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Elimination of soluble ^{123}I-labelled aggregates of human immunoglobulin G in humans; the effect of splenectomy

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SUMMARY

To study the role of the spleen in the elimination of immune complexes we examined mononuclear phagocyte system function in eight healthy controls and eight splenectomized patients, with soluble ^{123}I-labelled aggregates of human immunoglobulin G (AIgG). No differences were found between the two groups in elimination and degradation of AIgG. The loss of splenic function was compensated for by increased uptake of AIgG by the liver. With the dose of ^{123}I-AIgG used in this study (10 μg/kg body weight), significant generation of C3a was observed. No correlation was found between erythrocyte CR1 number and the fraction of aggregates that bound to erythrocytes.

Keywords ^{123}I-labelled IgG aggregates mononuclear phagocyte system function volunteers splenectomy

INTRODUCTION

The manifestations of immune complex diseases may be the result of deposition of circulating immune complexes (CIC) throughout the body. It has been proposed that high levels of CIC are the consequence of decreased removal of CIC by a dysfunctioning mononuclear phagocyte system (MPS). When MPS function was measured with IgG-sensitized erythrocytes, splenic dysfunction was indeed demonstrated in patients with systemic lupus erythematosus (SLE) (Frank *et al.*, 1979). However, studies with performed immune complexes (IC) in rabbits (Arend & Mannik, 1971), rats (Kijlstra *et al.*, 1979) and mice (Finbloom & Plotz, 1979) have shown that most IC are trapped in the liver, and very few are deposited in the spleen.

In primates, the clearance of IC is partially mediated by adherence of IC to erythrocytes (Cornacoff *et al.*, 1983). IC that activate complement and are coated with C3b can subsequently bind to the C3b-receptor (CR1) on erythrocytes to be transported to the MPS, where the erythrocytes are stripped of their IC load (Cornacoff *et al.*, 1983). Again, most IC are taken up by the liver, but there is also considerable splenic uptake. In baboons, for instance, 7–21% of injected IgG1–IC were deposited in the spleen (Waxman *et al.*, 1986).

We have recently described a method to measure the function of the mononuclear phagocyte system in humans, using radiolabelled soluble aggregates of human IgG (AIgG) as a probe (Lobatto *et al.*, 1987). AIgG has biological activities similar to naturally occurring IC: it activates complement, binds to erythrocytes after complement activation, and is preferentially cleared by the liver (Veerhuis *et al.*, 1986; Lobatto *et al.*, 1987). However, by external scanning appreciable uptake of AIgG by the spleen was demonstrated as well (Lobatto *et al.*, 1987).

In patients with rheumatoid arthritis and SLE, elimination of AIgG was found to be delayed (Lobatto *et al.*, 1988a; 1988b). In SLE patients this was associated with increased liver-spleen uptake ratios, suggesting decreased splenic uptake of AIgG. The present study was undertaken to investigate whether absence of the spleen would cause a delay in the clearance of IC, by examining the elimination of AIgG in splenectomized patients.

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MATERIALS AND METHODS

Procurement and radiolabelling of human AIgG

AIgG were prepared by the Central Laboratory of the Netherlands Red Cross Blood Transfusion (*A. A. Voetman*) and radiolabelled with Na^{123}I as previously described (Lobatto *et al.*, 1987). The amount of radioactivity administered to each subject averaged 8·2 ± 1·9 (s.d.) MBq (222 ± 51 μCi). The amount of polymeric IgG was assessed by counting the precipitate after treating a sample of the labelled material with polyethyleneglycol 6000 (PEG) at a final concentration of 3% for 30 min at 0°C. Of the administered radioactivity, 56·1 ± 6·4% consisted of polymers of IgG of various sizes, as was shown previously (Lobatto *et al.*, 1987).

Selection of healthy controls and patients

The study protocol was approved by the Ethical Committee of Leiden University Hospital. All subjects gave informed consent before participating in the study. IgA deficiency was excluded in all subjects in order to minimize the risk of an anaphylactic reaction, caused by antibodies directed against IgA, which is present in AIgG in trace amounts.

The healthy control population consisted of six men and two women aged 25·1 ± 5·2 (s.d.) years (range 20–34). In the splenectomized patient group there were six men and two women, aged 35·9 ± 15·5 years (range 22–63). The splenectomized group was studied 17–128 months after splenectomy for traumatic rupture (five patients), accidental laceration during abdominal surgery (two patients), and splenic cyst (one patient).

All subjects were treated with oral iodide to prevent thyroid uptake of $Na^{123}I$. Blood pressure and pulse rate were monitored throughout the experiment.

