Free Rad. Res. Comms., Vol. 4, No. 3, pp. 183–188
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Printed in Great Britain

CHANGES IN GLUTATHIONE PEROXIDASE ACTIVITIES AND THE OXIDATIVE BURST OF LEUKOCYTES DURING INFLAMMATION IN THE MOUSE AND RAT

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(Received February 27th, 1987)

The relationship between glutathione peroxidase (GSH-Px) activity and opsonized zymosan-induced chemiluminescence (CL) has been studied with exudate leukocytes obtained at different times after induction of inflammatory responses in the mouse peritoneal cavity with heat-killed *Corynebacterium parvum* and in the rat pleural cavity with λ-carrageenin. GSH-Px activity in mouse peritoneal exudate cells fell markedly after 2–4 h, returning to normal within 1–2 days. The lowered enzyme activity was associated with an increased ability of the cells to generate CL. Rat pleural exudate cells exhibited a slight fall in GSH-Px activity after 6 h which increased to supranormal levels within 1–2 days. During this period the ability of the cells to generate CL continually increased. The data indicate that during the early phase of increased generation of reactive oxygen species (ROS) by inflammatory leukocytes, the intracellular protective mechanism, represented by GSH-Px, is compromised. Subsequently, GSH-Px activity increases to or above initial levels possibly due to the presence of mononuclear cells and/or as a response to the increased generation of ROS.

KEY WORDS: Glutathione peroxidase, chemiluminescence, leukocytes, inflammation.

ABBREVIATIONS USED: BSS, balanced salt solution; CL, chemiluminescence; C. parvum, Corynebacterium parvum; GSH-Px, glutathione peroxidase; ROS, reactive oxygen species; SOD, superoxide dismutase.

INTRODUCTION

The generation of reactive oxygen species (ROS) by stimulated leukocytes has been the subject of intensive recent investigation and these reactive products are now considered to be important mediators of inflammatory responses.[1,2] Much less, however, is known about the changes in the activities of enzymes which scavenge ROS at sites of inflammation. In two reports on activities of scavenging enzymes in synovial fluid of patients with rheumatoid arthritis, superoxide dismutase (SOD) activities were low as were catalase activities,[3,4] though one group of authors found increased catalase activity in fluid from rheumatoid arthritis patients as compared to control fluid.[4] In this latter report, glutathione peroxidase (GSH-Px) activities were also slightly enhanced in rheumatoid synovial fluid, but remained biologically negligible.

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Several authors have studied the effects of selenium deficiency (and thereby a decrease in the activity of the selenium-containing endogenous GSH-Px) in inflammatory responses.[5] Recently we have reported that feeding mice a selenium-deficient diet results not only in a decrease in macrophage GSH-Px activity, but also increases H_2O_2 generation by these cells.[6] Furthermore, selenium levels have been reported to be lowered in a number of inflammatory and related diseases.[7] We have now investigated further the relationship between local ROS generation and GSH-Px activities at inflamed sites by comparing the production of chemiluminescence by and GSH-Px activities in leukocytes removed at different times from the inflamed mouse peritoneum and rat pleural cavity.

MATERIALS AND METHODS

Animals

Male inbred CBA or DBA mice (weighing approx. 20 g) were obtained at 6 weeks of age from Dr. Ivanovas, Kisslegg in Allgäu, F.R.G. and male Han Wistar rats (weighing approx. 120 g) from Hagemann, Extertal, F.R.G. All animals were kept in Makrolon cages and had access to food and water *ad libitum*.

Mouse Peritoneal Exudate Cells

Inflammatory exudate cells were obtained from mice by i.p. lavage with phosphate buffer (PBS), pH 7.4, either from normal mice (resident macrophages) or at different times after the i.p. injection of *Corynebacterium parvum* (1.4 mg in 0.2 ml; Deutsche Wellcome, Burgwedel, F.R.G.), as previously described.[8] Viability, determined by acridine orange fluorescence, was > 90%. Resident cells consisted of 95–99% macrophages, as determined by Pappenheim staining. Cell smears were also stained (Giemsa) after harvesting at different times after *C. parvum* and differentiated as described below.

