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Kupffer cell depletion *in vivo* results in preferential elimination of IgG aggregates and immune complexes via specific Fc receptors on rat liver endothelial cells

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SUMMARY

In the present study we have investigated the clearance kinetics and tissue distribution of monomeric (m) IgG and soluble aggregates of IgG (AIgG) and immune complexes (IC) in normal and Kupffer cell (KC) depleted rats. In normal rats, clearance of mIgG occurred in a biphasic manner with a first half-life ($T\frac{1}{2}$) (T1) of 36·3 ± 6·3 min and a second $T\frac{1}{2}$ (T2) of 168·4 ± 4·7 min. AIgG composed of 20–27 IgG molecules per aggregate were cleared significantly faster than mIgG with a T1 of 2·5 ± 0·1 min and a T2 of 32·5 ± 5·6 min. KC depletion did not have a significant effect on the clearance rate of mIgG (T1: 33·4 ± 8·9 min; T2: 159·5 ± 12·5 min), while clearance of AIgG was delayed significantly with T1 4·8 ± 0·7 min and T2 41·2 ± 3·2 min. Eight minutes after injection, 77% of AIgG was found in the liver in normal rats while 62% was found in the liver of KC-depleted rats. Double immunofluorescence studies indicated that AIgG in the liver was associated with KC and endothelial cells (EC) in normal rats. In KC-depleted rats, AIgG was strongly associated with EC. A similar staining pattern was observed when IgG-immune IC were administered. The clearance of AIgG in KC-depleted rats was inhibited fully by pre-administration of high concentrations of IgG but not by pretreatment with IgA, asialofetuin (ASFe) or ovalbumin (OVA). Aggregated $F(ab')_2$IgG was cleared with a comparable rate to mIgG from the circulation, again suggesting Fcγ receptor-mediated elimination of AIgG by EC. There was a reduced degradation of AIgG in rats depleted of KC as compared with normal rats. These data suggest binding and degradation of AIgG by EC *in vivo*.

Keywords IgG Kupffer cells endothelial cells liposomes Fc receptors

INTRODUCTION

The deposition of soluble immune complexes (IC) in different tissues has been implicated in the pathogensis of many diseases (Theofilopoulos & Dixon, 1979; Van Es, 1981; Schifferli & Taylor, 1989). Studies in humans and experimental models have established that the liver is primarily responsible for the removal of circulating IC from the circulation (Rifai & Mannik, 1984; Skogh *et al.*, 1985; Lobatto *et al.*, 1987; Halma *et al.*, 1989; Rifai *et al.*, 1989; Bogers *et al.*, 1989, 1990). Within the liver, the liver macrophages (Kupffer cells, KC) are considered to be responsible for IC clearance (Benacerraf, Sebestyen & Cooper, 1959; Rifai & Mannik, 1984; Bogers *et al.*, 1990).

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Very recently it has been reported that endothelial cells (EC) in rat and human liver sections are able to bind IgG-IC *in vitro* (Muro *et al.*, 1987). Furthermore, *in vivo* studies reported binding of IgG-IC to liver EC (Skogh *et al.*, 1985; Van Der Laan-Klamer *et al.*, 1985, 1986b; Muro *et al.*, 1987). Despite the lack of Fc receptors on EC of ordinary blood vessels (Ryan *et al.*, 1980; Shingu *et al.*, 1981), the binding of IgG-IC to liver EC seems to be mediated by Fc receptors (Pulford & Souhami, 1981; Van Der Laan-Klamer *et al.*, 1986a; Muro *et al.*, 1987). It is difficult to be precise concerning the relative contributions of EC and KC in the elimination of IgG-IC. Recently a method was developed to specifically eliminate KC *in vivo* by using drugs encapsulated in liposomes (Van Rooijen & Claassen, 1988). With this method, rodents can be fully depleted of liver and spleen macrophages without damaging other cell types (Van Rooijen & Claassen, 1988; Van Rooijen, 1989; Van Rooijen *et al.*, 1990; Bogers *et al.*, 1991). Using this approach, we have

investigated the contribution of liver EC and KC in the clearance and handling of IgG aggregates and IgG-IC.

