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THE EFFECT OF MANIPULATION OF RETICULOENDOTHELIAL SYSTEM ACTIVITY ON GLOMERULAR DEPOSITION OF AGGREGATED PROTEIN AND IMMUNE COMPLEXES IN TWO DIFFERENT STRAINS OF MICE

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Summary.—Glomerular uptake of intravenously administered aggregated albumen or immune complexes in mice appears to be inversely related to the activity of the reticuloendothelial system (RES). Stimulation of RES activity diminishes the amount of material appearing in the glomerulus whereas RES blockade enhances glomerular uptake. The possible relevance of these observations to experimental models of immune complex disease is discussed.

IT HAS BEEN shown previously that glomerular uptake of both immune complexes and of aggregated albumen differed in degree between various strains of mice. It was further shown that such differences appeared to be related to variation in activity of the systemic macrophage system, the ability of a strain to rapidly clear intravenously injected particulate matter being associated with a low degree of glomerular uptake of aggregated albumen or of immune complexes, the reverse being seen in a strain with less active clearance of particulate material (Ford, 1975*a*, *b*).

The conclusion drawn from these observations was that the amount of immune complex material or aggregated protein deposited in the glomerulus depended on the length of time such material was allowed to remain in the circulation and was thus, in part at least, inversely related to the activity of the systemic macrophage system. This idea was supported by the observation that blockade of the reticuloendothelial system (RES) resulted in an enhanced uptake of aggregated protein in the glomeruli of the strain, with a high rate of clearance of particulate matter from the circulation, whilst having little effect on the strain with a low rate of clearance.

This paper reports the effect of stimulation and of blockade of the RES on glomerular deposition of immune complexes or aggregated protein in 2 strains of mice, one with a naturally high degree of RES activity, the other with low activity.

MATERIALS AND METHODS

The mice used were of 2 inbred strains, ICR/IAR and DBA/2 both currently maintained in the animal house of the Medical Sciences Institute of the University of Dundee. The animals used were between 3 and 6 months old and weighed between 25 and 30 g. Bovine serum albumen obtained from Hoechst Pharmaceuticals Ltd was aggregated using the method of Ilo and Wagner (1963) as previously described (Ford, 1975*b*). The preparation was centrifuged at 1500 *g* for 30 min before intravenous administration. The dosage scheme used was 45 mg/100 g body wt.

Rabbit anti-BSA antibody–BSA immune complexes were prepared in 5× antigen excess as previously described (Ford, 1975*a*) and injected in a dose of 2 ml/100 g body wt. Systemic macrophage activity was determined by measurement of the rate of clearance of intraveneously injected carbon particles using

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the method of Biozzi, Benacerraf and Halpern (1953). Pelikan carbon C11/1431a (Gunther Wagner, Hanover, Germany) was used for both clearance studies and macrophage blockade.

The stock suspension was diluted in saline to 16 mg carbon/ml before being injected i.v. in a dose of 16 mg/100 g body wt. Serial blood samples were taken at 3, 6, 9, 12 and 15 min from the retro-orbital plexus into 20 μl heparinized capillary tubes. Each tube was washed out into 3 ml of distilled water, which was then shaken thoroughly and the optical density measured on a spectrophotometer at 650 nm. These results were expressed as a percentage of the 3-min value. In the stimulated group of ICR/IAR mice RES activity was so enhanced that clearance was almost complete by about 6 min; in these animals, and a control group, blood was therefore taken at 1-min intervals from 1 to 6 min and results expressed as a percentage of the 1-min figure.

Systemic macrophage blockade was effected by intravenous injection of 10 mg of colloidal carbon in 0·5 ml saline 24 h before subsequent injections.

Stimulation of the systemic macrophage system was carried out using the method of Dobson and Kelly (1973). 0·2 mg oestradiol dissolved in 0·2 ml olive oil was injected subcutaneously and 3 days later 0·05 mg endotoxin (Difco lipopolysaccharide O26 : B6) in 0·2 ml saline was given i.v. Subsequent investigations were performed a further 48 h later.

