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Hepatic uptake of circulating IgG immune complexes

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Summary. IgG antibodies were found to increase the uptake of circulating dinintrophenylated human serum albumin (DNP-HSA) preparations by the non-parenchymal liver cells in rats. Highly DNP-conjugated HSA was taken up by the Kupffer cells both when given alone and when complexed by IgG. More lightly DNP-conjugated HSA was taken up mainly by the liver endothelial cells. Here, IgG promoted the antigen uptake both by the Kupffer cells and by the endothelial cells. Uptake of IgG immune complexes (IgG-ICs) by the sinusoidal endothelial cells of the liver is a new aspect on the function of these cells. Whether or not this phenomenon is Fc receptor-mediated is discussed. A heat-labile serum factor was found to direct the ICs to the Kupffer cells. This implies that serum complement and hepatic C3 receptors are essential for the physiological clearance of circulating immune complexes.

INTRODUCTION

The liver has a very important role in removing circulating macromolecules, complexes and particles.

Abbreviations: CR, complement receptors; DNP, dinitrophenyl; FcR, Fc receptors; HSA, human serum albumin; IC, immune complex; Ig, immunoglobulin; NPC, non-parenchymal liver cells; NRS, normal rat serum; NRS 56°, heat-treated rat serum.

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The hepatic uptake occurs either in the parenchymal cells (hepatocytes) or in the non-parenchymal cells (NPC), i.e. the Kupffer cells or the sinusoidal endothelial cells. A third type of NPC, designated the stellate cells, is important for the storage of vitamin A (Wake, 1982). Adherence of circulating material to liver cells may occur owing to non-specific physicochemical forces such as electrostatic attraction and hydrophobic interaction (Kooistra *et al.*, 1980; Skogh, Magnusson & Stendahl, 1983b). Hepatic clearance may also be the result of highly specific receptor-mediated interactions. For instance, receptors specific for various carbohydrates can remove certain circulating glycoproteins and carbohydrate-exposing particles via the hepatocytes or NPC (Ashwell & Harford, 1982). In certain species, the hepatocytes expose 'secretory component' which efficiently transfers di- and polymeric IgA from the blood to the bile (Lemaitre-Coelho *et al.*, 1981). Whether or not this mechanism is also important for the removal of circulating IgA immune complexes (IgA-ICs) is debatable (Russel, Brown & Mestecky, 1981; Skogh, Edebo & Stendahl, 1983a). Like other macrophages (Unkeless, 1977; Leslie, 1980; Diamond, Birshtein & Scharff, 1979; Fearson, 1980; Ross, 1980), Kupffer cells possess receptors specific for the Fc parts of IgG (FcR) and for complement factor C3 (Munthe-Kaas, Kaplan & Seljelid, 1976; Crofton, Diesselhoff den Dulk & van Furth, 1978; Pulford & Souhami, 1981; Daha & van Es, 1984). It has also been reported that sinusoidal endothelial cells expose FcR which, in contrast to the FcR of Kupffer cells, do not mediate phagocytosis

(Crofton *et al.*, 1978; Pulford & Souhami, 1981). Complement receptors (CR) do not occur on liver endothelial cells (Pulford & Souhami, 1981). Hepatic CR may mediate the adherence of circulating particles (Nydegger & Kazatchkine, 1983), but have not been shown to enhance blood clearance of circulating ICs.

IgG-ICs are eliminated from the blood mainly by the liver (Mannik & Arend, 1971). The suggestion that the hepatocyte asialoglycoprotein receptor mechanism is responsible for the hepatic uptake of IgG-ICs (Thornburg *et al.*, 1980) has been put into serious doubt (Skogh, 1982; Rifai *et al.*, 1982). The phenomenon of IgG-mediated antigen uptake by hepatocytic FcR (Hopf, Meyer zum Büschenfelde & Dierich, 1976) is probably merely the result of preparation artefacts, since such 'FcR' can only be demonstrated on non-viable hepatocytes (Ramadori *et al.*, 1983). Although small amounts of circulating IgG-ICs may possibly be taken up by hepatocytes, the most important role in IgG-IC handling probably resides in the NPC (Skogh, 1982; Rifai *et al.*, 1982). IgG-mediated antigen clearance from the blood is probably mainly the result of FcR interaction in the liver (Kurlander, Ellison & Hall, 1984). The Kupffer cells are the intuitive candidates for such FcR-dependent uptake of IgG-ICs (Mannik & Arend, 1971). Whether the FcR of sinusoidal endothelial cells also contribute to the elimination of circulating IgG-ICs does not appear to have been previously considered.

Serum complement can mediate the attachment of immunoaggregates and ICs to macrophages and polymorphonuclear leucocytes *in vitro* (van Snick & Masson, 1978; Kijlstra, van Es & Daha, 1979; Taylor *et al.*, 1983), but has not been shown to increase the blood clearance of circulating ICs (Bockow & Mannik, 1981; Harkiss & Brown, 1981). On the contrary, complement may actually *decrease* the blood clearance rate of circulating ICs owing to reduced extrahepatic deposition, without affecting the hepatic uptake *quantitatively* (Skogh & Stendahl, 1983). This phenomenon is probably explained by a complement-mediated increase in IC solubility (Takahashi, Takahashi & Hirose, 1980; Schifferli, Woo & Peters, 1982) which prevents precipitation and non-specific deposition outside the reticuloendothelial system. In man, CR-mediated IC attachment can also occur on erythrocytes, which subsequently can deliver the complexes to the spleen (Cornacoff *et al.*, 1984).