MATERIALS AND METHODS

Rat IgG antibodies

Rat IgG was isolated from normal rat serum by 33% $(NH_4)_2SO_4$ precipitation followed by anion exchange chromatography on DEAE-Sephacel (Pharmacia LKB, Woerden, The Netherlands). The IgG preparations were shown by ELISA to contain less than 0·01% IgA and 0·05% IgM on a weight basis. IgG preparations were aliquoted and stored at −70 C.

Radioiodination

For clearance and tissue distribution studies, IgG was radiolabelled with ^{125}I ($Na^{125}I$, Radiochemical Centre, Amersham, UK), in the presence of Iodogen (1,3,4,6-tetrachloro3α,6α-diphenyl glycol uril, Pierce Chemical Co., Rockford, IL) as described previously (Bogers *et al.*, 1991).

Preparation and analysis of radiolabelled aggregated IgG

Purified radiolabelled IgG preparations (10 mg/ml) were heat aggregated for 20 min at 63 C (Kijlstra, Van Es & Daha, 1979). Insoluble aggregated IgG were removed by centrifugation (10 min, 1500 ***g***). Soluble aggregates of IgG (AIgG) were separated according to size by ultracentrifugation in sucrose gradients containing 0·5% bovine serum albumin (BSA) as described previously (Bogers *et al.*, 1989, 1990). For clearance and tissue distribution studies, ^{125}I-AIgG of a molecular composition between 42S and 51S, containing an average of 20–27 molecules of IgG per aggregate (as calculated from sedimentation rates (Kijlstra *et al.*, 1977)) were used.

To determine the site of localization in the liver, unlabelled IgG was aggregated and separated by size as described above. Fractions containing AIgG with a comparable molecular composition to that used for clearance studies (42–51S) were pooled, concentrated (1 mg/ml) and stored at −70 C until use.

Preparation of IgG immune complexes

Anti-bovine thyroglobulin (BTG) antibodies were raised in rabbits by intramuscular immunization with BTG in Freund's complete adjuvant. IgG anti-BTG was isolated by precipitation with 33% $(NH_4)_2SO_4$ followed by anion exchange chromatography on DEAE Sephacel. IgG containing fractions were pooled, concentrated and affinity purified using a BTG-immunosorbent column. Antigen-specific IgG anti-BTG was eluted with glycine-HCL (pH 2·8) and dialysed against phosphate-buffered saline (PBS). IgG anti-BTG (10 mg/ml) was mixed with BTG (5 mg/ml) and incubated for 30 min at 37 C and overnight at 4 C. Insoluble IC were removed by centrifugation during 10 min at 3500 ***g***. The soluble IC were separated according to size on a 10–30% sucrose gradient as described above. IC (>40S in size, concentration 1 mg/ml) were injected intravenously in rats. As a control, rabbit IgG was injected in rats.

Preparation of liposomes with entrapped CL2DMP

Multilamellar liposomes containing dichloromethylene diphosphonate (CL2MDP) were prepared as described earlier (Van Rooijen & Claassen, 1988; Van Rooijen, 1989). Rats were injected intravenously with 0·5 ml (containing about 5 mg of liposome entrapped CL2MDP) liposome suspension. After 24–48 h clearance and tissue distribution, studies were performed because no KC were present during this period (Van Rooijen *et al.*, 1990; Bogers *et al.*, 1991).

Some rats were injected with liposomes containing only PBS to see whether administration of liposomes does influence the number of KC. No differences in the number of KC per 100 nucleated liver cells were observed (Bogers *et al.*, 1991).

Animals

Studies were performed in normal male inbred Wistar rats (180 200 g) raised in our laboratory. For clearance and tissue distribution studies, the rats received 0·1% potassium iodide (KI) in their drinking water 24 h before the start of each experiment.

Clearance studies

Five micrograms of radiolabelled monomeric IgG (mIgG) (7S) or AIgG (42–51S) were administrated in a volume of 0·5 ml PBS via the penile vein. The rate of disappearance of radioactivity from the circulation was monitored by bleedings at 0·5, 1, 2, 4, 8, 16, 30, and 60 min following administration as described previously (Bogers *et al.*, 1989, 1990). The results are expressed as percentage of protein-bound activity and were determined by linear regression analysis of the data.

