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B cells in autoimmune diseases: Insights from analyses of immunoglobulin variable (Ig V) gene usage

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Abstract

The role of B cells in autoimmune diseases has not been fully elucidated. It is also unclear whether breaking of B cell tolerance in patients with autoimmune diseases is due to underlying defects in the molecular mechanisms involved in the arrangement of antibody genes or deficiencies in the subsequent selective influences that shape the antibody repertoire. Analysis of immunoglobulin (Ig) variable (V) gene usage is beginning to provide answers to some of these questions. Such analyses have identified some differences in the basic Ig V gene repertoire of patients with autoimmune diseases compared to healthy controls, even though none of these differences can be considered major. Defects in positive and negative selection, mutational targeting and, in some cases, receptor editing have also been detected. In addition, analysis of Ig V gene usage in target organs and tissues of patients with autoimmune diseases have clearly demonstrated that there is a highly compartmentalized clonal expansion of B cells driven by a limited number of antigens in these tissues. Great progress has been made in the structural and functional characterization of disease-associated antibodies, largely because of the development of the combinatorial library technique. Use of antibodies generated by this technique offers great promise in identifying B cell epitopes on known target antigens and in gaining greater insights into the pathogenic role of B cells in both B- and T-cell-mediated autoimmune diseases.

Keywords

Autoantibody; autoimmune; spectratyping; CDR3; somatic hypermutation

Take home messages

- According to the Witebsky-Rose-Koch criteria, an autoantibody is considered to be pathogenic if 1) transfer of the autoantibody induces disease, 2) the autoantibody can be isolated from disease-specific lesions, and 3) disease can be induced by immunization with the anti-idiotypic autoantibody.
- No obvious differences among the variable gene segment or the CDR3 region have been observed within B-cell and T-cell mediated autoimmune diseases.

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- Antigen presentation rather than antibody production may be the primary role of B-cells in contributing to the pathogenesis of some T-cell mediated autoimmune diseases.
- Using monoclonal antibodies in combination with spectratyping methods are invaluable tools in furthering today's understanding of the pathogenesis of autoimmune diseases.
- Overlapping inflammatory conditions such as rheumatoid arthritis or allergy need to be considered when investigating the antibody repertoire.
- Since high mutation frequency is not always necessary for the development of high-affinity monoreactive autoantibodies indicates that some germline genes encoding such antibodies fail to be deleted in patients with autoimmune diseases.

I. Introduction

The enigma of autoimmune disease etiology and pathology require the dissection of both the cellular and humoral compartments of the immune system. At the molecular level, the diversity of the antibody variable region has been investigated such as repertoire analysis of the variable region, including both the heavy chain and light chains. Additionally, various methods have been applied in exploring the structural and functional characteristics of the antibody variable region such as hybridomas, phage library display, and spectra-typing. Ultimately, the clues observed at the molecular level will shed some light in understanding the pathogenesis of autoantibodies.

II. Autoimmune Disease Research Progress

1. B cells in Autoimmune Diseases

In autoimmune diseases, the role of B cells and the antibodies (Abs) they produce is still incompletely understood. Some of these diseases are classified as B cell-mediated since pathogenic auto-Abs are clearly implicated in the destruction of the target tissue(s). According to the Witebsky-Rose-Koch criteria, an autoantibody is considered to be pathogenic if 1) transfer of the autoantibody induces disease, 2) the autoantibody can be isolated from disease-specific lesions, and 3) disease can be induced by immunization with the anti-idiotypic autoantibody. Classical autoimmune diseases fulfilling these criteria include myasthenia gravis (MG), pemphigus vulgaris, idiopathic autoimmune thrombocytopenic purpura (AITP), and Graves' disease. The glomerulonephritis that develops in a subset of patients with systemic lupus erythematosus (SLE) is also mediated by Abs, more specifically the deposition of certain types of anti-dsDNA Abs in the kidney.

Table 1 demonstrates that for B-cell mediated autoimmune disease such as SLE and MG, work has been done investigating the SLE variable region using different PCR techniques such as phage display library and hybridomas. These studies focused on several different cell types such as renal, splenocyte, blood, and follicular cells in SLE and thymus tissue in MG. The studies also demonstrated variable heavy (VH) gene segment biases such as VH4 and VH5 in SLE and VH3 in MG. Finally, no obvious differences are observed within the variable gene segments that would yield clues as to the source of gene dysregulation and autoimmune generation.

Other autoimmune diseases have been shown to be predominantly T cell-mediated, examples being rheumatoid arthritis (RA), type 1 diabetes (T1D), multiple sclerosis (MS), Sjögren's syndrome (SS), and primary biliary cirrhosis (PBC). Nonetheless, a vast majority of patients with such T-cell-mediated autoimmune diseases exhibits high affinity and titer autoAbs to a

variety of antigens, and B cells are seen in the infiltrate of the target tissues. In addition, a hallmark of MS is the presence in brain and cerebrospinal fluid of oligoclonal IgG, which is visualized as characteristics bands after electrophoresis. SS is frequently characterized by hypergammaglobulinemia and circulating immune complexes and is associated with lymphoproliferative diseases predominantly involving B cells.

The T-cell mediated autoimmune diseases such as RA, SS, PBC, anklyosing spondylitis, and celiac disease are described in Table 2. As with the B-cell mediated autoimmune disease studies, several groups have investigated the VH of BCR and both the heavy and light chains using limited patient populations. A VH gene family bias was reported, such as VH4 for Celiac Disease and RA. Another example is observed in the single ankylosing spondylitis, in which the VH5 is found to be over-represented, VH3 with normal representation, and VH4 under-represented. In addition, the CDR3 length for each autoimmune disease varied demonstrating no obvious differences in any disease population. Finally, one last study looking at the VH of liver portal cells from an overlapping B-&T-cell mediated autoimmune diseases such as PBC/SLE and PBC/Hashimoto/SS showed the CDR3 region expression to be oligoclonal.[6] In summary, as with the B-cell mediated autoimmune diseases, no obvious differences exist among the variable gene segment or the CDR3 length in the above T-cell mediated autoimmune diseases.

