Nephritogenic Properties of Nephrotoxic Guinea Pig Antibodies

I. Glomerulonephritis induced by Guinea Pig IgG₁ Antibody in Rats

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Summary. Guinea pig IgG₁ and IgG₂ were separated from guinea pig antisera against rat kidney using DEAE column chromatography with gradient elution. In vitro system, nephrotoxic IgG₁ antibody did not fix rat complement with rat kidney sediments, but did IgG₂. Nephrotoxic IgG₁ or IgG₂ was intravenously injected into rats respectively. Glomerulonephritis characterized by an immediate massive proteinuria was initiated by nephrotoxic IgG₁ antibody. Marked intraluminal fibrin formation and phagocytic accumulation of small numbers of neutrophils and monocytes were observed in the glomeruli. Rat β 1c globulin was only weakly seen along glomerular basement membrane of the biopsy specimens early in the development of nephritis. On the other hand, rats given a comparable dose of nephrotoxic IgG₂ antibody did not develop a significant proteinuria and glomerular changes despite localization of IgG₂ and rat β 1c in the glomeruli. These observations suggest that non-complement mediated glomerular injury is present at least in the initial phase of Masugi nephritis.

In the past few years, two classes of 7S immunoglobulins (IgG₁ and IgG₂) have been described and characterized in the guinea pig. The IgG₁ and IgG₂ immunoglobulins have different electrophoretic mobilities (Benacerraf *et al.*, 1963) and different biologic properties: IgG₁ antibody has a capacity to sensitize guinea pig for passive cutaneous or systemic anaphylaxis (Ovary *et al.*, 1963), but is incapable of fixing complement and lysing red blood cells (Bloch *et al.*, 1963; Kourilsky *et al.*, 1963). IgG₂, on the other hand, has been known to fix complement, lyse cells and be capable of provoking Arthus reaction, but not passive anaphylactic reaction.

Meanwhile, as to the mechanisms of the initiation of Masugi nephritis, a sequence of immunobiological events has been noticed. Especially, there is a general agreement that participation of the complement-leucocyte system is essential for initiation of glomerulonephritis (Cochrane *et al.*, 1965; Cochrane, 1969). On the other hand, it is found that rats injected with duck nephrotoxic antibodies developed an immediate proteinuria, despite little or no fixation of C3 (Unanue and Dixon, 1964) and the absence of neutrophil accumulation (Cochrane, 1965). Some experiments are also reported that Masugi nephritis could be induced in complement deficient rabbits (Rother *et al.*, 1967) and complement deficient mice (Unanue *et al.*, 1967).

The present investigation was undertaken to study further the immunobiological properties of the initiation of Masugi nephritis, using nephrotoxic guinea pig IgG₁ antibody which is found to be incapable of fixing complement in the classical pathway. Nephritogenicity of nephrotoxic IgG₂ antibody is also described.

Materials and Methods

Animals. Wistar rats of either sex weighing 150 to 200 g were used.

Antigens. Renal antigen for immunization was prepared by the method described by Fujimoto *et al.* (1964). Renal cortices were obtained from perfused rat kidneys and ground in a Waring blender. A 10% w/v suspension was prepared in saline. Soluble antigen of kidney was prepared by trypsinization of kidney sediment by the method of Cole (1951).

Preparation of Nephrotoxic Serum. Guinea pigs weighing 500 to 600 g were injected in footpads with 0.5 mgN of the antigen in an equal volume of incomplete Freund's adjuvant, followed by seven biweekly injections of the same antigen into the back muscle. They were bled 2 weeks after the last injection. Sera were absorbed with packed rat erythrocytes and a small quantity of rat serum until no hemagglutination and precipitin reactions were detected.