Organ distribution

At 8, 60 or 240 min after injection of 5 μg ^{125}I-AIgG, groups of rats were perfused with 100 ml PBS to remove the blood-associated radioactivity from the organs. Subsequently the liver, spleen, lungs, thymus, heart and kidneys were removed, weighed and assessed for radioactivity. Total blood volume of the rats was assessed as described previously (Veerhuis, Van Es & Daha, 1985).

Inhibition studies

To study the specificity of receptors involved in the clearance of ^{125}I-AIgG by liver EC, KC-depleted rats were injected with 30 mg asialofetuin (ASFe, Sigma Chemical, St Louis, MO) as ligand for galactose receptors, 30 mg ovalbumin (OVA, Chicken egg albumin, Koch Light Laboratories, Haverhill, UK) as ligand for mannose receptors, 100 mg rat IgG (as a specific immunoglobulin) or 100 mg human IgA (as immunoglobulin control, isolated as described previously (Bogers *et al.*, 1989)) in 1 ml PBS, 3 min before ^{125}I-AIgG administration.

To determine whether the clearance of ^{125}I-AIgG occurred via the Fc tail, $F(ab')_2$ fragments of IgG were prepared by pepsin digestion (Veerhuis, Van Es & Daha, 1985). Fc and $F(ab')_2$ fragments were separated by gelfiltration on HPLC. The $F(ab')_2$ fragments were radiolabelled and aggregated as described above and their clearance investigated in KC-depleted rats.

Immunohistochemical staining of liver biopsies

To determine the cellular site of deposition of mIgG and AIgG in liver sections, normal rats or KC-depleted rats were injected intravenously with either 1·0 mg mIgG, AIgG, rabbit IgG or rabbit IgG-IC (>40S). Liver biopsies were taken after administration and snap frozen in liquid nitrogen. Cryostat sections of 4 μm were made, and after air drying, acetone fixed for 10 min at room temperature.

KC in the present study were detected with the use of mouse anti-rat macrophage MoAb ED2 (Dijkstra *et al.*, 1985) followed

Table 1. Half-lives ($T\frac{1}{2}$) of mIgG and AIgG in normal and Kupffer cell depleted rats

Probe	$T\frac{1}{2}$	Clearance rate (min) Normal rats	KC-depleted rats	*P*
^{125}I-mIgG	T1	36·3 ± 6·3	33·4 ± 8·9	NS
	T2	168·4 ± 4·7	159·5 ± 12·5	NS
^{125}I-AIgG	T1	2·5 ± 0·1	4·8 ± 0·7	0·005
	T2	32·5 ± 5·6	41·2 ± 3·2	0·005

NS, not significant.

by tetramethyl rhodamin isothiocyanate (TRITC)-labelled goat anti-mouse IgG (Sigma). It has been shown previously that KC depletion of rats (ED2$^-$) after treatment with CL2MDP containing liposomes is a result of the absence of KC (assessed by light- and electronmicroscopy) and not due to loss of ED2 antigen (van Rooijen, 1989; Van Rooijen *et al.*, 1990).

EC were identified according to Muro *et al.* (1987) as latex free, endogenous peroxidase negative cells, and according to Leszcynski (1990) by expression of von Willebrand factor (vWF, factor VIII-related antigen) which was detected using a rabbit IgG anti-human vWF antibody (Nordic, Tilburg, The Netherlands) that cross-reacts with the rat antigen.

The injected mIgG and AIgG were detected with FITC conjugated rabbit anti-rat IgG (2 mg/ml, F/P ratio 2·4, working dilution 1:30) while rabbit IgG and IC were detected with goat anti-rabbit IgG-FITC conjugate (3 mg/ml, F/P ratio 3·3, working dilution 1:20). The polyclonal antibodies, goat anti-rabbit IgG and rabbit anti-rat IgG were raised in our laboratory. Between incubations (1 h, room temperature) of the slides with ED2 and combinations of conjugates, the slides were washed three times with PBS. A drop of 10% glycerol was added to cover the tissue sections. Photographs were taken on a Leitz microscope equipped with a 4 mm BG 38 + 5 mm BG 12 filter for FITC, and an interference 560 nm + 2 mm BG 12 filter for TRITC.

