *(82)*. Thus, clade-specific antigenic variations in gp120 molecules do not translate into strong functional differences at the level of SAg binding *(81)*, a feature reminiscent of the conservation of the CD4-binding site of gp120 between different viral clades.

To map the binding site, we utilized relatively long synthetic peptides in order to increase the chance that the peptides may mimic discontinuous epitopes and disulfide loops within the envelope. We found that the SAg-binding site is formed by protein sequences from two regions of the gp120 molecule *(81)*. The core motif is a discontinuous epitope spanning the fourth variable (V4) domain and the amino-terminal region flanking the fourth constant (C4) domain. In the BRU viral isolate, the V4 region starts at amino acid-position 385 and ends at amino-acid position 418, and forms a loop caused by the sulfide bonding between the Cyst at each end. The most critical residues appear to be Leu^{395}-Asp^{397} and Ile^{425}-Gln^{427}. Residues from the C2 constant domain (positions 252–272) also seem to play an accessory role in SAg binding of gp120 to normal human Igs *(81)*. Presumably, in the native gp120, both sites are spatially oriented in a suitable way. Whether allosteric changes in the Ig molecule also play a role cannot be ruled out. Additionally, conformational changes elicited by distal amino-acid positions may alter the specificity and/or surface accessibility of the target epitopes. These conclusions are consistent with the findings of others *(83)*. Since portions of the C2-, C3-, and C4-conserved regions of gp120 all contribute to the CD4 binding site, there seems to be an overlap between the CD4 and the SAg binding sites.

The V_H3^+ Ig Repertoire Engaged in SAg Interaction

The identification of this epitope on gp120 contributes to our understanding of the exposure of immunogenic determinants and functional domains of gp120. It was therefore critical to map the positions on human V_H3^+ Igs that interact with gp120 in a SAg manner. Here, it is useful to first summarize the mode of interaction of SpA, the prototypic B-cell SAg with human V_H3^+ Igs. SpA is a cell-wall component with a molecular weight of about 42,000. It is produced by over 95% of *S. aureus* strains, but not by other staphylococci. Among its many biologic and immunologic functions, SpA avidly binds mammalian IgG, contributes to the resistance of the organism to phagocytosis, activates complement, and elicits immediate and delayed hypersensitivity reactions. There are four IgG Fc-binding sites per SpA molecule, and approx 80,000 IgG-binding sites per organism. SpA is an important immunodiagnostic reagent because of its ability to bind the Fc fragment of a wide range of mammalian Igs. Cloning of the gene for *S. aureus* SpA revealed that the domain that acts as a Fc receptor comprises 58 amino acids and is repeated five times *(84)*. All of the five structurally related domains—*A* through *E*—can bind the constant and variable domains of Igs. The Fc-binding site of SpA can be destroyed by iodination, leading to a modified SpA. SpA binds 14–54% of human B cells *(58)*. When Igs were tested, SpA bound to roughly 22% of human polyclonal IgA and 15% of polyclonal human IgG $F(ab')_2$ fragments *(85)*. In a related study, 32% of polyclonal IgM, 13% of IgA1, and 22% IgA2 had SpA-binding activity *(86)*. Binding is more common among IgM-bearing than IgG-bearing B cells, and while virtually every germline-encoded IgM studied has displayed SpA-binding activity, only about 60% of hypermutated V_H3^+ IgG Fab did bind SpA *(87)*.

Great emphasis has rightly been placed on characterizing the mode of interaction of the SAg SpA with human Igs. Molecular studies showed that several human Ig gene products (3-23, 3-30, and 3-30.3) frequently bind to SpA *(88,89)*. It is also notable that IgM reactive with SpA can be divided into two groups, depending on the binding avidity for solid SpA, and that differences in the binding avidity correlated with the use of distinct sets of germline V_H genes *(88)*. While high-avidity binding involved 3-23, 3-15, 3-13, 3-49, and 3-74 V_H3 genes, low-avidity binding was attributable to 3-9, 3-30.3, 3-48, 3-21, 3-7, 3-30, 3-72, 3-73, and 3-74 V_H3 genes. In another study, the 3-23 V_H3 gene was also associated with the strongest SpA binding activity *(90)*. Recently, we found that gp120 binding is restricted to Igs from the V_H3 gene-family and that the two V_H genes 3-23 and 3-30, known to be overutilized during all stages of B-cell development, frequently impart gp120 binding *(91)*. We also provided evidence that the viral gp120 SAg can interact with only a subset of human V_H3^+ Igs that can convey binding to the prototypic bacterial B-cell SAg SpA (*see* Fig. 2).

