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# Hepatic and kidney uptake of soluble monomeric and polymeric IgA aggregates

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**Summary.** To investigate the handling of IgA by the mononuclear phagocytic system and by hepatocytes of mice, soluble, similar sized, heat-aggregated monomeric (A-mIgA) and polymeric IgA (ApIgA) were used as akin to IgA immune complexes. The half-life of the clearance from circulation decreased from 2·5 hr to 4·2 min and from 22 min to 1·8 min after aggregation of mIgA and pIgA, respectively. Tissue localization experiments indicated that the liver was the organ predominantly involved in the uptake and catabolism of the proteins injected. The rate of the liver catabolism and/or elimination of aggregated polymeric IgA was the slowest up to 24 hr of injection. Aggregated pIgA was deposited in the kidney in larger amounts than aggregated mIgA.

The participation of hepatocytes and nonparenchymal liver cells was determined after isolation and purification of these cells. The four substances injected, pIgA, A-pIgA, mIgA and A-mIgA, were predominantly localized in nonparenchymal cells when the uptake was expressed per volume of cells, due to their lower protein content. However, when the results were expressed cell to cell there was a high ratio of IgA aggregates associated with hepatocytes to nonparenchymal cells. It seems probable, therefore, that hepatocytes are almost exclusively responsible for clearance of IgA aggregates from blood.

Abbreviations: mIgA, monomeric IgA; p-IgA, polymeric IgA; A-mIgA, aggregated mIgA; A-pIgA, Aggregated pIgA; TBH, Tris-buffered Hanks's solution; TBH-BSA, TBH containing 0·5% bovine serum albumin; TBS, Tris-buffered saline.

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## INTRODUCTION

Most of the knowledge of the handling of immune complexes (IC) by the mononuclear phagocytic system (MPS) is based upon animal studies using either soluble IgG or IgM immune complexes or aggregates (Kijlstra *et al.*, 1978; Mannik, 1980; Harkiss & Brown, 1981). The liver uptake and processing of immune aggregates or IC was thought to be a function of Kupffer cells but the role of hepatocytes in the hepatic clearance of IC remained uncertain. Recently evidence has been published concerning the pivotal role of the liver, at least in some animal species, in the blood clearance of IgA, probably through the secretory component (SC) as a receptor (Hall & Andrew, 1980). However the specificity, affinity and the physiological role of the receptor for polymeric and monomeric IgA has not been fully determined. In a previous paper (Egido *et al.*, 1982), we have observed that (i) the blood elimination of aggregated multimeric IgA was significantly slower than that of aggregated IgG, and (ii) the aggregated IgA deposited in liver and kidney was removed significantly more slowly than aggregated IgG, probably due the impaired catabolism of aggregated IgA.

The purpose of the work reported here was to study the specificity and the mechanism of uptake and

handling of IgA by the liver. The use of aggregated and non-aggregated monomeric and polymeric IgA was intended to assess if the presence of polymers, with the known ability to interact with the SC on the hepatocyte membrane, could facilitate the uptake and processing by the liver and therefore have a unique behaviour compared to monomeric forms.

## MATERIAL AND METHODS

*Isolation of monoclonal monomeric and polymeric human IgA*

IgA myeloma serum containing high levels of both (pIgA) and (mIgA) was diluted 1/2 with phosphate-buffered saline (PBS) and precipitated with 50% $(NH_4)_2SO_4$(w/v). The precipitated proteins were centrifuged, redissolved and dialysed against 0·015 M phosphate buffer pH 8·0. The protein solution was chromatographed on a DEAE cellulose column (DE-52 Whatman) equilibrated with the same buffer. Unbound protein, mostly belonging to the IgG class, was discarded. Monomeric IgA (mIgA) was eluted with 0·05 M phosphate buffer pH 7·0 and polymeric IgA (pIgA) was eluted by a molarity gradient from 0·05 M phosphate to 0·2 M NaCl, in 0·05 M phosphate buffer pH 7·0. Protein solutions were thereafter dialysed with PBS, concentrated and chromatographed on a column (2·6 × 70 Pharmacia, Uppsala, Sweden) of Ultrogel AcA 22 (LKB Instruments Ltd, Browmma, Sweden). The two purified preparations were concentrated to 10 mg/ml and analyzed by immunoelectrophoresis. The molecular weight was estimated by gel filtration on Ultrogel AcA 22 column previously calibrated with marker proteins.