Statistical analysis

Results were analysed for statistical significance using Student's *t*-test for unpaired samples.

RESULTS

Clearance of IgG of different size in normal and KC-depleted rats

At day 2 after CL2aMDP treatment, groups of four rats were injected with 5 μg of radiolabelled mIgG or AIgG and assessed for clearance from the circulation. mIgG was cleared with a slow rate from the circulation in a biphasic manner with a first fast half-life ($T\frac{1}{2}$) (T1) and a second slow $T\frac{1}{2}$ (T2) (Table 1). AIgG was cleared with a T1 of 2·5 ± 0·1 min and a T2 of 32·5 ± 5·6 min.

The clearance of mIgG in KC-depleted rats was not significantly different from that in normal rats. On the other hand, the clearance of AIgG in KC-depleted rats was clearly delayed as compared with that in normal rats. Both T1 (4·8 ± 0·7 min) and T2 (41·2 ± 3·2 min) of AIgG in KC-depleted rats were prolonged ($P < 0·005$). To find out whether repopulaton of KC

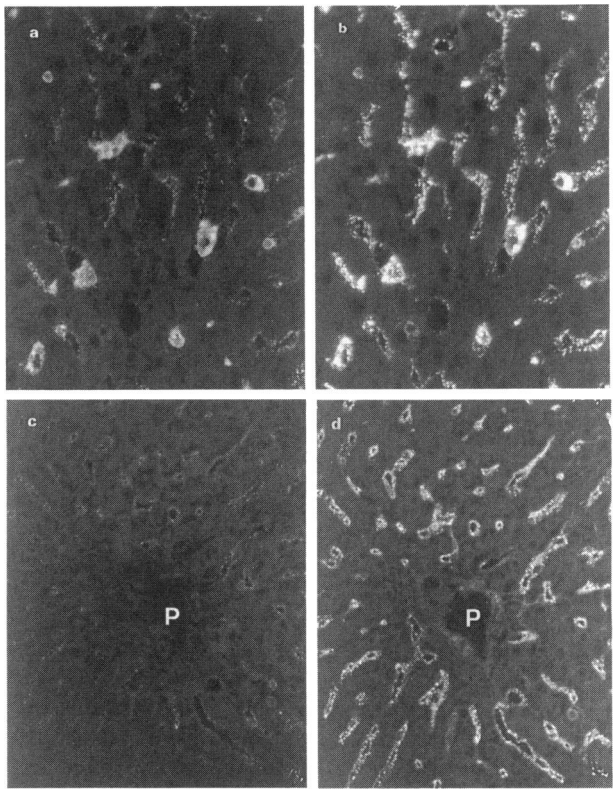

Fig. 1. Detection of IgG and KC in the liver of normal (a), (b) and KC-depleted (c), (d) rats. Normal and KC-depleted rats were injected with 1 mg AIgG and biopsies were taken at 8 min following injection. Immunofluorescence staining was performed to identify KC (a), (c) and IgG (b), (d). In normal rats AIgG is localized in KC and along sinusoidal EC. In KC-depleted rats AIgG is localized along sinusoidal EC (ED2$^-$; (c)). No positive IgG staining was found on EC from portal veins (P). In (a) and (c) a slight staining is visible along the sinusoidal EC. This is however the FITC dye for the IgG staining through the TRITC filter. Original magnification: ×400.

