cells were cultured under the same conditions as the resident macrophages, radioactive Clq was recorded in the culture fluid.

Differences observed in the kinetics of accumulation of radioactive Clq in the cells and in the external medium can evidently be explained on the grounds that after Clq formation *de novo* ceased, its continued accumulation in the external medium was due to release of protein from its intracellular depots.

The data of kinetic analysis of Clq biosynthesis by peritoneal macrophages, combined with the results of biosynthesis of total proteins by these cells suggest that biosynthesis of this protein is regulated by the feedback principle, and indeed that this process is regulated by Clq reaching the external medium. The signal is evidently transmitted into the cell through a specialized receptor for Clq, found *inter alia* on macrophages [3].

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EFFECT OF MYELOPEPTIDES ON CYTOLOGICAL ACTIVITY OF T LYMPHOCYTES

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UDC 616.155.3-097.35.32

KEY WORDS: myelopeptides; T lymphocytes; immunity.

The immune response to viral and cellular antigens and to lectins is depressed in cancer patients, especially after treatment (by radiotherapy or chemotherapy), and the total lymphocyte count falls. The proportion of T suppressor cells and of Ia^+ cells is increased under these circumstances, although their total number also is reduced [7].

Furthermore, unlike the majority of tumors induced by chemical carcinogens or by oncogenic viruses, spontaneous tumors of man and animals are often nonimmunogenic [8].

The use of immunomodulators to activate the T and B systems of cellular immunity thus assumes great importance in cancer patients. Attempts are now being made to use preparations based on hormones and mediators of the immune system as immunocorrective agents. Thymus peptides, which ensure normal functioning of T lymphocytes [3], have become widely familiar.

On the basis of mediators of bone marrow origin (myelopeptides) the preparation known as myelopeptin has been produced. Its action is aimed at correcting the B system of immunity when the level of antibody production in the immune organism is depressed [5]. The possibility cannot be ruled out that myelopeptin may also be effective in certain disturbances of the T system of immunity, in view of evidence showing its interaction with individual subpopulations of T lymphocytes [2].

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(Presented by Academician of the Academy of Medical Sciences of the USSR, R. V. Petrov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 101, No. 2, pp. 187-189, February, 1986. Original article submitted April 23, 1985.

0007-4888/86/0002-0215$12.50

TABLE 1. Effect of Myelopeptides on DNA Synthesis in Monoculture, Ordinary MLC, and Weakly Immunogenic MLC (number of counts per minute)

Expt. No.	Culture	Concentration of myelopeptides, μg/ml			
		0	30	60	90
1	Monoculture	5 927±134	5 870±262	6 840±931	—
	MLC	12 527±647	9 139±1 667	14 342±1 049	13 339±871
	Weakly immunogenic MLC	6 110±409	7 993±1 001	5 521±624	5 842±343
2	Monoculture	12 421±1 650	6 433±1 454	9 262±2 339	—
	MLC	19 765±2 370	12 754±2 671	—	—
	Weakly immunogenic MLC	20 580±1 270	14 871±4 348	14 011±118	13 585±394
3	Monoculture	7 129±1 171	6 381±442	6 797±1 436	—
	MLC	13 093±3 306	15 312±3 645	—	—
	Weakly immunogenic MLC	13 317±1 158	8 632±2 119	12 500±3 326	—

Legend. DNA synthesis determined as incorporation of ^{3}H-thymidine.

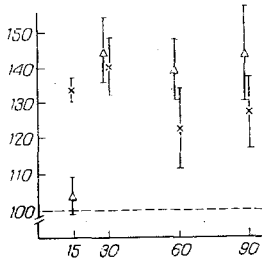

Fig. 1

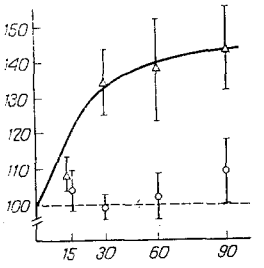

Fig. 2

Fig. 1. Increase in cytological activity of T lymphocytes of weakly immunogenic MLC under the influence of myelopeptides, added at different times. Abscissa, concentration of myelopeptides (in μg/ml), added to weakly immunogenic MLC at time 0 (triangles) and on 3rd day of incubation (crosses); ordinate, increase in cytolysis relative to control (in %, mean results of six experiments).

Fig. 2. Comparative data on effect of myelopeptides on cytolytic activity of lymphocytes from ordinary MLC and weakly immunogenic MLC. Myelopeptides added at time 0. Abscissa, concentration of myelopeptides (in μg/ml); ordinate, increase in cytolysis relative to control (in %) for ordinary MLC (circles) and weakly immunogenic MLC (triangles).

In the present investigation the effect of myelopeptides was studied on the cytolytic and proliferative activity of T lymphocytes.

EXPERIMENTAL METHOD

BALB/c (H-2^d) and C3H (H-2^k) mice aged 8-12 weeks were used. Cytolytic T lymphocytes (CTL) were obtained in mixed lymphocyte culture (MLC) by the method in [6]. Splenocytes of BALB/c mice, numbering $2 \cdot 10^6$ in 1 ml, were mixed with 10^5 or 10^6 stimulating spleen cells from C3H mice irradiated in a dose of 1000 R in medium RPMI-1640, containing $3 \cdot 10^{-5}$ M 2-mercaptoethanol, 10% embryonic calf serum, $2 \cdot 10^{-3}$ M L-glutamine, 5 mM HEPES, and 100 U/ml each of penicillin and streptomycin. The cells were cultured in plastic plates (Falcon Plastics, USA) in an atmosphere of 5% CO_2 at 37°C for 4 days.

