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Selective Alterations of the Antibody Response to HIV-1

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ABSTRACT

HIV infection leads to progressive alterations of humoral immune functions, including B-cell hyperplasia, hypergammaglobulinemia, elevated autoantibody titers, a poor response to neoantigens and mitogens, polyclonal B-cell activation, monoclonal gammopathies, and a significant deterioration of the antigen-specific humoral response. There is also an important isotypic imbalance of the antibody (Ab) response in the systemic compartment and a profound modification of mucosal immune functions. These abnormalities may contribute to disease progression and development of opportunistic infections, despite the presence of serum-neutralizing anti-HIV Abs. Equally important are the abnormal selection mechanisms of the Ab repertoire that seem to be responsible for B-cell clonal deletions. The V_H3 gene family, which encodes for approx 50% of immunoglobulins expressed by peripheral B-cells from normal adults, is underrepresented in human monoclonal antibodies to HIV-1 and in the peripheral B-cells of AIDS patients. These abnormalities, together with features of germinal center alteration, could be responsible for the clonal elimination of a subset of B-cells, and could contribute to HIV pathogenesis.

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

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INTRODUCTION

HIV infection leads to progressive alterations of immune functions. The timecourse of disease progression varies, depending on both viral factors and the host's immune system. During the initial infection phase, strong cellular and humoral responses to HIV are elicited. However, most infected individuals still progress to AIDS, as if the immune response to HIV were incapable of controlling the infection. It was soon realized that the principal target of HIV infection is the $CD4^+$ T-cell subset, which progressively and severely disappears from the lymphoid organs. This decline is accompanied by functional abnormalities, including unresponsiveness to recall antigens (Ags) and abnormal cytokine secretion. In addition to alterations of this cell population, the capacity of HIV-specific $CD8^+$ T-cells to undergo clonal expansion is reduced in AIDS patients. Thus, both the depletion of $CD4^+$ lymphocytes and the loss of HIV-specific cytolytic activity seem to contribute to clinical expansion of the disease. In addition to abnormalities at the T-cell level, a number of alterations of the humoral immune response are also detectable during HIV infection. This article discusses the isotypic imbalance of the antibody (Ab) response in the systemic and mucosal compartments, the biased variable (V) gene repertoire expressed at the systemic level, and the clonal elimination of a subset of B-cells during HIV infection.

IgG SUBCLASS IMBALANCE DURING HIV-1 INFECTION

A number of manifestations of B-cell dysfunction have been described in HIV-infected subjects. They include B-cell hyperplasia, hyperimmunoglobulinemia affecting all isotypes, elevated autoantibody titers, and a poor response to neoantigens and mitogens *(1–4)*. Despite the marked hyperimmunoglobulinemia, frequently there is a significant deterioration of the Ag-specific humoral response, hence the occurrence of opportunistic infections, i.e., *Pneumococcus* and *Cryptoccus*. Some of these abnormalities precede demonstrable peripheral $CD4^+$ T-cell defects, suggesting that they are intrinsic to the B-cells *(5)*. However, it remains possible that the B-cell changes might have been preceded by T-cell changes in other compartments of the immune system. Several studies reported the existence of polyclonal B-cell activation and a high incidence of oligoclonal or monoclonal immunoglobulins (Ig) in infected individuals *(2–7)*. For example, an oligoclonal B-cell expansion was detected in 25% of infected adults with a predominance of monoclonal IgG, and, to a lesser extent, IgM. Conversely, the IgA isotype was the only monoclonal serum Ig detected in a study of nine infected children *(6)*. Monoclonal gammopathies (MG) have also been observed with a much higher frequency