Rat Pleural Exudate Cells

Pleural leukocytes were obtained from rats following intrapleural injection of 0.1 ml 1% λ-carrageenin (Sigma, Munich). Animals were killed with ether at different times, the pleural cavity opened and exudate removed in 2 ml syringes, the volume determined and the exudate centrifuged for resuspension of cells. Control, peripheral blood polymorphonuclear leukocytes were obtained from normal rats by density centrifugation of citrated blood, as described previously.[9]

Glutathione Peroxidase Activity

After centrifugation at 300 g for 10 min, cells were resuspended in potassium phosphate buffer, pH 7, containing 1% Triton-X 100, to a final concentration of 3×10^6 cells/ml. Samples were stored overnight at −20°C before assay. Aliquots (0.5 ml) were taken for duplicate determination of GSH-Px activity by the method of Wendel[10] using 12 mmol/l t-butyl hydroperoxide (Fluka, Buchs, Switzerland) as substrate.

Leukocyte Chemiluminescence

Cells were resuspended to 5×10^6 cells/ml in balanced salt solution (BSS),[11] containing 2% bovine serum albumin. Aliquots (200 μl) of this cell suspension were added to tubes containing 100 μl BSS and 200 μl of the chemiluminescence generator, luminol (1×10^{-5} mol/l, end conc., Lumac, Schaesberg, The Netherlands), shaken and allowed to equilibriate at 37°C for 10 min in an LKB 1251 Luminometer. The reaction was initiated by the addition, with an automatic dispenser, of 100 μl opsonized zymosan (0.17 mg/ml, end conc.), preopsonized as described previously.[8] Chemiluminescence (CL), in mV, was recorded after 5 min. These responses were corrected by subtraction of background values obtained before addition of opsonized zymosan.

RESULTS

Mouse Peritoneal Exudate Cells

The peritoneal cavity of all mammalian species contains a resident population of macrophages. Resident macrophages from the mouse peritoneal cavity exhibited a relatively high activity of GSH-Px after cell breakdown with Triton X-100 and the intact cells generated little CL when stimulated with opsonized zymosan (Figure 1). Following the induction of an inflammatory response by the i.p. injection of heat-killed *C. parvum*, the GSH-Px activity of the peritoneal exudate cells fell markedly within the first 2-4 h, corresponding to an influx of polymorphonuclear leukocytes. Enzyme activity was still low after 24 h, but just failed to reach significance ($p = 0.057$) and gradually returned to normal within 2–3 days, despite the persistence of polymorphonuclear leukocytes in the exudate (Figure 1). In contrast, the generation of CL upon stimulation *in vitro* with opsonized zymosan, increased continuously after i.p. *C. parvum* injection, achieving a peak within 24 h, at which point the concentration of polymorphonuclear leukocytes in the peritoneal cavity was at its highest (Figure 1). CL generation fell slightly thereafter, but remained high for at least up to 8 days.

Rat Pleural Exudate Cells

The rat pleural cavity does not normally contain any leukocytes. After the intrapleural injection of λ-carrageenin a marked infiltration of polymorphonuclear leukocytes occurs. In comparison to similar cells isolated from normal rat peripheral blood, carrageenin-induced pleural exudate cells exhibited a markedly increased ability to generate CL in response to opsonized zymosan which continued to increase over the succeeding 48 h (Figure 2). When compared with peripheral blood cells, however, little change was observed in the GSH-Px activity of the pleural exudate cells within the first 3–6 h, except that a slight decrease was detected at 6 h, which was significant only in relation to the activity at 3 h (Figure 2). By 48 h after carrageenin injection, though, the GSH-Px activity of the pleural exudate cells was significantly higher than that of the peripheral blood polymorphonuclear leukocytes.

DISCUSSION

The data presented in this report clearly indicate that in the first few hours of an acute inflammatory response the intracellular GSH-Px activities of leukocytes fall in inverse