Fig. 1. A chromatographic pattern of 40% ammonium sulfate precipitate on DEAE cellulose column using gradient elution from 0.005 M, pH 8.5 to 0.59 M, pH 4.0 phosphoric acid-Tris buffers. IgG₁ fraction was mostly eluted in the second peak

Purification of IgG₁ and IgG₂ Fractions from Nephrotoxic Sera. Purification of immunoglobulin fractions was carried out according to the method described by Yagi *et al.* (1962) with diethylaminoethyl (DEAE) cellulose column chromatography. The serum was precipitated at 40% saturation of ammonium sulfate, and the precipitate was dissolved and dialyzed against 0.15 M saline and then a buffer consisting of 0.035 M Tris (hydroxymethyl) aminomethane (Tris) and 0.005 M phosphoric acid, pH 8.5. An aliquot of the sample was applied to a DEAE cellulose column which had been equilibrated with the same buffer. After the first peak was completely eluted with the buffer, the pH and salt gradient elution was started using the final buffer consisting of 0.5 M Tris and 0.59 M phosphoric acid, pH 4.0. IgG₂ was eluted in the first peak. The second peak contained most of IgG₁ (Fig. 1). When some contamination of IgG₂ was detected by immunoelectrophoresis, rechromatography of IgG₁ fraction was performed by the same procedure to devoid IgG₂. The preparation of IgG₁ fraction thus obtained was then passed through a Sephadex G 200 column (2.5 × 100 cm) to eliminate the possible contamination of IgM. The final preparation contained IgG₁ together with some α and β globulins.

Antisera. *Anti-guinea pig IgG₁.* Rabbit antibody specific for guinea pig IgG₁ was prepared by immunizing a rabbit with IgG₁ antibody specifically purified from hyperimmune guinea pig serum against hen's egg albumin (EA). Hyperimmune guinea pig serum was pooled and anti-EA was purified specifically by the method of Hong *et al.* (1965). The purified antibody containing guinea pig IgG₁ and IgG₂ was subjected to Starch block electrophoresis according to the method of Kunkel (1954). The fractions containing only IgG₁ were pooled, concentrated and used for the immunization of a rabbit. A rabbit received four injections of 400 μgN of this material emulsified in an equal volume of complete Freund's adjuvant with biweekly intervals and was bled 2 weeks after the last injection. This antiserum was absorbed with purified IgG₂ and was referred as anti-IgG₁.

Anti-Guinea Pig IgG₂. Normal IgG₂ for the immunization was prepared from normal guinea pig serum by the same chromatographic method as described above. Antiserum against IgG₂ was prepared by the same immunization procedure used for the preparation of anti-guinea pig IgG₁. The antiserum was absorbed with IgG₁ to render specific for IgG₂.

Anti-Rat β 1c Globulin. The antiserum against rat β 1c was prepared using zymosan according to the method described by Mardiney and Müller-Eberhard (1965).

Immunoelectrophoresis. Immunoelectrophoresis was carried out according to the method of Scheidegger (1955).

Quantitation of Kidney Fixing Antibody (KF Abs) in Nephrotoxic IgG₁ and IgG₂ Fractions in vivo. Radiiodination of IgG₁ and IgG₂ was carried out according to the method of Day *et al.* (1956). After extensive dialysis, the nitrogen content of the iodinated protein was estimated by nesslerization and its specific activity was determined. To calculate the rate of fixation of each nephrotoxic fraction to the kidneys and other organs, 150 μ gN of I¹³¹ labeled antibodies were injected into groups of 3 rats. Estimation of the radioactivity in each organ was carried out by the method described by Bale and Spar (1954). As 90 per cent or more of nephrotoxic antibodies are fixed to GBM within 1 hour after intravenous injection (Unanue and Dixon, 1965), all rats were sacrificed at 1 hour after the injection and organs were perfused with saline. Organs removed from the animals were blotted with filterpaper to remove excess fluids, and placed into test tubes which were then inserted into a well-type scintillation counter. The counts were corrected with background and decay of I¹³¹. As a control, 100 μ gN of I¹³¹ labeled normal guinea pig IgG₁ and IgG₂ were injected to groups of 3 rats and the degree of non-specific localization of immunoglobulins to various organs was estimated in a similar manner.

