Effects of Various Human Interleukin-2 Preparations and Lymphokine-Activated Killer Cells on the Production of Antibodies in Mice *In Vivo* and of Human Immunoglobulins *In Vitro*

M. P. Potapnev, T. S. Garbuzenko, A. V. Voznyuk, E. V. Ruksha, O. V. Shadrin, and S. N. Bykovskaya

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Comparison of the lymphocytic IL-2 (lIL-2, lymphokinin) and recombinant IL-2 (rIL-2) showed that lIL-2 is characterized by a more pronounced stimulating effect of low doses on antibody production in mice and on human immunoglobulin production. High doses of lIL-2, in contrast to rIL-2, do not cause hyperstimulation of antibody production or immunoglobulin production. Human lymphokine-activated killer (LAK) cells, induced by rIL-2 *in vitro* during 7 but not 3 days, enhance the production of human immunoglobulins. Monocytes activated by rIL-2 suppress the stimulating effect of LAK cells on immunoglobulin production.

Key Words: *interleukin-2; lymphokine-activated killer cells; humoral immunity*

Interleukin-2 (IL-2) is a broad-spectrum lymphokine [10]. At present IL-2 and LAK cells induced *in vitro* by IL-2 are used in immunotherapy of human oncological and infectious diseases involving severe T-cell immunodeficiency [4,7,9]. At the same time, preparations of human lIL-2 and rIL-2 are known to stimulate humoral immunity by enhancing antibody production *in vivo* and *in vitro* [8]. A few studies making use of LAK cells (a population enriched in $CD8^+CD16^+$) have revealed their suppressive effect on *in vitro* immunoglobulin (Ig) production [11].

The aim of our research was to compare the effects of lIL-2 and rIL-2 and of LAK cells on the formation of humoral immunity assessed *in vivo* and *in vitro*.

Research Institute of Hematology and Blood Transfusion, Ministry of Health of Belarus, Minsk; Oncological Research Center, Russian Academy of Medical Sciences, Moscow. (Presented by N. N. Trapeznikov, Member of the Russian Academy of Medical Sciences)

MATERIALS AND METHODS

Female CBA and BALB/c mice aged 14 to 16 weeks were used in experiments. The effects of rIL-2 (Cetus) and lIL-2 (lymphokinin manufactured at the Oncological Research Center, Russian Academy of Medical Sciences) on the formation of antibody-producing cells (APC) in mice immunized with sheep erythrocytes (SE) were studied as described previously [3]. The effects of rIL-2 and lIL-2 preparations on the formation of human cells secreting IgG were assessed in a culture of blood mononuclears of healthy donors by the reverse hemolytic plaque formation technique [1]. The levels of interleukin-6 (IL-6) and interleukin-1 (IL-1) in lymphokinin were assessed in a bioassay

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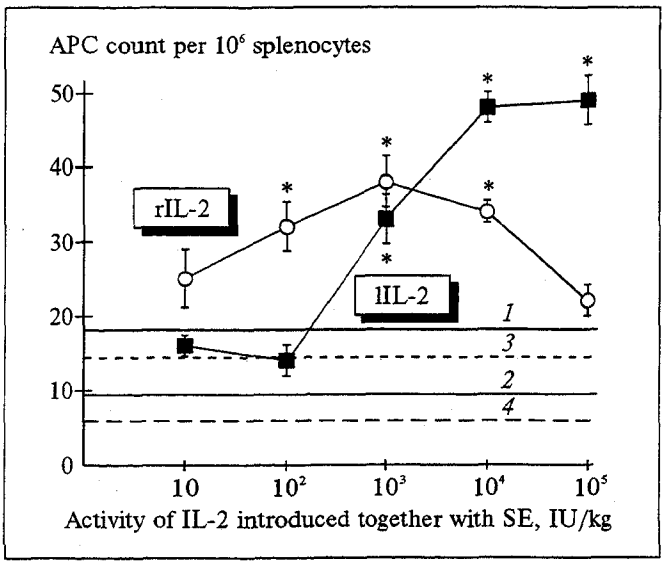

Fig. 1. Effects of various doses of lymphokinin (lIL−2) and rIL−2 on APC formation in BALB/c mice immunized with SE. Controls: response to administration of only SE (*1*), lIL−2 (*2*), rIL−2 (*3*), and normal saline (*4*). Here and in Figs. 2 and 3: an asterisk denotes $p<0.05$.

with cell lines B9.9 and D10G4.1, respectively, as described previously [5,6].

