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STIMULATION OF HUMAN PERIPHERAL LYMPHOCYTES WITH ENDOTOXIN AND RADIODETOXIFIED ENDOTOXIN

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The effects of parent endotoxin and radiodetoxified endotoxin on human peripheral lymphocytes were compared in experiments in vitro. Radiodetoxified endotoxin is able to exceed the degree of the stimulation induced by parent endotoxin and its stimulatory effect. At the same time, no change was observed in the presence of autologous serum. Radiodetoxified endotoxin did not inhibit the phytohaemagglutinin-induced proliferative response.

To our present knowledge, activation of B-lymphocytes and immunoglobulin synthesis is induced by polyclonal B-cell activators [1]. Endotoxins (LPS) of Gram-negative bacteria are known to have various effects on the immune system. They stimulate the B-lymphocytes, they can play an essential role in adjuvancy, and might elicit even a small synthesis of specific antibody [2-4].

In mice both spleen and peripheral lymphocytes respond to LPS stimulation by blastogenesis and antibody production. In humans the best stimulation by LPS was found in lymphoid cells from the spleen, lymph nodes, tonsils and the bone marrow, but it was ineffective in stimulating human, peripheral lymphocytes (HPL) to blast transformation [4-7].

The finding that LPS treatment might increase the antibody secretion, in spite of its slight effect on blast transformation in lymphocytes from human tonsils and in HPL points to an effect of LPS on a subpopulation of B-lymphocytes present among the lymphocytes.

LPS is known to be toxic, a fact greatly inhibiting its practical use. Promising are, however, the results obtained in experiments with radiodetoxified endotoxin (rdLPS), in vivo. Apart from a significant adjuvant activity, enhanced regeneration of the immune system could be observed under the influence of radiodetoxified endotoxin following radiation injury [8, 9]. The complement inactivating effect of parent endotoxin was much weaker when rdLPS was applied; its hypotensive effect was also considerably weaker

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[10]. The above mentioned advantageous changes together with other favourable features of rdLPS have been summarized by Bertok [11].

The present paper describes the stimulation of HPL in vitro, by parent LPS and rdLPS, detoxified with ionizing radiation.

Materials and methods

Cell suspension. Venous blood from healthy donors was used in the experiments. Samples were collected in 500 ml sterile blood transfusion bottles containing 10 000 U/litre heparin (National Institute for Haematology and Blood Transfusion). The mononuclear cells were obtained from blood diluted 1:1 with physiological saline and centrifuged in Ficoll-Uromiro gradient.

Cell culture, maintenance fluid and reagents. Lymphocytes were washed in PBS 3 times and cultured in Parker's 199 medium supplemented with penicillin (100 000 U/litre), 10% inactivated AB serum or autologous serum, and glutamine (100 mg/litre) in Falcon tubes in air containing 5% $C0_2$ for 3-6 days at 37 °C. Each culture contained 5x10" cells in 5 ml volume.

The sera were obtained from healthy humans of blood group AB. Following inactivation at 56 °C, they were stored at —20 °C.

Endotoxin preparation. Westphal extraction [12] from *Escherichia coli* 089 was used (LPS).

Radiodetoxified endotoxin. Westphal extraction was irradiated by a 50 kGy ^{60}Co gamma dose (rdLPS) [10, 11].

Phytohaemagglutinin. (PHA-P) Difco Bacto.

Stimulation. In 5 ml Parker's 199 medium $5X10^{G}$ lymphocytes were incubated together with 10-100 {ig LPS and rdLPS in the presence of 10% AB or autologous sera. Four hours before concluding the incubation, the cells were labelled with 1 juCi ^{3}H thymidine/culture (Prague, Czechoslovakia, 1 Ci/mmol). After labelling, the cells were washed in physiological saline, 5% TCA and 96% ethanol, then after extraction by hyamine-hydroxyde the radioactivity incorporated in the lymphocytes was measured in a Diotol cocktail, using a liquid scintillation spectrometer. Kesults and the standard error are given in dpm.

Results

The kinetics of LPS stimulation did not indicate any significant increase in DNA synthesis before the 5th day. After the 5th day the DNA synthesis enhancing effect of the tested LPS increased (Fig. 1). Stimulation by both unirradiated and irradiated LPS measured on the basis of the incorporation of tritiated thymidine given in dpm, reached its maximum at a dose of 10 цg LPS/culture and in the case of rdLPS at a dose of 50-100 /ug/culture on day 6, examined in the presence of AB serum (Fig. 1).

A study of the stimulatory effect of detoxified and non-detoxified LPS in the presence of AB and autologous sera (Table I) showed that it was highly dependent on the type of serum in the case of parent LPS. The stimulatory value obtained with AB sera was higher than in the presence of autologous serum in the case of parent LPS, while the stimulation by rdLPS seemed to be independent of the type of serum. Thus, in the presence of autologous serum, rdLPS in a dose of 100 /tig/culture elicited a stronger response than LPS in a dose of 10 ^g/culture.