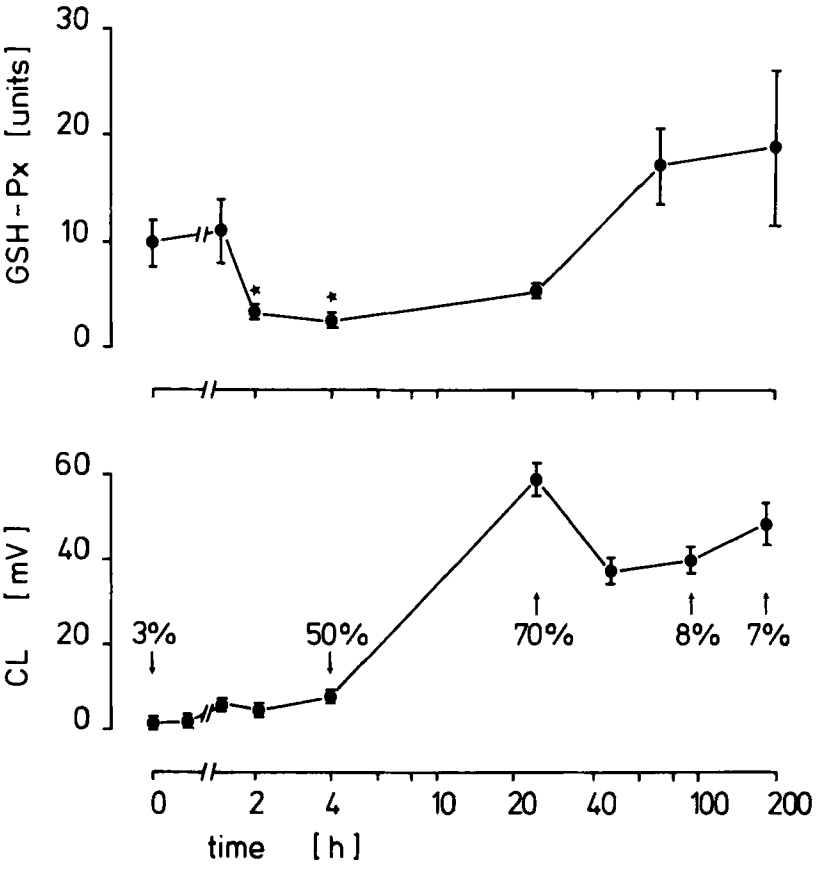

FIGURE 1 Changes with time in mouse peritoneal leukocyte GSH-Px activities and CL generation following i.p. injection of *C. parvum* at time zero. GSH-Px values are given as units per 1.5×10^6 cells, CL values as mV per 1×10^6 cells. The percentages indicate the proportion of polymorphonuclear leukocytes present in the cell suspensions at the points shown by the arrows. Values are means $\pm$ s.e.m. ($n = 3$–5)*$P < 0.05$ (vs. t_0; Mann-Whitney U test). All CL values are significantly different from that at time zero ($P < 0.05$). (The interruption in the x-axis is necessary because a log axis tends to but does not reach zero).

correlation to the generation of ROS by these cells, whether predominantly macrophages or polymorphonuclear leukocytes. The more precipitous and prolonged fall in GSH-Px in mouse peritoneal cells probably reflects the response of the resident macrophages to an inflammatory stimulus, since the predominantly polymorphonuclear leukocytic cells entering the inflamed rat pleural cavity exhibited a much less pronounced fall in GSH-Px activity and thus an increase in polymorphonuclear cells would not account for the fall in GSH-Px in the mouse cells. An early fall in the activities of scavenging enzymes appears to be a general response to local inflammation or injury since SOD activity has been reported to be decreased in phagocytozing human polymorphonuclear leukocytes[12] and SOD, catalase and GSH-Px activities are decreased at sites of myocardial ischaemia in the rat.[13]

After 2–3 days, in both mouse peritoneal and rat pleural leukocytes, GSH-Px activities had returned to normal or higher than normal levels. Particularly with the

