Mitogenic activity of different types of non-immunological inflammatory exudates on macrophages in culture

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As described in previous publications from our laboratories, rat peritoneal macrophages in culture could be stimulated to divide after treatment with an acute inflammatory exudate obtained 4 h after intrapleural injection of dextran (Adolphe, Fontagné, Pelletier & Giroud, 1975). Similar results were obtained on mouse macrophages treated with exudate obtained 4 days after implantation of diffusion chambers (Wynne, Spector & Willoughby, 1975).

The present report is an investigation into the effect of different types of acute inflammatory exudates on macrophage DNA synthesis in culture in order to confirm the presence of a 'macrophage mitogenic factor(s)' in other inflammatory reactions. For this purpose the activity of exudates obtained 4 h after intrapleural injection of dextran, λ-carrageenan, κ-carrageenan and calcium pyrophosphate were compared.

All experiments were performed on cultured normal rat peritoneal macrophages. After a 3-day culture period the medium was discarded and the macrophages treated with exudate diluted 50% in culture medium 199 supplemented with 20% calf serum. After a culture period of 3–4 days, macrophages DNA-synthesis was determined by autoradiography and the number of labelled cells counted.

The results show that of the four types of exudate tested all induced increased macrophage DNA-synthesis. However, differences were observed between the activity of these exudates. Firstly exudates induced by λ-and κ-carrageenan, and calcium pyrophosphate caused a greater increase in labelled cells than dextran exudates. This may be indicative of a lower level of 'mitogenic factor' in the dextran exudates, which contains relatively fewer cells and a lower exudate protein concentration compared with carrageenan and calcium pyrophosphate induced exudates. Secondly carrageenan exudates were also observed to stimulate higher binucleate cell formation than in control macrophage cultures.

It is concluded that the observed stimulation of macrophage DNA-synthesis induced by the four types of inflammatory exudate may be due to the release of an inflammatory mitogenic factor during the acute reaction, the production of which may be related to the presence of leucocytes in the exudate.

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Dose-dependent desensitization of lymphocyte beta-receptor function after long-term culture with isoprenaline

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Szentivanyi (1968) has proposed that asthmatics have an inherent partial blockade of β-adrenoceptor mechanisms. Several investigators (Parker & Smith, 1973; Logsdon, Middleton & Coffey, 1972; Alston, Patel & Kerr, 1974) have reported data from studies of lymphocytes or mixed leukocytes which would appear to support this theory. However, most of the asthmatics studied were receiving bronchodilators acting on β-adrenoceptors and possible effects of this therapy have not been fully considered.

Desensitization to the action of catecholamines by prior exposure to these agents has been shown in fibroblasts (Franklin, Morris & Twose, 1975), adipocytes (Ho & Sutherland, 1971; Smith, Isaakson, Nyberg & Sjöström, 1976), macrophages (Remold-O'Donnel, 1974) and C_6 astrocytoma cells (Browning, Brostrom & Groppi, 1976). The present study was designed to examine β-adrenoceptor function in lymphocytes after prolonged exposure to isoprenaline *in vitro*.

Lymphocytes from normal subjects on no medication were cultured in TC 199 for 24 h without and with isoprenaline 1×10^{-8} mol/l to 1×10^{-6} mol/l. The cells were washed, equilibrated with Hanks balanced salt solution and incubated for 15 min with isoprenaline 1×10^{-4} mol/l and theophylline

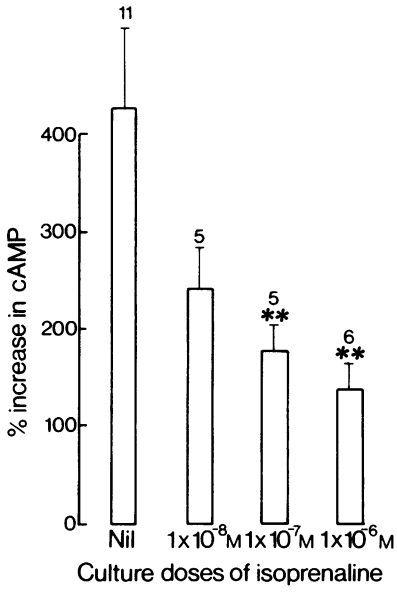

Figure 1 Mean percentage increase ± s.e. mean in cyclic AMP in response to isoprenaline (1×10^{-4} M) in lymphocytes cyltured without and with isoprenaline. Difference from control: ** significant at the 0.01% level compared to cells with no isoprenaline in culture (paired *t*-test). Figure at top of columns indicates number of experiments.

1×10^{-2} mol/l. Cyclic AMP was assay by the Gilman method (1970) as a measure of β-adrenoceptor function. Cells cultured with isoprenaline were significantly less responsive to β-adrenoceptor stimulation in the 15 min incubation and this desensitization increased as cells were cultured with higher concentrations of the drug (see figure). Lymphocytes from three asthmatics were studied similarly and showed no qualitative differences. Cells were also incubated with PGE_1. Despite prior culture with isoprenaline, the response to PGE_1 at 10.0 µmol/l was not changed, although there was a slight but not significant reduction in the response to PGE_1 at 0.5 µmol/l. Phosphodiesterase activity was examined, but was found not to be increased in cells cultured with isoprenaline.

It is concluded that lymphocyte β-adrenoceptor function, as assayed by cAMP production, can be specifically desensitized by prior exposure to β-adrenoceptor stimulant.

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