Clin. exp. Immunol. (1981) **46**, 581–588

The surface properties and antigen-presenting function of hepatic non-parenchymal cells

K. PULFORD & R. L. SOUHAMI *ICRF Human Tumour Immunology Group, University College Hospital Medical School, University Street, London*

(Accepted for publication 26 June 1981)

SUMMARY

Hepatic endothelial cells and Kupffer cells have been isolated after pronase digestion of mouse liver and separated by density-gradient centrifugation on Percoll. The endothelial cells had a central round nucleus with a pale cytoplasm which contained non-specific esterase. They had Fc receptors but no Ia antigen, C3 receptor or phagocytic ability. In contrast, the phagocytic Kupffer cell had an initial rounded appearance and possessed Fc and C3 receptors and Ia antigens. Kupffer cells were capable of functioning as antigen-presenting cells as shown by their ability to reconstitute a secondary *in vitro* antibody in spleen cell cultures depleted of adherent cells. This function was genetically restricted. There was progressive loss of antigen-presenting function of Kupffer cells as time in culture increased. This appeared to be related to the loss of Ia antigen. Hepatic endothelial cells did not restore the antibody response in macrophage-depleted spleen cell cultures. The experiments show that Kupffer cells can function as antigen-presenting cells in a secondary antibody response *in vitro* and that this function is not due to the presence of endothelial cells in the isolated sinus lining cells.

INTRODUCTION

When a particulate antigen such as sheep red blood cells (SRBC) is injected intravenously in mice the major part is taken up by liver macrophages (Souhami, 1972). However, the amount of antibody produced depends on the quantity of antigen reaching other sites such as the spleen and procedures which depress hepatic uptake increase the antibody response (Souhami, Addison & Bradfield, 1975). This suggests that liver macrophages have a mainly degradative role for this antigen *in vivo*.

Recent *in vitro* studies have shown that Kupffer cells can function as accessory cells in mitogen-induced (Rogoff & Lipsky, 1979) and antigen-specific T-cell proliferation (Richman *et al.*, 1979). However, the hepatic non-parenchymal-cell population is heterogeneous, consisting largely of endothelial cells. In man, endothelial cells from other sites have Ia antigen on their surface (Moraes & Stastny, 1977; Hirschberg, Moen & Thorsby, 1979) and can function as antigen-presenting cells (Hirschberg, Bergh & Thorsby, 1980). Like Kupffer cells, murine endothelial cells contain non-specific esterase (NSE) and some are adherent. They frequently contaminate Kupffer cell preparations during short-term culture.

Using a density-gradient technique for separating mouse hepatic non-parenchymal cells (NPCs) we have studied the surface properties of Kupffer cells and endothelial cells and their function as accessory cells in antigen-specific secondary antibody responses *in vitro*. Since we have noted the

Correspondence: R. L. Souhami MD, FRCP, ICRF Human Tumour Immunology Group, University College Hospital Medical School, University Street, London WC1E 6JJ.

0009-9104/81/1200-0581$02.00

disappearance of surface Ia antigen during culture we have also studied the effect of Ia-antigen loss on the antigen-presenting function of Kupffer cells.

MATERIALS AND METHODS

Animals. Male CBA and BALB/C Mice (ICRF Breeding Colonies) 4–6 weeks old and weighing 18–25 g were used.

Antigens. The antigens used were TNP-KLH (trinitrophenyl–keyhole limpet haemocyanin), DNP-Levan (dinitrophenyl–Levan) and TNP-CGG (trinitrophenyl–chicken γ-globulin) (all of which were kindly donated by Dr M. Rittenberg).

Immunization schedule. Mice were injected i.p. three times at weekly intervals with 100 μg TNP-KLH conjugated to bentonite. Spleen cells were obtained 1 week after the last injection.

Isolation and culture of non-parenchymal cells. The method used for the isolation and density-gradient separation of the NPCs has been described in detail elsewhere (Pulford & Souhami, 1980). For studies on cell morphology and enzyme content, the cell concentration was adjusted to 5×10^5 cells/ml in MEM buffered with HEPES containing 30% heat-inactivated fetal calf serum (FCS, Flow) and glutamine. One millilitre of this cell suspension was added to 16-mm wells of a Linbro plate each of which contained a sterile glass coverslip. After overnight incubation in 5% CO_2/air, the non-adherent cells were recovered and fresh medium added.