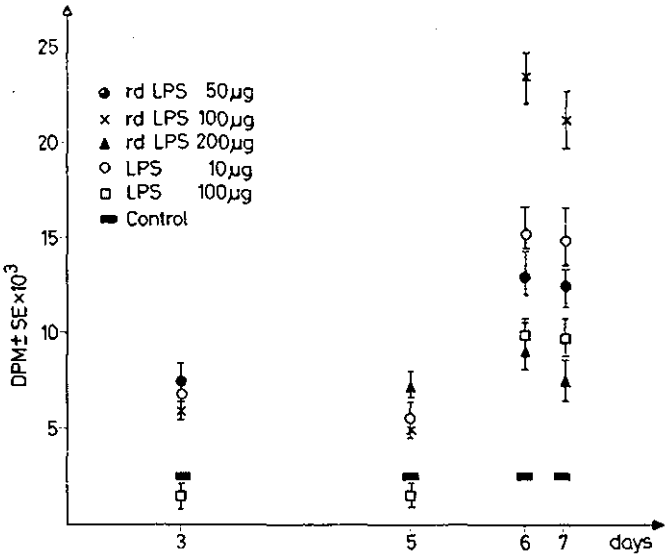

Fig. 1. Kinetics of the stimulatory effect of LPS. DNA synthesis by human peripheral lymphocytes in the presence of 10% AB serum was measured by ^{3}H-thymidine incorporation with different LPS doses

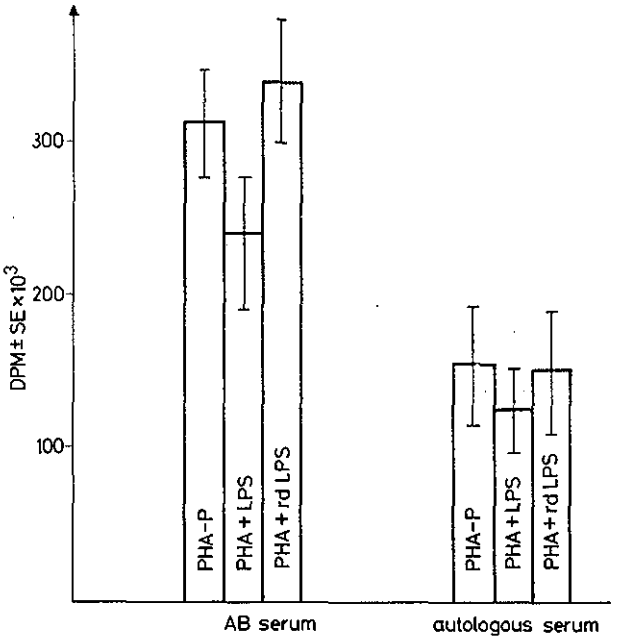

Fig. 2. Combined effect of PHA and LPS on the stimulation of human peripheral lymphocytes

The effect of LPS on the mitogenic response of lymphocytes induced by PHA in the presence of various sera, showed that rdLPS decreased the tritiated thymidine incorporation neither in the presence of AB nor of autologous sera. LPS decreased the PHA induced stimulation if used with AB or autologous serum (Fig. 2).

Table I

Effect of sera on the stimulatory characteristics of LPS

Serum	DPM ± S.E.		
	LPS	rdLPS	Control
AB	22.773±5.803	22.301±4.674	2.500±480
Autologous	14.613±2.584	21.239±4.145	2.841±700

Discussion

Several authors have reported on stimulation induced by LPS and on the degree of transformability in various lymphocyte cultures [2-6]. The kinetics of lymphocyte stimulation differs according to the origin of the lymphocytes (mouse or human). In humans it depends on their origin from a lymphoid organ, or pheripheral blood, and on the experimental circumstances [13]. Results showed maximal stimulation of HPL on days 6-7, while no stimulatory effect could be measured before the 5th day. These findings agree with several data in the literature [9, 13, 14].

In the case of parent endotoxin (LPS) the stimulation measured on the basis of tritiated thymidine incorporation was maximal in the presence of AB serum. The stimulatory effect of rdLPS, however, was independent of the type of the applied serum. This was probably due to the different toxicity of the two substances.

It is important to determine the optimal culturing conditions, to select a suitable serum. In order to obtain maximum stimulation, human serum must be added [9]. The stimulatory effect of LPS was more intensive in the presence of inactivated deep frozen AB serum than with autologous serum. Most authors have used commercially available calf or human sera [1-9]. These sera can promote the cell transformation induced by mitogens to reach the required level. Our experiments showed that mitogens exerted their maximal effect in the presence of inactivated freshly frozen AB serum. It is favourable that rdLPS showed a good stimulatory effect with both the applied serum types, and they did not inhibit the PHA induced proliferative response.

It is not easy to explain the results. It is possible that the lymphocyte stimulation elicited by LPS does not reach a measurable degree in the presence of autologous serum, only in the presence of AB serum.

Our results with various sera agree with the observation of Miller et al. [9] in that a better stimulation can be reached in the presence of pooled inactivated human sera. Using LPS in the presence of AB serum under the effect of LPS an inhibitory substance might be released by the macrophages which can prevent even the strong mitogenic effect of the PHA. It is considered a prostaglandin like effect [15], and the released substances inhibit T-helper cell activity [16].

Previous experiments have shown that the PHA-induced mitogenic response of lymphocytes of patients suffering from pseudomonas infection depends on the condition of the patient and on the applied serum of probably different endotoxin content [17, 18]. Our model experiments on lymphocytes from normal healthy humans were devised to find the explanation of these results. We have compared the effect of toxic endotoxin and detoxified endotoxin on the PHA induced transformation of lymphocytes in the presence of various sera. The activity of radiodetoxified endotoxin showed that it reaches and might even exceed the rate of stimulation of HPL induced by LPS, while it does not inhibit the PHA-induced stimulation in the presence of autologous sera.

These in vitro results might support the in vivo studies of Skarnes and HARPER [15] who reported on the prostaglandin release induced by LPS.

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