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CHEMOTHERAPY AND IMMUNITY IN RODENT MALARIA, I.  
DEPOSITS OF ANTIGEN AND AUTOLOGOUS IgM IN  
GLOMERULI IN MICE INFECTED WITH PLASMODIUM BERGHEI (NK65)  
FOLLOWING TREATMENT BY AN ANTIMALARIAL

INDEXED

Preliminary Report

by

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1. INTRODUCTION

Shaper et al. (1968) reported an immunological syndrome consisting of high malarial antibody and the presence of high levels of IgM and circulating autoantibodies to heart and other tissues in the peoples of Uganda. Ward & Kibukamusoke (1969) carried out immunofluorescent studies on renal tissues from needle-biopsy specimens on East African patients: their findings supported that the glomerular disease associated with quartan malaria is, at least in some cases, an immunological disorder characterized by deposits of soluble immune complexes. Their results were further confirmed by Allison, Houba et al. (1969). Simple experimental methods to produce such disorders in laboratory animals are desirable to clarify immunopathological aspects associated with malaria.

The present study concerns an experimental model to cause immune complex deposition in glomeruli in mice infected with Plasmodium berghei (NK65).

2. MATERIALS AND METHODS

(1) Strain of plasmodium. The NK65 strain of P. berghei (Yoeli, 1965) was used in the present work. The strain was generously supplied by Professor Meir Yoeli in 1969 and was kept by blood transfers and regular freezing at -70°C. The parasite maintained its vigorous nature and caused sporogonic development in Anopheles stephensi at the time when the present work was done.

(2) Animals. Five weeks old DDY female white mice (Shizuoka Farm, Japan) were used throughout the experiment. Female Wistar rats weighing about 50 g were prepared by the same farm to obtain hyperimmune sera (Diggs & Osler, 1969).

(3) Parasite inoculation. Infected blood for inoculation was harvested from mice infected 4 days previously by cardiac puncture in heparinized syringes. The pooled infected red blood cells were estimated from examination of Giemsa-stained blood film and the total red blood cell counting. Ten million of parasitized red blood cells were given by the intra-peritoneal route.

(4) Antimalarial, sulfamonomethoxine: DJ-1550. Previous experiments (Yoshinaga et al. 1970) revealed that the sulfamonomethoxine was one of the most potent antimalarial

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in P. berghei - white mouse system. ( $0.01 \leq SD_{50} < 0.04$  mg/kg  $2.5 \leq ED_{50} < 10$  mg/kg).<sup>1</sup> The drug was obtained from Daiichi Seiyaku Co. Tokyo in a liquid form. Dilutions of the drug were done with sterilized distilled water adjusted at pH 10.5 with 1N NaOH.

(5) Cryostat sections. Animals were bled to death by cutting the brachial arteries. Liver, spleen and kidneys from one mouse were placed together on a filter paper, and frozen in  $-70^{\circ}\text{C}$  n-Hexane. They were cut by a cryostat at 4-6  $\mu$  thickness, kept at  $4^{\circ}\text{C}$  after fixation by acetone and were examined within 48 hours.

(6) Detection of the plasmodial antigen. Rats were employed for the preparation of anti-plasmodial immune sera by the method of Diggs & Osler (1969). The harvested hyperimmune sera were pooled and absorbed with acetone-dried mice tissue powder at first at  $37^{\circ}\text{C}$  for 30 minutes and then at  $0^{\circ}\text{C}$  for the same period. The resultant serum and a fluorescent antibody against rat IgG (kindly supplied by Dr F. Shimizu - see Shimizu, 1970) were used in the usual way for indirect immunofluorescent technique.

(7) Demonstration of immunoglobulins in tissue. Fluorescent antibodies against mouse IgM and IgG as described in Nariuchi et al. (1971) were prepared by Dr H. Nariuchi. The staining titres of both anti-globulin conjugates were examined using highly immunized anti-plasmodial mouse serum and highly parasitized blood smears from a mouse infected 3 days before.

### 3. RESULTS

Doses at the rate of 40 and 10 mg/kg of sulfamonomethoxine were administered to groups of mice by subcutaneous route. Ten untreated control mice were used to monitor the virulence of the parasite used. All the animals in this control group died on the average on the 8th day. Drug treatments were started on the 3rd day (D3) after the parasite inoculation (D0) and were continued for 4 days successively (D3, D4, D5, D6). The treated mice showed no parasitaemia on the D7. Two mice from each group were sacrificed on the 3rd, 5th, 7th, 10th, 13th and 16th day respectively. Five mice in each group kept for the estimation of drug effectiveness, were sacrificed on the 60th day (D60).

