Title: SUPPORT FOR NEUTROPHIL-MEDIATED CYTOTOXICITY IN HALOTHANE HEPATITIS

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Introduction. Interest in the cytotoxic action of neutrophils stems from the hypothesis that certain disease states of the liver are the result of immune-mediated reactions. Because fulminant hepatic necrosis due to halothane ($CF_3CHBrCl$) has been associated with repeated exposures to the anesthetic (1), one current hypothesis proposes that the toxicity may be due in part to a hypersensitivity reaction. The biotransformation of halothane forms metabolites that bind covalently to cellular macromolecules (2,3), and it has been suggested that these metabolite adducts could act as haptens capable of provoking an immune response. Research has now demonstrated the presence of anti-halothane antibodies in patients diagnosed with halothane hepatitis (4). In addition, lymphocytes primed against halothane-derived haptens have been shown to kill hepatocytes *in vitro* (5).

It is important to determine whether neutrophils, activated by recognition of antibodies bound to the outer plasma membrane of hepatocytes or by release of hepatocyte-derived chemoattractant substances, are capable of damaging hepatocytes. For this purpose, we employed the potent neutrophil activator PMA, an agent demonstrated to cause the production of superoxide anion and specific degranulation in neutrophils.

Methods. Hepatocyte Isolation: Male Fisher 344 rats were utilized for all experiments. Hepatocytes were prepared by *in situ* perfusion of the liver with collagenase. Hepatocytes were added to collagen-coated 60 mm Lux culture dishes, and allowed to attach for 120 min at 37°C in 5% CO_2/95% air. The hepatocyte monolayers were then washed and covered with M-199. Cells at this point were more than 95% viable.

One hour prior to the commencement of the lysis experiments, 0.5 uCi of ^{51}Cr was added to each culture dish and incubated at 37°C in a 95% air/5% CO_2 atmosphere. The medium was then removed, and replaced with experimental medium. The experimental medium was either phenol-free complete M-199 or Hanks Balanced Salt Solution (HBSS), supplemented with $CaCl_2$ (1.8 mM) and $MgSO_4$ (1.0 mM).

Neutrophil isolation: The neutrophils were isolated according to the procedure of Markert (6).

Neutrophils were counted on a hemocytometer. Only those neutrophils that spread on the hemocytometer surface were counted (~90%); those that did not were assumed to be nonviable. The neutrophil yield was roughly $2.5\text{-}3x10^8$ neutrophils per 100 ml of donor blood. The neutrophils were assayed for superoxide generation and the values obtained were found to agree with the reported values of 3 nmol/min/10^6 cells (6).

Percentage cytotoxicity is expressed as the percentage of ^{51}Cr released over 4 h as determined by the formula % Lysis = [(A - S)/T] x 100; where A is the mean cpm in the supernatant of culture dishes containing activated neutrophils and hepatocytes, S is the mean cpm in the supernatant of culture dishes containing hepatocytes alone (spontaneous ^{51}Cr leakage), H is the mean cpm retained within the hepatocytes over 4 h, and T is the mean cpm internalized by the hepatocyte monolayers during the 1-2 h pre-incubation with ^{51}Cr (T = H + S).

Results and Discussion. Over a period of 4 h, neutrophils in serum-free medium and at effector-to-target ratios of 10:1 and 5:1 caused 60% and 30% lysis of hepatocytes, respectively. Hepatocyte lysis was considerably lessened in the presence of 10% (v/v) fetal calf serum: 19% at an effector-to-target ratio of 10:1 and 3% at an effector-to-target ratio of 5:1. The calcium ionophore A23187 (5 uM), in conjunctjon with PMA (1.6 uM), did not increase lysis over that of PMA alone. Neither PMA nor A23187 in the absence of neutrophils had noticeable detrimental effects on hepatocyte monolayers at the concentrations employed in these experiments. Similarly, neutrophils, in the absence of any activating agent, had no lytic effect. This *in vitro* study suggests that similar activation of neutrophils by immune complexes on the hepatocyte or chemoattractants could result in hepatotoxicity *in vivo*.

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