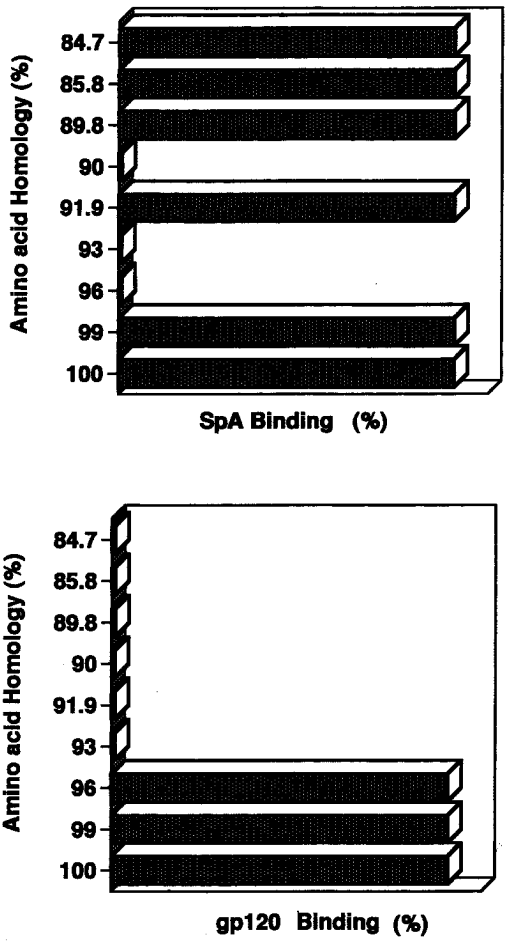

Fig. 2. Sensitivity of gp120 superantigen binding to somatic mutation of V_H3^+ immunoglobulins. The relative binding of a group of human 3-30 V_H3 gene-positive Igs to SpA and gp120 is represented together with their amino-acid sequence homology to the 3-30 V_H3 germline gene product (adapted from Ref. *91*).

Variable SAg-Interactive Sites on V_H3^+ Immunoglobulins

Studies using X-ray crystallography have demonstrated that structures of variable and constant domains of Ig from different sources are highly conserved, and that the FRs of these domains are essentially superimposable *(92)*. Generally, SAgs for T cells are thought to bind to the lateral surface of the Vβ chain and, in particular, to residues at the apex of the hypervariable region-4 (HVR4) *(93,94)*. In studies of the SpA SAg, the overall shape formed by the FR1, CDR2, and FR3 seems to be essential for binding. In particular, the residue at position 57 in CDR2 was found to be involved, in concert with adjacent residues, in SpA binding *(95–97)*. Using

molecular modeling programs and the coordinates for the crystal atomic structure of a V_H3^+ Ig, we constructed a space-filling model of the potential gp120 SAg interactions. The gp120-binding site of Igs consists of nonsequential residues, which can be modeled to fold in space into a solvent-exposed accessible compact region *(91)*. In this topological model for the sites that contact gp120, the binding site is complex, with residues likely to contact gp120 found in the FR1 and FR3 regions. The interaction involves a mixed assortment of hydrogen bonding, hydrophobic interactions, and salt bridges. This Ig site may dock into a conserved pocket bordered by several residues in gp120. These features are critical for the interaction with gp120 from different HIV strains, despite substantial sequence differences between them.

