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XANTHINE OXIDASE INCREASE IN POLYMORPHONUCLEAR LEUCOCYTES AND MACROPHAGES IN MICE IN THREE PATHOLOGICAL SITUATIONS

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Abstract—Polymorphonuclear leucocytes and peritoneal macrophages from mice infected with *S. aureus* or *P. berghei*, or inoculated with Ehrlich solid carcinoma show a significant increase in xanthine oxidase (XO) levels. Characteristic time curves, similar to the corresponding time curves observed with liver, have been obtained for each pathological situation. The magnitude of the increase in XO activity suggests that it may be a natural defence mechanism, although it does not appear to be specific to the pathological condition.

A substantial increase in xanthine oxidase (XO) activity has been reported in the liver of mice inoculated with Ehrlich carcinoma cells, or infected with either bacteria or protozoa [1]. This is a persistent, but not permanent, effect with a time course typical for each pathological situation studied. Exogenous XO administered subcutaneously (to mimic endogenous XO) to mice with bacterial infections results in increased survival time, whereas allopurinol, a specific inhibitor of XO, accelerates the course of the infection in a linear dose–effect relationship [2]. These findings support the concept previously suggested [2, 3] of a natural defence mechanism involving XO. To investigate the phenomenon further we studied XO levels in polymorphonuclear leucocytes (PMN) and in macrophages (MC) using the three pathological situations previously employed, i.e. bacterial or protozoal infection, or inoculation with Ehrlich carcinoma.

MATERIALS AND METHODS

Swiss CD_1 albino mice (Charles River) were injected with a culture of *Staphylococcus aureus* CN6538 (i.v.), *Plasmodium berghei* I.S.S. (i.p.) or Ehrlich hyperdiploid carcinoma cells (s.c. into the axillary cavity).

Polymorphonuclear leucocytes were elicited using the method of Drath and Karnowsky [4] by i.p. injection of 1.5 ml of 12 % (w/v) sodium caseinate. The cells were harvested 16 hr later by double lavage with 0.1 M phosphate buffer (pH 7.8), washed, resuspended in the same medium and counted. They were then disrupted by freezing and thawing according to the method of Quie *et al.* [5]. The preparation was clarified by centrifugation.

Macrophages were elicited using the same techniques; the cells were harvested 72 hr after i.p. injection of 1.5 ml of 1.2 % (w/v) sodium caseinate.

XO levels were determined spectrophotometrically using the method of Fried [6], and a standard curve was plotted as described by Nachlas [7]. Reactions were stopped using allopurinol 0.4 M. The effect of superoxide dismutase (SOD) in infected and control animals was determined in parallel experiments. XO activity in polymorphonuclear leucocytes was determined in the presence or absence of a fixed amount of SOD (768 U/ml) in groups of 5 mice infected i.v. 5 days earlier with *S. aureus* CN 6538 (2.5×10^8 microorganisms/0.1 ml/10 g), and in groups of 5 control animals. XO activity was determined, according to the method of Fried [6], as mcg of iodoformazan/10^6PMN/min at 37°.

XO levels in polymorphonuclear leucocytes were determined in 6 control animals, and in two groups of 6 mice (body wt 20 ± 1 g) infected (i.v.) with 2.5×10^8 *S. aureus*/0.1 ml/10 g or with 3.25×10^8 *S. aureus*/0.1 ml/10 g, respectively. Polymorphonuclear leucocytes were elicited as described above, on alternate days, collected and washed; XO content was determined and expressed as a percentage increase relative to the controls. Similarly, XO levels in peritoneal macrophages were determined in 8 control animals and 8 mice infected (i.v.) with 1.25×10^8 *S. aureus*/0.1 ml/10 g.

XO levels in polymorphonuclear leucocytes were determined both in controls and in mice (body wt 20 ± 1 g) infected i.p. with 6.2×10^4 erythrocytes/0.1 ml/10 g taken from mice on the 5th day after infection with *P. berghei*. PMN's were elicited on alternate days, collected and washed; XO levels were determined and expressed as a percentage increase relative to the controls. Each experimental group consisted of 12 animals, although in the infected group, due to coma and eventual death of some animals, measurements were restricted to 9 mice on day 9 and 4 mice on day 11.

Using the same techniques, XO levels in macrophages were determined in 6 control animals and in 6 animals infected with 6.0×10^4 erythrocytes/0.1 ml/10 g taken from mice on the 5th day after infection with *P. berghei*.

XO levels were measured both in polymorphonuclear leucocytes and in macrophages in groups of 8 mice inoculated s.c. with Ehrlich solid carcinoma (3.0×10^6 cells/0.1 ml/10 g) and in 8 control animals

(body wt 20 ± 1 g). As before, the polymorphonuclear leucocytes or macrophages were elicited on alternate days, collected and washed; XO levels were determined and expressed as a percentage increase relative to the controls.

