

The Story of the 16/6 Idiotype and Systemic Lupus Erythematosus

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Abstract

Idiotypic analyses of anti-DNA autoantibodies were widely reported a decade ago. More than 100 studies were conducted on one of the main analyzed idiotypes, the 16/6 Id of the anti-ssDNA monoclonal antibody. In this review we summarize current knowledge on the characteristics of the 16/6 Id, its link to infection and its target epitopes on other molecules known so far. This includes the modulation of T and B cell responses and gene expression by the 16/6 mAb *in vitro* and *in vivo*. We focus on the ability and mechanisms by which this idiotypic induces experimental lupus in naïve mice, manifested by autoantibody spread, kidney and brain involvement, and leukopenia associated with enhanced sedimentation rate. We also discuss various therapeutic modalities to treat 16/6 induced lupus in mice.

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Systemic lupus erythematosus is a multifactorial autoimmune disease with a diverse etiology [1]. The etiologies comprise genetic, hormonal, environmental (mainly infections, radiation, drugs) and immunological factors. One of the characteristics of the disease is the presence of elevated titers of more than 100 circulating autoantibodies [2]. Among the lupus-associated autoantibodies, anti-DNA and its idiotypes (located in the variable region of an immunoglobulin) were well characterized during the last 50 years, and represent the major family of antibodies that correlates with disease activity [3-5]. More than 100 studies were conducted on the 16/6 idiotypic of the anti-ssDNA monoclonal antibody. The human monoclonal anti-DNA antibodies were found to be polyspecific, targeting different molecules that belong to the cytoskeletal proteins, phospholipids, glycoproteins and various cells [5-10].

The characterization of anti-DNA 16/6 Id

In 1982, the exciting early days of the human-human hybridoma technology, an immunoglobulin M anti-ssDNA, defined as the 16/6 Id clone, was derived from a patient with a cold agglutinin disease [6]. The human IgM 16/6 monoclonal antibody was found to be encoded by a germline gene from the human VH4 gene family, with a high similarity to the germline gene VH4.21 that was previously shown to code for anti-DNA antibodies isolated from SLE patients. The VH4.21 germline gene was found also to code for most antibodies with cold agglutinin activity that were isolated from patients with cold agglutinin disease [11].

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Id = idiotypic

mAb = monoclonal antibody

Ig = immunoglobulin

SLE = systemic lupus erythematosus

Clinical studies addressing the incidence of the 16/6 Id in lupus patients showed an increased level of the 16/6 Id in the sera of 54% of patients with active lupus (40 of 74), compared to 25% of patients (6 of 24) at remission [12]. In a study with serial sera samples from lupus patients, elevated 16/6 Id concentrations were correlated with disease activity [8]. The presence of this idiotypic was detected also in the sera of relatives of lupus patients and in SLE patients with silica transplants [13]. The importance of the 16/6 Id in lupus was confirmed when depositions of this idiotypic were revealed in the skin, kidney and brain [10,14,15]. Offspring of mothers with SLE had circulating 16/6 Id even in the absence of such antibodies in the maternal serum [16].

Interestingly, the presence of circulating 16/6 Id was detected in other autoimmune diseases as well [17-19]. The idiotypic was found in the sera of 49% of patients with polymyositis (49%), 17% of patients with multiple sclerosis, 18% of those with primary Sjögren's syndrome, 23% with autoimmune thyroid diseases, 6% with myasthenia gravis, 15% with scleroderma, 65.9% with chronic liver diseases, 7% with rheumatoid arthritis, 8.7% of patients with monoclonal gammopathies, 60% of patients with active pulmonary tuberculosis, a few patients with schistosomiasis and filariasis, and in association with *Klebsiella pneumoniae* infection [17-20]. Interestingly, in patients with Waldenström's macroglobulinemia five of six monoclonal IgM that reacted with *Klebsiella* polysaccharides cross-reacted with the 16/6 Id anti-DNA antibody. The reaction between the macroglobulins and the 16/6 anti-idiotypic serum was specifically inhibited with *Klebsiella* polysaccharides, pointing to the presence of idiotypic determinant in the antigen-binding site of the macroglobulins. These results indicate that Waldenström's macroglobulinemia-derived monoclonal antibodies share a binding site variable region with bacterial polysaccharides and nucleic acids [20]. Shoenfeld and Mozes [9] proposed that the presence of this idiotypic in various autoimmune diseases points to a pathophysiological link between the diverse diseases.

The 16/6 Id anti-ssDNA mAb was found to be polyspecific and to bind: synthetic nucleotides, cytoskeletal proteins (vimentin), platelets, lymphocyte membranes, *Mycobacterium tuberculosis* glycoprotein, *Klebsiella* polysaccharides, brain glycolipids and tumor cells (e.g., L5178Y murine T cell lymphoma and human Raji cells) [5-10,21,22].

Pathogenicity of anti-ssDNA 16/6 Id mAb in an experimental model

According to Rose and Witebsky's autoimmune criteria, if a disease can be induced by a passive transfer of pathogenic

autoantibody or by active immunization with the autoantigen, it is an autoimmune disease. Jerne in 1974 [23] proposed that the immune response may be regulated by antigenic determinants of the immunoglobulin variable regions (complementarity-determining regions) (idiotypes).