This study was undertaken to investigate the intrahepatic distribution of three model antigens after intravenous injection either alone, or complexed by IgG in the presence or absence of serum complement.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing 250–300 g were used.

Antigens

Lyophilized human serum albumin (HSA) (Kabi, Stockholm, Sweden) was conjugated with dinitrophenyl (DNP) groups using 2,4-dinitrobenzene sulphonate (Eastman, Rochester, NY) essentially as described by Eisen (1964). The degree of DNP-conjugation was determined spectrophotometrically at 358 nm, using $1{\cdot}74 \times 10^4$ as the molar extinction coefficient of DNP. Commercially available $DNP_{35}HSA$ (Calbiochem, La Jolla, CA) was also used. The DNP-HSA preparations were labelled with ^{125}I (Institutt for Energiteknikk, Kjeller, Norway) using the lactoperoxidase method (Marchalonis, 1969). The different ^{125}I-DNP-HSA preparations [1·4 mg/ml phosphate-buffered saline (PBS)] were diluted 1:25 or 1:50 with PBS, or normal rat serum (NRS), or heat-treated (56°, 30 min) NRS (NRS 56°).

Antibodies

IgG-anti-DNP was prepared by immunizing rabbits subcutaneously with 2 mg DNP-Keyhole limpet haemocyanin (Calbiochem) together with Freund's complete adjuvant (Difco, Detroit, MI). A booster dose (1 mg in Freund's incomplete adjuvant) was given after 4 weeks. The animals were bled and serum prepared 1 week after the booster dose. IgG was purified from the serum by fractionation on a diethylaminoethyl cellulose column (Pharmacia Fine Chemicals, Uppsala, Sweden). The preparation gave a single precipitation line of identity in agarose double immunodiffusion against anti-rabbit IgG and anti-rabbit whole serum (Cappel, Cochranville, PA). The anti-DNP activity was titrated by precipitation of ^{125}I-activity with 40% saturated ammonium sulphate after reaction between the IgG preparation and ^{125}I-DNP_2HSA. A concentration of 0·35 mg IgG/ml PBS was used consistently.

Immune complexes

Equal volumes of IgG and ^{125}I-DNP-HSA (diluted 1:25 in PBS, NRS or NRS 56°) were mixed and incubated at 37° for 45 min. The immune complexes formed using PBS-diluted antigens were analysed by

ultracentrifugation in linear sucrose density gradients as described elsewhere (Skogh *et al.*, 1983c).

Administration of antigens and immune complexes
Using DNP_2HSA and DNP_8HSA, the rats were anaesthetized with ether, and 28 μg (1 ml) ^{125}I-DNP-HSA was injected, alone or complexed by IgG, into the right femoral vein. The animals were kept anaesthetized for the rest of the experiment. The experiments with $DNP_{35}HSA$ were performed with rats anaesthetized with barbiturate.

Plasma clearance and total hepatic uptake
Fifteen minutes after the intravenous injection of antigen or immune complex preparation, a 1-ml blood sample was taken from the inferior caval vein into a heparinized syringe. Radioactivity was measured in the plasma. The liver was perfused (see below) and the radioactivity in a ligated liver lobe was measured to estimate the total hepatic uptake. It was assumed that the liver contains 190×10^6 cells/g wet weight, and that the hepatocytes, endothelial cells, Kupffer cells and stellate cells constitute 66%, 19%, 10% and 5%, respectively (Blomhoff *et al.*, 1984b).

Preparation of liver cells
Immediately after the blood sample was taken, the liver was perfused via the portal vein with Ca-free buffer for 10 min, followed by perfusion with collagenase type IA (Sigma Chemical Company, St Louis, MO) as described in detail elsewhere (Tolleshaug *et al.*, 1977). The total liver cell suspension thus obtained was subjected to differential centrifugation to separate NPC from the hepatocytes. The hepatocyte preparation was contaminated with approximately 1·1% endothelial cells, 4·6% Kupffer cells and 6·4% stellate cells identified as described previously (Blomhoff, Eskild & Berg, 1984a). NPC were also prepared from the total liver cell suspension by incubation with enterotoxin from *Clostridium perfringens* (purchased from Dr P. E. Granum, Norwegian Food Research Institute, As, Norway) to selectively destroy the hepatocytes (Blomhoff *et al.*, 1984).

Separation of NPC
The NPC preparations were subjected either to centrifugational elutriation in a JE-6 elutriator rotor (Beckman Instruments, Palo Alto, CA), or to centrifugation in density gradients of Nycodenz (Nygaard & Co., Oslo, Norway). These procedures have been described in detail elsewhere (Blomhoff *et al.*, 1984a). In the rat liver, all Kupffer cells contain endogenous peroxidase, whereas all endothelial cells are peroxidase-negative (de Leeuw *et al.*, 1982). Cytochemical analysis was performed as described elsewhere (Blomhoff *et al.*, 1984a) to identify peroxidase-negative cells (sinusoidal endothelial cells and stellate cells) and peroxidase-positive cells (Kupffer cells) in the different fractions. Occasional hepatocytes in the preparations were identified by their distinctly different morphological appearance.