Preparation of adherent-cell populations for the reconstitution experiments. The method of Ackerman & Douglas (1978) was used to prepare the adherent cell populations. Petri dishes containing confluent layers of baby hamster kidney (BHK) cells (ICRF Laboratories) were treated with 10 mM EDTA to remove the cells and leave a coated surface. NPCs obtained from layers 1 and 2 of the Percoll gradient were then added in HEPES-buffered MEM (HMEM) with 10% FCS and allowed to adhere for 3 hr before they were removed with 3 mM EDTA.

Peritoneal macrophages. Peritoneal macrophages were used as controls for the effect of the isolation procedure as described previously (Pulford & Souhami, 1980).

Cell morphology and cytochemistry. May–Grünwald/Giemsa stain (MGG) was used for routine morphology. Enzyme activity was identified using the method of Yam, Li & Crosby (1971) for NSE and a modified Kaplow's (1965) technique to demonstrate peroxidase.

Candida phagocytosis and Fc receptor. Both techniques have been described in detail previously (Pulford & Souhami, 1980). *Candida guillermondeii* (Cd) was used as the test organism, 10^7 Cd being added to each coverslip, and the percentage of cells phagocytosing after 1 hr was counted. Fc receptors were demonstrated by rosette formation with 5% SRBC in the presence of rabbit anti-sheep IgG antiserum (Flow Laboratories). A cell was scored positive if it was viable and had either ingested one or more SRBC or had three or more adherent SRBC.

Demonstration of C3 receptor. A 5% suspension of SRBC was incubated for 30 min at 37°C with a suitable dilution of a rat IgM anti-SRBC antiserum. After washing, the cells were incubated for 30 min at 37°C with a 1:3 dilution of normal mouse serum as a source of complement. The SRBC suspension was then washed and adjusted to 0·5% suspension in HMEM and added to the adherent cell populations for 30 min at 37°C. Positive cells were scored as for the Fc-receptor assay. Suspensions of SRBC incubated only with the IgM antibody were included as controls.

Demonstration of non-specific receptor. Glutaraldehyde-treated sheep red blood cells (GSRBC) were prepared according to the method of Rabinovitch (1967). The concentration was adjusted to a 0·1% suspension and 1 ml added to the adherent cell populations. After incubation at 37°C for 30 min, the coverslips were removed, washed and stained with MGG and the number of cells forming rosettes observed.

All four of the tests mentioned above were performed in duplicate and 600 cells counted per pair of coverslips.

Demonstration of the Ia antigen. Three different Ia antisera of anti-H-2^k haplotype specificity were used. One was a heteroantiserum directed against the entire Ia region (prepared in ATH mice after immunization with cells from ATL mice) and the other two were obtained from mice immunized with the hybridoma cell lines 10-2-14 and 12/18 and were directed against the I-A^k and

I-E/C^k subregions of the Ia complex respectively (all of these sera were kindly donated by Dr D. Katz).

Twenty-five microlitres of a suitable dilution of the antisera were added to coverslips containing adherent cells and incubated at room temperature for 30 min. The coverslips were then rinsed and 25 μl of fluoresceinated rabbit anti-mouse $F(ab)_2$ serum was added for another 30 min at room temperature. After a final thorough washing, the coverslips were stained and fixed and mounted in glycerol. Using u.v. light, 500 cells were scored per coverslip.

Experiments to control for non-specific binding were carried out using PEM and NPCs obtained from BALB/c mice (of H-2^d haplotype) and by omission of the first layer in the studies on cells from CBA mice.

In vitro *antibody response*. Spleens from primed mice were aseptically removed and gently teased apart to produce spleen cell suspension. After washing, the spleen cell concentration was adjusted to $2{\cdot}5 \times 10^6$ cells/ml in bicarbonate-buffered RPMI containing 10% non-heat-inactivated FCS and 5×10^{-5} M 2-mercaptoethanol (2-ME). Two hundred microlitres of the cell suspension were added to each well of a flat-bottomed microtitre plate (Linbro) and 10 μl of a suitable concentration of TNP-KLH added. After 5 days' culture at 37°C in a humidified 5% CO_2/air incubator, the numbers of antibody-forming cells (AFCs) were assayed. Using the slide chamber method of Cunningham & Szenberg (1968), the numbers of direct IgM and indirect IgG AFCs were counted.