Even in B-cell mediated autoimmune diseases where antibodies have clearly been shown to play a pathogenic role, the production of these pathogenic antibodies alone may not be sufficient for disease development [15,16]. Instead, there is evidence suggesting that differential regulation of T cell responses to disease-specific antigens prevents a B cell response and the development of disease.

Antigen presentation rather than Ab production may be the primary role of B cells in contributing to the pathogenesis of some T cell-mediated autoimmune diseases. Several lines of evidence indicate that B cells in their role as APCs rather than antibody producing cells accelerate the initiation of diabetes in NOD mice [17,18], a spontaneous and rather faithful mouse model of human T1D. In other autoimmune diseases such as experimental autoimmune thyroiditis (EAT) and T1D, antigen-specific B cells may play an important role in the perpetuation of the autoimmune response due to of the high efficiency with which they can take up, process, and present antigen to autoreactive T cells. They may also contribute to determinant spreading and diversification of the autoreactive T cell response [19].

While no obvious differences exist among the several patient populations studied, published gene usage investigations still contribute greatly as a piece of the overall picture of the course of autoimmune disease. This includes identification of potential clonal expansion of specific gene segments in the variable region of the antibody molecule. The various gene segment combinations within the antibody variable region from autoimmune patient samples have provided clues in which VH gene families are preferred as compared with controls. Additionally, the gene segment utilization studies can provide additional clues such as the source of gene dysregulation and other factors in the etiology of autoimmune diseases.

2. Antibody diversity

There are several mechanisms for generating the enormous diversity of the antibody repertoire. The antibody molecule consists of two identical heavy (H) and a light (L) chains, each of which contains a variable domain for antigen recognition and a constant domain for effector functions. The variable domain of H chains is created by assembly of variable (V), diversity (D), and joining (J) segments, while L chains are assembled from V and J segments only. Within each variable domain, there are three areas of very high variability known as complementarity determining regions (CDR) embedded into four framework regions (FR). The entire FR 1, 2

and 3 and CDR1 and CDR2 are encoded by the V gene segments, while FR4 is encoded by the J segment. The center of the antigen-binding site is formed by the CDR3 of the heavy and light chains and its somatic rather than germline origin ensures that it is the most diverse of the CDR. For the assembly of H chains, there are 51 functional VH genes grouped into 7 families, ~30 D segments, and 5 J segments. The Vκ L chains encoded from chromosome 2 locus are grouped into 7 families, 6 of which contain a total of ~40 functional genes. The Vλ locus on chromosome 22 contains 51 genes, of which 30 are thought to be functional. These are grouped into 10 families and 3 clusters, with cluster A being Jλ-proximal and cluster B being Jλ-distal. The combination of different V(D)J segments creates considerable diversity, which is further enhanced by the pairing of different H and L chains. In the process of recombination, the available repertoire is further enlarged by imprecise joining, addition of P and N nucleotides, and exonuclease trimming. Finally, antigen binding induces somatic hypermutation, thereby further diversifying the antibody repertoire.

In total, a single BCR has 9 CDRs and 16 FR. Currently, the main identified purpose of FW is to provide the structure for the CDRs through peptide folding and binding interaction of the nucleotide atoms. Numerous publications have reported differing lengths of CDR3 in diseases. The CDR3 length depends on both somatic recombination and the nucleotide and palindrome additions which can range from 1 to 23 amino acids long. Since heavy chain CDR3 has proved to be the most diverse of the variable regions, several studies have focused on CDR3 gene usage in autoimmune disease in hopes of yielding clues of both etiology and persistence of autoantibody production.

3. Current Methods

The starting material for a majority of studies on Ig V gene usage is mRNA that is reverse transcribed into cDNA and amplified by PCR. Since activated B cells and plasma cells produce vastly increased amounts of mRNA compared to resting B cells, this introduces a major bias towards sequences expressed by these subsets. Use of genomic DNA reduces this bias and has the further advantage of allowing analysis of nonproductive rearrangements. Single-cell PCR has been shown to be a highly sensitive method for the analysis of the overall Ig V gene repertoire [20-23]. However, the analysis of hundreds of different B cells by this method is laborious and time-consuming. CDR3 spectratyping, a method that was originally used for the study of T cell receptors and is based on size variations within the CDR3, allows the more rapid characterization of V gene usage in a larger number of patients [24].

The development of hybridoma and EBV transformation techniques provided invaluable tools for analyzing the repertoire of B cells producing specific antibodies [25,26]. In humans, however, these methods suffer from low transformation frequencies and yield monoclonal Abs mostly of the IgM class, whereas the autoAbs of patients with autoimmune diseases are generally of the IgG class. The generation of high affinity IgG mAbs has become possible via the use of combinatorial libraries, in which separate H and L chain libraries are combined in order to allow expression of randomly reassorted H and L chains. Most commonly now, phage display libraries are used for this purpose. The procedure involves reverse transcription of mRNA into cDNA, which is then inserted into the phage genome for expression on the surface of each filamentous phage particle in association with a minor coat protein. Generally, either Fab or single chain variable fragments (scFv) are displayed. Selection of Abs with the specificity of interest is achieved through repeated panning with the appropriate antigen [27]. A major concern with this technique is that the random reassortments of H and L chains do not accurately recapitulate their in vivo rearrangements. However, comparisons with the reactivity and specificity of Abs in serum of patients with autoimmune diseases suggest that mAbs obtained from combinatorial libraries can reproduce the structural and functional properties of disease-related Abs [27]. Like hybridomas, this method does not allow distinction between

disease-relevant and irrelevant autoAbs, but the fact that IgG Abs can be created and that repeated panning can insure their high affinity for the antigen of interest increases the probability of isolating relevant autoAbs.