Animals receiving intravenous albumen or immune complexes were killed 6 h after injection. Portions of the kidneys were snap frozen in isopentane pre-cooled in liquid nitrogen; 5 μm air dried sections were washed for 30 min in phosphate buffered saline (PBS) pH 6·8 and then incubated for 30 min with the appropriate antiserum: for immune complex localization, FITC-goat anti-rabbit serum (Hoechst Pharmaceuticals Ltd); for albumen localization a "sandwich" technique was employed, incubation for 30 min with rabbit anti-BSA (Hoechst Pharmaceuticals Ltd) serum followed by a 15-min wash in PBS and then 30 min incubation with FITC-goat anti-rabbit serum. All sections were then further washed in PBS and mounted in buffered glycerol before examination under u.v. light using a Reichert Zetopan microscope with a B.G. 12/3 mm primary filter. Kodak high speed Ektachrome was used for photography.

Intensity of staining was scored 0–++++ as previously illustrated (Ford 1975*a*, *b*).

RESULTS

RES blockade

Figure 1 shows the change in optical density plotted against time for normal mice of each strain and for mice of each strain 24 h after 10 mg of i.v. particulate carbon. It can be seen that the steeper curve of carbon clearance in the ICR/IAR

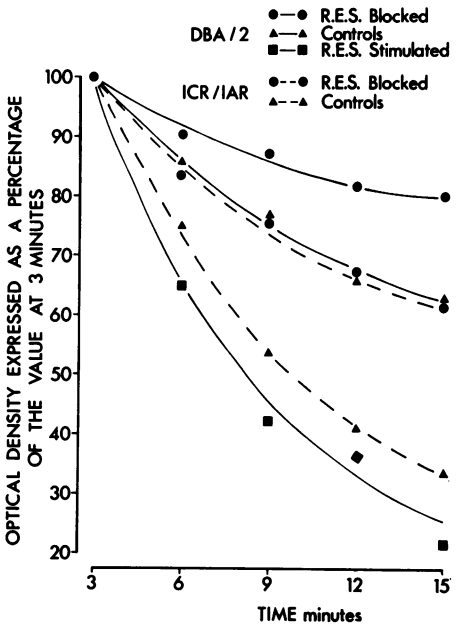

FIG. 1.—Graph to show percentage decrease in optical density against time for control and RES blocked ICR/IAR and control and RES stimulated and blocked DBA/2 mice. (The 3-min optical density value is taken as 100%.)

TABLE I.—*ICR/IAR Mice, Carbon Particle Clearance from the Blood of Control and RES Blocked Animals; Mean Results* (±1 *S.D.*) *Expressed as a Percentage of the Optical density at* 3 *Min*

ICR/IAR	No. of mice	Optical density as percentage of 3 min value			
		6 min	9 min	12 min	15 min
Control	9	75·1±13·7	54·1±14·9	41·0±17·5	33·4±15·1
RES blocked	9	83·9±8·9	76·6±9·4	66·7±5·6	61·7±5·9
P		<0·1	<0·005	<0·0025	<0·001

TABLE II.—*ICR/IAR Mice, Carbon Particle Clearance from the Blood of Control and RES Stimulated Animals; Mean Results* (±1 *S.D.*) *of* 2–6 *min Values Expressed as a Percentage of the Optical Density at* 1 *min* ($P = < 0{\cdot}001$ *for each set of figures*)

ICR/IAR	No. of mice	Optical density as percentage of 1 min value				
		2 min	3 min	4 min	5 min	6 min
Control	8	88·1±2·8	78·1±5·1	71·3±5·5	65·3±6·4	61·7±6·3
RES stimulated	9	68·3±11·9	49·7±12·7	36·7±15·4	30·2±7·1	29·7±8·6

TABLE III.—*DBA/2 Mice, Carbon Particle Clearance from the Blood of Control, RES Blocked and RES Stimulated Animals; Mean Results* (±1 *S.D.*) *of* 3, 6, 9 *and* 15 *min Values Expressed as a Percentage of the Optical Density at* 3 *min. Significance* (*P*) *for each Group is Compared with the Control*