Macrophage depletion was performed using a modification of the method of Sjöberg, Andersson & Möller (1972). Briefly, 50×10^6 spleen cells in 2 ml HMEM and 10% FCS were added to 50 mg carbonyl iron Grade E (GAF, UK) for 45 min at 37°C. Cells which had ingested or adhered to the carbonyl iron were then removed by the use of a strong magnet. Varying numbers of purified preparations of adherent cells from layers 1 and 2 of the gradient were then added to these macrophage-depleted spleen cell preparations and the numbers of AFCs measured after 5 days' culture.

RESULTS

Isolation and purification of NPCs

The average weight of the CBA mouse liver was 1·26 g which gave a yield of $25{\cdot}1 \pm 4{\cdot}1 \times 10^6$ NPCs/g. Twenty per cent of this population possessed the cytochemical and morphological characteristics of Kupffer cells.

After the Percoll density-gradient centrifugation step, NPCs could be separated into three compartments according to density (Pulford & Souhami, 1980). Layers 1 (density 1·03→1·055) and 2 (density 1·055→1·08) contained 96% of the total NPCs recovered from the gradient.

Morphological appearance of NPCs

Cytospin preparations on freshly isolated cells showed that the majority (83%) of the cells found in layer 1 contained a centrally located spherical nucleus in lightly staining basophilic cytoplasm. These cells contained NSE but no peroxidase and were regarded as endothelial cells. Approximately 8% of the cells present in layer 1 were identified as Kupffer cells, having oval or slightly indented nuclei in basophilic cytoplasm and containing both NSE and peroxidase. Kupffer cells accounted for 40% of cells in layer 2, the rest being endothelial cells (38%), NSE-negative mononuclear cells and a few granulocytes.

After overnight culture, 90% of the adherent cells from layer 1 were mononuclear, having an elongated, spindle-like appearance, often with long cytoplasmic processes, with a round or oval nucleus centrally located in pale-staining filamentous cytoplasm. These cells were NSE-positive and peroxidase-negative. For these reasons and because of their frequency in culture (approximately 10^5 cells per coverslip) and lack of phagocytic activity, they were identified as endothelial cells. The majority of the remaining adherent cells had a rounded appearance with intensely stained, basophilic, vacuolated cytoplasm and an oval or slightly indented eccentrically located nucleus. All of these cells contained NSE, and were regarded as Kupffer cells although only 50% contained peroxidase at this time.

Table 1. The distribution of Ia antigen on adherent endothelial cells and Kupffer cells in 3- and 24-hr cultures

	Endothelial cells				Kupffer cells					
	3 hr		24 hr		3 hr			24 hr		
	+*	−	+	−	++	+	−	++	+	−
Ia^k	—	100	—	100	8·3±1·7	70·4±4·3	21·3±3·4	29·5±6·4	53·5±3·5	17·0±1·0
$I\text{-}A^k$	—	100	—	100	10·0±7	67·5±7·8	22·5±10·6	26±3·5	63·8±3·8	10·2±5·9
$I\text{-}E/C^k$	—	100	—	100	6·2±3·4	10·8±1·0	83·0±3·2	9·0±1·0	18·7±2·1	72·3±3·1

* Intensity of staining: − = Ia-negative, + = Ia-positive, ++ = strongly Ia-positive.

Figures are mean % ± s.d. Kupffer cells were prepared from layer 2 and endothelial cells from layer 1 of the density gradient.

In contrast, 97% of the adherent cells in layer 2 were Kupffer cells having similar morphological characteristics to those found in layer 1, but in this case 90% contained peroxidase at 24 hr. Only 2% were found to be endothelial cells at 24 hr.

Characteristics of the adherent cell populations

Three hours after isolation, only 14% of Kupffer cells were phagocytic, 60% had Fc receptors and 22% C3 receptors. After 24 hr, the values were 93 and 70% for the Fc and C3 receptors respectively and 86% were phagocytic (all cells were phagocytic at 48 hr). This initial loss of surface receptors and phagocytic ability was due to the effect of pronase used during the isolation procedure. Control cultures of pronase-treated PEM demonstrated the same changes during the first 24 hr of culture but after 24 hr gave identical results to non-pronase-treated cells. In contrast to the findings with Kupffer cells, endothelial cells were not phagocytic and did not have C3 receptors. At 24 hr, 99% had Fc receptors and 100% showed 'non-specific' receptors for GSRBC.

