cytes, while its activity towards neutrophils increases considerably. It can be concluded that the catabolism of CRP in the focus of inflammation gives rise to products with new immunoregulatory properties. Free subunits can be regarded as endogenous pH-dependent immunoregulatory agents, which differ from native CRP in a greater selectivity towards phagocytic reactions upon inflammation [3,4,5]. Presumably, CRP subunits are one of the factors that determine the alternation of phases in the inflammatory response.

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Effect of β-Carotene on the Development of Adjuvant-Induced Arthritis and Production of Interleukin-1 in Rats

A. A. Potapova, O. V. Buyuklinskaya, T. L. Korsova, N. A. Morozova, L. I. Krivov, S. M. Yakushina, A. A. Poznanskaya, and E. A. Malakhova

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Adjuvant-induced arthritis (AA) of rats, an autoimmune disease caused by subcutaneous injection of mycobacteria, accurately reflects the pathological changes observed in human rheumatoid arthritis. Although the role of immunological factors in the development of AA has been studied intensively, numerous aspects of this problem still remain unclear. The findings that mycobacterial heat-shock protein and rat proteoglycan (a constituent protein of articular cartilage) have identical amino acid sequences [14], on the one hand, and the discovery of a heat-shock protein reactivity of arthritic patients [13], on the other, confirm that the AA model is a valuable tool in the search for anti-inflammatory and antiarthritic drugs. At the present time, application not only of immunodepressive, but also of immunostimulatory agents seems quite reasonable [2]. This approach is based on the fact that an immunological dysregulation is developed in autoimmune diseases, i.e., despite the hyperreactiv-

Vitaminy Industrial Complex, Moscow. (Presented by T. T. Berezov, Member of the Russian Academy of Medical Sciences)

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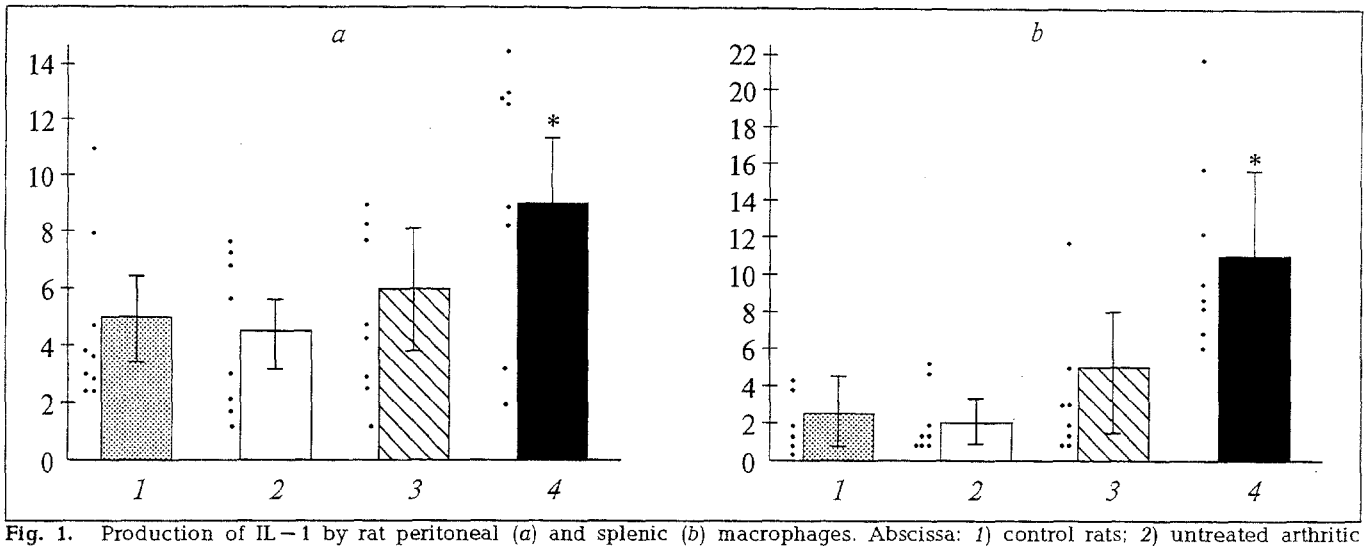

