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A comparative study of lysosomal enzyme activity in monocytes and Kupffer cells isolated simultaneously in a rat model of liver injury

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SUMMARY

Macrophages have been isolated and cultured *in vitro* from normal rat livers and from livers into which macrophages have been recruited *in vivo*, following an intravenous injection of killed *Corynebacterium parvum*. Simultaneously, peripheral blood monocytes have been isolated and cultured *in vitro*. After 24 hr in culture, supernatants and cell lysates were harvested and the activity of a lysosomal enzyme, *N*-acetyl-glucosaminidase (NAG), measured. NAG activity in the cell lysates of the recruited tissue macrophages was significantly higher than that measured in control tissue macrophages. Increased NAG activity was also observed in the supernatants from the recruited macrophages. In contrast, the NAG activity in cell lysates and supernatants of peripheral monocytes was not significantly changed after *C. parvum* injection. In this animal model, measurement of a lysosomal enzyme produced by peripheral monocytes did not reflect the magnitude of the changes observed for the tissue macrophages.

INTRODUCTION

Lysosomal *N*-acetyl-glucosaminidase activity is one of many parameters that have been measured in the assessment of macrophage activity (North, 1978; Karnovsky & Lazdins, 1978; Cohn, 1978). Several workers have studied production of acid hydrolases by peripheral monocytes cultured *in vitro* from subjects with chronic inflammatory diseases (Ganguly *et al.*, 1978; Mee *et al.*, 1980). However, the assumption that peripheral monocytes reflect the activity of tissue macrophages and hence their potential for promoting tissue damage remains uncertain.

We have utilized a rat model of liver inflammation, in which macrophages have been recruited into the liver following an intravenous injection with killed *Corynebacterium parvum* (Ferluga & Allison, 1978). By measuring the proteolytic enzyme NAG produced from both peripheral monocytes and tissue macrophages that have been isolated simultaneously and cultured *in vitro*, we hoped to determine whether peripheral monocytes accurately reflect tissue macrophage activity.

MATERIALS AND METHODS

Animals. Twelve adult female Wistar rats, each weighing 250–300 g, were used. Six rats comprised the control group and six received an intravenous injection into a tail vein of 1 ml of killed *C. parvum* (7 mg/ml, Wellcome) 6 days before killing.

Experimental procedure. Liver macrophages were isolated using a method described in detail

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elsewhere (Crofton *et al.*, 1978; Emeis & Planqué, 1976) with minor modifications as indicated below. All animals were anaesthetized with ether alone. A midline abdominal incision was made and the gut exteriorized. A loose ligature was applied to the portal vein, which was cannulated and perfused over 1 min with 10 ml of 0·2% pronase AS (Sigma) in Ca^{++}- and Mg^{++}-free Hanks' balanced salt solution (HBSS; GIBCO), supplemented with 2 ml HEPES buffer (Wellcome), streptomycin (100 μg/ml), benzyl penicillin (100 iu/ml) and amphotericin B (250 μg/ml) per 100 ml HBSS.

Simultaneous cannulation of the inferior vena cava was performed to obtain 10–15 ml of venous blood into a heparinized syringe. The liver was removed completely, weighed, minced thoroughly and suspended in 100 ml of the pronase solution. The suspension was mixed with a magnetic stirrer, maintained at 37°C and at a pH of $7{\cdot}35 \pm 0{\cdot}05$. At 15 and 40 min, DNase, 0·5 mg (type I Sigma) was added to the suspension. After incubation for 1 hr, the digested mixture was centrifuged at 400 ***g*** for 12 min and the sedimented cells washed in HBSS.

Liver macrophage and monocyte separation. This cell preparation was resuspended in 5 ml RPMI 1640 (GIBCO) and centrifuged on lymphocyte separation medium (Flow Laboratories), density 1·077 (Böyum, 1968), at 600 ***g*** for 15 min at 4°C. The interface non-parenchymal cells obtained were washed in HBSS and finally resuspended in 10 ml of RPMI 1640, containing 0·5% lactalbumin hydrolysate (LOH) (Sigma), together with antibiotics and amphotericin B. Liver macrophages were obtained by adherence overnight in plastic multiwell trays (Linbro) and non-adherent cells were discarded. Two separate wells containing sterile glass coverslips were prepared to assess the purity of the glass-adherent cell population by phagocytosis. After overnight culture and removal of non-adherent cells by washing with HBSS, the wells containing coverslips were incubated for 1 hr at 37°C with a suspension of 0·81-μm latex particles (DIFCO) in RPMI at an approximate particle–cell density of 50:1 and then inspected under phase-contrast microscopy. Cells containing more than three latex particles were regarded as phagocytic macrophages.