LAK cells were induced in a culture of blood mononuclears or lymphocytes from healthy donors by incubation with rIL-2 for 3 or 7 days as described previously [2]. After this the LAK cells were washed twice in RPMI-1640 medium with

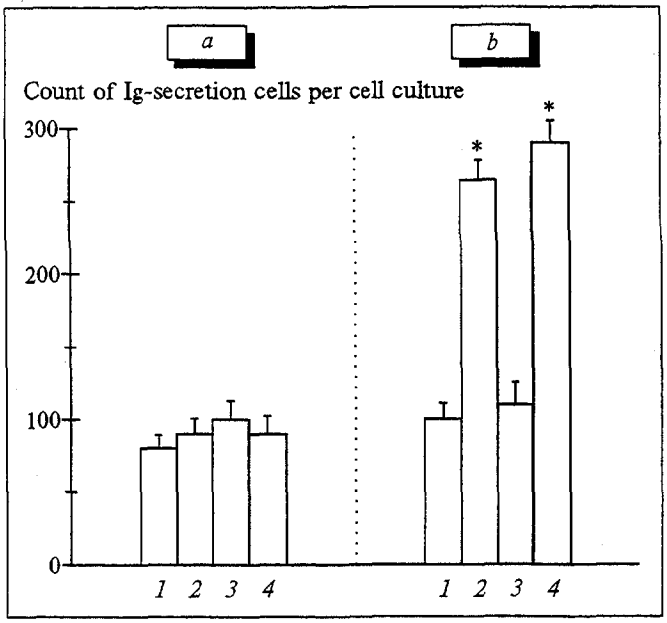

Fig. 2. Effects of 3−day (*a*) and 7−day (*b*) LAK cells on the formation of IgG−secreting cells in a culture of human autologous cells. *1*) test mononuclears+cultured intact mononuclears; *2*) test mononuclears + LAK cells induced in mononuclear culture; *3*) test mononuclears+cultured intact lymphocytes; *4*) test mononuclears+LAK cells induced in lymphocyte culture. The data of a typical experiment out of four are presented.

10% fetal calf serum and antibiotics. Some of the LAK cells were treated with 1% paraformaldehyde and washed again. The concentration of LAK cells was then brought to 2 mln/ml and mixed in a 1:1 ratio with autologous mononuclears preliminarily frozen in liquid nitrogen. *Phytolacca* mitogen (PM, Gibco) was added to some of the cultures in the suboptimal concentration 1:1000. The cells were incubated in the medium described above in 96-well microplates (Costar), 0.2 ml per well, at 37°C in an atmosphere with 7.5% CO_2. After 7 days the cells were washed and used to determine IgG-producing cells.

RESULTS

Earlier studies with rIL-2 [2] and preliminary experiments with lymphokinin revealed a stimulating effect of both IL-2 preparations in doses surpassing 10^2 IU/kg on the formation of APC in mice immunized with SE. The minimal and maximal effective doses of the preparations in these experiments manifested their immunostimulating action differently, as was demonstrated in a direct comparison of the dose-effect curves for preparations rIL-2 and lIL-2. Figure 1 shows that rIL-2 in a dose of 10^2 IU/kg fails to stimulate, and in doses of 10^3 to 10^5 IU/kg stimulates, to a progressive degree, the appearance of APC in mice immunized with SE. Lymphocytic IL-2 (lymphokinin) in a dose of 10^2 to 10^4 IU/kg raised the level of APC formation (10^3 IU/kg caused the maximal response), whereas in a dose of 10^5 IU/kg it caused no changes in the APC count after immunization with SE. When used in a dose of 10^5 IU/kg, lIL-2 did not cause ($p<0.05$) APC formation in nonimmunized mice, whereas rIL-2 did cause APC formation. In a culture of human mononuclears (Table 1) *in vitro* lIL-2 in the minimal effective concentration 10 IU/ml induced ($p<0.05$) the formation of IgG-producing cells in two out of the three experiments carried out, whereas rIL-2 had such an effect in only one experiment. Lymphocytic IL-2 in the maximally effective (nontoxic) concentration 1000 IU/ml induced in two experiments a lower level of formation of IgG-secreting cells than when used in the concentration 100 IU/ml. In the third experiment lIL-2 in concentrations of 500 and 100 IU/ml induced virtually the same level of formation of IgG-secreting cells. Recombinant IL-2 in the concentration 1000 IU/ml raised the level of formation of IgG-secreting cells in two experiments and inhibited this process (by 17%, $p<0.05$) in one experiment. We believe that the uniform differences in the effects of rIL-2 and lIL-2 observed *in vivo* and *in*

vitro are due to the presence in lIL-2 (lymphokinin) of other bioactive components which exert costimulating effects in low doses, whereas in high doses they suppress the formation of antibody (IgG)-producing cells. Additional studies revealed in lymphokinin substantial amounts of IL-1 (326-3816 IU/ml) and of IL-6 (from 2 to 55 IU/ml of the preparation). We attribute the reduction of the stimulating effect of high doses of lIL-2 to the presence of suppressor factors in the preparation.