*Preparation of aggregates of pIgA or mIgA*

Proteins were radiolabelled with $^{125}I$ to a specific activity of 0·1 mCi/mg by the chloramine-T method (McConahey & Dixon, 1966). Aggregation were performed by heating 10 mg/ml $^{125}I$-IgA for 150 min at 63° in 0·15 M Tris-buffered saline (TBS). Soluble aggregates of pIgA (A-pIgA) or mIgA (A-mIgA) of $1–2 \times 10^6$ mol. wt. were isolated from non-aggregated with TBS pH 7·4 containing 0·5% BSA to prevent self-aggregation (Knutson *et al.*, 1979).

*Blood clearance and organ localization of injected proteins*

ICR-Swiss mice weighing 15–20 g were injected via the jugular vein with 0·4 ml of buffer containing 5 μg of pIgA, mIgA A-pIgA of A-mIgA. The methods to determine blood clearance and organ localization of the different Igs have been previously described (Kijlstra *et al.*, 1978).

*Isolation of hepatocytes and non-parenchymal liver cells*

A modification of the method of Nishi *et al.* (1981) was used. Briefly, the liver was first perfused *in situ* through the portal vein for 2 min at a rate of 25 ml/min with warm (37°) $O_2$-saturated, $Ca^{+2}$-free Tris-buffered saline (TBS) (pH 7·4). The liver was transferred to a petri-dish and cut into small pieces and placed in the same buffer, centrifuged and resuspended in warm (37°), $O_2$-saturated TBH containing 2·5 mM $Ca^{+2}$ 0·5% (w/v), collagenase type I (Sigma, St. Louis, U.S.A.) and trypsin inhibitor at 0·04% (w/v) (Sigma, St. Louis, U.S.A.) pH 7·4. After 30 min, the liver was passed over on 100 mesh filter and the resulting suspension was resuspended in TBH-BSA 1% and centrifuged twice at 60 *g* for 1 min at 4°. The pellet was resuspended in 3 ml of TBH-BSA and the hepatocytes identified by their large size and characteristic morphologic appearance. Each mouse liver yielded about $6–7 \times 10^6$ hepatocytes with a viability of 85% measured by trypan blue exclusion. The supernatant of the first wash, which contained chiefly non-parenchymal cells, was centrifuged twice at 250 *g* for 10 min at 4° and resuspended in the same buffer. Total recovery ranged from 4 to $5 \times 10^6$ non-parenchymal cells. Contamination of hepatocytes in the non-parenchymal cell preparation and non-parenchymal cells in the hepatocyte preparation, as determined counting 100 cells from each preparation, was less than 1%.

## RESULTS

### Blood clearances of aggregated and non-aggregated monomeric and polymeric IgA

The blood clearance curves or the four proteins injected are shown in Fig. 1 and Table 1.

The disappearance of pIgA of mIgA without aggregation was a function of two exponential components whereas the disappearance of aggregates was a function of three exponential components.