Peripheral monocyte separation was performed using the method of Böyum (1968) and purified by adherence to plastic. Cell counts were performed using a Neubauer chamber and viability assessed using trypan blue. Phagocytosis by these cells was assessed as described above for tissue macrophages.

Culture system. The adherent cells ($1–2 \times 10^6$ cells/well) were obtained by washing the multiwell trays after overnight culture. The cells were then resuspended in 1·5 ml of RPMI supplemented with 0·5% LOH and incubated at 37°C in a 5% CO_2 humidified chamber. All cultures were done in duplicate. Twenty-four hours later, supernatants were collected and cell lysates harvested by adding 1·5 ml of sterile water to each well and scraping the wells thoroughly with a 'rubber policeman'.

NAG and protein assay. A sensitive assay for NAG, suitable for the enzyme in the cell lysates and supernatants, was developed from the spectrofluorometric method of Beutler *et al.* (1976) with modifications described by Ganguly *et al.* (1978). The cell suspensions were rapidly frozen and thawed six times before assay. All cell lysate results were expressed as a specific activity. The protein concentration of the cells was determined by the method of Lowry *et al.* (1951).

Esterase staining. Cytocentrifuge preparations of appropriate cells were stained for non-specific esterase, using the method as described by Horwitz *et al.* (1977).

Statistics. An independent *t*-test was utilized for all analyses.

RESULTS

Cell isolation

The number of non-parenchymal cells isolated from both normal rat livers and livers into which macrophages had been recruited following an intravenous injection of *C. parvum* demonstrate the increased yield from the latter group; normal livers, $10{\cdot}9 \pm 0{\cdot}8 \times 10^6$ cells/g digested liver weight; livers of *C. parvum*-injected animals, $41{\cdot}6 \pm 6{\cdot}9 \times 10^6$ cells/g digested liver weight. Of these cell populations, $71 \pm 2\%$ and $70 \pm 3\%$ respectively stained for non-specific esterase representing both macrophages (*ca* 28%) and endothelial cells. Viability of these cells as assessed by trypan blue exclusion was $89 \pm 2\%$ and $93 \pm 2\%$ for the respective cell populations. The purity of the adherent cell populations derived from this non-parenchymal cell preparation was assessed by latex phagocy-

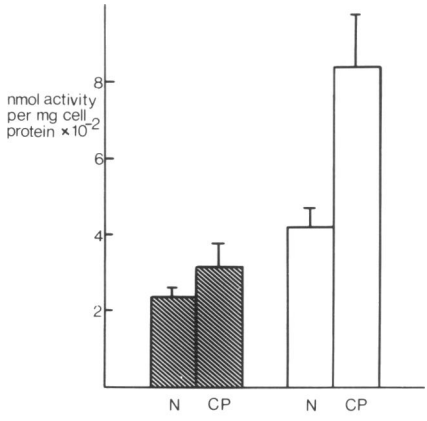

Fig. 1

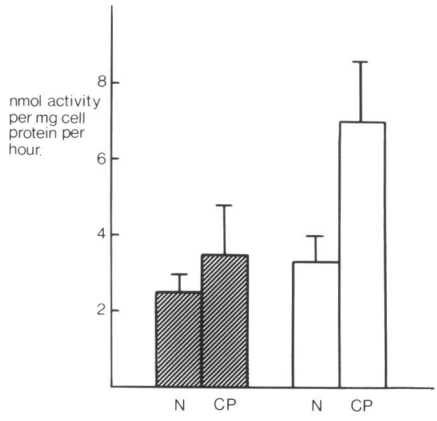

Fig. 2

Fig. 1. *N*-acetyl-glucosaminidase (NAG) activity (mean ± s.e.m.) in cell lysates expressed as a specific activity, nmol activity/mg cell protein. Hatched histograms represent the NAG activity from peripheral blood monocytes and open histograms represent the NAG activity from macrophages isolated from the liver. N = cells isolated from normal rats, CP = cells isolated from rats previously injected with *C. parvum*, $n = 6$ in each group.

Fig. 2. *N*-acetyl-glucosaminidase activity (mean ± s.e.m.) in the supernatants (nmol activity per mg cell protein per hr). A constant rate of release over the 24-hr period of culture has been assumed. See legend to Fig. 1 for explanation of symbols.

tosis. After overnight culture and washing, 89 ± 3% of the cells from control rats, compared to 84 ± 4% of the cells from *C. parvum*-injected rats, were phagocytic.