Fig. 1. Production of IL-1 by rat peritoneal (*a*) and splenic (*b*) macrophages. Abscissa: *1*) control rats; *2*) untreated arthritic rats; *3*) arthritic rats given placebo; *4*) β-carotene-treated arthritic rats. Ordinate: coefficient of IL-1 activity ($M \pm m$). The asterisk indicates values which are statistically significant ($p<0.05$) in comparison with group 2.

ity of some elements of the immune system, other elements are suppressed. This dysregulation may be normalized by immunostimulatory agents. Recent studies have shown that β-carotene is an effective stimulator of humoral and cellular immune reactions: it enhances proliferation of T and B cells and promotes secretion of factors which are necessary for the communication between immunocompetent cells [5]. In this connection we have analyzed the production of interleukin-1 (IL-1) by macrophages of AA rats and examined the effect of β-carotene on the development of AA and production of IL-1.

MATERIALS AND METHODS

Outbred white male rats weighing 190-230 g were used. The animals were divided into 4 groups. One group served as the control, and AA was induced in the other 3 groups [1]. The rats of one AA group received no β-carotene, while in 2 other groups they were given a placebo or 50 mg/kg body weight/day β-carotene 15 days prior to and 17 days after induction of AA. The rats were sacrificed on day 17 postimmunization. The intensity of AA was assessed by swelling of the hind and fore limbs and tail. The degree of swelling of each site was rated in points (0-4), and the arthritis index was calculated as their sum. Production of IL-1 by peritoneal and splenic macrophages was estimated as described [10]. The macrophages were incubated in 24-well plates (10^6 cells/cm^2) in RPMI-1640 medium (500 μl) containing 10 μg/ml lipopolysaccharide. After 24-h incubation, the levels of IL-1 in supernatants were measured by the extent of proliferation of thymocytes from C3H/HeJ mice. The thymocytes were cultured for 72 h in the presence or in the absence (control) of 1 μg/ml PHA. ^{3}H-thymidine (0.2 μCi) was added 4 h before the end of incubation. The intensity of thymocyte proliferation was estimated by ^{3}H-thymidine incorporation, and the coefficient of activity (CA) of IL-1 was calculated as the ratio of label incorporation in the experimental and control series. Spontaneous proliferation of splenic lymphocytes was evaluated after a 72-h culturing in 96-well plates (750,000 cells/well). ^{3}H-thymidine (0.2 μCi) was added to each well 4 h before the end of incubation, and its incorporation was measured in counts per minute. The content of β-carotene in the liver was measured by high-performance liquid chromatography [3], extraction of β-carotene was done by the method of Shapiro [12].

The preparation Cyclocar (β-carotene dispersed in water) was developed at the Vitaminy Industrial Complex and kindly provided by E. A. Malakhova. Cyclocar is a β-carotene-β-cyclodextrine molecular complex.

RESULTS

The rats developed edema of the left ankle, a primary response to adjuvant injection, 1 day postimmunization. At this stage we observed no difference between β-carotene-treated and untreated animals. Signs of a secondary reaction - swelling of the right noninjected hind paw, fore limbs and tail (polyarthritis) - developed 10-11 days postimmunization. Edema was weaker in rats administered β-carotene than in intact rats or in the placebo group. At 17 days postimmunization, the numbers of rats with signs of polyarthritis were essentially

TABLE 1. Indexes of Adjuvant-Induced Arthritis in Rats

Index	Control	Group		
		AA	AA+placebo	AA+β-carotene
Cases of polyarthritis	–	8/10	6/9	4/8
Arthritic index	–	7.5±1.1	7.1±3.8	4.5±0.8**
Changes in body weight, g/day	0.5±0.2	−2.1±0.5*	−2.1±0.4*	−0.9±0.8
ESR, mm/h	2.6±0.2	33.4±5.2*	30.4±4.2*	22.9±3.8*
Relative liver weight, %	4.0±0.2	4.6±0.3	4.6±0.3	4.3±0.4
Relative weight of adrenal glands, %	0.016±0.002	0.03±0.002	0.034±0.003	0.029±0.002*
Relative weight of thymus, %	0.12±0.01	0.09±0.02	0.07±0.01	0.10±0.01
Content of β-carotene in liver, μg/g	0.16±0.05	–	–	2.25±0.70*

Note. One asterisk indicates a statistically significant ($p<0.01$) difference from the control animals. Two asterisks indicate a statistically significant ($p<0.001$) diffrence from the group of untreated AA rats.

similar in the untreated and placebo groups, while in the β-carotene group the number of such animals was considerably lower (Table 1). The administration of β-carotene also reduced weight loss, the arthritic index, and the erythrocyte sedimentation rate but had no effect on the relative weight of the thymus, adrenal glands, or liver. From these observations it is obvious that β-carotene moderated the disease course.