4. The importance of B cell V gene usage analysis

It has been hypothesized that the production of high affinity monoreactive autoAbs in autoimmune diseases could arise from intrinsic abnormalities in the generation of Ig V genes. Such abnormalities could involve skewed use of particular gene families or individual genes for the encoding of the general antibody repertoire, preferential use of particular H and L chain combinations, defective receptor editing, and anomalies in the types and frequencies of somatic mutations [28,29]. Defects in these processes could be due to altered activity of enzymes involved in recombination and subsequent processes. One of the primary aims of analyzing the Ig V gene repertoire is to determine whether usage of particular gene families or individual genes for the creation of the general Ab repertoire is skewed in patients with autoimmune diseases compared to healthy subjects. Another important goal has been to determine whether there is antigen-driven clonal expansion of B cells in the target tissue(s), as would be evidenced by the use of the same CDR3 sequence in conjunction with a high ratio of replacement (R) to silent (S) mutations in the CDRs, but not in the FRs. The main identified purpose of FR is to provide the structure of the CDRs through the peptide folding and binding interaction of the nucleotide atoms. Much research has also focused on the V gene repertoire of specific autoAb-producing B cells with the objective of determining whether there are certain V gene combinations prone to mutations that confer high affinity binding to self proteins and of defining sequence characteristics associated with pathogenicity [30]. Patients with autoimmune diseases frequently produce autoAbs to a large variety of antigens. For many of these, it has not been possible to determine their relevance to the pathogenesis of the disease. In some cases, even autoAbs with the same specificity are not equally pathogenic. For example, not all SLE patients with anti-dsDNA Abs develop glomerulonephritis, and only some anti-dsDNA Abs isolated from SLE patients are able to induce proteinuria upon passive transfer to experimental animals [31]. The pathogenicity of different autoAbs with the same specificity cannot be determined with the polyclonal autoAbs present in the serum of individual patients. Knowledge of the sequence of a variety of autoAbs recognizing the same antigen enables the production of recombinant monoclonal Abs for the purpose of understanding the pathogenesis of autoimmune diseases. Such mAbs are also becoming increasingly valuable in mapping the epitopes they recognize, particularly conformational epitopes. [32]

The autoantibody conformational idiotype map will further confirm and/or improve the Witebsky-Rose-Koch's criteria of pathogenic autoantibody. This additional autoantibody information could result in the development of vaccines for autoimmune disease. In conclusion, B-cells play a dual, but prominent, role in autoimmune diseases and the BCR will provide critical clues in the characterization of both the idiotype and epitope. Thus using mAbs in combination with spectratyping methods are invaluable tools in furthering today's understanding of the pathogenesis of autoimmune diseases.

III. Differences in the overall repertoire

1. Comparison of nonproductive rearrangements

Only examination of both the productive and the non-productive repertoire allows distinction between the influences of the molecular events of recombination and the effects of positive and negative selection by antigens, since nonfunctional rearrangements are not shaped by selection processes. Only limited data are available on both functional and nonfunctional rearrangements in healthy subjects and patients with autoimmune diseases.

Analysis of productive and non-productive rearrangements in peripheral IgM^+ B cells of two healthy subjects indicated that human VH gene usage is intrinsically biased [33]. In particular, VH3 constitutes not only the largest VH family, but is used more frequently than expected from the proportion of genes it contributes to the total number of functional VH genes (see Table 3). In contrast, VH4 appears to be negatively selected as indicated by the significantly reduced representation in the productive compared to the nonproductive repertoire. Only seven VH genes mostly of the VH3 and VH4 families make up almost 60% of the productive peripheral B cell repertoire. There is also evidence of recombinatorial bias as well as positive and negative selection in the usage of Vκ and Vλ gene families and of individual genes within these families [20,34] (see Tables 4 and 5).

Comparison of peripheral B cells of a SLE patient with those of normal subjects did not reveal significant differences in the usage of VH families in the nonproductive rearrangements, but only 3 nonproductive SLE rearrangements were analyzed [29] (Table 3). A study that did not distinguish between the productive and nonproductive repertoire confirmed the absence of skewing in the usage of VH genes in patients with SLE [35]. Similarly, the VH3 and VH4 repertoire of peripheral B cells isolated from 4 RA patients did not differ significantly from that of 4 age- and sex-matched controls [36].

Analysis of nonproductive Vκ-Jκ gene rearrangements in peripheral B cells from 3 SS patients and 2 healthy controls did not identify major differences between the two groups in the distribution of Vκ gene families, although individual Vκ genes were differentially used in patients and controls [37] (Table 4). However, usage of Jκ2 was significantly greater in the productive repertoire of SS patients than in controls, while Jκ4 was underrepresented. In the same patients, usage of Vλ genes in the nonproductive rearrangement was similar to that of controls with the exception of overrepresentation of Vλ10, but there was a significantly higher usage of Jλ2/3 and less frequent usage of Jλ7 in the nonproductive as well as the productive repertoire of the patients compared to the normal controls [22] (Table 5). A study of a single patient with SLE also found usage of Vκ genes to be similar to that of normal adults [38]. In contrast to the SS patients, Jκ gene usage was also similar in the SLE patient and normal controls (Table 4). Similar to the SS patients, however, Jλ2/3 was significantly over-represented while usage of Jλ7 was decreased in the nonproductive rearrangements of this SLE patient [29] (Table 5).