DBA/2	No. of mice	Optical density as percentage of 3 min value			
		6 min	9 min	12 min	15 min
Control	11	86·2±5·0	77·0±9·0	65·8±13·2	63·2±14·6
RES blocked	10	90·5±7·1	87·5±9·3	81·9±9·4	80·4±8·8
P		<0·1	<0·025	<0·005	<0·005
RES stimulated	9	65·1±10·7	42·6±11·1	37·0±10·9	21·8±10·3
P		<0·001	<0·001	<0·001	<0·001

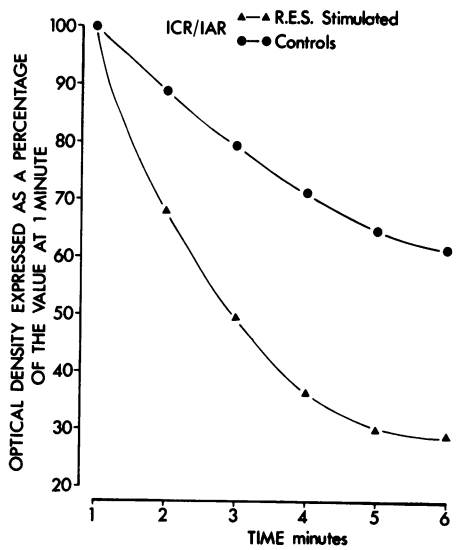

FIG. 2.—Graph to show percentage decrease in optical density against time for control and RES stimulated ICR/IAR mice. Note the different time scale from Fig. 1. (The 1-min optical density value is taken as 100%.)

mice flattens out following "blockade" and closely approximates that of the untreated DBA/2 mice, whilst carbon blockade of DBA/2 mice causes a smaller but still definite decrease in clearance. Optical density measurements for each set of animals are given in Tables I and III.

Table IV shows the degree of deposition of aggregated BSA 6 h after injection into carbon blocked mice. Both strains now showed an essentially similar intensity of staining as well as a similar pattern. Whilst there is little difference between normal and blocked DBA/2 mice, a very marked difference is seen in the ICR/IAR mice (Fig. 3*a*, *b*).

The staining intensity following i.v. immune complex injection in blocked and unblocked mice of both strains is also seen in Table IV. Again, there is a marked increase in staining intensity of blocked ICR/IAR mice compared with controls whilst no clear difference was seen between blocked and unblocked DBA/2 mice. It is interesting to note the protective effect of carbon blockade on the deaths from anaphylaxis in the ICR/IAR mice, a strain normally very sensitive to immune complex induced anaphylaxis (Ford, 1975*a*).

TABLE IV.—*To Show Staining Intensity in RES Blocked Mice and Controls. Column 3 shows Mortality from Anaphylaxis in Mice receiving Immune Complexes*

Strain of mouse	No. of mice	Mortality	RES block	Substance injected	Staining intensity
ICR/IAR	5	—	—	Agg. BSA	+
	5	—	+	Agg. BSA	+ + +
	10	8	—	Imm. complex	+
	6	1	+	Imm. complex	+ + +
DBA/2	5	—	—	Agg. BSA	+ + +
	5	—	+	Agg. BSA	+ + + +
	6	1	—	Imm. complex	+ + +
	6	0	+	Imm. complex	+ + + to + + + +

TABLE V.—*To show Staining Intensity in RES Stimulated Mice and Controls. Column 3 shows Mortality from Anaphylaxis in Mice receiving Immune Complexes*

Strain of mouse	No. of mice	Mortality	RES stimulation	Substance injected	Staining intensity
ICR/IAR	5	—	—	Agg. BSA	+ to + +
	10	—	+	Agg. BSA	0
	10	8	—	Imm. complex	+
	7	1	+	Imm. complex	0
DBA/2	5	—	—	Agg. BSA	+ + +
	10	—	+	Agg. BSA	+ to + +
	6	1	—	Imm. complex	+ + +
	8	0	+	Imm. complex	+ to + +

RES stimulation

Figures 1 and 2 show the change in optical density plotted against time for both normal and RES stimulated DBA/2 and ICR/IAR mice. Owing to an extremely rapid decrease in optical density in RES stimulated ICR/IAR mice, blood samples were taken at 1 min intervals in both this group and controls (Fig. 2). It can be seen that both strains show a marked increase in clearance of carbon particles, the rate of clearance in the stimulated DBA/2 animals is still less, however, than the rate in unstimulated ICR/IAR mice. The curves for both strains achieve statistical significance when compared with controls. Individual optical density measurements are shown in Table II and III.

