T cell subsets in experimental lupus nephritis: modulation by bacterial superantigen

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Abstract Chronic graft-vs-host disease (GvH), induced by injection of DBA/2 lymphocytes into (C57BL/6 × DBA/2)F1 hybrids, is a murine model for lupus nephritis, associated with a Th2-dependent polyclonal B cell activation. The development of glomerulosclerosis in this model is preceded by a glomerular influx of LFA-1⁺ T cells. We investigated whether exposure to bacterial superantigen would modulate the course of this autoimmune syndrome. Injection of the bacterial superantigen staphylococcal enterotoxin B (SEB) in mice has been shown to induce the activation of TcRV8⁺ T cells. Within 2 weeks after GvH induction, mice were injected twice with 20 μg of SEB and the following parameters were examined: cytokine and Ig profile, proteinuria and renal pathology. The second SEB injection induced in GvH mice an increased release of both interferon-γ (IFN-γ) and interleukin-10 (IL-10) as compared with control F1 mice. No differences were observed in IL-2 production. SEB-treated GvH mice demonstrated a delayed onset of proteinuria. Histological analysis of the kidney showed that SEB-challenged GvH mice displayed significantly more interstitial inflammation and mesangial proliferation together with more IgG2a deposits in glomeruli than non-injected GvH mice. From these results, we conclude that GvH mice are more responsive to SEB in terms of cytokine production and that bacterial infection can modulate the course of this renal disease from a membranous to a more proliferative type of nephropathy.

Key words: graft-vs-host disease; lupus nephritis; staphylococcal enterotoxin B; superantigen; cytokines

Introduction

The aetiology and the mechanisms at play in autoimmune diseases are multifactorial and still largely putative. However, among other hypotheses, the role of infection in the onset and/or exacerbation of autoimmunity is generally accepted. In this study, we investigated whether exposure to superantigen could modulate the course of a systemic autoimmune disease, namely the chronic graft-vs-host (GvH) disease in mice [1], which is a murine model for lupus nephritis [2]. Superantigens are products of microorganisms which can exert both immunostimulatory and immunosuppressive effects. They are involved in a large spectrum of human diseases such as food poisoning, toxic shock syndrome and several autoimmune diseases [3–6]. Bacterial superantigens bind on the one hand to the non-polymorphic part of the major histocompatibility complex (MHC) class II molecules, without being processed, and on the other hand to the variable part of the β chain (Vβ) of the T cell receptor (TCR), regardless of antigen specificity. This leads to activation of a large fraction of the T cell repertoire [7]. Staphylococcal enterotoxin B (SEB) induces activation and proliferation of Vβ8-positive T cells in BALB/c mice, and stimulates high systemic release of interleukin-2 (IL-2), tumour necrosis factor-α (TNF-α), interferon-γ (IFN-γ) and IL-10 [8,9]. Following this activation, SEB-reactive T cells become either apoptotic or anergic as defined by their unresponsiveness to subsequent SEB exposure in terms of IL-2 production and proliferation [10–12]. This defect in IL-2 production induced by a first SEB injection is associated with an enhanced production of IFN-γ and IL-10 after a second SEB challenge [13].

Here we studied the effects of SEB exposure on the course of GvH disease induced by injecting DBA/2 lymphocytes into unirradiated (C57BL/6 × DBA/2)F1 hybrids [1]. A defect in the donor CD8⁺ T cells leads to an uncontrolled stimulation of the host B cells, resulting in polyclonal B cell activation (PBA) and in the production of autoantibodies directed against nuclear antigens, erythrocytes, renal antigens, and other self-antigens [14]. This PBA is mediated by the Th2-type cytokine IL-4 [15–17]. Mice with GvH develop a lupus-like glomerulonephritis associated with high proteinuria and complicated by the late development of glomerulosclerosis [2]. In order to study the
consequences of SEB injections on the course of the GvH reaction, we followed the systemic release of cytokines, the serum levels and in vitro production of immunoglobulin (Ig), the development of proteinuria and the renal pathology in experimental mice.

Methods and experimental design

Chronic GvH was induced as described previously [18] by injection of lymphocytes from DBA/2 mice into (C57BL/6 × DBA/2)F1 hybrids. In the first week after GvH induction, mice were injected twice with an interval of 4 days with 20 μg of SEB (Sigma). Ninety minutes after SEB injection, blood was collected to measure serum IL-2, and 4 h after SEB injection to measure peak serum IL-4, IFN-γ and IL-10 by sandwich enzyme-linked immunosorbent assay (ELISA) (Endogen). In weeks 0, 4 and 6 after GvH induction, proteinuria was determined using Albustix. Mice were sacrificed in week 7. Week 7 sera were used to determine IgG1 and IgG2a levels by ELISA (Serotec). One kidney was snap-frozen for Ig, IgG1, IgG2a, and C3 detection by immunofluorescence and the other was fixed in formalin for histological analysis. In vitro production of immunoglobulins was determined after short-term culture of spleen cells as described by Florquin et al. [19].

Results and discussion

Firstly, we analysed the profile of cytokine secretion by GvH mice after SEB injections, since chronic GvH reaction is considered to be IL-4 mediated [15–17]. As shown in Table 1, and as expected from the literature, serum IL-2 was reduced to background values after the second injection of SEB in both groups of mice as a consequence of anergy [10,20]. Unexpectedly, GvH mice were able to produce more IL-10 and IFN-γ after the second injection of SEB than control F1 mice. Serum IL-4 was below the detection level in all mice in this study. These results show that the T cell response of mice with GvH is not completely irreversibly committed to a Th2-type response. The in vitro secretion of cytokines by spleen cells isolated 7 weeks after GvH induction was comparable in both groups, demonstrating that the observed priming for IL-10 and IFN-γ induced by SEB was transient.

Secondly, we compared the immunoglobulin profile (namely IgG1 as an IL-4-dependent isotype and IgG2a as an IFN-γ-dependent isotype) in GvH mice either with or without injection of SEB. According to the observed cytokine profile, GvH mice injected with SEB displayed higher serum IgG2a and lower serum IgG1 than un.injected GvH mice. Spleen cells of GvH mice injected with SEB produced in vitro significantly more IgG1 and IgG2a than spleen cells of control GvH mice. It is likely that the transient high serum IL-10 encountered after SEB injections contributed to this observation since IL-10 is a well-known B cell activation and differentiation factor [21].

Thirdly, we investigated whether the SEB-induced modification of serum Ig might correlate with the Ig deposits observed in the glomeruli of those mice. As expected from the Ig serum profile, we found a significant increase of IgG2a in immune complex deposits along the capillary walls of the glomeruli of SEB-injected GvH mice. No clear differences could be detected in IgG, IgG1, and C3 glomerular deposits.

Fourthly, SEB-challenged GvH mice displayed significantly more interstitial inflammation and mesangial proliferation as well as hypertrophy of the glomeruli as compared with control GvH mice. The instrumental role of IFN-γ in the pathogenesis of lupus nephritis in mice has been widely documented [22–25]. In these models, the development of a severe proliferative glomerulonephritis associated with high glomerular deposits of IgG2a, IgG3 and C3 was due to IFN-γ. Here, we hypothesized that the same mechanism might be at play in GvH mice injected with SEB.

Finally, the injection of SEB delayed the onset of proteinuria for 2 weeks in GvH mice but the development of proteinuria was not prevented. The involvement of IL-4 in the development of proteinuria is largely unexpected, although recently this cytokine has been detected in glomeruli from patients with proliferative glomerulopathies [26]. The results presented herein provide evidence that bacterial infection can modulate the course of an ongoing autoimmune disease.

References

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