In these studies, we have also identified 16 amino-acid positions present primarily in the FR1 and FR3 of the Ig heavy chain, which correlate with gp120 binding *(91)*. Among these, only residues 28, 75, and 82a overlap with the SpA binding site for V_H3^+ Igs, which encompasses at least eight residues. Remarkably, 13 of the 16 amino-acid positions identified map outside the conventional antigen-binding site, in the FR1 (residues 10, 13, 19, and 23) and the FR3 regions (residues 59, 64, 65, 75, 79, 81, 82a, 83, and 85). In addition, three positions localized in the hypervariable loops were identified that might influence gp120 binding. In this model, the points of contact between the two molecules represent a virtually continuous area, flanked by four basic side chains, and surrounded by amino-acid residues 54 and 13 on either side. Overall, if charge and shape complementarity are to be observed, the contact surface presented by gp120 should be concave and of an acidic character. Furthermore, the similarity in structures with the TcR would allow the V_H FR3 region (corresponding to the hypervariable loop 4 in Vβ) to make direct contacts with gp120 residues essential for binding. The contact between the viral glycoprotein and the Ig might therefore include a set of ionic interactions flanking a central hydrophobic patch. However, it is possible that the actual Ig residues that mediate SAg binding to gp120 include additional residues beyond those identified, which may interfere slightly with the correct fit of Igs to gp120. Importantly, the binding site on gp120 is more complex than the site for SpA. Another B-cell SAg, called pFv (Fv fragment binding), is a gut-derived molecule exhibiting a more commonly expressed V_H specificity than SpA. The binding of SpA and pFv can involve the same site(s) in the V_H3 domain and recognize conformationally sensitive structures in the Ig *(98)*. Ultimately, three-dimensional structure analysis by X-ray crystallography is required to elucidate the structure-function relationships in Ig–gp120 SAg interactions.

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References

1. Coffin, J. (1995), *Science* **267,** 483–489.
2. Levy, J. A. (1993), *Microbiol. Rev.* **57,** *183–289.*
3. Legendre, C., Gras, G., Krzysiek, R., Galanaud, P., Richard, Y., and Dormont, D. (1996), *FEBS Lett.* **381,** 227–232.
4. Biagiotti, R., Giudizi, M. G., Almerigogna, F., Mazzetti, M., Alessi, A., Del Prete, G. F., Rafanelli, D., Fiorilli, M., Morfini, M., and Romagnani, S. (1986), *Clin. Exp. Immunol.* **63,** 354–358.
5. Fauci, A. S., Schnittman, S. M., Poli, G., Koenig, S., and Pantaleo, G. (1991), *Ann. Intern. Med.* **114,** 678–693.
6. Miedema, F., Petit, A. J., Terpstra, F. G., Schattenkerk, J. K., de Wolf, F., Al, B. J., Roos, M., Lange, J. M., Danner, S. A., Goudsmit, J., et al. (1988), *J. Clin. Investig.* **82,** 1908–1914.
7. Pahwa, S., Pahwa, R., Saxinger, C., Gallo, R., and Good, R. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 8198–8202.
8. Crapper, R. M., Deam, D. R., and Mackay, I. R. (1987), *Am. J. Clin. Pathol.* **88,** 348–351.
9. Amadori, A., Zamarchi, R., Ciminale, V., Del Mistro, A., Siervo, S., Alberti, A., Colombatti, M., and Chieco-Bianchi, L. (1989), *J. Immunol.* **143,** 2146–2152.
10. Zouali, M. (1997), in "HIV-Associated B-Cell Abnormalities," Guenounou, M., ed., pp. 329–336, INSERM, Paris, France.
11. Muller, S. and Kohler, H. (1997), *Int. Rev. Immunol.* **14,** 339–349.
12. Corre, J. P., Fevrier, M., Chamaret, S., Theze, J., and Zouali, M. (1991), *Eur. J. Immunol.* **21,** 743–751.
13. Fevrier, M., Boudet, F., Corre, J. P., Chamaret, S., Theze, J., and Zouali, M. (1992), *J. Gen. Virol.* **73,** 2561–2568.
14. Zouali, M. (1996), *Appl. Biochem. Biotechnol.* **61,** 149–155.
15. Muller, S., Margolin, D., Nara, P., Alvord, W., and Kohler, H. (1998), *Proc. Natl. Acad. Sci. USA* **95,** 276–281.
16. Karray, S. and Zouali, M. (1997) in "Probing human antibody diversity in HIV infection," Shoenfeld Y., Kennedy R. C., and Ferrone S., eds., Elsevier Science B.V., Amsterdam, 1997, pp. 269–277.
17. Adderson, E. E., Shackelford, P. G., Insel, R. A., Quinn, A., Wilson, P. M., and Carroll, W. L. (1992), *J. Clin. Investig.* **89,** 729–738.
18. Andris, J. S., Johnson, S., Zolla-Pazner, S., and Capra, J. D. (1991), *Proc. Natl. Acad. Sci. USA* **88,** 7783–7787.
19. Andris, J. S., Ehrlich, P. H., Ostberg, L., and Capra, J. D. (1992), *J. Immunol.* **149,** 4053–4059.
20. Bagley, J., Dillon, P. J., Rosen, C., Robinson, J., Sodroski, J., and Marasco, W. A. (1994), *Mol. Immunol.* **31,** 1149–1160.
21. Barbas, C. F., 3d., Collet, T. A., Amberg, W., Roben, P., Binley, J. M., Hoekstra, D., Cababa, D., Jones, T. M., Williamson, R. A., Pilkington, G. R., et al. (1993), *J. Mol. Biol.*, **230,** 812–823.
22. Burton, D. R., Barbas, C. F., 3d, Persson, M. A., Koenig, S., Chanock, R. M., and Lerner, R. A. (1991), *Proc. Natl. Acad. Sci. USA* **88,** 10,134–10,237.
23. Felgenhauer, M., Kohl, J., and Ruker, F. (1990), *Nucleic Acids Res.* **18,** 4927.
24. Ikematsu, H., Harindranath, N., Ueki, Y., Notkins, A. L., and Casali, P. (1993), *J. Immunol.* **150,** 1325–1337.
25. Marasco, W. A., Bagley, J., Zani, C., Posner, M., Cavacini, L., Haseltine, W. A., and Sodroski, J. (1992), *J. Clin. Investig.* **90,** 1467–1478.
26. Moran, M. J., Andris, J. S., Matsumato, Y., Capra, J. D., and Hersh, E. M. (1993), *Mol. Immunol.* **30,** 1543–1551.
27. van der Donk, E. M., Schutten, M., Osterhaus, A. D., and van der Heijden, R. W. (1994), *AIDS Res. Hum. Retrovir.* **10,** 1639–1649.
28. van der Heijden, R. W., Bunschoten, H., Pascual, V., Uytdehaag, F. G., Osterhaus, D. M., and Capra, J. D. (1990), *J. Immunol.* **144,** 2835–2839.
29. Wisnewski, A., Cavacini, L., and Posner, M. (1996), *J. AIDS* **11,** 31–38.

