Processes of Homeostatic Proliferation in the Pathogenesis of Autoimmune Glomerulonephritis Induced by Chronic Graft-Versus-Host Reaction

E. V. Goiman, O. T. Kudaeva, N. A. Ilyina, V. I. Borisov, V. S. Kozhevnikov, O. P. Kolesnikova, and V. A. Kozlov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 149, No. 1, pp. 60-63, January, 2010
Original article submitted May 7, 2009

Lymphopenia developing at the early stage of chronic graft-versus-host reaction is associated with increased content of IL-7 in the peripheral blood and leads to an increase of the $CD4^+$ and $CD8^+$ cell subpopulations in the spleen of the recipient. After 3 months, some animals develop autoimmune glomerulonephritis (*lupus* recipients). High levels of IL-7 and T-cells with the memory cell phenotype ($CD4^+CD45RB^{low}$ and $CD8^+CD45RB^{low}$) persist in these animals, in contrast to *nonlupus* recipients without signs of autoimmune disease. This can attest to the involvement of homeostatic proliferation processes in the formation of autoimmune disease in this model.

Key Words: *homeostatic proliferation; IL-7; $CD4^+$ and $CD8^+$ subpopulations; chronic graft-versus-host reaction*

Homeostatic proliferation (HP), compensatory repair of quantitative deficit of lymphocytes by triggering their proliferation at the periphery, is now regarded as a possible mechanisms of the development of autoimmune disease [9]. HP decreases the variety of antigen recognizing receptors and leads to the appearance of an appreciable amount of autoreactive effector cells [1,5,6,11]. Induction of chronic graft-versus-host reaction (GVHR) in the semiallogenic DBA/2→(C57Bl/6×DBA/2)F_1 system causes the formation of immunocomplex glomerulonephritis of autoimmune nature in some recipients, similar by some signs to nephritis in human autoimmune disease (systemic lupus erythematosus) [2,10,14]. Since the early stages of chronic GVHR development are associated with pronounced lymphopenia [4], it is essential to study the involvement of HP processes in the development of autoimmune disease in chronic GVHR induced in the DBA/2→(C57Bl/6×DBA/2)F_1 model.

Institute of Clinical Immunology, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk, Russia. ***Address for correspondence:*** L.Goiman@mail.ru. E. V. Goiman

MATERIALS AND METHODS

The study was carried out on 2-month-old female DBA/2 mice and (C57Bl/6×DBA/2)F_1 (B6D2F1) hybrids from experimental biological laboratory animal clinic of Siberian Division of the Russian Academy of Medical Sciences. The animals were kept in accordance with the regulations adopted by European Convention for Protection of Animals Used for Experimental and Other Research Purposes (Strasbourg, 1986).

Chronic GVHR was induced by transplantation of parental DBA/2 lymphoid cells to B6D2F1 hybrids. The lymph node and splenic cells were injected to recipients intravenously in a dose of 60-70×10^6 cells, 2 injections at 5-day interval [10]. Intact sex- and age-matched animals of the same genotype served as the control.

The counts of $CD4^+$, $CD8^+$, and $CD45RB^{low/high}$ lymphocyte subpopulations were evaluated by FACS-Calibur flow cytometer (Becton Dickinson) using the CellQuest software (Becton Dickinson).