It was formerly postulated that β-carotene is not absorbed and accumulated in the rat body in an unaltered form and that the effect of β-carotene is due to its conversion into retinoids. However, recent studies employing more sensitive methods have shown that β-carotene is absorbed unchanged in the rat organism [6, 12]. In our experiments, the administration of β-carotene for 32 days markedly increased its liver content (Table 1), indicating that the effects observed in this study were produced not only by retinoids, but also by β-carotene.

Figure 1 illustrates the production of IL-1 by rat peritoneal and splenic macrophages. It can be seen from the figure that AA induced no statistically significant changes in the synthetic activity of macrophages: the values of CA IIL-1 for stimulated macrophages obtained from intact and arthritic rats were 5.13±0.89 and 4.58±0.76, respectively (Fig. 1, *a*). It should be mentioned that the published data on this matteer are contradictory. It was reported that the production of IL-1 by stimulated macrophages from arthritic rats either remains unchanged [11] or is increased [8]. We observed almost a 2-fold (CA IL-1 9.36±1.44) statistically significant (compared with the placebo and intact groups) increase in the production of IL-1 by peritoneal macrophages from β-carotene-treated rats. Similar results were obtained in the experiments with stimulated splenic macrophages (Fig. 1, *b*). The macrophages from arthritic and intact rats secreted the same amounts of IL-1, CA IL1 being 2.27±0.51 and 2.43±0.5, respectively. The production of IL-1 by macrophages from β-carotene-treated rats was increased almost 3-fold (CA IL-1 10.76±2.01), the increase being statistically significant. These findings indicate that β-carotene stimulated the production of IL-1 by peritoneal and splenic macrophages of rats. Increased secretion of IL-1, however, did not result in aggravation of the inflammatory process in the joints. Previously it was shown that there is no direct correlation between increased production of IL-1 by macrophages located outside the inflammatory focus and a more intense inflammatory reaction in the joints [7,9].

It was to be expected that increased production of IL-1 in arthritic rats given β-carotene would affect the function of immunocompetent cells. In fact, the spontaneous proliferative activity of splenic lymphocytes in AA was decreased ($367\pm84\times10^2$ cpm versus $989\pm234\times10^2$ cpm normally After administration of β-carotene it rose to the normal level ($1132\pm220\times10^2$ cpm), which was significantly higher than in diseased untreated animals. This effect was not observed in the placebo group, where the proliferative activity was $512\pm\pm149\times10^2$ cpm. Obviously, β-carotene can increase not only mitogen-induced proliferation of lymphocytes, as was shown previously [4], but also their spontaneous proliferation.

Our results provide more information regarding the immunomodulating activity of β-carotene and point to the relevance of investigating this naturally occurring immunomodulator not only in immune deficient states observed in oncological diseases, but also in other pathologies of the immune system.

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EXPERIMENTAL GENETICS

Modeling the Genetic Correction of Familial Hypercholesterolemia by Generating Cell Clones with Stable Expression of the Receptor for Low Density Lipoproteins

N. B. Dolzhanskaya, E. L. Patkin, O. L. Runova, A. L. Shvartsman, and V. S. Gaitskhoki

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At the present time, familial hypercholesterolemia (FH) is one of the most widespread autosomal dominant inherited human diseases [1]. In most populations the occurrence of homozygous and heterozygous FH is 1:1,000,000 and 1:500, respectively [2].

The primary biochemical defect in this disease is depletion of the receptor for low density lipoproteins (rLDL) [3,4]. Insertion of the normal gene coding for rLDL and providing for its stable and effective expression in the cells of FH patients might be a radical approach to the treatment of FH.

The aim of the present study was to model genetic correction of FH by generating clones of animal cells with stable expression of the human rLDL gene inserted into the cells by genetic engineering techniques.

MATERIALS AND METHODS

CHOLA3 cells were cultured in HAM'S F12 medium supplemented with 10% fetal calf serum.

Institute of Experimental Medicine, Russian Academy of Medical Sciences, St. Petersburg. (Presented by A. N. Klimov, Member of the Russian Academy of Medical Sciences)

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