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The role of innate immunity in the induction of autoimmunity ☆

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ABSTRACT

The autoimmune diseases are a diverse group of conditions characterized by abnormal B and T cell reactivity in association with autoantibody production. Among these diseases, systemic lupus erythematosus (SLE) is notable for the expression of antibodies to DNA, with these antibodies representing diagnostic markers. While mammalian DNA is immunologically inert, DNA from bacteria can potently stimulate the innate immune system, activating both toll-like receptors (TLRs) as well as non-TLR internal receptors. Since the sera of normal humans contain antibodies specific for bacterial DNA, this molecule appears to be immunogenic during infection. In pre-autoimmune mice, immunization with bacterial DNA can induce anti-DNA autoantibody production, suggesting a role in initiating this response. The immune properties of DNA are mutable, however, since mammalian DNA can acquire immunological activity when bound to certain proteins or anti-DNA antibodies to form immune complexes. In SLE, these immune complexes can drive the production of interferon by plasmacytoid dendritic cells, thereby intensifying autoimmunity. Together, these observations suggest that DNA can induce innate as well as adaptive immune responses and promote the pathogenesis of SLE because of its intrinsic immunostimulatory activity.

Contents

1. Introduction

The autoimmune diseases are a diverse group of conditions characterized by abnormal immune reactivity in association with autoreactive B and T cells responses. Among these diseases, systemic lupus erythematosus (SLE) is a prototype for generalized autoimmunity and displays the abundant production of autoantibodies to nucleic acids. As shown in studies of patients and animal models, pathogenic mechanisms operative in SLE provide important paradigms to understand the way in which stimulation of innate immunity can culminate in autoimmunity.

The innate immune system constitutes an important defense system to respond rapidly to both endogenous and exogenous

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molecules. Exogenous stimuli are products of bacteria and viruses and have been termed PAMPs (pathogen associated molecular patterns). Endogenous stimuli include cellular macromolecules that emanate from injured or dying tissue. Such endogenous molecules have been termed DAMPs for damage (or death) associated molecular patterns. Both PAMPs and DAMPs can interact with the toll-like receptors (TLRs) and powerfully stimulate B cells, T cells, and antigen presenting cells [1,2].

While stimulation by PAMPs and DAMPs promotes autoreactivity via effects on immune activation and antigen presentation, in SLE, these molecules may have a special role in pathogenesis since they can serve as targets of autoreactivity as well as inducers. Among these molecules, DNA is a central autoantigen in SLE and, depending on structure and context, can stimulate innate immunity. This review will discuss the role of DNA in activating the immune system in SLE as well as promoting autoantibody responses.

2. Stimulation of innate immunity by DNA

Among macromolecules, DNA was long viewed as unique because of its seeming inertness to the immune system. As shown in serological studies, antibodies to DNA occur essentially only in SLE and serve as diagnostic markers. These antibodies bind to various determinants on DNA, although antibodies to double stranded (ds) DNA are the most characteristic. Importantly, antibodies to dsDNA are difficult to induce by immunization of normal mice. These features suggested that the generation of anti-DNA antibodies requires an exceptional immunological setting [3].

While free mammalian DNA lacks immunological activity, the immune properties of DNA are heterogeneous and mutable, with bacterial DNA as a potent immune stimulant. This activity relates to sequences, known as CpG motifs, which occur much more commonly in bacterial DNA than mammalian DNA and lead to recognition as foreign [4]. As such, bacterial DNA represents a PAMP and can stimulate immune responses via TLR9. Since TLR9 resides on the inside of cells, to trigger responses, foreign DNA must arise from an internal source (e.g., intracellular infection) or enter the cell by endocytosis or transport [5,6].

The ability of DNA to stimulate innate immunity has been extensively documented in animal models although many of these studies have involved synthetic oligonucleotides (ODN) that have been optimized for use as therapeutics by backbone modification. While these compounds stimulate TLR9, they differ from conventional DNA in their resistance to nucleases as well as ability to enter cells [7]. Establishing the role of natural DNA in infection is difficult since microorganisms frequently display more than one PAMP. Table 1 summarizes immune stimulation by bacterial DNA.