The eventual macrophage yield has been derived from the percentage of phagocytic cells of the adherent population, since non-specific esterase staining does not distinguish macrophages from endothelial cells in this situation (Emeis & Planqué, 1976). The adherent cell populations were counted after removal of the cells from the wells with 0·7% lignocaine in RPMI. The respective yields of liver macrophages were $3{\cdot}1 \pm 0{\cdot}2 \times 10^6$ cells/g digested liver weight for the normal rats and $11{\cdot}9 \pm 0{\cdot}9 \times 10^6$ cells/g digested liver weight for the *C. parvum*-injected rats.

The yield of mononuclear cells from the blood of either group of rats was not different; $4{\cdot}3 \pm 0{\cdot}4 \times 10^6$ cells/ml of blood from normal rats, $4{\cdot}5 \pm 0{\cdot}4 \times 10^6$ cells/ml of blood from *C. parvum*-injected rats, and the percentages of these cells staining for non-specific esterase were also similar, 38 ± 4% and 42 ± 3% respectively. After overnight culture, > 95% of adherent cells phagocytosed latex particles.

NAG activity in cell lysates and supernatants from macrophages and monocytes

After 24 hr in culture, NAG activity in cell lysates expressed as a specific activity/mg cell protein (Fig. 1) of the recruited macrophages was significantly higher than in control tissue macrophages ($P < 0{\cdot}05$). Increased NAG activity in the supernatants (Fig. 2) was also observed for the recruited macrophages. Since these results have been expressed as specific activities, variations in cell adherence and counts at the termination of the cultures have been excluded as a source of error. As expected for a lysosomal enzyme, the cellular activity of NAG was greater than that observed in the supernatant. The respective NAG values obtained from cell lysates and supernatants of peripheral blood monocytes isolated from *C. parvum*-injected animals showed some increases compared to normal blood monocytes – 35% increase for cell lysates (Fig. 1) and 40% increase for supernatants (Fig. 2) – but these increases were minor in comparison to the differences observed for the tissue macrophages (97% increase in cell lysates and 112% increase in supernatants for recruited cells).

DISCUSSION

There are many parameters that have been measured to assess monocyte–macrophage function. These include production of lysosomal enzymes and neutral proteases, phagocytosis, bacterial killing and chemotaxis (North, 1978; Karnovsky & Lazdins, 1978; Cohn, 1978; Territo & Cline, 1977).

A role in the pathogenesis of chronic inflammation by lysosomal proteolytic enzymes has been proposed by Davies & Allison (1976). A recent study by Ganguly *et al.* (1978), evaluating subjects with a variety of chronic inflammatory states, demonstrated a rise in the NAG activity of supernantants and cell lysates from monocytes exposed *in vitro* to endotoxin and zymosan. Mee *et al.* (1980) examined monocytes exposed *in vitro* to immune complexes and endotoxin in subjects with inflammatory bowel disease. Stimulation with immune complexes produced a fall in cell lysate NAG activity without alteration in supernatant values; after endotoxin, cell lysate values were unchanged, but supernatant levels rose over a period of time. These results are difficult to interpret, but both studies have relied on the assumption that peripheral monocyte lysosomal acid hydrolase output reflects tissue macrophage activity at the site of inflammation.

The model of hepatic inflammation used in this study has been previously described by Ferluga & Allison (1978). Intravenous injection of *Corynebacterium parvum* results in a mononuclear cell portal tract infiltrate together with epithelioid granulomas scattered throughout the lobule. The results for our cell isolations and yields reflect the increased number of non-parenchymal cells present within the liver of the experimental model.

The aim of this study was to determine whether measurement of the NAG activity produced by peripheral monocytes in *in vitro* culture accurately reflects the activity of this enzyme at the tissue level, where resident macrophages may promote continuing tissue damage in a specific or non-specific manner (Davies & Allison, 1976). We have measured a significant rise in the cellular NAG activity of recruited liver macrophages and a rise in the corresponding supernatant NAG activity. However, the peripheral monocytes from these injected rats, although demonstrating minor rises in NAG activity, did not reflect the large changes observed for the recruited tissue macrophage.

In this model, therefore, observations on the recently recruited cell population present at the site of tissue damage provide a more accurate reflection of the potential for tissue damage. Although only one function of macrophage activity has been measured in this study, assessment of other tissue macrophage functions may provide more precise information on the pathogenesis of tissue injury. In this study, assessment of peripheral monocytes has provided some information on the state of activity of macrophages at the site of inflammation but has not indicated the magnitude of change observed in the tissue macrophages.

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