Mod Rheumatol (2002) 12:300–304

REVIEW ARTICLE

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Mechanisms of anti-DNA antibody production

Abstract The nucleosome has attracted attention because it is a possible primary antigen that initiates autoimmune reactions and also seems to play a key role in glomerular injury in systemic lupus erythematosus (SLE). It is not immunogenic in nonautoimmune individuals, however, and many other protein or peptide antigens inducing anti-DNA antibodies in normal animals have been reported recently. Various mechanisms have been proposed to account for this, including idiotype-anti-idiotype, hapten-carrier, molecular mimicry, and others. In addition to the initial immune responses to such antigens, breakdown of self-tolerance influenced by genetic factors or hormonal dysregulation may be necessary preconditions for occurrence of the disease.

Key words Anti-DNA antibodies · Systemic lupus erythematosus (SLE) · DNA-binding protein · Molecular mimicry · Idiotypic network

Introduction

It was in 1948 that Hargraves and coworkers observed LE cells in bone marrow preparations of patients with systemic lupus erythematosus (SLE). To find LE cells in peripheral blood, however, blood samples have to be incubated for some time. Today, we know that increased numbers of apoptotic cells are present in SLE bone marrow, and even the peripheral blood mononuclear cells of the patients tend to apoptose at higher rates than normal in vitro.[1,2] Increased formation of apoptotic bodies bound by high titer antibodies specific for nuclear antigens such as DNA and histones facilitates neutrophil engulfment; thus, the LE cell phenomenon can be observed exclusively in active SLE.[3]

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Antibodies to DNA and histones are members of an anti-nucleosome antibody family, which includes antibodies specific for each component as well as specific for the tertiary structure of the nucleosome. Despite these advances over the last half century, the mechanisms of antibody production in SLE remain elusive. Recently, however, many models have been proposed in which researchers succeeded in inducing anti-dsDNA antibodies in normal animals lacking the particular genetic background of autoimmunity. This review introduces studies related to the mechanisms of production and pathogenicity of anti-DNA antibodies published mainly in the last 5 years.

Idiotype-anti-idiotype mechanism

The first paper on the idiotypic mechanism of anti-DNA antibody production was published in 1988 by Shoenfeld and colleagues,[4] in which they observed anti-DNA antibody production by normal BALB/c mice immunized with a 16/6 idiotype-positive human monoclonal anti-DNA antibody. 16/6 is one of the idiotypes widely expressed on nephritogenic antibodies in SLE, and these mice were described to show many features characteristic of SLE, including glomerulonephritis. Thereafter, these investigators produced many models of autoimmune disorders using a similar strategy; e.g., immunization of mice with anti-cardiolipin antibodies, which led to production of anti-cardiolipin antibodies associated with thrombocytopenia, prolonged activated thromboplastin time, and fetal loss; or immunization of mice with anti-neutrophil cytoplasmic antibodies (ANCA) purified from patients with Wegener's granulomatosis, which caused ANCA production and granulomatous lesions.[5] To explain the underlying mechanism of these intriguing models, they have adopted Jerne's idiotypic network theory. Immunization of naïve mice with autoantibodies (Ab 1) leads to production of anti-idiotype antibodies (Ab 2), and later, the mice produce anti-anti-idiotype antibodies (Ab 3), which simulate the binding characteristics of the original autoantibodies (Fig. 1).

Recently, we reproduced and further characterized these models using monoclonal anti-dsDNA antibodies that had been purified either with or without a high-salt wash to remove bound nucleosomal antigens.[6] BALB/c mice immunized with these antibodies certainly produced IgG anti-dsDNA antibodies. Of importance, the monoclonal antibodies purified with the high-salt wash were more effective in anti-DNA antibody induction than those that were purified without the high-salt wash. Even when bound to these monoclonal antibodies, none of dsDNA, nucleosome, or double-stranded synthetic polynucleotides exerted significant antigenicity, suggesting that anti-DNA antibody molecules per se, and not any DNA/nucleosome components carried by them, play a role in this model. These results are compatible with the explanation of idiotype-anti-idiotype mechanism mentioned earlier. However, this theory does not explain how the initial anti-DNA antibodies necessary to trigger the system arise.

p53 protein, when activated by damaged DNA, acts as a tumor suppressor by inducing growth arrest and DNA repair, or apoptosis of aberrant cells. Autoantibodies to this DNA-binding protein have been detected in SLE as well as in MRL/lpr mice.[7] Furthermore, immunization of normal BALB/c mice with an anti-p53 monoclonal antibody has been reported to induce anti-DNA antibodies.[8] The authors suggested that a kind of idiotypic mechanism operates in these experiments; i.e., the DNA-binding domain of p53 acts as Ab 1, anti-p53 autoantibodies act as Ab 2, and therefore anti-anti-p53 antibodies (Ab 3) may have DNA-binding activities (Fig. 2). Because p53 is involved in apoptosis, which is increased significantly in SLE, efforts to search for pathogenic molecules among apoptosis-related proteins would seem to be worthwhile.

