The Potential Role of Lyn Kinase in Systemic Lupus Erythematosus and Autoimmunity

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Systemic lupus erythematosus (SLE) is a multifactorial autoimmune disease characterized by a loss of tolerance to self antigens, deposition of immune complexes, and tissue inflammation and destruction. The disease occurs nine times more often in women than in men, particularly in women of childbearing age (15 to 35 years). Ethnicity is another factor since SLE is more common in African-Americans and Asians compared to people of Caucasian origin. It is characterized by high morbidity and increased mortality compared to the healthy population; SLE can affect vital organs such as the central nervous system, kidneys, heart and lungs, as well as joints, skin and blood vessels. It is known that SLE patients have an impaired immune response to exogenous antigens reminiscent of anergy. The T cells in SLE are hyperactive and the resulting enhanced activation of B cells may lead to the production of autoantibodies. The immune complexes that are formed (in situ or elsewhere) are deposited in end organs such as the kidneys and blood vessels and can cause tissue damage and perhaps multiple organ failure.

ROLE OF LYN KINASE IN PHYSIOLOGY

Lyn is a kinase, a member of the Src family of protein tyrosine kinases. Src kinases are associated with cell membrane molecules [1]. The gene that encodes for Lyn is located at chromosome 12q13, and the protein is identified as a doublet of a non-phosphorylated protein with a molecular weight of 53 kD and a phosphorylated form of p56 kD. Lyn is expressed in all types of human leukocytes, apart from T cells [1].

Lyn is both essential and critical for B cell function. In the resting state, Lyn is found in an inactive form, but can be activated by B cell activation via a variety of receptors and/or stimuli including the B cell antigen receptor (BCR), CD40, lipopolysaccharide (LPS), and others [2].

Lyn is a kinase critical to the function of B cells

Lyn is constantly found in close proximity to the BCR and is important in BCR-mediated signaling. Although the role of Lyn in BCR-initiated signaling was previously unclear, it is currently thought that Lyn can function as both an enhancer and an inhibitor of BCR-mediated signal transduction depending on the type of stimulus, developmental stage of the cell, and extracellular environment.

The role of Lyn as a positive transducer of BCR-initiated signaling is regulated by Lyn-mediated phosphorylation of ITAMs (immunoreceptor tyrosine-based activation motifs) of membrane proteins such as Igα/β and CD19, which recruit proteins such as Syk and phospholipase Cγ2, resulting in the transduction and amplification of BCR-mediated signaling. However, knockout models have clearly shown that the role of Lyn in B cell activation and the relevant signal transduction is redundant [1-5].

In contrast, when Lyn phosphorylates tyrosine residues within ITIMs (immunoreceptor tyrosine-based inhibitory motifs), present in inhibitory proteins such as CD22, FcγRIIB1 and PIR-B, it clearly functions as a signaling repressor [3-5].

Even though Lyn is found in the majority of leukocytes, the only effect of the Lyn null mutation on leukocyte development is seen strictly in the B lymphocyte lineage, underscoring its significant role in B cell physiology [6]. $Lyn^{-/-}$ mice present with peripheral B cell lymphopenia due to the inability of B cells to proliferate [6]. Lyn plays a critical and necessary role in the physiological B cell proliferation and is a significant part of the BCR activation complex [6,7].

DATA FROM ANIMAL MODELS

To address the role of Lyn in physiology and perhaps pathophysiology, knockout experimental animals were generated. Murine $Lyn^{-/-}$ models develop a disease that has several features in common with the systemic autoimmune disease developing in humans with SLE. This lupus-like disease of Lyn-deficient mice is characterized by hyper-gammaglobulinemia, the appearance of circulating antinuclear antibodies, and deposition of pathogenic immune complexes in the kidney [8]. The glomerulonephritis developing in the $Lyn^{-/-}$ animals is interesting principally because it is a uniquely, gradually worsening, severe

glomerulonephritis very similar to the renal disease of SLE; these mice eventually die because of the deposition of autoantibodies in vital organs such as the kidneys. Finally, apart from the renal disease, $Lyn^{-/-}$ mice also develop pancytopenia, another component of the systemic nature of SLE. It is interesting that in the $Lyn^{-/-}$ mouse the BCR-initiated signaling is enhanced instead of diminished as one might expect.

