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Studies on rheumatoid factor: I. The effect of rheumatoid factor on the clearance of preformed immune complexes in mice

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SUMMARY

The blood clearance of passively transferred immune complexes (IC), preformed at different antigen-antibody ratios, was measured in mice with LPS-induced rheumatoid factor (RF) and in normal controls. RF had a differential effect on IC clearance, significantly enhancing clearance of complexes formed in antibody excess and slight antigen excess but inhibiting the clearance of complexes formed in moderate ($\times 10$) antigen excess. The results are discussed with regard to the mechanisms of IC clearance and suggest that RF may play an important role in governing the behaviour of immune complexes *in vivo*.

Keywords rheumatoid factor LPS immune complexes

INTRODUCTION

Circulating immune complexes (IC) are found in a number of different diseases, and tissue deposition of IC has often been implicated in the disease pathology. Elevated levels of rheumatoid factor (RF), an auto-antibody to the Fc region of IgG, are found in rheumatoid arthritis and other chronic diseases and are usually of the IgM isotype, although IgG and IgA RF have also been described (Lemm, Hohendahl & Wamatz, 1984). In addition, RF production has been described in healthy humans (Kissick, 1961) and animals (Van Snick & Masson, 1980) and has recently been shown to be a component of the normal secondary immune response (Coulie & Van Snick, 1983; Nemazee & Sato, 1983). Clearance of IC from the circulation is mediated primarily by the mononuclear phagocyte system (MPS), particularly the Kupffer cells of the liver, and is influenced by a variety of factors, including the size and solubility of the complex (Weigle, 1958; Haakenstad, Striker & Mannik, 1982), the isotype of the antibody (Mannik, 1980), antibody affinity (Devey & Steward, 1980) and interaction with the complement system (Bockow & Mannik, 1981; Skogh & Stendall, 1983). In some disorders, defective MPS function has been demonstrated (Frank *et al.*, 1979) and the ability of the MPS to remove complexes from the circulation can be saturated, albeit transiently (Haakenstad & Mannik, 1974).

The interaction between RF and circulating IC is of interest as it may profoundly influence IC processing and tissue deposition. Van Snick *et al.* (1978) have demonstrated that RF enhances the attachment and ingestion of IC *in vitro* by macrophages and this was most marked with small complexes formed in antigen excess. On the other hand RF has been reported to inhibit the uptake

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of insoluble IC by neutrophils (Ward & Zvaifler, 1973) and has also been shown to inhibit activation of complement by IC (Doekes *et al.*, 1985).

Immune complexes, preformed *in vitro*, have been shown to induce RF formation in mice and this was shown to be dependent on the antigen-antibody ratio and the presence of antigen primed B and T cells (Coulie & Van Snick, 1985). This, together with the finding that an IgM monoclonal RF binds significantly better to IC than to free antibody *in vivo* (Coutelier & Van Snick, 1985), supports the view that IC may be involved in the induction of RF synthesis and that RF may be of physiological importance in IC handling *in vivo*.

The purpose of the work described here was to assess the effect of elevated levels of RF induced by injections of bacterial lipopolysaccharide on the clearance of preformed IC from the circulation of mice and to determine the influence of immune complex size and antigen-antibody ratio on this.

MATERIALS AND METHODS

Mice. Low affinity NM female mice (Devey *et al.*, 1984) at 10–12 weeks of age were used in all experiments.

RF assay. Serum samples (10 μl) were diluted in veronal-buffered saline (VBS) and incubated with a ^{125}I-labelled mouse monoclonal IgG1 protein in VBS with 0·2% casein and 0·05% Tween 20 for 1 h at room temperature and 16 h at 4°C. The monoclonal IgG1 was chosen from a panel of different monoclonals with anti-DNP specificity. Other monoclonals of the same or different IgG subclass and specificity were just as effective in the RF assay. RF–IgG1 complexes were precipitated after incubation for 1 h at 4°C with 7% (final concentration) polyethylene glycol (PEG, mol. wt 8,000) using ^{22}Na as a volume marker. At this concentration of PEG none of the radiolabelled mouse IgG1 protein was precipitated. Precipitates were counted in a gamma counter and RF binding activity was expressed as a percentage.