(1) Malarial antigen in the glomeruli. Plasmodial antigen in the glomeruli was demonstrated in 7 mice out of 15 which were examined within 16 days (D16) after parasite inoculation. Nine mice sacrificed on the 60th day exhibited no antigen deposit in the glomeruli. Those infected mice without drug administration thus far examined also showed no antigen deposits in the glomeruli on the 3rd (D3) day and 6th day (D6) except for mouse No. 61 (D3). Control mice showed no significant fluorescence in glomeruli except for slight staining in mesangium in 2 cases (Table 1).

(2) Deposits of autologous immunoglobulins in glomeruli. Deposits of autologous IgM in glomeruli were demonstrated in all mice sacrificed within 16 days (early period). Significant deposits were detected in the glomeruli in 4 mice out of 9 which were kept for 60 days (late period). In the glomeruli from mice in early period, generalized diffuse or disseminated deposits of autologous IgM were found. In one case, which was sacrificed on the 10th day, slight beaded deposits along the peri-tubular capillaries were seen (Fig. 1-A). The glomeruli from mice examined in the late period were marked by focal, disseminated nodular or granular deposits (Fig. 1-C) or local dense continuous deposits along the peripheral capillary walls (Fig. 1-D). In both cases, the typical linear pattern was not observed. Slight deposits in the mesangium sometimes mixed with scattered granules were often found in controls (see footnotes to Table 1).

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<sup>1</sup>  $SD_{50}$  denotes drug dose to cause 50% suppression of parasitaemia in mice on the 5th day after giving  $1 \times 10^7$  parasitized red blood cells followed by 4 successive days drug administration.  $ED_{50}$  indicates the dose to give 50% survival of the same mouse after 60 days observation.

IgG was not found in the glomerular deposits in any of the mice as long as this experiment was followed.

Parasites in the organs. Spleens and livers were examined for surviving parasites using the indirect fluorescent technique. Many parasites were detected in the organs from untreated mice, from mice sacrificed on the 5th day (D5) and from one mouse that showed recrudescence on the 16th day (D16) (No. 54). In all the treated mice, parasites were detected neither in the organs nor in the peripheral blood specimens (Table 1). Examinations of brain, lungs, heart, thymus, spleen, pancreas, liver, kidneys, lymphnodes, the large intestine, bone marrow and adipose tissue in the same experimental system, demonstrated that no parasites could be detected by indirect fluorescent technique except where there was a recrudescence.<sup>1</sup>

#### 4. DISCUSSION

The methods whereby soluble immune complexes can induce immunopathological renal lesion have been well documented (Unanue & Dixon, 1967). In mice infected with P. berghei, malarial antigen appears in the serum during the stage of acute parasitaemia (Cox et al., 1968). Such antigen, naturally may be able to combine with the antibody to provide circulating soluble immune complex in the infected mice. Sudden increase of the soluble antigen caused by rapid disintegration of the proliferated parasites following the administration of an antimalarial may be a trigger for the immune complex to deposit in the glomerular capillaries. Indeed, the patterns of the renal deposits of autologous IgM shown in this experiment seems to be typical for the immune complex disorders. Attempts to demonstrate the plasmodial antigen in the deposit were successful. In the same renal specimen, generalized deposits of autologous IgM were seen to occur diffusely in the glomeruli (Fig. 1-A,E). In the late period, significant disseminated or local deposits of autologous IgM were observed. The absence of the antigen in this case resembles the similar event which occurs in the third phase of serum sickness referred to as the immune phase of the antigen elimination. Thus, the results presented in this report suggest that the renal disorder was caused by soluble malarial antigen-antibody complex. If so, the experimental system would be an acceptable model for the investigation of immune complex diseases not to mention for the research in immune renal disorders in malaria infection.

Deposits of autologous IgG were not observed in the glomeruli during the course of this experiment. Using the same experimental system, more recent work has shown that IgG type antibody to the Plasmodium could be demonstrated in the peripheral blood for more than 1 month by indirect fluorescent technique (Waki, S. & Suzuki, M., in preparation). This finding seems to imply that IgG has less avidity to fix in the tissues than the IgM.

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<sup>1</sup> Suzuki, M. (1971) Recrudescence in mice infected with P. berghei (NK65) following the treatment by a long-acting sulfonamide: Sulfamonomethoxine, presented in Seminar on the Epidemiology, Prevention and Control of the Endemic Diseases in South-East Asia and the Far East. (Held in Tokyo and Osaka).

## SUMMARY

Immunofluorescent studies were carried out to detect immune complex deposits in glomeruli in mice infected with Plasmodium berghei (NK65). Following rapid disintegration of the proliferated parasites with an antimalarial, remarkable IgM deposits in the glomeruli were seen. The patterns were characterized by irregular nodular, granular and beaded deposits, and the typical linear pattern was not observed. The deposits were generalized, diffuse or disseminated in mice sacrificed soon after the completion of 4 days drug treatment. In the glomeruli in some of the mice, malarial antigen was detected. The IgM deposits were clearly demonstrated in mice which were examined on the 60th day, although antigen was no longer detected. IgG was never found in the glomerular deposits in any of the mice throughout the course of the experiment.

