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Human Autoantibodies and Their Genes

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ABSTRACT

We undertook an analysis of the B cell repertoire at both the germline and somatic levels. To assess the content and organization of the IgH-V and IgK-V loci in SLE, endonuclease-generated polymorphisms were used to characterize individual variations within the human V gene segments. The results are compatible with the conclusion that this disease is not caused by major abnormalities in the structure, size, or organization of the IgV loci. We propose that hyperproduction and lupus-associated autoantibodies arises through a two-stage mechanism whereby a general activation of the multireactive preimmune B-cell repertoire precedes oligoclonal expansion of selected B cell clonotypes.

Index Entries: Human antibody genes; SLE; B-cell repertoire.

INTRODUCTION

Mature B-cells respond to antigen stimulation, yet must avoid high affinity with self-antigens that may lead to aggressive autoimmunity. Although formulation of this central paradigm goes back to the early days of immunology and many interpretations have been posited, no mechanism fully accounts for this phenomenon; likewise the origin of autoimmune diseases remains unknown.

Lupus, a prototype of systemic autoimmune diseases, occurs in humans, dogs, and mice with several common pathological features. The autoantibody profile observed in the three species is also conserved with a marked reactivity with conserved structures. Over 35 years ago, when researchers discovered that lupus patients produce serum antibodies to DNA, they also uncovered an antoantibody specificity so unexpected immunologists are still scratching their heads over it. Finding an antibody activity to a nucleic acid in a human disease was the first surprise. The next shock followed quickly: normal individuals may also produce antibodies to DNA. In the past decade, a number of other autoantibodies to nucleus antigens have been fingered as the culprits in a series of systemic

autoimmune diseases (*1*), and that will not be the end of the list. The immunologists community is currently excited about observations that antibodies to DNA may exhibit a catalytic activity (2). As a result, this autoantibody specificity has gone from being solely a disease-linked anomaly to being one of the hottest topics in research.

HUMAN ANTIBODY VARIABLE GENES

The variable region of the antibody molecule heavy chain is encoded by variable (V_H), diversity (D_H), and junctional (J_H) gene segments that are somatically assembled in a site-specific manner. How V_H genes are selected from the libraries of germline genes remains unknown. To probe the available, functional V_H repertoire, we have used peripheral cells stimulated with mitogen so that a large proportion of the B-cells would be stimulated. *In situ* hybridization allows analysis of a large sampling of untransformed B-cells for V_H gene expression (3). The approach offers an advantage over other methods, since one can obtain, on relatively small samples, information about the frequency of cells expressing a specific mRNA, as well as about the amount of mRNA per cell. The specificity of the method was demonstrated by the failure of control probes to hybridize to cells and the ability of Ig gene probes to label positive cells. The results indicated that the mitogen-induced adult repertoire appears nonrandom in terms of V_H gene expression. The V_H3 family was clearly the predominant family in the adult, and the expressed repertoire is essentially in strict correlation with the genomic complexity. It is possible that regulatory factors influence the expressed repertoire and that the proportions of pseudogenes also play a role. In this regard, a group of investigators noticed a structural heterogeneity in human V_H pseudogenes (4). They found that, although some pseudogenes are conserved and have few mutations, others are diverged and have drastic changes. They also observed that some pseudogenes are conserved, and many of them are not very different from active V_H gene segments. In the conserved pseudogenes, the relative distribution of mutations in framework (FRW) and complementarity determining regions (CDRs) was similar to those of functional genes. The features led to the proposal that some selection constraints operated in these pseudogenes (*4*). That pseudogenes are evolutionary conserved suggests that they may play a role in shaping the V_H gene repertoire. It will be of interest to explore the evolution of the human V_H cluster in the human population and to test the hypothesis that conservation of germline sequences confers an evolutionary advantage.

SELECTION OF AUTOANTIBODY VARIABLE GENES

To examine the genetic repertoire from which the V_H regions of DNA binding antibodies are derived, we have produced a panel of antibodies

that display high binding to DNA in solution. At low concentrations, unlabeled inhibitor causes 50% inhibition of binding. Although it is not possible to obtain an affinity constant of anti-DNA antibodies, these antibodies display apparent antigen binding constants that are, in fact, similar to those of antigen-induced human antibodies. In addition, they are highly specific for DNA, and exhibit no crossreactivity with a panel of other autoantigens and exogenous antigens. They also express the 0-81 idiotype (Id), a marker present in SLE patients with active disease (*5*). Their Ig variable region genes were amplified by PCR, cloned, and the complete sequences were determined (*6,7*). The V_H gene segments are derived from the V_H3, V_H4, and V_H2 gene families. When compared with germline genes, sequence variation found in the V_H segments of the antibodies can be explained by somatic mutations leading to single base substitutions. The fact that a high amount of mutations was found raises the questions of whether the rate of somatic mutation in the corresponding B-cells is similar to that of B-cells producing conventional antibodies, and whether these mutations alter the affinity for self or the potential of the antibodies for internal connectivity. That accumulation of mutations shifts the specificity toward a pathogenic autoantibody is an intriguing possibility. It is also remarkable that all the lupus anti-DNA V_H genes that we (*8*) and others (*9–11*) have analyzed are hypermutated. This somatic diversification may suggest that B-cell clonotypes expressing germline V_H genes that impart high auto-antibody affinity or 0-81 idiotype expression are suppressed.

