THE INFLUENCE OF IN VIVO PRETREATMENT OF CYCLOPHOSPHAMIDE ON PHAGOCYTIC ACTIVITY OF MOUSE MACROPHAGES IN VITRO

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Cyclophosphamide (CY) is a nitrogen mustard which has been extensively used as an immunosuppressive agent. There is no doubt that this drug can affect both the cell-mediated and the humoral response of the host. An apparent deficiency in the ability of the alveolar macrophages of the rat to destroy ingested bacteria as a result of immunosuppressive treatment with CY has been reported. These studies also showed a marked decrease in the intracellular acid phosphatase of alveolar macrophages from treated animals. Earlier studies reported an impairment of function of the reticuloendothelial system in immunosuppressed animals.

Pretreatment of guinea pigs with CY enhances some forms of delayed-type hypersensitivity. The basis of this phenomenon appears to be elimination of the suppressor B lymphocytes modulating these reactions. Moreover, recently it has been demonstrated that CY treatment and whole body x-irradiation of mice reduced the in vitro phagocytic capacity of their peritoneal macrophages towards Escherichia coli bacteria, and several reports attest the compromising activity of this drug on host immunity, including alterations in serum immunoglobulins and complement levels. On the other hand, it must be pointed out that low doses of CY did not decrease the titre of circulating hemagglutinating antibody.

We conducted a study on the effect of in vivo CY pretreatment on phagocytic activity of in vitro cultured mouse peritoneal macrophages, in the presence of serum from untreated animals, in order to obtain further information on the influence of immunosuppressive agents on the phagocytic process. In addition, the activity of CY on suppression of the macrophage population was examined.

Key-words: Cyclophosphamide; Kolmogorov and Smirnov test; Macrophages; Mouse peritoneal macrophages; Phagocytic activity; Phagocytosis.

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MATERIALS AND METHODS

Animals - The studies were performed on male albino mice (Swiss strain) weighing about 38-40 g.

CY pretreatment - The mice were treated with 8 mg/kg of CY administered daily by the i.m. route for 7 days.

Cell cultures - Mouse peritoneal macrophages were obtained by a modification of the method of Mallucci*. The treatment with CY was discontinued for 2 days. After this time the mice received 2 ml i.p. of a starch suspension in sterile saline (1.5 g/dl). One day later the animals were killed by cervical dislocation, and the peritoneal exudate was removed via abdominal washing with Parker’s 199 medium. The suspension was diluted to a concentration of 1.5 x 10^6 cells/ml in Leighton tubes containing a coverslip (2 ml/tube). The cells were cultured at 37°C in Parker’s 199 medium enriched with 15% fetal calf serum and 10% lactalbumin hydrolysate. More than 95% of the adherent cell population was judged to be mononuclear phagocytes by microscopic evaluation of hematoxylin-eosin stained preparations.

Experiments - On the 8th day the cell cultures were washed three times with Parker’s 199 medium to remove traces of serum. The medium was replaced in one group by 2 ml opsonized zymosan 1.5 x 10^7/ml suspended in Parker’s 199 medium with 15% of homologous complemented serum from untreated animals, in a second group by 2 ml of opsonized zymosan in the medium with 15% of homologous inactivated serum from untreated animals, in a third group by 2 ml of opsonized zymosan in the medium without serum. After 1 h of contact the coverslips were extracted from the tubes and stained with hematoxylin-eosin by the routine procedure. Controls were carried out by replacing the nutrient medium with plain zymosan suspension. The peritoneal macrophage population was evaluated by direct count in a Burker hemocytometer.

Biometrical analysis - For each experiment 50 microscopic fields were examined at random using a 100x oil immersion lens. The intracellular particles of each cell were counted and the average number of phagocytized zymosan particles per cell, the variance and the standard error were determined by a routine procedure. The ratio between this value for the pretreated cell cultures and the corresponding value of control cell cultures was expressed as an index of phagocytosis. Student's t test would not be reliable as the behavior of the intracellular particles did not follow a normal distribution. It was necessary to use a global non-parametric test for a comparison between the experimental and the control findings, i.e. the Kolmogorov and Smirnov test. A p value < 0.05 was considered significant.

RESULTS

The results of experiments designed to examine the effect of CY pretreatment on the ability of macrophages to ingest zymosan particles are summarized in tab. 1. In the control cell cultures the presence of opsonized zymosan with homologous serum with complement had a positive effect on the phagocytic