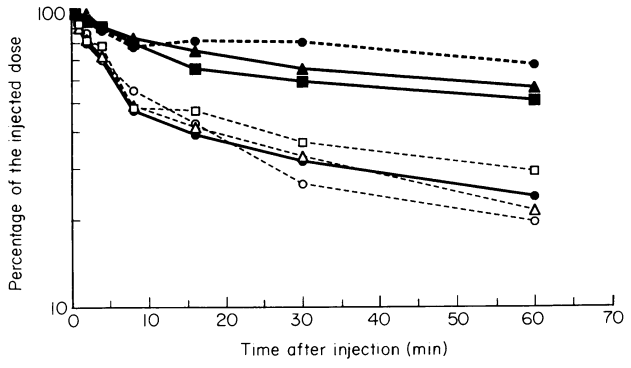

Fig. 2. Clearance of ^{125}I-AIgG and ^{125}I-F(ab')$_2$ AIgG in KC-depleted rats. Groups of four rats were not pretreated (-●-), or pretreated with 100 mg IgG (■), 100 mg IgA (○) 30 mg ASFe (△) or 30 mg OVA (□) intravenously 3 min before administration of ^{125}I-AIgG. The clearance of ^{125}I-F(ab')$_2$ IgG (▲) and control monomeric ^{125}I-IgG (- -●- -) in KC-depleted rats is also shown. Each point represents the mean of four rats (for the sake of clarity no standard deviations are depicted; s.d.s do not exceed 10%).

Table 2. Effect of pretreatment of Kupffer cell-depleted rats with IgA, IgG, ASFe and OVA on the clearance of ^{125}I-AIgG

Half-life	No pretreatment (min)	Pretreated with* IgA (min)	IgG (min)	ASFe (min)	OVA (min)
T1	4·8±0·7†	5·8±1·1	29·1±9·7‡	5·2±1·0	5·5±0·3
T2	41·2±3·2†	41·8±1·1	103·4±11·5‡	47·3±4·8	45·3±3·8

* KC-depleted rats were pretreated intravenously with IgA, IgG, ASFe or OVA 3 min before AIgG administration.

† These data are derived from Table 1.

‡ $P<0·001$, pretreated *versus* non-pretreated, and as compared with the groups pretreated with IgA, ASFe and OVA.

Table 3. Degradation products in the circulation at various times following administration of ^{125}I-AIgG in Kupffer cell-depleted rats either with or without pretreatment with IgA, IgG, ASFe and OVA

Time after administration (min)	No pretreatment	Pretreated with* IgA	IgG	ASFe	OVA
8	2·1±2·1†	ND	1·5±1·5	ND	ND
16	3·5±3·5	3·0±1·0	1·3±1·2	2·0±1·5	1·7±1·7
60	23·2±6·8	24·1±8·8	1·5±1·4‡	25·8±5·2	22·1±1·4

* KC-depleted rats were administered intravenously with IgA, IgG, ASFe or OVA 3 min before administration of AIgG.

† Percentage of non-TCA precipitable radioactivity.

‡ $P<0·001$, pretreated *versus* non-pretreated, and as compared with the groups pretreated with IgA, ASFe and OVA.

ND, not detectable.

in the liver resulted in reversal of the rate of clearance of AIgG, four rats were first depleted of KC, and on day 5 when the number of KC had returned to 50% of the original value (Bogers *et al.*, 1991), assessed for clearance of AIgG. In these rats the delayed clearance of AIgG was restored to normal (T1: 3·4±0·5 min; T2: 35·7±8·0 min, $P>0·1$ as compared to normal rats).

Organ distribution of AIgG in normal and KC-depleted rats

Groups of three rats were injected with ^{125}I-AIgG and sacrificed 8, 60 or 240 min after administration. Eight minutes after injection most of the AIgG (76·7±4·4% of the initial dose) was found in the liver of normal rats. Less than 4% of AIgG was found in spleen, heart, thymus, lungs or kidneys. The amount of AIgG in the liver decreased with time (28·3±2·7% after 60 min and 5·0±0·8% after 240 min).

In KC-depleted rats, 8 min after injection there was significantly less AIgG in the liver and more AIgG in the circulation as compared with normal rats ($P<0·02$). Also in KC-depleted rats the liver remained the main organ involved in the clearance of AIgG (61·5±4·2%, 8 min after administration), after which a gradual decrease in time was observed (25·5±5·1%, 60 min after administration; 5·0±0·4% after 240 min). Again less than 4% of the injected dose was recovered in other organs.

Site of localization in the liver

Immunohistochemical studies of liver biopsies from normal rats receiving 1 mg AIgG showed that KC and sinusoidal EC are responsible for the removal of AIgG (Fig. 1). No AIgG were detected in hepatocytes or in or around the EC within the central or portal vein areas.

