Available online http://arthritis-research.com/content/4/2/080

Commentary

Clues to the etiology of autoimmune diseases through analysis of immunoglobulin genes

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Received: 24 August 2001
Revisions requested: 27 September 2001
Revisions received: 18 October 2001
Accepted: 19 October 2001
Published: 12 November 2001

Arthritis Res 2002, **4**:80-83

(Print ISSN 1465-9905; Online ISSN 1465-9913)

Abstract

The role of autoantibodies in the etiology of autoimmune diseases remains unclear. However, an examination of the sequences of these autoantibodies can be informative. Antibody sequences that violate constraints normally imposed during ontogeny and during development point to a failure of regulation. The existence of clonally related sequences indicates that production of these antibodies may frequently be driven by self-antigen. A better understanding of the mechanisms that normally constrain the composition of the antibody repertoire and of the nature of the inciting and/or driving antigens may yield new insights into both the pathogenesis and potential treatment of these crippling diseases.

Keywords: autoantibody, B cell, development, immunoglobulin genes, sequence analysis

Introduction

Antibodies directed against self-antigens are frequently found in the serum of patients suffering from autoimmune diseases. The presence of autoantibodies has raised a series of fundamental questions for those who seek to understand the pathogenesis of these disorders. Are the processes that regulate the production of mature B-cell receptors functioning aberrantly in autoimmunity? If dysregulation is present, are there additional processes of B-cell proliferation and/or antigen response that serve to augment the deleterious properties of autoantibodies? Moreover, are these autoantibodies the product of nonspecific B-cell stimulation (a bystander effect) or of an antigen-driven response? If the latter, what is the identity of the inciting antigen(s) and/or driving antigen(s)?

Antibody assembly and B-cell development

Antibodies are the product of a complex set of gene rearrangements, somatic hypermutation, and receptor-driven selection. These processes are carefully regulated during development, during ontogeny, and during the response to antigen. Each of these processes can alter the composition or the sequence of the antibody; hence, when studied as a population, or even individually, analysis of autoantibody sequences can indicate whether they lie outside the normal range exhibited by conventional antibodies and thus are intrinsically aberrant. Sequence analysis of individual antibodies can indicate the stages of development through which the antibody-producing B cell has passed. An analysis of a population of sequences can demonstrate whether the response is polyclonal, pointing to nonspecific stimulation, or oligoclonal, suggesting antigen drive.

B cells develop in the bone marrow and fetal liver, and mature in the peripheral lymphoid organs [1,2]. The immunoglobulins that they produce contain two heavy (H) and two light (L) polypeptide chains. Each chain includes

CDR = complementarity determining region; DH = diversity gene segments; H = heavy; J = joining; L = light; V = variable.

a variable (V) domain that helps define the antigen specificity of the mature antibody. In the bone marrow and fetal liver, B-cell progenitors initiate creation of an H chain V domain by joining one of 27 diversity (DH) gene segments (belonging to one of seven families) to one of six joining (JH) gene segments, and then adding one of approximately 50 variable gene segments (from one of seven families) to the formed DJ join [3,4]. Creation of a translatable H chain allows the developing B cell to express the H chain associated with a surrogate chain to form the pre-B-cell receptor, and in a later stage the H chain associates with a translatable light chain V → J rearrangement product to form a mature immunoglobulin. Expression of immunoglobulin on the cell surface allows receptor-based selection of the B cell. The organization of the κ and λ L chain loci is conducive to repeated cycles of V → J rearrangement, enabling the system to edit deleterious receptors by replacing the L chain [5,6]. Editing of the H chain is also possible [7].

Immunoglobulin V domains each contain three β loops of highly variable sequence (the complementarity determining regions [CDRs]) and four β sheets of conserved framework sequence [8]. Of these, the HCDR3 and, to a lesser extent, the LCDR3 are the most critical because they are created directly by V(D)J joining and they lie at the center of the antigen binding site, where they typically play a critical role in defining the antigen specificity of the antibody (reviewed in [9]). Within HCDR3, DH gene segments have the potential to be read in any one of six reading frames, which magnifies the potential for combinatorial diversity in the H chain [10]. Additional diversity is introduced by flexibility in the site of gene segment rearrangement and by the somatic addition of nontemplated (N regions) and templated (P junctions) nucleotides at the rearrangement junctions [9].

