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Lessons from an anti-DNA autoantibody

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Abstract

R4A is an anti-DNA antibody derived by hybridomas technology from a BALB/c mouse. The study of its properties has helped to elucidate aspects of the physiopathology of Systemic Lupus Erythematosus (SLE). It has been useful to decipher the mechanisms implicated in breaks in B cell self tolerance by B cell extrinsic or B cell intrinsic factors. Furthermore, it illustrates the importance of molecular mimicry in understanding the cellular and molecular effects relevant to some manifestations of neuropsychiatric lupus, as it cross-reacts with the D/E W D/E YS/G peptide sequence present in subunits of the N-methyl D-aspartate receptor (NMDAR).

Keywords

anti-DNA antibody; peptide mimotope; B cell tolerance; BCR signaling

Introduction

One of the specific markers of Systemic Lupus Erythematosus (SLE) is a high titer of autoantibodies directed against nuclear antigens especially double stranded (ds)DNA (Satoh et al., 2007). Anti-dsDNA antibodies are present in around 75% of diagnosed lupus patients. Whether these autoantibodies are pathogenic or not was a long lasting debate for more than 50 years, but it is now well established that they can be nephritogenic, either directly by binding to kidney protein or indirectly by binding to dsDNA present in nucleosomes which are sequestered on glomerular basement membrane (Fenton et al., 2009; Yung and Chan, 2008). Several experiments in mice prove that high affinity anti-dsDNA antibodies are sufficient to induce glomerulonephritis in non-spontaneously autoimmune mice (Ehrenstein et al., 1995; Tsao, 1992). In the 1980's we proposed that these autoantibodies could be generated by a response to bacterial challenge in a non-autoimmune context, through somatic mutation of an anti-microbial antibody (Diamond and Scharff, 1984). Subsequently, we demonstrated that approximately 40% of germinal center B cell making antibodies to phosphorylcholine (PC), the dominant epitope on pneumococcal cell wall polysaccharide, cross-react with DNA but are eliminated by apoptosis prior to joining the memory B cell compartment or becoming plasma cells (Ray et al., 1996). In the course of these studies, we identified some high affinity, germline encoded anti-dsDNA antibodies (Shefner et al.,

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1991). One of these antibodies, R4A, reacts against the consensus pentapeptide sequence D/E)W(D/E)Y(S/G (Katz et al., 1994).

Generation and sequence of R4A antibody

Previous studies showed that a single amino acid substitution in the PC-binding site of the VH1-encoded myeloma cell line S107, resulted in the production of the U4 antibody, which no longer bound PC but had acquired reactivity for dsDNA (Diamond and Scharff, 1984). BALB/c mice were immunized with PC coupled to human gamma globulin (PC-HGG) in an attempt to isolate U4-like anti-dsDNA antibodies that might arise by in vivo somatic mutation of anti-PC antibodies encoded by an S107 VH gene. The S107 gene family has four members (VH1, VH3, VH11 and VH13) and is known to be important in the protective immune response to pneumococcal bacteria (VH1) and to influenza (VH11) in BALB/c mice.

In the course of these studies, the IgG3 hybridoma R4A.12 was generated (Shefner et al., 1990). The antibody is encoded by germline VH11, a short D region trimmed by N diversity and JH4; the light chain is germline Vk1 and Jk1. This antibody has no or low reactivity to PC or influenza, high reactivity for dsDNA, and some reactivity for ssDNA and cardiolipin. Its apparent affinity for dsDNA is around 10^9 – 10^{10} (Shefner et al., 1991). An IgG2b class switch variant of R4A.12 has been used for further studies. This antibody was tested for its pathologic potential and shown to deposit in kidney glomeruli when injected into mice (Katz et al., 1994).