0007-4888/10/1491-0054

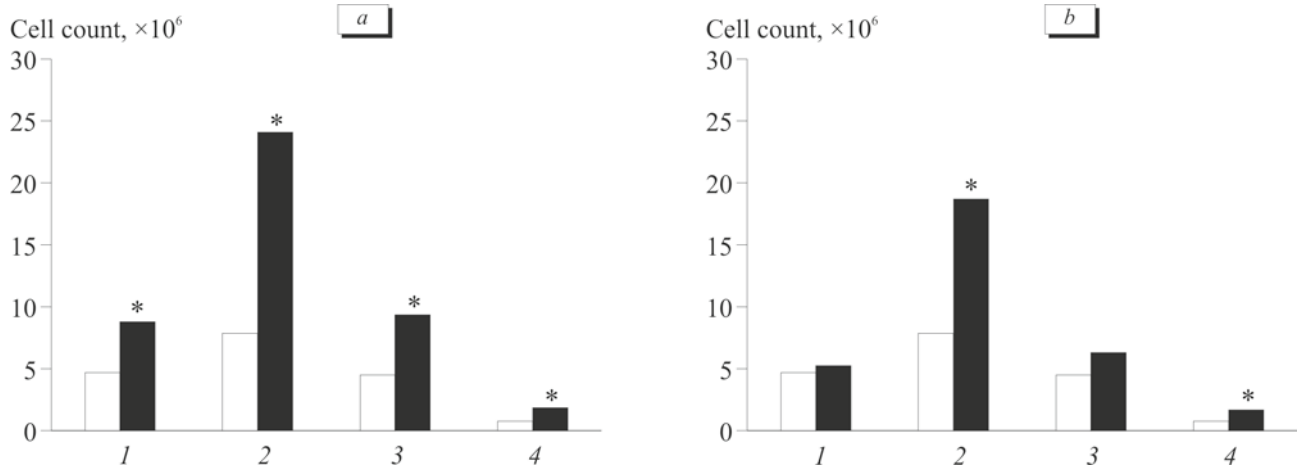

Fig. 1. Subpopulations of splenic cells at the early stages of chronic GVHR development. *a*) 1 week after GVHR induction; *b*) 1 month after GVHR induction. *1*) $CD4^+CD45RB^{high}$; *2*) $CD4^+CD45RB^{low}$; *3*) $CD8^+CD45RB^{high}$; *4*) $CD8^+CD45RB^{low}$. Light bars: control group (n=16); dark bars: experimental group (n=16). *$p<0.05$ compared to the control.

The concentration of IL-7 in the peripheral blood was measured by ELISA (R&D Systems).

Urinary protein was measured by colorimetry with Kumsai Brilliant Blue stain (Loba Feinchemie) at λ=570 nm [7]. The calibration curve was plotted by BSA (100-1000 μg/ml), the results were expressed in mg/ml.

The results were statistically processed by nonparametric methods. The differences were considered significant at $p<0.05$.

RESULTS

Acute GVHR is associated with a drop of lymphocyte count. Chronic GVHR is called immunostimulatory, because of lymphoproliferative reaction characteristic of it [14]. However, measurements of lymphocyte counts in the recipient peripheral blood over the course of experiment revealed its drastic decrease at the early stage of chronic GVHR development. This decrease persists throughout the first two weeks and then is replaced by lymphocytosis followed by normalization of lymphocyte count in the peripheral blood against the background developing splenomegalia [4]. Since HP is associated with changes in the proportion of lymphocyte subpopulations, primarily disproportional increase in memory cell count, we evaluated the counts of naive cells and memory cells among splenic $CD4^+$ and $CD8^+$ lymphocytes during different periods of chronic GVHR.

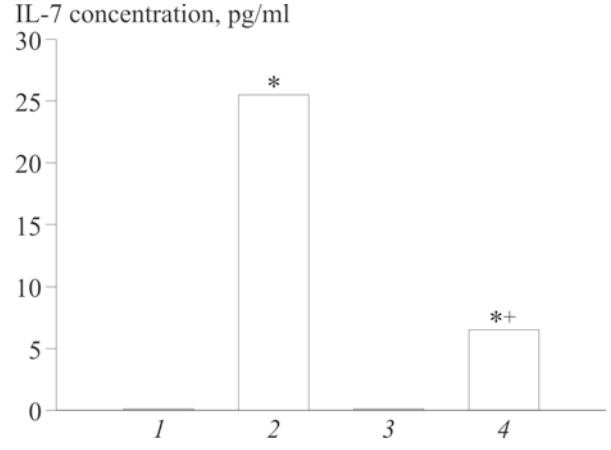

Fig. 2. Concentration of IL-7 in the peripheral blood of experimental animals after induction of chronic GVHR. *1*) control (n=16); *2*) lymphopenia (n=16); *3*) *nonlupus* recipients (n=8); *4*) *lupus* recipients (n=8). $p<0.05$ compared to: *control, $^+$*nonlupus* recipients.

An increase in the absolute counts of $CD4^+$ and $CD8^+$subpopulations in the spleen was detected at the initial stages of GVHR. This increase involved $CD4^+CD45RB^{high}$ and $CD8^+CD45RB^{high}$ naive cells and $CD4^+CD45RB^{low}$ and $CD8^+CD45RB^{low}$ memory cells (Fig. 1, *a*). Then, the counts of $CD4^+CD45RB^{low}$ and $CD8^+CD45RB^{low}$ memory cells remained high, while the counts of $CD4^+CD45RB^{high}$ and $CD8^+CD45RB^{high}$ naive cells started to decrease after their elevation (Fig. 1, *b*). The increase in the subpopulation counts during this period can be caused by transplantation of many cells (GVHR induction) and by proliferation of donor antigen-reactive lymphocytes and HP of recipient cells.

HP is a common property of T-, B-, and NK cells in response to reduction of their counts; HP of different lymphocyte populations requires different signals. An obligatory condition for HP of naive $CD4^+$ T-cell is high concentration of IL-7 and recognition of MNC complexes with autopeptides, the intensity of proliferation directly depends on avidity of this interaction. It is assumed that memory T-cell HP is supported by IL-7 and IL-15 and less depends on recognition of autopeptides [6,8,12,13,15].

The content of IL-7 in the peripheral blood of recipients sharply increased during the early period, paralleled by lymphopenia (Fig. 2).