Complement Fixation Tests. Two-dimensional complement fixation tests were carried out by the method of Mayer (1961). Equal volumes (0.4 ml) of rat kidney sediments, nephrotoxic IgG₁ or IgG₂ antibody and 0.5 ml of fresh rat serum containing 5 CH₅₀ units complement were incubated at 37° C for 1 hour. The mixture was centrifuged at 3000 rpm for 20 minutes, and then to the supernatant, 0.2 ml of EA containing 10⁸ cells were added. After incubation at 37° C for 1 hour the degree of hemolysis was read macroscopically.

Induction of Glomerulonephritis by Nephrotoxic IgG₁ Antibody. Rats with urinary protein less than 1.0 mg/24 hr were used for the experiments. Nephritis was induced by a single intravenous injection of various amounts of IgG₁ or IgG₂ antibody. The amounts of urinary protein during 24 hr period were estimated according to the Kingsbury-Clark's method. In order to follow the dynamics of renal lesions, renal biopsy and autopsy were carried out at various times from 6 hr to 3 weeks after the injection. In control studies rats were given intravenously either 1 ml of normal guinea pig serum or 2 mgN of IgG₁ fraction of normal guinea pig serum.

Immunohistochemical Studies. Specimens obtained at renal biopsy and autopsy were snap frozen in liquid n-hexane at -70° C. Frozen sections cut at 4 μ in a cryostat were stained with a fluorescent microscope (Type S-F, Nippon Kogaku). Conjugation of anti-rat β 1c, anti-guinea pig IgG₁ and anti-guinea pig IgG₂ with Fluorescein isothiocyanate (FITC) was carried out according to the method of Wood *et al.* (1965). As controls of fixation of rat β 1c in glomeruli, the blocking test was performed and normal rat kidney was stained with FITC-conjugated anti-rat β 1c.

Histological Studies. For electron microscopy small blocks obtained at renal biopsy and autopsy were immersed in 3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4 for 1 to 2 hr at 4° C. The tissues were then post-fixed in 1% osmium tetroxide in 0.1 M phosphate buffer of pH 7.4 for 1.5 hr. After dehydration with graded ethanol, they were embedded in Epon 812. The ultrathin sections were cut on a Porter-Blum MT-1 microtome, doubly stained with uranyl acetate and lead citrate, and examined with a Hitachi HS-8 or 11Ds electron microscope. Semithin sections (1 μ) were stained with PAS and Toluidine blue after removing the resin by the method by Imai *et al.* (1968) for light microscopic examination. Other tissue blocks were fixed in 10% formalin and embedded in paraffin for usual microscopic examination.

Results

Purity of IgG₁ Fraction. The purity of the final preparation of nephrotoxic IgG₁ fraction was tested by immunoelectrophoresis. As shown in Fig. 2, the

Fig. 2. Immuno-electrophoresis analyses of IgG₁ and IgG₂ fractions. 500 μ gN of IgG₁ or IgG₂ fraction was applied into each well. IgG₁ fraction shows a precipitin band in the γ 1 region (1-a). This IgG₁ fraction developed with soluble kidney antigen forms a single distinct precipitin line corresponding to γ 1 region but not γ 2 (1-b)

Table 1. Localization of I¹³¹-nephrotoxic IgG₁ and IgG₂ antibodies to various organs *in vivo*

Materials injected	Doses injected (μ gN)	Per cent localized					
		Kidney	Heart	Lung	Liver	Spleen	Blood ^a
Nephrotoxic IgG ₁ fr. ^b	150	2.536 ± 0.511	0.078 ± 0.017	0.569 ± 0.104	9.303 ± 1.297	0.830 ± 0.091	2.714 ± 0.073
Normal IgG ₁ fr.	100	1.625 ± 0.354	0.044 ± 0.014	0.171 ± 0.112	5.721 ± 1.228	0.190 ± 0.019	3.385 ± 0.145
Nephrotoxic IgG ₂	150	2.338 ± 0.150	0.093 ± 0.012	2.003 ± 1.227	13.202 ± 2.988	0.743 ± 0.135	1.958 ± 0.139
Normal IgG ₂	100	0.725 ± 0.091	0.016 ± 0.006	0.113 ± 0.035	4.700 ± 0.311	0.139 ± 0.042	1.636 ± 0.190

All results are average of 3 rats. Localization of each fraction to tissues was estimated at 1 hour after injection.