Table 1
Immune stimulation by DNA

Stimulation of TLR and non-TLR receptors
Stimulation requires uptake into cells
Depends on sequence and backbone structure
CpG motifs important for stimulation by natural DNA
Binding to a carrier or antibody can modify activity

Table 2
Properties of antibodies to bacterial DNA

Specific for bacterial DNA
Specificity for both single stranded and double stranded DNA
IgG2 isotype
Increased ratio of κ/λ expression
Inducible in normal animals by immunization

Perhaps the best evidence that bacterial DNA impacts on the normal immune system relates to the presence of antibodies to bacterial DNA in the serum of normal humans (Table 2). These anti-DNA differ in specificity from anti-DNA autoantibodies found in SLE sera, which bind the DNA backbone. In contrast, antibodies to bacterial DNA from normal individuals bind to non-conversed sequence determinants found on the DNA of some, but not all, bacterial species; these differ from the CpG motifs which are widely shared on all bacteria [8–10]. Of note, immunization of normal mice with bacterial DNA can induce the expression of antibodies that bind to bacterial but not mammalian dsDNA [11]. These findings suggest that antigens that can stimulate a TLR may have enhanced immunogenicity since the adjuvant is present on the same structure as the antigenic determinants.

3. The role of internal DNA receptors in innate immunity

Although TLR9 can mediate signaling by foreign DNA, other internal receptors may recognize DNA to activate innate immunity [12]. Evidence for these receptors derives from studies in which DNA is used to stimulate cells in the presence of transfecting agents. Such agents facilitate entry of DNA into cells via endocytosis. With DNA in transfection agents, the usual rules regarding the necessity for CpG motifs may not pertain as both mammalian and bacterial are active in this context; the downstream pathways activated also differ when DNA is incubated in a free form or with a transfection reagent [13,14].

The internal nucleic acid receptors include molecules such as DAI (DLM-1/ZBP1) and may stimulate innate immunity from an internal microorganism or from extracellular DNA brought into cells with a carrier [15–17]. Beyond synthetic agents such as transfection reagents, carriers may include DNA binding molecules such as the anti-bacterial protein LL37 or the nuclear protein HMGB1. HMGB1 is a non-histone nuclear protein that can function as an alarmin to activate innate immunity. Both LL37 and HMGB1 may form complexes with DNA that has been released from dead or dying cells for delivery into cells to stimulate internal receptors or TLR9 [18,19].

4. Stimulation of innate immunity by immune complexes in SLE

As shown in studies of patients with SLE as well as animal models, interferon α/β (IFN), a key mediator of innate immunity, plays a prominent role in disease pathogenesis [20]. The clearest evidence for this role derives from microarray analyses showing that peripheral blood cells of patients with SLE display an “interferon signature,” as manifest by increased expression of IFN-responsive genes. Since conventional assays for IFN are often unsatisfactory using patient blood, the interferon signature exemplifies the

activation of innate immunity in SLE and the impact of IFN on immune cell populations in the periphery [21,22].

Although many factors could activate innate immunity in SLE, immune complexes containing nucleic acids play a major role. Thus, SLE sera contain a factor that can induce IFN production by plasmacytoid dendritic cells (PDC). The factor is comprised of complexes of antibodies to DNA in association with bound DNA and can be mimicked by incubating lupus sera with media from apoptotic cells. Since apoptotic cells release DNA as they die, such media provides an abundant source of nucleic acids to form complexes. Subsequent studies have shown that antibodies to RNA-binding proteins can show the same activity [23–25].

Studies in human SLE and murine models have indicated that activation by immune complexes depends on both antibody and antigen and may involve both TLR9 as well as non-TLR9 receptors. In addition, other receptors may play a role, including the Fc receptor and RAGE (receptor for advanced glycation end-products) [26,27]. In the stimulation of RAGE by complexes, HMGB1 may contribute to the response. HMGB1 binds chromatin and can be released from apoptotic as well as necrotic cells. Since clearance of apoptotic material may be disturbed in SLE, cell death may represent a setting in which the extracellular content of DAMPs rises and pathogenic complexes form.

According to this model, the stimulation of innate immunity in SLE involves distinct receptor systems (i.e., TLR9, Fc receptor, RAGE as well as non-TLR internal nucleic acid receptor) that interact with one or more components of the complex. Inhibiting this activation may utilize agents that block any of these ligand receptor interactions. Among these, ODN that inhibit TLR9 signaling can block the progression of SLE in animal models [28,29]. Inhibiting HMGB1 interactions by either anti-HMGB1 or anti-RAGE also a promising therapeutic approach.

5. Induction of immune response to DNA in SLE

The stimulation of IFN production by immune complexes containing nucleic acids represents a novel mechanism by which the activation of the innate immune system can promote autoreactivity. Its role in initiating SLE is less clear since this pathway requires the presence of antibodies to DNA (or RNA or RNA-binding proteins). Without these antibodies, the circuitry fails since free DNA by itself is unable to stimulate response.

