Effects of Preparations Modifying Th1/Th2 Ratio on the Incidence of Clinical Variants of Chronic Graft-versus-Host Reaction

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Induction of chronic graft-versus-host reaction in a semiallogenic DBA/2→(DBA/2×C57Bl/6) F_1 system leads to the development of Th1- or Th2-dependent immunopathologies. Modification of the Th1/Th2 ratio during induction with preparations acting on the immune system cells via different mechanisms and shifting the Th1/Th2 balance towards Th2 (bisphenol A, pentoxifylline, muramyl dipeptide) increases the incidence of Th2-dependent autoimmune lupus-like glomerulonephritis.

Key Words: *chronic graft-versus-host reaction; Th1/Th2; bisphenol A; pentoxifylline; muramyl dipeptide*

Transplantation of parental lymphoid cells to F_1 hybrid mice induces graft-versus-host reaction (GVHR). The course of GVHR depends on many factors: differences between the donor and recipient by the main histocompatibility complex, number and phenotype of transplanted cells, *etc.* Intravenous transplantation of 100×10^6 cells from the spleen and lymph nodes at 5-day interval in the DBA/2→(DBA/2×C57Bl/6)F_1 system induces the development of chronic GVHR paralleled by an autoimmune disorder (lupus-like glomerulonephritis) in females [6].

When inducing GVHR by the above scheme we found that, despite genetic homogeneity of (DBA/2×C57Bl/6)F_1 recipients, the reaction can develop by two pathways: some mice develop autoimmune glomerulonephritis (lupus$^+$), as described previously, while in others humoral response to T-dependent antigen is deeply suppressed (lupus$^-$) in the absence of renal involvement. In the former case the reaction runs mainly by the Th2 route, in the latter one by the Th1 route [2].

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We previously showed that insertion of a plasmid stimulating IL-12 synthesis in the body modifies the ratio of lupus$^+$/lupus$^-$ mice towards the latter type, thus directing the reaction by the Th1-dependent route [1]. Similar data were obtained on (C57Bl/10×DBA/2) F_1 mice [11].

Here we studied the possibility of modifying the course of reaction towards activation of Th2-dependent processes. Preparations with different mechanism of action shifting the Th1/Th2 ratio towards Th2 were used as modifiers.

MATERIALS AND METHODS

The study was carried out on 2-4-month-old female (C57Bl/6×DBA/2)F_1 and DBA/2 mice from Rassvet Breeding Center (Tomsk) and Experimental Biological Clinic of Laboratory Animals, Siberian Division of Russian Academy of Medical Sciences (Novosibirsk). The mice were kept in accordance with the regulations adopted by the European Convention for Protection of Animals Used for Experimental and Other Research Purposes (Strasbourg, 1986). Chronic GVHR was modeled by transplanting lymphoid cells of parental DBA/2 strain to B6D2F1 mice. Lymph node and sple-

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nic cells isolated *ex temporo* in sterile RPMI-1640 (Vektor Firm) were transplanted. Each recipient mouse received two intravenous injections of 65×10^6 cells in 0.5 ml medium at 5-day interval [6].

The development of glomerulonephritis was seen from stable proteinuria (>3 mg/ml at least 3 times running at everyday testing), which correlated with morphological verification of disease [3]. We tried to change the incidence of autoimmune glomerulonephritis development during induction of chronic GVHR in a semiallogenic DBA/2→(DBA/2×C57Bl/6)F_1 model by modulating the Th1/Th2 ratio during induction. Compounds with different mechanisms of action shifting the Th1/Th2 ratio towards Th2 cells were used as immunomodulators: muramyl dipeptide (MDP), bisphenol A, and pentoxifylline. Bisphenol A (ICN) was given orally in a dose of 2.5 μg/kg daily for 2 weeks. MDP (ICN) was injected intraperitoneally in doses of 0.5 or 1.0 mg/kg (twice at 10-day interval); pentoxifylline (Aventis Pharma Ltd.) was given orally in a daily dose of 1 mg for 1 week. All drugs were used only during the reaction induction (weeks 1 and 2 after cell transplantation). The results were evaluated by changes in the ratio of lupus$^+$/lupus$^-$ mice at the end of the experiment 3 months after semiallogenic cell transfer.

The results were statistically processed by nonparametric statistical methods.

RESULTS

Th2 cells play a central role in the pathogenesis of autoimmune glomerulonephritis induced by chronic GVHR [4,9]. Th1 and Th2 are characterized by different sensitivity to many regulatory factors (corticosteroids, norepinephrine, prostaglandins, *etc.*) [8,5]. Different levels of these bioactive substances in recipients leads to predominant stimulation of T-helpers of different classes and hence, to different ratio of cytokines in the body, which can result in different course of the immune process.