than in the normal population *(7)*, but their occurrence in HIV-positive subjects seems to be without prognosis value *(8)*. In a study of 31 Caucasians with lymphadenopathy syndrome (LAS), IgG levels, particularly IgG1, were increased in 87% of the cases, elevated IgA and IgM were observed in 13% and 16% of cases, respectively *(9)*. In HIV-positive subjects, the mean concentration of IgG2 and IgG4 was slightly decreased, and there was a negative correlation between IgG1 and IgG2 levels and a positive correlation between those of IgG2 and IgG4. A different isotype distribution is found in AIDS, in which IgG levels were increased in only 55% of 11 patients, and, in contrast to LAS, IgA levels were often high (73% of cases) *(9)*. The IgG subclass distribution was reminiscent of that found in patients with LAS, although IgG1 levels were lower in AIDS than in LAS. Similar observations were made in another study of 10 AIDS patients, in whom this global increase was caused by IgG1 *(10)*. As is the case in adults, most infected children (88%) show elevated serum Ig levels (IgA, 87%; IgM, 74%; and IgG, 60%). IgG subclass serum levels are also significantly altered, with a marked augmentation of the IgG1 fraction, and to a lesser extent, the IgG3 isotype *(11)*.

This isotype imbalance may be related to the abnormal Ab response of HIV^+ subjects to vaccination. For example, IgG2 directed to capsular polysaccharides form an essential component in the defense against infections with encapsulated bacteria such as *Streptococcus pneumoniae* and *Haemophilus influenzae* type b, and their production is deficient in a number of well-defined immunodeficiency syndromes, including AIDS and some forms of lymphoid malignancies. To determine whether HIV^+ adults are capable of mounting an effective immune response when immunized with a polyvalent pneumococcal vaccine, 28 HIV-infected patients and 11 healthy volunteers were immunized with Pneumovax II *(12)*. Titers of IgG1 and IgG2 Abs were measured before and 1 mo after immunization. The magnitude of the antipneumococcal response was significantly higher in the normal volunteers, with a significant IgG2 response in 11/11 controls and 14/28 HIV seropositives. Poor Ab responses were unrelated to the $CD4^+$ T-lymphocyte count at the time of immunization. Thus, half of HIV Ab seropositive individuals failed to mount an adequate immune response to polyvalent pneumococcal vaccine.

The isotypic expression of Ab responses to self-determinants and to pathogens is, in general, dominated by certain isotypes *(13–15)*. For example, the IgG2 predominance of Ab to bacterial carbohydrate Ags has been described by several authors (see review in ref. *16*). Because most HIV-infected individuals often progress to AIDS despite the presence of neutralizing Abs in their serum *(17–19)*, many studies focused on the analysis of the isotype of the Ab response to HIV Ags, including *env*, *gag*, and *pol* gene products, and various profiles of Ab responses have been observed in

AIDS patients and in asymptomatics. For example, in contrast to AIDS patients, who have a high serum reactivity with the gp41 transmembrane protein and a low reactivity with the p24 core protein, asymptomatic individuals show a predominant reaction with p24 *(20,21)*. Other reports documented a correlation between low Ab titers to gp120, p24, and the p51/65 reverse transcriptase, and disease progression *(22)*. Circulating IgG Abs capable of inhibiting the catalytic activity of the viral polymerase have also been found in HIV-infected subjects, and a correlation was also observed between the decrease of this activity and disease progression *(23,24)*. Analysis of the isotype of the Ab response to *gag* gene products demonstrated a polyisotypic pattern; Abs to *env* and *pol* gene products were predominantly of the IgG1 isotype. The isotypic distribution was influenced by the route of infection, gag-specific IgG4 and IgE being elicited in the hemophiliac group *(25)*. Another study concluded that there is an association between the clinical stage of HIV-1 infection and the presence of Abs against p17 and p24 proteins *(26)*. IgG1 was found to be the dominant anti-HIV-1 subclass, and to decline with disease progression. In contrast, IgG3 Abs which were predominantly directed against gag proteins, persisted with disease progression *(27)*. The IgG2 subclass was also represented in response to *gag* and *env* gene products *(28)*. Significantly lower levels of IgG2 anti-gp41 Abs were observed in patients at Walter Reed stages 5 and 6 (5% of patients), when compared to those at Walter Reed stages 1 and 2 (88% of patients), suggesting that lack of IgG2 response to gp41 correlates with clinical manifestations of the disease *(28)*. In another study, the human IgG1 subclass response to an epitope of gp41 (positions 583–599) was in correlation with an absence of symptoms *(29)*. A parallel distribution of IgG subclass reactivity to an immunodominant gp41 epitope was seen in maternal and pediatric sera *(30)*. Sera from infected children showed *de novo* synthesis of anti-HIV IgG1 and/or IgG3, but most children with a rapid disease progression failed to produce IgG1 and/or IgG3 to the third variable region (V3) of gp 120.