The divergent role of DNA on innate and adaptive immunity can be reconciled in a model in which initiation of autoreactivity depends on foreign nucleic acid while its maintenance depends on self nucleic acid. Since foreign DNA has adjuvant properties, it can promote response to its own structural determinants. In normal individuals, these responses would be directed to non-conserved determinants (sequences). In contrast, in patients with SLE, disturbances in the composition of the immune repertoire likely cause a skewing to autoreactive precursors, including antibodies to the DNA backbone [30].

When confronted with foreign DNA, patients with SLE may produce antibodies that bind conserved determinants which are present on both foreign and self DNA [31]. Once produced, anti-DNA autoantibodies could then bind endogenous DNA, form immune complexes and stimulate IFN. IFN in turn could affect the signaling threshold of B cells and allow the response to intensify.

6. Summary

The prototypic autoimmune disease SLE may represent a unique convergence of the innate and adaptive immune systems in which DNA promotes responses to itself by virtue of its intrinsic immunostimulatory activity.

Take-home messages

- DNA can stimulate the innate immune system via TLR and non-TLR mechanisms
- Immune stimulation by DNA depends on structure and context
- Normal individuals have antibodies specific for bacterial DNA
- Patients with lupus have antibodies broadly reactive to DNA
- Immune complexes containing DNA can stimulate interferon production

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Assessment of Pulmonary Arterial Hypertension in Patients with Systemic Sclerosis: comparison of Noninvasive Tests with Results of Right heart Catheterization.

Lung disease is the leading cause of morbidity and morality in patients with systemic sclerosis (SSc). Pulmonary hypertension (PH), which affects 15-20% of SSc patients and interstitial lung disease are the main causes of lung disease in those patients. Early identification of pulmonary involvement in SSc is of great importance, but subtle symptoms may be missed and diagnosis is sometimes made only at a very late stage of disease. Right heart catheterization (RHC) is the definitive method for diagnosis of PH, but it is impractical as a screening test. Therefore, Hsu VM *et al* **(The Journal of Rheumatology 2008; 35: 458-465)** aim was to assess the reliability of 3 noninvasive tests, namely Doppler echocardiography (echo), cardiac-MRI and pulmonary function tests (PFT) in the diagnosis of PH. 49 patients with SSc were evaluated for PH based on clinical findings, dyspnea and PFT. All patients underwent RHC followed by echo and cardiac-MRI preformed within 4 hours of RHC. PH was defined as mean PA pressure ≥25 mmHg or 30 mmHg after exercise. The noninvasive cut-points were: right ventricular systolic pressure > 47mmHg by echo, main pulmonary artery diameter >28mm by MRI and FVC/DLCO >2 by PFT. 24/49 (49%) of patients were diagnosed with PH based on RHC. Relatively, echo had a sensitivity of 58% and specificity of 96%, MRI had a sensitivity of 68% and specificity of 71%, and PFT had a sensitivity of 71% and specificity of 72%. The negative predictive value of these non invasive tests was enhanced by combining their results, in other words, no patients having all normal values for echo, MRI and PFT had PH. Thus, the authors concluded that individually non invasive testing has limited value and RHC should remain the gold standard in evaluation of PH. Nevertheless, in patients with SSc echo appeared to be the most reliable noninvasive method for diagnosis of PH due to its high positive predictive value, and RHC may not be necessary when all 3 noninvasive tests are found to be below cut-points.

High levels of endoglin in systemic sclerosis

Vascular endothelia growth factor (VEGF) is a potent substance implicated in angiogenesis. Patients with systemic sclerosis hava high levels and increased expression of it in their serum and tissue. The vascular effects of VEGF are mediateb by endoglin, a coreceptor of TGF-beta expressed on endothelial cells. In this line, Wipff *et al.* **(Rheumatology 2008;47:972-5)** have studied serum levels of endoglin, by ELISA, in 187 systemic sclerosis patients and compared with 48 controls. The authors found high serum levels of endoglin and BEGF in patients in comparison to controls. Moreover, endoglin levels were associated in multivariate analysis with skin ulceration, positivity to anticentromere antibody, and abnormal relation of diffusing capacity for carbon monoxide per alveolar volume. This study showed that patients with systemic sclerosis and ischemic phenomena (pulmonary and cutaneous) have increased levels of endoglin, and this mediator may play a role in scleroderma pathogenesis.