MDP, a bacterial cell wall derivative characterized by adjuvant effect, causes polyclonal activation of B-cells, and potentiates the stimulatory effect of IL-4 on activated B-lymphocytes [12]. MDP injected in a dose of 0.5 mg/kg increased the incidence of glomerulonephritis development in recipient mice to 75% *vs.* 18% in the control group ($p<0.05$). MDP in a dose of 1.0 mg/kg produced a more pronounced effect: the incidence of glomerulonephritis increased to 83.3% ($p<0.05$). The time course of proteinuria development in B6D2F1 mice after induction of chronic GVHR during treatment with MDP in different doses was different: during treatment with MDP in a dose of 0.5 mg/kg the development of glomerulonephritis was delayed, but then reached the values observed after treatment with a dose of 1.0 mg/kg (Fig. 1).

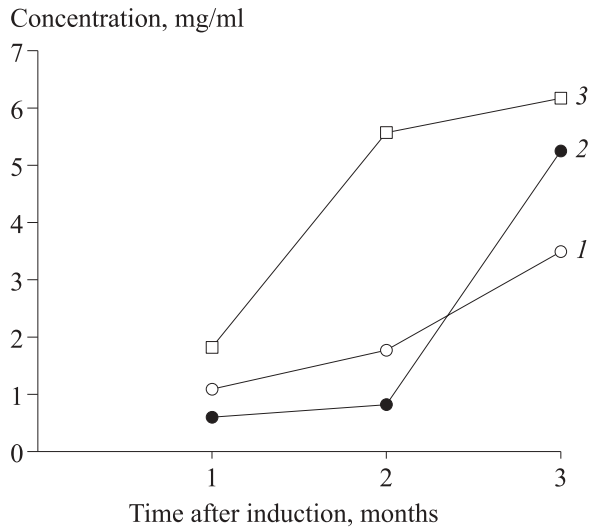

Fig. 1. Effect of treatment with muramyl dipeptide (MDP) on protein concentration in the urine of B6D2F1 mice after induction of chronic graft-versus-host reaction (GVHR). *1*) chronic GVHR (n=7); *2*) chronic GVHR+MDP (0.5 mg/kg; n=6); *3*) chronic GVHR+MDP (1.0 mg/kg; n=6).

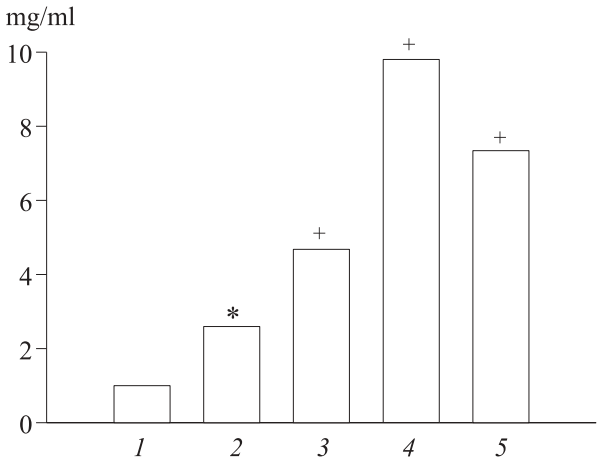

Fig. 2. Proteinuria in B6D2F1 mice treated with different drugs 3 months after induction of chronic GVHR. Ordinate: protein concentration in the urine compared to intact control. *1*) intact mice (n=8); *2*) chronic GVHR (n=21); *3*) chronic GVHR+bisphenol A (n=11); *4*) chronic GVHR+MDP (1.0 mg/kg; n=6); *5*) chronic GVHR+pentoxifylline (n=4). $p<0.05$ compared to: *intact control; $^+$chronic GVHR.

Bisphenol A widely used in the industrial manufacture of plastics binds to estrogen receptors despite structural differences from the hormone. This is paralleled by decreased production of IFN-γ and IgG2a [10]. In our experiments oral treatment of the recipient mice with bisphenol A in low doses (2.5 μg/kg) was associated with higher incidence of glomerulonephritis (63.6% *vs.* 33.3% in the control group; $p<0.05$).

Pentoxifylline (drug improving microcirculation and blood rheology) is characterized by immunomo-

dulating effects: inhibits production of proinflammatory cytokines (including IFN-γ) and suppresses the development of Th1-dependent experimental allergic encephalomyelitis in mice [7]. Treatment with pentoxifylline increased the incidence of glomerulonephritis in recipient mice to 75% *vs.* 25% in the control group.

Protein concentration in the urine increased under the effects of the above drugs (Fig. 2), which attests to the development of nephritis [3].

Hence, the results confirm our hypothesis that the direction of the development of immunopathological process is determined by the Th1/Th2 cells ratio at the earliest stages of chronic GVHR induction, when the direction of the process can be still modified by exogenous factors.

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