The progress of chronic GVHR in this model leads to the formation (after 3 months) of autoimmune glomerulonephritis in some animals (*lupus* recipients); this was seen from the development of stable proteinuria (more than 3 mg/ml) correlating with morphologically verified pathological changes in the renal tissue [2,3]. These *lupus* recipients retain high levels of IL-7 in the peripheral blood and high counts of $CD4^+$ and $CD8^+$ subpopulations in the spleen in comparison with control animals and *nonlupus* recipients without signs of autoimmune disease (Figs. 2 and 3). In addition, the counts of naive $CD4^+$ and $CD8^+$ cells in the spleen are elevated in *lupus* recipients, presumably due to stimulation of migration from the thymus, which remains intact in chronic GVHR [1]. Despite the increase in both $CD4^+$ subpopulations, the proportion is shifted towards memory cells ($CD4^+CD45RB^{low}$ in *lupus* recipients is 75.8% constitute 62.1% in the control, $p<0.001$). The T-cell chimerism at this stage of chronic GVHR in recipients in the studied model is just 2% and is presented almost exclusively by $CD4^+$ cells [14], and hence, the $CD4^+CD45RB^{low}$ and $CD8^+CD45RB^{low}$ cells in the spleens of *lupus* mice are mainly recipient cells. It seems that lymphopenia characteristic of the early stages of chronic GVHR causes lymphocyte HP in some recipients, which, in turn, disturbs the maintenance of immune cell tolerance and stimulates their autoaggression, thus leading to the development of autoimmune disease in this model.

Hence, HP processes are involved in the formation of autoimmune disease induced by chronic GVHR in the DBA/2→(C57Bl/6×DBA/2)F_1 system, which can serve as an experimental model for studies of the regularities of HP development and possibility of its regulation.

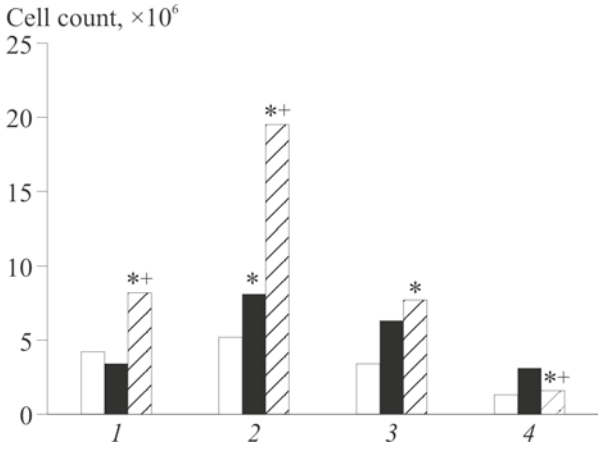

Fig. 3. Splenic cell subpopulation counts in chronic GVHR. *1*) $CD4^+CD45RB^{high}$; *2*) $CD4^+CD45RB^{low}$; *3*) $CD8^+CD45RB^{high}$; *4*) $CD8^+CD45RB^{low}$. Light bars: control group (n=8); dark bars: *nonlupus* recipients (n=8); cross-hatched bars: *lupus* recipients (n=8). $p<0.05$ compared to: *control group, $^+$*nonlupus* recipients.

REFERENCES

1. V. A. Kozlov, *Immunologiya*, No. 6, 378-382 (2006).
2. V. A. Kozlov, O. T. Kudaeva, O. P. Kolesnikova, *et al.*, *Ibid.*, No. 3, 143-146 (2002).
3. O. P. Kolesnikova, O. T. Kudaeva, V. A. Loginov, *et al.*, *Vestn. Akad. Med. Nauk SSSR*, No. 12, 13-16 (1991).
4. V. O. Tkachyov, E. V. Goiman, A. P. Lykov, *et al.*, *Immunologiya*, No. 3, 168-171 (2006).
5. A. A. Yarilin, *Ibid.*, No. 5, 312-320 (2004).
6. R. Baccala and A. N. Theofilopoulos, *Trends Immunol.*, **26**, No. 1, 5-8 (2005).
7. M. M. Bradford, *Anal. Biochem.*, **72**, 248-254 (1976).
8. T. J. Fry and C. L. M'kall, *J. Immunol.*, **174**, No. 11, 6571-6576 (2005).
9. A. Khoruts and J. M. Fraser, *Immunol. Lett.*, **98**, No. 1, 23-31 (2005).
10. M. Kimura, K. Shimada, and Y. Kanai, *Clin. Exp. Immunol.*, **69**, No. 2, 385-393 (1987).
11. A. M. Marleau and N. Sarvetnick, *J. Leukoc. Biol.*, **78**, No. 3, 575-584 (2005).
12. M. V. Soares, N. J. Borthwick, M. K. Maini, *et al.*, *J. Immunol.*, **161**, No. 11, 5909-5917 (1998).
13. C. D. Surth and J. Sprent, *Immunity*, **29**, No. 6, 848-862 (2008).
14. C. S. Via and G. M. Shearer, *Immunol. Today*, **9**, Nos. 7-8, 207-213 (1988).
15. R. T. Woodland and M. R. Schmidt, *Semin. Immunol.*, **17**, No. 3, 209-217 (2005).