^a Values are percent dose in 1 ml of blood.

^b IgG₁ fraction.

fraction, 500 μ gN of which were applied into a well, showed an only precipitin band in the γ 1 region with rabbit anti-guinea pig immunoglobulins in the immuno-electrophoresis. When the preparation was subjected to the immuno-electrophoresis with soluble antigen of trypsin digested kidney sediments, a precipitin band having a distinctly fast electrophoretic mobility developed. These results imply that the IgG₁ fraction contained mainly IgG₁ and the contamination of IgG₂ is negligible.

Table 2. Two-dimensional complement fixation tests with rat kidney sediments and nephrotoxic IgG₁ and IgG₂ antibodies

IgG ₁ antibody system							
IgG ₁ (μ gN)	Rat kidney sediments (μ gN)						
	100	50	25	12.5	6.25	3.13	0
10	2 ^a	3	4	4	4	4	4
5	2	4	4	4	4	4	4
2.5	2	4	4	4	4	4	4
1.25	2	4	4	4	4	4	4
0.63	2	4	4	4	4	4	4
0	2	4	4	4	4	4	4

IgG ₂ antibody system							
IgG ₂ (μ gN)	Rat kidney sediments (μ gN)						
	100	50	25	12.5	6.25	3.13	0
10	0	0	1	1	2	2	4
5	0	1	2	3	4	4	4
2.5	1	2	3	3	4	4	4
1.25	1	3	3	4	4	4	4
0.63	2	3	4	4	4	4	4
0	2	4	4	4	4	4	4

Dotted line represents zone of complement fixation.

0 means no lysis, 4 means complete lysis.

^a Anti-complementary effect.

Quantitation of Kidney Fixing Antibodies. The IgG₁ fraction was tested for its kidney fixing activity by the method described above. As shown in Table 1, 0.91 per cent of nephrotoxic IgG₁ fraction was fixed specifically to the kidney during 1 hr after the intravenous injection. On the other hand, 1.6 per cent of nephrotoxic IgG₂ was fixed to the kidney. It is worthy of note that 1.9 per cent of nephrotoxic IgG₂ which was over the dose of kidney fixing IgG₂ was fixed to the lung, while only 0.4 per cent of nephrotoxic IgG₁ was.

Complement Fixation Tests. Table 2 shows the results of two-dimensional complement fixation tests. In IgG₁ antibody system, IgG₁ antibody did not fix rat complement at all, whereas IgG₂ did in IgG₂ system.

Induction of Glomerulonephritis by Nephrotoxic IgG₁ Antibody. Table 3 represents the results of urinalysis in the rats with nephrotoxic IgG₁ fraction. All rats given more than 1 mgN of nephrotoxic IgG₁ containing 9.1 μ gN or more of KFAbs developed an immediate proteinuria within 24 hr after the injection. Especially in rats injected with more than 2.7 mgN of nephrotoxic IgG₁, a massive proteinuria during the first 24 hr appeared. This proteinuria persisted for 2 to 3 months of the observation period. On the other hand, rats given less than 1.5 mgN of

Table 3. Induction of glomerulonephritis by nephrotoxic guinea pig IgG₁ or IgG₂ antibody