30. Demaison, C., Chastagner, P., Theze, J., and Zouali, M. (1994), *Proc. Natl. Acad. Sci. USA* **91,** 514–518.
31. Dawood, M., Conway, B., Patenaude, P., Janmohamed, F., Montaner, J., O'Shaughnessy, M., and Hammond, G. (1998), *J. Clin. Immunol.* **18,** 235–240.
32. Berberian, L., Valles-Ayoub, Y., Sun, N., Martinez-Maza, O., and Braun, J. (1991), *Blood* **78,** 175–179.
33. Bessudo, A., Rassenti, L., Havlir, D., Richman, D., Feigal, E., and Kipps, T. (1998), *Blood* **92,** 1317–1323.
34. Steger, K., Dykhuizen, M., Mitchen, J., Hinds, P., Preuninger, B., Wallace, M., Thomson, J., Montefiori, D., Lu, Y., and Pauza, C. (1998), *J. Virol.* **72,** 1600–1605.
35. Finkel, T., Tudor-Williams, G., Banda, N., Cotton, M., Curiel, T., Monks, C., Baba, T., Ruprecht, R., and Kupfer, A. (1995), *Nat. Med.* **1,** 129–134.
36. Berberian, L., Goodglick, L., Kipps, T. J., and Braun, J. (1993), *Science* **261,** 1588–1591.
37. Susal, C., Kirschfink, M., Kropelin, M., Daniel, V., and Opelz, G. (1994), *J. Immunol.* **152,** 6028–6034.
38. Amariglio, N., Vonsover, A., Hakim, I., Neumann, Y., Mark, Z., Brok-Simoni, F., Ben-Bassat, I., and Rechavi, G. (1994), *Acta Haematol.* **91,** 103–105.
39. Amariglio, N. and Rechavi, G. (1996), *Leuk. Lymphoma* **22,** 237–243.
40. White, J., Herman, A., Pullen, A. M., Kubo, R., Kappler, J. W., and Marrack, P. (1989), *Cell* **56,** 27–35.
41. Laurence, J. Hodtsev, A. S., and Posnett, D. N. (1993), *Ann. NY Acad. Sci.* **685,** 746–755.
42. Imberti, L., Sottini, A., Bettinardi, A., Puoti, M., and Primi, D. (1991), *Science* **254,** 860–862.
43. Akolkar, P. N., Gulwani-Akolkar, B., Chirmule, N., Pahwa, S., Kalyanaraman, V. S., Pergolizzi, R., Macphail, S., and Silver, J. (1995), *Clin. Immunol. Immunopathol.* **76,** 255–265.
44. Akolkar, P. N., Chirmule, N., Gulwani-Akolkar, B., Pahwa, S., Kalyanaraman, V. S., Pergolizzi, R., Macphail, S., and Silver, J. (1995), *Scand. J. Immunol.* **41,** 487–498.
45. Weber, G. and Cantor, H. (1993), *Med. Hypotheses* **41,** 247–250.
46. Laurence, J., Hodtsev, A. S., and Posnett, D. N. (1992), *Nature* **358,** 255–259.
47. Posnett, D. N., Kabak, S., Hodtsev, A. S., Goldberg, E. A., and Asch, A. (1993), *AIDS* **7,** 625–631.
48. Boyer, V., Smith, L. R., Ferre, F., Pezzoli, P., Trauger, R. J., Jensen, F. C., and Carlo, D. J. (1993), *Clin. Exp. Immunol.* **92,** 437–441.
49. Choi, Y., Lafferty, J., Clements, J., Todd, J., Gelfand, E., Kappler, J., Marrack, P., and Kotzin, B. (1990), *J. Exp. Med.* **172,** 981–984.
50. Sutkowski, N., Thorley Lawson, D., and Huber, B. (1996), *Trends Microbiol.* **4,** 89–91.
51. Garcia, S., Dadaglio, G., Cilote, V., Chena, l. H., Bondurand, A., and Gougeon, M. (1996), *Blood* **88,** 2151–2161.
52. Trentin, L., Zambello, R., Facco, M., Sancetta, R., Cerutti, A., Milani, A., Tassinari, C., Crivellaro, C., Cipriani, A., Agostini, C., and Semenzato, G. (1996), *AIDS* **10,** 729–737.
53. Boldt Houle, D., Jamieson, B., Aldrovandi, G., Rinaldo, C. J., Ehrlich, G., and Zack, J. (1997), *AIDS Res. Hum. Retrovir.* **13,** 125–134.
54. Martensson, C., Ifversen, P., Borrebaeck, C., and Carlsson, R. (1995), *Immunology*, **86,** 224–230.
55. Torres, B., Tanabe, T., Yamamoto, J., and Johnson, H. (1996), *Biochem. Biophys. Res. Commun.* **225,** 672–678.
56. Torres, B. A., Tanabe, T., Subramaniam, P. S., Yamamoto, J. K., and HM, J. (1998), *Alcohol Clin. Exp. Res.* **22,** 188S–192S.
57. Zouali, M. (1995), *Immunol. Today*, **16,** 399–405.
58. Zouali, M. (1996), *Human B Cell Superantigens*. R. G. Landes, Austin, TX, p. 270.
59. Silverman, G. J. (1997), *Immunol. Today* **18,** 379–386.
60. Pirofski, L., Lui, R., DeShaw, M., Kressel, A., and Zhong, Z. (1995), *Infect. Immun.* **63,** 3005–3014.