Radioactivity in the fractions was measured in the different liver cell fractions. It was always possible to achieve pure peroxidase-negative cells. The Kupffer cell fraction was, however, also contaminated by some peroxidase-negative cells. This was probably mainly due to the adherence of Kupffer cells to endothelial cells. For calculation of the total amount of radioactivity associated with the different NPC, we assumed that the amount of radioactivity per peroxidase-negative cell was the same both in the pure endothelial cell fractions and in the Kupffer cell enriched fractions.

RESULTS

Immune complex characterization

The sucrose density gradient ultracentrifugation profiles of the different IC preparations formed in PBS are shown in Fig. 1. The complexes formed using DNP_8HSA or $DNP_{35}HSA$ were large (> 50 S), but soluble as determined by centrifugation at 3500 ***g*** for 10 min (data not shown). The complexes formed using DNP_2HSA were much smaller.

Distribution of radioactivity after intravenous injection of antigen and immune complex preparations

$DNP_{35}HSA$. About 80% of the radioactivity was recovered from the total liver 15 min after the intravenous injection of ^{125}I-$DNP_{35}HSA$ alone (data not shown). The intrahepatic distribution of radioactivity after the injection of the antigen and the different IgG/$DNP_{35}HSA$ complexes, respectively, is illustrated in Fig. 2. Seventy-five to eighty percent of the hepatic radioactivity was recovered from the NPC in all instances. The Kupffer cells were consistently responsible for the bulk of this uptake, e.g. 67% of the injected radioactivity from uncomplexed $DNP_{35}HSA$ was recovered from Kupffer cells and 8% from endothelial cells; 69% of the radioactivity from the IgG-ICs formed in PBS alone was found in the

Table 1. Distribution of radioactivity (percentage of administered dose) 15 min after intravenous injection

	DNP_8HSA (PBS)	IgG/DNP_8HSA (PBS)	IgG/DNP_8HSA (NRS 56°)	IgG/DNP_8HSA (NRS)	DNP_2HSA (PBS)	IgG/DNP_2HSA (NRS 56°)	IgG/DNP_2HSA (NRS)
Plasma	66	44	33	32	ND*	ND	ND
Total liver	7	21	22	20	2	11	17
Hepatocytes	1	4	5	5	ND	5	5
NPC	6	17	17	15	ND	6	12
Endothelial cells	5	10	8	4	ND	4	3
Kupffer cells	1	7	9	11	ND	2	8

* ND, not determined.

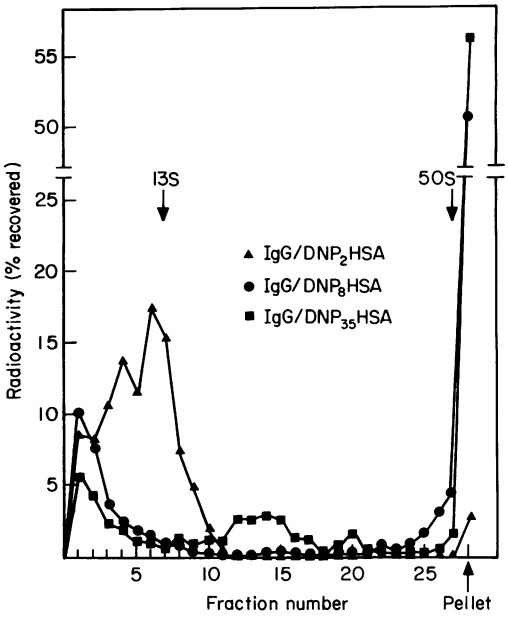

Figure 1. Sucrose density gradient ultracentrifugation profiles of radioactivity illustrating the size distribution of immune complexes formed between IgG-anti-DNP, and DNP$_2$HSA (▲), or DNP$_8$HSA (●) or DNP$_{35}$HSA (■).

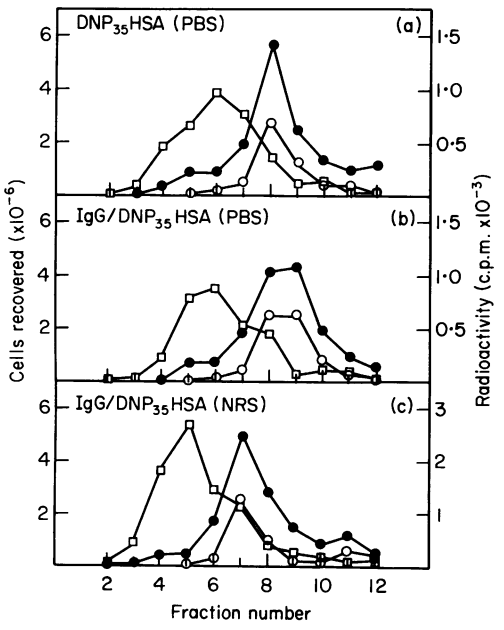

Figure 2. Nycodenz gradient fractionation profile illustrating the distribution of peroxidase-negative NPC (□), peroxidase-positive NPC (○) and radioactivity (●) 15 min after the intravenous injection of ^{125}I-labelled DNP$_{35}$HSA given alone (a) or complexed by IgG either in PBS (b) or in NRS (c).

Kupffer cells and 6% in the endothelial cells; when the ICs formed in the presence of fresh rat serum were given, 75% of the radioactivity was recovered from Kupffer cells and 4% from the endothelial cells. The experiments illustrated in Fig. 2 were performed by Nycodenz centrifugation of NPC prepared by differential centrifugation.