Rat No ^a	Nephrotoxic immunoglobulins	Total doses injected (mgN)	Amounts of kidney fixing antibodies (μgN)	Days after injection	Proteinuria (mg/day)	Immunofluorescent studies			
						GPIgG ₁	GPIgG ₂	Ratβ1c	RatIgG
801	IgG ₁	13.3	121	1 14	140 256	3+ ^b 3+	— —	1+ 3+	— 3+
802	IgG ₁	4.0	36.4	1 died on 4	137 120	3+ not done	—	1+	—
851	IgG ₁	2.7	24.6	1 14	66 210	3+ 3+	— —	1+ 3+	— 3+
803	IgG ₁	1.0	9.1	1 14	11.5 5.0	3+ 3+	— —	1+ 3+	— 3+
813	IgG ₂	2.6	41.6	died at 20 min		not done			
814	IgG ₂	1.9	30.6	died at 30 min		not done			
811	IgG ₂	1.5	24.2	1 9	0.4 0.1	— —	3+ 3+	3+ 3+	— 3+
815	IgG ₂	1.1	17.7	1 14	0.1 0.6	— —	3+ 3+	3+ 3+	— 3+

^a These rats included in Table 3 are representative cases.

^b Grade (3+) to (—) represents the intensity of fluorescene in glomeruli.

nephrotoxic IgG₂ containing 24.2 μgN of KFABs survived but did not develop a significant proteinuria. Furthermore, if rats were given 30.6 μgN or more of KFABs of IgG₂, they all died of the acute pulmonary edema within 1 hr after the injection as seen in rats given large amounts of nephrotoxic rabbit IgG (Shigematsu and Kobayashi, 1972).

Immunohistochemical Studies. In biopsy specimens obtained between 6 to 24 hr after the injection of nephrotoxic IgG₁, IgG₁ was observed to be localized along GBM in a linear pattern (Fig. 3), whereas IgG₂ was not detected. Rat β1c globulin was seen only weakly and usually locally along the same site in a linear pattern (Fig. 4). In the blocking test, rat β1c was negative in glomeruli of these biopsy specimens. In the survived group of rats given nephrotoxic IgG₂, IgG₂ together with β1c was distinctly stained despite no proteinuria and little histologic changes.

Histological Findings. As early as 6 hr after the injection, the glomerular endothelial linings detached themselves broadly from the basement membrane (Fig. 5).

At the same time small numbers of migrant neutrophils and/or monocytes accumulated locally in the glomerular loops, particularly in their peripheral parts (Fig. 5, 8). Local fusions of epithelial foot processes were also seen. In the 24 hr biopsy specimen an accumulation of the materials with a staining property of fibrin was prominent in the glomeruli (Fig. 6). The fibrin mass often clung to the

Fig. 3. Fluorescent micrograph of a glomerulus from the biopsy specimen obtained 24 hr after the injection of NTIgG₁. Guinea pig IgG₁ is brilliantly seen along GBM in a linear pattern

Fig. 4. Fluorescent micrograph of a glomerulus from the same specimen as that of Fig. 3
Rat β 1c globulin is slightly seen along GBM

basement membrane and some capillary lumina were entirely occupied by these materials. In such areas the endothelial linings were usually absent or lifted up by the depositions of fibrinoid material (Fig. 9). Cell debris or osmiophilic granular materials were intermingled with the fibrin masses. The glomerular changes on the 2 to 3 day were similar to those found in the 24 hr-biopsy specimen, but, at this stage, infiltration of small numbers of monocytes or macrophages were noted in the glomeruli. Some glomerular loops were occluded by massive fibrin thrombi with an occasional partial necrosis of the glomerular tissues. Eleven to 14 days after the injection, the thrombotic changes of the glomeruli rather regressed, but the persistence of fibrinoid materials was still observed in the loops in varying degrees. The glomerular capillary wall showed local edematous thickening with the moderate increase of the mesangial matrix (Fig. 7).

Discussion

Present investigation demonstrated that glomerulonephritis characterized by massive formation of fibrin in the glomerular capillary lumen with minimum phagocytic reaction was induced by nephrotoxic guinea pig IgG₁ fraction.

