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COMMENTARY

The Genetic Origin of Human Autoantibodies

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Between 1968 and 1974, several papers emerged from the Kunkel laboratory describing cross-idiotypic specificities among human autoantibodies, specifically cold agglutinins (1) and monoclonal rheumatoid factors (2, 3). These reports provided some of the first insights into the genetic origin of autoantibodies, demonstrating that all members of one of the cross-idiotypic groups of human rheumatoid factors (the Wa group), contained V_KIIIb L chains by serologic analysis (3). These discoveries were followed by primary structural analyses of human rheumatoid factors that defined their structural relatedness (4–8). It is important to recall that these early studies (as well as most studies until recently), used monoclonal rheumatoid factors derived from patients with B cell malignancies. Few of these patients had manifestations of rheumatoid disease. The relationship of these monoclonal rheumatoid factors to the polyclonal rheumatoid factors characteristic of rheumatoid arthritis has never been entirely established. Nonetheless, these studies were interpreted to suggest that proteins with similar idiotypes contained similar structures in their hypervariable regions. Not appreciated at the time (because relatively few human proteins had been sequenced) was that the extraordinary similarity of the structures of these molecules suggested that they might derive from similar if not identical germ-line genes.

During the past 10 years, a large body of experimental evidence has accumulated, largely emanating from the laboratory of Dennis Carson, defining in precise serologic and structural terms the relationships and genetic origins of this most interesting group of human antibodies. The most recent of these studies is published in this issue of *The Journal* by Crowley et al. (9). It reports the interrelatedness of a large panel of monoclonal paraproteins with a series of precisely defined serologic reagents largely directed toward monoclonal human rheumatoid factors. The study concludes that the vast majority of rheumatoid factors bear the serologic markers of V_KIII L chains although certain important exceptions exist.

Our present understanding of the genetic origin of human autoantibodies represents a significant advance for modern immunochemistry. Carson and his group have used mAb to define the serologic determinants on this group of human autoantibodies. In addition, they have produced anti-peptide sera to the various hypervariable regions of both the H and L polypeptide chains of the various serologic groupings of these molecules. The reactivity of these anti-peptide sera with polyclonal rheumatoid factors, monoclonal rheumatoid factors, and, as illustrated by Crowley et al., with large panels of paraproteins and with normal sera demonstrates the precision with which one can define antibody molecules at the present time. These studies have nicely dovetailed with the cloning of the germ-line L chain gene segments that give rise to both V_KIIIa and V_KIIIb rheumatoid factors, thus contributing greatly to our understanding of the molecular basis of autoantibodies and autoimmunity (10–15).

The genetic origin of these molecules has long remained an enigma. This question lies at the heart of a fundamental understanding of autoimmunity. Do autoantibodies directly derive from germ-line genes? Do all of us carry the same complement of Ig germ-line genes? Is the distinction between individuals who get autoimmune disease and those who do not based on differences in their Ig V_H or V_L genes or are somatic and/or regulatory factors involved? These issues are being addressed in a fundamental way by a number of scientists using several different systems.

Studies of murine rheumatoid factors by Weigert and his group (16) as well as by the Scripps group of Dixon and Theofilopoulos (17) as well as studies of murine anti-DNA antibodies by Barrett's group (18) provide evidence that there are not fundamental structural differences between autoantibodies that arise in various murine models of autoimmunity and autoantibodies that can be induced and/or selected in normal mice. More importantly, the sequences of many such autoantibodies show numerous somatic mutations, the distributions of which suggests positive selection by Ag (19).

In the human system it has been much more difficult to address these issues largely because of the difficulty in producing human-human hybridomas and because except for the model system of mixed cryoglobulinemia, there are relatively few human situations in which monoclonal autoantibodies are readily available. However, within the last year several groups have developed systems to address this issue. Livneh et al. (20), for example, defined the so-called 8.12 idiotype among human lupus antibodies and recently Hoch and Schwaber (21) identified and sequenced the V_H gene elements encoding a human anti-DNA antibody. Dersimonian et al. (22) have published an important paper concerning the structure of various human anti-DNA hybridomas documenting that the structures of two human anti-DNA antibody V_H regions, one derived from a patient with leprosy and one derived from a patient with lupus, were absolutely identical. These data argue that autoantibodies need not require the somatic mutation of a germ-line gene. An important difference between the majority of human and

mouse studies is that, by and large, the murine antibodies sequenced were of the IgG class (23) whereas the human antibodies were IgM (22).

