Mechanisms of Destruction of Human Rhesus Sensitized Red Blood Cells

Phagocytosis of anti-D coated red cells has been noticed morphologically by several authors[1–4]. However, attemps to show extracellular cytotoxicity[5–8] using conventional techniques were invariably unsuccessful unless the red cells were trypsinized, presensitized and cultured in the absence of serum or by using mononuclear cells from a highly immune subject. It was of interest therefore to examine what contribution was made to red cell destruction by extracellular cytotoxicity as compared with phagocytosis and in addition what effect serum had on these systems.

Materials and methods. Human incomplete anti-D sera were obtained from women sensitized during pregnancy. Control serum consisted of a pool from healthy AB Rh-positive donors. The antibody content of anti-D sera was tested by haemagglutination in albumin. Leukocyte rich plasma (buffy coat cells) from healthy Rh-positive donors was separated by 3.5% dextran sedimentation. Human monocytes from healthy Rh-positive donors were purified by the method of HUBER and FUDENBERG[9] with minor modifications. Human 0 Rh-positive red blood cells were labelled with 50 μCi sodium-chromate (Radiochemical centre, Amersham) at 37 °C for 1–2 h.

The cytotoxicity assay using chromium release was carried out as follows: 2.5×10^5, mononuclear leukocytes or purified monocytes were incubated with 10^4 labelled red cells in the presence of serial dilutions of anti-D serum for 18 h at 37 °C in a humid atmosphere of 5% CO_2 in air, the total volume being 0.3 ml. The radioactivity of the cell-free supernatant is expressed as a percentage of the total radioactivity of the tube and is a measure of the extracellular haemolytic cell damage (HOLM[6]). The phagocytosis assay was a modification of the isotope method of KLAUS[10]. In addition we used cytochalasin B (Ralph N. Emmanuel Ltd.) to inhibit the phagocytosis of Rhesus sensitized red cells by monocytes or buffy coat cells. A sample of 2.5×10^6 lymphocytes or monocytes were incubated with 6×10^6 ^{51}Cr-red cells in the presence of anti-D serum in a total volume of 0.3 ml. Control samples containing cytochalasin B 80 μg per ml were also prepared. The mixture was incubated for 11 h at 37 °C in a humid atmosphere of 5% CO_2 and air. After incubation 1 ml 0.83% NH_4Cl was added to each culture for 6 min to lyse the extracellular red cells. The pellet was washed 3 times and then counted in a well type gamma counter. Control consists of pre-sensitizing red cells with normal AB serum.

Results and discussion. In our experimental system the phagocytic index was calculated by the ratio of counts in the experimental samples to the cytochalasin B controls. A ratio greater than 1.0 indicates that erythrophagocytosis has occurred. In the control experiments the ratio varied insignificantly from 1.0. Moreover, as described by

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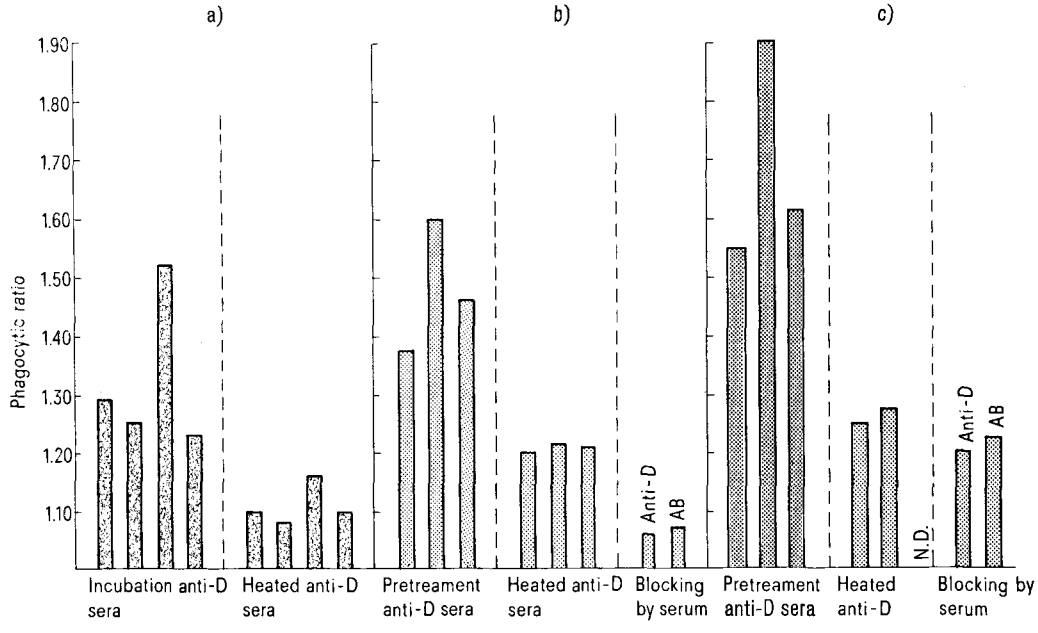

a) Increase in phagocytic ratio of Rhesus positive red cells by buffy coat leukocytes in the presence of anti-D sera. Heat inactivation of sera reduced the phagocytosis. Bars represent different anti-D sera and each column represents the mean of triplicate observations. b) Increase in phagocytosis of presensitized red blood cells by buffy coat leukocytes is seen in the absence of serum in the culture. There was inhibition of phagocytosis when heat inactivated anti-D sera were used and more effective blocking occurred by adding either anti-D or AB serum to the culture for the duration of the incubation. c) Increase in phagocytosis of erythrocytes is seen after their pretreatment with anti-D sera and incubation with monocytes in the absence of serum in the culture medium. Heat inactivation of the serum reduced the phagocytosis. When presensitized red cells were cultured in the presence of serum either anti-D or AB, phagocytosis was disminished although it was still significantly greater than control cultures. N.D. = Not done.

