



Review

B Cell Tolerance to Self in Systemic Autoimmunity

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Abstract. After a century of research and despite intensive scrutiny, the origin of autoantibody production remains an enigma. Recently, the essential role of B cells in promoting systemic autoimmunity in mice seems more important than previously thought: self-reactive B cells can be subject to positive selection and a deficiency in serum IgM predisposes to the development of IgG antibodies to autoantigens. Studies of the B cell repertoire expressed in systemic autoimmune diseases have provided important clues. In human lupus, quantitation of this repertoire reveals the presence of an expansion of IgG clonotypes that impart reactivity with disease-related autoantigens. The nucleotide sequences of autoantibodies derived from these patients and expressing nephritogenic idiotopes (present in immune complexes and renal eluates of subjects with active disease) show features of diversification with a high rate of replacement/silent mutations and clustering of the mutations in the hyper-variable regions, suggesting that an antigen-driven process plays a role in the generation of pathogenic autoantibodies. Currently, the contributions of apoptosis and of cell receptor signaling to this triggering are being appreciated. Pursuing these and related issues will have an important impact on autoimmune research.

Key words: autoimmunity; B cells; systemic lupus erythematosus.

Introduction

Interest in self-reactivity was initiated in the early days of immunology. Despite the genetic potential of normal individuals to mount an immune response to self-epitopes, autoimmunity remains a rare event. After a century of research and despite intensive scrutiny, the origin of pathogenic autoantibody production remains an enigma. The issue of how an “aggressive” autoimmune reaction is initiated and sustained in diseased subjects has provoked the formulation of a number of theories, including a role for autoantigen, pathogen-related antigen, molecular mimicry, increased expression of major histocompatibility complex (MHC) class II antigens, polyclonal activation, and altered antigen processing and/or presentation. None of these mechanisms

fully accounts for all the findings in autoimmune diseases, and it is conceivable that multiple processes may act simultaneously or in temporal succession in a single autoimmune disorder, and that distinct processes operate in a single autoimmune subject^{36, 39}. The case of anti-DNA antibodies is even more provocative because, even though nucleic acid antigens are usually poorly immunogenic, a subset of these autoantibodies is a hallmark of a systemic human disease, systemic lupus erythematosus (SLE)¹².

The Primary Repertoire

Several lines of evidence indicate that the human preimmune repertoire is produced by CD5⁺ B cells. In

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recent years, it has also become clear that most CD5⁺ B cells from healthy subjects can produce anti-DNA antibodies. This observation led to the proposal that CD5⁺ B lymphocytes give rise to CD5⁻ B cells producing antibodies with selective binding activity through somatic mutation, antigen clonal selection and loss of surface CD5⁺ molecules^{17, 35}. We have analyzed the B cell precursors that secrete anti-DNA antibodies and found them to secrete multireactive antibodies. A notable feature of the preimmune repertoire is that the great majority of the hybridomas and B cell clones derived from humans and mice are directed to intracellular structures rather than to cell-surface antigens. This may be due to the density of target epitopes on these molecules. For example, the antibodies generated are directed to the intracellular antigens tubulin, actin, myosin and DNA-molecules exhibiting repetitive, highly charged motifs, which may allow the binding of low-affinity multivalent IgM antibodies. In contrast, surface molecules such as receptors generally do not exhibit this repetitive structure and are generally bound by high-affinity antibodies. However, it is also possible that the frequency of B cell clonotypes to cell-surface motifs may be too low in the primary, unmutated repertoire to be detected.

Mechanisms of B Cell Tolerance to Self

In recent years, progress has been made in understanding the mechanisms of induction of self tolerance of B and T cells. Both clonal deletion by cell death and clonal anergy, a state in which autoreactive cells are functionally inactive, have been described in experiments where transgenic mice have been instrumental. When mice expressing anti-lysozyme antibody transgenes were mated with transgenic mice expressing soluble lysozyme, B cells of the double transgenics resulting from the backcross became anergic. However, when mice expressing anti-lysozyme antibody transgenes were mated with transgenic mice expressing a membrane-bound form of lysozyme, B cells specific for this antigen were deleted. Parallel findings were obtained with the MHC H-2K^k transgene expressed in either a membrane-bound or a soluble molecule. These observations favor the view that cross-linking interactions of B cells with multivalent surface antigens result in clonal deletion, whereas interactions with soluble antigens induce anergy in the same cells. These mechanisms of tolerance occur in developing B cells of the bone marrow. They also take place in peripheral B cells. More recently, a novel mechanism, termed re-

ceptor editing, was discovered. It enables B cells to undergo a form of receptor processing that markedly alters their immunoglobulin variable-region genes and, consequently, changes the specificity of the B cell receptor expressed at their surface. Receptor editing exploits the capacity of variable light and heavy genes to undergo successive rearrangements. This novel mechanism not only diversifies the repertoire, but also may allow B cells to avoid high-affinity autorecognition. Hence, receptor editing has implications with regard to the maintenance of B cell tolerance and, possibly, to the induction of pathogenic autoimmunity^{5, 6, 11, 13, 15, 25, 27, 30}.