Anti-DNA and anti-peptide reactivity

The use of a peptide display phage library allowed identification of peptides that react with R4A. Twenty three phages selected for their interaction with R4A had the sequence DWEYSVWLSN and 23 others had the sequence WCEADYGRCP. The consensus sequence derived from this analysis was D/E - W - D/E - Y - S/G (Gaynor et al., 1997). Micromolar concentrations of the L or D form of the DWEYS peptide were able to inhibit R4A binding to dsDNA. Reciprocally, dsDNA could inhibit the binding of R4A to the phage expressing DWEYSVWLSN. In vivo, the D-form of the DWEYS peptide was injected into SCID mice and inhibited the glomerular deposition that follows intraperitoneal injection of R4A antibody (Gaynor et al., 1997).

Other peptides have been described as mimotopes for dsDNA and possess negatively charged amino acids that mimic the DNA double stranded helix phosphate backbone (mimotope XXXDCTXNT-Aro-Aro-CQ(L/Y/D)XE recognized the F14.6 anti-dsDNA antibody or mimotope (V/L/I)TGDC(W/G)(Y/G)W(G/Q)XXXX recognized by the J20.8 anti-dsDNA antibody, both from NZB/W mice (Sibille et al., 1997). The DWEYS peptide does not mimic the DNA backbone but may a mimotope of the small groove. The RLTSSLRYNP that is recognized by 80% of SLE human sera with anti-DNA or anti-RNA reactivity has also been reported to recognize the small groove (Sun et al., 2001), but a subsequent report suggested it was actually not a mimotope of DNA but a DNA-binding peptide (Dieker et al., 2005).

The sequence DWDYS is present in the NR2A subunit of the NMDA receptor (position aa283–287) and the sequence EWDYG is present in the NR2B subunit of the NMDA receptor (position aa284–288).

H-2^d dependence of the DWEYS peptide immunization

The DWEYS peptide can be octamerized on a poly-lysine backbone, MAP-DWEYS. Syngenic BALB/c, DBA2, and C57BL6 mice were immunized with MAP-DWEYS peptide. Only BALB/c mice, which are H-2^{d/d}, mounted an antibody response anti-DWEYS peptide and to dsDNA, showing that DWEYS is a true mimotope of dsDNA and has the ability to induce a break of self tolerance. The immunogenicity of DWEYS is MHC restricted as wild type C57BL6 mice, which are (H-2^{b/b}) are unresponsive to MAP-DWEYS peptide immunization while congenic C57BL6 mice that are H-2^{d/d} are able to mount an anti-DWEYS, anti-dsDNA antibody response. This response is CD4+ T helper cell dependent and restricted to I-E^d; it is characterized by production of IgG1 and IgG2a anti-DWEYS, anti-dsDNA antibodies (Khalil et al., 2001). The presence of the DWEYS sequence in the choline kinase of several different species of bacteria (Eubacterium, Streptococcus, Legionella, Clostridium, Lactobacillus-acidophilus, -rumini or -brevi, Hemophilus influenza) suggests the possibility of an immunization by infection. In mice, the immunization with streptococcus choline kinase also elicits an anti-dsDNA antibody titer.

B cell tolerance and the anti-DNA response

SLE is a disease of B cell dysregulation that depends on genetic predisposition and is triggered and exacerbated by various events, which can be hormonal, infectious, environmental or a combination of the three. For H-2^d expressing strains, the penetrance of the break in tolerance to dsDNA varies as a function of the BCR signaling strength. In particular, DBA/2 mice fail to mount a robust antibody response despite a strong T cell response to MAP-DWEYS (Wang et al., 2003). Further studies demonstrated that the failure of DBA/2 mice to mount an antibody response to MAP-DWEYS reflects a stronger BCR mediated signal and, therefore, stronger negative selection of DWEYS and DNA-reactive B cells (Wang et al., 2003).