The possibility of suppressor cell induction by IL-2 was verified *in vitro,* when we assessed the effect of LAK cells (induced by rIL-2 in a concentration of 1000 IU/ml) on the production of human immunoglobulin-secreting cells. Figure 2, *a* shows that LAK cells induced during 3 days did not by themselves cause the formation of immunoglobulin-secreting cells for cocultivation with autologous mononuclears. After a 7-day induction LAK cells caused a reliable increase in the formation of immunoglobulin-secreting cells in three out of four experiments (Fig. 2, *b*). The degree of LAK cell stimulating effect did not depend on the culture in which they were induced - whether it was a mononuclear culture or a culture of lymphocytes free of monocytes. Three-day LAK cells enhanced the formation of IgG-secreting cells in mononuclear cultures stimulated with PM, in comparison with the added intact cells (Fig. 3, *a*). LAK cells induced in a lymphocyte culture were the most active in this respect. Seven-day LAK cells induced in a lymphocyte culture similarly caused a reliable increase of the formation of IgG-secreting cells in a culture of mononuclears stimulated with PM (Fig. 3, *b*). Seven-day LAK cells induced in a mononuclear culture reduced the level of formation of IgG-secreting cells in mononuclear cultures stimulated with HPM (Fig. 3, *b*). Paraformaldehyde-treated 3- and 7-day LAK cells diminished the stimulating effect by 15-25% in comparison with the effect of intact cells, this implying the participation of both membrane-bound and secreted transmitters in the function of LAK cells.

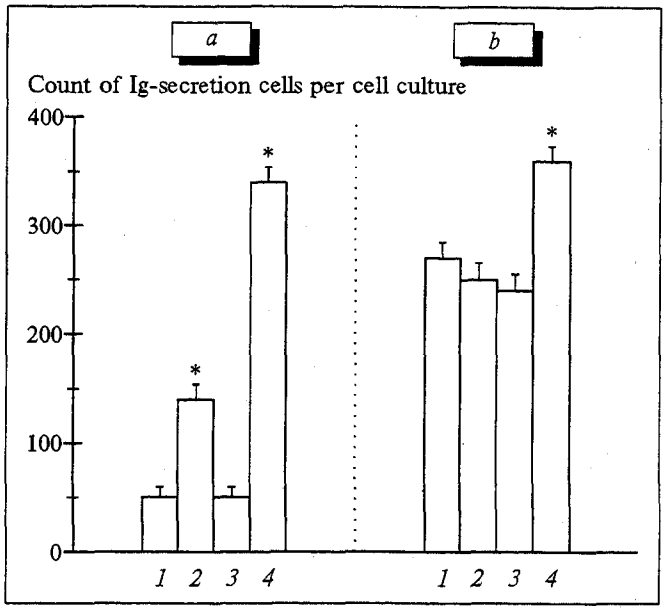

Fig. 3. Effects of 3-day (*a*) and 7-day (*b*) LAK cells on the formation of IgG-secreting cells in a culture of human autologous cells stimulated with PM. Notation as in Fig. 2.

Our data indicate a pronounced stimulating effect of IL-2 on antibody (Ig) production *in vivo* and *in vitro*. Lymphocytic IL-2 (lymphokinin) is characterized by a more pronounced effect of low doses due to its enrichment with other cytokines (IL-1 and IL-6, among others). High doses of lIL-2 do not stimulate the formation of APC and IgG-secreting cells, due to the presence of suppressor factors in the preparation. Equivalent rIL-2 doses cause a further increase of APC and IgG-secreting cell production, which may induce autoimmune reactions [2]. Human LAK cells induced by rIL-2 during 7 (but not 3) days cause the formation of IgG-secreting cells in a culture of intact autologous lymphoid cells. In a PM-stimulated culture of lymphoid cells LAK cells generated in the absence of monocytes induced the formation of IgG-secreting cells. LAK cells obtained in the presence of monocytes were capable of enhancing the formation of IgG-secreting cells only in some experiments (3-day LAK cells) or even suppressed the formation of these cells