Two types of MLC were used: weakly immunogenic, in which the ratio of reacting to stimulating cells was 20:1, and ordinary, in which this ratio was 2:1. On the day of the experiment the number of living cells was counted by staining with trypan blue.

Myelopeptides were isolated from the supernatant to pig bone marrow cell cultures by gel-chromatography on Sephadex G-25 [3]. The protein concentration in the preparation was determined by Lowry's method and antibody-stimulating activity was estimated in culture *in vitro* [4]. Myelopeptides were added to the culture either immediately after mixing of the cells (time 0) or on the 3rd day of incubation of the MLC.

To determine DNA synthesis, lymphocytes from MLC on the 3rd day of incubation were introduced into 96-well plates (from Linbro, England) at the rate of $5 \cdot 10^5$ cells per well in a volume of 0.2 ml. Next, ^{3}H-thymidine was added in a dose of 1 μCi per well for 6 h. Prolif-

erative activity relative to incorporation of ^{3}H-thymidine was determined in a Mark 2 β-spectrometer. The results were assessed as the arithmetic mean of four separate determinations of the number of counts per minute.

The cytolytic activity of MLC was determined by the use of mouse L fibroblasts of syngeneic C3H mice as targets. The L-cells were labeled with ^{51}Cr (100 μCi per 10^6 cells for 45 min) and introduced into flat-bottomed plates (Falcon Plastics, USA) overnight at the rate of $4 \cdot 10^4$ cells per well. Lymphocytes taken on the 5th day of MLC were added to the targets for 3 h in ratios of lymphocytes to target cells of 10:1, 5:1, and 2:1 and incubated at 37°C in an atmosphere of 5% CO_2.

The incubation medium was carefully transferred into plastic ampuls. Radioactivity was measured in a γ-spectrometer (Nuclear Chicago, USA). The specific outflow of chromium in per cent, reflecting the degree of lysis of the target cells, was calculated by the equation:

$$\% \text{ of cytolysis} = \frac{\text{Experiment} - \text{control}}{\text{Total lysis} - \text{control}} \times 100,$$

where experiment denotes the number of counts per minute in the presence of lymphocytes, and control denotes the number of counts per minute without CTL. Total lysis was determined by adding detergent to the targets.

EXPERIMENTAL RESULTS

Myelopeptides, added to the weakly immunogenic MLC at time 0 stimulated cytolytic activity of T killer cells (Fig. 1). An increase in cytolysis by 5-10% compared with the control was observed when the concentration of myelopeptides was 15 μg/ml, it rose to 35-50% when the concentration was 30 μg/ml, and remained at that level until the concentration reached 90 μg/ml. In a concentration of 120 μg/ml the myelopeptides increased activity of CTL relative to the control by only 10-15%. Addition of the myelopeptides on the 3rd day of incubation to weakly immunogenic MLC also increased the cytolytic activity of the T lymphocytes, but their effect in this case was weaker. Within the concentration range from 30 to 90 μg/ml lysis of the target cells was increased only 20-35% compared with the control.

An attempt was made to assess the effect of myelopeptides on proliferation of lymphocytes in MLC. The preparation was added to the culture at time 0, and DNA synthesis was determined by measuring incorporation of ^{3}H-thymidine on the 3rd day of incubation. Within the concentration range from 30 to 90 μg/ml the myelopeptides caused no increase in cell proliferation in either normal or weakly immunogenic MLC (Table 1). When monocultures of splenic lymphocytes of BALB/c mice were used as the control, the preparation likewise had no activating action.

When myelopeptides are used *in vitro*, correction of the cytolytic activity of weakly immunogenic MLC can thus be observed. As the experiments showed, the number of precursor cells responding to antigens of the principal histocompatibility complex, or to virus-associated or tumor antigens, is strictly determined [9, 10].

In an immunodeficiency state induced by weak antigenic action or by the immunodepressive effect of a tumor, a virus infection, or certain other causes, not all precursor cells of CTL may thus be involved in the immune response.

In the present experiments a weakly immunogenic MLC, the level of whose cytolytic activity was enhanced after addition of myelopeptides to the incubation medium, was used as the model system. The effect of the preparation was optimal when it was added to the original culture, and weaker when added on the 3rd day of MLC. These data suggest that myelopeptides can recruit those precursor cells of CTL which did not participate in the immune response. This hypothesis is also confirmed by the fact that the cytotoxicity of lymphocytes of normal MLC is unchanged.

The increase in cytolytic activity of the weakly immunogenic MLC ought to be accompanied by an increase in DNA synthesis also. However, no increase in proliferative activity was observed on the 3rd day of MLC, when DNA synthesis in mouse MLC reached a peak. These data suggest that myelopeptides act on differentiation of CTL precursors without activating DNA synthesis.

Myelopeptides can thus evidently be successfully used to stimulate or correct the immune response.

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