DNP$_8$HSA. The radioactivity recovered from plasma, total liver, and the different cells 15 min after intravenous injection of DNP$_8$HSA, or IgG/DNP$_8$HSA prepared in PBS, or IgG/DNP$_8$HSA prepared in the presence of serum, is shown in Table 1. The proportion of radioactivity in the different NPC was estimated from the results shown in Figs 3 and 4, illustrating the radioactivity distribution in liver cells after centrifugation in Nycodenz. Figure 5 shows the results of the centrifugational elutriation experiments. In the absence of complement (i.e. in PBS or NRS 56°), IgG increased the total hepatic uptake of DNP$_8$HSA from about 7% to about 21% (17% in the NPC and 4% in the hepatocyte fractions). Although the IgG-mediated increase in hepatic antigen uptake was largest in the Kupffer cells, a marked increase was also noted in the endothelial cells. Roughly 50% of the NPC-located ICs were recovered from the liver endothelial cells as compared to the Kupffer cells. A slight uptake by the stellate cells too cannot be excluded. IgG-ICs prepared in the presence of NRS were taken up to approximately the same extent (about 20%) as those prepared in the absence of complement, but the hepatic uptake was efficiently directed towards the Kupffer cells (Figs 3, 4 and 5).

DNP$_2$HSA. When DNP$_2$HSA alone was injected intravenously, less than 2% of the radioactivity was recovered from the total liver after 15 min. No analysis of the intrahepatic distribution of DNP$_2$HSA was performed. In the absence of active complement, IgG increased the hepatic uptake of DNP$_2$HSA to about 11% (6% in the NPC and 5% in the hepatocyte fraction). About 60% of the NPC-located radioactivity was recovered from the endothelial cells and 40% from the Kupffer cells. About 17% (11% in the NPC and 5% in the hepatocytes) of the IgG/DNP$_2$HSA complexes preincubated with fresh serum were recov-

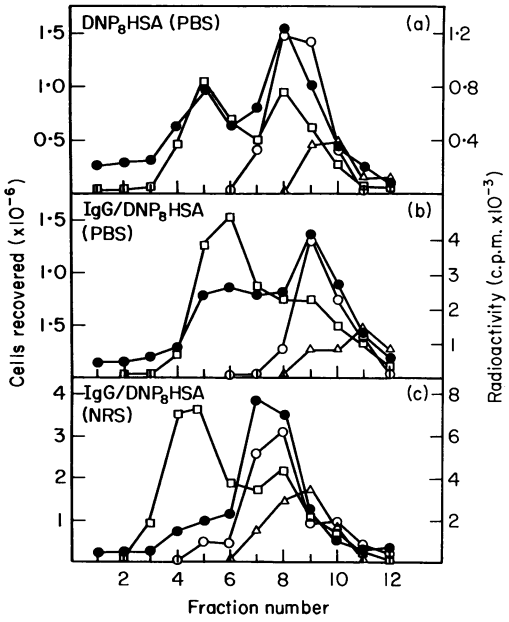

Figure 3. Nycodenz gradient fractionation of peroxidase-negative NPC (□), peroxidase-positive (○) NPC, hepatocytes (△) and radioactivity (●) 15 min after the intravenous injection of ^{125}I-DNP$_8$HSA alone (a) or complexed by IgG either in PBS (b) or in NRS (c).

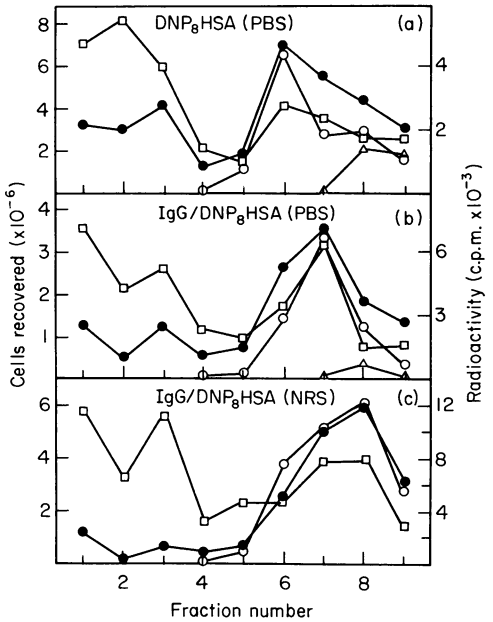

Figure 5. Centrifugational elutration fractionation profile of peroxidase-negative NPC (□), peroxidase-positive NPC (○), hepatocytes (△) and radioactivity (●) 15 min after the intravenous injection of ^{125}I-DNP$_8$HSA alone (a) or complexed by IgG, either in PBS (b) or in NRS (c).

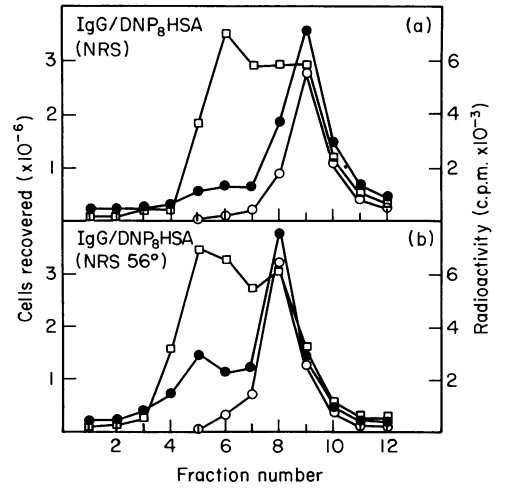

Figure 4. Nycodenz gradient fractionation profiles illustrating the distribution of peroxidase-negative NPC (□), peroxidase-positive NPC (○) and radioactivity (●) 15 min after the intravenous injection of IgG/DNP$_8$HSA immune complexes preincubated in NRS (a) and NRS 56° (b).