Studies in healthy controls and patients

Patients and controls received a dose of ~10 μg/kg body weight of ^{123}I-AIgG intravenously over 30 sec. Radioactivity over hepatic and splenic areas was registered continuously for 1 h after injection with a Toshiba GCA 40A gamma camera. Serial blood samples were drawn from the opposite forearm into chilled tubes 1, 2, 3, 5, 8, 15, 30, 60 and 120 min after injection and kept on ice until processed to prevent complement-mediated release of AIgG from erythrocytes. Residual radioactivity was counted in whole blood, plasma and in precipitates of plasma, after treating the samples with 10% (final concentration) trichloroacetic acid (TCA) and 3% PEG, as described above, to measure protein-bound and aggregate-bound activity, respectively. Erythrocyte-bound activity (E-AIgG) was measured after washing the blood samples twice with 2 ml of PBS at 0°C. In order to compare E-AIgG between subjects, erythrocyte-bound counts were corrected for haematocrit by multiplication with (measured haematocrit/0·45), and the maximal number of counts was expressed as a percentage of whole blood radioactivity (E-AIgG%). Radioactivity was measured in a Packard Autogamma Scintillation Spectrometer 5221.

Complement activation by AIgG was assessed by measuring C3a-levels in plasma in a sandwich ELISA as described previously (Lobatto *et al.*, 1987). Leukocyte count and CH50 were measured at 0, 5 and 120 min after injection. C3 was measured by radial immunodiffusion. To detect CIC the ^{125}I-C1q binding assay (Zubler *et al.*, 1976) and the IgG–PEG assay (Valentijn *et al.*, 1984) were used. As the AIgG preparation contains aggregates of different sizes, the clearance of AIgG was also studied with sucrose density gradient ultracentrifugation, as previously described (Lobatto *et al.*, 1987). The number of CR1 on erythrocytes was calculated from a radio-ligand binding assay, using the anti-CR1 monoclonal antibody (2A8 (Lobatto *et al.*, 1988a).

Statistical analysis

Residual radioactivity time curves were analysed with a non-compartmental method based on statistical moment theory (Gibaldi & Perrier, 1982). This method was used because not all curves seemed biexponential. It has a theoretical advantage in the fact that no assumption regarding a pharmacokinetic model is required (Yamaoka, Nakagawa & Uno, 1978). The zero moment of the radioactivity time curve is the area under the curve (AUC), which was calculated by numerical integration (lin-log trapezoidal rule) of a finite time course (0–120 min). The first moment of the activity time curve, mean residence time (MRT), is the statistical moment that is analogous to half-life ($t_{\frac{1}{2}}$). Clearance of radioactivity was calculated according to the standard pharmacokinetic formula:

$$\text{Clearance} = \frac{\text{Administered dose}}{\text{AUC.}}$$

To calculate the clearance of PEG-precipitable AIgG and erythrocyte-bound activity, the PEG-precipitable fraction of the total dose was substituted in the formula. This fraction had been determined in four healthy controls and in six splenectomized subjects. The activity time curves, calculated as percentages of the highest level of activity, were averaged. Of the mean curves $t_{\frac{1}{2}}$ were calculated, using linear regression analysis (least squares estimates). Comparisons were made using Student's *t*-test, Mann-Whitney *U*-test, Wilcoxon's rank sum test and Spearman's rank correlation test.

RESULTS

Removal of ^{123}I-AIgG from the circulation

Activity time curves for whole blood, TCA-precipitable (not shown), PEG-precipitable and erythrocyte-bound radioactivity showed no differences between patients and controls (Fig. 1). Similarly, no differences were found in AUC, MRT and clearances (Table 1).In contrast to some individual curves, the mean curves were clearly biexponential. For PEG-precipitable radioactivity $t_{\frac{1}{2}}$ of the first component was 7·8 min in healthy controls and 6·8 min in splenectomized patients while $t_{\frac{1}{2}}$ of the second component was 154·6 and 192·8 min in healthy controls and splenectomized patients; respectively. For erythrocyte-bound activity, $t_{\frac{1}{2}}$ of the first component was 3·6 and 5·4 min, and for the second component 80·7 and 97·4 min in healthy controls and splenectomized subjects, respectively. At 120 min the remaining radioactivity of whole blood, TCA-precipitate, PEG-precipitate and E-AIgG was not significantly different in the two groups. As ^{123}I-AIgG is taken up by macrophages and degraded into free ^{123}I and Ig fragments, TCA-nonprecipitable radioactivity, i.e. free ^{123}I, was calculated by subtracting TCA-precipitable radioactivity from total plasma activity (Fig. 2). In both groups, TCA-non-precipitable activity rose, with no difference between the two groups. Radioactivity associated with monomeric IgG or its fragments, calculated as TCA- min PEG-precipitable activity, remained constant and was not different in the two groups (data not shown). Sucrose-density gradient ultracentrifugation showed that the larger aggregates disappeared most rapidly in both groups (data not shown), as was also demonstrated previously (Lobatto *et al.*, 1987).