The distribution of Ia antigen on the adherent cells from layers 1 and 2 is shown in Table 1. Endothelial cells were consistently Ia-antigen-negative, both in 3- and 24-hr cultures. In 24-hr cultures of Kupffer cells, 83% stained for Ia^k, 89·8% for $I\text{-}A^k$ but only 27·7% for $I\text{-}E/C^k$. The proportion of Kupffer cells showing Ia antigen at 3 hr in culture was somewhat smaller. In

Table 2. The secondary *in vitro* antibody response to TNP-KLH by whole and macrophage-depleted spleens

Culture	Antigen added *in vitro*	PFCs/culture IgM	PFCs/culture IgG
TNP-KLH-primed spleen	TNP-KLH	74±3	483±45
TNP-KLH-primed spleen	None	7±4	7±3
TNP-KLH-primed spleen	TNP-CGG	6±2	14±3
TNP-KLH-primed macrophage-depleted spleen	TNP-KLH	26±2	184±10
TNP-KLH-primed macrophage-depleted spleen	None	6±3	3±2
TNP-KLH-primed spleen	TNP-Levan	46±10	—
TNP-KLH-primed macrophage-depleted spleen	TNP-Levan	38±5	—

Antigen concentration was 1 μg/ml (0·01 μg/culture). Each result represents mean (± s.d.) of triplicate cultures.

experiments where the anti-Ia serum was omitted or where Kupffer cells were obtained from BALB/c mice, there was no positive staining, indicating that the reaction was with the Ia antigen of cells of H-2^k haplotype and not due to non-specific Fc binding. In tests on peritoneal macrophages, treatment with pronase decreased the number of Ia^+ cells found in 24-hr cultures, by 37%.

The secondary in vitro *response to TNP-KLH*

TNP-KLH-primed spleen cells cultured with TNP-KLH gave a secondary IgM and IgG anti-TNP response (Table 2). The antigen specificity of this response was demonstrated by the absence of a response when TNP-CGG was used as antigen. Treatment with carbonyl iron resulted in macrophage depletion of the spleen so that the cultures contained less than 2% NSE-positive cells. This led to a decrease in both the secondary IgM and IgG response by 67·8 and 60% respectively. The 2-ME was therefore not replacing the accessory function of macrophages.

Carbonyl iron, however, does cause a loss of 40% of the original spleen cell population. The decreased *in vitro* response in the macrophage-depleted cultures could have been due to loss of antibody-producing B cells. To exclude this possibility, the ability of the macrophage-depleted cultures to give a primary *in vitro* response to the T-cell-independent antigen TNP-Levan was assayed. It can be seen from Table 2 that sufficient B cells remained in the macrophage-depleted spleen to mount a response against TNP-Levan comparable to that obtained from a whole spleen suspension, indicating that macrophage depletion was probably the cause of the reduction of the TNP-KLH response rather than loss of B cells.

Reconstitution experiments

The secondary immune response to TNP-KLH in macrophage-depleted spleen cell cultures was measured after the addition of purified populations of either Kupffer cells from layer 2 or endothelial cells from layer 1. The Kupffer cells were 98% NSE-positive and 75·2% I-A^k-positive, the endothelial cell population was 99% NSE-positive and 2·1% I-A^k-positive. A result from a typical experiment is shown in Fig. 1.

Kupffer cells were able to restore the ability of the macrophage-depleted spleen to give both IgG and IgM antibody responses. In repeated experiments it was found that a concentration of 2·5% of

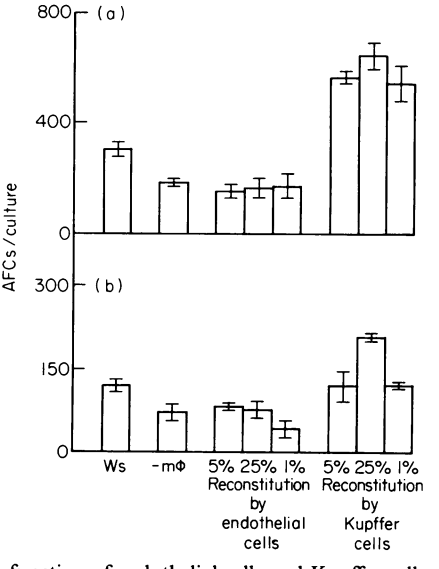

Fig. 1. The antigen-presenting function of endothelial cells and Kupffer cells. Each column represents mean (±s.d.) of triplicate cultures. (a) IgG plaques, (b) IgM plaques. WS = response of whole spleen, −MØ = response of macrophage-depleted spleen.