In KC-depleted rats, AIgG can be seen localized on sinusoidal EC (Fig. 1c, d). No positive AIgG staining was found within central vein areas. The same pattern of fluorescence in both normal and KC-depleted rats was observed after administration of IgG-IC. Liver biopsies taken at later time intervals (30, 60 and 240 min after administration) showed a decrease in the intensity of the fluorescent staining both in normal and KC-depleted rats. At 4 h after IgG-IC or AIgG administration all positive IgG staining had disappeared both from KC and sinusoidal EC. No detectable IgG was observed on hepatocytes, KC or EC in liver sections from both normal and KC-depleted rats after mIgG or rabbit IgG administration (data not shown).

Inhibition of clearance of AIgG in KC-depleted rats

To determine whether specific receptors are involved in the clearance of AIgG by liver EC, KC-depleted rats were pretreated with IgG, IgA, ASFe or OVA and subsequently assessed for clearance of ^{125}I-AIgG (Fig. 2). Pretreatment with IgG

resulted in inhibition of clearance of AIgG, with T1 of 29·1 ± 9·7 min and T2 of 103·4 ± 11·5 min (Table 2). These values are not different from the clearance of mIgG (Table 1). Pretreatment of KC-depleted rats with IgA, ASFe or OVA had no significant effect on the rates of clearance of AIgG (Fig. 2, Table 2).

Since these experiments suggested that the clearance by EC *in vivo* is Fcγ receptor-mediated, clearance of $F(ab')_2$ aggregates was assessed as well. These aggregates were cleared with a comparable rate to mIgG from the circulation in KC-depleted rats, with a T1 of 28·9 ± 2·4 min and a T2 of 123·3 ± 12·2 min ($P > 0.05$) (Fig. 2).

Degradation of AIgG in normal and KC-depleted rats

As a measure for AIgG degradation, non-TCA precipitable radioactivity was determined in normal and KC-depleted rats after administration of ^{125}I-AIgG. There was a time-dependent increase of degradation products of ^{125}I-AIgG in normal rats. In normal rats the amount of non-TCA precipitable radioactivity was 7·5 ± 3·5%, 16 min after ^{125}I-AIgG administration. After 30 min and 60 min, 28·1 ± 6·0% and 39·5 ± 3·1% of non-TCA precipitable activity was measured respectively. The amount of degradation products was significantly lower in KC-depleted rats (3·5 ± 3·5% after 16 min; 11·5 ± 4·4% after 30 min ($P < 0.01$) and 23·2 ± 6·8% after 60 min ($P < 0.01$)). Following administration of ^{125}I-mIgG, maximal levels of 3% non-TCA precipitable radioactivity in the circulation was found both in normal and KC-depleted rats up to 1 h after injection.

Degradation products of ^{125}I-AIgG were also measured in KC-depleted rats which were pretreated with IgA, IgG, ASFe or OVA (Table 3). A significant decrease in the amount of non-TCA precipitable radioactivity was found in KC-depleted rats pretreated with IgG ($P < 0.001$). No differences in the amount of degradation products were observed after pretreatment with IgA, ASFe or OVA up to 1 h after ^{125}I-AIgG administration.

Only 5·1 ± 3·2% non-TCA precipitable activity was found in KC-depleted rats up to 1 h after aggregated ^{125}I-$F(ab')_2$ IgG fragments.

DISCUSSION

In the present study we investigated the contribution of rat liver EC in the clearance of soluble IC and soluble aggregates of IgG (AIgG). IC and AIgG of 42–51S in size were chosen because these agents do not enter the fenestrae between the liver EC because of their size (Phillips *et al.*, 1983), and are therefore mainly cleared by cells between the sinusoidal walls, e.g. KC and EC. Because aggregates of IgG are handled in a similar way to IC (Knutson, Kijlstra & Van Es, 1977) and because they remain stable, AIgG can be used as a tool to obtain information on the mechanism of the handling of IC.

