Inhibition of DNA synthesis in concanavalin A stimulated rat lymph node cells by serum from arthritic rats

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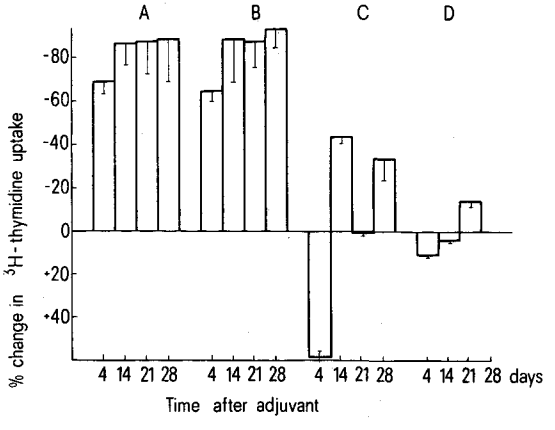
Summary. Serum from adjuvant arthritic rats inhibits the mitogenic response of rat lymph node cells to concanavalin A, leaving unaffected the response to phytohemagglutinin. This activity is already evident 4 days after adjuvant administration and persists for at least 28 days.

Several recent reports have been concerned with specific and non-specific depression of T-lymphocyte responses by various serum factors. These factors include products elaborated in different conditions, such as acute inflammation[1] and cancer[2–4], and have been characterized as low molecular weight peptides[5], immune complexes[6,7] or products of activated lymphocytes[8].

In rats with adjuvant arthritis, a disease considered to involve thymic-dependent cell-mediated immunity[9,10], comprehensive investigations have demonstrated a complex pattern of alterations of blood proteins, as well as the appearance of an acute phase globulin not present in normal rat serum[11,12]. In addition, in adjuvant induced arthritis, the response of lymphocytes from various lymphoid organs[13] and from peripheral blood[14] to different mitogens was found to be altered in widely different ways. We wish to report here results suggesting the presence in serum from arthritic rats of factor(s) able specifically to inhibit the responsiveness of rat lymph node cells to the mitogenic effect of concanavalin A.

Materials and methods. Adjuvant arthritis was induced in female inbred Lewis rats (body weight 150 g) by injecting 0.3 mg of heat-killed Mycobacterium butyricum (Difco) suspended in 0.1 ml of mineral oil into the right hind paw. Uninjected animals matched for sex and body weight served as controls. On days 4, 14, 21 and 28 after adjuvant administration, at least 3 rats from each group were anaesthetized with ether, the blood was collected by cardiac puncture and allowed to clot at room temperature for 30 min. The serum was then separated by centrifugation (500 × g, 10 min) and used immediately or after storage overnight at 4 °C. From the same animals, the axillary and cervical lymph nodes were removed and cell suspensions of 10^6 cells/ml were prepared in bicarbonate buffered RPMI 1640 (Grand Island Biological Co.), supplemented with 2 mM glutamine, 100 IU/ml of penicillin and 100 µg/ml of streptomycin. Concanavalin A (Con A, 1 µg/ml, Sigma, grade IV) or phytohemagglutinin P (PHA, 100 µg/ml, Difco) were added to 1 ml samples of cell suspension, together with 100 µl of normal rat serum, arthritic rat serum or fetal calf serum. The samples were then incubated for 48 h at 37 °C in an atmosphere of 5% CO_2 in air. 1 µCi of ^{3}H-thymidine (5000 mCi/mmole, Amersham) was added to each sample before stopping the incubation by cooling and precipitation of acid-insoluble material with trichloroacetic acid (TCA). The precipitates were washed 3 times with TCA, solubilized in 1 ml of Soluene (Packard Instruments) and the samples were counted in a liquid scintillation counter. The viability of the cells was assessed just prior to the addition of the radioactive label by the eosin exclusion test. The results were calculated as dpm/10^6 viable cells. In some experiments, the lymphocytes were incubated in RPMI 1640, supplemented with 50 µl of normal rat serum and 1 µg/ml of Con A, and additional 50 µl of either normal or arthritic rat serum were added at different times after the beginning of the culture.