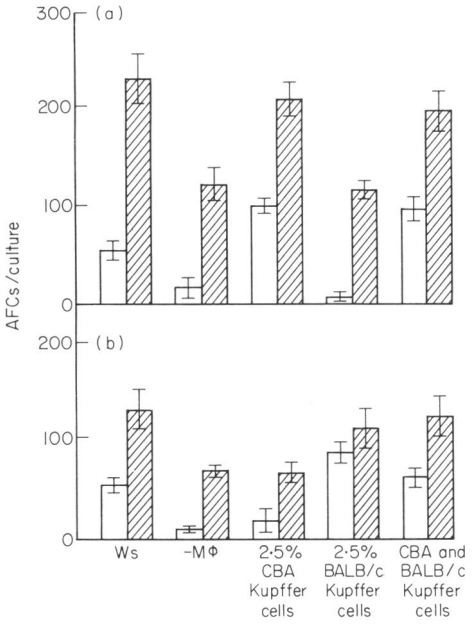

Fig. 2

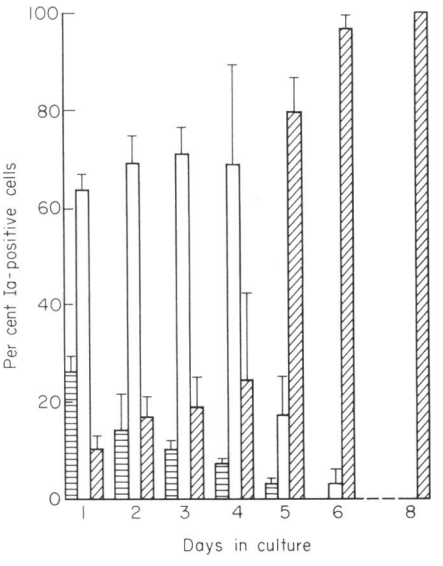

Fig. 3

Fig. 2. Genetic restriction of the antigen-presenting function of Kupffer cells. Each column represents mean (±s.d.) of triplicate cultures. (a) CBA spleen, (b) BALB/c spleen. WS = response of whole spleen, −MØ = response of macrophage-depleted spleen. (□) IgM response, (■) IgG response.

Fig. 3. Loss of I-A^k on cultured Kupffer cells. Results are mean per cent (±s.d.). (■) Strongly Ia-positive cells, (□) less strongly Ia-positive cells, (■) Ia-negative cells.

Table 3. Loss of antigen-presenting function of Kupffer cells with increasing time in culture

Age of added Kupffer cells	No. of AFCs/culture (±s.d.)			
	IgM		IgG	
	Reconstituted spleen / Whole spleen	% Reconstitution	Reconstituted spleen / Whole spleen	% Reconstitution
3 hr	211± 5 / 121±12	176	631±56 / 311±15	202
1 day	63±12 / 148±15	45	349±10 / 402±50	87
2 days	47± 6 / 123±21	38	183±16 / 305±40	60
7 days	17± 2 / 92± 7	19	84±14 / 465±30	18

Reconstitution was with 2·5% syngeneic Kupffer cells in each case. Values represent means (±s.d.) of triplicate cultures.

Kupffer cells gave the optimal response. In contrast, endothelial cells from layer 1 were unable to restore the secondary response in macrophage-depleted spleen cell cultures.

Purified Kupffer cell populations were prepared from both CBA and BALB/c mice and were used to reconstitute *in vitro* antibody responses in macrophage-depleted cultures of primed CBA and BALB/c spleen cells. Fig. 2 shows that addition of syngeneic Kupffer cells to macrophage-depleted spleen cells was alone capable of restoring the secondary response, allogeneic Kupffer cells being without effect. A mixture of allogeneic and syngeneic Kupffer cells did, however, result in restoration of the immune response, indicating that there was no suppressive effect generated by allogeneic cells.

Effect of time in culture on antigen-presenting function

Kupffer cells maintained in culture showed a loss of antigen-presenting ability. After culture for 48 hr before addition to the macrophage-depleted spleen cells, the Kupffer cells possessed negligible antigen-presenting function (see Table 3). After 7 days, they exerted an inhibitory effect on the antibody response.

