in vitro are factors which lead to an increase in SCE in cells in culture. It can be postulated that it is the actual procedures of isolation of the cells and obtaining of a primary culture which lead to an increase in the number of SCE in cells in culture compared with the number estimated in vivo. Evidence in support of this conclusion is given by the fact that the frequency of SCE in lymphocytes during culture of whole blood averages 8.5 per cell [3], but when the additional procedure of isolating the cells with Ficoll is used the number of SCE increases to 11.4 exchanges per cell [5].

LITERATURE CITED

1. S. V. Stuaklov and A. N. Chebotarev, Byull. Éksp. Biol. Med., No. 4, 74 (1983).
2. A. N. Chebotarev, G. T. Selezneva, and V. I. Platonova, Byull. Éksp. Biol. Med., No. 2, 242 (1978).
3. P. E. Crossen, Sister Chromatid Exchanges, New York (1982), p. 706.
4. R. J. Du Frain, Sister Chromatid Exchanges, New York (1984), p. 891.
5. B. Santesson, K. Lindahl-Kiessling, and A. Mattsson, Clin. Genet., 16, 133 (1979).

SYNERGIC ACTION OF LIPOPOLYSACCHARIDE AND MURAMYL DIPEPTIDE IN IMMUNOTHERAPY OF DBA/2 MICE WITH MASTOCYTOMA P815

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KEY WORDS: lipopolysaccharide; muramyl dipeptide; immunotherapy.

One of the most promising and effective methods of tumor immunotherapy is considered to be the use of combinations of immunomodulators with different mechanisms of action on cells of the immune system, and for that reason capable of potentiating each other's effect [8]. The writers previously demonstrated the synergic action of immunomodulators of bacterial origin, namely lipopolysaccharide (LPS) and muramyl dipeptide (MDP), on activation of the production of tumor necrosis factors (TNF) and of interleukin-1 in mice in vitro [4]. It was shown that the combined use of LPS and MDP can induce necrosis and regression of subcutaneous nodes of highly immunogenic syngeneic tumors: sarcoma Meth A in BALB/c mice [6] and sarcoma SA-1 in A/Sn mice [5]. This paper describes a study of the effect of a combination of LPS and MDP on growth of syngeneic mastocytoma P815, which has low immunogenicity, in DBA/2 mice. The cyclo-oxygenase inhibitor indomethacin and the cytostatic cyclophosphamide also were used for immunotherapy.

EXPERIMENTAL METHOD

Male DBA/2 (H-2^d) mice weighing 18-20 g were used. Injection of 10^6 mastocytoma P815 (H-2^d) cells, maintained in vivo by weekly passages in DBA/2 mice, was given subcutaneously into the right side. After 5 days, either LPS of E. coli 055:B5 ("Difco," USA) in a dose of 5 μg per mouse or MDP (obtained from N. V. Bovin, Institute of Bioorganic Chemistry) in a dose of 15 μg per mouse, or a combination of LPS and MDP, in 0.2 ml of medium 199 was injected at the site of injection of the tumor cells; some mice were injected with tumor cells only. Immunotherapy was given 5 times at intervals of 2-3 days (experiments of series I). In series II the mice were divided into seven groups with 10 animals in each group. A mixture

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TABLE 1. Synergism of Action of LPS and MDP on Regression of Subcutaneous P815 Tumors in DBA/2 Mice

Group	Number of mice	Immunomodulator	Mean diameter of tumor, mm	Percentage of mice with regression of tumor	Survival rate, %
1	13	—	17,5	0	15
2	10	MDP	16,8	0	20
3	10	LPS	11,5	25	40
4	15	LPS + MDP	6,0	56	60

Legend. Data for 20th day after inoculation of tumor cells and 2 days after fifth injection of preparations are given.

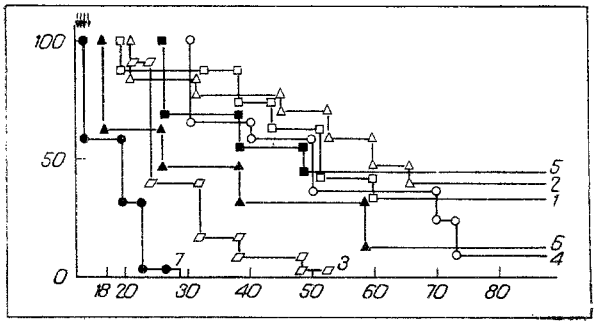

Fig. 1. Increase in length of survival of DBA/2 mice with mastocytoma P815 after injection of a combination of LPS and MDP into the tumor. Abscissa, time after injection of tumor cells (in days); ordinate, % of surviving animals. Arrows indicate time of injection of preparations. 1-7) Groups of mice.

