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Nonrandom Features of the Human Immunoglobulin Variable Region Gene Repertoire Expressed in Response to HIV-1

MONCEF ZOUALI

Département d'Immunologie, Institut Pasteur, 28, rue du Dr Roux, 75015 Paris, Cédex 15, France

ABSTRACT

Characterization of the immune response toward HIV is important for understanding the basic mechanisms of the disease and may give essential information for development of an anti-HIV vaccine. Paradoxically, although HIV infection is associated with a strong antibody response to structural and nonstructural HIV proteins, this immune response does not seem to halt disease progression. Both quantitative and qualitative B-cell abnormalities are associated with disease progression. The immunological abnormalities in HIV-1 infection include abnormal cytokine production and expansion of HIV-1-specific B-cell precursors that may reach 40%. There is also evidence that gp120 exerts a B-cell superantigen-like activity on human B-cells through binding to gene products of the third heavy-chain variable region family (VH3). This property of gp120 may induce abnormal mechanisms of selection of the antibody repertoire. It may also account for the apparent paucity of anti-gp120 antibodies expressing VH3 genes and for the polyclonal activation seen in the early stages of HIV infection. This expansion would reflect specific stimulation of VH3 B-cells, but not all B-cells. It would then be followed by a significant deletion of this B-cell subset. Finally, autoimmune phenomena have been described in HIV infection, and several hypotheses have been put forward to account for such associations. On the basis of the superantigen concept discussed above, one may suggest that gp120 may trigger B-cell subsets bearing receptors with specificities for self-components. This would explain the multiplicity of autoantibody specificities seen in this disease.

Index Entries: B-cell superantigens; human antibodies to HIV; humoral immunity; immunoglobulin variable genes.

*Author to whom all correspondence and reprint requests should be addressed.

INTRODUCTION

Following HIV infection, although people repeatedly exposed to the virus do not appear to become infected, others, despite being infected with HIV in the late 1970s, remain healthy for many years and do not progress to AIDS. Furthermore, some individuals may die within 1 yr of infection, whereas others remain asymptomatic for over 10 yr. This disease variability may be related to heterogeneity of the biological properties of this retrovirus. For example, the mixtures of HIV-1 quasispecies that exist in vivo differ in their infectious potential, and in a single patient, variant isolates may exhibit differential tropism for target cells. Because of this variability, even if the immune response can cope with the initial infecting isolate, it must keep diversifying its repertoire constantly to neutralize mutant viruses. During the initial phases of infection, vigorous humoral and cellular immune responses can be detected against HIV-1. In addition, elevated levels of serum immunoglobulin (Ig)G and/or IgA and of spontaneous Ig-secreting cells are seen in AIDS. However, there is no consensus on whether neutralizing antibodies correlate with disease progression (reviewed in ref. *1*).

Since Ig genes may serve as markers for selection and diversification of the antibody repertoire, and in as much as they may influence susceptibility to infection and disease progression, we and others have initiated studies of the variable (V) Ig repertoire during HIV infection. Here, recent findings on Ig V gene expression in this disease and data on V gene utilization of human antibodies to HIV antigens compared to that of antibodies to other pathogens will be reviewed. Since it is reasonable to assume that the anti-HIV immune responses are related to the stimulating properties of both B-cell and T-cell superantigens, the potential role of B-cell superantigens will also be discussed.

HUMAN IG VARIABLE REGION GENES

In developing lymphocytes of higher vertebrates, a series of site-specific recombination events culminate in assembly of the variable gene elements that encode functional antibody molecules. Combinatorial joining of multiple variable (V), diversity (D), and joining (J) heavy-chain gene segments and multiple V and J light-chain gene segments is, together with additional somatic mechanisms, responsible of generating the diverse array of the immunoglobulin repertoire (*2*). Elucidation of the mechanisms that govern selection of the V gene elements from the inheritable library of germline genes is the focus of considerable interest. Murine and human heavy-chain V genes (V_H) have been classified into families of nucleotide sequence-related members (*2–4*). In the mouse, where V_H families map as discrete clusters within the heavy-chain locus (*2*), a combination of experimental approaches showed that assembly of (V_H-D_H-J_H)

transcriptional units is a temporally ordered process whereby members of the 3′ V_H gene families are expressed early during ontogeny. This biased rearrangement is then normalized in the adult where V_H gene utilization becomes proportional to the genomic complexity of the corresponding families. In humans, where V_H genes exhibit a high degree of intermingling among members of the different families (*3–5*), recent evidence indicates that there is also a preferential utilization of a limited number of immunoglobulin V_H gene segments during human fetal life. There is then a shift toward a probabilistic usage of V_H genes in the adult (reviewed in *6*).