The composition of the antibody repertoire is regulated and constrained

Although at first glance the diversity of the antibody repertoire appears random, a closer inspection reveals evidence of bias, constraints, and restrictions. Gene segment utilization is nonrandom, with a preference in the adult for a small set of HV and LV gene segments; for example, in the heavy chain members of the DH3, DH2 and DH6 families, and JH4, JH5 and JH6 families are most prevalent (reviewed in [11,12]). These preferences can change during ontogeny, with D7, JH3 and JH4 gene segments over-represented during the second trimester of fetal life. Preference for sets of gene segments in a population of cells from a given disease that do not reflect the bias seen in healthy individuals can result from abnormal regulation of B-cell development, from outgrowths of cells derived from early ontogeny, or from antigen-driven selection of a rare subset of the normal repertoire. Nonrandom use of V gene segments in autoantibodies produced by a subset of B-cell chronic lymphocytic leukemia cells raised the possibility that transformation had occurred in the fetal period [13]. However, a more current view holds that the bias in gene segment utilization in these autoimmune tumor cells reflects antigen selection [14].

Autoantibodies may violate constraints placed on the normal antibody repertoire

In antibodies from healthy individuals, HCDR3 sequences are enriched for neutral, hydrophilic amino acids, including tyrosine, glycine, and serine (reviewed in [15]). Hydrophobic and charged residues, especially those with a positive charge such as arginine, lysine, and histidine, are quite rare. This bias is due, in part, to a preference for a single DH reading frame, RF1, which is enriched for these amino acids. However, use of charged amino acids is quite rare even in antibodies with extensive N addition. Antibodies against double-stranded DNA that contain arginine in HCDR3 [16] are thus not only abnormal in their specificity, but also in their composition; they potentially reflect a failure to limit reading frame preference during rearrangement, a possible factor in enhancing susceptibility for the development of autoantibodies [17].

The length distribution of LCDR3 and HCDR3, and hence the structure of the antigen binding site, also appears to be regulated. Human κ chain LCDR3s, for example, are typically nine amino acids long, whereas HCDR3 lengths range from six to 20 amino acids in the fetus and from six to 28 amino acids in the adult [12,18,19]. The mechanisms that control length are incompletely understood. Accumulations of B cells are found in the diseased synovium of patients with rheumatoid arthritis and these cells often produce significant quantities of antibody, much of which has rheumatoid factor activity. In these patients, the frequency of κ chain LCDR3s with lengths outside the normal range is increased, which could be the product of an abnormal pattern of production [18,20].

Receptor editing

Receptor editing is a mechanism that could be used to prevent the production of abnormal antibodies. Conversely, it has the potential to introduce abnormalities at an intermediate stage in B-cell development. Evidence of an enhancement of receptor editing in autoimmune disease has been sought through analysis of J utilization in L chains on the presumption that enrichment for downstream J gene segments would reflect repeated rearrangement and editing [21]. Other studies have sought evidence of prolonged activity of the recombination machinery [20]. Proof of abnormal receptor editing function in autoimmune disease has been elusive, although it remains an attractive possibility.

All of the aforementioned ontological and developmental constraints in the creation of the mature B-cell repertoire

play a crucial role in shaping the population of antibodies in healthy individuals, and each process is a potential target for dysregulation. Mechanisms used for affinity maturation in an antigen-driven response also have the potential to create aberrant or pathogenic antibodies. It should be noted that these possibilities are not mutually exclusive.

The role of affinity maturation

In peripheral lymph nodes, B cells with antibodies specific for antigen can, with appropriate T-cell help, form germinal centers. The variable domains in these organs typically undergo somatic hypermutation, which is the basis for affinity maturation. The mutations initially appear to be scattered throughout the variable domain, although with an emphasis on RGYW hot spots [22]. B cells that have gained the highest affinity for antigen subsequently outcompete B cells with lower affinity for antigen presented on the surface of the follicular dendritic cells from which the B cells receive signals necessary for survival. Associated with this process is enrichment for replacement mutations in the CDRs with conservation of sequence in the frameworks, which maintains the structural stability of the antigen binding site [23]. One product of sequential mutation is an accumulation of sequences that share common mutations, yet differ at other sites. Sequences derived from a common ancestor that contain distinguishing mutations are termed 'clonally related' and are another marker of an antigen-driven humoral response.