of LPS and MDP in the above-mentioned doses was given 5 days after subcutaneous injection of the P815 cells (10^6), at the site of injection (group 1). The same preparations were used in groups 2-6. The mice of group 2 drank a solution of indomethacin ("Sigma," initial solution 2.5 mg/ml in ethanol) throughout the experiment in a final concentration of 7 μg/ml. In group 3, 2 h before the first injection of the preparations, a solution of cyclophosphamide ("Serva," West Germany) was injected intraperitoneally in a dose of 100 mg/kg. In group 4, a combination of LPS and MDP in the above doses was injected intravenously 1, 3, and 5 days after inoculation of the tumor cells. As an additional measure, simultaneously with LPS and MDP intact syngeneic splenocytes were injected into the tumor in a dose of 5×10^6 per mouse (group 5) or the same dose of splenocytes, treated for 24 h with LPS and MDP and subsequently washed (group 6) were injected into the tumor. None of the preparations was given to control mice with tumors (group 7). Preliminary experiments showed that injection of medium 199 into the tumor did not affect growth of mastocytoma P815 in DBA/2 mice. In all groups a mixture of LPS and MDP was injected 5 times into the tumor, 5, 8, 11, 14, and 17 days after injection of the tumor cells. Two perpendicular diameters of the subcutaneous tumors were measured by means of calipers and the mean value calculated. In the experiments of series III, in order to locate the immunologic memory, DBA/2 mice were given a second subcutaneous injection of 10^6 mastocytoma P815 cells after 8 months, and the duration of survival of the animals was counted.

EXPERIMENTAL RESULTS

In the experiments of series I the effect of injection of LPS, MDP, and a mixture of the two into the tumor on growth of subcutaneous nodes of mastocytoma P815 was investigated in DBA/2 mice. The results obtained 20 days after inoculation of the tumor cells and 2 days after the fifth injection of the preparations are given in Table 1. In most mice receiving both preparations by injection into the tumor, necrosis and regression of the nodes were observed and the mortality of the animals was reduced. In one of 10 mice regression of the tumor was observed after injection of LPS alone. Immunotherapy with MDP alone did not affect

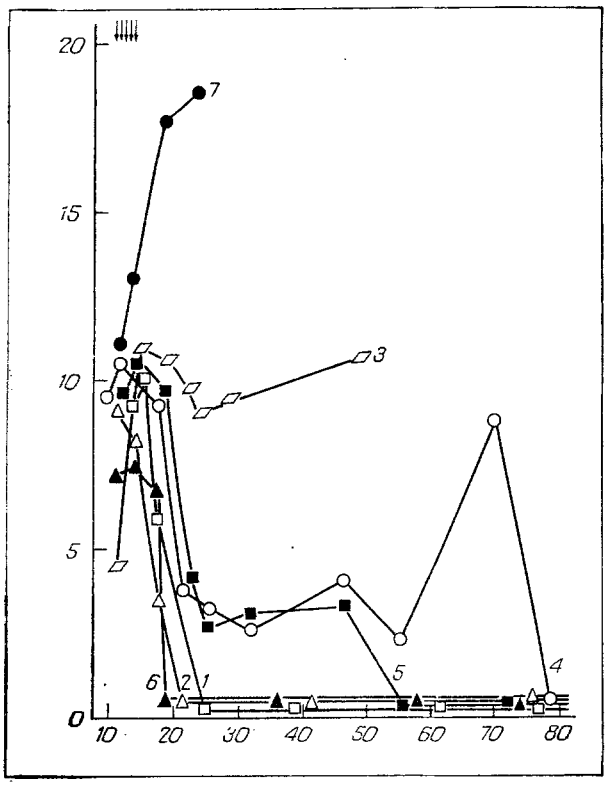

Fig. 2. Regression of subcutaneous nodes of mastocytoma P815 in DBA/2 mice after injections of a combination of LPS and MDP into the tumor. Ordinate, mean diameter of tumor (in mm). Remainder of legend as to Fig. 1.

growth of the tumor or survival. This is evidence of the synergic immunotherapeutic action of LPS and MDP when injected into the tumor in DBA/2 mice with subcutaneous nodes of mastocytoma P815. Meanwhile, systemic injection of a combination of the preparations had no significant effect on growth of this tumor [2].

In the experiments of series II the effect of preliminary sensitization of the animals with LPS and MDP (group 4), of inhibition of function of suppressors by indomethacin (group 2) or by cyclophosphamide (group 3), and of intact and activated lymphoid cells (groups 5 and 6) on the therapeutic action of injections of a combination of LPS and MDP into the tumor, was investigated. All the control mice died 20-30 days after injection of tumors cells. The highest survival rate (up to 40%) was observed in groups 1, 2, and 5, i.e., no increase in the efficacy of a combination of LPS and MDP was observed under the influence of the additional factors mentioned above. The treated animals survived 8 months (period of observation).