Comparative genomic PCR analysis showed that the substitutions seen were acquired by somatic mutations of germline variable genes. Although this point still calls for a formal proof by germline gene cloning, it is also evident that somatic point mutations are also present in the D_H and J_H gene segments. The pattern of mutations in the V_H genes sequences is characteristic of antigen-driven selection. In addition, all the expressed V_H genes have a replacement/silent mutation ratio higher in the CDRs than in the framework regions. This high load of mutations and the pattern of the substitutions seen are consistent with an antigen-driven B-cell activation and clonal expansion process via T helper cell-dependent mechanisms.

The analysis of fine specificity of the repertoire in different individuals showed the dominance of subtle clonotypic patterns. Clonotypic variation has been observed even in mouse inbred strains (*12*). The subtle variations seen in V_H and V_L genes probably reflect the heterogeneity of random utilization and various combinatorial associations of genes derived from various V_H and V_L gene families. Our findings indicate that V_H genes belonging to different families may be used in constructing DNA-specific antibodies and that their variable regions are composed of various V_H, D_H, and J_H gene elements, with unique junctional or N-region sequences. If every known mechanism can be used to generate diversity in the anti-DNA antibody repertoire, what determines production of aggressive autoantibodies? It is possible that, in addition to exogenous stimuli, genetic

factors may play a role in determining the relative expansion of a subset of B-cell clones specifying pathogenic lupus antibodies. If DNA-specific B-cells undergo selection in vivo through interactions with antigens, our data reveal that affinity *per se* does not directly correlate with clonal dominance. It is possible that different related antigens promote the selective expansion of idiotype-positive pathogenic clonotypes.

AUTOANTIBODIES AND MEMORY B-CELLS

Studies of the antibody response in several systems have revealed that, although in the primary immune response the serum antibody population is usually restricted, the memory response is more heterogenous. Although the general phenotypic characteristics of immunologic memory are known, its detailed mechanism at the cellular and molecular levels is less well understood. In the last few years, experiments have been performed to address questions concerning how memory cells are generated and what signals are required for memory cell generation after encounter with antigen. If the immune system is viewed as being composed of layers of lymphocytes that arose in phylogeny at different times and function at different times during an individual life-span, the data on anti-DNA antibodies do not support the idea that this immune response is derived from a separate cell lineage than is the primary response (*13,14*).

It is of interest to consider this separate lineage hypothesis in relation to autoimmunity. On the surface, studies on $CD5^+$ B-cells may lend support to the separate lineage hypothesis (*15*). However, quantitation analysis of the B-cell repertoire (*16*) and characterization of the variable genes utilized by autoantibody-secreting cells in autoimmune conditions (*8,17*) also suggests that the corresponding cell precursors may arise directly from the primary response. In the process of evolutionary development, essentially similar ends have been achieved by a diversity of means. Therefore, it would not be too surprising if autoantibody memory cells may be generated both from a separate lineage and also via progenies of primary precursors that undergo differentiation together with Ig gene somatic hypermutation. One hypothesis is that pathogenic autoantibodies represent a small portion of the autoantibody repertoire, but they may have higher affinity for antigen binding and higher efficiency for clonal expansion. This would allow for their selective advantage and codomination in the serum autoantibody response. The dominance of certain idiotopes in the antibody response may be the result of:

1. Efficiency of clonal expansion caused by, for example, affinity differences between Id-positive and Id-negative clones;

2. Id interactions promoting dominance;
3. Effects of Id-specific T helper cells; and
4. In vivo dominance of Id-expressing B-cell precursor clones that arose from pre-B-cells without any regulatory environmental influence.

Whether the extensive somatic mutations we have found in the lupus autoantibodies allowed for a higher affinity for DNA and thus an advantage in selection during the immune response remains an attractive possibility. It will be of interest to test this idea at the molecular level, and ascertain what gene families are important and what is the extent of somatic mutation occurring during the generation of memory B-cells and B-cells secreting pathogenic autoantibodies.

