prodigiosin costimulation (see Fig.2), allowing us to conclude merely that there is a tendency toward a stimulation of O_2 formation by Mn.

The study confirmed the ability of taftsin like peptides from the CRP molecule to the formation of active forms of oxygen in Mn and Nph of human peripheral blood regardless of the presence of a second stimulating signal. This conclusion is confirmed by the reliable correlation coefficients between the peptide effects on intact and stimulated Nph ($r = 0.86+0.11$) and Mn ($r = 0.85+0.11$); $p<0.001$ in both cases. In addition, the influence of the peptides from the CRP molecule was found to be not inferior to that of taftsin, a well-known activator of blood phagocytes. Therefore, the limited CRP proteolys occurring in the inflammation focus under the influence of intra- and extracellular enzymes of neutrophilic origin may be assumed to regulate phagocyte activity by means of the removal of peptides of different composition.

Peptides split off from the CRP molecule differ in their tropism with respect to phagocytes. While TKPQ and TKPL showthe greatest stimulating effect with respect to Nph, GKPR and TKRL are the most effective with respect to monocytes. Based on differences in effective concentrations on can evaluate the range of peptide influence. The fact that TKPQ show maximum activity in high concentrations (10^{-8} M) is assumed to be evidence for its activity within the bounds of the inflammation focus, whereas GKPR and TKPL are most effective in concentrations ranging from 10^{-10} to 10^{-11}M and probably have a more systemic effect. The alternative explanation of this phenomenon may consist in the differences in the affinity of the sites of different peptides on phagocytes.

Consequently, the immunoregulatory properties of CRP are not only associated with its native molecule or its subunits but are also influenced by the nature of limited proteolysis occurring in the inflammation focus. The removal of taftsin like peptides from the CRP molecule may therefore be assumed to cause the involvement of new cells in protective reactions and act on the distribution of functions between them.

LITERATURE CITED

1. I.Yu.Ivanov and O.N.Pogorelyuk, Statistical Processing of Results of Medical-Biological Investigations by Microcomputer Software. [in Russian], Moscow (1990).
2. A.N.Mayanskii and D.N.Mayanskii, Essays on Neutrophils and Macrophages. [in Russian], Novosibirsk (1989).
3. P.G.Nazarov and B.N.Sofronov, Immunologia, No. 4, p. 12-18 (1986).
4. A.G.Pearse, Histochemistry, Theoretical and Appliet, 4th ed.,Churchill (1980).
5. A.Boyum, Clin.Lab.Invest., **21**, suppl., p,97 (1968).
6. P.Eggleton, R.Gargan, and D.Fisher, J.Immunol.Meth., Vol. 121. No. 1, p. 105-113 (1989).
7. V.Kolb-Bachofen, Immunobiology, **183**, No. 1-2. p. 133-145 (1991).
8. K.Miyazawa and K.Inoue, J.Immunol., **145**, No. 2, p. 650-655 (1990).
9. K.Piganelli-Parker, W.R.Benjamin, K.L.Kaffka, and P.L.Kilian, J.Immumol., **142**, No. 2, p. 537-542 (1989).
10. L.A.Potempa, J.N.Siegel, B.A.Fiedel, et al., Mol.Immunol., **24**, No. 2, p. 531-542 (1987).
11. F.A.Robey, K.Ohura, S.Futak, et al., J.Biol.Chem. **262**, No. 15, p. 7053-7057 (1987).
12.G.A.W.Rook, J.Steele, S.Umar, et al.,, J.Immunol.Meth., **82**, No. 1, p. 161-167 (1985).

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ONCOLOGY

Contribution of Tumor Necrosis Factor to the Lethality of Mice with Endotoxin Shock Presensitized by Serum from Tumor-Bearing Mice

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A combination of lipopolysaccharide (LPS) and muramyl dipeptide (MDP) administered to tumor-bearing mice has been shown to cause delayed growth and rejection of the tumor [2]. To achieve this effect, however, LPS and MDP have to be given in near-toxic doses, which interferes with their use as

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antineoplastic agents. In addition, tumor-bearing animals display increased susceptibility to the toxic actions of bacterial immunomodulators [4,5].

In mice with growing tumors, such heightened susceptibility is due to the presence of certain high-molecular-weight substances in their serum [3] and to the activation of reticuloendothelial cells which produce increased amounts of tumor necrosis factor (TNF) when exposed to LPS [12]. Moreover, tumor-bearing animals exhibit increased sensitivity to the toxic action of this factor which is an important endogenous transmitter of the effects produced by LPS [10].

In this study, an attempt was made to assess the contribution of TNF to the lethality of mice sensitized by factors contained in the sera of tumor-bearing mice, and to find out which cells of the mice thus sensitized are responsible for the augmented TNF production in response to stimulation by a combination of LPS and MDP.