During culture the Kupffer cells gradually lost Ia antigen (Fig. 3) until after a period of 8 days in culture, all were I-A^k-negative. The same loss was observed using anti-Ia^k and anti I-E/C^k sera. This change was not due to cell loss since during this time the numbers of cells in culture were increasing due to cell division. A similar loss of Ia antigen and of antigen-presenting function was found in PEM, whether or not they were treated with pronase.

DISCUSSION

Kupffer cells after isolation and culture for 24 hr are NSE-positive and have Fc receptors. The majority also have C3 receptors and possess Ia antigens. The present study shows evidence of heterogeneity in that 10% are Ia^k-negative and 72% are I-E/C^k-negative. With increasing time in culture, Ia antigen is lost and we have previously shown a gradual loss of Fc receptor and phagocytic ability accompanied by morphological changes (Pulford & Souhami, 1980). The I-A and I-E/C^k subregion antigens have been shown to be necessary for accessory cell function (Ahmann, Sachs & Hodes, 1978; Cowing *et al.*, 1978).

Endothelial cells are the major component of isolated hepatic NPCs and we have shown that they are NSE-positive, have Fc receptors and 'non-specific' receptors. The presence of an Fc receptor on non-phagocytic NPCs has been mentioned previously by Crofton, Diesselhoff den Dulk & van Furth (1978). We have been unable to demonstrate Ia antigens on these cells. Some endothelial cells are adherent after 24 hr in culture.

There is a considerable amount of clinical and experimental evidence to suggest that, *in vivo*, Kupffer cells have a major role in antigen sequestration (Bradfield, 1974; Souhami, 1972; Bjørnboe & Prytz, 1976) and that antigen taken up by the liver is not available for immunization. Recent studies have shown, however, that hepatic non-parenchymal cells can function as accessory cells *in vitro* in mitogen- and antigen-induced T-cell proliferation (Rogoff & Lipsky, 1979, 1980; Richman *et al.*, 1979; Richman, Strober & Berzofsky, 1980).

The present study also shows that Kupffer cells are able to reconstitute the secondary immune response in cultures of primed spleen cells depleted of adherent cells. We have found, as have others using T-cell-proliferative assays (Rogoff & Lipsky, 1980; Richman *et al.*, 1980), that the accessory function is genetically restricted. This restriction is not due to the formation of suppressor cells in the allogeneic cell culture (Erb & Feldmann, 1975).

The antigen-presenting property is not due to contamination with endothelial cells since purified populations of endothelial cells do not restore the immune response in spleen cells depleted of adherent cells. This finding contrasts with other studies which have shown that some human endothelial cells possess the equivalent of Ia (Moraes & Stastny, 1977; Hirschberg *et al.*, 1979) and can function as antigen-presenting cells (Hirschberg *et al.*, 1980). Our studies do not rule out the possibility that hepatic endothelial cells can also function in antigen presentation. The degree of macrophage depletion which we achieved was sufficient to reduce the antibody response by

approximately 60%. Further depletion leads to loss of B cells. In this situation, a relatively weak effect of endothelial cells in antigen presentation might not be demonstrated even when they were added back to form 5% of the total spleen cell population. The finding that hepatic endothelial cells have Fc receptors raises interesting questions as to the role of these cells in clearance of substances such as immune complexes from the bloodstream, and further investigation of this aspect of endothelial cell function is needed.

The antigen-presenting function of the Kupffer cells was lost with increasing duration of culture; indeed, we found that aged Kupffer cells caused a reduction in the antibody response below the background levels found in macrophage-depleted spleen cultures. This suppressive effect was consistent and has been observed before in mitogen-stimulated cultures (Munthe-Kaas, 1980).

Studies on isolated Kupffer cells must therefore take the age and purity of the cultured cells into account. The fact that Kupffer cells can function in antigen presentation *in vitro* is evidence against their *in vivo* differentiation into cells with a purely degradative function. It seems more probable that failure of antigen taken up by the liver to immunize is due to the environment of the hepatic sinusoid being unfavourable for contact with immunocompetent cells.

This work was supported by a grant from the Cancer Research Campaign.

REFERENCES

ACKERMAN, S.K. & DOUGLAS, S.D. (1978) Purification of human monocytes in microexudate coated plates. *J. Immunol.* **120,** 1372.

AHMANN, G.B., SACHS, D.H. & HODES, R.J. (1978) Genetic analysis of Ia determinants on Con A reactive cells. *J. Immunol.* **121,** 159.

