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INCREASED ANTIBODY PRODUCTION FOLLOWING DEPRESSION OF HEPATIC PHAGOCYTOSIS

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SUMMARY

The influence of Kupffer cell blockade on the humoral immune response to suboptimal doses of intravenous sheep red blood cells has been measured in mice. Hepatic phagocytosis was suppressed using dextran sulphate. Direct (IgM) and indirect (IgG) plaque-forming cells were measured in spleens from treated and untreated mice at varying times after the antigen. The results show that after Kupffer cell blockade both IgM and IgG responses correspond to the responses seen after a 10-fold greater dose of cells in control animals. The implications of this are discussed.

INTRODUCTION

We have shown previously that the intravenous injection of carbon and dextran sulphate profoundly depresses the hepatic uptake of a particulate antigen subsequently injected intravenously (Souhami, 1972; Bradfield, Souhami & Addison, 1974). The depressed hepatic uptake of antigen results in increased uptake by the spleen and bone marrow and an increase in production of humoral IgM antibody and in numbers of splenic direct plaque-forming cells (PFC). The increase in immune response can be accounted for entirely by the diversion of antigen from liver to other, immunizing, sites.

Hyperglobulinaemia is a well recognized feature of chronic liver disease in man. It is associated with increased production of antibody (Havens, Schaffner & Hopke, 1951) and is not due to decreased catabolism (Havens *et al.*, 1954). All classes of immunoglobulin are increased in amount (Eliakim, Zlotnick & Slavin, 1972). The probable explanation for this increased antibody production is that in patients with chronic liver disease, bacterial antigens, which would normally be sequestered by the liver, are taken up by the spleen and other phagocytic sites (Triger & Wright, 1973).

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This paper describes the effect of blockade of hepatic uptake of antigen on production of antibodies of the IgG class.

MATERIALS AND METHODS

The methods have mostly been described previously (Bradfield *et al.*, 1974). Doses of sheep red blood cells (RBC) which give a submaximal immune response were injected intravenously (i.v.) into BALB/c mice, some of which had received dextran sulphate (mol. wt 900,000) 4 hr previously (500 μg i.v.) to block hepatic phagocytosis. Direct and indirect plaque-forming cells (PFC) were assayed (Cunningham & Szeneberg, 1967) in spleens removed at varying times after the sheep RBC. The developing antiserum was made by immunizing rabbits with purified mouse IgG. On immunoelectrophoresis against mouse whole serum it reacted only with IgG. It slightly reduced the PFC count on day 2 when the response comprises mainly IgM (Wortis, Taylor & Dresser, 1966) but considerably increased the count from day 5 onwards when the response would be predominantly IgG. The time course of the PFC developed by this serum resembled that for IgG PFC reported elsewhere (Nossal, Lewis & Warner, 1971).

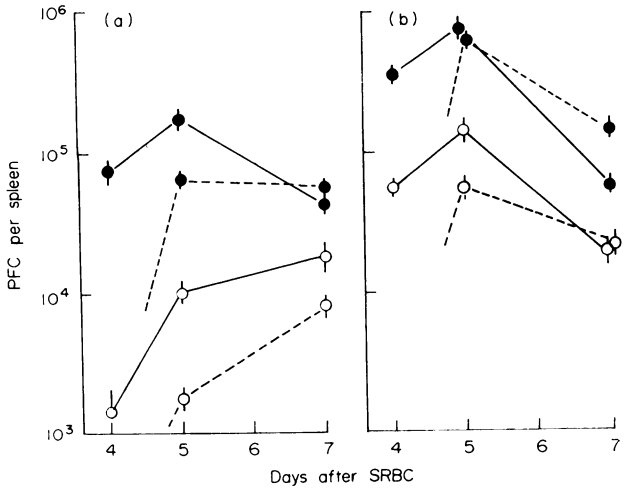

FIG. 1. The number of plaque-forming cells (PFC) after the injection of (a) 1×10^6 and (b) 1×10^7 sheep RBC, in the spleens of normal mice and mice which had received dextran sulphate (500 μg i.v.) 4 hr before antigen. Direct PFC (IgM) in control (○——○) and dextran sulphate-treated (●——●) mice. Indirect PFC (IgG) in control (○ - - ○) and dextran sulphate-treated (● - - ●) mice. Groups of six mice were used. Points represent geometric means and bars show standard errors.

RESULTS

Effect of Kupffer cell blockade on the immune response to a suboptimal i.v. dose of sheep RBC

Intravenous sheep RBC were injected into groups of mice in one of two suboptimal doses (1×10^6 or 1×10^7). Half of each group received dextran sulphate i.v. 4 hr previously. PFC were measured in spleens removed 4, 5 and 7 days later. The results (Fig. 1) show that

with the smaller dose of sheep RBC dextran sulphate increased both the IgM and IgG responses to the same degree. The delay in the peak IgM and IgG responses seen after the smaller dose was eliminated in the dextran sulphate-treated group. It can be seen, therefore, that in both these respects dextran sulphate caused mice to respond to a dose of 1×10^6 sheep RBC as if they had been given 1×10^7. This is consistent with the earlier finding that dextran sulphate causes a 10-fold increase in the number of sheep RBC taken up by the spleen (Bradfield *et al.*, 1974). The results shown in Fig. 1 show that animals receiving the larger dose of cells also manifested an increased immune response.