The advent of molecular amplification of Ig V genes made it possible to quantitate the repertoire of V gene families rearranged in peripheral B-cells of patients. This method permits analysis of a large number of cells in a single experiment. It also circumvents the potential bias of cell cloning, Epstein-Barr transformation, and somatic fusion of B-cells.

UNRESTRICTED UTILIZATION OF IG VH GENES FAMILIES IN ANTIBODIES TO INFECTIOUS AGENTS

A number of studies have examined the V gene segments used in antibodies to pathogens. In experimental models, there are examples of both random and skewed utilization of V gene families in antibody-producing cells. Similarly, when human antibody-producing B-cell clones were analyzed with regard to V gene family utilization, depending on the pathogens studied, some investigators found evidence for family overrepresentation of V gene families, whereas others obtained data that support a stochastic model of V gene usage (reviewed in ref. *7*). A VH-restricted response to conventional antigens has been described for the response to the polysaccharide of *Haemophilus influenzae* type b (Hib) (*8*). However, there is a preponderance of the V_H3 gene segment DP-58 in insulin-specific antibodies. The antibody response to rabies virus also shows a preferential use of VH3 segments (*6–8*).

BIASED VH GENE FAMILY UTILIZATION IN HUMAN ANTIBODIES TO HIV-1

Sequence analysis of the rearrangements expressed by human antibodies to HIV revealed that anti-HIV B-cells exhibit all mechanisms that generate Ig H-chain diversity, and that anti-HIV antibodies exhibit some remarkable characteristics. First, certain VH segments were overrepresented, but the preference for certain VH regions has been described in other immune responses (*9,10*). Second, the distribution of clones expressing a particular VH family reflected a nonrandom utilization of the different families when compared to the estimated complexity of each

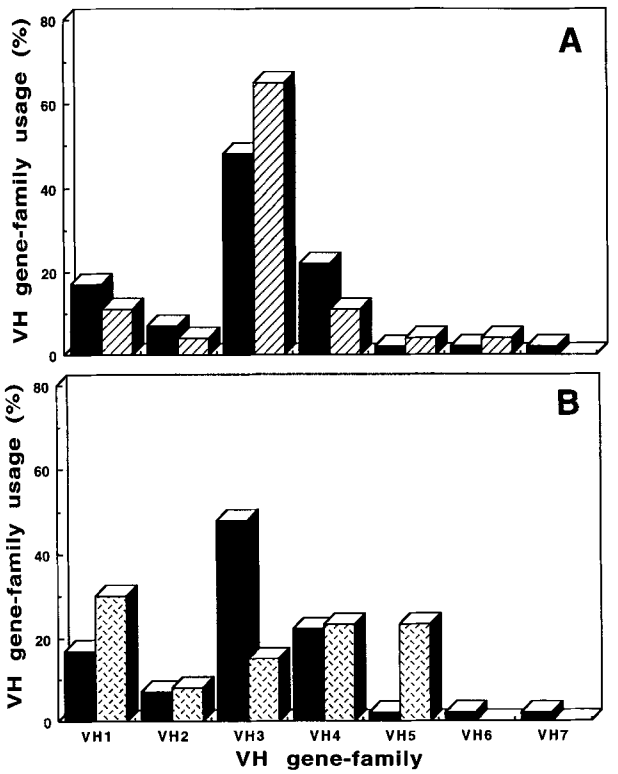

Fig. 1. Biased usage of V_H gene families in human antibodies to gp120 of HIV-1 compared with antibodies to other pathogens. (**A**) VH gene utilization in antibodies to pathogens. Genomic complexity (black bars) represents the proportions of functional genes in the germline repertoire (3,4). Antibodies to other pathogens (n = 21) are from refs. (*19, 22–25*) (hatched bars). (**B**) VH gene utilization in antibodies to gp120 of HIV. Genomic complexity (black bars) represents the proportions of functional genes in the germline repertoire (3,4). Antibodies to gp120 of HIV-1 (n = 13) are from refs. (*26–30*) (crosshatched bars).

family, and the VH3 gene family was underexpressed. This VH family has been reported to be predominantly expressed in antibodies to pathogen-related antigens and to self-antigens (Fig. 1). Within the VH3 family, preferential utilization of VH gene elements was observed. By contrast, the VH1 family was overrepresented in the anti-HIV clones when compared to the complexity of these families. Third, when the configuration of the VH genes expressed was considered, although there were a number of silent mutations (S), most nucleotide changes led to amino acid replacement mutations (R) and were nonconservative, leading to high R/S ratios. The striking high R/S ratios is highly suggestive of positive selection by antigen (*10*). Fourth, the third hypervariable regions (CDR3) are remarkable in several respects. In most cases, the DH segments cannot be explained by germline sequences. The DH-DH fusion, proposed initially by Kurosawa and Tonegawa in 1982, constitutes another important mechan-

