IMMUNOLOGY AND MICROBIOLOGY

Contribution of Interleukin-1 to Th1- and Th2-Dependent Variants of Chronic Graft-Versus-Host Reaction

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Semiallogenic transfer of lymphoid cells from parental DBA/2 strain to (C57Bl/6×DBA/2)F_1 hybrids induces two variants of chronic graft-versus-host reaction with predominant activation of donor Th1 or Th2 cells [2] in genetically homologous recipients: first, severe inhibition of the humoral immune response, or second, autoimmune disease (lupus nephritis) against the background of suppressed cell and humoral immunity. These variants of chronic graft-versus-host reaction are characterized by different changes in interleukin-1 level.

Key Words: *chronic graft-versus-host reaction; interleukin-1; glomerulonephritis*

Induction of chronic graft-versus-host reaction (GVHR) in (C57Bl/6×DBA/2)F_1 mice by transfer of lymphoid cell from DBA/2 mice led to the development of autoimmune lupus nephritis against the background of suppressed cell and humoral immunity in some animals, while in others pronounced suppression of humoral immune response was not paralleled by suppression of cell response and the formation of autoimmune disease [2]. In order to elucidate the mechanisms leading to the development of different immunopathological states we studied production of interleukin-1 (IL-1) playing an important regulatory role in GVHR [5].

MATERIALS AND METHODS

Female DBA/2 and (C57Bl/6×DBA/2)F_1 mice aged 2 months (Stolbovaya Breeding Center, Moscow) were used. Chronic GVHR was induced by transfer of lymphoid cells from DBA/2 mice (5×10^6 lymph node cells, 15×10^6 thymocytes, and 30×10^6 splenocytes) to (C57Bl/6×DBA/2)F_1 mice. The cells were injected intravenously twice with a 5-day interval [7]. The development of glomerulonephritis was monitored by urinary protein excretion starting from 6 weeks after GVHR induction. The animals were divided into 2 groups: with stable proteinuria and without proteinuria (urinary protein level as in the control group). Glomerulonephritis was diagnosed by the presence of at least 3 mg/ml protein in the urine (protein was measured colorimetrically on a Titertec Multiscan at 570 nm using Coumassi blue stain; BSA calibration curve 100-1000 μg/ml), which correlated with morphological verification of the disease [3]. Proliferative response of splenocytes to T- and B-cell mitogens concanavalin A (ConA), pokeweed mitogen (PWM), lipopolysaccharide (LPS), and alloantigens was evaluated by ^{3}H-thymidin incorporation into cultured cells *in vitro* [4]. Splenocyte stimulation index (SI) was estimated as the ratio of mitogen-induced to spontaneous proliferation (cpm).

Production of IL-1 by macrophagal cells was evaluated by the capacity of this cytokine to stimulate thymocyte proliferation in response to suboptimal mitogen dose [10]. Thymocyte SI was estimated as the ratio of proliferative responses in the presence or absence of macrophages supernatant. Control group comprised sex- and age-matched animals of the same genotype as the recipients. The results were processed by the methods of nonparametric statistics [1].

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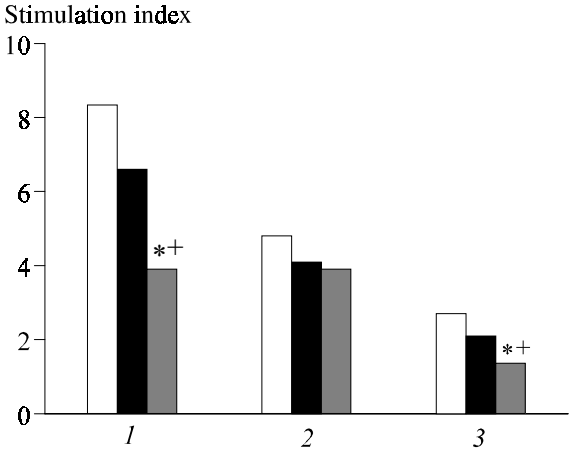

Fig. 1. Mitogen-induced proliferation of splenocytes of (C57Bl/6× DBA/2)F_1 mice after induction of chronic graft-versus-host reaction. *1*) control (*n*=17); *2*) mice without proteinuria (*n*=19); *3*) mice with stable proteinuria (*n*=11). Light bars: concanavalin A (2 μg/ml); dark bars: lipopolysaccharide (15 μg/ml); shaded bars: pokeweed mitogen mitogen (1 μg/ml). Here and in Fig. 3 $p<0.05$: *compared to the control, +compared to mice without proteinuria.