Cellular Hyperactivity in SLE

Increased helper cell activity has been considered as a possible alternative or complementary explanation to the defective T cell suppressor cell function and the increased B cell function. B cells are overactivated in SLE and secrete increased amounts of autoantibodies. They seem to respond to excessive signals provided by T cells and produce autocrine factors that promote their own activation. With regard to T cells, several groups found that peripheral cells of lupus patients produce decreased amounts of interleukin 2 (IL-2) when stimulated *in vitro* with certain mitogens. High IL-2 levels were detected in the sera of 50% of active SLE. One possibility is that an on-going endogenous *in vivo* stimulation induces both constitutive IL-2 production and compensatory suppressor activity. Other data suggest that there is an on-going *in vivo* production of IFN- γ in SLE and that this cytokine has local pathogenic effects. In addition, the levels of IL-6, a cytokine with multiple effects in several biological cell systems, are elevated in some of the sera and the cerebrospinal fluid of patients with SLE²¹. In sum, there is an aberrant expression of cytokines in SLE and this abnormality could reflect direct or complex modulatory events.

At the level of the expressed autoantibody repertoire, we addressed the question of how disease-related clonotypes are selected and expanded. We probed, at the single cell level, the repertoire of B cell paratopes expressed in normal and diseased subjects. In healthy controls, we found that a relatively large proportion of IgM⁺ cell precursors are committed to the production of autoantibodies whose immunoglobulin variable regions impart binding to exogenous antigens and to self antigens, including intracellular and cell surface antigens. Notably, the frequency of high-affinity IgG⁺ B cells in the human preimmune repertoire was below

the level of detection. This finding, together with related observations in the murine system³⁸, is consistent with recent evidence obtained using transgenic mice showing that, in addition to clonal deletion, anergy is an important mechanism of B cell tolerance. We then extended this analysis to autoimmune patients and found that the absolute numbers of clone precursors of the immune repertoire expressing IgM receptors whose paratopes confer affinity to self- and exogenous determinants were higher than in controls. Additionally, IgG antibody-forming cell precursors with binding specificity for autoimmunity-associated antigens were detectable in the repertoire of these patients. Based on these results and those reported in murine models of autoimmunity, we have proposed that hyperproduction of human lupus-associated autoantibodies arises in a two-stage mechanism, whereby a general activation of the multireactive preimmune B cell repertoire precedes an oligospecific expansion of selected B cell clonotypes.

Genetic Susceptibility in SLE

Since lupus is now emerging as a clinically and serologically heterogeneous disorder in which a variety of genes important to immune responsiveness plays a central role, it is useful to highlight these important genetic factors. Approximately 10–12% of lupus patients will have a first-degree relative with SLE. Concordance for SLE is higher (25–70%) in monozygotic than in dizygotic twins (1–3%). However, the time intervals between the dates of disease onset in relatives with SLE are smaller than their age differences at onset. This, together with the lack of complete concordance in monozygotic twins, calls into question a purely genetic basis for the disease and implies that other factors are also important. At the level of the MHC, the majority of the genes which have been associated with a predisposition to SLE map within the HLA region: the Caucasian HLA-B8, DR3, DRw52, DQw2.1 haplotype also bears a deletion of the C4A gene in the class III region. It now appears that partial deficiencies of C4, especially in the C4A isotype, are a risk factor for SLE. Homozygous C2 deficiency also predisposes to a cutaneous form of lupus in which anti-Ro/SSA antibodies are common, and this is probably related to ineffective clearance of immune complexes. Moreover, 15–20% of asymptomatic relatives of lupus patients have abnormal levels of circulating autoantibodies²⁸. To assess the content of the immunoglobulin variable loci in autoimmunity, we used endonuclease-generated

polymorphisms to characterize individual variations within the human variable gene segments. In the course of these studies we described a high number of restriction site polymorphisms of these loci and concluded that these gene complexes are much more polymorphic than previously thought^{37, 40}. We also analyzed germ-line DNAs isolated from tissues of patients with idiopathic or drug-induced lupus. The results were parallel to those reported by others in murine models of lupus²⁰ and are compatible with the conclusion that this disease is not caused by major abnormalities in the structure, size or organization of the variable loci. These findings exclude the possibility that the disease is directly related to major abnormalities of the size, structure or the organization of immunoglobulin variable loci.