Lyn deficiency may be associated with SLE, as depicted by molecular, functional and genetic studies

Histologically, a proliferative and focally sclerotic and even crescentic glomerulonephritis was seen. Mesangial deposits were present, and occasionally necrotic glomeruli and capillary vasculitis were recorded. Severe glomerulonephritis associated with IgG-containing immune complexes was correlated with renal failure resulting in the death of some Lyn-deficient animals [8].

The $Lyn^{-/-}$ mouse has a B cell lymphopenia, the marginal zone of B cells is absent, the plasma cells are increased, the levels of IgM, IgA and IgE are increased, and the B cells in the knockout animal are hyperactive, influencing the BCR signaling.

DATA FROM PATIENTS WITH SLE

Patients with systemic lupus erythematosus have B cells with an abnormal cascade of early transduction events that are mediated by an antigen receptor [9]. We sought to investigate the molecular background of these enhanced BCR-initiated signaling events in patients with SLE, and whether Lyn levels might be affected. To address this question we analyzed levels of B cell Lyn with Western immunoblotting. It was evident that in two-thirds of these patients (all Caucasian), circulating resting B cells were obviously deficient in Lyn kinase. Our initial results were confirmed a few years later by another independent group of investigators. Finally, it was recently reported by another group of researchers that Lyn deficiency does affect the B cells of two-thirds of patients with SLE from a totally different ethnic background. Moreover, these authors further dissected the mechanism(s) responsible for lupus B cell Lyn deficiency and presented data on the role of miR-30a. The higher the levels of miR-30a in B cells the lower the levels of Lyn protein [10].

The glomerulonephritis of Lyn-deficient models is one of the best models for the study of SLE renal disease

Lyn kinase is also an integral part of the CD22-initiated signaling inhibitory complex; however, the expression of CD22 itself is not deficient on the surface of resting peripheral B cells of patients with SLE. Therefore, although CD22 expression is not altered, the disease-specific deficient expression of Lyn in B cells from patients with SLE may represent a molecular background which explains, at least in part, the aberrant BCR-mediated signaling that characterizes lupus B cells [11].

The increased degradation of Lyn, initially observed in SLE patients by a group of investigators from England, may reduce the Lyn-mediated inhibitory signaling. This, in turn, may enhance B cell responses to autoantigens and the production of autoantibodies [12]. The same group of investigators performed experiments showing that SLE B lymphocytes with decreased levels of Lyn proliferated more when compared with B lymphocytes from healthy donors, and produced IgG anti-dsDNA autoantibodies. Such data are quite reminiscent of data produced in the Lyn knockout mice. One may postulate that such a deficiency of Lyn may result, correlate, or underlie a potentially reduced phosphorylation of CD22, SHP2 and FcRII [12-15]. An additional piece of evidence indicating that Lyn may be involved in SLE pathogenesis is the identification of Lyn as a disease-susceptibility gene in two different genome-wide association scans in large cohorts of lupus patients [16,17].

In another human systemic autoimmunity syndrome, the lupus-like disease developing in a small number of patients treated with tumor necrosis factor-alpha (TNFα) blockers, we recently reported that treatment with such agents resulted in an almost linear induction of Lyn in B cells of patients who suffered from systemic rheumatic diseases other than SLE, perhaps explaining, at least in part, the anti-TNFα-induced lupus-like autoimmunity occasionally encountered in such patients. In such patients Lyn increased gradually after treatment initiation and remained fully functional, as judged by the ability of this induced Lyn to undergo tyrosine phosphorylation itself. Moreover, Lyn remained functional based on the results of experiments demonstrating that one specific Lyn substrate did indeed undergo increased phosphorylation, correlating with increased levels of Lyn protein [18]. Although Lyn deficiency has been clearly associated with systemic lupus-like autoimmunity in animal studies and may also be involved in the systemic autoimmunity encountered in the SLE patient, it is of interest that Lyn overexpression may be related to lupus-like autoimmunity as well. Mice overexpressing Lyn did indeed develop systemic lupus-like autoimmunity. Such genetically manipulated $Lyn^{up/up}$ mice develop circulating autoantibodies and severe glomerulonephritis with autoimmunity features frequently leading to death, suggesting that enhanced positive signaling may overcome the well-established continuous inhibitory Lyn-mediated signaling in B cells.

CONCLUSIONS

It may be reasonable to conclude that Lyn in B cells must be constantly and closely regulated in order to avoid too little or too much Lyn-mediated signaling. Enhanced or decreased Lyn function may eventually lead to lupus-like autoimmunity [19].

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