61. Domiati Saad, R. and Lipsky, P. (1997), *Int. Rev. Immunol.* **14,** 309–324.
62. Townsley Fuchs, J., Neshat, M., Margolin, D., Braun, J., and Goodglick, L. (1997), *Int. Rev. Immunol.* **14,** 325–338.
63. Domiati-Saad, R. and Lipsky, P. (1998), *J. Immunol.* **161,** 1257–1266.
64. Patella, V., Giuliano, A., Bouvet, J., and Marone, G. (1998), *J. Immunol.* **161,** 5647–5655.
65. Patarca, R., Wei, F., Iregui, M., and Cantor, H. (1991), *Proc. Natl. Acad. Sci. USA* **88,** 2736–2739.
66. O'Rourke, A., Mescher, M., and Webb, S. (1990), *Science* **249,** 171–174.
67. Chirmule, N., McCloskey, T., Hu, R., Kalyanaraman, V., and Pahwa, S. (1995), *J. Immunol.* ***155,*** *917–924.*
68. Phipps, D., Reed-Doob, P., MacFadden, D., Piovesan, J., Mills, G., and Branch, D. (1995), *Clin. Exp. Immunol.* **100,** 412–418.
69. Goldman, F., Jensen, W., Johnson, G., Heasley, L., and Cambier, J. (1994), *J. Immunol.* **153,** 2905–2917.
70. Hubert, P., Bismuth, G., Korner, M., and Debre, P. (1995), *Eur. J. Immunol.* **25,** 1417–1425.
71. Townsley Fuchs, J., Kam, L., Fairhurst, R., Gange, S., Goodglick, L., Giorgi, J., Sidell, N., Detels, R., and Braun, J. (1996), *J. Clin. Investig.* **98,** 1794–1801.
72. Berberian, L., Shukla, J., Jefferis, R., and Braun, J. (1994), *J. Acquir. Immune. Defic. Syndr.* **7,** 641–646.
73. Muller, S., Wang, H., Silverman, G. J., Bramlet, G., Haigwood, N., and Kohler, H. (1993), *Scand. J. Immunol.* **38,** 327–334.
74. David, D., Demaison, C., Bani, L., Zouali, M., and Theze, J. (1995), *Eur. J. Immunol.* **25,** 1524–1528.
75. Margolin, D., Reimann, K., Sodroski, J., Karlsson, G., Tenner-Racz, K., Racz, P., and Letvin, N. (1997), *J. Virol.* **71,** 8582–8591.
76. Juompan, L., Lambin, P., and Zouali, M. (1998), *FASEB J.* **12,** 1473–1480.
77. Zouali, M. (1994), *Nat. Genet.* **7,** 118–120.
78. Juompan, L., Lambin, P., and Zouali, M. (1998), *Appl. Biochem. Biotechnol.* **75,** in press.
79. Korber, B., Allen, E., Farmer, A., and Myers, G. (1995), *AIDS*, **9,** S5–S18.
80. Gao, F., Morrison, S. G., Robertson, D. L., Thornton, C. L., Craig, S., Karlsson, G., Sodroski, J., Morgado, M., Galvao-Castro, B., von Briesen, H., Beddows, S., Weber, J., Sharp, P. M., Shaw, G. M., and Hahn, B. H. (1996), *J. Virol.* **70,** 1651–1657.
81. Karray, S. and Zouali, M. (1997), *Proc. Natl. Acad. Sci. USA* **94,** 1356–1360.
82. Moore, J., McCutchan, F., Poon, S., Mascola, J., Liu, J., Cao, Y., and Ho, D. (1994), *J. Virol.* **68,** 8350–8364.
83. Goodglick, L., Zevit, N., Neshat, M. S., and Braun, J. (1995), *J. Immunol.* **155,** 5151–5159.
84. Uhlen, M., Guss, B., Nilsson, B., Gatenbeck, S., Hilipson, L., and Lindberg, M. (1984), *J. Biol. Chem.* **259,** 1695–1702.
85. Sasso, E. H., Silverman, G. J., and Mannik, M. (1991), *J. Immunol.* **147,** 1877–1883.
86. Ibrahim, S., Kaartinen, M., Seppala, I., Matoso, F. A., and Makela, O. (1993), *Scand. J. Immunol.* **37,** 257–264.
87. Silverman, G. (1996) in *B-Cell Superantigens and the Somatic Diversification of Immune Repertoires: Molecular and Cellular Implications*, Zouali, M., ed., R.G. Landes, Austin, TX, pp. 67–88.
88. Hakoda, M., Kamatani, N., Hayashimoto-Kurumada, S., Silverman, G., Yamanaka, H., Terai, C., and Kashiwazaki, S. (1996), *J. Immunol.* **157,** 2976–2981.
89. Rassenti, L. and Kipps, T. (1997), *J. Exp. Med.* **185,** 1435–1445.
90. Sasano, M., Burton, D. R., and Silverman, G. J. (1993), *J. Immunol.* **151,** 5822–5839.
91. Karray, S., Juompan, L., Maroun, R., Isenberg, D., Silverman, G., and Zouali, M. (1998), *J. Immunol.* **161,** 6681–6688.
92. Chothia, C., Lesk, A. M., Tramontano, A., Levitt, M., Smith, G. S., Air, G., Sheriff, S., Padlan, E. A., Davies, D., Tulip, W. R., Colman, P. M., Spinelli, S., Alzari, P. M., and Poljak, R. (1989), *Nature* **342,** 877–883.
93. Li, H., Llera, A., Tsuchiya, D., Leder, L., Ysern, X., Schlievert, P., Karjalainen, K., and Mariuzza, R. (1998), *Immunity* **6,** 807–816.

