Clin. exp. Immunol. (1970) **7**, 793–803.

SUPPRESSION OF AUTOIMMUNE DISEASE IN NEW ZEALAND MICE ASSOCIATED WITH INFECTION WITH MALARIA

I. (NZB × NZW) F_1 HYBRID MICE

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(*Received* 4 *May* 1970)

SUMMARY

Female (NZB × NZW) F_1 hybrid (B/W) mice were infected with *Plasmodium berghei* at the age of 1 month. In contrast to the high mortality from renal disease observed in control female mice of this strain, all of the malaria-infected female mice were alive at 12 months of age and none had any clinical features of severe renal disease. This finding gives some support to the hypothesis that a background of repeated parasitic infection may modify the development of autoimmune disease in man.

INTRODUCTION

Autoimmune diseases, including systemic lupus erythematosus, are uncommon in Western Nigeria and probably in some other parts of tropical Africa (Greenwood, 1968). Nevertheless autoimmune disease is seen frequently among American Negroes and it has been (reported Siegel *et al.*, 1962) that in New York systemic lupus erythematosus occurs more frequently among American Negroes, many of whom are of West African origin, than among white Americans. These observations suggest that environmental factors may play some part in protecting the indigenous population of parts of tropical Africa from autoimmune disease. It has been shown that a background of repeated parasitic infection, particularly with malaria, induces a number of immunological changes and it has, therefore, been suggested (Greenwood, 1968) that repeated parasitic infection might be one of these environmental factors.

In order to investigate this hypothesis we have studied the effect of infection with the rodent malaria parasite *P. berghei* on the spontaneous autoimmune disease of NZB and (NZB × NZW) F_1 hybrid mice. This paper describes the effect of malaria infection on the autoimmune disease of (NZB × NZW) F_1 hybrid mice. Our preliminary findings have been briefly recorded elsewhere (Greenwood, Herrick & Voller, 1970).

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MATERIALS AND METHODS

Mice

(NZB × NZW) F_1 hybrid mice (B/W mice) from the Taplow Colony were studied. Only female animals were used in test and control groups as the development of renal disease is more predictable in female than in male mice of this strain. All the results in this paper refer to those obtained in female mice. Control data have been obtained from mice studied at Taplow over a period of several years. However, a small group of four control animals was studied in parallel with the malaria-infected mice. The course of their illness closely followed the pattern previously observed in larger control groups.

Malaria infection

P. berghei yoelii (Landau & Killick-Kendrick, 1966) was used in our experiments. This strain of rodent malaria was not contaminated with *Eperythrozoon coccoides*. Infection was transmitted by intraperitoneal injection of parasitized red cells obtained from an infected mouse of the same strain. Parasitaemia was monitored by regular examination of tail blood films. The degree of parasitaemia was recorded as the number of parasites present per 10^4 red blood cells.

Malaria antibody levels were assayed by a fluorescent method (Voller, 1964) using mouse blood films heavily infected with *P. berghei yoelii* as the antigen. A rabbit anti-mouse globulin conjugate was employed (Nordic Pharmaceuticals Ltd, Tilburg).

Haematocrits

The packed cell volume (PCV) was determined at monthly intervals in malaria-infected mice. Control values were obtained for uninfected mice in different age groups.

Antinuclear factor

Sera from malaria-infected and control mice were examined for the presence of anti-nuclear factor (ANF) by an indirect immunofluorescent method. Cryostat sections of rat liver were treated with test serum diluted 1:10, washed and then treated with a rabbit anti-mouse globulin conjugate at a dilution of 1:10.

Renal disease

Clinical Features. Mice were examined every few days for the presence of ascites and weighed at monthly intervals. Mice with clinical features of severe renal disease were killed when they appeared to be moribund.

Proteinuria. 24-hr urine collections were obtained in metabolism cages at monthly intervals and the protein concentration of an aliquot determined by the method of Kingsbury *et al.* (1926) using bovine serum albumin as a standard. 24 hr urine protein excretions were then calculated.