2. Productive rearrangements

Screening of the entire phage display IgG Fab libraries obtained from peripheral blood of a SLE patient and her healthy twin and revealed that the VH5 gene family was represented in 42% and 37% of the gamma rearrangements of the SLE and the healthy twin, respectively, whereas it usually accounts for <1% of rearrangements in libraries from healthy adults and ~2% in the productive repertoire of peripheral B cells [1] (and see Table 1). In another SLE patient, VH3 was used significantly more frequently than in controls and VH4 was underrepresented, while VH5 was not detected [29] (Table 3). JH gene usage was similar to that in healthy adults. In the same patient, the productive rearrangements involving Vλ and Jλ also differed significantly from those of controls, with greater usage of Vλ6 and Vλ8, less frequent use of Vλ4, and overrepresentation of Jλ7 [29] (Table 5). In addition, usage of Vκ1 was significantly increased, as was usage of Vκ4, whileVκ2 and Vκ3 tended to be underrepresented [38] (Table 4). In a patient with primary SS, the frequencies of VH family genes in her productive repertoire did not differ significantly from those reported for healthy adults [39] (Table 3). Similar to the results in the SLE patient, Vκ1 was significantly over-represented in productive Vκ-Jκ gene rearrangements of 3 SS patients, while Vκ3 family members were found with significantly decreased frequency compared to controls [37].

3. Receptor editing

Receptor editing refers to the secondary rearrangement in which an H chain combines with a new L chain. This process is thought to be important in preventing the persistence of autoreactive B cells. As yet, little is known about the frequency of receptor editing in healthy subjects or patients with autoimmune diseases. Several groups of researchers have provided indirect evidence suggesting that receptor editing was defective in patients with autoimmune diseases. For example, the preferential use of J-proximal Vκ and Vλ genes for encoding Abs against thyroid peroxidase (TPO) and the low mutation frequencies detected within these genes suggest defective receptor editing in patients with autoimmune thyroid disease [40]. A similar defect in SLE patients is suggested by the highly skewed use of proximal Vκ genes and the infrequent utilization of downstream Jκ segments in anti-DNA Abs produced by EBV-transformed B cell clones [41]. Another study found evidence that receptor editing prevented the expression of mRNA derived from the A30 gene in healthy subjects, whereas some SLE patients rearrange and transcribe this gene, which encodes the κ chain of a cationic anti-DNA Ab [42].

In marked contrast to these findings, usage of Vλ genes of the most Jλ-proximal cluster was decreased in a patient with untreated SLE, compared to normal controls, while that of the most distal cluster was increased, suggesting an increased occurrence of receptor editing [29]. Receptor editing involving Vλ genes occurred before somatic hypermutations, indicating that it must have taken place centrally, i.e., in the bone marrow. Indications of increased receptor editing were also found in the Vκ gene usage of this patient, but analysis of the mutational frequency in the Vκ genes indicated that it occurred in the periphery [38]. Others, however, did not find evidence of increased receptor editing in CD19+ B cells from SLE patients [43].

In patients with SS, over-representation of Jλ2/3 and a decreased frequency of Jλ7 were noted in rearrangements in peripheral B cells, suggesting decreased receptor editing [22]. Analysis of the VH and VL repertoire in CSF of 4 MS patients by performing single B cell PCR provided evidence of receptor editing in 2 clones of one of the patients [23]. In particular, one clone characterized by a specific H chain CDR3 sequence was paired with several different L chain sequences, most of which were highly homologous and one of which was identical with its germline sequence. In addition, the difference in the mutational frequency between H and L chain sequences was significantly greater in this clone compared to the other B cell populations identified in the same patient. Nonetheless, the absence of indications of receptor editing in the other three patients suggests that this is a fairly rare phenomenon in MS.

Both productive and nonproductive rearrangements also differ significantly within the normal patient group. The observed productive repertoire for VH4 is lower for Normal CD5-/IgM+ B cells[33] but higher for Vk3[34]. However, only the SS and SLE group demonstrated significant differences between a productive and nonproductive repertoire such that SLE had decreased productive rearrangement for Vλ4 and Vλ5 while increased for Vλ6. The SS repertoire demonstrated increased productive rearrangement for Vλ7 and decreased for Vλ10.

In summary, the tables outlined in this section reflect only the B-cells in the peripheral blood within an extremely limited patient population size. The two autoimmune diseases, SLE and SS are identified as systemic diseases with several affected organs such as kidney, skin, heart, joints, lungs, circulation, brain (SLE) and salivary glands (SS). Additionally, both SS and SLE are identified as B-cell mediated autoimmune diseases which qualify both as reasonable models for the BCR variable region study. Since no obvious differences between the BCR gene segments between these two autoimmune diseases and normal controls have been shown thus far, additional factors need to be considered like overlapping inflammatory conditions such as rheumatoid arthritis or allergy. Finally, further studies need to be done with autoantigen-specific B-cells, both naïve and memory, to confirm these findings.

4. Mutational frequency and targeting

The overall mutational frequency in productive and non-productive Vκ and Vλ rearrangements of peripheral B cells did not significantly differ between 3 patients with SS and healthy controls [22,37]. However, the frequency of G nucleotide mutations was significantly higher in the nonproductive, but not in the productive, Vκ repertoire of these patients [37]. In addition, the mutational targeting of the RGYW and WRCY motifs, which have been identified as major mutation hot spots in normal subjects, was essentially absent in SS patients. However, positive selection of these mutations was also obvious in the patients.

A high frequency of G mutations in the Vκ and Vλ as well as the VH rearrangements was also reported for a patient with SLE [44-46]. In contrast to the 3 SS patients described above, targeting of mutation to the RGYW and WRCY motifs was normal, but there was evidence that positive selection of RGYW mutations was absent from the Vκ repertoire [38]. Also unlike the observations in SS patients, the overall mutational frequency was not only higher than normal in this SLE patient, but was significantly increased in the nonfunctional compared to the functional Vκ repertoire, which is the opposite of what is seen in normal subjects [44]. Furthermore, there was a markedly reduced frequency of nonfunctional VH rearrangements compared to healthy subjects, and the R/S ratios in the FRs were similar in the productive and the nonproductive repertoire in this SLE patient, whereas they are lower in the productive rearrangements of normal controls [46]. Together, these results suggest that both positive and negative selection of mutated V gene rearrangements is defective in this SLE patient. A high R/S ratio in FRs and a low frequency of nonproductively rearranged VH genes was also reported for another SLE patient, providing further evidence for a reduced negative selection [4]. Interestingly, whereas SLE patients were reported to have an increased mutational frequency and normal targeting of mutation to the RGYW sequences, lupus antibodies positive for the anti-DNA associated F4 idiotype exhibited a normal mutational frequency, but decreased targeting of these mutational hot spots [47].