Pathogenic Autoantibodies

The main immunological event in the pathogenesis of SLE is B cell hyperactivity. In both human and murine lupus, the number of B cells that secrete immunoglobulins spontaneously is dramatically increased. It is not known whether B cells are intrinsically defective. It has long been debated whether aggressive autoantibodies are the product of polyclonally active activated B cells that express variable genes with a limited number of mutations distributed throughout the variable region, or of antigen-selected B cells expressing variable genes with an accumulation of mutations that are not randomly distributed throughout the variable region³³. We have therefore adopted the view that studying the variable genes expressed by SLE B cells may help in understanding the origin of the disease. To address this issue, we cloned and sequenced the variable-region gene elements expressed by a panel of human autoantibodies that may be considered representative of the pathogenic subset of lupus antibodies. Overall, the data indicate that the molecular configuration of the variable genes is similar to that found in the secondary response to exogenous antigens, with somatic mutations and a pattern of variation typical of antigen selection and clonal expansion. We also used the polymerase chain reaction and nucleotide sequencing to generate information regarding the fine specificity, clonal and genetic characteristics of these aggressive antibodies. The sequence data, together with intraclonal and interclonal comparisons, were instrumental in delineating the clonotypic origin of the corresponding B cells. This led us to speculate that the production of pathogenic autoantibodies occurs through a two-stage mechanism, whereby preactivation of the overall repertoire precedes the

conversion of a non-pathogenic autoantibody of the primary repertoire into a mutant autoantibody with a pathogenic potential by virtue of either its affinity, its fine specificity and/or its idiotype^{3, 4, 7, 8, 34}. Other investigators reached a similar conclusion^{23, 32}. Finally, analysis of the κ -chain genes prompted us to hypothesize that an alteration in receptor editing may be responsible for the production of pathogenic autoantibodies^{1, 27}. Other converging findings support this view^{26, 29}.

Conclusions

In sum, studies of the B cell repertoire expressed in systemic autoimmune diseases have provided important clues. In human lupus, quantitation of this repertoire reveals the presence of an expansion of IgG clonotypes that impart reactivity with disease-related autoantigens. The nucleotide sequences of autoantibodies derived from these patients and expressing nephritogenic idiotopes (present in immune complexes and renal eluates of subjects with active disease) show features of diversification with a high rate of replacement/silent mutations and clustering of the mutations in the hypervariable regions. These characteristics imply that a purely polyclonal activation mechanism¹⁹ cannot be the only mechanism responsible for autoantibody production. More likely, an antigen-driven process plays a role in the generation of pathogenic autoantibodies.

Even though genetic factors are important for disease development, the environmental contribution to clinical expression cannot be ignored. It is likely that different mechanisms could lead to the loss of self-tolerance characteristic of SLE. More than one factor could play a role in a single patient and it is conceivable that the combination of factors varies throughout the disease. This is supported by the observation that different SLE patients produce different spectra of autoantibodies. Environmental stimuli, cytokines, multiclonal antigen-driven processes and viruses are potential candidates. The EBV prevents human B cells from undergoing apoptosis. The contributions of endogenous retroviruses, apoptosis^{10, 17, 24} and cell receptor signaling^{16, 18, 22, 31, 35} are being investigated. Finally, the role of B cells in autoimmunity is perhaps more important than previously thought. Only recently, it was realized that B cells can be subject to positive selection generated and maintained on the basis of their autoreactivity¹⁴ and that B cells are essential in promoting systemic autoimmunity². Also important is the observation that a deficiency in serum IgM predisposes to the

development of IgG antibodies to autoantigens⁹. Pursuing these and related issues will have an important impact on autoimmune research.

Acknowledgment. The author is a senior investigator of the Institut National de la Recherche et de la Santé Médicale (INSERM).