MATERIAL AND METHODS

C57BL/6(H-2^b) and BALB/c (H-2^d) mice of both sexes aged 2-3 months were used. C57BL/6 mice were injected with syngeneic leukemia EL-4 cells subcutaneously at $1x10^6$ cells per mouse.

Sera were obtained from intact mice and those with subcutaneous tumor nodes (on day 7 of tumor growth) and stored at -20°C.

Sera from the tumor-bearing mice were passed through filters with different pore sizes - UM2, UM10, PM30, XM50, XM100A, and XM300 - that allow passage of substances having molecular weights of less than 1, 10, 30, 50, 100, and 300 kDa, respectively. The resulting fractions were stored at - 70°C and examined for their ability to elicit sensitiza-tion to endotoxin shock and thus induce TNF hyperproduction in response to the subsequent administration of LPS plus MDP.

TABLE 1. Effect of Serum Fractions from Tumor-Bearing Mice on TNF Level and Lethality of Recipient Mice Given LPS or MDP (Mean values ± SD)

Pretreatment	Lethality, %	TNF, units/ml
None	20	29,3±9,9
Phosphate-buffered saline	33,3	124,2±68,7
Intact serum	20	18,4±5,0
Tumor serum	100*	15990,6±6031,3*
Serum fractions from tumorbearing mice, kD		
<1	13,3	248,8±161,3
1-10	33,3	43,3±16,8
10-30	80*	8103,1±3236,5*
30-50	53,3	92,8±38,9
50-100	26,6	90,0±43,1
100-300	100*	21477,1±4993,1*
>300	100*	34384,8±14376,2

Note: *$p<0.05$.

In each of the experiments, sera or their fractions were injected intravenously into the retro-orbital sinus in an amount of 0.2 ml. After 24 h, a proportion of the mice received an intravenous injection of LPS (20 μg) and MDP (20 μg) or various doses of a recombinant human TNF-α (kindly donated by V.G. Korobko, senior researcher at the Institute of Biological Chemistry, Russian Academy of Medical Sciences) in 0.2 ml of phosphate-buffered saline (PBS) to evaluate their sensitivity to the toxicity of these agents. Lethality among these mice was determined 48 h thereafter.

The remaining mice were used to study TNF hyperproduction in vivo and in vitro. In these experiments, LPS and MDP were used in combination, as it had been shown in our laboratory that their combinations are more potent in eliciting TNF production than either of them alone [1]. In the in vivo experiments, sera obtained from mice 2 h after the injection of these two immunomodulators were tested for TNF. In the in vitro experiments, plastic-adherent and nonadherent splenocytes (5×10^6 cells of each type) as well as unseparated splenocytes (1×10^6) were incubated with various doses of LPS and MDP, and the supernatants harvested 2, 6, and 24 later were tested for TNF activity on L-929 target cells [8]. The cytotoxic activity of TNF in the sera was defined as their dilution at which 50% of the target cells were lysed. The cytotoxic index (CI) in the supernatants of cells incubated with the immunomodulators was calculated by the formula $CI = (a - b)/b \times 100$, where a is the optical density (absorbance) of the incubation medium-containing wells and b is the optical density of the supernatant-containing wells.

RESULTS

We have shown previously that the state of sensitization to the endotoxin shock induced in tumor-bearing animals by a combination of LPS and MDP can be produced, and transferred to intact animals, with high-molecular-weight substances contained in the serum [3]. In this study, we further fractionated these substances and tested them for their ability to induce TNF hyperproduction in parallel with the examination of their impact on the susceptibility of mice to endotoxin shock. As seen in Table 1, a good correlation was observed between lethality and TNF activity in the sera from mice presensitized by various fractions of sera from tumor-bearing mice.

These findings are in accord with those of other authors reporting a correlation between the severity of some parasitic [15] or infectious [11] diseases and