THE PREIMMUNE REPERTOIRE AND PATHOGENIC AUTOANTIBODIES

Why a subset of autoantibodies is only expanded in subjects prone to disease is one of the most baffling questions immunologists are facing. Answering it would, of course, help explain how the disease arises. Finding out the relationship between antibodies of the preimmune repertoire and those that cause tissue injury might also provide a better understanding of the mechanisms that the immune system uses to maintain tolerance. Studies of their variable genes are revealing some clues. When nucleotide sequences of natural antibodies were compared to those of antigen-induced antibodies, it was found that both groups use the same V_H/V_L pairs. Importantly, natural and antigen-induced antibodies differ markedly in their V_HCDR3 with respect to length and amino acid sequence (*18*). This suggests that there are structural constraints for CDR3 of monospecific antibodies rather than natural antibodies.

In humans, it has been suggested that the difference in length between the D_H segments utilized by polyreactive antibodies (36–45 bp) and those utilized by monoreactive high-affinity antibodies (15–24 bp) may be related to specificity of the antibodies (*19*). We found that there is some degree of uniformity in the CDR3 region of some natural antibodies and pathogenic antibodies (*8*), suggesting a clonal relationship between the two sets of antibodies. On the other hand, the germline origin of natural antibodies (*20*) contrasts with that of pathogenic autoantibodies, which are usually IgG and which have generally accumulated a high proportion of replacement mutations in the CDRs (*6,7*). It has been proposed that the latter antibodies derive from precursors that are present in the preimmune repertoire, providing a head start for antibody diversification (*20*).

CONCLUSIONS

When all experimental evidence has been presented and discussed, it is customary to indulge in some degree of speculation, if for no other purpose than to advance testable scientific hypothesis. Several major questions are related to the production of pathogenic autoantibodies:

1. How does T-cell help influence the somatic generation of B-cell autoantibody specificities?
2. What are the precise links between the preimmune repertoire, recognition of self, internal connectivity of the immune system through idiotype interactions (*21*), first-line defense against external invaders, and aggressive autoimmunity?
3. What is the precise genetic composition of the B-cell repertoire involved in aggressive activity against self and responsible of autoimmune diseases?

It must be recognized that this antibody response is antigen-driven in diseased subjects. The clinical diversity in patients with apparently similar profiles may suggest that muliple forces, in addition to antigen, act on selection and expansion of the anti-DNA antibody repertoire. It is also possible that several stimuli may lead to induction of these antibodies, which could account for the difficulties in finding a common structural, molecular, and genetic feature of these antibodies. Finally, the possibility that alterations in the developmental program of V-gene utilization may provide the predisposing cellular and molecular template on which antigen-specific selection will act to induce aggressive autoimmunity (*22*).

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DISCUSSION

M. Zouali

Polanovsky: You talked about groups of V_H genes that are preferentially connected with distinct J_H segments. Is there a correlation between the linear location of V_H genes and J_H segments and their occurrence in the antibodies?

Zouali: Most of these V_H genes have not been mapped precisely on the locus. We do not know where exactly they are located.

Alekberova: Do you have the same results in female and male lupus because lupus is predominantly a woman's disease? You studied renal material. Was that biopsy material or postmortality material?

Zouali: This was postmortality material. Of the five patients we have analyzed, four were women and one was a male, although it is difficult to make statistics here.

Gabibov: Because it is hard work?

Zouali: No, because the sample size is too small.

Paul: Professor Stollar indicated that the specificity of antibodies raised by immunization with DNA becomes similar to that of the autoantibodies by introducing a few mutations. This implies, I assume, that there may not be a fundamental difference in the way the antibodies are generated. You find differences in the way the genes for the two types of antibodies undergo rearrangements. Do you think that this difference is related to the pathogenetic potential of the two kinds of antobodies?

Zouali: For light chain or for heavy chain?

Paul: I think it was the light chains.

Zouali: Yes. I think this is a very important phenomenon. I do not know what the immunogen is, whether it is an autoantigen or whether it is a pathogen, whether it is an idiotype or anti-idiotope. On encounter with this immunogen, it seems to me that these cells are unable to undergo these secondary light-chain rearrangements that usually allow B-cells to escape deletion or pathways that lead to B-cells with other specificities. This may be why these B-cells secreting pathogenic autoantibodies arise.

Hansen: Could you comment on the complete absence of utilization of V_H3?

Zouali: First of all, it seems that there is sequence homology between the third framework region of human V_H genes and a portion of the gp120. This could be one explanation. The other more speculative explanation is that the virus could produce a B-cell superantigen. We know that like T-cells, B-cells also have superantigens. This superantigen could be responsible for deletion of V_H3 genes, for example.

Hansen: As the virus evolves to evade the immune system, there could be this relationship between gp120 and the gene family that was selected for throughout evolution.

Zouali: Or it could be just by chance, because we do not know how old the virus is.