Recent studies from our own laboratory confirm these observations. In collaboration with Paolo Casali and Abner Notkins, we have examined the V_H nucleotide structures of several monoclonal multiple organ-reactive antibodies and have found some that had identical nucleotide sequences although derived from genetically distinct individuals. Similarly, in collaborative studies with Howard Dang and Norman Talal (24), we have determined the complete V_H structure of an anti-Sm antibody derived from a patient with SLE. In this instance, the nucleotide sequence of the V_H gene segment was identical to a cDNA clone recently published from Perlmutter's group and obtained from a fetal liver cDNA library (25). This result shows 1) that the V_H gene used by this SLE-specific autoantibody is most likely germ-line encoded; 2) that these genes are without significant polymorphism in the general population and, therefore, could play an important physiologic role inasmuch as evolutionary pressure is undoubtedly acting to preserve these structures; and 3) that at least some of these autoreactive V_H genes are expressed early in the development of the B cell repertoire. Collectively these studies further suggest that autoantibodies derived from normal individuals (those without disease) and autoantibodies derived from patients with disease may be structurally identical.

The protein studies of the 1970s led authors to conclude that there were three and only three human V_H gene families. However, a large body of evidence now argues that as many seven different V_H gene families exist. The laboratories of Honjo (26), Alt (27, 28), Perlmutter (25), and Tucker (29), as well as our own (24), have documented the presence of important, relatively small, V_H gene families (1 to 10 germ-line genes) that are present in the germ-line in addition to the 20 to 60 germ-line genes of the V_HI, II, and III subgroups. More importantly, it appears that many of the autoantibodies deriving either from EBV-transformed PBL or by human-human fusions of PBL utilize these relatively small V_H families. This has allowed and will continue to allow a comparison of the germ-line and expressed repertoires in an individual as the screening of relatively few germ-line genes that give rise to potential autoantibodies is manageable.

That the human V_H and V_L genes used in many autoantibodies derive from relatively small V_H and V_L gene families may be of crucial importance. One of our multiple organ reactive antibodies is the first example of a functional V_HIV gene and an antiinsulin antibody studied in our laboratory by Tom Thomas is the first example of a functional V_HV gene. Kipps et al. (10) have shown that a significant number of patients with chronic lymphatic leukemia utilize the V_KIIIb L chain gene, and Humphries et al. (30) report that almost a third of patients with lymphocytic leukemia rearrange genes of the V_HV variable region family. This latter observation is particularly remarkable in view of the fact that there are a maximum of three V_HV germ-line genes representing at most 3% of the entire human V_H gene repertoire. The predominant utilization of some V_H families in specific B cell tumors, along with the predominance of the "classical" families (V_HI, V_HII, and V_HIII) in paraproteins produced by plasma cell tumors arising in the bone marrow, suggests that B cells from distinct subsets at different stages of maturation and/or in different compartments with different microenvironments may utilize different sets of V_H genes.

Out of these studies have come two fundamental points. First, the polymorphism in the human appears to be dramatically less than the polymporphism that distinguishes the various inbred murine strains and, second, at least among the IgM autoantibodies, their V_H and V_L gene segments appear to be direct copies of the germ-line genes.

Why, then, do autoantibodies in patients with autoimmune disease appear "aggressive" whereas "normal" autoantibodies appear relatively benign? Two main structural explanations come to mind. 1) The D segments of these two groups of antibodies appear significantly different and have no known germ-line counterparts. This suggests that either there are additional germ-line D segments or novel mechanisms are involved in the generation of D segments in autoantibodies, a notion that has been described in detail by Meek et al. (31). 2) The pathogenic autoantibodies still remain largely unknown and few have been subjected to structural analysis. It may well be that somatic events operate upon physiologic autoantibodies and transform them either by increasing their affinity or by changing the idiotype, which in turn may allow them to escape idiotype control.

Should the genetic origin of autoantibodies be no different in normal individuals and in patients with autoimmune disease, we are left with the relatively uninspiring conclusion that autoimmunity has no genetic component amongst the Ig genes. This would fly in the face of a large body of data suggesting that idiotypes of both rheumatoid factors and anti-DNA antibodies are heritable as well as considerable statistical evidence suggesting that a second or a third genetic system in addition to the MHC is involved in human autoimmunity. One way to reconcile the information is to postulate that the difference lies in complex regulatory pathways of the immune system (timing of expression in ontogeny, selection of T cell repertoire, etc.). All those factors, along with MHC products and environmental agents could interact to expand, in autoimmune patients, clones that in normal individuals are also present but are down-regulated. Studies such as the Crowley analysis published in the current issue of *The Journal*, go a long way toward addressing such issues and providing important tools for the clinical scientist to address these and other fundamental problems.

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