Mutations in autoantibody sequences suggest that the B cells that produced them have passed through a germinal center. Random mutations in the sequence, as evidenced by a moderate amino acid replacement to silent site substitution ratio in the affected codons, would support a bystander effect in the stimulation and growth of autoreactive B cells. In sequences derived from cloning of transcripts from diseased rheumatoid synovial tissue [18] and in sequences obtained by single cell analysis from germinal center-like structures in rheumatoid synovium [24] there is evidence of preservation of the frameworks with divergence in the CDRs, supportive of an antigen-driven response. Clonally related sequences have also been found in synovial fluid and in the synovium, further supporting antigen drive as the impetus for B-cell proliferation and immunoglobulin production in the diseased synovium [24,25].

Studies of the type described have been carried out in a number of autoimmune diseases. Together, the evidence supports the view that autoantibody production in autoimmune disease is antigen driven. The observation that many of these autoantibody sequences carry features that are uncommon in antibodies from healthy individuals also supports the view that regulation of the repertoire is abnormal in a number of autoimmune diseases.

Conclusion

Although there is strong evidence that the antibody response in many autoimmune diseases is antigen driven, a full understanding of the mechanisms that normally constrain the repertoire and knowledge of the defects in these mechanisms that allow the production of autoantibodies, as well as the nature of the inciting and driving antigen(s), has yet to be achieved. Elucidation of these mechanisms as well as identification of the inciting antigen through use of techniques such as single cell analysis and phage display systems may provide new insight into the pathogenesis, treatment, and prevention of these puzzling diseases.

Acknowledgements

This work was supported, in part, by NIH AI07051, AI47322, and AI48115.

References

1. Burrows PD, Cooper MD: **B cell development and differentiation.** *Curr Opin Immunol* 1997, **9**:239-244.
2. Loder F, Mutschler B, Ray RJ, Paige CJ, Sideras P, Torres R, Lamers MC, Carsetti R: **B cell development in the spleen takes place in discrete steps and is determined by the quality of B cell receptor-derived signals.** *J Exp Med* 1999, **190**:75-89.
3. Yancopoulos GD, Alt FW: **Regulation of the assembly and expression of variable-region genes.** *Annu Rev Immunol* 1986, **4**:339-368.
4. Matsuda F, Ishii K, Bourvagnet P, Kuma K, Hayashida H, Miyata T, Honjo T: **The complete nucleotide sequence of the human immunoglobulin heavy chain variable region locus.** *J Exp Med* 1998, **188**:2151-2162.
5. Tiegs SL, Russell DM, Nemazee D: **Receptor editing in self-reactive bone marrow B cells.** *J Exp Med* 1993, **177**:1009-1020.
6. Pelanda R, Schwers S, Sonoda E, Torres RM, Nemazee D, Rajewsky K: **Receptor editing in a transgenic mouse model: site, efficiency, and role in B cell tolerance and antibody diversification.** *Immunity* 1997, **7**:765-775.
7. Wilson PC, Wilson K, Liu YJ, Banchereau J, Pascual V, Capra JD: **Receptor revision of immunoglobulin heavy chain variable region genes in normal human B lymphocytes.** *J Exp Med* 2000, **191**:1881-1894.
8. Kabat EA, Wu TT, Perry HM, Gottesman KS, Foeller C: *Sequences of proteins of immunological interest.* Bethesda, National Institutes of Health; 1991.
9. Kirkham PM, Schroeder HW Jr: **Antibody structure and the evolution of immunoglobulin V gene segments.** *Semin Immunol* 1994, **6**:347-360.
10. Padlan EA: **Anatomy of the antibody molecule.** *Mol Immunol* 1994, **31**:169-217.
11. Schroeder HW Jr, Ippolito GC, Shiokawa S: **Regulation of the antibody repertoire through control of HCDR3 diversity.** *Vaccine* 1998, **16**:1363-1368.
12. Shiokawa S, Mortari F, Lima JO, Nunez C, Bertrand FE III, Kirkham PM, Zhu S, Dasanayake AP, Schroeder HW Jr: **IgM heavy chain complementarity-determining region 3 diversity is constrained by genetic and somatic mechanisms until two months after birth.** *J Immunol* 1999, **162**:6060-6070.
13. Kipps TJ, Tomhave E, Pratt LF, Chen PP, Carson DA: **Developmentally restricted VH gene is expressed at high frequency in Chronic Lymphocytic Leukemia.** *Proc Natl Acad Sci USA* 1989, **86**:5913-5917.
14. Damle RN, Fais F, Ghiotto F, Valetto A, Albesiano E, Wasil T, Batliwalla FM, Allen SL, Schulman P, Vinciguerra VP, Rai KR, Gregersen, PK, Ferrarini M, Chiorazzi N: **Chronic lymphocytic leukemia: a proliferation of B cells at two distinct stages of differentiation.** *Curr Topics Microbiol Immunol* 2000, **252**:285-292.
15. Ivanov IJ, Link JM, Ippolito GC, Schroeder HW: **Constraints on hydropathicity and sequence composition of HCDR3 are conserved across evolution.** In *The Antibodies.* Edited by Capra JD,