In both strains given i.v. aggregated BSA a reduced intensity of glomerular staining was noted in the RES stimulated groups when compared with controls (Table V). It is of interest to note that although RES stimulated DBA/2 mice show a reduction in staining intensity, deposition is still greater than in the ICR/IAR controls.

Following injection of immune complexes, a similar pattern of reduction of deposits in RES stimulated mice is seen in both groups (Table V) although again this is less marked in DBA/2 mice. RES stimulation is shown to have a markedly protective effect against anaphylaxis in ICR/IAR mice. DBA/2 mice, although there was only one death in the control group, showed less distress in the RES stimulated group following immune complex injection compared with the controls.

DISCUSSION

The role of the reticuloendothelial system in clearing circulating immune complexes was clearly shown by Benacerraf, Sebestyen and Cooper (1959*a*). It has also been shown that intravenously injected colloidal carbon in mice is not taken up by the glomeruli unless the RES has previously been " overloaded "

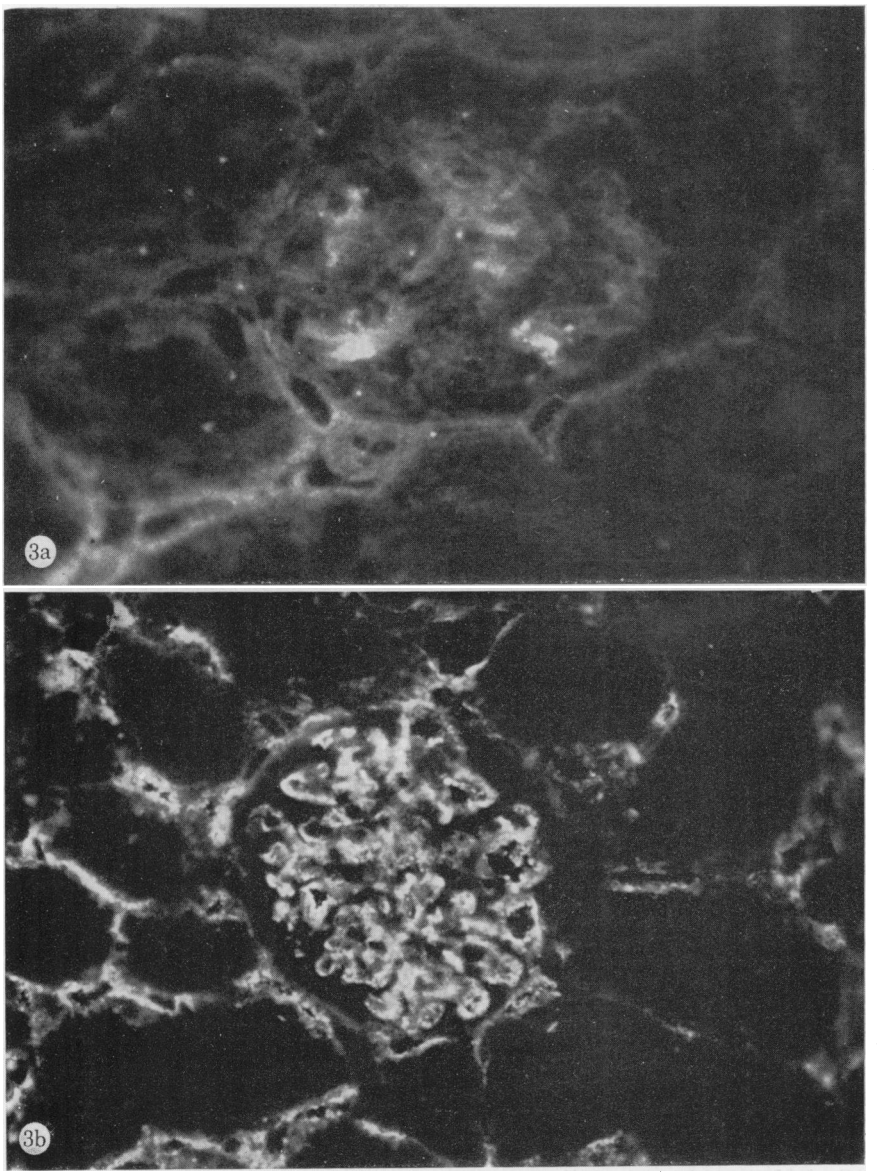

FIG. 3*a*.—Glomerulus from a control ICR/IAR mouse which had received 45 mg/100 g body wt aggregated BSA 6 h previously.

FIG. 3*b*.—Glomerulus from a " carbon blocked " ICR/IAR mouse given the same dose of aggregated BSA.

Both preparations (× 340) stained with a " sandwich " of rabbit anti-BSA serum and FITC-goat, anti-rabbit.

by repeated doses of carbon (Benacerraf *et al.*, 1959*b*).

The results reported above would appear to support these previous observations and further suggest that the uptake of particulate material by the mesangial cells of the glomerulus, be it aggregated protein or immune complexes, is inversely related to the activity of the systemic macrophage system (Ford, 1975*b*). The most obvious explanation for these observations is that more rapid removal of particulate material from the circulation allows less opportunity for contact with the glomerular capillaries and that a less rapid removal of material allows a greater chance of glomerular deposition, due either to the greater concentration presenting or the longer period of time over which such material passes through the glomerular blood vessels.

A similar mechanism might also explain the observation that in mice splenic macrophages take up a greater quantity of injected sheep red cells after carbon blockade (Fisher, 1966). It has also been shown that in rats the phagocytic cells in the bone marrow show enhanced uptake of injected foreign red cells after RES blockade (Keene and Jandl, 1965).