It is reported that guinea pig IgG₂ antibody is capable of fixing complement and provoking passive Arthus reaction, whereas IgG₁ antibody is not (Bloch *et al.*, 1963). A question arises as to whether the glomerulonephritis induced by nephro-

Fig. 5. 6 hr after the injection. Focal accumulation of neutrophils in the peripheral capillary loops. Fibrin (*F*) is also observed in the capillary lumen. PAS and toluidine blue. 650 $\times$

Fig. 6. 24 hr after the injection. Massive intravascular fibrin formation is found in the peripheral part of the capillary loops. PAS and Toluidine blue. 650 $\times$

toxic IgG₁ fraction may be caused by IgG₂ antibody contaminating into IgG₁ fraction. However, the contamination of IgG₂ in IgG₁ preparation seemed to be little, if any: In the glomerulus of biopsy specimens of rats injected with nephrotoxic IgG₁ fraction, IgG₂ was not detected by fluorescent antibody methods. When a rat was injected with 1.0 mgN of nephrotoxic IgG₁ fraction containing 9.1 μ gN of KFABs, it developed an immediate proteinuria, while a rat injected with 1.5 mgN of nephrotoxic IgG₂ containing 24.2 μ gN of KFABs did not. These

Fig. 7. 14 days after the injection. Intraluminal fibrinoid material is still present (arrows). Foam cells (FC) are observed in some capillary loops. The subendothelial space is seen to be edematous (Ed). PAS and toluidine blue. 650 $\times$

findings imply that nephrotoxic IgG₁ antibody are responsible for this glomerulonephritis.

If this is so, then, the next question is posed as to whether the complement system had a share in the initiation of the glomerulonephritis by IgG₁ antibody. It is unclear whether guinea pig IgG₁ antibody can not fix rat complement, although it is incapable of fixing guinea pig complement in the classical sequence of reactions (Bloch *et al.*, 1963; Keller *et al.*, 1968). Recently, Osler *et al.* reported that preformed immune aggregates composed of IgG₁ antibodies and antigens did interact with not only guinea pig, but also rat C3 and later components, despite little utilization of C1, C4 and C2, although IgG₁ antibody failed to activate the complement system in the conventional assays in which the order of addition was antibody, C and antigen (Osler, *et al.*, 1969; Sandberg *et al.*, 1970). However, the significance of the alternate pathway of complement fixation in the immunologic tissue injury is still unclear. In this experiment, nephrotoxic IgG₁ antibody did not fix rat complement with rat kidney sediments, whereas IgG₂ did. Moreover, in the initial glomerular changes induced by nephrotoxic IgG₁, rat β 1c was only weakly seen along GBM, and an accumulation of only small numbers of neutrophils and monocytes was observed, while the marked intraluminal fibrin deposition was the most dramatic feature. These are apparently different from the prominent phagocytic changes in rat Masugi nephritis initiated by nephrotoxic rabbit IgG antibody with an ability of fixing complement (Shigematsu, 1970). On the basis of these results, it is probable that the characteristic changes induced by nephrotoxic IgG₁ antibody is caused by non-complement mediated pathway at least in the classical sense of complement activation.

As mentioned above, the paucity of cellular reactions is another characteristic feature of IgG₁ nephritis. A small number of neutrophils accumulated in the

Fig. 8. 6 hr after the injection. Note broad exfoliation of endothelial lining from the glomerular basement membrane (arrow). Fibrin formation (*F*) and migrant monocyte (*MO*) are seen in the capillary lumen. Partial detachment of epithelial cytoplasm from the meandering basement membrane is found at double arrows. Urinary space (*US*) Bowman's capsule (*BC*). 5200 $\times$

early stage of the nephritis disappeared almost completely at 24 hr after the injection. The number of mononuclear cells was also few which appeared instead of neutrophils in the glomerulus. The deposition of fibrin, on the other hand, was