The half-life of the fast component of mIgA decreased from 2·5 hr to 4·2 min and the proportion of protein uptake with this clearance rate increased from 65% to 80·1% after aggregation. The half-life of the fast component of pIgA decreased from 22 min to 1·8

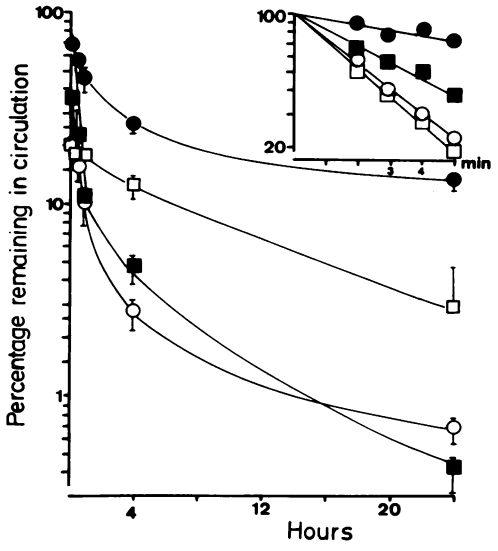


**Figure 1.** Rate of blood clearance from mice injected with (●—●) mIgA, (○—○) A-mIgA, (■—■) pIgA, (□—□) A-pIgA. Each point represents the mean ±SD from three mice.

min, and the proportion of protein eliminated with this clearance rate decreased from 89 to 80·7%. Thus, our results showed that the aggregation of proteins resulted in a significant increase in their clearance rates. Of the four proteins injected, A-pIgA was cleared most rapidly.

On the basis of previous studies (Mannik, 1980) the second component was thought to represent the equilibration phase of the remaining aggregates. The clearance of A-mIgA in this period was faster than A-pIgA ($t_{\frac{1}{2}}$ 1·65 hr *vs* 2·5 hr) with a higher proportion of protein eliminated (17·1% *vs* 2·17%).

The slowest (third) component, called the metabolic or elimination phase, for pIgA and mIgA had a mean half life of 12·38 hr and 22·3 hr with 10·6 and 21% of the radioactivity eliminated from the circulation with these half-lives, respectively. The half-life at this period of A-mIgA and A-pIgA was not significantly different. However, the proportion of material having this half-life was higher for A-pIgA than for A-mIgA, suggesting a lower rate of catabolism.

Because it was possible that the late clearance of large aggregates could in fact reflect clearance of aggregates of smaller size, we examined the size of aggregates remaining in the circulation up to 24 hr for A-pIgA and A-mIgA. By that time the terminal slope had been reached for both aggregates. The sedimentation pattern of A-pIgA of A-mIgA was not altered during the period of observation (results not shown). It appears therefore that the aggregates remain in the original size at least until cleared from the serum.

### Organ uptake and catabolism

Since the observation that pIgA is removed by an active mechanism by the liver, appearing in the bile (Hall & Andrew, 1980), we studied tissue deposition of human pIgA and mIgA either aggregated or not, in order to assess the specificity of that mechanism (Fig. 2). After 5 min of injection the percentage of radioactivity deposited in the liver was between 50 and 60% for both polymeric and monomeric aggregates, with no significant differences between them. By contrast, the

**Table 1.** Calculated exponential components describing the disappearance of the proteins injected in mice

| | First component* | | Second component* | | Third component* | |
|---|---|---|---|---|---|---|
| Protein injected | $t_{\frac{1}{2}}$(hr) | % | $t_{\frac{1}{2}}$(hr) | % | $t_{\frac{1}{2}}$(hr) | % |
| A-pIgA | 0·03 | 80·73 | 2·50 | 2·17 | 10·09 | 17·10 |
| A-mIgA | 0·07 | 80·10 | 1·65 | 17·1 | 10·03 | 2·1 |
| pIgA | 0·37 | 89·0 | — | — | 12·38 | 10·6 |
| mIgA | 2·5 | 65·0 | — | — | 22·3 | 21·0 |

* A parametric fitting of data based on least squares regression analysis by computer program yielded calculated values which provided the best fit for the experimental values. The half-life and the percentage of injected material having this half-life were computed for each component for groups of three mice receiving each preparation.