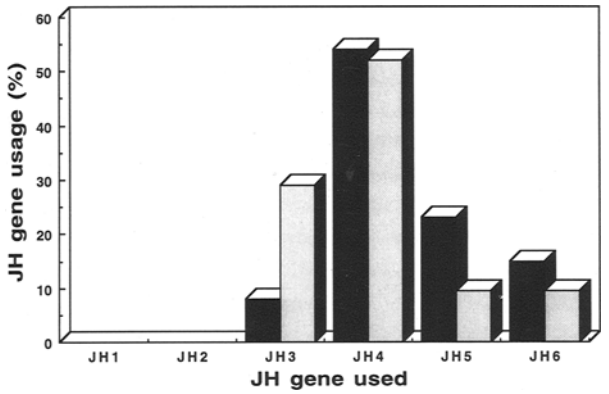

Fig. 2. J_H gene expression in human antibodies to gp120 of HIV-1 compared with antibodies to other pathogens. Antibodies to gp120 of HIV-1 (n = 13) are from refs. (*26–30*) (black bars). Antibodies to other pathogens (n = 21) are from refs. (*19, 22–25*) (Gray bars).

ism for generating the desired amino acid sequences of the CDR3 (*2*). The sequences of anti-HIV heavy chains suggest that DH-DH fusions have taken place between DH gene segments and, in some cases, the inverted complement of the other DH. The presence of possible P sequences was also observed in a number of cases, primarily at the VH to DH junctions. Additional nucleotides at the 5′ and 3′ ends of the DH segments are most likely owing to N segment addition by terminal transferase (*2*), leading to additional sequence diversity. Studies in the murine system have shown that the load of N sequences flanking the DH sequences in cells from adult animals is in general heavier at the VH-DH border than at the DH-JH border (*7*), and this feature may represent the footprint of antigen selection. Anti-HIV CDR3 regions exhibit a variety of sequences and lengths. However, additional anti-HIV antibodies must be sequenced and their CDR3 regions compared to one another to determine the significance of CDR3 length and sequence on antigen binding. Finally, utilization of JH elements does not deviate from that of human antibodies to other antigens (Fig. 2).

THE ROLE OF B-CELL SUPERANTIGENS

Recently, a new type of specific Ig ligands, referred to as B-cell superantigens, have been described. This idea has been proposed to account for the binding of VH3 Ig to *Staphylococcus aureus* protein A (*11*). In this mode of interaction, the VH region determines nearly exclusively the specificity of the Ig for the B-cell superantigen. Although conventional antigens stimulate a small proportion of B-cells, the proportion of B-cells responsive to B-cell superantigens can be orders of magnitude higher. Because the B-cell super-

antigen interacts primarily with the VH portion of the Ig molecule, it can, in principle, trigger all B-cells bearing the appropriate VH, regardless of the other JH, D, JL, and VL segments. Since there are a limited number of VH genes, this property results in stimulation of a large proportion of the repertoire. For example, the bacterial membrane protein, *Staphylococcal* protein A (SpA), has sites that interact with the Fab of many IgM, IgA, IgG, and IgE, and this interaction is restricted to the VH 3 gene family (*12–16*). In addition, there is a hierarchy within the binding affinities of VH3 Fab to SpA based on V gene usage (*14*). This unconventional mode of interaction with B-cells, which is comparable to that of known superantigens with T-lymphocytes, is being investigated for HIV antigens, and it has been shown that the pattern of VH family restriction of Ig reactive with gp120 is also reminiscent of the properties of B-cell superantigens (*17*).

CONCLUSION

In recent years, the number of laboratories involved in the study of the human antibody repertoire expressed in response to HIV infection has grown rapidly, in part because the subject offers prospects for understanding disease pathogenesis and for immunointervention. Of the various principles described in this article, the following are worthy of emphasis. The VH gene repertoire of anti-gp120 antibodies in seropositive subjects is dramatically biased and markedly deficient in genes of the VH3 family, which represents 50% of the VH genes available to the human immune system (*18,19*). *Ex vivo* studies of the VH gene repertoire suggest the following scenario. Soon after in vivo exposure to HIV antigens, there is a slight expansion of VH3-positive B-cells in the periphery of seropositive subjects. This stimulation is followed by a progressive decline of this B-cell subset, which seems to follow a deletional pathway of development. By virtue of the B-cell superantigen property of gp120, this deletion mechanism is reminiscent of the effect of injection of T-cell superantigens to experimental animals (*20*), and may reflect the continuous interaction between VH3 positive B-cells and gp120 (*17,19,20*). It is of further interest that deletion of VH3-positive B-cells, which was initially observed in the peripheral blood of seropositive individuals (*18,19*), is also an active process in their lymph nodes. Recent experiments show that the degree of apoptosis in all compartments of the lymph node obtained from HIV-seropositive persons is three to four times higher than that observed in HIV-negative persons, and that B-cells undergo apoptosis (*21*). Understanding the mechanism of superantigenicity of gp120 and of B-cell death may lead to designing means to counteract these pathological effects (*31*).