Renal pathology. Kidneys from sacrificed mice were examined by light microscopy, electron microscopy and fluorescent microscopy. Formalin fixed sections were stained with haematoxylin and eosin and with periodic acid Schiff (PAS). Electron micrographs were prepared from 1 mm cubes of kidney fixed in cold 3% glutaraldehyde and subsequently

treated as described by Denman, Russell & Denman (1969). Fluorescent microscopy was performed on cryostat cut sections from snap frozen kidneys. These were stained with an anti-mouse globulin conjugate, a rabbit anti-mouse IgG conjugate and a conjugated antiserum to mouse β_1 C prepared by the method of Mardiney & Müller-Eberhard (1965) and repeatedly absorbed with mouse IgG globulin prepared by DEAE chromatography.

RESULTS

Malaria infection

Thirteen female B/W mice were infected when 4 weeks old with *P. berghei yoelii* by intra-peritonal injection of 1×10^6 parasitized red cells obtained from a heavily infected mouse of the same strain. All thirteen mice developed a malaria parasitaemia which lasted for a mean of 12·6 days (range 12–14 days). Heavy infections occurred with a mean peak parasitaemia of 1920 parasites/10^4 red blood cells (range 718–5350 parasites/10^4 red blood cells). Although no parasites were seen in peripheral blood films taken 3 weeks or more after infection, it was thought to be important to determine as far as possible whether a low-grade malaria parasitaemia was persisting. Blood samples were, therefore, taken 3 months after infection, pooled and injected into young BALB/c mice. No parasitaemia developed in the recipient mice.

TABLE 1. Malaria antibody levels in B/W mice infected with *P. berghei yoelii* when 1 month old.

Age (months)	No. tested	Reciprocal of antibody titre			
		<50	50	100	200 or >
3	6	0	0	0	6
6	9	0	1	5	3
12	9	0	2	4	3

Fluorescent malaria antibody levels were determined in nine of the malaria infected mice, 3, 6 and 9 months after malaria infection. Although a fall in titre had occurred, malaria antibodies were still detectable many months after infection (Table 1).

The spleens of five mice sacrificed 11 months after infection contained large amounts of pigment, some of which was probably malarial pigment.

Haematocrits

The PCV level of the malaria-infected mice fell sharply during the period of parasitaemia but 2 months after infection the haematocrit had returned to a normal value (mean 50%). A slight fall in mean PCV occurred with increasing age but at 12 months the mean PCV was still 47%. In contrast, many control B/W mice became anaemic at the time they developed features of renal disease. At 8 months, the mean PCV of the malaria infected mice (48%) was significantly higher than the mean PCV of ten control mice of the same age (40%) ($t = 4{\cdot}2$; $P = <0{\cdot}001$).

Serum antinuclear factor

Control female B/W mice developed homogeneous ANF at a titre of 1:10 from the age of 5 months onwards and at 7–8 months sera from eleven out of thirteen mice (85%) were positive. In contrast, homogeneous ANF was not found in the sera of any of the malaria-infected mice on monthly testing until the age of 12 months, when 3-weakly positive tests were recorded for the first time. Sera from three of the malaria-infected mice gave weak speckled staining of live rat nuclei at irregular intervals following malaria infection.