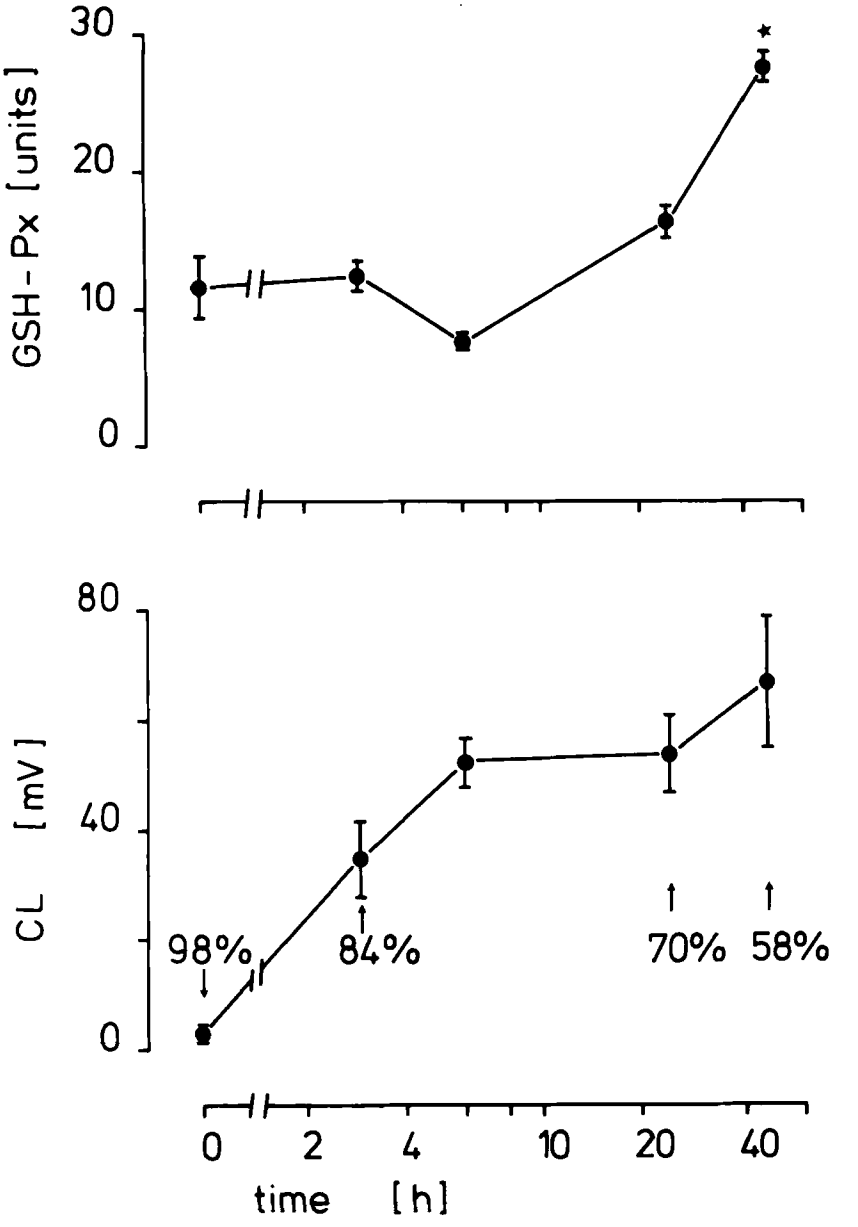

FIGURE 2 Changes with time in rat pleural leukocyte GSH-Px activities and CL generation following intrapleural injection of λ-carrageenin at time zero. GSH-Px values are given as units per 1.5×10^6 cells, CL values as mV per 1×10^6 cells. Values at time zero were obtained from rat peripheral blood polymorphonuclear leukocytes. The percentages indicate the proportion of polymorphonuclear leukocytes present in the cell suspensions at the points shown by the arrows. Values are means ± s.e.m. (n = 3–6)*$P < 0.05$ (vs. t_0; Mann-Whitney U test). All CL values are significantly different from that at time zero ($P < 0.05$). The GSH-Px activity at 6 h is also significantly different from that at 3 h ($P < 0.05$). (The interruption in the x-axis is necessary because a log axis tends to but does not reach zero).

rat pleural cells, this would appear to be a homeostatic response to increased ROS generation, as reflected by the enhanced sensitivity of the cells to CL generation by opsonized zymosan. This positive homeostatic reponse of leukocytic GSH-Px and probably catalase may lead to increased levels of hydroperoxide scavenging enzymes during prolonged inflammation. This may explain the reports of increased activities of GSH-Px and catalase in synovial fluid of patients with rheumatoid arthritis[4] and the reversal with time of the depression by prolonged selenium deficiency of lymphocyte proliferation in mice.[6] An alteration in the predominant leukocyte type with differing GSH-Px activities in later phases of inflammation, however, cannot be ruled out as an interpretation of the late rise in the activity of this enzyme.

The increased ability of mouse peritoneal macrophages to mount an oxidative burst following activation with *C. parvum* or other immunological activators is well documented.[6,14,15] and is confirmed by our present data in both mice and rats. Interestingly, a peak of CL is achieved 24 h after *C. parvum* injection, when the concentration of polymorphonuclear leukocytes in the peritoneal cavity is at its highest. This is paralleled by the continual increase over the first 48 h after carrageenin injection of the generation of CL by pleural exudate leukocytes, which are predominantly polymorphonuclear. These findings confirm data of other authors showing increased sensitivity to CL generation of polymorphonuclear leukocytes from the inflamed rat pleural cavity,[16] the rheumatoid joint[17] and sites of renal allograft rejection.[18]

In conclusion, we have shown that during acute inflammation in the mouse peritoneal cavity local leukocytic GSH-Px activity decreases in inverse correlation to and presumably as a result of the increase in the generation of ROS by these cells. A similar trend was observed with rat pleural cells. However, particularly in the pleural cavity, exudate leukocytes respond with a subsequent increase in GSH-Px activity, either as a homeostatic response to the continued generation of ROS and/or as a result of an increase in the proportion of mononuclear cells entering the inflamed area.

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Accepted by Prof. H. Sies