In an analysis of VH6 rearrangements in peripheral B cells of T1D patients and in splenocytes of AITP patients, the frequency of somatic mutations was found to be significantly increased in the non-functional VH6 repertoire, but markedly decreased in the functional repertoire from both patient groups compared to healthy controls [48]. However, T1D patients exhibited an increased number of mutations per gene in the functional rearrangements of VH6, whereas the number of mutated genes, but not the number of mutations per gene, was increased in AITP patients in this family of VH genes.

IV. Differences in Ig V gene usage in other compartments

The preceding indicates that there is no major skewing in the nonproductive repertoire, but skewed usage of certain VH and Vκ and Vλ genes in the productive rearrangements of essentially all patients with autoimmune diseases analyzed to date. These studies used peripheral blood mononuclear cells (PBMC) as starting material. Peripheral B cells contain a population of recirculating memory cells that have encountered a large variety of antigens over the subject's lifetime. In contrast, B cells from germinal centers (GC) in secondary lymphoid tissues represent that subset of B lymphocytes responding to antigen and undergoing antigen-driven hypermutation and selection at the time the sample is obtained.

An analysis of splenic GC B cells of a SLE patient suggested a possible bias toward expression of VH5 family genes and complete absence of VH1 genes in addition to overutilization of J4 and underutilizatian of J1 and J2 [4]. In contrast, no deviation from the normal repertoire was seeded in another study of SLE patients [35]. Of particular interest, GC-like structures, containing B cells, T cells, and follicular dendritic cells, have been detected within non-lymphoid target tissues in several autoimmune diseases. The first demonstration that such GC-

like structures were able to support antigen-driven B cell expansion and somatic hypermutation came from the finding of clonally related B cells in consecutive sections of synovial tissue from patients with RA [49]. These results have been confirmed [50] and extended by the finding the GC-like structures in RA synovium also sustain isotype switching [51]. Of note, hybridomas from synovial tissue of RA patients exhibited the characteristics of an antigen-driven expansion, but were negative for RF. This suggests that RF is not the only, and maybe not even the most important, antigen involved in the local B cell expansion repeatedly observed in RA synovium [52]. GC-like structures were also detected in the thymus of a patient with MG [5]. The population of B cells isolated from GCs containing acetylcholine receptor (AChR)-specific plasma cells was found to be highly heterogeneous in terms of their Ig VH gene usage. Nonetheless, there was evidence that a subpopulation of these B cells was undergoing antigen-driven clonal expansion, somatic hypermutation and selection. The salivary gland of SS patients is another target organ of autoimmune reactions that was found to contain GC-like structures, and a dominant B cell clone with stepwise accumulation of mutations could be isolated from these structures [53]. The finding of highly limited numbers of rearrangements in the VH genes of B cells isolated from lymphoid aggregates in portal tracts of the liver of PBC patients suggests that these aggregates also support oligoclonal expansion of B cells [6].

Several other studies have provided evidence for limited V gene usage and clonal expansion in the target tissues of a variety of autoimmune diseases. Analysis of the IgG heavy chain sequences obtained from the brain of a patient with acute MS revealed a striking over-representation of the VH4, which was used in ~60% of the sequences analyzed, including those that exhibited evidence of clonal expansion [54]. The CDR spectratyping profile of B cells from brain lesions of another group of MS patients deviated markedly from the profile obtained with peripheral blood lymphocytes (PBL) of normal subjects in almost all Ig VH families, with VH1 and VH4 showing the most obvious signs of clonal expansion [24]. Cloning and sequencing of VH1 and VH4 genes confirmed the usage of one or both of these genes in clonally related B cells of each of the 10 patients examined. Similarly, clonally expanded B cells obtained from cerebrospinal fluid (CSF) of 4 MS patients all use members of the VH4 gene family [55].

Analysis of brain tissue from two other MS patients, though revealing skewed usage of VH genes compared to the VH family distribution in normal peripheral B cells, did not confirm this striking over-representation of VH4 [56]. Instead, the results indicated that the pattern of VH gene usage was unique in each patient, even though clonally related B cells with extensive somatic mutations and accumulation of replacement mutations mainly in the CDRs, but not in the FRs, were found in both of these patients. Similar repertoire heterogeneity despite the detection of clonally expanded B cell populations has also been reported from the analysis of cerebrospinal fluid in MS patients [23,57].

Of note, clonally related B cells are rarely found in adjacent GCs of the same tissue [4,53], suggesting that these cells do not seed new GCs. Nonetheless, there are reports of clonally related sequences in different joints of the same RA patient [58,59] and in contralateral biopsy specimens obtained from patients with SS [11]. These findings not only indicate that B cell clones recirculate, but also suggest that mature B cells can undergo repeated rounds of somatic hypermutation.