Induction of RF in mice. Thirty-two mice received intraperitoneal (i.p.) injections of 0·1 mg bacterial lipopolysaccharide (LPS, phenol extract from *Salmonella minnesota*, Sigma) in sterile PBS twice a week for 6 weeks. Thirty-three control mice received similar injections of sterile PBS.

Preformed IC. Preformed IC were prepared by mixing appropriate amounts of a saturated ammonium sulphate (SAS) fraction of pooled sera from mice hyperimmunized with HSA (producing moderate to high affinity antibody) with ^{125}I-labelled HSA. Equivalence was determined by a modification of the Farr assay to determine the point of antibody excess and by an ELISA to determine the point of antigen excess. Complexes were prepared at $\times 2$, $\times 5$, and $\times 10$ antigen excess and at $\times 2$, $\times 5$ and $\times 10$ antibody excess.

Blood clearance of IC. ^{125}I-HSA or preformed ^{125}I-HSA IC (0·1 ml at 1×10^6 ct/min) were injected into the tail vein of mice. Blood was taken from the retro-orbital plexus after 1, 5, 10 and 30 min and 1, 2, 4 and 24 h. The total ct/min injected was calculated by counting the syringe before and after each injection. Sera were precipitated with SAS and, taking the total ct/min injected as 100%, the % of SAS precipitable counts (IC) in the circulation was calculated, assuming that serum weight was 5% of the body weight.

Statistics. The significance of differences between means was tested using Student's *t*-test.

RESULTS

Levels of RF in LPS injected and control mice. In LPS injected mice, serum RF levels rose with the increasing number of injections, reaching a plateau by 6 weeks (Table 1). In saline injected mice RF, levels remained low and were comparable to those found in uninjected mice.

Blood clearance of HSA in LPS injected and control mice. In both LPS and control mice, HSA was cleared slowly from the circulation after i.v. injection, with a half life of 3·1 and 3·2 h respectively by regression analysis, and there was no significant difference between the two groups of mice.

Blood clearance of antibody excess IC in LPS injected and control mice. Preformed, passively

Table 1. Serum RF binding activity in LPS injected and control mice after 6 weeks of injection

Treatment group	Number	Mean RF binding activity (% + s.d.)
LPS	32	16·3 + 4·0 (range 11·5–27.4)
Saline	33	6·9 + 1·5 (range 4·1–9.4)
t		12·69 ($P < 0·001$)

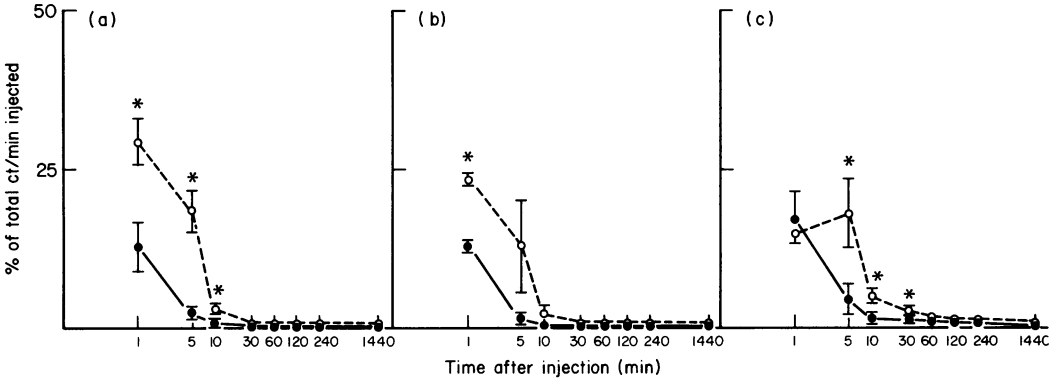

Fig. 1. Clearance of antibody excess IC in LPS stimulated (●——●) and control mice (○——○) injected with preformed IC at (a) × 10, (b) × 5, and (c) × 2 antibody excess. Points represent the mean serum level in three mice ± s.d. *$P < 0·01$.

Table 2. Time to clear 90% of the injected IC from the circulation of control mice and mice with LPS-induced RF.