There are nine women for every one man with SLE, with an age of onset after puberty and before menopause, leading some to speculate that hormones might help regulate DNA-reactive B cells. In order to study the role of estrogen in B cell selection, we generated mice with the heavy chain of R4A antibody was introduced as an IgG2b transgene under the control of the B cell specific IgM promoter (mu promoter) (Offen et al., 1992). In either the BALB/c or C57BL/6 background, the mice did not generate high affinity anti-DWEYS, anti-dsDNA antibodies because high affinity autoreactive R4A expressing B cells are negatively selected during development. When BALB/c R4A transgenic female mice are treated with a continuous high physiological level of estrogen or prolactin, they develop titers of anti-DWEYS, anti-dsDNA autoantibodies, demonstrating the ability of sex hormones to impair B cell negative selection and suggesting that estrogen and prolactin may contribute to the gender bias observed in SLE (Bynoe et al., 1999; Grimaldi et al., 2002; Peeva et al., 2003). Alternatively, if BALB/c mice expressing the R4A heavy chain transgene are deficient in the FcγRIIB receptor, they spontaneously break tolerance (Venkatesh et al., 2009). These results illustrate that multiple different pathways, B cell receptor signaling, hormone response, the FcRIIB pathway, can abrogate B cell tolerance.

The study of this mouse model has also added to our understanding of B cell depletion in SLE. While B cell depletion has been suggested as a therapeutic goal in SLE, we have shown that a consequence of the therapeutic B cell depletion is a rise in the serum concentration of soluble BAFF to levels that promote the rescue of de-novo autoreactive B cells during the reconstitution of the B cell repertoire (Kawabata et al., 2010). This observation illustrates the necessity to monitor BAFF levels during such therapy and may help us understand why B cell depletion was not successful in clinical trials.

Interaction with the NMDA Receptor

The sequence of the consensus peptide is present in some self- and nonself-proteins; in particular, it is found in a solvent exposed extracellular loop of both the NR2A and NR2B subunits of the N-methyl D-aspartate receptor (NMDAR), an ionotropic glutamate receptor expressed mostly in the brain. This observation suggested that anti-DWEYS, anti-dsDNA antibodies might display NMDAR reactivity and raised the possibility that such anti-dsDNA antibodies might interfere with the function of this neuronal ion channel, and so with brain function. The NMDAR is composed of a homo- or heterodimer of NR2 subunits and two NR1 subunits. NR2A or NR2B subunits are detected by Western blotting of brain lysates with the R4A antibody and R4A recognizes NMDAR expressing cells by immunohistochemistry on brain slices (DeGiorgio et al., 2001; Faust et al., 2010). The strongest proof of the interaction of R4A with NMDAR is functional and comes from electrophysiology studies that show that R4A affects NMDAR function only in presence of glutamate by prolonging its signaling (Faust et al., 2010). Moreover, mouse or human monoclonal or polyclonal IgG with DWEYS, anti-dsDNA reactivity can induce excitotoxic death of neurons and cause *in vivo* cognitive and behavioral anomalies relevant to neuropsychiatric syndromes observed in some lupus patients. Moreover, when these antibodies are present in pregnant mice, they cause an altered development of the fetal brain *in utero* with long-term cognitive consequences (Lee et al., 2009). Thus, the regulation of these antibodies seems of clinical importance.

Adult neuronal effects depend of the breaches of the blood brain barrier

The mouse model allowed us to decipher the mechanism of neurotoxicity of anti-DWEYS anti-dsDNA antibodies. In particular, the antibodies induce neuronal death by apoptosis *ex-vivo* on brain slices or *in-vivo* when they are directly injected in the hippocampus of a living mouse (DeGiorgio et al., 2001). These antibodies are co-agonists and their modality of action is direct. Their effects are reproduced solely by their Fab'2 fragments but they require the presence of glutamate to affect NMDAR signaling and promote apoptosis by excitotoxicity. A similar neuronal death is obtained when anti-DWEYS antibodies purified from serum or cerebrospinal fluid (CSF) of neurolupus patients are injected into mouse brain (DeGiorgio et al., 2001; Kowal et al., 2006).