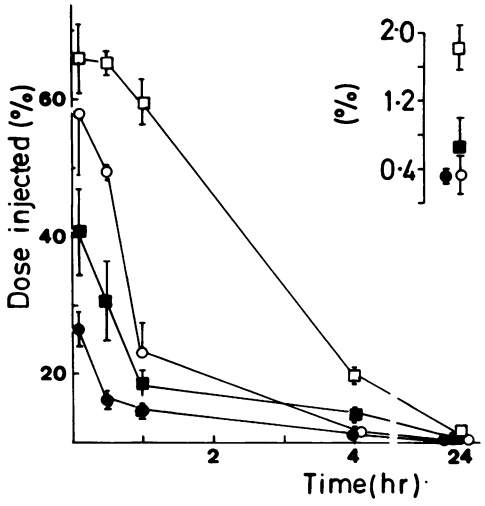


**Figure 2.** Uptake and removal of (□—□) A-pIgA, (○—○) A-mIgA, (■—■) pIgA and (●—●) mIgA, from liver after injection of 5 μg of protein. Each point represents the mean ±SD from three mice. The insert indicates the hepatic localization of proteins at 24 hr after injection. The hepatic uptake of A-pIgA and A-mIgA was equal at 5 min but the A-mIgA uptake was significantly lower than A-pIgA at 0·5, 1, 4 and 24 hr (at least $P < 0.05$, Student's unpaired $t$-test). The uptake of pIgA and mIgA was significantly different at 5 and 30 min ($P < 0.05$). Also, the mean values of mIgA and A-mIgA uptake at 5 and 30 min were significantly different ($P < 0.01$ and $P < 0.001$, respectively). There were not significant differences between the hepatic uptake of pIgA, mIgA and A-mIgA at 1, 4 and 24 hr.

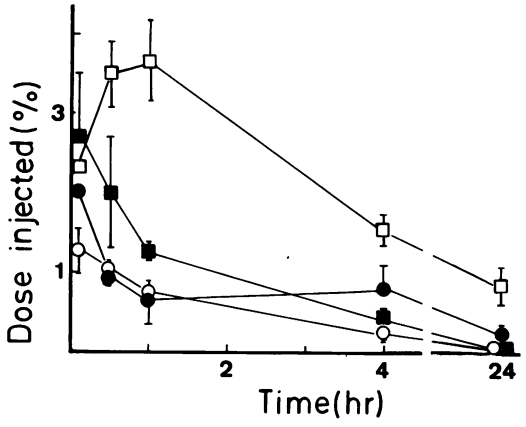


**Figure 3.** Uptake and removal of (□—□) A-pIgA (■—■) pIgA, (●—●) mIgA and (○—○) A-mIgA from kidney after injection of 5 μg of protein. Each point represents the mean ±SD from three mice. The kidney uptake or removal of A-pIgA was significantly higher than the uptake of the other three proteins at 1, 4 and 24 hr (at least $P < 0.05$, Student's unpaired $t$-test). The pIgA uptake at 5 and 30 min was significantly higher than A-mIgA kidney uptake ($P < 0.05$). Also the differences between pIgA and mIgA were significant at 30 and 60 min ($P < 0.01$ and $P < 0.05$, respectively). Kidney uptake of mIgA and A-mIgA was not statistically significant at any time.

uptake of non-aggregated pIgA was significantly higher than mIgA (≥2 SD). The rate of elimination and/or catabolism expressed by the decay of c.p.m. deposited in the liver after 5 minutes of injection was significantly slower for the A-pIgA up to 24 hr.

Kidney uptake of proteins is detailed in Fig. 3. Five minutes after injection, pIgA, either aggregated or not, was deposited in larger amount than mIgA though these differences were not statistically significant. Much more important was the fact that the elimination and/or catabolism of A-pIgA was significantly slower than the other proteins up to 24 hr after injection. The fact the blood levels of mIgA were always higher than A-pIgA but its deposition minor suggest that the size and probably the physicochemical (or the combination of both) differences between pIgA and mIgA (e.g. charge) could be important for the kidney deposition. In this sense, pIgA uptake by the kidney was higher than mIgA or A-mIgA uptake within 1 hr (Fig. 3).

