CONTROLLING INFLUENCE OF SUPPRESSOR T CELLS ON CELL PROLIFERATION IN VARIOUS TISSUES

N. A. Kraskina, E. M. Veger,
N. M. Gutorova, M. S. Blyakher,
A. G. Babaeva, G. V. Kharlova,
I. V. Markelova, and N. V. Yudina

UDC 612.6.014.2-06:612.112.94.017.1

KEY WORDS: suppressor T cells; regeneration; proliferation.

The ability of the immune system to undergo autoregulation has attracted the special attention of investigators. An important role in regulation of immunoreactivity is played by suppressor T cells, which control and limit antibody synthesis, hypersensitivity reactions, the response to transplanation antigens, and so on.

The results of previous investigations aimed at a combined study of changes in the immune system induced by injection of bacterial antigens [4] have led to the suggestion that the biological functions of suppressor T cells are much wider than this, and that not only immunocompetent cells are under their "surveillance." It was shown previously that high mitotic activity of regenerating tissues can be achieved in intact recipient animals by transfer of lymphocytes from donors undergoing operations to them [1, 2].

The aim of this investigation was to test experimentally the hypothesis that suppressor T cells control cell proliferation in different meristems.

EXPERIMENTAL METHODS

Experiments were carried out on (CBA × C57Bl/6)F_1 mice weighing 18-20 g.

Accumulation of suppressor T cells in the spleen of the mice was induced by intravenous injection of 25 μg of the surface polysaccharide of meningococci belonging to the A serogroup [5]. As a result, immunologic tolerance was created in the mice, due to suppressor T cell activity [6]. The source of the suppressor T cells in this investigation was the spleens of mice 15-20 days after injection of the polysaccharide. To eliminate suppressor T cells, specific serum against mouse suppressor T cells (ASS), the method of obtaining and the prop-

TABLE 1. Effect of Suppressor T Cells on Proliferation

Substance injected	No. of mice	Number of mitoses, ‰					
		bone marrow	spleen	reticulo-endothelial sys. of liver	renal epithelium	intestinal epithelium	cornea
Control group	30	10,47 (9,77±11,17)	9,57 (7,35±11,77)	0,81 (0,21±1,41)	0,05 (0±0,1)	53,9 (47,2±60,6)	6,4 (5,5±7,3)
ASG	20	19,58 (16,34±22,82)	15,62 (11,9±19,3)	3,96 (3,47±4,45)	0,29 (0,1±0,48)	64,4 (61,6±77,2)	7,8 (6,63±8,97)
NRG	20	9,3 (7,74±10,86)	6,35 (4,28±8,42)	1,0 (0,15±1,85)	0,09 (0,04±0,14)	52,8 (45,5±60,1)	5,0 (3,94±6,06)
Cells of tolerant spleen	20	3,85 (3,0±4,7)	5,68 (4,54±6,82)	0,78 (0,01±1,55)			
Cells of intact lymph node	18	9,45 (8,49±10,4)	—	1,41 (0,64±2,18)			

Legend. Limits of variations shown in parentheses.

Laboratory of Immunology, G. N. Gabrichevskii Moscow Research Institute of Epidemiology and Microbiology. Laboratory of Growth and Development, Research Institute of Human Morphology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 105, No. 4, pp. 464-466, April, 1988. Original article submitted April 20, 1987.

 0007-4888/88/0004- 0556$12.50

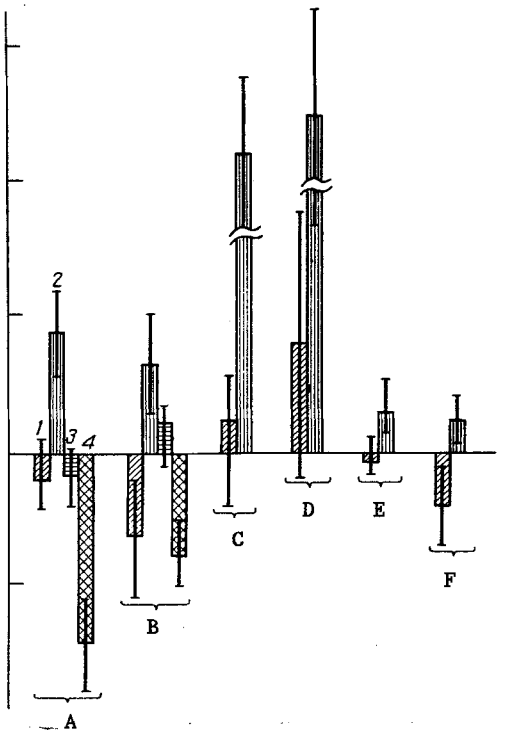

Fig. 1. Changes in proliferation in various tissues in response to a change in number of suppressor T cells. A) Bone marrow; B) spleen; C) reticuloendothelial system of liver; D) renal epithelium; E) intestinal epithelium; F) cornea. 1) Mice receiving NRG; 2) mice receiving ASG; 3) lymph node cells; 4) tolerant spleen cells.