BALB/c mice immunized with MAP-DWEYS display neuropathology only if there is a breach of the blood brain barrier (BBB). A regional specificity of the compromise of the BBB depends of the BBB opening agent. After treatment with bacterial lipopolysaccharide (LPS), which mimics an infection, a massive neuronal death occurs in the hippocampus. This neuronal loss correlates with behavioral effects, in particular, memory impairment (Kowal et al., 2006). Epinephrine has a strong and transient effect; it increases intracranial blood pressure and destabilizes the BBB. When epinephrine is administered to mice with high serum titers of these neurotoxic antibodies, neuronal death is observed in the amygdala (Huerta et al., 2006). This neuronal damage correlates with abnormal fear responses in the treated mice.

Fetal neuronal effects are long lasting

During gestation, the fetal brain is not protected by a BBB and the maternal antibodies are actively transferred to the fetus through the placenta. It was not clear if anti-neuronal antibodies like R4A, which are neurotoxic in adult brain if there is a breach of the BBB, could affect fetal brain development. The question was motivated by clinical observations: children born to mothers with SLE show a high incidence of learning disorders, and the mechanism for these disorders remain unknown (Lahita, 1988; McAllister et al., 1997; Neri et al., 2004; Ross et al., 2003; Tincani et al., 2006). In contrast, children born to a healthy

mother and a father suffering from SLE have no increased incidence of learning disorders. To know if maternal neurotoxic autoantibodies could be responsible of abnormal brain development and later of behavioral disorders for the adult offsprings, BALB/c mice female were immunized with MAP-DWEYS and female mice with high anti-DWEYS, anti-dsDNA antibody titer were allowed to become pregnant. Embryos were studied and offspring were tested for behavior, as neonates and adults. The study established that the autoantibodies are transported from the mother to the embryo and their presence in the brain, and that they cause congenital brain injuries in a dose dependent manner in the fetal neocortex. These injuries are most probably due to the triggering of excitotoxicity by interaction with NMDAR (Lee et al., 2009). Finally, these studies also established that subsequent long term cognitive changes are observed in the offspring.

Anti-DWEYS reactivity and neuropsychiatric lupus in patients

Clinical studies performed worldwide have allowed us to better know the symptoms of neuropsychiatric lupus and have revealed a high frequency of cognitive dysfunctions, particularly memory impairment and depression in the SLE patient population, symptoms that could be explained by the action of anti-NMDAR autoantibodies. Several groups have investigated whether titers of anti- DWEYS anti-dsDNA autoantibodies in the serum would correlate with the intensity or the frequency of neuropsychiatric manifestation (Aranow et al., 2010; Hanly et al., 2006; Steup-Beekman et al., 2007). These studies yielded inconsistent results. Contrary to the serum studies, studies of antibody in the CSF gave consistent results and established a strong correlation between the presence of anti-DWEYS, anti-dsDNA autoantibodies in the CSF of lupus patients and the occurrence of neuropsychiatric manifestations (Arinuma et al., 2008; Fragoso-Loyo et al., 2008; Yoshio et al., 2006).

As the presence of these autoantibodies in serum may be a prerequisite for their accumulation in the CSF where they exercise their deleterious effects, the detection of a serum titer of these antibodies can be considered as a potential for neuropsychiatric symptoms during the course of the disease. The determinant event would be a breach of the BBB, which allows the transfer of the autoantibodies to the brain. Ongoing work in the laboratory is establishing ways to neutralize these antibodies and to monitor the integrity of the BBB.

Conclusion

It is clear that the study of anti-DNA antibodies continues to yield valuable information regarding both B cell tolerance and clinical manifestations of systemic lupus. It is important to note that R4A is a single antibody and antibodies with other affinities for DNA and other cross-reactivities to either self or microbial antigen will yield different results in studies such as those described above and will provide new insights into B cell tolerance and disease mechanism.

Research Highlights

1. a subset of anti-DNA antibodies contributes to CNS lupus.
2. B cell receptor signal strength is governed by B cell intrinsic and extrinsic factors.
3. Low B cell receptor signal strength in transitional B cells predisposes to autoimmunity.

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