RESULTS

The effect of bacterial infection on XO levels in polymorphonuclear leucocytes and peritoneal macrophages are shown in Figs. 1 and 2. In PMN's an approximately 4-fold increase in XO levels was observed on the 4th or 5th day after infection, depending upon the number of microorganisms administered. This peak was followed by a progressive decrease until the original levels were reached on day 15 (Fig. 1).

A significant increase in XO levels was also observed in macrophages, although it was less pronounced, but prolonged compared with the response in polymorphonuclear leucocytes.

Similar elevated values for XO were observed both in PMN's and in macrophages taken from mice infected with *P. berghei* (Fig. 3). No conclusions can be made, however, on the persistence of the effect, as the animals became severely ill after day 7, and death eventually occurred.

In mice inoculated with Ehrlich carcinoma cells (Fig. 4), significant induction of XO occurred in macrophages. The response in polymorphonuclear leucocytes was negligible.

To determine any possible correlation between XO induction in liver and in phagocytes, the correlation coefficients were calculated from time curves of XO induction (following infection with *S. aureus* or *P. berghei*) in PMN's and macrophages, and compared with those previously obtained in the liver [1] (Table 1). Correlations using data from mice inoculated with Ehrlich carcinoma were not made, as different tumour types were used in the two experiments (ascitic and solid type).

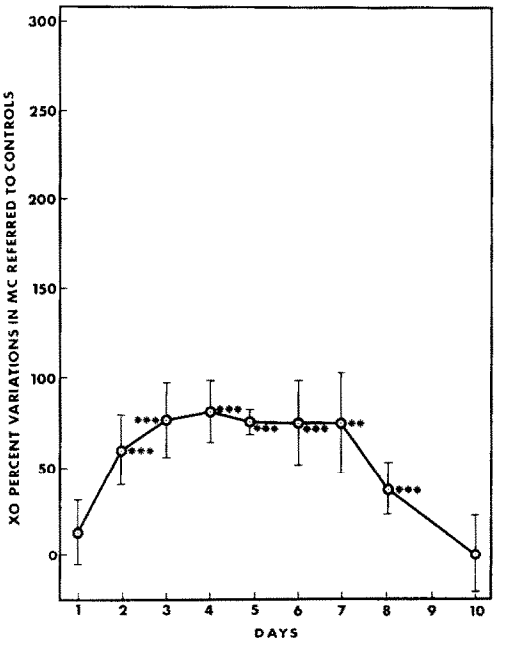

Fig. 2. XO levels in peritoneal macrophages in mice infected i.v. with *S. aureus*. *** $P < 0.001$, ** $P < 0.01$.

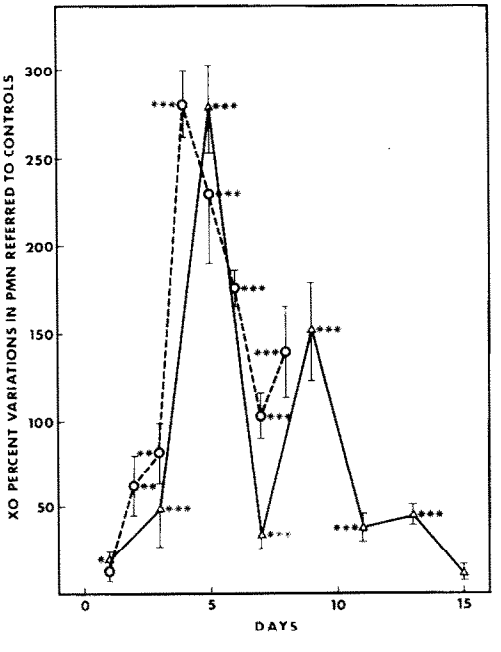

Fig. 1. XO Levels in PMN In mice infected i.v. with *S. aureus*. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$. Dotted line refers to a second experiment.

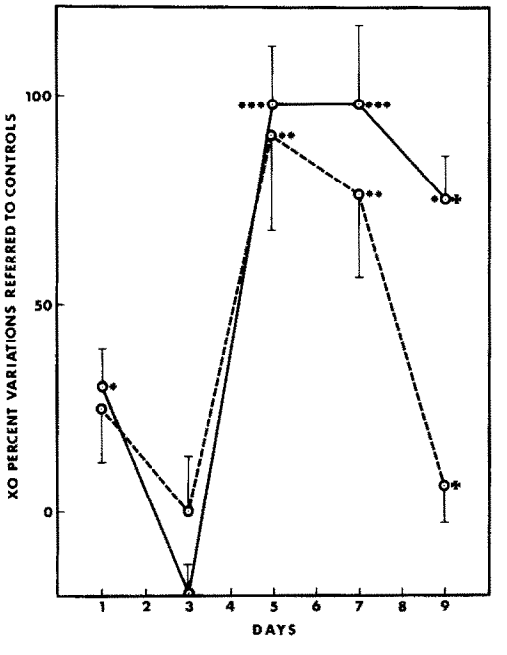

Fig. 3. XO levels in PMN (straight line) and macrophages (dotted line) in mice infected i.p. with *P. berghei*. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, ✠ Death occurred on following day.