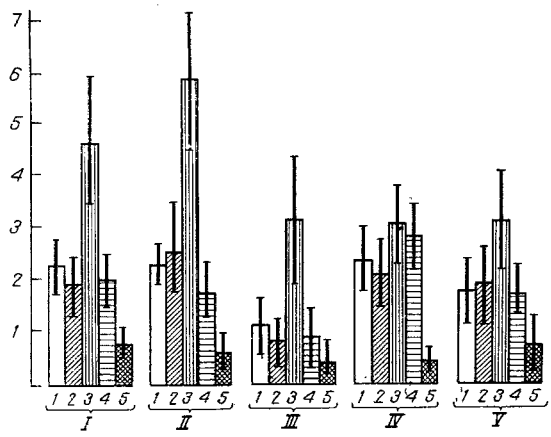

Fig. 2. Effect of suppressor T cells on proliferation of various cells in mouse bone marrow. 1) Intact mice; 2) recipients of NRG; 3) recipients of NSG; 4) recipients of lymph node cells; 5) recipients of tolerant spleen cells; I) transitional cells; II) blasts; III) lymphoid series; IV) erythroid series; V) myeloid series. Vertical axis indicates numbr of mitoses (in %).

erties of which were fully described previously [3], was used. To prepare it, rabbits were immunized with spleen cells of mice tolerant to meningococcal polysaccharide ($5 \cdot 10^8$ cells, three injections); the specificity of the serum was attained by absorption with mouse erythrocytes and lymph node cells. The serum acted selectively only on suppressor T cells and not on other lymphocyte subpopulations. The globulin fraction was isolated by salt fractionation from the ASS and from normal rabbit serum (NRS). Globulin isolated from ASS (ASG) or from NKS (NKG) was injected into mice in a single dose of 5 mg protein per animal.

In adoptive transfer experiments a cell suspension was prepared by carefully crushing the spleen or lymph nodes in a glass homogenizer, followed by washing and subsequent dilution of the suspension with medium 199. Recipient mice received $5 \cdot 10^7$ spleen cells or lymph node cells intravenously.

All the recipient mice were killed (between 10:30 a.m. and 12 noon) and were investigated 48 h after injection of serum or cells.

Films were prepared from the bone marrow and spleen of the mice, fixed with methanol, and stained with azure-eosin. The suspension of spleen cells was centrifuged beforehand, the supernatant removed, the cells resuspended in whole serum, centrifuged again, and films were prepared from the residue.

When the films were studied the number of mitoses was counted in 2000-4000 nucleated cells, and cells undergoing mitosis were differentiated. Pieces of the organs were fixed in Carnoy's fluid. Total preparations were made from the cornea. The liver, kidney, and a segment of the small intestine (6-6.5 cm from the stomach) were treated histologically in the usual way. Paraffin sections 4 μ thick were stained with hematoxylin and eosin. In each mouse 14,500 cells in the proximal portions of the urinary tubules, 2500-3000 reticuloendothelial cells in the liver, 3000 epithelial cells in the intestine (in 50-55 longitudinally divided crypts), and 10,000-20,000 corneal cells were counted. The mitotic index, the number of mitoses per 1000 cells, was calculated in each case (‰). The results were subjected to statistical analysis by Student's or Lord's tests [7].

EXPERIMENTAL RESULTS

The results of experiments in which the number of suppressor T cells in the animals was modified by special experimental procedures – reduced by injection of ASG or increased by transfer of a cell suspension enriched with suppressor T cells – are summarized in Table 1 and Fig. 1. A single injection of ASG led to a significant increase in the number of mitoses in all the tissues studied. The intensity of the effect depended on the tissue studied. In the bone marrow and spleen the number of mitoses was increased by 60-90%; in the reticuloendothelial system of the liver and in the renal epithelium, where mitotic activity normally is very low, it was increased by 390-480%. In tissues with a high level of proliferation (intestinal and corneal epithelium) the numbr of mitoses was increased by 22-29%. Injection of the control preparation (NRG) did not stimulate mitotic activity.

Transfer of a suspension of spleen cells from animals tolerant to meningococcal A polysaccharide (suspension enriched with suppressor T cells) into intact mice led to a sharp decrease in the number of mitoses in the recipients' hematopoietic tissue. For instance, the number of mitoses in the bone marrow in this case was reduced almost by two-thirds, and in the spleen by half. Transfer of cells of intact lymph nodes, among which there are hardly any suppressor T cells, did not inhibit proliferation.