Fig. 9. 24 hr after the injection. Massive fibrin formation (F) in the capillary lumen. Deposits (DP) of fibrinoid material are seen beneath the endothelial cytoplasm (EN). Mesangial cells (MS). Cell debris (DB). 9600 $\times$

most striking in the glomerular capillary lumen, often clinging along glomerular basement membrane. The formation of fibrin thrombi was found most evident in 2 to 3 day biopsy specimens and also seen to persist for 2 to 3 weeks after the injection. The fibrin deposition and/or thrombotic changes in the early phase of Masugi nephritis were particularly noticed by Vassalli *et al.* (1964) with respect

to its role in the development of glomerular changes. The acceleration of the blood coagulation might have been initiated by an early endothelial lesion caused by the nephrotoxic antibodies (Winemiller *et al.*, 1961) or local release of some blood coagulation activator as a consequence of the antigen-antibody interaction. The massive deposition of fibrin and the persistence of thrombotic changes in glomeruli observed in the present study may be partly due to the weakness of the cellular reaction of neutrophils and monocytes which have phagocytic-reparative effects in the primary phase of rat Masugi nephritis, as has been suggested by Shigematsu (1970).

On the other hand, nephrotoxic IgG₂ antibody which is capable of fixing complement had only a weak nephritogenicity at a comparable dose. Doses of over 30.6 µgN of KFABs caused death from pulmonary complications. Guinea pig IgG₂ antibody in the preparation used may be lethal at nephrotoxic doses. It serves to confirm this possibility that the dose of lung fixing antibody in the IgG₂ preparation is much more than that of kidney fixing antibody. Although the reason why the kidney fixed IgG₂ antibody did not initiate complement mediated glomerular injury at a comparable dose is unexplained, it is suggested that non-complement mediated pathway may be of crucial importance in the initiation of at least some glomerular tissue injury.

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References

- Bale, W. F., Spar, I. L.: In vivo localization of rat organ antibodies in ovaries, adrenals and other tissues. *J. Immunol.* **73**, 125-133 (1954)
- Benacerraf, B., Ovary, Z., Bloch, K. J., Franklin, E. C.: Properties of guinea pig 7S antibodies. I. Electrophoretic separation of two types of guinea pig 7S antibodies. *J. exp. Med.* **117**, 937-950 (1963)
- Bloch, K. J., Kourilsky, F. M., Ovary, Z., Benacerraf, B.: Properties of guinea pig 7S antibodies. III. Identification of antibodies involved in complement fixation and hemolysis. *J. exp. Med.* **117**, 965-981 (1963)
- Cochrane, C. G.: Immunologic tissue injury mediated by neutrophilic leucocytes. *Advanc. Immunol.* **9**, 97-162 (1969)
- Cochrane, C. G., Unanue, E. R., Dixon, F. J.: A role of polymorphonuclear leucocytes and complement in nephrotoxic nephritis. *J. exp. Med.* **122**, 99-116 (1965)
- Cole, L. R., Cromartie, W. J., Watson, D. W.: A specific soluble substance involved in nephrotoxic nephritis. *Proc. Soc. exp. Biol. (N.Y.)* **77**, 498-501 (1951)
- Day, E. D., Planinsek, J., Korngold, L., Pressman, D.: Tumor-localizing antibodies purified from antisera against Murphy rat lymphosarcoma. *J. nat. Cancer Inst.* **17** 517-532 (1956)
- Fujimoto, T., Okada, M., Kondo, Y., Tada, T.: The nature of masugi nephritis. *Histo- and Immunopathological studies. Acta path. jap.* **14**, 275-310 (1964)
- Hong, R., Palmer, J. L., Nisonoff, A.: Univalence of half-molecules of rabbit antibody. *J. Immunol.* **94**, 603-610 (1965)
- Imai, Y., Sue, A., Yamaguchi, A.: A removing method of the resin from epoxy-embedded section for light microscopy. *J. Electron Microscopy* **17**, 84-85 (1968)
- Kabat, E. A., Mayer, M. M.: *Experimental immunochemistry* (2nd ed), p. 133. Springfield: Thomas 1961
- Keller, H. U., Nussenzweig, V., Sorkin, E.: Studies on chemotaxis. VIII. The role of 7S γ 1 and γ 2 guinea pig antibodies for chemotaxis in granulocytes. *Immunochemistry* **5**, 293-295 (1968)