Dextran sulphate alone caused no increase in the background numbers of PFC in animals given no sheep RBC.

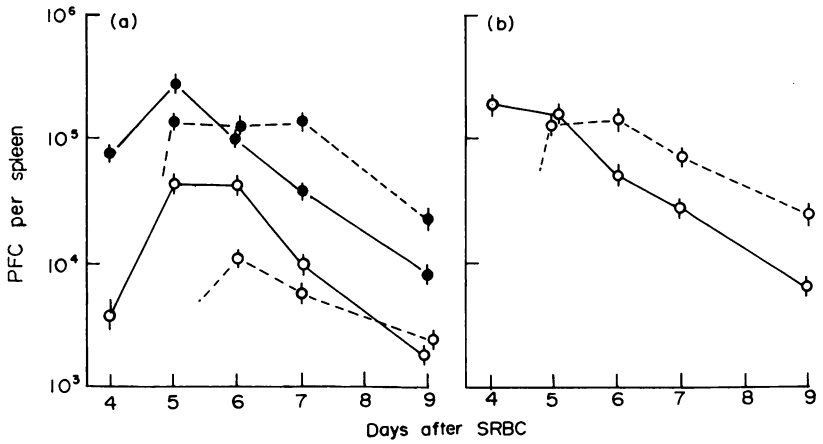

FIG. 2. The number of plaque-forming cells (PFC) after the injection of (a) 5×10^6 and (b) 1×10^8 sheep RBC, in the spleens of normal mice and mice which had received dextran sulphate (500 μg i.v.) 4 hr before antigen. Direct PFC (IgM) in control (○——○) and dextran sulphate-treated (●——●) mice. Indirect PFC (IgG) in control (○ – – ○) and dextran sulphate-treated mice (● – – ●). Groups of six mice were used. Points represent geometric means and the bars show the standard errors.

Time course of the immune response to sheep RBC after previous Kupffer cell blockade

When direct and indirect PFC were measured day by day after 5×10^6 or 1×10^8 sheep RBC, it was confirmed that previous Kupffer cell blockade caused the animals to respond to the smaller dose of cells exactly as if they had been injected with the greater dose (Fig. 2). Although the peak IgM response was maintained 1 day longer after dextran sulphate than in controls, this did not happen in a further experiment (not shown here), even though the expected adjuvant effects were obtained.

DISCUSSION

Many cirrhotic patients have a reduced ability to clear colloid from the blood (Biozzi & Stiffel, 1965) with increased uptake by the spleen (Castell & Johnson, 1966). In addition, antibody titres to gut-associated bacteria and dietary proteins are increased in cirrhosis (Triger, Alp & Wright, 1972) and in patients with a porto-caval shunt (Bjørneboe, Prytz &

Oskov, 1973). These findings suggest that the increased immune responses found in cirrhosis are due to failure of the liver to sequester antigen, resulting in spillover of antigen to immunizing sites such as the spleen.

Several animal models have now been made. In rats carbon tetrachloride-induced cirrhosis is associated with a defective ability of the liver to trap blood-borne *Salmonella adelaide* and immune complexes, and there is increased uptake by the spleen (Thomas, McSween & White, 1973). This applies to bacteria introduced into the portal or the systemic circulation. Establishment of a porto-caval shunt in rats is followed by increased circulating levels of 7S immunoglobulin and increased titres of antibody to *E. coli* lipopolysaccharide (Meyers, 1973). In mice, specific blockade of Kupffer cells using carbon causes a decreased clearance of i.v. sheep RBC associated with increased splenic uptake and a corresponding increase in the humoral immune response (Souhami, 1972). A corollary of this is that, *in vivo*, Kupffer cells do not contribute to the immune response to particulate antigens and probably have only a degradative role. However cell transfer experiments have shown that when antigen is associated with Kupffer cells there may be either increased (Howard, 1970) or decreased (Inchley, 1969) immunogenicity.

The present experiments have shown that after Kupffer cell blockade both IgM and IgG responses correspond to the responses seen after a 10–20-fold greater dose of cells in control animals, a result which extends previous studies (Bradfield *et al.*,1974) where only IgM production was measured. These new results are of interest for two reasons. First, they show that the adjuvant effect of agents which block hepatic phagocytosis requires no explanation in terms of T or B lymphocytes but can be accounted for entirely by an effect on mononuclear phagocytes. Hence assessment of organ distribution is an essential requirement in the investigation of any adjuvant effect when intravenous particulate antigens are used. Secondly, in humans, failure of the liver to filter particles can allow presentation of these to other sites in the body with a wide variety of clinical consequences (Bradfield, 1974). Spillover of particulate antigen from the liver into the spleen appears to be an important example of this.

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