An *in vitro* effect of an acute non-specific inflammatory serum on rat lymphocytes proliferative response to PHA

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## Abstract

This study has demonstrated that serum obtained from animals undergoing an acute inflammatory reaction induced by an intrapleural injection of dextran is able to modulate the proliferative response to PHA of lymph node and spleen cells *in vitro*. This response is dependent on the concentration of the inflammatory serum and on the time of collection of the serum during the acute inflammatory process. At low concentrations of serum (0.5%) stimulatory activity was observed at all time points. At higher concentrations (1%) inhibitory activity was present in 24 and 72 h sera.

These results support the previous observation that the acute non-specific inflammatory reaction is able to modulate cell functions. For example, sera obtained from such animals reduced the chemotactic responsiveness of polymorphonuclear leukocytes [4]. In addition, inflammatory serum has been shown to contain a mitogenic factor that is capable of inducing both DNA synthesis and proliferation of macrophages *in vitro* [2]. It was suggested that the mitogenic activity of inflammatory serum could be related to both thymic and bone marrow cells and that this cellular activity is enhanced when inflammation is present [5]. The question arose whether inflammatory serum contained mitogenic factors for lymphoid cells.

In the present study the effects of inflammatory sera, harvested after an intrapleural injection of dextran, on lymphoid cells (spleen, lymph node) in their response to PHA were investigated. The sera have been collected at various times after the induction of the acute non-specific inflammatory reaction in order to characterize the kinetics of the formation of this mitogenic activity.

## Introduction

The acute inflammatory process is a well-known phenomenon which is initiated when the body is subjected to some injurious stimulus. Previously the effects of this process were considered to be predominantly confined to the site of inflammation. However, recent studies have indicated that an acute inflammatory event is able to modify the functions of various cell types not only at the site of inflammation but at sites remote from it [1]. It has been found that pleural exudates induced by calcium pyrophosphate, dextran or carrageenan are capable of inducing macrophage proliferation *in vitro* [2]. Additional studies using inflammatory exudate from calcium pyrophosphate injected rats demonstrated that the exudate, when cultured with spleen cells, was able to modulate their response to the mitogen phytohaemagglutinin (PHA) [3]. Serum from animals undergoing an acute inflammatory reaction is also able to modulate cell functions. For example, sera obtained from such animals reduced the chemotactic responsiveness of polymorphonuclear leukocytes [4]. In addition, inflammatory serum has been shown to contain a mitogenic factor that is capable of inducing both DNA synthesis and proliferation of macrophages *in vitro* [2]. It was suggested that the mitogenic activity of inflammatory serum could be related to both thymic and bone marrow cells and that this cellular activity is enhanced when inflammation is present [5]. The question arose whether inflammatory serum contained mitogenic factors for lymphoid cells.

In the present study the effects of inflammatory sera, harvested after an intrapleural injection of dextran, on lymphoid cells (spleen, lymph node) in their response to PHA were investigated. The sera have been collected at various times after the induction of the acute non-specific inflammatory reaction in order to characterize the kinetics of the formation of this mitogenic activity.

## Materials and methods

Male Sprague-Dawley rats, 200–250 g (Depré, St-Doulchard, France), were used in these studies.

## Materials

RPMI 1640 (Gibco, Grand Island, NY) containing 2 mM glutamine, streptomycin (100 μg/ml) and penicillin (100 units/ml) was used throughout. This medium was supplemented with either normal rat serum at eight concentrations (5%, 1%, 0.5%, 0.1%, 0.05%, 0.01%, 0.005% and 0.001%) or inflamed serum at two concentrations (1% and 0.5%). PHA was purchased from Wellcome (UK).
Preparation of Inflammatory sera

Rats were injected intrapleurally with 1 ml of 6% dextran (Pharmacia, France, 40,000 daltons) according to the technique previously described [6]. Briefly, rats were lightly anaesthetized, using ether, and then given an injection into the right pleural cavity between the fifth and sixth intercostal space using a 1 ml Gillette syringe fitted with a size 21 G needle reduced in size to 4 mm long with the end rounded off.

Blood was collected 1 h, 24 h and 72 h later from the carotid artery and centrifuged at 4°C (200 g x 10 min and 400 g x 10 min). The sera were passed through a millipore filter (0.22 pm) and stored until required at -20°C. Serum from untreated animals was obtained in the same way, but prior to storage it was heated at 56°C for 30 min.

Lymphocyte culture

Individual normal rat spleen and lymph nodes were aseptically removed. Single-cell suspensions were prepared by using a Teflon and glass hand-homogenizer and then passed through nylon mesh. Cells were washed twice and resuspended in RPMI 1640 supplemented with different concentrations of normal rat serum or inflamed serum. Lymphoid cell suspensions were cultured in round-bottomed microplates (Falcon, Oxnard, USA) containing 2 x 10^6 cells/well in triplicate with different doses of PHA (10 pg, 5 pg, 2.5 pg, 1.2 pg, 0.6 pg). Cells were cultured for 3 days. Eighteen hours before the end of the incubation they were pulsed with 1 μCi of 3H-thymidine. Cells were harvested by an automated sampler harvester and the amount of 3H-thymidine incorporation was evaluated by liquid scintillation counting. The results were expressed by the average values in counts/min (c.p.m.).

Results

The effect of normal rat serum on PHA-induced proliferation of spleen and lymph node cells

In preliminary studies the response of rat lymph node and spleen cells to PHA in the presence of various concentrations of normal rat serum were studied. From these studies, both in the case of lymph node and spleen cells, a dose-dependent relationship between the concentration of normal rat serum and the proliferation of the cells in response to PHA was observed. From this data the optimal concentration of normal rat serum was 0.5%. For the studies of the effect of inflammatory serum on the proliferation of lymph node and spleen cells in response to PHA doses of 0.5% (optimal) and 1% (supra optimal) were used. Over the range of concentrations of PHA that were studied a dose-dependent relationship was seen. The optimal concentration was 1.2 μg, suboptimal doses of 0.6 μg and supra-optimal doses of 2.5, 5 and 10 μg showed reduced effects. For the following experiments concentrations of PHA were used within this range (i.e. 0.6 to 10 μg).

The effect of inflammatory sera on PHA-induced proliferation of spleen cells

Figures 1 and 2 demonstrate the effect of 0.5% and 1% inflammatory serum on PHA-induced proliferation of spleen cells. Using a dilution of 0.5% (Fig. 1) inflammatory serum collected 1 h after initiation of the acute inflammatory reaction shows a marked enhancement of the response to PHA (final concentration of 0.6 and 2.5 μg/ml) when compared to normal rat serum. Sera obtained 24 and 72 h after induction of the inflammation are not significantly different from the control. A somewhat different picture is observed using a serum dilution of 1% (Fig. 2). In this case 1 h inflammatory serum is no different from control values. However, inflammatory sera collected at 24 and 72 h demonstrate an inhibition of the proliferative response to PHA. These results were observed for all concentrations of PHA used (2.5, 5 and 10 μg/ml final concentration).

The effect of inflammatory sera on PHA-induced proliferation of lymph node cells

The effects of 0.5% and 1% inflammatory sera on the PHA-induced proliferation of lymph node cells may be seen in Figs 3 and 4. In Fig. 3, using a serum dilution of 0.5%, all the inflammatory sera (1 h, 24 h and 72 h) show an