The AIgG used in this study has been shown to activate complement *in vitro* and *in vivo* (Lobatto *et al.*, 1987). *In vivo* parameters of complement activation were determined before and after injection of AIgG. Baseline levels of CH50 and C3 were similar in the two groups and no CIC were detected. CH50 did not change significantly after injection. Baseline levels of C3a were similar in the two groups. Within 5 min of injection of AIgG, the levels rose sharply: in the controls with 154·1 ± 86·6% (s.d.) and in the patients with 135·9 ± 102·5% ($P < 0·02$, compared with initial levels, with no difference between the two groups). At the end of the experiment, C3a levels had returned

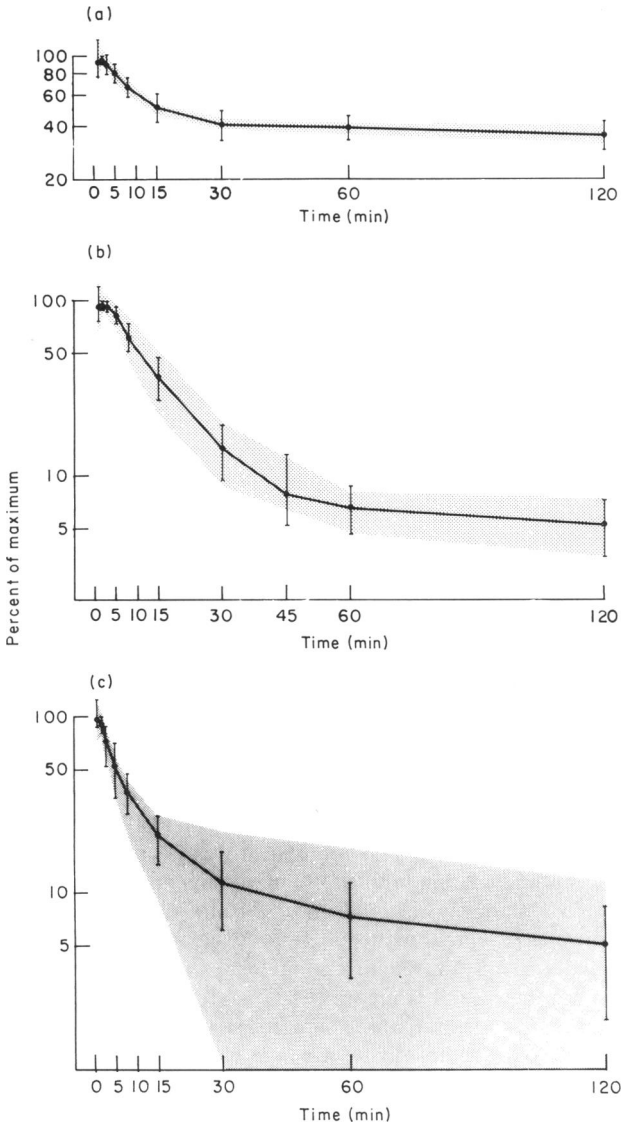

Fig. 1. Radioactivity-time curves for whole blood (a); PEG-precipitable (b); and erythrocyte-bound ^{123}I-AIgG (c); expressed as percent of maximum in eight splenectomized patients (mean ± s.d.). Shaded area represents mean ± 1 s.d. of healthy controls. There are no differences between the two groups.

to baseline values in all subjects. In both groups the white cell counts dropped to 50% of baseline values 5 min after injection. They returned to normal in all subjects at the end of the experiment.

Of the 16 subjects who received ^{123}I-AIgG, two patients reported chest tightness, headache, anxiety and flushing. The symptoms started within 2 min of the injection and lasted less than 3 min. In one patient this was associated with a transient rise in pulse rate and blood pressure. The parameters of complement activation in these two patients were not different from those of the other subjects, who had no complaints or changes in vital signs.

There was no correlation between the number of CR1 per erythrocyte (range 240–1063) and maximal E-AIgG% (range 2·0–32·8%). Erythrocyte-bound radioactivity showed a peak within 2 min of injection in all subjects. Maximal E-AIgG% correlated negatively with the mean residence time of erythrocyte-bound radioactivity ($r = -0{\cdot}79$, $P < 0{\cdot}005$).