A flow cytometry-based assay was used to compare the isotype distribution of serum anti-HIV Abs from 14 mothers giving birth to HIV-1 seropositive infants with those of 25 females whose infants were not infected *(31)*. Sera from transmitting mothers contained a broader distribution of class and subclass Abs compared to those of nontransmitting women, and the single most frequent Ab–Ag combination in the transmitting mother was IgG2–gp160. IgG subclass levels were estimated in the cerebrospinal fluid (CSF) of 10 patients with AIDS and 10 controls, and the local (intrathecal) synthesis was determined for each subclass *(10)*. In AIDS patients, all IgG subclasses were increased in the CSF (about five-fold for IgG1 and 10-fold for IgG3), with a local production of IgG1, IgG3, and IgG4, but rarely IgG2 (3/10). The variations of each IgG subclass in the

serum and in CSF were not in correlation. The reactivities of intrathecal IgG and IgM, and IgG1–4 subclass Abs with various HIV-1 proteins were assessed by immunoblotting at various stages of HIV-1 infection *(32)*. In early infection, the occurrence of anti-gag Abs was higher than that of anti-pol Abs among all IgG subclasses. In late infection, however, the occurrence of anti-p65 IgM and IgG2-4 Abs of both CSF and serum was higher than in early stages. Regarding the anti-env antibody response, patients with advanced infection had a restricted IgG1 anti-gp120 response in the CSF and serum. These results indicate an association between decrease in anti-pol p32 and anti-env gp120 Abs and disease severity.

ALTERATIONS WITHIN THE MUCOSAL COMPARTMENT

The mucosal system plays an important role in the immune response. Secretory IgA predominates in external secretions, and it is thought that it probably protects against pathogen invasion by blocking attachment of Ag particles and neutralizing them. For instance, the presence of mucosal virus- and bacteria-specific secretory IgA is associated with viral clearance and bacterial resistance *(33–35)*. Because sexual transmission is the predominant mode of HIV infection, the virus is transmitted through the mucosal surface of the genitourinary tract and the rectum, where the virus has been found either free or cell-associated in HIV-infected men and women *(36–38)*. However, the role of the mucosal immune system in HIV infection is not as well documented as that of the systemic immune response.

Reminiscent of what is seen in the systemic compartment, increased levels of total IgA and IgG have been observed in many secretions of HIV^+ subjects, including salivary, intestinal, and cervicovaginal fluids, and semen. The predominance of IgG over IgA could result, in part, from plasma transudation *(39,40)*. However, an elevated number of mucosal IgG^+ plasma cells, with a low number of IgA^+ plasma cells, have been described *(39)*, suggesting a local IgG synthesis. During HIV infection, distinct but related virus variants have been detected in the blood and mucosal secretions of infected subjects *(41,42)*. These variants have been shown to elicit various anti-HIV Abs. Since their specific anti-HIV activity is higher in mucosal secretions than in the serum *(39)*, the protective role of the immune response at the mucosal surface could be important. Furthermore, an increased IgG response to HIV Ags in infected and immunized subjects, and in experimental animals, has been reported. For example, an increased IgG anti-gp160 Ab response has been detected in the cervicovaginal secretions of HIV-infected women *(43)*, in the saliva of AIDS patients *(44)*, and in the saliva and nasal secretions of volunteers immunized by intramuscular injection of rgp160 *(45)*. The salivary anti-HIV-gp160 activity was carried largely by the IgG isotype, but the salivary