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REFERENCES

1. Levy, J. A. (1993), *Microb. Rev.* **57,** 183–289.
2. Tonegawa, S. (1983), *Nature* **302,** 575–581.
3. Cook, G. P., Tomlinson, I. M. Walter, G., Riethman, H., Carter, N. P., Buluwela, L., Winter, G., and Rabbitts, T. H. (1994), *Nature Genet.* **7,** 162–168.
4. Cook, G. P. and Tomlinson, I. M. (1995), *Immunol. Today* **16,** 237–242.
5. Berman, J. E., Pollock, R., Smith, C., Such, H. Y., Heinke, B., Kowal, U., Chess, L., Cantor, C., and Alt, F. (1988), *EMBO. J.* **7,** 727–738.
6. Zouali, M. (1994), *Nature Genet.* **7,** 118–120.
7. Pascual, V. and Capra, J. D. (1991), *Adv. Immunol.* **49,** 1–74.
8. Adderson, E. E., Shackelford, P. G., Quinn, A., Wilson, P. M., Cunningham, M. W., Insel, R. A., and Carroll, W. L. (1993), *J. Clin. Invest.* **91,** 2734–2743.
9. Zouali, M. (1992), *Immunol. Rev.* **128,** 73–99.
10. Demaison, C., Chastagner, P., Theze, J., and Zouali, M. (1994), *Proc. Natl. Acad. Sci. USA* **91,** 514–518.
11. Sasso, E. H., Silverman, G. J., and Mannik, M. (1991), *J. Immunol.* **147,** 1877–1883.
12. Hillson, J. L., Karr, N. S., Oppliger, I. R., Mannik, M., and Sasso, E. H. (1993), *J. Exp. Med.* **178,** 331–336.
13. 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.
14. Sasano, M., Burton, D. R., and Silverman, G. J. (1993), *J. Immunol.* **151,** 5822–5839.
15. Silverman, G. J. (1992), *Int. Rev. Immunol.* **9,** 57–78.
16. Silverman, G. J., Sasano, M., and Wormsley, S. B. (1993), *J. Immunol.* **151,** 5840–5855.
17. Berberian, L., Goodglick, L., Kipps, T. J., and Braun, J. (1993), *Science* **261,** 1588–1591.
18. Berberian, L., Shukla, J., Jefferis, R., and Braun, J. (1994), *J. Acquired Immune Defic. syndrome* **7,** 641–646.
19. Zouali, M. (1995), *Immunol. Today* **16,** 399–405.
20. Lafon, M., Scott-Algara, D., Marche, P. N., Cazenave, P.-A., and Jouvin-Marche, E. (1994), *J. Exp. Med.* **180,** 1207–1215.
21. Muro-Cacho, C. A., Pantaleo, G., and Fauci, A. S. (1995), *J. Immunol.* **154,** 5555–5566.
22. Andris, J. S., Ehrlich, P. H., Ostberg, L., and Capra, J. D. (1992), *J. Immunol.* **149,** 4053–4059.
23. Andris, J. S., Brodeur, B. R., and Capra, J. D. (1993), *Mol. Immunol.* **30,** 1601–1616.
24. Adderson, E. E., Shackelford, P. G., Quinn, A., and Carroll, W. L. (1991), *J. Immunol.* **147,** 1667–1674.
25. Huang, D. F., Olee, T., Masuho, Y., Matsumoto, Y., Carson, D. A., and Chen, P. P. (1992), *J. Clin. Invest.* **90,** 2197–2208.
26. Andris, J. S., Johnson, S., Zolla-Pazner, S., and Capra, J. D. (1991), *Proc. Natl. Acad. Sci. USA* **88,** 7783–7787.
27. Marasco, W. A., Bagley, J., Zani, C., Posner, M., Cavacini, L., Haseltine, W. A., and Sodroski, J. (1992), *J. Clin. Invest.* **90,** 1467–1478.
28. Moran, M. J., Andris, J. S., Matsumato, Y., Capra, J. D., and Hersh, E. M. (1993), *Mol. Immunol.* **30,** 1543–1551.
29. Bagley, J., Dillon, P. J., Rosen, c., Robinson, J., sodroski, J., and Marasco, W. A. (1994), *Mol. Immlunol.* **31,** 1149–1160.
30. van der Donk, E. M., Schutten, M., Osterhaus, A. D., and van der Heijden, R. W. (1994), *Aids Res. Hum Retroviruses* **10,** 1639–1649.
31. Zouali, M., ed. (1996), *Human B-Cell Superantigens,* Molecular Biology Intelligence Unit, Austin, TX, Springer Verlag, in press.