Zanetti M. Amsterdam: Harwood Academic Publishers; 2002 (in press).
16. Shlomchik MJ, Mascelli MA, Shan H, Radic MZ, Pisetsky DS, Marshak-Rothstein A, Weigert MG: **Anti-DNA antibodies from autoimmune mice arise by clonal expansion and somatic mutation.** *J Exp Med* 1990, **171**:265-297.
17. Klonowski KD, Primiano LL, Monestier M: **Atypical VH-D-JH rearrangements in newborn autoimmune MRL mice.** *J Immunol* 1999, **162**:1566-1572.
18. Bridges SL Jr, Lee SK, Johnson ML, Lavelle JC, Fowler PG, Koopman WJ, Schroeder HW Jr: **Somatic mutation and CDR3 lengths of immunoglobulin kappa light chains expressed in patients with rheumatoid arthritis and in normal individuals.** *J Clin Invest* 1995, **96**:831-841.
19. Brezinschek HP, Brezinschek RI, Lipsky PE: **Analysis of the heavy chain repertoire of human peripheral B cells using single-cell polymerase chain reaction.** *J Immunol* 1995, **155**: 190-202.
20. Meffre E, Davis E, Schiff C, Cunningham-Rundles C, Ivashkiv LB, Staudt LM, Young JW, Nussenzweig MC: **Human B cells expressing surrogate light chains show increased receptor editing and accumulate in rheumatoid synovia.** *Nat Immunol* 2000, **1**:187-188.
21. Hansen A, Dorner T, Lipsky PE: **Use of immunoglobulin variable-region genes by normal subjects and patients with systemic lupus erythematosus.** *Int Arch Allergy Immunol* 2000, **123**:36-45.
22. Dorner T, Foster SJ, Farner NL, Lipsky PE: **Somatic hypermutation of human immunoglobulin heavy chain genes: targeting of RGYW motifs on both DNA strands.** *Eur J Immunol* 1998, **28**: 3384-3396.
23. Shlomchik MJ, Marshak-Rothstein A, Wolfowicz CB, Rothstein TL, Weigert MG: **The role of clonal selection and somatic mutation in autoimmunity.** *Nature* 1987, **328**:805-811.
24. de Boer BA, Voigt I, Kim HJ, Camacho SA, Lipp M, Forster R, Berek C: **Affinity maturation in ectopic germinal centers.** *Curr Topics Microbiol Immunol* 2000, **251**:191-195.
25. Randen I, Brown D, Thompson KM, Hughes-Jones N, Pascual V, Victor KD, Capra JD, Forre O, Natvig JB: **Clonally related IgM rheumatoid factors undergo affinity maturation in the rheumatoid synovial tissue.** *J Immunol* 1992, **148**:3296-3301.