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Divergent members of a single autoreactive B cell clone retain specificity for apoptotic blebs

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Abstract

Specificity for double-stranded DNA can arise due to somatic mutations within one of the branches of an autoreactive B cell clone. However, it is not known whether a different autospecificity predates anti-dsDNA and whether separate offshoots of an expanding B cell clone retain or evolve alternative specificities. We compared 3H9, an anti-dsDNA IgG, to 4H8 and 1A11, antibodies produced by hybridomas representing an alternative branch of the 3H9 B cell clone. All three IgG bound chromatin in ELISA and apoptotic cells in confocal microscopy, yet only 3H9 bound dsDNA, as measured by plasmon resonance. Moreover, we demonstrate that despite the unique specificity of 3H9 for dsDNA, all three clone members exhibited indistinguishable binding to chromatin. The binding to chromatin and apoptotic cells was unaffected by *N*-linked glycosylation in L chain CDR1, a modification that results from a replacement of serine 26 with asparagine in 4H8 and 1A11. These data provide the first evidence that specificity for nucleosome epitopes on apoptotic cells provides the initial positive stimulus for somatic variants that comprise a B cell clone, including those that subsequently acquire specificity for dsDNA. Conversely, selection of autoreactive B cells for binding to apoptotic cells leads to clonal expansion, antibody diversification, and the development of linked sets of anti-nuclear autoantibodies.

Keywords: Anti-DNA; Autoantibody; Autoimmunity; Apoptosis; B lymphocytes; Somatic mutations; Systemic lupus erythematosus

1. Introduction

Nucleosomes, the complexes of DNA and histones that package genomic DNA into the repeating structure of chromatin, are the predominant target of autoantibodies in systemic lupus erythematosus (SLE) and murine models for SLE (Monestier and Kotzin, 1992; Burlingame et al., 1993; Jovelin et al., 1998). Autoantibodies to DNA, histones, or the native nucleosome particle have been identified and characterized at the molecular level (Shlomchik et al., 1990; Losman et al., 1992; Tillman et al., 1992). Nevertheless, a clear insight into the mechanisms that lead to the production of autoantibodies to these abundant nuclear antigens remains elusive.

The genetic analysis of anti-DNA and anti-histone autoantibodies from murine models for SLE argues that B cells derive a selective advantage from improvements in binding to nuclear antigens (Radic and Weigert, 1994; Stollar, 1994; Monestier and Novick, 1996; Rahman, 2004). The binding to DNA, histones, and their complexes benefits from the use of specific H and L chain combinations, a suitable arrangement of contact residues within the combining site, and the introduction of cationic residues (mostly arginines) within the gene junctions that code for complementarity-determining region 3 (CDR 3). The consistent choice of particular H and L chain V genes, recurrent motifs within the H and L chain junctions, and independent parallel mutations yielding the same amino acid replacements in different autoantibodies strongly suggest antigen-specific selection of autoreactive B cells. In addition, the positive selection of B cells with Ig receptors that recognize the native nucleosome particle (Losman et al., 1993; Seal et al., 2000) implies direct contacts between B cells and nuclear antigens.

Abbreviations: AP, alkaline phosphatase; ANA, anti-nuclear antibody; PNGase F, protein *N*-glycosidase F; SLE, systemic lupus erythematosus; ScFv, single chain variable fragment; SPR, surface plasmon resonance

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An opportunity for nuclear autoantigens to exit the confines of the nucleus and gain access to the extracellular space arises during the energy-dependent modification and redistribution of cellular contents that characterize apoptosis, or programmed cell death (Casciola-Rosen et al., 1994). Nucleosomes are released from the apoptotic nucleus by the caspase-dependent activation of nucleases that cleave DNA between adjacent nucleosomes (Widlak and Garrard, 2005). Thereafter, nucleosome core particles associate with the outer membrane of the fragmenting nucleus (Radic et al., 2004). As nuclear fragments emerge from the plasma membrane, nucleosomes become exposed at the cell surface (Radic et al., 2004). The fact that surface blebs containing nuclear fragments constitute a site of enhanced autoantibody reactivity, suggests that apoptotic cells are the source of nuclear autoantigens (Cocca et al., 2002). Consistent with this view, genetic defects leading to increased abundance of apoptotic cells are among the most potent risk factors for the development of systemic autoimmunity (Botto et al., 1998; Bickerstaff et al., 1999; Lu and Lemke, 2001; Scott et al., 2001; Cohen et al., 2002).