The same groups of experiments are demonstrated in Fig. 2, but the dynamics of the diameters of the tumor cells throughout the experiment was used as the criterion of efficacy of the preparations. Combined injection of cyclophosphamide intraperitoneally and LPS + MDP into the tumor (group 3) gave unfavorable results. In the remaining groups the tumors progressively decreased in size and at about the same rate. Morphological and histochemical investigations showed that massive necrosis in the subcutaneous P815 nodes was associated with the formation, initially, of juxtamural and later of occlusive fibrin thrombi in capillaries and venules supplying the tumor. This is in agreement with the morphological picture after injection of TNF [9]. Evidence that TNF generated under the influence of LDS and MDP in vivo [5] and in vitro [4] has an indirect action on tumor cells is given by the absence of its cytostatic effect on tumor P815 cells in vitro [3].

The experiments of series III showed that the thrombonecrotic stage of the action of LPS and MDP is followed by a stage of development of antitumor immunity. A second injection of P815 cells (10^6) subcutaneously into DBA/2 mice successfully treated for tumor and surviving 8 months (from groups 1, 2, and 5 of the experiments of series II) led to growth followed by rejection of the tumors, and to survival of about 50% of the mice for 7 weeks

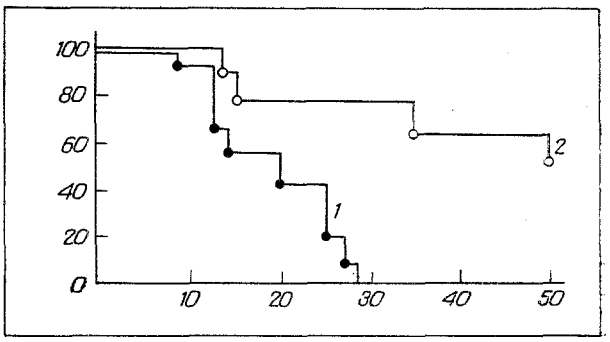

Fig. 3. Increase in survival time of DBA/2 mice receiving a second injection of P815 cells 8 months after regression of primary tumors. Abscissa, time after subcutaneous injection of 10^6 P815 cells (in days); ordinate, % of surviving animals. 1) Intact DBA/2 mice, 2) DBA/2 mice treated 8 months previously for primary P815 tumor by a combination of LPS and MDP. Each group consisted of 10-12 animals.

(Fig. 3). Development of tumor-specific immunity in the mice after immunotherapy with TNF was demonstrated previously [10]. During combined administration of LPS and MDP to mice with tumors, activation of tumor-specific killer T cells and inhibition of suppressor function were revealed.

It can be tentatively suggested that the immunosuppressor function of these tumor cells in necrotic zones of P815 tumors is depressed [1, 11] and that recirculating T lymphocytes are not inactivated but, on the contrary, they are immunized under the influence of tumor antigens and with the indirect booster action of LPS and MDP.

LITERATURE CITED

1. A. E. Medvedev, Byull. Éksp. Biol. Med., No. 6, 701 (1988).
2. A. L. Rakhmilevich, M. S. Rakhimova, O. V. Bogomolov, et al., All-Union Conference on Cell Pathology [in Russian], Moscow (1987), p. 100.
3. A. L. Rakhmilevich and M. S. Rakhimova, Factors in Cellular and Humoral Immunity in Various Physiological and Pathological States [in Russian], Chelyabinsk (1988), pp. 105-106.
4. A. L. Rakhmilevich and M. S. Rakhimova, Byull. Éksp. Biol. Med., No. 4, 483 (1988).
5. B. B. Fuks, A. L. Rakhmilevich, A. A. Pimenov, and A. G. Dubrovskaya, Byull. Éksp. Biol. Med., No. 10, 497 (1987).
6. N. Bloksma, F. M. A. Hofhuis, and J. M. N. Willers, Cancer Immunol. Immunother., 19, 205 (1985).
7. B. B. Fuks (B. B. Fuchs), A. L. Rakhmilevich, A. A. Pimenov, and V. V. Deev, Period. Biol., 85, Suppl. 1, 192 (1987).
8. J. W. Hadden and F. Spreafico, Springer Semin. Immunopathol., 8, 321 (1985).
9. T. Kawai, N. Satomi, N. Sato, et al., Virchows Arch. Abt. B, Zellpath., 52, 489 (1987).
10. M. A. Palladino, M. R. Shalaby, S. M. Kramer, et al., J. Immunol., 138, 4023 (1987).
11. S. Tsunawaki and C. F. Nathan, J. Exp. Med., 164, 1319 (1986).