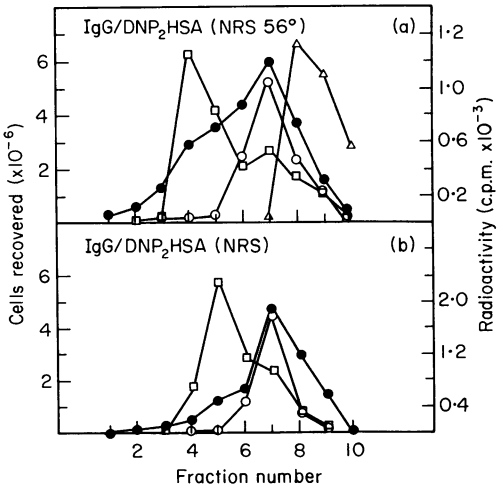

Figure 6. Nycodenz gradient fractionation profiles illustrating the distribution of peroxidase-negative NPC (□), peroxidase-positive NPC (○), hepatocytes (△) and radioactivity (●) 15 min after the intravenous injection of IgG/DNP$_2$HSA immune complexes.

ered from the total liver 15 min after intravenous injection. Here too, a heat-labile serum factor clearly promoted uptake by the Kupffer cells (Table 1 and Fig. 6).

DISCUSSION

In earlier studies, we found that $DNP_{35}HSA$ was efficiently eliminated from the blood by the liver, and that IgG-anti-DNP increased the hepatic uptake further (Skogh, 1982). In this study, we showed that the hepatic uptake of $DNP_{35}HSA$, as well as the uptake of IgG/$DNP_{35}HSA$ immune complexes, occurred mainly in the Kupffer cells. Serum complement apparently did not affect the intrahepatic distribution of IgG/$DNP_{35}HSA$. DNP_8HSA was taken up by both Kupffer cells and by the sinusoidal endothelial cells, although the blood clearance of DNP_8HSA was less efficient as compared to $DNP_{35}HSA$. Both of these preparations may adhere to the non-parenchymal liver cells by non-specific physicochemical attraction, for instance hydrophobic interaction (Skogh *et al.*, 1983b, c). In addition, the highly DNP-conjugated preparation could have become bound to complement factor C1q exposed on the surface of the Kupffer cells (Loos, 1982). Although highly DNP-conjugated HSA activates the complement system both via the classical and the alternative pathways (König *et al.*, 1974; Loos & König, 1977), serum complement does not have any effect on the elimination of intravenously injected $DNP_{35}HSA$ (Skogh, 1982; Skogh *et al.*, 1983b). The predominant localization of $DNP_{35}HSA$ to the Kupffer cells contrasts with the intrahepatic distribution of formaldehyde-treated HSA, which is efficiently cleared from the blood by the sinusoidal endothelial cells alone (Blomhoff *et al.*, 1984a). Further studies on this matter are underway.

The hepatic uptake of DNP_2HSA was very limited within 15 min of intravenous injection, but was markedly increased by IgG-anti-DNP. Surprisingly, most of the ICs formed between IgG and DNP_2HSA in the absence of complement were recovered from the sinusoidal endothelial cells, although a large proportion was also taken up by the Kupffer cells. Similarly, IgG-anti-DNP increased the hepatic uptake of DNP_8HSA. The increase was most evident in the Kupffer cells, but nevertheless about 50% of the IgG/DNP_8HSA ICs were recovered from the sinusoidal endothelial cells. Since both Kupffer cells and sinusoidal endothelial cells have been reported to express FcR for IgG (Crofton *et al.*, 1978; Pulford & Souhami, 1981), it is possible that the NPC uptake of IgG-ICs was FcR-mediated. However, the IgG-mediated increase in uptake of DNP_2HSA and DNP_8HSA by the NPC must not necessarily be the result of FcR-interaction alone. Exposed Fc parts of antigen-bound IgG are hydrophobic, and therefore promote non-specific adherence to hydrophobic surfaces (van Oss, 1978; Stendahl *et al.*, 1977). There is no reason to doubt the existence of specific IgG FcR on the surface of Kupffer cells analogously with the properties of other mononuclear phagocytes (Unkeless, 1977; Leslie, 1980; Diamond *et al.*, 1979). The nature of the FcR on sinusoidal endothelial cells can be questioned, however. Until the specificity of these 'receptors' have been proven by means other than inhibition with aggregated IgG, the possibility of a merely non-specific IgG-mediated phenomenon cannot be excluded.