References

1. BENSIMON C., CHASTAGNER P. and ZOULI M. (1994): Human lupus anti-DNA autoantibodies undergo essentially primary V kappa gene rearrangements. *EMBO J.*, **13**, 2951–2962.
2. CHAN O. T. M., MADAIO M. P. and SHLOMCHIK M. J. (1999): B cells are required for lupus nephritis in the polygenic Fas-intact MRL model of systemic autoimmunity. *J. Immunol.*, **163**, 3592–3596.
3. CHASTAGNER P., DEMAISON C., THEZE J. and ZOULI M. (1994): Clonotypic dominance and variable gene elements of pathogenic anti-DNA autoantibodies from a single patient with lupus. *Scand. J. Immunol.*, **39**, 165–178.
4. CHASTAGNER P., THEZE J. and ZOULI M. (1991): Cloning of a gene encoding a lupus-associated human autoantibody VK region using the polymerase chain reaction and degenerate primers. *Gene*, **101**, 305–306.
5. CHEN C., NAGY Z., RADIC M. Z., HARDY R. R., HUSZAR D., CAMPER S. A. and WEIGERT M. (1995): The site and stage of anti-DNA B-cell deletion. *Nature*, **373**, 252–255.
6. CYSTER J. G., HEALY J. I., KISHIHARA K., MAK T. W., THOMAS M. L. and GOODNOW C. C. (1996): Regulation of B-lymphocyte negative and positive selection by tyrosine phosphatase CD45. *Nature*, **381**, 325–328.
7. DEMAISON C., CHASTAGNER P., THEZE J. and ZOULI M. (1994): Somatic diversification in the heavy chain variable region genes expressed by human autoantibodies bearing a lupus-associated nephritogenic anti-DNA idiotype. *Proc. Natl. Acad. Sci. USA*, **91**, 514–518.
8. DEMAISON C., RAVIRAJAN C. T., ISENBERG D. A. and ZOULI M. (1995): Analysis of variable region genes encoding anti-Sm and anti-cardiolipin antibodies from a systemic lupus erythematosus patient. *Immunology*, **86**, 487–494.
9. EHRENSTEIN M. R., COOK H. T. and NEUBERGER M. S. (2000): Deficiency in serum immunoglobulin (Ig) M predisposes to development of IgG autoantibodies. *J. Exp. Med.*, **191**, 1253–1258.
10. EMLIN W., NIEBUR J. and KADERA R. (1994): Accelerated *in vitro* apoptosis of lymphocytes from patients with systemic lupus erythematosus. *J. Immunol.*, **152**, 3685–3692.
11. GOODNOW C. C., BRINK R. and ADAMS E. (1991): Breakdown of self-tolerance in anergic B lymphocytes. *Nature*, **352**, 532–536.
12. HAHN B. H. (1998): Antibodies to DNA. *N. Engl. J. Med.*, **338**, 1359–1368.
13. HARTLEY S. B. and GOODNOW C. C. (1994): Censoring of self-reactive B cells with a range of receptor affinities in transgenic mice expressing heavy chains for a lysozyme-specific antibody. *Int. Immunol.*, **6**, 1417–1425.
14. HAYAKAWA K., ASANO M., SHINTON S., GUI M., ALLMAN D., STEWART C., SILVER J. and HARDY R. (1999): Positive selection of natural autoreactive B cells. *Science*, **285**, 113–116.