TABLE 1. Formation of Ig-Secreting Cells in a Culture of Human Blood Mononuclears ($M \pm m$) under the Effects of lIL-2 (Lymphokinin) or rIL-2 (Cetus)

№. of experiment	Control	lIL-2, IU/ml			rIL-2, IU/ml		
		10	100	1000	10	100	1000
1	200±29	717±44*	1567±88*	1117±44*	317±73	1767±60*	1467±17*
2	367±44	467±73	2917±142*	567±73	667±60*	2417±73*	2567±44*
3	53±6.7	110±10*	183.3±6.7*	223.3±6.7**	73.3±3.3	113.3±6.6*	196.7±8.8**

Note. In experiments Nos. 1 and 2 the data are presented as the count of IgG-secreting cells per ml of cell culture; in experiment No. 3 they represent the count of IgG-secreting cells per 0.2 ml of cell culture. One asterisk: $p<0.05$; two asterisks: in experiment No. 3 the data are presented for IL-2 activity 500 IU/ml.

(7-day LAK cells). The detected suppressor effect of rIL-2 - activated macrophages - appears to play a significant role in the regulation of the biological activity of LAK cells.

REFERENCES

1. M. P. Potapnev and N. N. Voitenok, *Immunologiya,* № 6, 61-65 (1983).
2. M. P. Potapnev, V. D. Zobnin, and E. V. Ruksha. *Ibid.,* № 3, 23-26 (1991).
3. M. P. Potapnev and D. V. Pechkovskii, *Byull. Eksp. Biol. Med.,* **114**, № 12, 641-643 (1992).
4. N. N. Trapeznikov, V. V. Yavorskii, A. B. Syrkin, *et al., Vestn. Vsesoyuz. Onkol. Nauch. Tsentra,* № 1, 31-34 (1990).
5. L. A. Aarden, E. R. De Groot, O. L. Scharp, *et al., Europ. J. Immunol.,* **17**, 1411-1416 (1987).
6. S. J. Hopkins and M. Humphrey, *J. Immunol. Meth.,* **120**, № 2, 271-276 (1989).
7. G. Kaplan, Z. A. Cohn, and K. A. Smith, *Biotechn. Bioeng.,* **10**, 157-162 (1992).
8. A. O'Garra, S. Umland, T. De France, and J. Christiansen, *Immunol. Today,* **9**, № 2, 45-54 (1988).
9. R. C. Rees and R. H. Wiltrout, *Ibid.,* **11**, № 2, 36-38 (1990).
10. F. W. Ruscetti, in: *Immunophysiology. The Role of Cells and Cytokines in Immunity and Inflammation,* Eds. J. J. Oppenheim and E. M. Shevach, Oxford (1990), pp. 46-66.
11. H. Suzuki, N. Yamashita, M. Maruyama, *et al., Clin. Exp. Immunol.,* **78**, 406-411 (1989).

Rheumatoid Factors of the Blood and Cerebrospinal Fluid in Patients with a Complicated Spinal Injury

N. V. Petrova, G. S. Yumashev, V. A. Aleshkin, and A. M. Ponomareva

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The levels of rheumatoid factors (RF) of IgM-RF and IgG-RF classes were measured by enzyme immunoassay (EIA) in the sera of patients with spinal injuries. Analysis showed that 46% of patients with spinal injuries were seropositive for IgG-RF and 40% for IgM-RF; no IgG-RF was detected in the cerebrospinal fluid (CSF), whereas the IgM-RF content was 37.6 μg/ml. Results of RF measurements in the sera make it possible to qualify the systemic involvement of the body in the late period of a spinal injury as a rheumatic disease. The findings point to the presence of independent mechanisms of immune control in the CSF.

Key Words: *spinal injury; rheumatoid factors*

Neurological deficit in a spinal injury is regarded as the cause of impaired motor activity and systemic visceral involvement. Spinal injuries are associated with disorders in the hemo- and CSF dynamics. Changes in the basic parameters of the blood and immunological shifts during an injury are sufficiently well documented in the literature [1]. Traumatic disease of the spine in the long term is classified by many signs among the autoimmune diseases. The presence of chronic inflammations and disturbances in the immune system suggest that antibodies to the patient's own immunoglobulins (RF) contribute to the development of the disease.

To elucidate the role played by RF in the pathogenesis of traumatic spinal disease, we measured RF levels in the serum and CSF.

Department of Traumatology, Orthopedics, and Military Surgery, I. M. Sechenov Medical Academy, Moscow

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