The idiotype network theory of Jerne provided the basis for the development of the idiotype/anti-idiotypic network, in which the anti-anti-Id mimics the internal image of the epitope used for immunization. Thus, the idiotypic determinants of each antibody molecule would be complemented by those of another, creating an idiotypic network through which immunoglobulin expression may be controlled. Immunization of naïve mice with the human anti-DNA 16/6 Id Ab (Ab1) resulted in an experimental lupus that presented with immune complex deposition in the glomerular mesangium and sclerosis of the glomeruli associated with proteinuria, leukopenia, increased sedimentation rate, and autoantibody spread (e.g., mouse 16/6, anti-Ro, anti-La, anti-RNP, antiphospholipid) [9,24,25]. Briefly, immunization with the human 16/6 Id anti-DNA (Ab1) induced elevated titers of mouse anti-16/6 anti-idiotypic Ab (Ab2), followed by mouse anti-anti-16/6 Ab (Ab3). Ab3 (mouse anti-DNA 16/6 Id) had the internal image of Ab1 and shared the same binding specificities [9,24]. To study the role of anti-Id antibodies in the induction of the disease, a murine monoclonal antibody against the 16/6 Id was prepared and injected into C3H.SW mice [24]. The mouse anti-16/6 Id antibody induced experimental SLE similarly to the 16/6 Id, with an impressive kidney pathology. The 16/6 Id located on other autoantibodies from other sources also induced experimental lupus [26]. The pathogenicity of the 16/6 Id region in the immunoglobulin was proved by constructing a single-chain-Fv 16/6 Id anti-DNA and exchanging the heavy chain between the 16/6 Id and antiphospholipid antibodies [27]. Only the scFv bearing the CDR with the 16/6 Id induced experimental lupus in an animal model, while exchanging the 16/6 heavy chain with heavy chain of an anti-beta-2-glycoprotein-I, reduced the anti-ssDNA binding properties and failed to cause a lupus-like picture in an animal model [27]. A milder disease and a decrease in the capacity to respond to the pathogenic 16/6 Id was observed in aged mice [28].

T cells derived from lupus patients proliferated in the presence of the 16/6 Id [29]. The final proof for the importance of T cells bearing the 16/6 Id raised from the studies in which experimental lupus T cells reacted with the 16/6 id and the disease could be developed in naïve mice passively transferred with T cell lines specific for the 16/6 Id [30]. T cell lines specific for 16/6 Id-bearing antibodies from various sources induced experimental SLE in naïve mice [31]. The latter is additional support for the pathogenic role of 16/6 Id in SLE.

From 16/6 Id pathogenicity to lupus treatment

Having a lupus-like model induced in naïve mice by immunization with the pathogenic idiotype enabled the study of mechanisms

and the development of diverse approaches to treat experimental lupus models. Various treatments were found to be effective in down-regulating the serological and clinical picture of the experimental lupus induced by 16/6 Id [summarized in ref. 32]. Disease manipulations include administration of T suppressor cells specific for the 16/6 Id, and bone marrow transplantation. Successful treatments using antibodies included also anti-CD4⁺ T cells, anti-16/6-anti-Id antibodies and intravenous immunoglobulin [32]. Amelioration in the serological and clinical picture of experimental lupus was achieved by employing antibodies directed to interferon-gamma, M-20 and interleukin-1 inhibitor [32,33]. Since lupus is mostly prevalent in females, hormonal treatments were studied, e.g., estrogen depletion and T suppressor cell induction by bromocriptine treatment. Immunomodulatory compounds (SA101, M-20, AVE-MAR, cyclosporine A, linomide), diet with different polyunsaturated fatty acid contents, and retroviral infection decreased the lupus picture in the 16/6 Id immunized mice. Immunization of diabetes NOD mice with the 16/6 Id induced experimental lupus and inhibited the development of diabetes [32,34-37].

16/6 Id and brain cross-talk

Previously, elevated titers of anti-ssDNA 16/6 Id were detected among neuropsychiatric SLE patients, indicating a possible role for anti-DNA 16/6 Id antibodies in neuropsychiatric involvement in SLE [38]. Exposure of the 16/6 Id antibodies to healthy mouse brain sections showed specific binding to the choroid plexus, the cortex, the piriform cortex, the hippocampus and the thalamus (unpublished data). Naïve mice, intraventricularly subjected to the 16/6 Id antibodies, exhibited depressive behavior as compared to mice injected with control IgG and to non-injected mice, as previously shown by us for anti-ribosomal-P antibodies intraventricular passive transfer [39]. When employing the forced swimming test, the immobility time of 16/6 mice was significantly higher compared to control mice, indicating a depressive-like behavior in the 16/6 group [40]. There was no significant difference between 16/6 and control mice in motor skill tests, indicating that the immobility of the mice was not due to motor dysfunction. In a smell threshold detection test a tendency to increased threshold among 16/6 mice was observed [submitted for publication]. We concluded that passive transfer of anti-ssDNA 16/6 Id antibodies had induced central nervous system involvement manifested as cognitive impairment and particularly depression-like behavior.

Conclusions

The 16/6 Id is a pathogenic idiotype found in high prevalence in lupus patients, patients with other autoimmune diseases, as well as in patients with different infections. This idiotype correlates with disease flares. The idiotype can induce experimental lupus in naïve mice by idiotypic manipulation exemplified as autoantibody spread and a clinical picture that resembles that of human lupus. Being a pathogenic idiotype, the 16/6 Id derivative may be used as a preventive molecule for induction of lupus in mouse models. Thus, 16/6 Id may be an important future idiotype-targeted treatment for lupus patients.

scFv = single-chain Fv

CDR = complementarity-determining regions

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