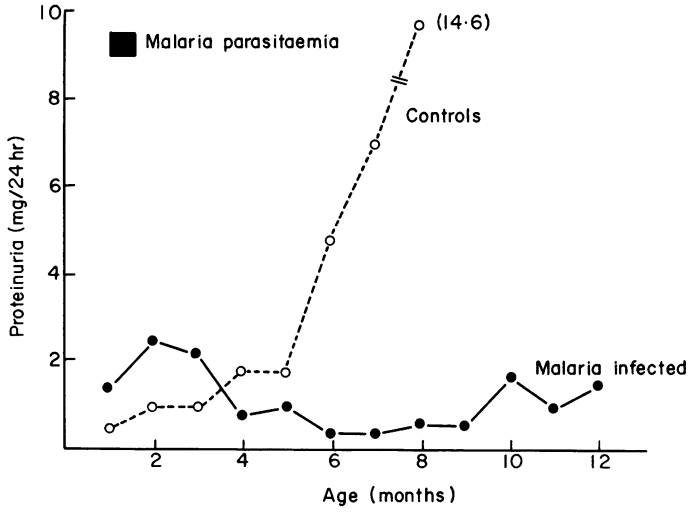

FIG. 1. Mean 24-hr urine protein excretion in thirteen malaria-infected female B/W mice and in six to twelve control B/W mice in different age groups. ●, malaria-infected; ○, controls.

Early renal disease

Malaria-infected B/W mice were studied for features of a complex nephritis precipitated by the malaria infection. Serial estimations of 24-hr urine protein excretion showed a slight increase in mean 24-hr protein excretion following malaria infection (Fig. 1.) The mean 24-hr urine protein excretion (2·6 mg/24 hr) 3 weeks after malaria infection was significantly higher than the mean 24-hr urine protein excretion (1·0 mg/24 hr) of ten control B/W mice of the same age ($t = 3{\cdot}1$; $P<0{\cdot}001$) and higher than the mean 24-hr urine protein excretion (1·7 mg/24 hr) prior to infection, although this latter difference is not statistically significant.

Two malaria-infected B/W mice were killed 1 month after malaria infection and their kidneys compared with those obtained from six control female B/W mice of the same age. On light microscopy a few small aggregates of round cells were seen in kidney sections from one of the malaria infected mice but similar small aggregates were also seen in the kidneys of two of the six controls. Immunofluorescent staining showed small, localized deposits of γ-globulin within the mesangium of the kidneys of the malaria-infected mice, but similar small deposits were also seen in control mice of the same age. Staining of the basement membrane was not seen. Electron micrographs of the kidneys of the two malaria-infected

mice showed some fusion of the basement membrane foot-processes and a few areas of homogeneous expansion of the basement membrane but no electron dense deposits were seen.

Terminal renal disease

Proteinuria. Control B/W mice developed severe proteinuria from the age of 6 months onwards (Fig. 1) and by the age of 8 months all of forty female controls had a urinary protein excretion of greater than 10 mg/24 hr (Fig. 2). In contrast the malaria-infected B/W mice showed only a slight increase in mean 24-hr protein excretion as they aged (Fig. 1) and at 12 months none had a protein excretion of greater than 5 mg/24 hr.

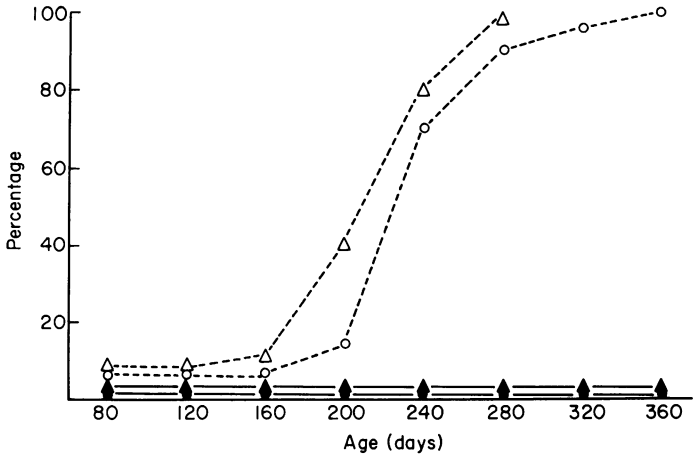

FIG. 2. Cumulative incidence of proteinuria greater than 10 mg/24 hr and cumulative mortality of thirteen malaria-infected B/W mice and of B/W controls. ▲, proteinuria in malaria-infected mice; ●, cumulative mortality in malaria-infected mice; △, proteinuria in controls, n = 40; ○, cumulative mortality in controls, *n* = 101.