15. HEALY J. and GOODNOW C. (1998): Positive versus negative signaling by lymphocyte antigen receptors. *Annu. Rev. Immunol.*, **16**, 645–670.
16. HIBBS M. L., TARLINTON D. M., ARMES J., GRAIL D., HODGSON G., MAGLITTO R., STACKER S. A. and DUNN A. R. (1995): Multiple defects in the immune system of Lyn-deficient mice, culminating in autoimmune disease. *Cell*, **83**, 301–311.
17. HUCK S., JAMIN C., YOUINOU P. and ZOULALI M. (1998): High-density expression of CD95 on B cells and underrepresentation of the B-1 cell subset in human lupus. *J. Autoimmun.*, **11**, 449–455.
18. HUCK S. and ZOULALI M. (1997): Expression of the tyrosine kinase Lyn during B-cell receptor engagement and apoptosis. *Ann. NY Acad. Sci.*, **815**, 122–123.
19. KLINMAN D. M. and STEINBERG A. D. (1987): Systemic autoimmune disease arises from polyclonal B cell activation. *J. Exp. Med.*, **165**, 1755–1760.
20. KOFLER R., PERLMUTTER R. M., NOONAN D. J., DIXON F. J. and THEOFILOPOULOS A. N. (1985): Ig heavy chain variable region gene complex of lupus mice exhibits normal restriction fragment length polymorphism. *J. Exp. Med.*, **162**, 346–351.
21. LINKER-ISRAELI M., DEANS R. J., WALLACE D. J., PREHN J., OZERI-CHEN T. and KLINENBERG J. R. (1991): Elevated levels of endogenous IL-6 in systemic lupus erythematosus. *J. Immunol.*, **147**, 117–123.
22. LIOSSIS S. N., DING X. Z., DENNIS G. J. and TSOKOS G. C. (1998): Altered pattern of TCR/CD3-mediated protein-tyrosyl phosphorylation in T cells from patients with systemic lupus erythematosus. Deficient expression of the T cell receptor zeta chain. *J. Clin. Invest.*, **101**, 1448–1457.
23. MANHEIMER-LORY A., KATZ J. B., PILLINGER M., GHOSSEIN C., SMITH A. and DIAMOND B. (1991): Molecular characteristics of antibodies bearing an anti-DNA-associated idiotype. *J. Exp. Med.*, **174**, 1639–1652.
24. MYSLE E., BINI P., DRAPPA J., RAMOS P., FRIEDMAN S. M., KRAMMER P. H. and ELKON K. B. (1994): The apoptosis-1/Fas protein in human systemic lupus erythematosus. *J. Clin. Invest.*, **93**, 1029–1034.
25. NEMAZEE D. (1993): Promotion and prevention of autoimmunity by B lymphocytes. *Curr. Opin. Immunol.*, **5**, 866–872.
26. QIN X., SCHWERS S., YU W., PAPAVALIOU F., SUH H., NUSSENZWEIG A., RAJEWSKY K. and NUSSENZWEIG M. (1999): Secondary V(D) J recombination in B-1 cells. *Nature*, **397**, 355–359.
27. RADIC M. Z. and ZOULALI M. (1996): Receptor editing, immune diversification and self-tolerance. *Immunity*, **5**, 505–511.
28. REVEILLE J. D., MACLEOD M. J., WHITTINGTON K. and ARNETT F. C. (1991): Specific amino acid residues in the second hypervariable region of HLA-DQA1 and DQB1 chain genes promote the RO (SS-A)/LA (SS-B) autoantibody responses. *J. Immunol.*, **146**, 3871–3876.
29. SUZUKI N., HARADA T., MIHARA S. and SAKANE T. (1996): Characterization of a germline V κ gene encoding cationic anti-DNA antibody and role of receptor editing for development of the autoantibody in patients with systemic lupus erythematosus. *J. Clin. Invest.*, **98**, 1843–1850.
30. TIEGS S. L., RUSSELL D. M. and NEMAZEE D. (1993): Receptor editing in self-reactive bone marrow B cells. *J. Exp. Med.*, **177**, 1009–1020.
31. VASSILOPOULOS D., KOVACS B. and TSOKOS G. C. (1995): TCR/CD3 complex-mediated signal transduction pathway in T cells and T cell lines from patients with systemic lupus erythematosus. *J. Immunol.*, **155**, 2269–2281.
32. WINKLER T. H., FEHR H. and KALDEN J. R. (1992): Analysis of immunoglobulin variable region genes from human IgG anti-DNA hybridomas. *Eur. J. Immunol.*, **22**, 1719–1728.
33. ZOULALI M. (1992): Development of human antibody variable genes in systemic autoimmunity. *Immunol. Rev.*, **128**, 73–99.
34. ZOULALI M. (1997): The structure of human lupus anti-DNA autoantibodies. *Methods*, **11**, 27–35.
35. ZOULALI M. (1998): Signaling in human lupus T lymphocytes. *Lupus*, **7**, 499–502.
36. ZOULALI M. (2000): Molecular dissection of the human antibody repertoire in systemic autoimmunity. In ZANETTI M. and CAPRA J. (eds.): *The antibodies*. Hardwood Academic Publishers, Amsterdam, **6**, 105–121.
37. ZOULALI M., CHASTAGNER P., ABADIE A. and THEZE J. (1990): Organization of the immunoglobulin heavy chain variable region gene complex in human lupus. *Immunogenetics*, **32**, 451–455.
38. ZOULALI M., FOURNIE G. J. and THEZE J. (1991): Quantitative clonal analysis of the B cell repertoire in human lupus. *Cell. Immunol.*, **133**, 161–177.
39. ZOULALI M., KALSI J. and ISENBERG D. (1993): Autoimmune diseases – at the molecular level. *Immunol. Today*, **14**, 473–476.
40. ZOULALI M., MADAIO M. P., CANOSO R. T. and STOLLAR B. D. (1989): Restriction fragment length polymorphism analysis of the V kappa locus in human lupus. *Eur. J. Immunol.*, **19**, 1757–1760.

Received in July 2000

Accepted in September 2000