We have examined interactions between autoantibodies and apoptotic cells in order to establish relevant criteria for the activation of autoreactive B cells (Cocca et al., 2001, 2002; Cline and Radic, 2004b; Radic et al., 2004, 2006). Autoantibodies to DNA, individual histones, and epitopes of the native nucleosome core particle, showed consistent and characteristic binding to apoptotic blebs (Radic et al., 2004). One autoantibody in particular, 3H9, can be viewed as a typical representative of these anti-nuclear autoantibodies (ANA). This antibody binds DNA (Shlomchik et al., 1990), chromatin (Radic et al., 1991), and intact nucleosomes (Seal et al., 2000) and reacts with large apoptotic blebs containing nuclear fragments (Radic et al., 2004). The precise epitope of 3H9 on the nucleosome is the H2A/H2B/DNA complex (DN and MM, unpublished data). The H2A/H2B dimer occupies opposite, exposed sides of the nucleosome core particle that are available for interactions with adjacent nucleosomes in chromatin (Davey et al., 2002) or with viral proteins that specifically recognize the nucleosome (Barbera et al., 2006). These studies lead to the conclusion that nucleosomes arrayed on the surface of apoptotic blebs are accessible to ANA or anti-nuclear Ig receptors and may thus constitute a direct antigenic stimulus for B cells expressing ANA (Radic et al., 2004).

The B cell producing 3H9 arose as a member of an expanded B cell clone. This was concluded from the genetic analysis of 3H9 and its clonal relatives, 4H8 and 1A11, that were captured along with 3H9 as hybridomas in the same B cell fusion (Shlomchik et al., 1987). These B cells express the same Ig gene segments and VDJ and VJ rearrangement junctions. Because of their shared and unique mutations, the 4H8, 1A11, and 3H9 hybridomas could be linked in a genealogical dendrogram (Fig. 1) that approximates the evolution of the expanded B cell clone.

The comparison of binding between 3H9, 4H8 and 1A11 revealed that somatic mutations in 3H9 are responsible for its unique specificity for dsDNA (Shlomchik et al., 1990). The key role in DNA binding of the glycine to arginine mutation in V_H3H9 was tested by reverting arginine 53 back to glycine: The R53G mutant lost specificity for dsDNA (Radic et al., 1993b). This result established that somatic mutations give rise to dsDNA specificity and that selection for anti-dsDNA B cells accounts for the expression of anti-dsDNA antibodies in murine models of SLE. However, this result also suggested that additional autospecificities must mediate positive selection of other members of this autoreactive B cell clone.

In continuation, we characterize the similarities and differences between 3H9 and its relatives and examine their specificity for DNA, chromatin, and apoptotic cells. Using surface plasmon resonance (SPR), we found that only 3H9 shows significant binding to dsDNA, whereas all three antibodies bind chromatin in ELISA and apoptotic cells in confocal microscopy.

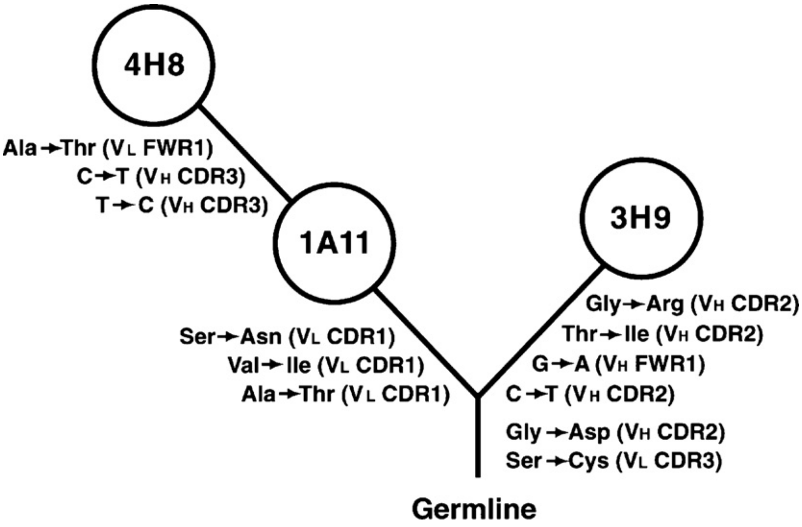

Fig. 1. Dendrogram illustrating clonal relation between 3H9, 1A11, and 4H8. The diagram was constructed using shared and unique mutations in each antibody, assuming the minimum number of mutations (Shlomchik et al., 1990). Mutations were identified by comparison to the germline sequences of the closest VL (Thiebe et al., 1999) and VH genes (Haines et al., 2001). Silent mutations are listed as nucleotide changes, replacements as amino acid substitutions. The location of mutations is indicated in parentheses.