## RESUME

Des études ont été faites pour détecter par immunofluorescence l'existence de dépôts d'immuno-complexes dans les glomérules de souris infectées avec Plasmodium berghei (NK65). Après destruction rapide au moyen d'un antipaludique des parasites qui avaient proliféré, on a observé un dépôt notable d'IgM. Les dépôts sont irréguliers, nodulaires, granulaires et à billes; on n'a pas observé le type linéaire. Les dépôts étaient particulièrement remarquables dans les souris sacrifiées immédiatement après le traitement médical de 4 jours. L'antigène palustre a été détecté dans les glomérules de certaines souris. Les dépôts d'IgM ont été clairement démontrés dans les souris examinées après 60 jours, mais l'antigène n'a pas été détecté. Les dépôts des glomérules d'aucune souris examinée n'ont jamais révélé la présence d'IgG.

REFERENCES

- Allison, A. C. et al. (1969), Lancet 21 June, 1232
- Cox, H. W., Milar, R. & Patterson, S. (1968) Amer. J. trop. Med. Hyg., 17, 13
- Diggs, C. L. & Osler, A. G. (1969) J. Immunol., 102, 298
- McCluskey, R. T. & Vassalli, P. (1969) Experimental glomerular diseases, In: The Kidney, vol. 2, Ed. Rouiller, C. & Muller, A. F., Acad. Press, New York and London, P. 83
- Nariuchi, H. et al. (1971) Int. Arch. Allergy, 40, 590
- Shaper, A. G. et al. (1968) The Lancet, i, 1342
- Shimizu, F. (1970) Japan J. Exp. Med., 40, 227
- Unanue, E. R. & Dixon, F. J. (1967) Experimental Glomerulonephritis; Immunological Events and Pathogenic Mechanisms, In: Advances in Immunology, vol. 6, Ed. Dixon, F. R., jr & Humphrey, J. H., Acad. Press, New York and London, p. 1
- Ward, P. A. & Kibukamusoke, J. W. (1969) The Lancet, i, 283
- Yoeli, M. (1965) Trans. roy. Soc. trop. Med. Hyg., 59, 255
- Yoshinaga, T. et al. (1970) Arzneim. Forsch., 20 (9), 1206

TABLE 1. ANTIGEN AND IMMUNOGLOBULIN DEPOSITS IN GLOMERULI

Animal number	Doses mg/kg <sup>a</sup>	Parasitaemia			Glomerular		deposits IgG	Parasites in spleen and liver
		D3	Dx <sup>b</sup>	Dx <sup>b</sup>	Antigen	IgM		
32	10	12.8	D5	16.5	+	+++	-	++
34		7.8	D10	0 <sup>c</sup>	-	++	-	-
35		5.9	D10	0	++	+++	-	-
38		2.2	D13	0	-	++	-	-
39		1.5	D16	0	+	++	-	-
40		1.7	D16	0	++	+++	-	-
46	40	1.4	D5	1.2	-	+++	-	++
47		4.7	D5	6.0	-	++	-	++
50		9.6	D7	0	-	++	-	-
59		1.3	D7	0	+	+++	-	-
48		1.2	D10	0	+	+++	-	-
51		8.7	D10	0	+	++	-	-
52		2.6	D13	0	-	++	-	-
53		7.6	D13	0	-	+++	-	-
54		4.6	D16	0.02	+	++	-	+
41	10	0.4	D60	0	-	- <sup>d</sup>	-	-
42		0.2	D60	0	-	-	-	-
43		2.4	D60	0	-	++	-	-
44		3.9	D60	0	-	++	-	-
45		7.1	D60	0	-	-	-	-
56	40	2.0	D60	0	-	-	-	-
57		9.3	D60	0	-	+++	-	-
58		0.9	D60	0	-	-	-	-
60		3.1	D60	0	-	++	-	-
61	0	0.3	D3		+	-	-	+++
62		5.8	D3		-	-	-	+++
66		7.4	D6	34.9	-	-	-	+++
C1 <sup>e</sup>	10	0	D7	0	-	-	-	-
C2	10	0	D7	0	-	-	-	-
C3	40	0	D7	0	-	-	-	-
C4	40	0	D7	0	-	-	-	-
C5	10	0	D14	0	-	-	-	-
C6	10	0	D14	0	-	-	-	-
C7	40	0	D14	0	-	-	-	-
C8	40	0	D14	0	-	-	-	-
C9	0	0		0	-	-	-	-
C10	0	0		0	-	-	-	-
C11	0	0		0	-*	-	-	-
C12	0	0		0	-*	-	-	-