Mortality. The onset of proteinuria in control mice was usually followed by the development of oedema and ascites, reflected by a marked increase in body weight. The cumulative mortality of control female B/W mice, nearly all of whom died from renal disease, is indicated in Fig. 2. By 12 months nearly all of 101 control mice were dead. In contrast all eleven malaria-infected mice were alive at 12 months and none had any clinical features of renal disease. The difference in mortality rates for malaria-infected and control mice is highly significant ($\chi^2 = 121$; $P<0{\cdot}001$). Five of the eleven malaria infected mice were killed at 1 year. The six survivors were still alive and well at the age of 17 months.

Terminal renal pathology. Kidneys from control mice dying from renal disease showed thickening of the basement membrane, deposits within the mesangium and infiltration with round cells as previously described (Howie & Helyer, 1968). Kidney sections from five malaria-infected mice killed at 1 year all showed some basement membrane thickening, some accumulation of PAS positive material within the mesangium, and occasional small infiltrates of round cells. The extent of these changes was, however, much less marked than in the kidneys of control mice and only one of the five kidneys contained sclerotic glomeruli.

The extent of the lesions varied considerably from glomerulus to glomerulus and some had a normal structure.

On fluorescent microscopy heavy deposits of immunoglobulin and complement were demonstrated in the mesangium and basement membrane of the glomeruli of control mice

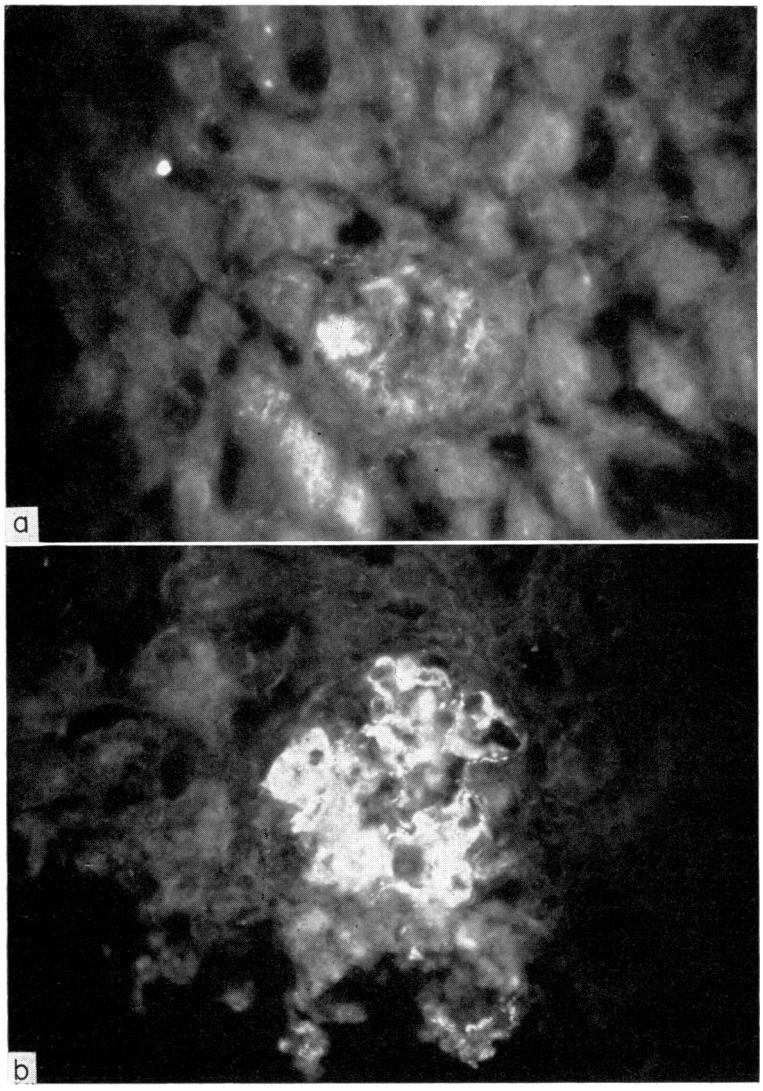

FIG. 3. Renal glomeruli stained by immunofluorescence for mouse IgG from (a) malaria-infected B/W mouse aged 12 months, (b) control B/W mouse with renal disease killed when 7 months old.