Organ uptake of ^{123}I-AIgG

Radioactivity was registered continuously over hepatic and splenic areas for 60 min after injection of ^{123}I-AIgG. After correction for background, a liver/spleen (L/S) ratio was calculated. Maximal L/S ratio was 2·4 ± 0·4 (s.d.) at 7·1 ± 3·3 min. It decreased to 0·7 ± 0·3 at 60 min, when remaining circulating PEG-precipitable activity was 6·5 ± 1·7%. In order to compare healthy controls and splenectomized subjects, liver/background (L/B) ratios were calculated after subtraction of background radioactivity, corrected for surface area, and expressed as counts/pixel/sec (Table 2). Significantly more activity per pixel (= surface unit) was registered in the hepatic area of the splenectomized subjects when compared with the healthy controls ($P < 0{\cdot}05$). However, hepatic uptake per pixel in splenectomized subjects was less than the sum of hepatic and splenic uptakes in healthy controls ($P < 0{\cdot}05$).

DISCUSSION

Using radiolabelled human AIgG as a probe to measure MPS function, we found no differences in elimination of AIgG between healthy controls and patients who had undergone splenectomy. Radioactivity time curves and clearance parameters (AUC, MRT and clearances) were very similar. Degradation of ^{123}I-AIgG was also similar in both groups. Free ^{123}I in plasma rose within 30 min after injection, coincident with a decline in hepatic radioactivity. The averaged activity time curves of the PEG-precipitable material showed a biexponential decline, with a fast $t_{\frac{1}{2}}$ of 7 min and a slow $t_{\frac{1}{2}}$ of 150–190 min. Taken together with the organ uptake results, these data suggest that the first rapid component of the disappearance curve of the PEG-precipitable material represents the uptake by the liver.

In experimental animals AIgG is chiefly taken up by the liver (Mauer *et al.*, 1972; Brown *et al.*, 1973; Jimenez & Mannik, 1982; Veerhuis *et al.*, 1986). In mice, splenic uptake is 3–4% of the injected dose, while liver uptake has been reported to be 42% (Jimenez & Mannik, 1982). In rats hepatic uptake of AIgG was found to be 72·6%; from the spleen, 4% of the injected dose was recovered (Veerhuis *et al.*, 1986).

In primates the elimination of AIgG possibly proceeds differently from rodents because of the erythrocyte-CR1 transport system (Cornacoff *et al.*, 1983). IC that fix complement acquire C3b and subsequently bind to the erythrocyte CR1 to be transported to the fixed macrophages in liver and spleen, where the erythrocytes are stripped of their load. In baboons 7–21% of preformed IgG1 IC were found to be deposited in the spleen in this way (Waxman *et al.*, 1986), a rate much higher than the ≤1% IC that are found in mouse spleens (Finbloom & Plotz, 1979). In our study, 2·0–32·8 of ^{123}I-AIgG detected in the circulation was bound to erythrocytes. In the healthy controls we found appreciable splenic uptake of ^{123}I-AIgG with spleen-background ratios as high as L/B ratios, showing no significant decrease in the first 60 min after injection (Table 2). Possibly, activity accumulates in the spleen because of trapping of erythrocytes bearing ^{123}I-AIgG. The accumulation of radioactivity in the spleen indicates that even if degradation of AIgG takes place, free ^{123}I and IgG fragments are not quickly released into the circulation. This may be explained by the fact that

Table 1. Elimination kinetics of ^{123}I-AIgG in eight healthy controls (HC) and eight splenectomized patients (SP), 0–120 min after administration.

	Whole blood		TCA		PEG		ery	
	HC	SP	HC	SP	HC	SP	HC	SP
AUC	1330±227	1455±400	1594±325	1823±526	330±77	415±146	47±18	59±32
MRT	53·29±1·6	53·34±1·3	53·49±1·7	52·58±1·6	29·05±2·6	28·72±2·2	31·63±9·5	32·65±8·6
Clearance (ml/min)	339±53	299±58	217*±35	183±49	675†±185	624*±199	4300†±908	4666*±1738
Activity (% of max)	36·3±4·3 at t=120	36·0±7·0	41·6±7·2	37·8±8·9	5·5±2·0	5·4±1·9	5·5±6·2	5·1±3·2

All results are expressed as mean±s.d. No significant differences are found between patients and controls for all parameters ($P>0·05$).

TCA: TCA-precipitable radioactivity; PEG: PEG precipitable activity; ery: erythrocyte bound radioactivity; AUC: area under the curve (10^3 ct × min per ml); MRT: mean residence time (min)

* $n=6$.

† $n=4$.