<sup>a</sup> Timing of the drug administration was scheduled on D3, D4, D5, D6

<sup>b</sup> Dx denotes the day when the animals were sacrificed

<sup>c</sup> 0 parasitized red blood cells/10 000 counted cells

<sup>d</sup> There were insignificant mesangial deposits in which scattered granules were sometimes seen

<sup>e</sup> Mice in control groups (C1-C8) were given healthy red blood cells on D0

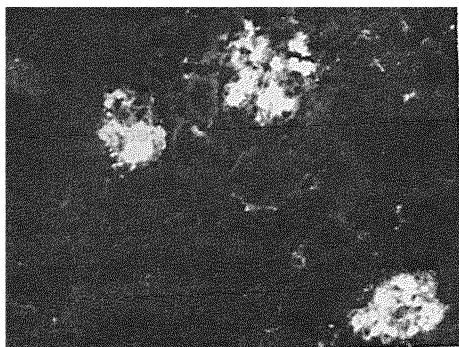
\* C11 and C12 mice showed mesangial deposits in the glomeruli

Explanation for Fig. 1

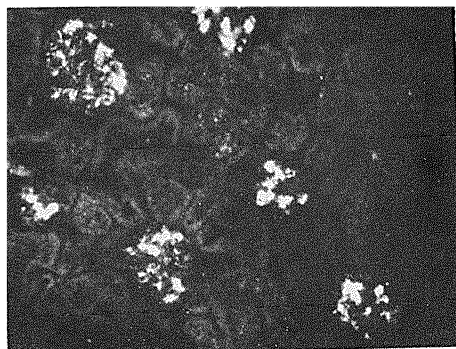
- A. Fluorescence micrograph of the glomeruli from mouse No. 35 (D10). Generalized diffuse deposits of autologous IgM can be observed in three glomeruli. Very faint granular deposits on peritubular capillaries are seen. (X100)
- B. Fluorescence micrograph of glomeruli from mouse No. 48 (D10). Note the generalized, disseminated nodular pattern of IgM deposits. (X100)
- C. Fluorescence micrograph of glomeruli from mouse No. 57 (D60). Focal disseminated nodular form of autologous IgM distributions are observed. (X200)
- D. Fluorescence micrograph of glomeruli from mouse No. 44 (D60). Focal and local dense, continuous IgM deposits along the capillary walls are shown. (X400)
- E. Fluorescence micrograph of a glomeruli from mouse No. 35 (D10) with deposits of plasmodial antigen. (X200)
- F. A glomerulus from mouse C9 (control, untreated) with very faint deposit of autologous IgM. (X200)

FIG. 1.

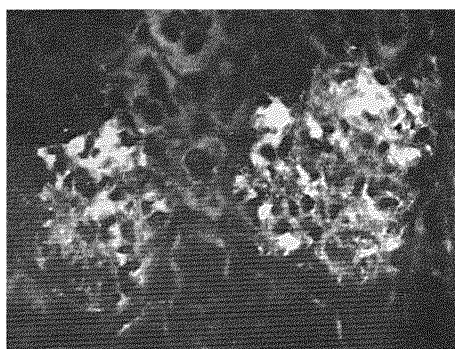
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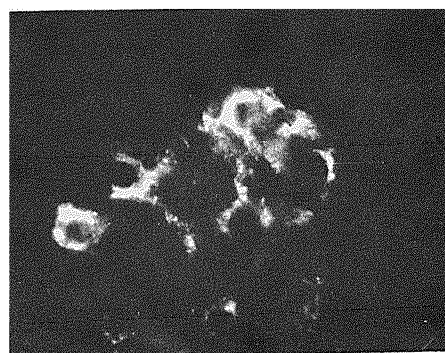
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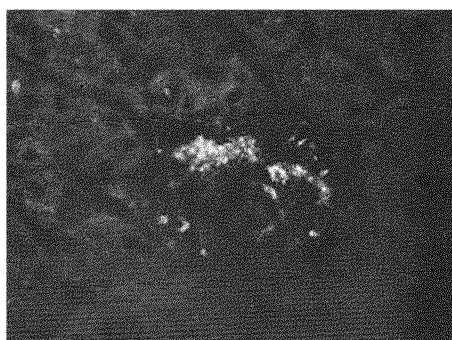
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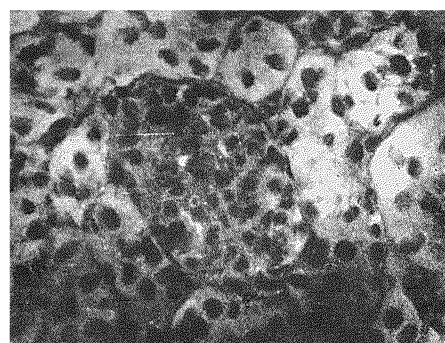
D



E



F



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