BJØRNBOE, M. & PRYTZ, H. (1976) The mononuclear phagocytic functions of the liver. In *Immunological Aspects of the Liver and Gastrointestinal Tract (ed. by A. Ferguson and R. N. M. MacSween), p. 251. MTP Press, England.*

BRADFIELD, J.W.B. (1974) Control of spillover. The importance of Kupffer cell function in clinical medicine. *Lancet,* **ii,** 883.

COWING, C., PINIUS, S.H., SACHS, B.H. & DICKLER, H.B. (1978) A subpopulation of adherent accessory cells bearing both I-A and I-E or C subregion antigens is required for antigen specific murine T-lymphocyte proliferation. *J. Immunol.* **121,** 1680.

CROFTON, R.W., DIESSELHOFF DEN DULK, M.M. & VAN FURTH, R. (1978) The origin, kinetics and characteristics of the Kupffer cells in the normal steady state. *J. exp. Med.* **148,** 1.

CUNNINGHAM, A.J. & SZENBERG, A. (1968) Further improvements in the plaque technique for detecting single antibody forming cells. *Immunology,* **14,** 599.

ERB, P. & FELDMANN, M. (1975) The role of macrophages in the generation of T-helper cells. II. The genetic control of the macrophage–T-cell interaction for helper cell induction with soluble antigens. *J. exp. Med.* **142,** 460.

HIRSCHBERG, H., BERGH, O.J. & THORSBY, E. (1980) Antigen presenting properties of human vascular endothelial cells. *J. exp. Med.* **152,** 249S.

HIRSCHBERG, H., MOEN, T. & THORSBY, E. (1979) Complement and cell-mediated specific distinction of human endothelial cells treated with α-DRW antisera. *Transplant. Proc.* **11,** 776.

KAPLOW, L.S. (1965) Simplified myeloperoxidase stain using benzidine dihydrochloride. *Blood,* **26,** 215.

MORAES, J.R. & STASTNY, P. (1977) A new antigen system expressed in human endothelial cells. *J. clin. Invest.* **60,** 449.

MUNTHE-KAAS, A.C. (1980) The accessory cell effects of liver macrophages in concanavalin A stimulation of rat spleen lymphocytes. *Scand. J. Immunol.* **11,** 522.

PULFORD, K. & SOUHAMI, R.L. (1980) Cell division and giant cell formation in Kupffer cell cultures. *Clin. exp. Immunol.* **42,** 67.

RABINOVITCH, M. (1967) The dissociation of the attachment and ingestion phases of phagocytosis by macrophages. *Exp. Cell. Res.* **46,** 19.

RICHMAN, L.K., KLINGENSTEIN, R.J., RICHMAN, J.A., STROBER, W. & BERZOFSKY, J.A. (1979) The murine Kupffer cell. I. Characterization of the cell serving accessory function in antigen specific T cell proliferation. *J. Immunol.* **123,** 2602.

RICHMAN, L.R., STROBER, W. & BERZOFSKY, J.A. (1980) Genetic control of the immune response to myoglobin. III. Determinant-specific, two Ir gene phenotype is regulated by the genotype of reconstituting Kupffer cells. *J. Immunol.* **124,** 619.

ROGOFF, T.M. & LIPSKY, P.E. (1979) Characterization of isolated guinea-pig Kupffer cells. Accessory cell function in mitogen-induced T lymphocyte activation. *J. Immunol.* **123,** 1920.

ROGOFF, T.M. & LIPSKY, P.E. (1980) Antigen presentation by isolated guinea-pig Kupffer cells. *J. Immunol.* **124,** 1740.

SJÖBERG, O., ANDERSSON, J. & MÖLLER, G. (1972) Requirement for adherent cells in the primary and secondary immune response *in vitro*. *Eur. J. Immunol.* **2,** 123.

SOUHAMI, R.L. (1972) The effect of colloid carbon on the organ distribution of sheep red blood cells and the immune response. *Immunology,* **22,** 685.

SOUHAMI, R.L., ADDISON, I.E. & BRADFIELD, J.W.B. (1975) Increased antibody production following depression of hepatic phagocytosis. *Clin. exp. Immunol.* **20,** 155.

YAM, L.T., LI, C.Y. & CROSBY, W.H. (1971) Cytochemical identification of monocytes and granulocytes. *Am. J. clin. Pathol.* **55,** 287.