antibacterial activity (anti-*Streptococcus sobrinus;* anti-LPS from *Escherichia coli*) remained in the IgA isotype, as is usually observed with infectious agents *(44)*. Salivary IgG carried a specific anti-gp160 activity 25-fold higher than that of serum IgG. Thus, significant local synthesis of specific IgG by the oral mucosa is a characteristic feature of HIV immunization. In some of these cases, a high relative concentration of anti-HIV IgG-producing cells was observed in the mucosal tissue or lymph nodes *(39,46,47)*. In contrast to the IgG response, which is directed against several HIV proteins, the IgA response seems to be restricted to a smaller subset of viral proteins *(40,48)*. Thus, by producing specific anti-HIV Abs mucosal immune cells are actively involved in the anti-HIV response. Nevertheless, serum anti-HIV IgA from infected individuals has been shown to possess both neutralizing and enhancing activity in vitro. This enhancing activity, which is mediated by the Fcα receptors, can be blocked by anti-HIV IgG *(49)*. Nonimmune IgA has also been shown to inhibit the in vitro infection of macrophages by binding to Fcα receptors *(50)*. Since an important property of an HIV virus transmitted by the mucosal route might be its ability to infect macrophages *(36)*, anti-HIV IgA with enhancing activity could play a role in this process.

Secretory IgAs, which play a critical protective role at the mucosal surface, are rarely triggered by immunization via the systemic route. In order to improve the immune response to pathogens, different immunization strategies have been tested to target the mucosal route. Indeed, previous work demonstrated that mucosal immunization at one anatomical location induces either a local immune response at the site of immunization and at distant mucosal tissue, or a systemic immune response *(51)*. For example, intranasal immunization of mice with a hybrid C4/V3 peptide (derived from HIV and cholera toxin) induced both elevated serum and vaginal antipeptide Abs *(46)*. In the macaque simian immunodeficiency virus (SIV) model, prevention of rectal infection with SIV was achieved by intramuscular and intravenous exposure with either inactivated or live SIV cultured in human T-cells *(52)*. Since no detectable Ab was observed, the protective response seems to be mediated by T-cells *(53)*. However, immunization by targeting the iliac lymph nodes, which drain the genitorectal mucosa, with SIV gp120 and gp27 has been shown to elicit a broad local immune response with specific secretory Abs *(45,54)*. This was associated with an increase of the numbers of IgA Ab-secreting cells to p27 in iliac lymph nodes *(47)*. Moreover, infection by mucosal exposure has been shown to be less efficient than intravenous injection because of the mucosal environment and virus-specific factors *(36,55)*. Therefore, the generation of a mucosal immune response to HIV can be achieved by systemic immunization, but an optimal immune response may be mediated by both the systemic and the mucosal immune systems *(56)*.

Taken together, an elevated local production of Igs of either an undefined specificity or an anti-HIV specificity was described in HIV-infected subjects. This suggests that a profound modification of mucosal immune functions occurs early in HIV infection. The role of anti-HIV Abs particularly Igs of the IgA isotype, in promoting or limiting HIV infection must be considered. This should be taken into account in designing mucosal vaccine preparations, in order to elicit secretory Abs with a neutralizing activity. However, by preventing primary HIV infection, nonspecific IgA, which binds to Fcα-receptors, could also play an important functional role. In association with neutralizing anti-HIV Abs, it might impair HIV infection, at least at the mucosal level.

BIASED EXPRESSION OF ANTI-HIV ANTIBODY VARIABLE REGION GENES

Although the relationship between the isotype imbalances described above and the humoral deficiency is not immediately apparent, the abnormal selection mechanisms of the Ab repertoire seem to be responsible for B-cell clonal deletions. Currently, it is recognized that human V_H genes can be classified into seven families (V_H1–V_H7). The V_H3 gene family comprises approx 26 functional genes and codes for approx 50% of Ig expressed by peripheral B-cells from normal adults *(57–59)*. Initially, analysis of lymph node lymphocytes from HIV-infected subjects revealed a selective depletion of V_H3^+ B-cells *(60)*. This restricted V_H gene-family usage does not depend on V_L utilization *(61)*. In contrast to the normal adult repertoire, the V_H3 gene family is also underrepresented in human MAbs to HIV-1 *(62–64)*. Further studies at the polyclonal level showed that the V_H3 gene family is overexpressed in asymptomatic subjects and underutilized in AIDS patients *(65,66)*.