Other DNA-binding proteins or peptides

The nucleosome consists of two turns of dsDNA wound around an octameric histone core. The presence of nucleosome-reactive $CD4^+$ T cells, even before the elevation of anti-DNA antibody titer has been demonstrated in lupus-prone mice, and histone-derived peptide epitopes recognized by those T cells, has also been identified.[9] In analogy to these murine studies, epitopes for autoreactive T cells in lupus patients were finally detected after overcoming difficulties inherent in assays using a limited number of activated autoreactive T cells from peripheral blood samples.[10] Thereafter, the nucleosome was proposed as a source of primary antigen necessary to induce anti-DNA antibodies.[11] However, the nucleosome is not immunogenic under normal circumstances and there are many candidates for DNA-binding proteins other than histones that potentially induce anti-DNA antibody production.

Nucleolin, which is a 110-kDa nucleolar DNA- and RNA-binding phosphoprotein associated with biogenesis of ribosomal RNA, was shown to be one of the early targets of autoantibodies in MRL/lpr mice.[12] In that study, antibodies that react with nucleolin were detected in MRL/lpr and NZB/NZW F1 mice as young as 4–8 weeks of age, preceding anti-histone antibodies, and thereby suggesting that the nucleosome may not be an initial target antigen in SLE. T-cell epitopes on nucleolin have not been identified so far.

Several studies have succeeded in inducing anti-DNA antibodies in animals by administration of DNA coupled with some DNA-binding proteins or peptides (Fig. 3). By immunization of preautoimmune NZB/NZW F1 mice with DNA and/or DNase I, IgG anti-dsDNA antibodies were induced in 50% of the mice given DNA–DNase I complexes, in 25% given DNase I alone, and in 6% given free DNA.[13] dsDNA bound to a 52-mer DNA-binding peptide derived from ubiquitin in *Trypanosoma cruzi* has been shown to induce anti-DNA antibodies in normal mice. When this experiment was carried out using a homologous mouse peptide, no significant anti-DNA antibody was observed, suggesting that the DNA-binding proteins or peptides have to be immunogenic to the host to induce anti-

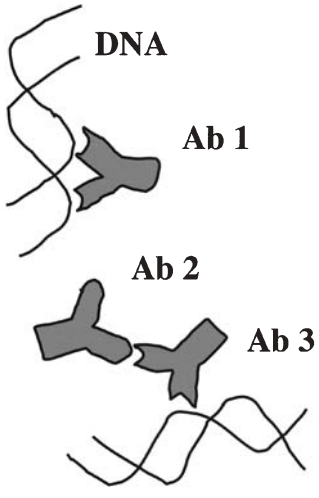

Fig. 1. Anti-DNA antibody production caused by anti-DNA antibodies. Once anti-DNA antibodies (*Ab 1*) are produced or administered, they would trigger production of anti-idiotype antibodies (*Ab 2*), which in turn induce anti-anti-idiotype antibodies (*Ab 3*). Ab 3 may have similar antigen-binding specificity as Ab 1

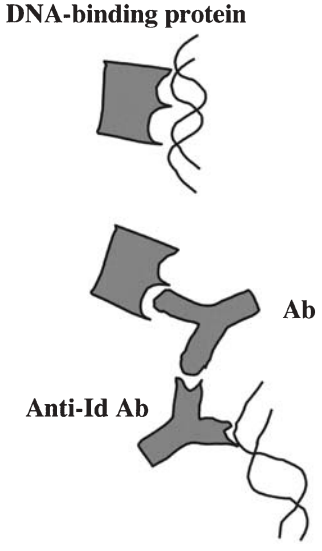

Fig. 2. Anti-DNA antibody production caused by DNA-binding proteins or peptides. If the proteins or peptides are highly immunogenic to the host, as is the case with some bacterial or viral antigens, antibodies (*Ab*) to the DNA-binding protein lead to anti-idiotype antibodies (*Anti-Id Ab*) that react with DNA