The results given in Fig. 2 show the identity of the cells in the hematopoietic tissue whose proliferation is under suppressor T-cell control. It will be evident that injection of the control preparation (NRG) did not affect the level of proliferation of any of the cell series. Conversely, injection of ASG significantly increased the number of mitoses both among the least mature forms (transitional and blast cells) and among cells of the lymphoid and myeloid series. The stimulating effect on ASG on cells of the erythroid series was weaker. Transfer of tolerant spleen cells (i.e., containing a large quantity of suppressor T cells) to the recipients led to a sharp decrease (by 2.5-5 times) in the number of mitoses in all series studied. In this form of the experiment cells of the erythroid series exhibited high sensitivity to the effect of suppressor T cells.

It can be concluded from these results that suppressor T cells induced by injection of bacterial polysaccharide control cell proliferation in a wide variety of tissues. Controlled elimination of suppressor T cells leads to intensification of proliferation, whereas an in-

crease in the number of suppressor T cells in the body leads to inhibition of proliferation. A new biological function of the regulatory subpopulation of lymphocytes has been discovered: "surveillance" of cellular proliferation. The mechanism of realization of this function is not yet known, and experimental investigation is therefore necessary. It can be tentatively suggested that this function of the suppressor T cells plays an important role in the maintenance of homeostasis, and its study will open up new prospects for the correction of various pathological states.

LITERATURE CITED

1. A. G. Babaeva N. A. Kraskina, and L. D. Liozner, Tsitologiya, No. 12, 1511 (1969).
2. A. G. Babaeva, N. A. Kraskina, and N. V. Yudina, Byull. Éksp. Biol. Med., No. 1, 69 (1980).
3. N. A. Kraskina, T. K. Lopatina, and A. I. Mokrenko, Immunologiya, No. 4, 50 (1980).
4. N. A. Kraskina, M. S. Blyakher, T. K. Lopatina, et al., Cellular and Molecular Mechanisms of Anti-infectious Immunity [in Russian], Moscow (1983), pp. 27-27.
5. V. I. Kuvakina, M. A. Smirnova-Mutusheva, and N. V. Kholchev, Zh. Mikrobiol., No. 4, 63 (1974).
6. A. I. Mokrenko, Byull. Éksp. Biol. Med., No. 9, 339 (1980).
7. G. W. Snedecor, Statistical Methods in Application to Research in Agriculture and Biology, Iowa State Univ. Press, Ames, Iowa (1956).

MODEL TO LOOK FOR AFFERENT SIGNALS FROM THE IMMUNE TO THE NERVOUS SYSTEM

G. I. Chipens, E. A. Korneva,*
S. N. Sklyarova, V. M. Klimenko,
and R. É. Vegner

UDC 612.8-06:612.017.1

KEY WORDS: brain; immunity; afferent information; oligopeptides.

Numerous investigations have demonstrated close interaction of the immune and nervous systems in the response of an organism to an antigen [3, 13]. Factors acting on particular structures of the CNS have been shown to modify the microenvironment of lymphoid cells, the number of circulating antibodies, the intensity of cellular reactions of immunity, and the ratio between numbers of lymphocytes in subpopulations [4, 10]. In turn, deep brain structures react to injection of antigens by persistent and reproducible restructuring of neuronal activity, the level of excitability, and the time course of bioelectrochemical potentials [1, 2]. Methods and channels of transmission of information from the immune system (IS) to the brain constitute the least studied aspect of interaction between the nervous and immune systems.

There is evidently not only one method of transmission of information from the IS to the CNS, but the chemical nature of the carriers of the afferent signals also may be different. The greatest interest from this point of view is aroused by regulators, synthesized in the IS and CNS, such as histamine, serotonin, prostaglandins, endorphins, enkephalins, and ACTH. In the opinion of some workers the role of carrier of afferent information may be performed by interferon, which has a common amino-acid sequence with ACTH and β-endorphin [11, 12], by interleukin-1 [10], and by a myelopeptide, isolated from bone marrow, which possesses not only immunologic activity, but also various neurotropic properties [5]. A possible role of substance P and of somatostatin in this process also has been suggested [14, 15].

*Corresponding Member of the Academy of Medical Sciences of the USSR.

Department of Peptide Chemistry, Institute of Organic Synthesis, Academy of Sciences of the Latvian SSR, Riga. Department of Pathophysiology and General Pathology, Research Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad.

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 105, No. 4, pp. 466-469, April, 1988. Original article submittted July 14, 1987.

0007-4888/88/0004- 0559$12.50