In contrast to the ICs formed between IgG and DNP_2HSA or DNP_8HSA, the relative amount of IgG/$DNP_{35}HSA$ taken up by the sinusoidal endothelial cells was very small. This finding contradicts the hypothesis that uptake of IgG-ICs by the liver endothelial cells is FcR-mediated, although it could be argued that the great tendency of the antigen to interact with the Kupffer cells governed the fate of the IgG/$DNP_{35}HSA$ ICs. Other studies have shown that the properties of the antigen may be of considerable importance for the fate of circulating ICs (Skogh, 1982; Rifai *et al.*, 1982; Kijlstra *et al.*, 1979), and it is likely that the intrahepatic distribution of the ICs used in this study (at least those containing DNP_8HSA and $DNP_{35}HSA$) was largely influenced by the antigens.

Although the proportion of IgG/$DNP_{35}HSA$ taken up by the liver endothelial cells was very small as compared to the uptake by the Kupffer cells, the total uptake of these ICs by the endothelial cells (6%) was comparable to the total uptake of IgG/DNP_2HSA (6%) and IgG/DNP_8HSA (8–10%) by the liver endothelial cells at the doses given in this study. It is, therefore, still possible that liver endothelial cells possess specific FcR for IgG, but that the number of receptors may be smaller than on the Kupffer cells. Clearance of circulating IgG-ICs by liver endothelial cells, FcR-mediated or not, is a new aspect of the function of these cells. Although IgG-ICs adhere to both Kupffer cells and liver endotelial cells, it is possible that endocytosis and the subsequent degradation of the complexes is restricted to the Kupffer cells. We are presently examining these questions.

In a recent publication by Sancho *et al.* (1984), it was suggested that the blood clearance of IgG immune complexes is mainly the result of FcR-mediated adherence to the hepatocytes. This was based upon their claim that heat-aggregated IgG was taken up mainly by hepatocytes after intravenous injection in mice, and that the *in vitro* adherence of IgG aggregates to hepatocytes was not inhibited by IgA aggregates. We think that these results and interpretations must be regarded with great caution. Sancho *et al.* (1984) studied mechanically isolated liver cells. This procedure should result in cells of low viability, and adherence of IgG aggregates to such hepatocytes is not FcR-mediated (Ramadori *et al.*, 1983). Furthermore, it has been shown by others that heat-aggregated IgG, in contrast to IgG-ICs, is more likely to adhere non-specifically rather than via specific FcR (Whaley *et al.*, 1983). The failure of aggregated IgA to inhibit attachment does by no means prove the specificity of the interaction between IgG aggregates and hepatocytes, since IgA is less hydrophobic and consequently less prone to non-specific (hydrophobic) interaction than is IgG (Edebo *et al.*, 1981). Also in our study, however, we found limited amounts of radioactivity in the hepatocyte fractions 15 min after intravenous injection of IgG-ICs. This was probably to some extent due to small radioactive degradation products, and to some extent owing to contamination of NPC in the hepatocyte preparations. However, it cannot be excluded that small amounts of our IgG-IC preparations were also taken up by the hepatocytes, probably by non-specific mechanisms.

Perhaps the most exciting finding in this study was that a heat-labile serum factor—most probably complement factor C3b—caused a profound alteration of the intrahepatic distribution of IgG-ICs without affecting the total hepatic uptake. The IgG-ICs prepared in the presence of active serum complement were taken up almost exclusively by the Kupffer cells. The requirement to preincubate (37°, 45 min) the ICs to demonstrate this effect in normal rats is not surprising, considering that complement activation is a time-consuming process (Takahashi *et al.*, 1980). Many attempts have been made to demonstrate that the complement-mediated attachment of ICs to phagocytes, known to occur *in vitro* (van Snick & Masson, 1978; Kijlstra *et al.*, 1979; Taylor *et al.*, 1983), is important for the removal of circulating ICs. These investigations have all failed, however, to show that complement increases the clearance of circulating ICs (Bockow & Mannik, 1981; Harkiss & Brown, 1981). In fact, complement may actually decrease the blood clearance rate owing to reduced non-specific IC deposition outside the RES, without affecting the hepatic uptake quantitatively (Skogh & Stendahl, 1983). The results of the present study nevertheless support the hypothesis that complement is very important for the quality of hepatic IC handling, directing the complexes almost exclusively to the liver cells possessing C3b receptors, i.e. the Kupffer cells (Munthe-Kaas *et al.*, 1976; Crofton *et al.*, 1978; Pulford & Souhami, 1981; Daha & van Es, 1984), and thereby perhaps increasing the specificity of IC uptake by the liver.

Low levels or defective function of serum complement in disease states such as systemic lupus erythematosus (Schur & Sandson, 1968; Sliwinsky & Zwaifler, 1972) and rheumatoid arthritis (Naama *et al.*, 1983) may result both in poor ability to keep ICs soluble and in defective IC opsonization. This could lead to increased extrahepatic IC deposition with the consequent onset of inflammation at these sites. Although the quantitative uptake of ICs by the liver appears to be normal in the absence of complement, the quality of hepatic IC handling may be seriously impaired if opsonization by complement is insufficient.

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REFERENCES

ASHWELL G. & HARFORD J. (1982) Carbohydrate-specific receptors of the liver. *Ann. Rev. Biochem.* **51,** 531.

BLOMHOFF R., ESKILD W. & BERG T. (1984a) Endocytosis of formaldehyde-treated serum albumin via scavenger pathway in liver endothelial cells. *Biochem. J.* **218,** 81.

BLOMHOFF R., HOLTE K., NAESS L. & BERG T. (1984b) Newly administered H retinol is transferred from hepatocytes to stellate cells in liver for storage. *Exp. Cell Res.* **150,** 186.