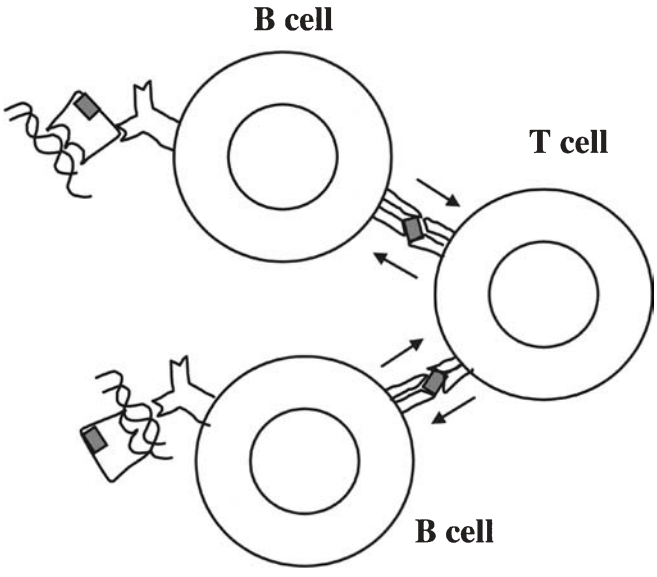

Fig. 3. Anti-DNA antibody production caused by high-affinity complexes of DNA-binding proteins and DNA. In this case, the protein acts as a carrier and the DNA plays the role of a hapten. T cells that react with the processed protein provide help to B cells that recognize the DNA in the complexes as well as those that recognize a part of the protein

DNA responses in this kind of model.[14] DNA vaccination has also been investigated in one study, in which BALB/c mice injected intramuscularly with an expression plasmid for the DNA-binding domain of the glucocorticoid receptor (nonimmunogenic self protein) linked to green fluorescent protein (immunogenic non-self protein) produced anti-DNA antibodies.[15]

Complexes of immunogenic DNA-binding proteins and DNA can also be used as a tool to make antibodies that specifically recognize the sequence of the DNA. BALB/c mice immunized with a 1:1 mixture of the C-terminal DNA-binding domain of the E2 protein from human papillomavirus strain-16 and the oligonucleotide corresponding to the E2 binding site produced antibodies that discriminated the specific nucleotide sequence.[16] The sequence-specific anti-DNA response generated in this study has been explained as the result of the high stability of the protein–DNA complex conferring a long lifetime to the DNA.

Epitope spreading

Several studies have succeeded in producing anti-DNA antibodies using non-DNA-binding peptides. One assessed the effects of immunizing New Zealand White rabbits with the Sm B/B′-derived peptide PPPGMRPP, which had been identified as a major epitope of anti-Sm antibodies in human and murine SLE.[17] After rabbits developed antibodies to the peptide, additional boosting resulted in production of antibodies reactive with the whole molecules of Sm and RNP, and anti-dsDNA antibodies as well, resembling

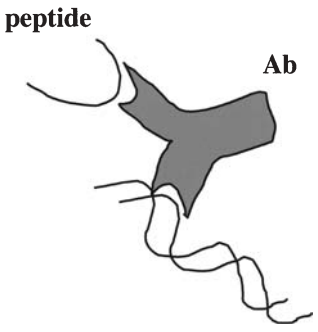

Fig. 4. Anti-DNA antibody production caused by peptides that mimic the structure of DNA. The epitopes formed by particular amino-acid sequences are thought to resemble the structure of DNA

human SLE. Recently, this interesting model was applied to mice in a genetic analysis.[18] The same short peptide as described earlier was used for immunization of various strains of mice. As a result, all of the strains tested produced antibodies reactive with the immunizing peptide; in addition, sera of some of the strains (A/J, AKR/J), but not the others, bound Sm, RNP, and dsDNA. These strain-specific autoimmune responses were suspected to be dependent on the H-2 haplotype of the mice. However, further analysis showed that this hypothesis was not correct. For example, replacing the H-2 locus of the nonresponder C57BL/6J strain with the H-2 locus from the responder AKR/J strain did not alter the resistance of C57BL/6 mice, suggesting the importance of other background genes. Baboons immunized with the PPPGMRPP peptide also produced antibodies not only to the peptide, but also to Sm, RNP, and dsDNA.[19] The authors speculated that one of the potential etiologic agents that contain similar sequences to this peptide is the Epstein-Barr virus, the EBNA-1 of which contains the sequence PPPGRRP. According to the intermolecular epitope spreading theory, antibodies that bind non-self epitopes with sequence similarity emerge in the early immune response, but once initiated, the whole protein or whole spliceosomal complex of the host could be an autoimmunogen.