Few studies to date have compared the Ig V gene repertoire in peripheral blood and either secondary lymphoid tissues or target tissue(s) in the same patients. CDR3 spectratyping showed differential usage of Vκ and Vλ genes in synovium and peripheral blood of RA patients, but each patient exhibited a unique pattern [60]. Another spectratyping study indicated progressive narrowing of the repertoire from peripheral blood to synovial fluid and finally to

synovial tissue [61]. Similarly, none of the clonally expanded or distinct VH sequences that were expressed in plaque of a MS patient could be detected in PBL of the same patient [62]. Others found only a single identical sequence in CSF and PBMC of a MS patient, whereas numerous other molecular clones in PBMC were unrelated to those identified in the CSF [57]. Together, these results suggest that there is strong compartmentalization of the antigen-driven B cell response within the CNS of MS patients. Such compartmentalization is also obvious in patients with PBC, in whom no common sequences could be detected in the portal area of the liver and peripheral blood [6]. In a 76-year old SS patient, usage of individual Vλ and Vκ genes was found to differ between the patient's parotid gland and peripheral blood [63]. In particular, Vλ2E was used with markedly higher frequency in the productive repertoire of the parotid gland compared to peripheral blood while not being detected in any of the nonproductive rearrangements. Among Vκ genes, higher usage of A27 was found in the nonproductive and even more so in the productive repertoire of the parotid gland compared to peripheral blood. In addition, several Vκ rearrangements from the parotid gland were clonally related. Interestingly, this is one of the few studies in which members of a B cell clone were detected both in the peripheral blood and in the target tissue. However, those from the parotid gland contained 7 and 18 mutations, respectively, whereas those from blood were unmutated. This, together with the fact that the differences between the peripheral and the glandular repertoire were most pronounced in the productive rearrangements, indicates that B cells infiltrating the salivary glands are positively selected and constitute a unique population. This is further evidenced by the finding that replacement mutations appeared to be negatively selected in the parotid repertoire compared to that of peripheral blood [63], which agrees with the observation of decreased R/S ratios in VH gene rearrangements of B cells obtained form the salivary gland of other SS patients [53,64]. Another example of restricted V gene usage in SS is provided by the finding that a total of 11 B cell clones obtained from salivary gland biopsy samples from 8 different SS patients (identified as clonal bands on electrophoresis after nested PCR) used only two VH gene loci, namely V1-69 and V3-7 [11].

The pronounced compartmentalization of the antigen-driven clonal expansion of B cells in the target tissue noted in all of these studies, including that of a SS patient [63], is in marked contrast to findings in several other SS patients. Comparison of VH rearrangements in peripheral blood and parotid gland $CD19^+$ B cells of a SS patient, analyzed by single-cell PCR, revealed similar VH gene usage in blood and glandular B cells and polyclonal B cell infiltration of the gland with the exception of only two clonal expansions [39]. Distribution of VH genes in peripheral blood of this patient did not differ significantly from that previously reported in healthy subjects. Analysis of VH gene usage in the salivary gland and submandibular lymph node of two other SS patients also indicated that the infiltrate was polyclonal, although clonally related B cells were found in the salivary gland of one of the subjects [64].

When GC-like structures that included AChR-specific B cells were microdissected from the thymus of a MG patient and their VH gene usage analyzed, numerous small clonally related B cell populations were identified [5]. Whereas the overall VH gene repertoire was strikingly heterogeneous, 14 of 18 clonally related sets expressed VH3 genes. Furthermore, replacement mutations resulting in common amino acid substitutions were identified not only in several distinct B cell clones from different GCs, but had previously been reported in some anti-AChR-specific mAbs. Shared amino acid residues encoded by N-nucleotides at the VH-D junction were also identified in 3 different sets of clonally related B cells isolated from salivary gland biopsy tissue from two different SS patients, all of which used the V3-7 VH [11]. These findings suggest that a common antigen directs the clonal proliferation and somatic hypermutation in B cells of different MG and SS patients. In other autoimmune diseases, however, the oligoclonal nature of the B cell infiltrate of target organs are suggestive of the involvement of several different antigens in the expansion of some of these B cells.

V. Auto-antibodies

1. Gene usage

AutoAbs of different specificities appear to differ in the degree of restriction in their Ig V gene usage. For example, there is no evidence for preferential usage of VH and Vκ family members in anti-DNA mAbs [47,65,66]. A possible exception is the Vλ gene 2a2, which was reported in 5 of 6 IgG anti-DNA Abs that used lambda chains [66], but the overall number of Abs whose Ig VL genes have been analyzed is as yet insufficient to conclude that there is preferential usage of Vλ2a2. A markedly increased frequency of Vλ3 has been reported in RFs [67]. In contrast, others found the VL repertoire of RFs to be quite heterogeneous, whereas usage of VH genes was restricted to members of the VH1 and VH3 families [68]. Interestingly these are the same VH families that were found to predominate in anti-TPO Abs [40]. Analysis of ~180 anti-TPO Abs, predominantly obtained through the use of combinatorial libraries derived from B cells infiltrating the thyroid of mainly Graves' and only a few Hashimoto's disease patients indicated that VH1 predominated in patients with Graves' disease, whereas VH3 was the most frequently used gene family in Hashimoto's disease. These Abs also showed preferential use of certain L chains, with Vκ1 and Vλ1 predominating. A single VH3 gene (VH3-30) was identified in almost all of the 39 platelet-reactive clones isolated from phage display libraries constructed from splenocytes of 2 patients with chronic idiopathic thrombocytopenic purpura [69].

There are indications that, although Vλ genes encode between 30% and 40% of L chains in the Ig repertoire of healthy subjects, they are found more frequently than κ L chains in a variety of autoAbs, including RF and Abs with specificity for dsDNA, LA/SS-B and Ro/SS-a, phospholipids, histone A2, laminin, and collagen [1,68,70]. In contrast, the most reactive auto-Abs positive for the anti-dsDNA-associated idiotype F4 were found to use κ light chains, although $F4^+$ H chains were capable of associating with λ L chains [47]. For the analysis of TPO-specific Abs, most research groups used only Lκ phage libraries because κ chains predominated in the anti-TPO Abs in the serum of the patients from whom they were isolated [40]. Nonetheless, inclusion of a λ chain library resulted in the isolation of numerous λ anti-TPO scFv.