The increased deposition of immune material seen in RES "blocked" mice would support the suggestion by Oldstone and Dixon (1969) that in the mouse infected with lymphocytic choriomeningitis virus deposition of immune complexes in the kidney occurs only after the chronic viraemia has produced RES depression. A similar chain of events has been postulated for experimental serum sickness in the rabbit (Wilson and Dixon, 1971). Haakenstad and Mannik (1974) have, more recently, shown in mice that with increasing doses of passively administered immune complexes apparent saturation of the RES occurs, allowing persistence of immune complexes in the circulation.

The protective effect of RES blockade, which has been noted before (Wistar, Treadwell and Rasmussen, 1960) and of RES stimulation against anaphylactic shock may possibly be explained by the observation that, at least in dogs and rabbits, injection of either particulate carbon or endotoxin i.v. may be followed by a marked thrombocytopenia (Cohen, Braunwald and Gardener, 1965), the rate of subsequent recovery of platelet numbers being related to the initial dose of carbon or endotoxin administered. It has been shown by Humphrey and Jaques (1954) that in rodents the platelets contain 5-hydroxytryptamine (5-HT) and it is known that depletion of 5-HT in mice by pharmacological agents protects against the anaphylactogenic effect of intravenously administered immune complexes (Tokuda and Weiser, 1961). A thrombocytopenia induced by carbon particles or by endotoxin might therefore deplete the available 5-HT to an extent sufficient to protect against anaphylaxis. It is also possible that such depletion might affect immune complex deposition as release of vasoactive amines is thought to be an important factor. Indeed, Kniker (1972) has shown that in chronic serum sickness in rabbits antagonists of both histamine and 5-HT reduce the amount of immune material deposited in glomeruli. However, in the experiments reported here it is unlikely that any 5-HT depletion was responsible for the results as similar changes were seen with aggregated BSA which is not thought to provoke release of vasoactive substances and the deposition of which in the glomeruli should therefore be unaffected by changes in available 5-HT.

The results reported here, together with the observations of Passwell, Steward and Soothill (1974), who showed that variations in RES activity influenced antibody affinity, would tend to suggest that the RES might have a significant role in the pathogenesis of immune complex induced glomerulonephritis. It would therefore be of interest to examine the effect of stimulation of the systemic macrophage system in some of the available animal models of immune complex

nephritis such as acute and chronic serum sickness.

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