Molecular mimicry and/or cross-reaction

Peptides containing a YYGS(G)S motif, which were originally derived from the VH CDR3 segment of anti-phosphorylcholine antibodies and anti-DNA antibodies, have been shown to induce anti-dsDNA antibodies in normal mice.[20] To explain this observation, the authors have proposed not the idiotype-anti-idiotype mechanism, but mimicry between the structure of the peptides and DNA; i.e., the peptides induce anti-DNA antibodies directly according to this hypothesis (Fig. 4). Because phosphorylcholine is an immunodominant component of the pneumococcal cell wall polysaccharide, it is probable that some bacterial antigens that mimic DNA induce anti-DNA autoantibodies during the course of a normal immune response. In this regard, it is worth noting that polyclonal anti-dsDNA antibodies purified from SLE sera often cross-react with phosphorylcholine.[21] Some other examples of peptides that mimic DNA have also been reported.

Immunization of BALB/c/NZW F1 mice with a peptide KGRFTISRDNAKSTLYL, originating from the framework 2 region of a NZB/NZW F1 monoclonal anti-DNA antibody, induced anti-DNA antibodies.[22] A monoclonal antibody, and some polyclonal antibodies from patients, were found to recognize both dsDNA and a 44-mer peptide, the protein of origin of which is unknown.[23]

The issue of cross-reactivity of anti-DNA antibodies with peptide antigens also relates to pathogenic roles of the antibodies. Using a phage display peptide library method, a monoclonal anti-DNA antibody was found to react with peptides containing a D/E-W-D/E-Y-S/G consensus sequence. Such peptides act as surrogates for DNA and elicit anti-DNA antibodies in BALB/c mice.[24] Interestingly, this consensus sequence has been found in the extracellular, ligand-binding domain of mouse and human NMDA (N-methyl-D-aspartate) receptors NR2 expressed on brain neurons.[25] Antibodies cross-reacting with DNA and NR2 were actually detected in the cerebrospinal fluid of a patient with neuropsychiatric lupus, and they did induce neuronal death when injected into mouse brain. Another study showed the presence of histone H1 at the surface of neuronal cells in the brain.[26] Because anti-DNA antibodies frequently emerge in association with anti-histone antibodies, these findings could provide a novel clue to understand the mechanisms of neurological manifestations of SLE.

Cross-reaction of anti-DNA antibodies with glomerular antigens may be one of the causes of lupus nephritis. In one study, hybridoma clones secreting anti-DNA, anti-histone, and anti-nucleosome monoclonal antibodies were administered intraperitoneally or subcutaneously to immune deficient nonautoimmune mice.[27] The results showed that some anti-DNA antibodies, but not anti-histone or anti-nucleosome antibodies, significantly bound kidney tissue, resulting in proteinuria. Western blotting of glomerular lysates with the pathogenic anti-DNA antibodies revealed a reactive component of 100 kDa, which was later demonstrated to be α-actinin. This protein is overexpressed in mice with active lupus nephritis.[27,28]

Breakdown of self-tolerance

For many years, numerous hormonal studies have been performed to elucidate the preponderance of SLE in women. Recent advances have been achieved using BALB/c mice transgenic for the heavy chain of a pathogenic anti-DNA antibody. Serum anti-DNA antibodies in these mice were negligible, but they have a B-cell population with high-affinity DNA binding. Implanted with pellets designed to release estradiol for up to 60 days, those mice started to produce high-titer anti-DNA antibodies.[29] ELISPOT analysis of their splenocytes revealed increased survival of high-affinity anti-DNA antibody-secreting B cells, which usually undergo deletion or anergy induction. However, it is not clear whether functional estrogen receptors are expressed in lymphocytes. On the other hand, it is known that estrogen is a physiological prolactin-stimulating agent, and the prolactin receptor is known to be present on T cells and B cells. A current study using the same transgenic mice treated with bromocriptine, which inhibits secretion of prolactin by the pituitary, suggested that the effect of estrogen on survival and activation of autoreactive B cells is mediated by prolactin.[30] In accord with those studies, a clinical benefit of bromocriptine for some patients with SLE has been reported.[31]

Conclusion

Native forms of DNA per se are not immunogenic; however, when bound to proteins that are immunogenic for the host, antibodies to not only the proteins, but also to the DNA can be generated. In some studies, purified DNA-binding proteins or peptides alone are sufficient to induce anti-DNA antibody production. However, other studies are discordant and indicated that DNA itself is necessary. Thus far, there is no consensus explanation for the underlying mechanisms responsible: hapten-carrier, idiotype-anti-idiotype, or molecular mimicry mechanisms are all under discussion. Whatever the mechanisms are, we now know that normal animals do initiate anti-DNA antibody production under certain circumstances. However, for this to continue and become augmented so that full-blown lupus results, further factors are required to break self-tolerance.

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