		90% clearance time (min)	
Preformed immune complex		Control	LPS
Antibody excess	× 10	7·6	2·0
	× 5	6·2	1·9
	× 2	8·0	3·2
Antigen excess	× 2	7·5	2·4
	× 5	13·3	4·7
	× 10	43·1	76·4
Free HSA		3229·9	3093·3

transferred IC at × 2, × 5 and × 10 antibody excess were cleared rapidly from the circulation in both LPS and control mice (Fig. 1). In each case, clearance was significantly more rapid in LPS injected mice during the first 30 min after IC injection, with 90% of the injected complexes cleared within 1–3 min compared to 6–8 min for the controls (Table 2). There was essentially no difference in the clearance kinetics between any of the IC formed in different amounts of antibody excess,

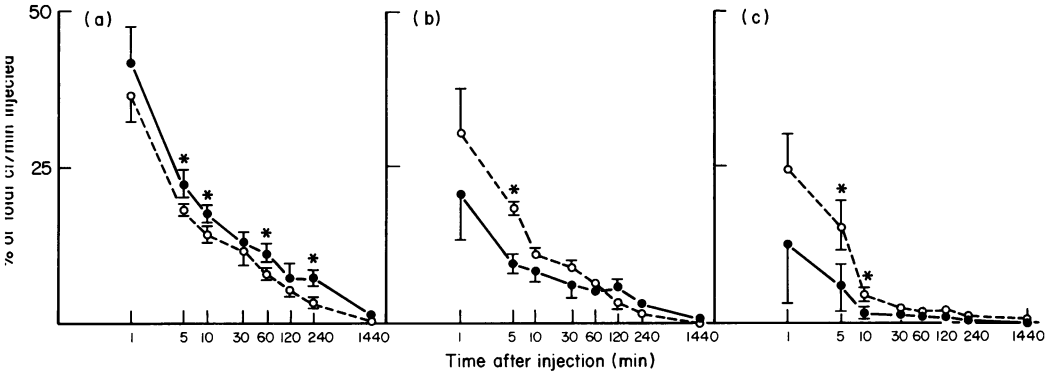

Fig. 2. Clearance of antigen excess IC in LPS stimulated (●——●) and control mice (○ - - - ○) injected with preformed IC at (a) × 10, (b) × 5, and (c) × 2 antigen excess. Points represent the mean serum level in three mice ±s.d. $*P<0{\cdot}01$.

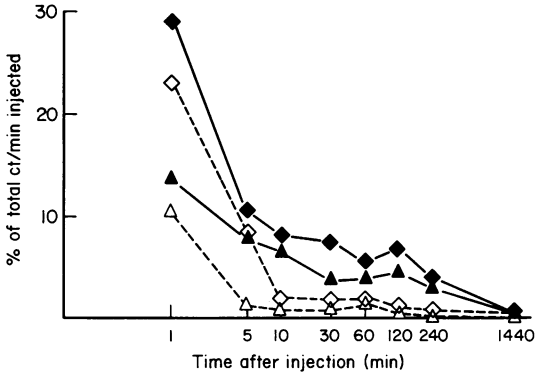

Fig. 3. Clearance of IC in individual LPS stimulated mice with different levels of RF. Solid lines: × 5 antigen excess IC with RF level 19·0% (▲) and RF level 14·2% (♦). Broken lines: × 2 antigen excess IC with RF level 21·3% (△) and RF level 12·1%(◇).

except for × 2 antibody excess complexes which were still detected at low levels in the serum of control mice 30 min after injection.

Blood Clearance of antigen excess IC in LPS injected and control mice. Preformed, passively transferred IC at × 2, × 5 and × 10 antigen excess were cleared progressively less rapidly in both LPS injected and control mice (Fig. 2). With × 2 and × 5 antigen excess complexes, clearance was again more rapid in LPS injected mice although the differences became less marked with the increasing amount of excess antigen. With × 2 and × 5 antigen excess, 90% of the injected complexes were cleared in 2·4 and 4·7 min respectively in LPS injected mice compared to 7·5 and 13·3 min in control mice (Table 2). However with × 10 antigen excess complexes, clearance was significantly slower in LPS injected mice compared to control mice (Fig. 2a), with 90% of the injected dose cleared in 76·4 min compared to 43·1 min for the controls (Table 2). The clearance kinetics of × 2 antigen excess complexes were very similar to those of × 2 antibody excess complexes in both groups of mice. However with × 5 and × 10 antigen excess complexes, complete clearance was markedly delayed and complexes persisted for up to 24 h after injection.

