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BRIEF COMMUNICATION

Complement receptor of the immunoglobulin superfamily reduces murine lupus nephritis and cutaneous disease☆

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Abstract Complement activation takes place in autoimmune diseases and accounts for tissue inflammation. Previously, complement inhibition has been considered for the treatment of SLE. Complement receptor of the immunoglobulin superfamily (CRIg) is a selective inhibitor of the alternative pathway of complement and a soluble form reverses established inflammation and bone destruction in experimental autoimmune arthritis. We asked whether specific inhibition of the alternative pathway could inhibit autoimmunity and/or organ damage in lupus-prone mice. Accordingly, we treated lupus-prone MRL/lpr mice with a soluble form of CRIg (CRIg-Fc) and we found that it significantly diminished skin lesions, proteinuria and pyuria, and kidney pathology. Interestingly, serum levels of anti-DNA antibodies were not affected despite the fact that serum complement 3 (C3) levels increased significantly. Immunofluorescent staining of kidney tissues revealed a reduction in staining intensity for C3, IgG, and the macrophage marker Mac-2. Thus our data show that inhibition of the alternative pathway of complement controls skin and kidney inflammation even in the absence of an effect on the production of autoantibodies. We propose that CRIg should be considered for clinical trials in patients with systemic lupus erythematosus.

1. Introduction

The complement system is composed of more than thirty serum and membrane-bound proteins and plays an important role in host defense, inflammation, and clearance of apoptotic cells [1]. While genetic deficiencies in early components of the complement pathway (C4, C1q) are associated with an increased risk for the development of systemic lupus erythematosus (SLE), excessive, uncontrolled activation of the

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complement system leads to tissue inflammation and damage [2].

Treatment of NZB/WF1 mice with an anti-C5 blocking monoclonal antibody was first reported to diminish lupus-related manifestations [3]. Administration of recombinant soluble complement receptor I-related gene/protein y (Crry-Ig) or decay accelerating factor (DAF) also have been reported to be protective against renal disease in MRL/lpr mice [4,5]. While immune complex-mediated complement activation is known to initiate the classical pathway in SLE, substantial complement activation through the alternative complement pathway (AP) plays a crucial role in SLE-related tissue injury. Later reports suggested that the AP pathway is activated in a drug (adriamycin)-induced glomerulopathy [6] and the genetic absence of the alternative pathway component complement factor B (fB) improved lupus cerebritis in MRL-lpr mice [7]. Furthermore, the targeted delivery of the alternative pathway inhibitor fH by a hybrid molecule (complement receptor (CR) 2-fH) to MRL/lpr lupus-prone mice decreased autoimmunity and kidney pathology more extensively than CR2-Crry which has a more general effect on complement activation [8]. These studies have collectively shown that the alternative pathway is involved in the expression of kidney inflammation whereas as an intact classical pathway does not seem to oppose the effect of inhibition of the alternative pathway component.

A novel transmembrane complement receptor, complement receptor of the immunoglobulin superfamily (CRIg), has been described and found to be an intrinsic inhibitor of complement activation of the alternative pathway. CRIg is involved in the internalization of circulating C3-opsonized particles and is located primarily on the surface of tissue macrophages, binds C3 cleavage components and blocks C5 convertase [9]. Recently, a soluble form, CRIg-Fc (CRIg conjugated to Fc portion of IgG), has been shown to significantly alleviate inflammation and reduce bone destruction in mice injected with collagen or mice infused with anti-collagen antibodies by limiting the presence of local joint C3a and the production of inflammatory cytokines [10]. However, these arthritis models did not allow the evaluation of a possible effect of CRIg on the production of autoantibodies.

We asked whether administration of CRIg-Fc can prevent the progression of autoimmunity and organ damage in MRL/lpr mice. We report herein that administration of CRIg-Fc to MRL/lpr mice results in decreased skin and kidney inflammation, decreased proteinuria and pyuria, as well as decreased C3 consumption in the absence of an effect on the levels of serum anti-dsDNA autoantibodies.

2. Materials and methods

2.1. In vivo drug treatment

The use of animals and experiment protocols were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committees (IACUC) at Beth Israel Deaconess Medical Center (Boston, MA) under an approved protocol. Five-week old female MRL/lpr lupus-prone mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and housed in our sterile pathogen-free animal facility. Four to ten female MRL/lpr animals were injected intraperitoneally three times per week with 100 μg/mL of the Fc-fusion protein murine CRIg (STIgMA)-mIgG1 or with the isotype control murine α-HIV gp120-IgG1 antibody for eight weeks. Fc-fusion proteins CRIg-Fc and the isotype control were kind gifts from Dr. Menno van Lookeren Campagne (Genentech, Inc., San Francisco, CA). The mice were monitored for lymphadenopathy, skin lesions, proteinuria, hematuria and pyuria during treatment.

2.2. Urinary protein

Urine samples were collected weekly beginning at 6 weeks of age using Nalgene metabolic cage systems (Braintree Scientific, Braintree, MA) for 16 h before assay. Proteinuria and pyuria were assessed semi-quantitatively by dipstick analysis on the ClinTek STATUS Bayer HealthCare instrument per manufacturer's instructions.

2.3. Serum profiling assays

Serum was collected from the mice prior to drug dosing at 8 weeks of age, again at 12 weeks of age, and at the time of sacrifice (16 weeks of age). Anti-dsDNA IgM/IgG (Alpha Diagnostic International, San Antonio, TX), total IgG (Bethyl, Montgomery, TX) and C3 (GenWay Biotech Inc., San Diego, CA) levels were measured by ELISA according to manufacturer's protocols.

2.4. Histopathology

Skin, spleen, liver, lung, lymph node and kidneys were collected from CRIg-Fc treated and control animals and were fixed with 10% formalin for 24 h at 4 °C. Paraffin sections were prepared and stained with Hematoxylin and Eosin (H&E) or Periodic Acid Schiff (PAS) for histopathological assessment and evaluated by light microscopy. Kidney pathology was closely examined and glomerular, tubular, and perivascular damage and cell accumulation was determined semi-quantitatively by scoring. The severity of glomerulonephritis was graded on a 0–3 scale (0, normal (35–40 cells/glomerulus); 1, mild (41–50 cells/glomerulus); 2, moderate (50–60 cells/glomerulus); 3, severe (>60 cells/glomerulus)) [11]. At least 20 glomeruli and 10 tubular and vessel areas in low power field were scored and calculated an average in each tissue section. To evaluate skin lesions we used the following scoring: degree of acanthosis, none (0) to marked thickened dermis (2); hyperkeratosis, none (0) to markedly increased keratin (2); inflammation, sparse (0) to heavy lymphocytic infiltrates (2); fibrosis, dermal collagen with normal (0) to markedly thickened (2); vessels, normal (0) to diffuse dilated (2); ulcer, absence (0) or presence (1) [12]. After sections were cut they were subjected to deparaffinization, oxidation blocking with hydrogen peroxide (H_2O_2), and antigen retrieval with Retrievagen A (BD Pharmingen, San Jose, CA).

To detect infiltrating macrophages, kidney sections were incubated with anti-galectin3 (Mac2, M3/38, BioLegend, San Diego, CA) antibody overnight at 4 °C, and then incubated with horseradish peroxidase (HRP)-conjugated anti-rat IgG for 60 min at room temperature. Development was performed with NovaRED (vector laboratories), counterstained with hematoxylin, and dehydrated.