Analysis of the Ab repertoire can be achieved by probing the corresponding mRNA by hybridization *(64)*, or by using V_H gene-family-specific Abs *(66)*, as shown in Figs. 1 and 2. A related work suggested that the V_H3 clonal deficit observed in AIDS patients could be caused by a superantigenic-like activity of gp120 toward V_H3^+ Ig *(67)*. Indeed, gp120 from highly divergent isolates binds specifically to V_H3^+ immunoglobulins. Recently, the superantigen-binding site of gp120 has been identified as a discontinuous epitope spanning the fourth V domain (V4) and the amino-terminal region flanking the fourth constant domain (C4), with an accessory participation of residues from the C2 domain *(68)*. In addition to these peripheral B-cell abnormalities, germinal centers, which are the site of B-cell maturation, show many features of alteration during HIV infection, including histological abnormalities of the B-cell zones, an increased proportion of immature circulating cells and a high rate of apoptotic B-cells

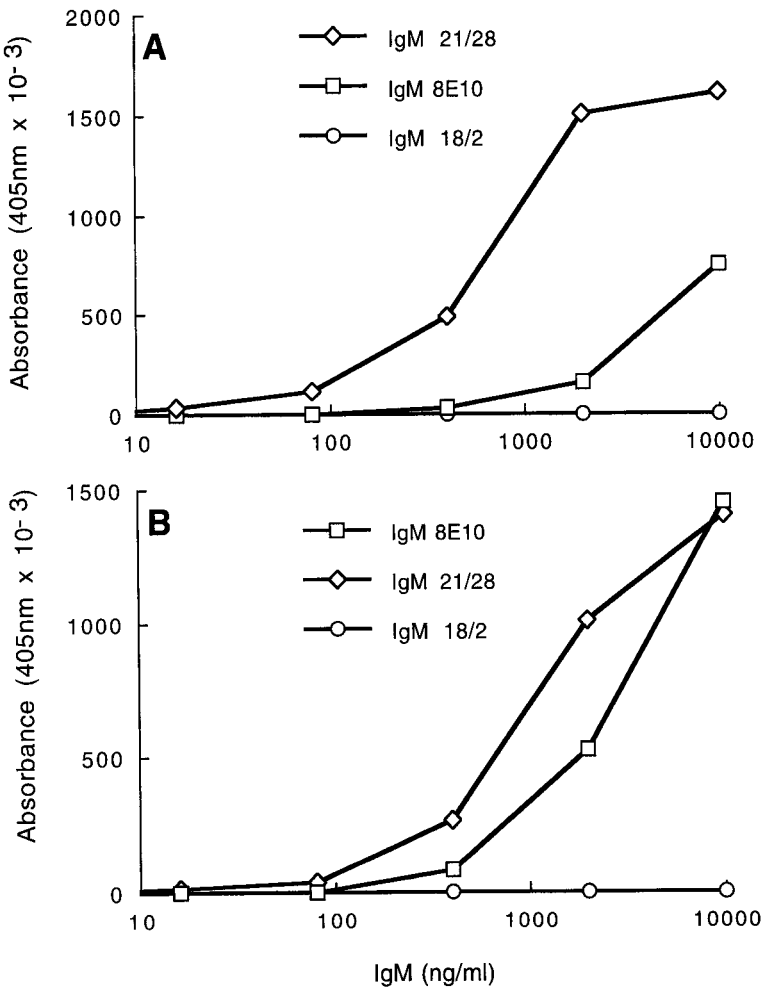

Fig. 1. Binding of anti-V_H1 G8 and G6 MAbs to human IgM. Microplates were coated overnight at 4°C with goat antihuman IgM (500 ng/well). After washing and saturation, 100 μL of IgM dilutions were added to the microplate. After 2 h at 37°C, 100 μL of a 1/1000 diluted anti-idiotype MAb G8 **(A)** or G6 **(B)** were added. Bound Abs were revealed with goat antimouse IgG conjugated with alkaline phosphatase (1/1000 diluted). IgM 18/2 is a V_H3^+ Ab that served as a negative control. Human Igs (21/28, 8E10, and 18/2) are the products of hybridoma cells obtained by fusing human B-cells and a human lymphoblastoid cell line. Their molecular characteristics were described elsewhere *(77,78)*. Mouse anti-idiotypes G8 and G6 were used as reported previously *(79,80)*.

