NATURAL KILLER CELLS AND INTERLEUKIN-2 IN MRL/MpJ-lpr/lpr MICE

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UDC 612.112.94.017.4+612.112.94.015.2:612.6

KEY WORDS: autoimmune states; natural killer cells; interleukin-2

A new strain of inbred mice MRL/MpJ-lpr/lpr (MRL/1), which, at a certain stage of ontogeny, develops massive T-cell lymphoproliferation and generalized B-cell hyperactivity leading to hypergammaglobulinemia, the production of autoantibodies against many endogenous antigens, the formation of immune complexes, and so on, has been bred comparatively recently [4]. In other words, these animals developed an autoimmune process similar, in many of its features, with systemic lupus erythematosus in man [2, 6].

This paper gives the results of a study of natural killer cells (NKC) and interleukin-2 (IL-2) in MRL/1 mice, which constitute a unique model for the study of genetically predetermined autoimmune disorders.

EXPERIMENTAL METHOD

Mice of inbred line CBA ($H\text{-}2^k$) and MRL/1 ($H\text{-}2^k$) were used. The MRL/1 mice were obtained from Dr. E. D. Murphy (Bar Harbor, USA) and maintained in the animal house of the Institute of Immunology, Ministry of Health of the USSR. NKC activity in the spleen and lymph nodes was determined by the direct cytotoxic test with ^{51}Cr [3], using YAC-1 lymphoma cells. To obtain IL-2, mouse spleen cells ($5 \cdot 10^6$ cells in 1 ml) were incubated with 5 g/ml of concanavalin A (con A, from "Pharmacia," Sweden) in medium RPMI-1640 with all additives for 24 h in a CO_2 incubator. The supernatant of the cultures was used as the source of IL-2 after addition of 20 mg/kg of α-methyl-D-mannoside.

Activity of IL-2 was tested by the use of 96-h T-blast cells, activated with con A under the same conditions as were used to obtain the IL-2. The cells were washed, made up to the necessary concentration (10^6 cells/ml), and transferred into wells in flat-bottomed 96-well plates in a volume of 50 µl. IL-2 was added to the wells and, after incubation for 14 h, ^3H-thymidine was added in a volume of 50 µl (0.5 µCi, specific activity 26 Ci/mole). The cells were then washed, deposited on filters, and the radioactivity of the samples was measured with a β-counter ("Packard").

TABLE 1. Splenic NKC Activity in MRL/1 Mice (M ± m)

Expt. No.	Line of mice	Age of mice	Cytotoxicity, % effector–target ratio 100:1	50:1	20:1
1	MRL/l	1 months	0	0	0
	MRL/l	3 months	0	0	0
	MRL/l	5 months	0	0	0
	CBA	1 months	4,8±2,3	1,5±0,5	0
	CBA	3 months	9,4±2,0	6,7±0,8	0
2	MRL/l	7–10 days	9,1±2,0	7,6±1,1	3,9±1,0
	MRL/l	2 months	2,4±1,5	3,8±0,7	0,7±0,5
	MRL/l	6 months	3,1±0,3	5,9±0,6	2,4±0,4
	CBA	2 months	39,1±4,9	38,0±1,4	27,7±3,8

TABLE 2. Lymph Node NKC Activity of MRL/1 Mice

Line of mice	Age of mice	Cytotoxicity, % effector–target ratio 100:1	50:1	20:1
MRL/l	7–10 days	7,0	1,0	2,0
MRL/l	2 months	19,2	7,8	3,8
MRL/l	6 months	0	0	0
CBA	2 months	1,2	0,1	0

Institute of Immunology, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR, R. V. Petrov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 8, pp. 197-198, August, 1987. Original article submitted October 10, 1986.

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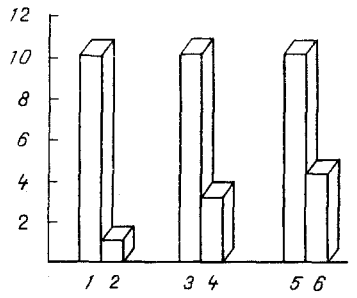

Fig. 1. IL-2 levels in MRL/1 mice at different ages. Ordinate, stimulation index (experiment/control, in relative units). 1, 3, 5) IL-2 production by spleen cells of CBA mice in 1st, 3rd, and 5th months of life respectively (control); 2, 4, 6) IL-2 production by spleen cells of MRL/1 mice at age of 1, 3, and 5 months, respectively.

EXPERIMENTAL RESULTS

Table 1 gives the results of experiments to study NKC in the spleen of MRL/1 mice at different times of life, using YAC-1 cells as target cells. At virtually all times of the investigation NKC activity was absent in mice of the autoimmune MRL/1 line (experiment 1), or was extremely weak (experiment 2). Meanwhile, splenocytes of CBA mice, under the same experimental conditions, exhibited marked natural cytotoxicity against YAC-1 lymphoma cells.

The study of NKC activity in the lymph nodes of MRL/1 mice yielded somewhat unexpected results. NKC activity in lymph nodes of nonautoimmune inbred lines of mice is known to be negligible [5]. However, lymph node cells of MRL/1 mice exhibited perceptible NKC activity as early as the 7th-10th day after birth, and peak values were reached by the 2nd month of life (Table 2). Later, however, the level of NKC activity evidently fell, and by the 6th month of life virtually none could be detected. In other words, the above data, together with results published previously [1], indicate that MRL/1 mice can be characterized as a line of mice with inborn immunodeficiency, and in particular, with deficiency of NKC activity. However, this conclusion must be qualified to some degree because high NKC activity is recorded in the lymph nodes of these mice in the early stages of postnatal development.

The level of NKC activity is known to be controlled by a family of mediator molecules, namely lymphokines and monokines. Among them the T-cell factor, or IL-2, plays the key role in maintaenance of cytotoxic lymphocyte function. The complexly structured intercellular interactions (in particular, between NKC and target cells) also are known to be realized through a lymphokine cascade, one of the nodal stages of which is IL-2 production by a class IL-2 production by MRL/1 mice as they developed autoimmune disorders. It was found that IL-2 production by spleen cells of MLR/1 mice was sharply inhibited as early as in the 1st month of life (Fig. 1). A basically similar picture was found also at the later stages of the investigation (for comparison, the time course of IL-2 production by CBA mice of the corresponding age was studied).

It can thus be concluded from the data given above that MRL/1 mice are characterized by a combination of marked disturbances of production of IL-2, the most important cytokine of the immune system, and by a consequently marked inhibition of NKC activity.

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