Influence of RF level on IC clearance in individual mice. In the LPS-injected mice there was considerable variation in the RF level between individual mice. With all preformed IC, except for × 10 antigen excess, there was a significant correlation between the individual RF level and the rate

of IC clearance, with more rapid clearance occurring in mice with the highest RF levels (Fig. 3). In each experimental group the mean RF levels were, however, comparable.

DISCUSSION

It has been known for many years that the blood clearance of passively transferred IC becomes progressively less rapid when they are prepared with increasing amounts of excess antigen (Weigle, 1958; Mannik *et al.*, 1971). Clearance rate is directly related to complex size and to lattice formation (Mannik, 1980; Haakenstad *et al.*, 1982). These findings were confirmed in the present study, where increasing impairment of IC clearance was demonstrated when preformed antigen excess complexes were injected into mice.

We have found that stimulation of mice with LPS resulted in a differential effect on the rate of clearance of passively transferred IC *in vivo*. Complexes prepared in antibody excess and slight antigen excess showed enhanced clearance in LPS-stimulated mice but clearance of IC prepared in moderate ($\times$ 10) antigen excess was significantly delayed. LPS has a broad effect on the immune system including polyclonal activation of B cells with production of a wide range of autoantibodies. However, as RF is the major and probably most important autoantibody specificity (Dresser & Popham, 1976) and as, in individual mice, IC clearance showed a direct correlation with the level of RF in the serum (Fig. 3), we suggest that the difference in IC clearance kinetics was due to the presence of RF. LPS may also affect MPS function (McGee *et al.*, 1979) and it is possible that this may have influenced IC clearance kinetics, although MPS function was presumably similar in all the LPS-stimulated mice which showed differential clearance of antibody excess and antigen excess complexes. In addition, preliminary studies with IC preformed in the presence and absence of RF and passively transferred into normal mice has confirmed the findings reported here (D. N. Hogben, unpublished results) and RF isolated from sera of LPS-stimulated mice has been shown to significantly alter immune complex handling in an experimental model of chronic serum sickness (Devey & Hogben, in preparation).

Complexes formed in antibody excess or slight antigen excess are large in size and would be expected to interact well with RF. Enhancement of the clearance of these IC by RF may be most readily explained by the increase in size which occurs on interaction with RF (Doekes *et al.*, 1985) and which has been shown to enhance phagocytosis by macrophages *in vitro* (Van Snick *et al.*, 1978). The role of complement in IC clearance is still controversial and, although there is quite a lot of experimental evidence to suggest that it does not play an important role in the blood clearance of preformed soluble IC (Mannik *et al.*, 1971; Bockow & Mannik, 1981), it may influence the fate of IC formed in its presence by inhibiting precipitation (Skogh & Stendahl, 1983; Malasit, Bartolotti & Humphrey, 1983). IgM RF may, therefore, interfere with complement activation by soluble IC (Doekes *et al.*, 1985) if it is unable to undergo a conformational change to the complement activating 'staple' configuration (Lachmann & Hughes-Jones 1984). It is likely that the preformed antibody excess and slight antigen excess IC used in these experiments, although not formed in the presence of complement, would be able to activate it efficiently *in vivo* and, as RF appeared to enhance blood clearance of these IC, either it did not affect complement interaction or complement-mediated clearance was not an important mechanism.

The effect of RF on $\times$ 10 antigen excess IC clearance could not be explained by a simple failure of RF to interact with these small soluble IC (although it might be expected to interact less well), as their clearance was significantly delayed in mice with RF compared to controls, suggesting that RF was actively responsible for this. The mechanism by which this occurred is not immediately obvious, particularly as IC formed in moderate antigen excess probably interact less well with complement than those formed in antibody excess. It is possible that low-affinity mice produced low-affinity RF which interacted in a different way with small soluble IC compared to larger IC, although our own preliminary studies and those of others (Steward *et al.* (1973), suggest that RF-binding affinities are usually low compared to other antigen-antibody reactions. If clearance of $\times$ 10 antigen excess IC was primarily Fc mediated, RF may have caused steric hindrance of Fc receptor interaction as well as preventing complement activation and therefore in delayed clearance.

These results suggest that RF influences the behaviour of IC *in vivo* and support the view that it may be of physiological importance in immune complex diseases. Work is in progress to determine if RF influences the site of IC deposition and to assess the role of complement on the effect of RF using preformed IC and IC formed *in vivo*.

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