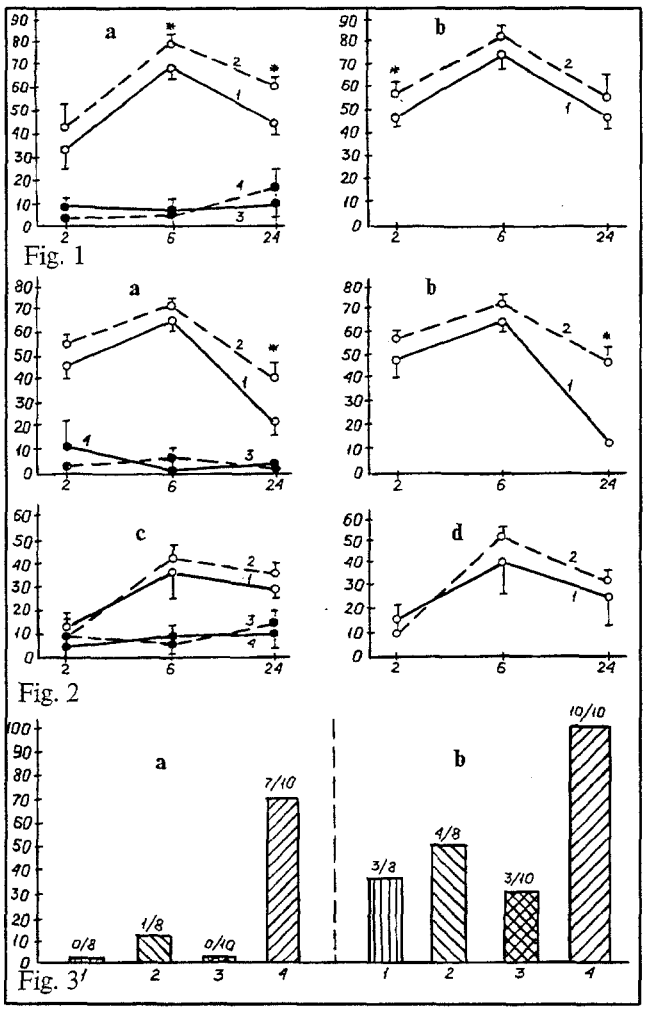

Fig. 1. Effect of sera from tumor-bearing mice on TNF production by splenocytes from recipient mice. Abscissa: incubation time (hours) of splenocytes with immunomodulators. Ordinate: LPS concentration: *a*) 1 ng/ml; *b*) 10 ng/ml. *1* and *3*, Mice given intact sera; *2* and *4*, mice given sera from tumor-bearing mice; *3* and *4*, splenocytes not exposed to LPS or MDP. Here and in Figs. 2 and 3 the asterisk shows values for which $p<0.05$.

Fig. 2. TNF production by adherent and nonadherent spleno-cytes from mice sensitized with sera from tumor-bearing mice. LPS concentration: *a* and *c*, 1 ng/ml; *b* and *d*, 10 ng/ml. *a* and *b*, Adherent splenocytes; *c* and *d*, nonadherent splenocytes. *1* and *3*, Mice given intact sera; *2* and *4*, those given sera from tumor-bearing mice; *3* and *4*, cells not exposed to LPS and MDP. For other designations see Fig. 1.

Fig. 3. Effect of sera from tumor-bearing mice on the susceptibility of intact mice to the toxic action of recombinant TNF. Ordinate: % lethality. At 24 h before TNF injection (*a*, 5 μg/mouse; *b*, 10 μg.mouse) BALB/c mice received the following: *1*, nothing (control); *2*, PBS; *3*, sera from intact C57BL/6 mice; *4*, sera from C57BL/6 mice with subcutaneous EL-4 tumors. Figures under columns: numerator, number of dead animals; denominator, number of test animals.

C57BL/6(H-2^b) and BALB/c (H-2^d) mice of both sexes aged 2-3 months were used. C57BL/6 mice were injected with syngeneic leukemia EL-4 cells subcutaneously at 10×10^6 cells per mouse.

TNF levels in sera from patients. It has even been claimed by some that the serum level of TNF in patients with meningitis is a predictor of death from this disease [9].

Peripheral blood monocytes from cancer patients are known to produce increased amounts of TNF in response to LPS [12]. and this has led us to suggest that the major contribution to TNF hyperproduction in our model was made by cells of the macrophage lineage. It can be seen in Fig. 1 that the splenocytes from mice sensitized by sera from tumor-bearing mice elaborated more TNF when stimulated by LPS and MDP that did those from mice pretreated with sera from intact mice. Sera from tumor bearers also exerted a sensitizing effect on adherent spleen cells. These, as well as the unseparated spleen cell population of mice sensitized by sera from tumor bearers for 24 h, were found to produce TNF in increased amounts (Fig. 2). In contrast, the nonadherent splenocytes from mice sensitized with such sera failed to elaborate increased amounts of TNF when stimulated with LPS plus MDP. It should be noted that peritoneal cells from mice that had received sera from tumor-bearing animals produced TNF in the same amounts as did those from the control mice pretreated with intact sera. It remains to be seen what contribution, if any, is made to this effect by adherent cells of other organs, particularly liver and lung.

The factors contained in sera from tumor bearers are thus able to sensitize adherent spleen cells and augment their capacity for induced production of TNF. The susceptibility of experimental animals to endotoxin shock can be increased by some of the substances, such as glucan and BCG, that activate cells of the reticuloendothelial system [14]. It is noteworthy that TNF was originally identified in the sera of BCG-activated animals [7]. Substances that sensitize cells of the macrophage series therefore promote TNF production in the presence of LPS, thereby increasing lethality among mice with endotoxin shock. Some of the macrophage functions are also activated during tumor growth [5, 6].