- Kourilsky, F. M., Bloch, K. J., Benacerraf, B., Ovary, Z.: Properties of guinea pig 7S antibodies. V. Inhibition by guinea pig γ 1 antibodies of passive immune lysis provoked by γ 2 antibodies. *J. exp. Med.* **118**, 699–709 (1963)
- Kunkel, H. G.: Zone electrophoresis. *Meth. biochem. Anal.* **1**, 140–170 (1954)
- Mardiney, M. R., Müller-Eberhard, H. J.: Mouse β 1c-globulin: Production of antiserum and characterization of the complement reaction. *J. Immunol.* **94**, 877–882 (1965)
- Osler, A. G., Oliveira, B., Shin, H. S., Sandberg, A. L.: The fixation of guinea pig complement by γ 1 and γ 2 immunoglobulins. *J. Immunol.* **102**, 269–271 (1969)
- Ovary, Z., Benacerraf, B., Bloch, K. J.: Properties of guinea pig 7S antibodies. II. Identification of antibodies involved in passive cutaneous and systemic anaphylaxis. *J. exp. Med.* **117**, 951–964 (1963)
- Rother, K., Rother, U., Vassalli, P., McCluskey, R. T.: Nephrotoxic serum nephritis in C'6-deficient rabbits. I. Study of the second phase of the disease. *J. Immunol.* **98**, 965–971 (1967)
- Sandberg, A. L., Osler, A. G., Shin, H. S., Oliveira, B.: The biologic activities of guinea pig antibodies. II. Modes of complement interaction with γ 1 and γ 2 immunoglobulins. *J. Immunol.* **104**, 329–334 (1970)
- Scheidegger, J. J.: Une micro-methode de l'immunoelectrophorese. *Int. Arch. Allergy* **7**, 103–110 (1955)
- Shigematsu, H.: Glomerular events during the initial phase of rat Masugi nephritis. *Virchows Arch. Abt. B* **5**, 187–200 (1970)
- Shigematsu, H., Kobayashi, Y.: Pulmonary involvements in the initial phase of rat Masugi nephritis. *Virchows Arch. Abt. B* **11**, 111–123 (1972)
- Unanue, E. R., Dixon, F. J.: Experimental glomerulonephritis. IV. Participation of complement in nephrotoxic nephritis. *J. exp. Med.* **119**, 965–982 (1964)
- Unanue, E. R., Dixon, F. J.: Experimental glomerulonephritis. V. Studies on the interaction of nephrotoxic antibodies in tissues of the rat. *J. exp. Med.* **121**, 697–714 (1965)
- Unanue, E. R., Mardiney, M. R., Dixon, F. J.: Nephrotoxic serum nephritis in complement intact and deficient mice. *J. Immunol.* **98**, 609–617 (1967)
- Winemiller, R., Steblay, R. W., Spargo, B.: Electron microscopy of acute antibasement membrane serum nephritis in rats. *Fed. Proc.* **20**, 408–408 (1961)
- Wood, B. T., Thompson, S. H., Goldstein, G.: Fluorescent antibody staining. III. Preparation of fluorescein isothiocyanate-labeled antibodies. *J. Immunol.* **95**, 225–229 (1965)
- Vassalli, P., McCluskey, R. T.: The pathogenic role of the coagulation process in rabbit Masugi nephritis. *Amer. J. Path.* **45**, 653–677 (1964)
- Yagi, Y., Maier, P., Pressman, D.: Two different anti-insulin antibodies in guinea pig antisera. *J. Immunol.* **89**, 442–451 (1962)

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