There appear to be autoAbs whose specificity is determined mainly by the H chain, with the L chain contributing only to affinity [71,72]. In other autoAbs, the L chain is most important for establishing specificity. When both contribute equally to antigen recognition, one would expect to find only particular H and L chain combinations in Abs specific for that antigen. Restrictions on H/L chain pairings have been reported in Abs with several different specificities, including dsDNA [1], platelet antigens [69], and TPO [73]. Note, however, that a review of ~180 anti-TPO mAbs did not find evidence of restricted H/L pairing [40]. Importantly, pairing of H chains with specific κ or λ L chains was not evident in peripheral B cells of healthy subjects [74].

Occasionally, clonally related B cells expressing V genes that have also been reported in disease-related autoAbs have been isolated from target tissues of patients with autoimmune diseases, such as SLE [1,4,75] or SS [53,76]. However, the number of patients whose Ig V genes have been analyzed in detail is far too small and the individual patterns of V gene usage are too divergent as to allow any correlations to be established with gene utilization of disease-specific antibodies.

2. Somatic hypermutation

It has been suggested that autoantibodies may arise from somatic hypermutation of Ig genes that have minimal capacity to encode autoantigen-binding Abs in their germline configuration

[45]. Consistent with this hypothesis, the Vλ repertoire of SLE patients has been found to exhibit several similarities with that of fetal spleen cells as well as adult $CD5^+$ B cells, the latter being known to produce natural autoantibodies [43]. Several other lines of evidence also suggest that somatic mutations play an important role in increasing the affinity of autoAbs. Such autoAbs frequently are highly mutated, and back mutation of mutated human V genes to the germline sequence has been reported to result in loss of antigen binding [65,68,70]. That is, however, not a consistent finding. For example, reversion to the germline configuration did not significantly alter the affinity with which a monoclonal anti-DNA Ab derived from B-1 cells of a SLE patient [77]. The authors suggested that selection of the point mutations in the VH and Vκ gene segments of this mAb might have been attributable to an antigen other than DNA. In addition, the mutational frequency does not necessarily correlate with increased affinity [78]. Furthermore, there are examples of high-affinity autoantibodies derived from V genes that are in the germline configuration or only minimally mutated [25,30,68]. The fact that a high mutation frequency is not always necessary for the development of high-affinity monoreactive autoAbs indicates that some germline genes encoding such Abs fail to be deleted in patients with autoimmune diseases.

3. Use of phage libraries for characterizing autoantibody epitopes

Phage display libraries allow the generation of recombinant mAbs [27], which can then be used for the characterization of the epitopes recognized by these Abs [79,80]. In one of these studies, peptide phage display libraries together with molecular modeling were used to more narrowly define a conformational epitope of a monoclonal Ab that recognizes IA-2, a major target of both B and T cell responses in T1D [80]. Of note, this B cell epitope was found to overlap two repeatedly identified T cell epitopes on IA-2. Overlap of T and B cell epitopes has also been reported for GAD65, another major autoantigen in T1D [32], for the E2 subunits of the pyruvate dehydrogenase complex in PBC [81,82], and for myelin basic protein in MS [83]. It has been hypothesized that binding of autoAbs to autoantigens could alter their proteolytic processing, e.g., by protecting them from degradation and facilitating their uptake, processing and presentation to autoreactive T cells. Some support for this hypothesis has come from experiments using GAD65-specific B cells and GAD65-specific T cell hybridomas [32]. Interestingly, however, presentation of T cell epitopes located fully within B cell epitopes was markedly inhibited.

VI. Conclusion

Additional investigations into the average repertoire from each patient need to be done in order to gain a global picture of the patient population. Spectratyping of both productive and nonproductive arrangements of both heavy and light chains of antigen-specific BCR for both B-cell and T-cell mediated autoimmune will provide additional clues as to how and when B-cell participation in disease occurred. An unbiased and efficient approach in studying antibody gene usage will not only confirm that some auto-immune diseases are an antigen-driven process, and may reveal the fine characterization of the antibody idiotype repertoire.

VII. References

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Table 1

B-cell Mediated Autoimmune Diseases & Autoantibody Variable Region Information

Autoimmune Disease	Auto-Ag	V chain	Technical approach (primers)	VH gene family bias	CDR3	Sample.. (# pat.)	Type of Cells sampled	Isotype	Ref.
SLE	a-dsDNA, a-smAb		Phage display library	VH5	-	-	-	IgG	[1]
SLE	a-dsDNA, a-smAb	VH		VH4-21	-	11	Renal	-	[2]
SLE	a-dsDNA, a-smAb	VH & VL	Hybridoma; 5' VH4, VkII, VkIII primers	VH4-21	Arginine-rich sequences; predominantly basic	2	1 splenocyte; 1 blood	IgM & IgG	[3]
SLE	a-dsDNA, a-smAb	VH	7 VH leader & universal JH primers from rearranged DNA (36 sequences obtained)	VH5-51, VH3 (16% each)	CDR1 & CDR2 only observed. Serine usage bias.	1	a-CD20 & a-Follicular Dendritic Cells	-	[4]
MG	AChR	VH	7 VH leaders & JH cocktail (18 sequences obtained)	VH3	No conservation observed	1	Rearranged thymus tissue specific for a-AChR	N/A	[5]