The ability of mice to produce TNF, α/β-interferon, and interleukin-6 in response to endotoxin has been shown to increase considerably after subcutaneous tumor implantation [13].

In addition to eliciting hypersensitivity to the toxic action of LPS and MDP, sera from mice with growing tumors also led in our experiments to augmented lethality among mice given the recombinant TNF (Fig. 3). The latter's dose of 5 μg/mouse, while being nontoxic to the control mice, caused a 70% lethality in the group of mice preinjected with sera from tumor bearers.

It looks as if TNF itself, which is a major mediator of endotoxic shock, can induce the production of other factors that, too, are involved in the development of pathological changes following endotoxin administration.

In conclusion, we have shown that augmented TNF production correlates with increased lethality among mice with endotoxin shock after their sensitization with high-molecular-weight substances contained in the sera of tumor-bearing mice. Such TNF hyperproduction is shown by cells of the macrophage lineage from sensitized animals. Intact animals injected with sera from tumor-bearing mice become more susceptible not only to the toxic activity of LPS and MDP but also to that of the recombinant TNF. With the substances contained in the sera of tumor-bearing animals, hypersensitivity to LPS and MDP resulting in TNF hyperproduction can therefore be transferred to macrophagal elements of intact animals and, in addition, the sensitivity of the body systems acted upon by the TNF itself can be increased in these animals.

LITERATURE CITED

1. A.L. Rakhmilevich and M.S. Rakhimova, Byull. Eksp. Biol. Med., No. 4, 483-485 (1988).
2. B.B. Fuks, A.L. Rakhmilevich, A.A. Pimenov, et al., Byull. Eksp. Biol. Med., No. 10, 497-499 (1987).
3. B.B. Fuks, A.I. Shapoval, and I.M. Grzhebin, Byull. Eksp. Biol. Med., No. 7, 78-80 (1991).
4. J. Bartholeyns, M. Freudenberg, and C. Galanos, Infect. Immun., **55**, 2230-2233 (1987).
5. M.J. Berendt, M.F. Newborg, and R.J. North, Infect.Immun., **28**, 645-647 (1980).
6. J.W.B. Bradfield, T. Whitmarsh-Everiss, and D.B. Palmer, Brit. J. Cancer, **42**, 900-907 (1980).
7. E. Carswell, L. Old, R. Kassel, et al., Proc. Nat. Acad. Sci. USA, **72**, 3666-3670 (1975).
8. H. Fisch and G.E. Gifford, Int. J. Cancer, **32**, 105-112 (1983).
9. G.E. Grew, J.E. Teylor, D.O. Trop, et al., New Engl. J. Med., **320**, 1586-1591 (1989).
10. J.A. Krosnic, J.K. McIntosh, J.J. Mule, et al., Cancer Immunol. Immunother., **30**, 133-138 (1989).
11. M.M. Mustafa, O. Ramilo, X. Saer-Llorens, et al., Pediat. Infect. Dis., **8**, 921-922 (1989).
12. K. Nara, H. Odagiri, M. Fujii, et al., Cancer Immunol. Immunother., **25**, 126-132 (1987).
13. A.L. Rakhmilevich and R.J. North, Cytokine, **3**, 398-406 (1991).
14. E. Suter, G. Ullman, and R. Hoffman, Proc. Soc. Exp. Biol., **99**, 167-169 (1958).
15. T. Takashima, C. Ueta, I. Tsuyugchi, et al., Infect. Immun., **58**, 3286-3292 (1990).

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Mammary Carcinogenesis Suppression by Ginseng Tissue Culture Biomass Tincture

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Much attention has been paid of late to search for agents capable of suppressing carcinogenesis, since such agents appear to be promising in the primary prevention of cancer [1]. Some authors have reported that a natural ginseng root tincture inhibited the development of pulmonary adenomas induced by various carcinogens in mice [5,17]. An epidemiologic survey from South Korea, where ginseng preparations are widely used, has demonstrated by the chance control method that patients with malignancies use ginseng much more seldom than control patients with nononcologic diseases [16]. Hence, further research of the oncoprophylactic or anticarcinogenic characteristics of ginseng seems to hold promise.

The present research was aimed at experimental study of ginseng potentialities in the prevention of mammary carcinoma. Bioginseng, an officinal drug (Provisional Pharmacopeial Article No 42-1890.89 as of April 21, 1989), obtained from cultured cells of Panax ginseng C.A.Mey, was used in the study. The cultural bioginseng preparation is characterized by the same pharmacologic activity as natural ginseng root galenics [4], but it is more readily available for clinical practice.

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