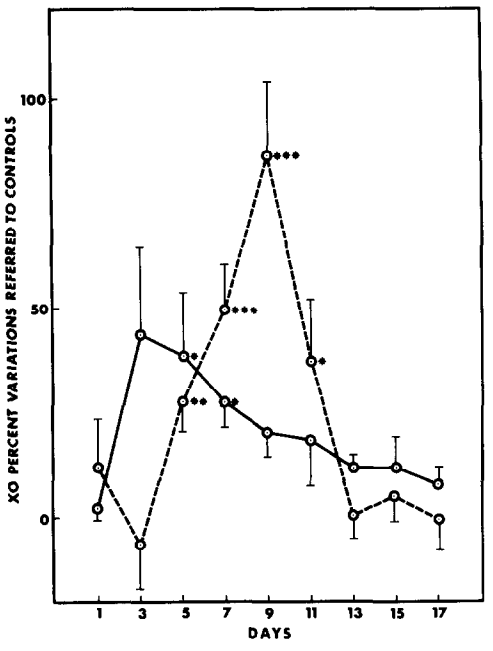

Fig. 4. XO levels in PMN (straight line) and macrophages (dotted line) in mice inoculated with Ehrlich solid carcinoma. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

In order to repeat the findings of our previous studies in liver [1], that the enzyme activity occurred mainly as the oxidase, XO determinations were carried out in disrupted polymorphonuclear leucocytes from normal and infected animals, in the presence or absence of superoxide dismutase (Table 2).

Both in the control and in the infected animals, SOD significantly inhibited XO activity, higher values of enzyme activity being observed in the infected group. Thus, the results obtained in liver confirmed that the enzyme activity occurs principally as the oxidase. For polymorphonuclear leucocytes at least, this appears to eliminate the possibility that the increase in enzyme activity is due to conversion of dehydrogenase into oxidase in response to infection.

DISCUSSION

The results indicate that a bacterial or protozoal infection produce significant increases in XO levels both in polymorphonuclear leucocytes and in macrophages; inoculation of carcinoma cells produces a corresponding effect in macrophages but not in PMN. Each pathological condition appears to have a characteristic time curve for XO induction similar to the corresponding time curve observed in the liver.

Statistical analysis of XO induction curves in phagocytes, following either bacterial or protozoal infection, shows a correlation with XO induction curves in the liver. The delayed peak XO values in some of the phagocyte curves compared with those of the liver indicate the possible passage of the enzyme from the liver to the phagocytes.

The significant increases in XO activity both in macrophages and, particularly, in polymorphonuclear leucocytes following bacterial infection of mice is in accord with the known role of polymorphonuclear leucocytes during bacterial infection. Similarly, the increase in XO activity, observed only in macrophages from mice inoculated with Ehrlich hyperdiploid carcinoma cells, is not unexpected since it is well known that macrophages play a role in tumour resistance. This is modified by serum inhibiting or enhancing factors [8, 9] and may be demonstrated by the use of antimacrophage agents acting as promoters of tumour growth [10].

It is known that destruction of bacteria by phagocytes is due to the generation of O_2^-, H_2O_2 or the product of the reaction between them [11]; this may be shown using SOD or catalase which suppresses bacterial destruction by phagocytes without interfering with phagocytosis itself. Although the final

Table 1. Correlation coefficients from time curves of XO induction

Type of infection	Comparisons*	Regression coefficient	t
	Liver vs PMN	0.89	4.74†
S. aureus	PMN vs MC	0.49	1.40
	Liver vs MC	0.36	0.94
	Liver vs PMN	0.93	5.54†
P. berghei	PMN vs MC	0.79	3.35†
	Liver vs MC	0.91	4.84‡

* Comparisons between curves obtained in the experiments using *S. aureus* were made by superimposing the curves in order to abolish the 2 day differences in the maxima.
† $P < 0.01$.
‡ $P < 0.001$.

Table 2. Effect of SOD on XO levels in disrupted PMN's

Experimental group	XO Activity		Inhibition (%)
	No external SOD	+ SOD	
Controls	0.53 ± 0.07	0.13 ± 0.03	75.5
Infected animals	1.22 ± 0.28	0.28 ± 0.09	77.1

stages of the oxidative pathway are known to depend upon SOD and catalase, the initial stages, i.e. the major physiological sources, are yet to be identified [12]. Our results suggest that XO, considered until now only as a possible producer of O_2^- and H_2O_2 [13], might participate in the oxidative processes during phagocytosis.

The magnitude of the increase in XO activity in polymorphonuclear leucocytes and macrophages suggests that this may be a natural defence mechanism; further, as the effect occurs in response to three different challenges, it appears to be non-specific in nature.

It seems possible that an increase in the oxidative activity of phagocytes may be a non-specific defence mechanism against infection which occurs during the lag phase of the immunological response.

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