BLOMHOFF R., SMEDSROD B., ESKILD W., GRANUM P.E. & BERG T. (1984c) Preparation of isolated liver endothelial cells and Kupffer cells in high yield by means of an enterotoxin. *Exp. Cell Res.* **150,** 194.

BOCKOW B. & MANNIK M. (1981) Clearance and tissue uptake of immune complexes in complement-depleted and control mice. *Immunology*, **42,** 497.

CORNACOFF J.B., HEBERT L.A., SMEAD W.L., VAN AMAN M.E. & WAXMAN L.A. (1983) Primate erythrocyte-immune complex clearing mechanism. *J. clin. Invest.* **71,** 236.

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.

DAHA M.R. & VAN ES L.A. (1984) Fc- and complement receptor-dependent degradation of soluble immune complexes and stable immuno aggregates by guinea pig monocytes, peritoneal macrophages, and Kupffer cells. *J. Leuk. Biol.* **36,** 569.

DIAMOND B., BIRSHTEIN B.K. & SCHARFF M.D. (1979) Site of binding of mouse IgB2b to the Fc receptor on mouse macrophages. *J. exp. Med.* **150,** 721.

EDEBO L., NORMANN B., SKOGH T. & STJERNSTRÖM I. (1981) Properties of antibodies of different immunoglobulin class in relation to opsonisation. *Monogr. Allergy*, **17,** 48.

EISEN H.N. (1984) Preparation of purified anti-2,4-dinitrophenyl antibodies. In: *Methods in Medical Research* (ed. H. N. Eisen), p. 94. Year Book Medical Publishers, Chicago.

FEARON D.T. (1980) Identification of the membrane glycoprotein that is the C3b receptor of the human erythrocyte, polymorphonuclear leukocyte, B lymphocyte and monocyte. *J. exp. Med.* **152,** 20.

HARKISS G.D. & BROWN D.L. (1981) Clearance kinetics of soluble preformed immune complexes containing IgM antibodies in normal and complemented rabbits. *Immunology*, **42,** 297.

HOPF U., MEYER ZUM BUSCHENFELDE K.H. & DIERICH M.P. (1976) Demonstration of binding sites for IgG-Fc and the third component of complement (C3) on isolated hepatocytes. *J. Immunol.* **117,** 639.

KIJLSTRA A., VAN ES L.A. & DAHA M.R. (1979) The role of complement in the binding and degradation of immune aggregates and on their processing by macrophages. *J. Immunol.* **123,** 2488.

KIJLSTRA A., KNUTSSON D.W., DAHA M.R. & VAN ES L.A. (1979) Clearance and glomerular localization of preformed DNP anti-DNP immune complexes. *Scand. J. Immunol.* **10,** 421.

KÖNIG W., BITTER-SUERMAN D., SCHORLEMMER H.-V. & HADDING U. (1974) DNP-antigens activate the alternative pathway of the complement system. *J. Immunol.* **113,** 501.

KOOISTRA T., DUURSMA A.M., BOUMA J.M.W. & GRUBER M. (1980) Effect of size and charge on endocytosis of lysozyme derivatives by sinusoidal rat liver cells *in vivo*. *Biochem. biophys. Acta*, **631,** 439.

KURLANDER R.J., ELLISON M.E. & HALL J. (1984) The blockade of FC receptor-mediated clearance of immune complexes *in vivo* by a monoclonal antibody (2.4.G2) directed against Fc receptors on murine leukocytes. *J. Immunol.* **133,** 855.

DE LEEUW A.M., SLEYSTER E.C., QUIST M.J. & KNOOK D.L. (1982) Rat and mouse Kupffer and endothelial cells: a comparison of structural and functional characteristics. *Kupffer Cell Bull.* **4,** 4.

LEMAITRE-COELHO I., ALTAMIRANO G.A., BARRANCO-ACOSTA C., MEYKENS R. & VERMAN J.-P. (1981) *In vivo* experiments involving secretory component in the rat heptatic transfer of polymeric IgA from blood into bile. *Immunology*, **43,** 261.

LESLIE R.G.Q. (1980) The binding of soluble immune complexes of guinea pig IgG_2 to homologous peritoneal macrophages. Determination of the avidity constants at 4°C. *Eur. J. Immunol.* **10,** 317.

LOOS M. (1982) The functions of endogenous C1q, a subcomponent of the first component of complement, as a receptor on the membrane of macrophages. *Molec. Immunol.* **19,** 1229.

LOOS M. & KÖNIG W. (1977) Interaction of DNP-antigens with the first component of complement (C1). *J. Immunol.* **118,** 223.

MANNIK M. & AREND W.P. (1971) Fate of preformed immune complexes in rabbits and Rhesus monkeys. *J. exp. Med.* **134,** 19S.

MARCHALONIS J.J. (1969) An enzymic method for the trace iodination of immunoglobulins and other proteins. *Biochem. J.* **113,** 229.

MUNTHE-KAAS A.C., KAPLAN G. & SELJELID R. (1976) On the mechanism of internalization of opsonized particles by rat Kupffer cells *in vitro*. *Exp. Cell. Res.* **103,** 201.

NAAMA J.K., MITCHELL W.S., ZOMA A., VEITCH J. & WHALEY K. (1983) Complement-mediated inhibition of immune precipitation in patients with immune complex diseases. *Clin. exp. Immunol.* **51,** 292.