Results and discussion. 100 µl of serum obtained from adjuvant arthritic rats at different stages of disease development almost completely inhibited the Con A-stimulated ^{3}H-thymidine incorporation in lymph node cells from normal (normal LNC) and adjuvant arthritic rats (arthritic LNC) (figure, A and B). The arthritic LNC obtained 4 days after adjuvant administration showed an increased responsiveness to Con A when incubated in normal rat serum, while decreased ^{3}H-thymidine incorporation was observed when the LNC were obtained 14 and 28 days after adjuvant administration (figure, C). The same LNC, however, when incubated in fetal calf serum did not show any variation in responsiveness to



Concanavalin A-stimulated ^{3}H-thymidine incorporation by rat lymph node cells (LNC). *A* Normal LNC cultured in arthritic rat serum. *B* Arthritic LNC cultured in arthritic rat serum. *C* Arthritic LNC cultured in normal rat serum. *D* Arthritic LNC cultured in fetal calf serum. Each value represents the mean of 3–9 separate determinations, calculated as per cent change in ^{3}H-thymidine incorporation relative to the appropriate control. Normal LNC in normal rat serum: 159,063 ± 18,196 dpm/10^6 cells (n = 20). Normal LNC in fetal calf serum: 320,945 ± 22,318 dpm/10^6 cells (n = 20).

Table 1. Absence of inhibitory effect of arthritic rat serum on PHA stimulated ^{3}H-thymidine incorporation in LNC from normal or arthritic rats. Arthritic LNC and sera were obtained from rats injected with adjuvant 14 days previously. Each value is the mean ± SE of at least triplicate determinations

Serum	LNC	dpm/10^6 cells
Normal	Normal	762,603 ± 41,421
Arthritic	Normal	685,566 ± 69,361
Normal	Arthritic	666,898 ± 55,797
Arthritic	Arthritic	779,826 ± 83,465

Table 2. Inhibitory effect of arthritic serum on Concanavalin A stimulated ^{3}H-thymidine incorporation in lymph node cells from normal rats: influence of different times of serum addition. 1 µg/ml of Con A and 50 µl of normal rat serum were added at the beginning of the culture, and 50 µl of normal rat serum or arthritic serum were added at the indicated times

Interval (h) between Con A and arthritic serum	Inhibition of ^{3}H-thymidine incorporation (± SD) as percent of control
0	88.2 ± 7.5
1	78.5 ± 10.2
18	6.8 ± 1.1

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Con A (figure, D). The basal, non-stimulated ^{3}H-thymidine incorporation was unmodified in all experimental conditions (results not shown).

This inhibitory effect seems to be specifically directed towards the Con A induced mitogenesis since, as shown in table 1, the arthritic serum did not decrease the PHA induced ^{3}H-thymidine incorporation in either normal or arthritic LNC. Moreover, this normal pattern of stimulation with PHA excludes any non-specific action of the arthritic serum on membrane function, thymidine uptake and intracellular thymidine pools.

It is evident from the results reported in table 2 that the inhibition of the Con A stimulated mitogenesis by arthritic serum occurs in the early phases of the process. In fact the ^{3}H-thymidine incorporation was reduced by 88% when the serum was added together with Con A, and by 78% when serum was added 1 h later. No inhibition occurred when the serum was added 18 h after Con A. These observations exclude the possibility of interference of the arthritic serum with binding of Con A to the cell membrane, since this binding is completed within 15 min from the mitogen addition[15].

It should be noted, in addition, that in these experiments only 50% of the serum was arthritic. 50 µl of normal rat serum were added from the beginning of the incubation to allow normal growth until supplementation at the appropriate times with 50 µl of normal or arthritic serum (see 'materials and methods').

The alterations in composition and concentration of the various blood proteins found in adjuvant arthritic rats show a widely different pattern in the different stages of the disease[11,12]. It therefore seems unlikely that variable changes in serum composition may be responsible for an inhibitory effect observed quantitatively unchanged in the different phases of the disease.

The nature of this factor is unknown. It is noteworthy, however, that it is present in the arthritic serum in the very early stages of the disease, before the appearance of the delayed systemic response, and that there is a specific inhibition of the LNC response to Con A. Both Con A and PHA are T-cell mitogens. However, it has recently been reported that the response to Con A is a characteristic of a distinct subpopulation of T-cells isolated from normal mice spleen[7].

Effect of methyl palmitate on the survival of skin semi-allografts in rats

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13 September 1976

Summary. In experiments with rats, the immunosuppressive effect of methyl palmitate on survival of skin semi-allografts was proved. Methyl palmitate was applied in a single dose 1 day prior to transplantation.

Relatively little is known about the inhibitory effect of methyl palmitate (MP) on RES. MP was found to cause lowering of phagocytic activity[1–6] as well as inhibition of primary and secondary antibody response [4–7], while the mechanism of MP action has not yet been fully elucidated. It is not distributed in the organism as other colloids which are phagocyted, and it is quickly hydrolyzed[1]. The effect of MP is manifested mostly in the decreased hepatic and splenic activity of RES, though no substantial damage to these organs could be proved[3,4,6]. According to some authors, the protracted inhibitory

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