### Cell separation studies

To document better the fate of aggregates within the liver, we separated liver cells into parenchymal (hepatocytes) and non parenchymal (Kupffer and endothelial cells) fractions after injecting a dose of radiolabelled test substance. The ratio between the uptake by the two cell types (Table 2) is expressed both per $10^6$ cells and per volume of cells, which correlates with presenting data as radioactivity per milligram of cell protein (Steer & Clarenburg, 1979).

When expressed per $10^6$ cells, the specific uptake by hepatocytes was higher than by non-parenchymal cells. When expressed per volume of cells, however, the nonparenchymal cells appeared to have a much higher binding capacity due to their lower volume. These data were used to calculate the contribution of each cell type to the uptake by the total liver (Fig. 4). Since two-thirds of the liver cells are hepatocytes, the contribution of this cell type to the clearance of all substances is very high.

**Table 2.** Recovery of infused $^{125}$I-labelled proteins in isolated hepatocytes and non-parenchymal cells

| Substance | Cell type | | A/B† | B × 27/A‡ |
|---|---|---|---|---|
| | Hepatocyte (A) | Non-parenchymal (B) | | |
| $^{125}$I-mIgA | 405 ± 66* | 162 ± 38* | 2·5 | 10·8 |
| $^{125}$I-pIgA | 1528 ± 75 | 617 ± 189 | 2·47 | 10·89 |
| $^{125}$I-A-mIgA | 1321 ± 580 | 721 ± 33 | 1·83 | 14·74 |
| $^{125}$I-A-pIgA | 1957 ± 632 | 1228 ± 99 | 1·6 | 16·94 |

* c.p.m./$10^6$ cells (mean ± SD).

† Radioactivity per hepatocytes/radioactivity per nonparenchymal cell.

‡ Radioactivity per non-parenchymal cell × 27/radioactivity per hepatocyte cell. Since hepatocytes are 27-fold larger in volume than non-parenchymal cells, the data were calculated from the radioactivity per volume of cells, which correlates well with presenting the data as radioactivity per mg of cell protein.

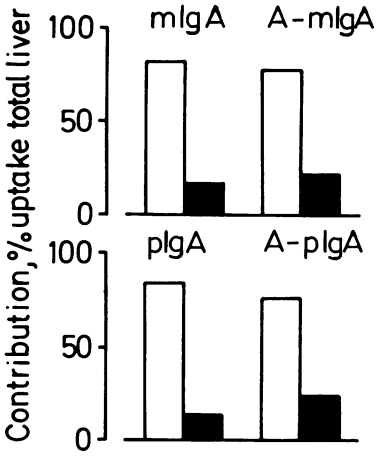


**Figure 4.** Contribution of each cell type to the clearance of infused $^{125}$I-labelled proteins by the total liver. Mice were injected with 5 μg of proteins and killed at 5 min after injection, when the liver uptake was maximum (see Fig. 2). Per cent radioactivity in both cells in total liver was based on the uptake per $10^6$ cells (Table 2) and the observed average cell ratio of two hepatocytes per one non-parenchymal cell in liver (Steer & Clarenburg, 1979), considering the liver uptake at 5 min as 100%. (□) Hepatocytes; (■) non-parenchymal cells.

## DISCUSSION

In the work present here, it has been shown that pIgA was quickly removed from the circulation. In the studies on the organ uptake *in vivo*, pIgA was also deposited and rapidly eliminated by the liver consistent with the previous observations of the active transport of pIgA in some species (Hall, Gyure & Payne, 1980). The blood clearance and liver uptake of mIgA remained lower than pIgA throughout the 24 hr period examined. However, using stable aggregates of mIgA or pIgA of similar size, akin to IgA immune complexes, we have observed an increased blood clearance and liver uptake of both aggregates, being A-pIgA the most rapidly cleared, with a half-life of only 1·8 min.