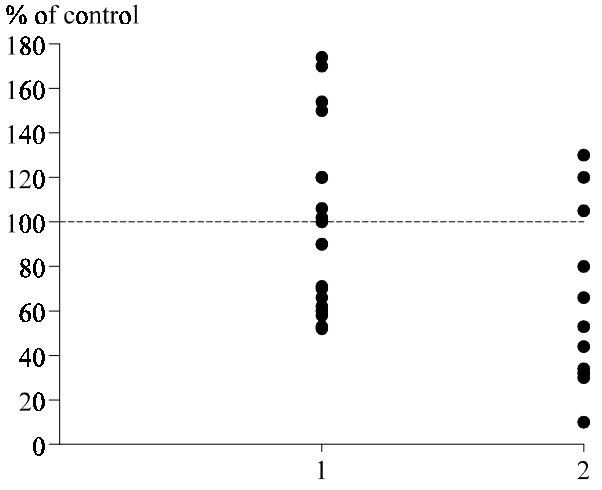

Fig. 2. Distribution of individual parameters of proliferative response to allogenic stimulation of splenocytes from (C57Bl/6×DBA/2)F_1 mice after induction of chronic graft-versus-host reaction. *1*) mice without proteinuria (*n*=19); *2*) mice with stable proteinuria (*n*=11).

RESULTS

We previously showed that animals differing by clinical manifestations of GVHR demonstrated different immune responses to T-dependent antigen: primary humoral immune response (number of IgM- and IgG-producing cells in the spleen) was suppressed in all animals, but this suppression was more pronounced in animals without proteinuria; cell immunity (DTH response) was decreased only in mice with autoimmune disease [2].

Evaluation of the splenocyte proliferation in response to T- and B-cell mitogens showed a significant decrease of ConA-stimulated proliferation of splenocytes in mice with proteinuria compared to mice without proteinuria and controls (Fig. 1). T cell reactions were evaluated by splenocyte response in mixed lymphocyte culture (Fig. 2). In animals without proteinuria, splenocyte proliferative response did not differ from the control (98% of control), while in animals with proteinuria the response was significantly decreased in comparison with animals without proteinuria and controls (64%, both $p<0.05$).

Hence, the development of autoimmune lupus glomerulonephritis in mice is associated with suppression of both *in vivo* [2] and *in vitro* T cell immunity.

One-three months after induction of chronic GVHR, the spontaneous and LPS-stimulated production of IL-1 in mice without proteinuria increased significantly ($p<0.05$) comparised to intact mice, while in animals with signs of autoimmune disease these parameter did not differ from the control (Fig. 3).

Interleukin-1 is essential for proliferation and activation of Th1 [9,11,12]. Reduced content of IL-1 in

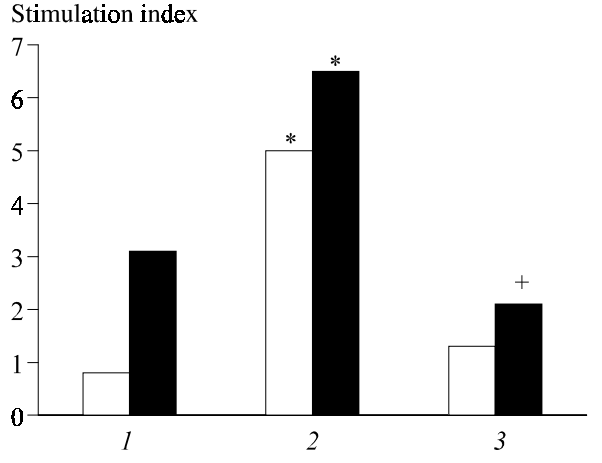

Fig. 3. Spontaneous (light bars) and lipopolysaccharide-stimulated (dark bars) production of interleukin-1 by macrophages of (C57Bl/6× DBA/2)F_1 mice after induction of chronic graft-versus-host reaction. *1*) control (*n*=8); *2*) mice without proteinuria (*n*=9); *3*) mice with stable proteinuria (*n*=5).

mice with proteinuria was paralleled by suppression of T cell immunity. Proinflammatory cytokines IL-1 and TNF-α play an important role in the development of acute Th1-dependent GVHR. IL-1 production sharply increased in the course of GVHR; injection of soluble IL-1 receptors or their antagonists reduced the severity of transplantation disease [5,6,13]. Moreover, the presence of IL-1 is essential for inhibition of Th2 mediated by interferon-γ [8]. It can be hypothesized that enhanced production of IL-1 in recipients at early stages after transplantation promotes stimulation of Th1 and suppression of Th2 cells, thus inhibiting polyclonal proliferation of B-lymphocytes and development of autoimmune disease. If IL-1 production is not increased, T-cell reactions *in vivo* [2] and *in vitro* are decreased, the

Th1/Th2 ratio is shifted towards Th2, host B-lymphocytes are activated, and lupus nephritis develops.

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