as previously recorded (Howie & Helyer, 1968). The glomeruli of the five malaria-infected mice killed at 1 year also contained some immunoglobulin and complement but the degree of deposition was not as marked as in the controls, and the deposits were largely restricted

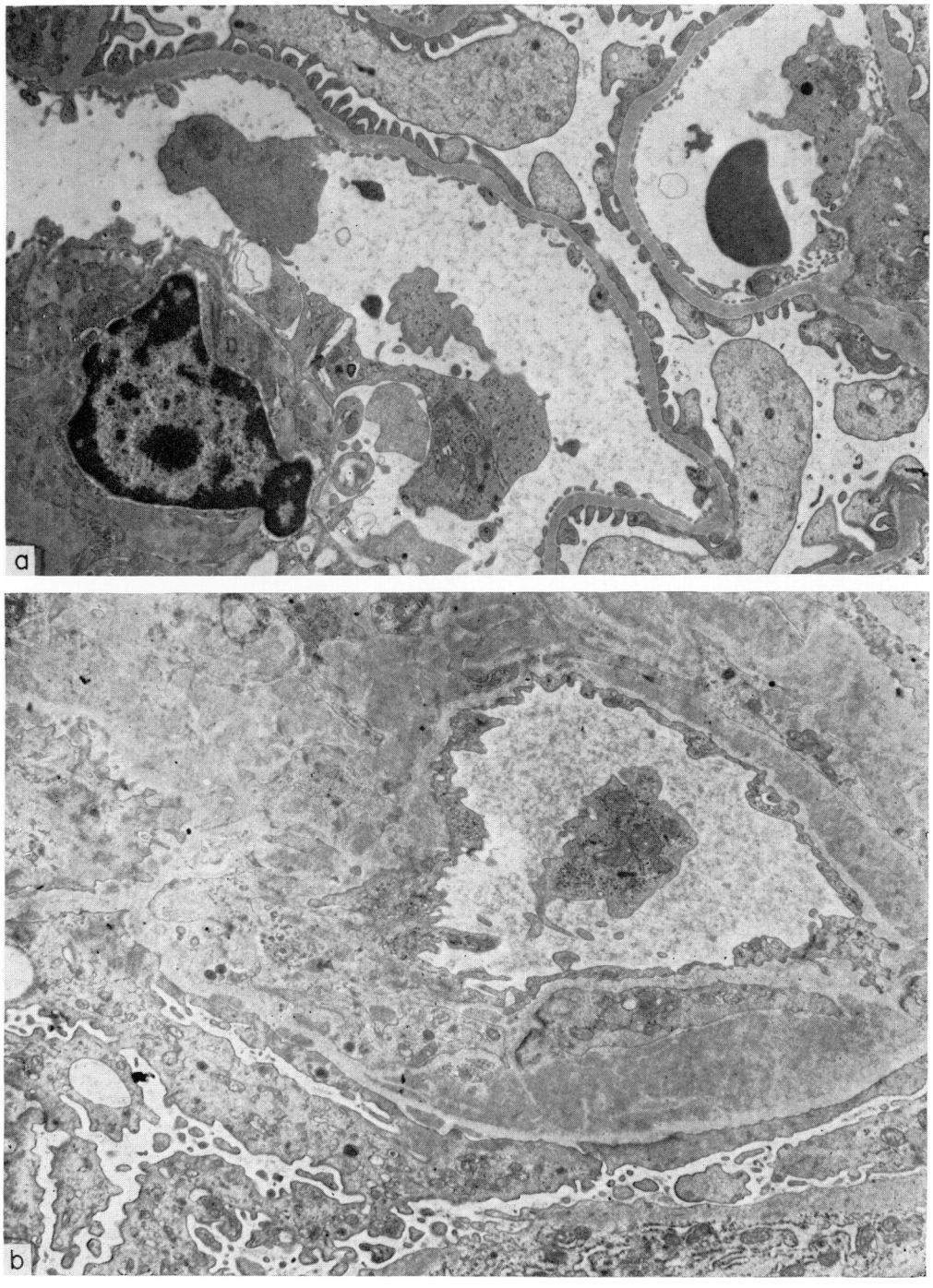

FIG. 4. Electron micrographs of renal glomeruli from (a) malaria-infected B/W mouse aged 12 months and (b) control B/W mouse with renal disease killed when 8 months old, ×6825.

to the mesangium. Considerable variation in the extent of the immunoglobulin and complement deposits was observed between glomeruli. A representative glomerulus is shown in Fig. 3.

Electron micrographs prepared from the kidneys of the five malaria-infected mice killed at 1 year showed patchy fusion of the foot processes and some areas of irregular thickening of the basement membrane but in many areas the glomerular basement membrane was normal (Fig. 4). The extensive electron dense deposits seen on the sub-epithelial surface of the basement membrane of control mice dying from renal disease were not observed. Virus particles were demonstrated in electron micrographs of the kidneys of two of the malaria-infected mice.

DISCUSSION

Infection of female B/W mice at the age of 1 month with the rodent malaria parasite *P. berghei* has been found to have a marked suppressive effect on the spontaneous development of autoimmune disease in mice of this strain. In contrast to the very high mortality in female control mice at 1 year, as noted in several other studies (Russell & Hicks, 1968; Lambert & Dixon, 1968) the malaria-infected mice were all alive and well at that age and none had heavy proteinuria. Kidneys from five malaria-infected mice killed at 1 year showed abnormalities on light and fluorescent microscopy of a similar nature to those seen in control mice but the changes were very much less severe and electron dense deposits were not seen in electron micrographs.