94. Papageorgiou, A., Collins, C., Gutman, D., Kline, J., O'Brien, S., Tranter, H., and Acharya, K. (1999), *Eur. Mol. Biol. Organ. J.* **18,** 9–21.
95. Hillson, J. L., Karr, N. S., Oppliger, I. R., Mannik, M., and Sasso, E. H. (1993), *J. Exp. Med*. **178,** 331–336.
96. Randen, I., Potter, K. N., Li, Y., Thompson, K. M., Pascual, V., Forre, O., Natvig, J. B., and Capra, J. D. (1993), *Eur. J. Immunol.* **23,** 2682–2686.
97. Potter, K. N., Li, Y., and Capra, J. D. (1996), *J. Immunol*. **157,** 2982–2988.
98. Silverman, G. J., Pires, R., and Bouvet, J. P. (1996), *J. Immunol.* **157,** 4496–4502.
99. Rathmell, J. C., Townsend, S. E., Xu, J. C., Flavell, R. A., and Goodnow, C. C. (1996), *Cell* **87,** 319–329.
100. Rothstein, T. L., Wang, J. K., Panka, D. J., Foote, L. C., Wang, Z., Stanger, B., Cui, H., Ju, S. T., and Marshak-Rothstein, A. (1995), *Nature* **374,** 163–165.
101. Mandik, L., Nguyen, K. A., and Erikson, J. (1995), *Eur. J. Immunol.* **25,** 3148–3154.
102. Garrone, P., Neidhardt, E. M., Garcia, E., Galibert, L., van Kooten, C., and Banchereau, J. (1995), *J. Exp. Med*. **182,** 1265–1273.