In our study, in normal rats both IC and AIgG were found mainly in KC and partially in EC, but not in hepatocytes. Others have shown that heterologous IgG-IC are taken up by both KC and EC in rats (Skogh *et al.*, 1985; Van Der Laan-Klamer *et al.*, 1986a, 1986b; Muro *et al.*, 1987). The contribution of either KC or EC alone in the clearance of IgG-IC has been difficult to approach until now. Elimination of KC resulted in a decrease of uptake of ^{125}I-AIgG in the liver, but the liver remained the main organ involved in the clearance of AIgG. The methods used in the present study also result in elimination of macrophages in the spleen. However, all the other organs together, including the spleen, contained less than 4% of the injected dose both in normal and liposome-treated rats. Therefore these organs probably do not play a major role in the clearance of AIgG or soluble IC. Immunofluorescence studies showed that in KC-depleted rats, AIgG and IC were observed associated only with sinusoidal EC (Fig. 1) and not with EC from portal or central veins, which is in agreement with the detection of Fc receptors *in vitro* on liver sections (Muro *et al.*, 1987). From these observations we conclude that the clearance of large sized AIgG and IC in KC-depleted rats is mediated by liver EC. Recently we also described a role for EC in the clearance and degradation of IgA aggregates and IC (Bogers *et al.*, 1991).

It has been demonstrated that both rat (Van Der Laan-Klamer *et al.*, 1986b; Muro *et al.*, 1987) and human liver EC bind IC via Fc receptors (Muro *et al.*, 1987). In the present study we could show that clearance of ^{125}I-AIgG was only inhibited after IgG administration. Similar clearance kinetics to mIgG were observed after administration of aggregated ^{125}I-$F(ab')_2$ IgG fragments (Fig. 2), and therefore we conclude that the clearance of AIgG by liver EC is Fcγ receptor mediated.

Liver EC are able to endocytose a large variety of exogenous and endogenous particles and molecules (Steffan *et al.*, 1986; Brouwer, Wisse & Knook, 1988). Because they also contain high levels of lysosomal enzymes (Brouwer *et al.*, 1988) it is suggested that EC may play a role in uptake and degradation of proteins. In KC-depleted rats, in which localization of AIgG was found in the EC, we saw a decrease in the amount of AIgG in the liver in time and an increase in the amount of degradation products of AIgG in the circulation, suggesting degradation of AIgG by EC. Whether ingestion is essential for degradation of AIgG or IC by EC still remains to be investigated.

The amount of degradation products in KC-depleted rats was significantly lower as compared with normal rats. This may be explained by differences in rate of processing or enzyme activities between KC and EC (Brouwer *et al.*, 1988). Various lysosomal and metabolically involved enzymes of KC have a higher activity as compared with EC enzymes, which might explain the lesser amount of degradation products of AIgG in KC-depleted rats.

Clearance of circulating IgG aggregates or IC and the subsequent degradation by liver EC is a new aspect of the function of these cells. Although as we and others have shown IgG-IC bind both to KC and EC (Skogh *et al.*, 1985; Van Der Laan-Klamer *et al.*, 1986b; Muro *et al.*, 1987), the question whether phagocytosis and the subsequent degradation of the aggregates or IC are restricted to the KC in normal situations is not answered yet. It is possible that the Fcγ receptors on EC may act as 'standby' receptors for IgG aggregates or IC under normal conditions but with functional activity during an immune response. This mechanism was already suggested for the Fcγ RII present on human granulocytes (Tax & van de Winkel, 1990). Another possibility might be that binding and degradation of IC both by KC and EC occur at the same time. A third possibility might be that there are two separate clearance mechanisms: one mediated by KC and the second mediated by liver EC. Further studies are required to analyse these possibilities.

Whether human liver EC express Fc receptors *in vivo* is not clear at present. Furthermore, it is not known whether human liver EC are able to internalize and degrade IC. The observation of binding of heterologous IC to human liver sections *in vitro*

(Muro *et al.*, 1987) might indicate that human liver EC are able to bind IC *in vivo*.

Considering the great total surface area of liver EC, which in rats is 3·6 times greater than that of KC (Blouin, Bolender & Weibel, 1977), the presence of Fcγ receptors on EC, and their ability to bind IgG aggregates or IC from the circulation, suggests that liver sinusoidal endothelium may participate in the clearance and metabolism of IC.

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