NYDEGGER U.E. & KAZATCHKINE M.D. (1983) The role of complement in immune clearance of blood cells. *Springer Semin. Immunopathol.* **6,** 373.

VAN OSS C.J. (1978) Phagocytosis as a surface phenomenon. *Ann. Rev. Microbiol.* **32,** 19.

PULFORD K. & SOUHAMI R.L. (1981) The surface properties and antigen presenting function of hepatic non-parenchymal cells. *Clin. exp. Immunol.* **46,** 581.

RAMADORI G., LENZI M., DIENES H.P. & MEYER ZUM BUSCHENFELDE K.H. (1983) Binding properties of mechanically and enzymatically isolated hepatocytes for IgG and C3. *Liver*, **3,** 358.

RIFAI A., FINBLOOM D.S., MAGILAVY D.B. & PLOTZ P.H. (1982) Modulation of the circulation and hepatic uptake of immune complexes by carbohydrate recognition systems. *J. Immunol.* **128,** 2269.

ROSS G.D. (1980) Analysis of the different types of leukocyte membrane complement receptors and their interaction with the complement system. *J. immunol. Meth.* **37,** 197.

RUSSEL M.W., BROWN T.A. & MESTECKY J. (1981) Role of serum IgA. Hepatobiliary transport of circulating antigen. *J. exp. Med.* **153,** 968.

SANCHO J., GONZALEZ E., ESCANERO J.F. & EGIDO J. (1984) Binding kinetics of monomeric and aggregated IgG to Kupffer cells and hepatocytes of mice. *Immunology*, **53,** 283.

SCHIFFERLI J.A., WOO P. & PETERS K. (1982) Complement-mediated inhibition of immune precipitation. I. Role of the classical and alternative pathways. *Clin. exp. Immunol.* **47,** 555.

SCHUR P.H. & SANDSON J. (1968) Immunologic factors and clinical activity in systemic lupus erythematosus. *New Engl. J. Med.* **278,** 533.

SKOGH T. (1982) Tissue distribution of intravenously injected dinitrophenylated human serum albumin. Effects of specific IgG and IgA antibodies. *Scand. J. Immunol.* **16,** 465.

SKOGH T., EDEBO L. & STENDAHL O. (1983a) Gastrointestinal uptake and blood clearance of antigen in the presence of IgA antibodies. *Immunology*, **50,** 175.

SKOGH T., MAGNUSSON K.-E. & STENDAHL O. (1983b) Hydrophobic interaction between DNP-HSA and different tissue structures *in vivo* assessed by indirect immunofluorescence microscopy. *J. immunol. Meth.* **61,** 385.

SKOGH T. & STENDAHL O. (1983) Complement-mediated delay in immune complex clearance from the blood owing to reduced deposition outside the reticuloendothelial system. *Immunology*, **49,** 53.

SKOGH T., STENDAHL O., SUNDQVIST T. & EDEBO L. (1983c) Physicochemical properties and blood clearance of human serum albumin conjugated to different extents with dinitrophenyl groups. *Int. Archs Allergy appl. Immunol.* **70,** 238.

SLIWINSKY A.J. & ZWAIFLER N.J. (1972) Decreased synthesis of the third component of complement (C3) in hypocomplementemic systemic lupus erythematosus. *Clin. exp. Immunol.* **4,** 21.

VAN SNICK J.L. & MASSON P.L. (1978) The effect of complement on the ingestion of soluble antigen–antibody complexes and IgM aggregates by mouse peritoneal macrophages. *J. exp. Med.* **148,** 903.

STENDAHL O., TAGESSON C., MAGNUSSON K.-E. & EDEBO L. (1977) Physico-chemical consequences of opsonization of *Salmonella typhimurium* 395 MS sensitized with anti-MS IgG and complement. *Infect. Immun.* **18,** 261.

TAKAHASHI M., TAKAHASHI S. & HIROSE S.-I. (1980) Solubilization of antigen–antibody complexes: a new function of complement as a regulator of immune reactions. *Prog. Allergy*, **27,** 134.

TAYLOR R.P., BURGE J., HORGAN C. & SHASBY D.M. (1983) The complement-mediated binding of soluble antibody/dsDNA immune complexes to human neutrophils. *J. Immunol.* **130,** 2656.

THORNBURG R.W., DAY J.F., BAYNES J.W. & THORPE S.R. (1980) Carbohydrate-mediated clearance of immune complexes from the circulation. *J. biol. Chem.* **255,** 6820.

TOLLESHAUG H., BERG T., NILSSON M. & NORUM K.R. (1977) Uptake and degradation of ^{125}I-labelled asialo-fetuin by isolated rat hepatocytes. *Biochem. biophys. Acta*, **499,** 73.

UNKELESS J.C. (1977) The presence of two Fc-receptors on mouse macrophages. Evidence from a variant cell line and different trypsin sensitivity. *J. exp. Med.* **145,** 931.

WAKE K. (1982) The sternzellen of von Kupffer—after 106 years. In: *Sinusoidal Liver Cells* (eds D. L. Knook and E. Wisse), p. 1. Elsevier Biomedical Press, Amsterdam.

WHALEY K., LAPPIN D., MCPHADEN A.R., RICHES D.W.H. & SANDILANDS G.P. (1983) A comparison of the effect of heat-aggregated and chemcally cross-linked IgG on monocyte C2 production. *Immunology*, **49,** 457.