Discussion

Kohler: Have you actually seen apoptosis in your SCID mouse model?

Zouali: No, not yet. We are in the early stages of these experiments, and we have done very little, as you have seen. We don't have much data. But it is known in the literature that there is a high rate of apoptosis in the lymph node of HIV-positive subjects.

Gololobov: The superantigen-binding site on gp120 looks like it overlaps with the CD4-binding site of the molecule. Can you comment?

Zouali: The CD4-binding site is a more complex site, because it involves several portions of the gp120 molecule. This site is lost when the gp120 is denatured. So you need the native molecule, while the superantigen-binding site is not lost with denaturation of the gp120. There might be some overlap, but I don't think that the two sites are identical.

Paul: Do all V_H3 antibodies bind equally to gp120?

Zouali: Only a limited number of VH3 positive genes bind gp120. Some immunoglobulins that express V_H3 genes do not bind gp120. But the V_H3-positive genes that bind gp120 are very frequently expressed in the repertoire. Therefore, the consequences of this recognition are important.

Paul: Does gp120 use its superantigen activity to either stimulate or block the synthesis of antibodies—V_H3 or otherwise?

Zouali: From Jonathan Braun's experiment, it seems that superantigen-binding stimulates immunoglobulin production.

Paul: Yet the V_H3-positive cells decrease with disease progression.

Zouali: Yes, but that's in vivo. You see, in Braun's experiment, the superantigen concentrations are not the same as those in vivo. The encounter does not take place in the same way. Perhaps it takes place in the lymph node. So the consequences may be different.

Kohler: We have proposed that the gp120 superantigen activity can cause clonal restriction and persistence of clonal dominance. We believe these are very important factors in the failure to eradicate HIV-1 infection. Do you share our hypothesis?

Zouali: Yes, of course; I agree with you.