Table 2

T-cell Mediated Autoimmune Diseases & Autoantibody Variable Region Information

Autoimmune Disease	Auto-Ag	V chain	Technical approach (primers)	VH gene family bias	CDR3 result	Sample (# pat.)	Type of Cells sampled	Isotype	Ref.
Celiac Disease		VH & VL	Phage library display	VH4	-	1	Peripheral Blood Lymphocyte & Ileum BL???	IgA	[7]
RA	RF	VH & VL	Human/mouse hetrohybridoma; 5' leader degenerative sequence, 6VH	VH4-18 & VH1	VH4 sequences CDR3 identical	1	Rheumatoid synovial tissues	IgM	[8]
RA	RF	VH & VL	Hybridoma 5' leader sequence degenerate	VH1	9& 16 aa long	1	Rheumatoid synovial tissues	IgM	[9]
Sjogren's Syndrome	a-Ro-SSA, a-La-SSB, RF	VH & VL	-	-	VH CDR3 shortest	-	Glandular rearrangements	-	[10]
Ankylosing Spondylitis			70 PCR results from 6 VH family primers & known JH primers. DNA used.	VH5 over-represented; VH3 normal; VH4 under-represented	Median of 27.2 nt (~9aa)	1	Rearranged synovial membrane		[12]
PBC	PDC-E2, BCKD-E2, OGDC, ProteinX, PDC-E1a	VH & VL	2 Hybridoma & phage-combinatorial library; RT-PCR with random hexamers; PCR 5' VH/VL leader & FW1 and 3' Constant Region	-	-	5	Regional lymph node specific for 2-OADH autoAg	IgM & IgG	[13]
PBC	2-OADH family	VH & VL	5 Hybridoma clones (1 IgM & 4 IgG). RT-PCR using oligo-dT; PCR using 5' VH leader 1-6 & VL leader and 3' Constant Region.	VH3 & VH4 families	3 VH clones > Replacement mutations in CDR1 & CDR2; 2 CDR3 clones longer (lypoylate-specific) than 3 CDR3 clones that recognize both (lypoylate & un) confusing paragraph	2	Total RNA from peripheral B-cells specific for Anti-PDC	IgM & IgG	[14]

Table 3

VH gene family distribution in peripheral B cells from SLE and SS patients and healthy controls

	Expected frequency	2 Normal CD5-/IgM$^+$ B cells [33] non-productive (%)	Productive (%)	1 SLE patient [29] non-productive (%)	Productive (%)	1 SS patient [39] non-productive (%)	Productive (%)
VH1	21.6	4.0	13.1[a]	0	2.4	16.7	24.7
VH2	5.9	8.0	1.9	16.7	7.3	-	3.1
VH3	43.1	36.0	53.9[a]	66.7	82.9[b]	44.4	46.4
VH4	21.6	44.0[a]	**24.8**	16.7	7.3[b]	22.2	15.5
VH5	3.9	4.0	2.9	0	0	5.6	9.3
VH6	2.0	4.0	2.4	0	0	11.1	1.0
VH7	2.0	0	1.0	0	0	-	-

Bold numbers indicate significant differences between the productive and the non-productive repertoire Note that there were no significant differences between the productive and the non-productive repertoire of the SS patient or between either of these repertoires and those of healthy controls

[a] significantly different from expected frequency

[b] significantly different from normal values

Table 4

Vκ and Jκ gene family distribution in peripheral B cells from SLE and SS patients and healthy controls

		2 Normal CD5$^-$/IgM$^+$ B cells [34]		1 SLE patient [38]		3 SS patients [37]	
	genome	non-productive (%)	Productive (%)	non-productive (%)	Productive (%)	non-productive (%)	Productive (%)
Vκ1	47.5	47	44	65	~63,[ab]	~60[a]	64[b]
Vκ2	22.5	29	19	~25[a]	~9,[ab]	~20[a]	~12[a]
Vκ3	17.5	13	**29**	~12[a]	~18[a]	~5[a]	15[b]
Vκ4	2.5	6	5	0	14[b]	~10[a]	~10[a]
Vκ5	2.5	4	2	0	0	~2[a]	0
Vκ6	7.5	1	<1	0	0	0	0
Vκ7						~2[a]	
Jk1		21	30	18	24	5	~30,[ab]
Jk2		40	36	53	32	64	~64,[ab]
Jk3		4	6	12	5	0	~2
Jk4		20	17	6	2[b]	7	~5,[ab]
Jk5		15	11	12	36[b]	~24[a]	~10[a]

Bold numbers indicate significant differences between the productive and the non-productive repertoire

[a] ~ indicates that numbers are estimated from graphic data

[b] significantly different from the respective repertoire in normal B cells

Table 5

Vλ and Jλ gene family usage in peripheral B cells from SLE and SS patients and healthy controls

	Expected frequency	2 normal $CD5^-$ /IgM^+ [20] non-productive (%)	Productive (%)	1 SLE patient [29] non-productive (%)	Productive (%)	3 SS patients [22] non-productive (%)	Productive (%)
Vλ1	16.7	25.4[a]	29.1[a]	19	29	20.0	12.8[b]
Vλ2	16.7	30.9	33.1[a]	24	26	37.5	51.1[b]
Vλ3	26.7	5.4[a]	15.7[a]	2	8	2.5	3.2[b]
Vλ4	10.0	18.2[a]	5.8	21	**0**[b]	7.5	4.3
Vλ5	10.0	7.3	3.5[a]	9	**2**	0	0
Vλ6	3.3	3.6	3.5	9	17[b]	7.5	4.3
Vλ7	6.7	1.8	4.1	3	2	2.5	**18.1**[b]
Vλ8	3.3	1.8	1.2	7	13[b]	5.0	2.1
Vλ9	3.3	1.8	0.6[a]	5	3	0	2.1
Vλ10	3.3	3.6	3.5	2	0	17.5[b]	**2.1**
Jλ1	25	5.5[a]	7.0[a]	5.2	3.8	5	0
Jλ2/3	50	34.5[a]	39.0[a]	58.6[b]	28.8	70[b]	72[b]
Jλ7	25	60.0[a]	54.1[a]	36.2[b]	**67.3**[b]	18[b]	22[b]

Bold numbers indicate significant differences between the productive and the non-productive repertoire

[a] significantly different from expected

[b] significantly different from the respective repertoire in normal B cells