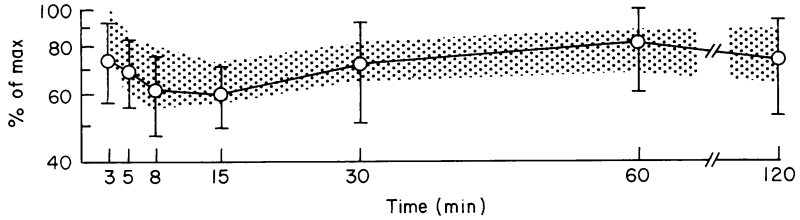

Fig. 2. Radioactivity time curves of non-TCA precipitable ^{123}I, calculated as radioactivity in plasma minus TCA-precipitable counts in eight splenectomized patients (percent of maximal level, mean±s.d.). Shaded area represents mean±s.d. of eight healthy controls. Between t=15 and t=60 min, free ^{123}I rises significantly ($P<0·05$) in both groups, but no differences are seen between patients and controls.

Table 2. Organ uptake of ^{123}I-AIgG in eight healthy controls (HC) and eight splenectomized patients (SP)

	Tmax (min)	ratio Tmax	ratio t=15	ratio t=60
HC				
L/BG	16·3±5·2	2·1±0·4*	1·9±0·4*	0·6±0·2*
S/BG	28·8±16·3	1·9±0·3	1·7±0·3	1·5±0·4
L+S/BG	17·5±4·8	3·8±0·6†	3·6±0·5†	2·1±0·5†
SP				
L/BG	15·1±3·0	2·9±0·7*†	2·7±0·7*†	0·8±0·2*†

All values are ratios of radioactivity expressed as counts/pixel/sec, after subtraction of background activity from hepatic and splenic counts.

L: radioactivity in hepatic area; S: radioactivity in splenic area; BG: background radioactivity; Tmax: point in time when maximal ratio is calculated.

* $P<0·05$ when HC and SP are compared.

† $P<0·05$ when L+S/BG in HC and L/BG in SP are compared.

splenic macrophages are not in such close contact with the circulation as Kupffer cells.

Interestingly, the loss of splenic function seemed to be mostly but not completely compensated for by an increase in hepatic uptake of AIgG. The number of hepatic counts per pixel in the splenectomized patients was higher than the number of hepatic counts in the healthy controls, but lower than the sum of hepatic and splenic counts. It may be that sites other than the liver compensate for the loss of splenic function. Animal studies have suggested a role for the lungs in clearance of AIgG (Brown *et al.*, 1973).

The ^{123}I-AIgG preparation used in this study activated complement. The transient reduction in peripheral leukocyte counts was probably caused by trapping of granulocytes in the pulmonary microcirculation, as a result of complement activation. The same phenomenon has been described in haemodialysis patients, in whom transient pulmonary leukostasis is caused by complement activation by the dialyser membrane (Craddock *et al.*, 1977). In two patients a transient reaction was observed, similar to the ones sometimes seen after administration of i.v. Igs. These reactions have been ascribed to the presence of aggregates with complement-activating properties (Barandun, Morell & Skvaril, 1979). The dose of AIgG (10 μg/kg of body

weight), although found to be safe in previous studies, may still be too high.

In a recent study it was reported that the percentage of binding of preformed IC to red blood cells after injection into volunteers and patients with SLE correlated with the number of CR1 on the erythrocytes (Schifferli *et al.*, 1988). We did not find a correlation between erythrocyte CR1 number and E-AIgG%, probably because of the vast excess of CR1 as compared with the small amount of ^{123}I-AIgG administered. Part of AIgG probably also binds to Fc-receptors of white cells. However, *in vivo* experiments in baboons have shown that there is relatively little binding of IC to leukocytes as compared with binding to erythrocytes (Waxman *et al.*, 1984).

The amount of erythrocyte-bound AIgG correlated inversely with the mean residence time of the erythrocyte-bound radioactivity. Under the conditions of our study, the rate of removal of ^{123}I-AIgG from erythrocytes by hepatic macrophages was apparently independent of the amount of E-AIgG presented to the liver. Thus, with the dose of ^{123}I-AIgG used, the liver does not play a limiting role in the elimination of E-AIgG in healthy subjects.

In patients with SLE delayed clearance of ^{123}I-AIgG was found, associated with increased L/S ratios (Lobatto *et al.*, 1988a), suggesting diminished splenic uptake. This study shows that splenectomy has no effect on the elimination of AIgG from the circulation. Thus, decreased clearance of ^{123}I-AIgG in patients with SLE probably reflects dysfunction of the MPS as a whole, rather than loss of splenic function.

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