A recent report (Rifai & Mannik, 1983) described similar clearance kinetics of mouse IgA immune complexes prepared with monomeric or dimeric IgA. Interestingly, 60 min after administration of these complexes, the amount of heavy oligomers ($1·2 \times 10^6$ mol. wt.) of dimeric IgA accumulated in the kidney was higher than heavy oligomers of mIgA (0·54 ± 0·07% *vs* 0·36 ± 0·03%). Although it is not known if these differences were statistically significant, these authors did not determine the kidney deposition at longer periods after injection of complexes. Five minutes after the injection of our aggregates we have observed a similar accumulation of pIgA or mIgA aggregates in the kidney. The differences were significant after 30 min, suggesting faster catabolism of monomeric IgA aggregates. We have also observed that IgA or IgG aggregates of identical size have similar potential for tissue deposition (Egido *et al.*, 1982). The higher persistence of IgA aggregates in the kidney or liver tissue was governed by the lower catabolism of these complexes by the mononuclear phogocytic system, suggesting that IgA or IgG IC

deposition is influenced by variables other than size and lattice structure of complexes (Mannik, 1980).

Thus the presence of polymeric IgA in the aggregates or immune complexes seems to be important for the deposition at sites susceptible to injury. This was evidenced in an experimental model of IgA nephropathy where polymeric IgA-IC were crucial for the induction of nephritic histological changes. Immune complexes formed either *in vivo* or *in vitro* with monomeric IgA failed to induce glomerulonephritis (Rifai *et al.*, 1979). In the same way in three human nephropaties such as Berger's disease, Schönlein-Henoch syndrome and some cases of alcoholic liver disease characterized by the predominance of IgA deposits in the glomeruli, we have found high levels of polymeric IgA, partially as immune complexes, in the serum and kidney tissue (López Trascasa *et al.*, 1980; Egido *et al.*, 1980; Sancho *et al.*, 1982).

Although the hepatic uptake and sequestration of immunological aggregates is thought to be a function of Kupffer cells (Mannik, 1980), evidence has been accumulated in the last few years concerning the potential role of hepatocytes in the liver clearance. In this context various receptors have been well described: (i) receptors for the Fc portion of IgG and the third complement component (C3) (Hopf, Meyer Zum Buschenfelde & Dierich, 1976; Frommel & Rachman, 1976); (ii) receptor for the Fc portion of IgA (Hopf *et al.*, 1978); (iii) SC receptor with the ability to bind and transport polymeric IgA from blood to bile (Hall & Andrew, 1980), and (iv) asialoglycoprotein receptor that could bind IgA, since this immunoglobulin has a high carbohydrate content (Stockert *et al.*, 1982).

Recently we have shown that hepatocytes have 10–15 times more Fcγ receptors than Kupffer cells, although the affinity of the receptor was not different between both cell types (Sancho *et al.*, submitted). Also we have observed '*in vivo*' that heavy aggregates of IgG were, unexpectedly, primarily taken up by hepatocytes. Since there is another report suggesting that hepatocytes participate in the removal of IgG immune complexes (Thornburg *et al.*, 1980), we have examined the cellular distribution of bound IgA aggregates to determine if this could be attributed to the preferential uptake by hepatocytes or Kupffer cells. When expressed per volume of cells the Kupffer and endothelial cells appeared to have a much higher binding capacity (Table 2), due to their lower protein content. However, when the results were expressed cell to cell, the high ratio of IgA aggregates associated with parenchymal to non-parenchymal cells indicated that hepatocytes made the highest relative contribution to the clearance of IgA aggregates. Thus, it appears that the assumption that Kupffer cells would be almost exclusively responsible for clearance of IgA complexes from blood (Rifai & Mannik, 1983) does not seem to hold from our data. The available information suggest that there are several mechanisms whereby IgA, depending of the presence of polymers and its aggregation or not, can be taken up by the liver. The relative contribution of the different mechanisms of uptake of IgA immune complexes remain to be elucidated.

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