GREENBERG 1974[11], there was a good correlation between the phagocytic index and morphological evidence of erythrophagocytosis in the presence or absence of cytochalasin B. In control experiments, cytochalasin B was not found to cause any metabolic effect, at the concentration used, except for inhibition of phagocytosis. However, the phagocytosis induced by a series of different anti-D sera tested was readily detected, the phagocytic indices varied from 1.23 to 1.52. Heat inactivation of anti-D sera (56 °C for 30 min) led to less phagocytosis (Figure a). As various authors (HOLM[8], HUBER and FUDENBERG[9]) have shown that the presence of free IgG in the incubation mixture can block effector cells, a further series of experiments were performed in the absence of serum, pretreating the erythrocytes (CDe/cDE) with anti-D serum for 30 min, washing and then adding the phagocytic cells. Phagocytosis of these presensitized red cells by buffy coat leukocytes was increased compared to experiments where serum was present for the whole incubation period (phagocytic indices varying from 1.37 to 1.60). Nevertheless, if either anti-D or normal serum was added to the culture of buffy coat cells and presensitized red cells for the whole period of experiments, phagocytosis was inhibited and this reduction was even greater than that when anti-D serum was heat inactivated (Figure b).

In the series of experiments where monocytes were used as phagocytic cells in culture with presensitized red blood cells, phagocytosis was greater than that seen with buffy coat cells (ratio 1.55 to 1.90) (Figure c). It was also observed that purified monocytes in the presence of anti-D serum during the whole incubation period were still able to phagocytose Rh-positive red cells (phagocytic ratio 1.58; not shown). As in the previous experiments with buffy coat leucocytes, the presence of anti-D or AB serum in the incubation mixtures of monocytes and presensitized red cells reduced phagocytosis. An explanation for this could be that when the binding sites of the target cells are saturated, the addition of free IgG antibodies or complexes would block the Fc receptors of the effector cells. Some other authors[12] have also reported that this inhibitory effect of IgG seems to depend on the degree of red cell sensitization. Moreover, this phagocytic activity was partly dependent on the presence of complement, since heat inactivated sera were less effective. An alternative explanation for this could be that aggregated IgG blocks the Fc receptors of the monocytes. However, in the series of experiments performed in order to show extracellular lysis of anti-D sensitized red blood cells, poor chromium was released from target cells as compared with control pooled AB serum. Nevertheless, in accordance with HOLM[8], more significant haemolytic activity was detected by pretreatment of red cells with anti-D serum and culturing in the absence of serum thus avoiding its inhibitory effects (unpublished observations).

However, the mechanism by which anti-D coated red cells are removed from the circulation still remains unclear. We believe that both mechanisms of haemolysis extracellular and intracellular are involved in the process, and our data support the concept that with IgG antibody coated red cells, one of the main mechanism of removal is by phagocytosis.

Summary. In order to determine the phagocytosis of human anti-D coated red cells we adopted a quantitative technique for its measurement using human erythrocytes labelled with ^{51}Cr-chromate to support the assumption that erythrophagocytosis is one of the main mechanisms by which IgG-anti-D-antibody coated red cells are destroyed.

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The Effect of Luteineizing Hormone on Chicken Testis and Pineal Gland Uptake of ^{32}P[1]

An increasing number of investigations of the pineal gland have been reported since the discovery of a potential pineal hormone (melatonin)[2] and the demonstration of a relationship between ambient lighting and the biosynthetic activity of the pineal parenchyma[3]. The existence of one or more gonad inhibiting polypeptides in the mammalian pineal gland has been established[3–7]. Various pineal indoleamines such as melatonin have also been shown to possess anti-gonadotropic qualities[3,8,9]. This effect may vary with age. For example, RATHKAMP[10] has shown that the pineal of the White Leghorn cockerel stimulates gonadal activity between 9 and 17 days post-hatching and inhibits gonadal activity after 17 days post-hatching. It has also been observed that the pineal organ may be a target organ of testicular hormones[11–14].

The glandular uptake of ^{32}P, administered carrier-free, has been used in our laboratory as an indicator of increased or decreased organ metabolism[15,16]. This method was used in these studies to determine the effect of luteinizing hormone on the pineal gland and testis of 3-, 10- and 13-day-old White Leghorn cockerels (*Gallus domesticus*).

Materials and methods. White Leghorn cockerels were purchased from the Indiana Farm Bureau Co-op, Indianapolis, Indiana, within 12 h of hatching. All birds used in these studies were housed in brooders under controlled temperature (21–24 °C) and lighting (14 h light: 10 h dark). Food and water were available ad libitum until 12 h before autopsy at which time all birds were taken off feed.

Eighty 13-day-old cockerels were divided into 5 groups of 16 birds each. An injection of 30 μg LH (NIH-LH-S18)[17] was administered to each bird of a given group at either 1.0, 2.0, 4.0 or 8.0 h before autopsy. As a control the 5th group did not receive LH treatment (Table I).

In a 2nd experiment, sixty-four 13-day-old cockerels were divided into 4 groups of 16 birds each. An injection of 5, 10 or 20 μg LH (NIH-LH-S18) was administered to