(69–73). Taken together, these observations suggest that the perturbation of the Ab repertoire during HIV infection could be a consequence of the B-cell dysfunction, and could contribute to HIV pathogenesis *(64,74–76)*.

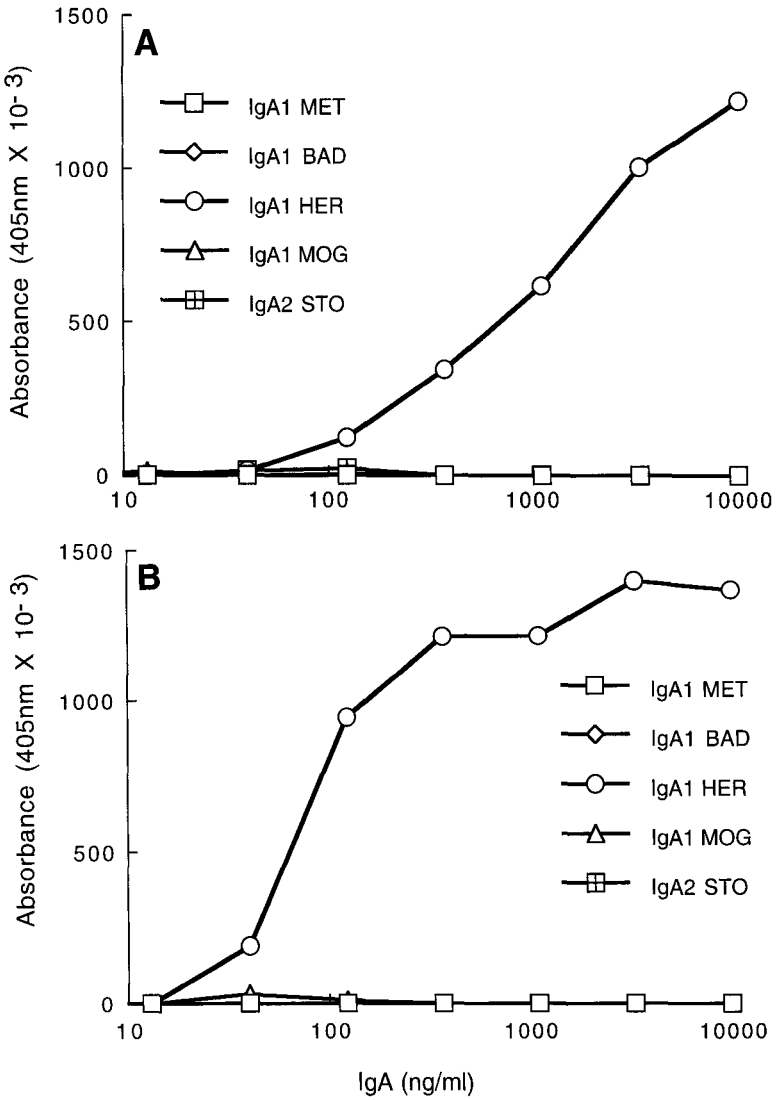

Fig. 2. Binding of anti-V_H3 7B4 and D12 MAbs to human IgA. Microplates were coated overnight at 4°C with goat antihuman IgA (500 ng/well). After washing and saturation, 100 μL of IgM dilutions were added to the microplate. After 2 h at 37°C, 100 μL of a 1/1000 diluted anti-idiotype MAb 7B4 or D12 were added. Bound Abs were revealed with goat antimouse IgG conjugated with alkaline phosphatase (1/1000 diluted). Human Igs (MET, BAD, HER, MOG, and STO) were isolated from the serum of patients with multiple myeloma or Waldenström macroglobulinemia, as described elsewhere *(81)*. In **(A)**, 7B4 mouse anti-idiotype Ab *(79)* was used, and in **(B)** D12 mouse anti-idiotype Ab *(66,80)* was used.

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