The way in which malaria infection exerted a protective effect on the development of autoimmune disease in B/W mice is uncertain but a number of possibilities can be considered. Any postulated mechanism of protection must take into account the fact that the period of detectable malaria parasitaemia was only short-lived. However, when mice were killed 11 months after infection, large amounts of pigment were still present in the spleen and it is possible that malaria antigen may have persisted in the reticuloendothelial system for a prolonged period.

Virus particles have been observed in the tissues of NZB and B/W mice (Prosser, 1968) and it has been suggested that virus infection plays a critical role in the development of their autoimmune disease (Mellors & Huang, 1967; Mellors, Aoki & Huebner, 1969). Malaria in rodents is associated with the production of interferon (Huang, Schultz & Gordon, 1968) and it is possible that a pathogenic virus could have been destroyed or severely inhibited during the period of malaria parasitaemia by this mechanism. The demonstration of virus particles in the kidneys of two of five malaria-infected mice killed at 1 year does not exclude this possibility as the mice may have carried more than one virus. However, it has been shown that the interferon inducers, Statolon (Russell, 1968) and polyinosinic-polycytidylic acid (Steinberg, Baron & Talal, 1969) do not suppress the autoimmune disease of New Zealand mice and indeed the latter was found to precipitate a premature onset of renal disease in B/W mice. The recent studies of Remington & Merigan (1960) with *Toxoplasma gondii* suggest that this intracellular parasite may be able to protect mice against virus infection by mechanisms other than interferon induction. It is possible that *P. berghei* may sometimes have a similar virus repressive effect.

Treatment of B/W mice with cyclophosphamide suppresses the development of renal disease (Russell & Hicks, 1968; Horowitz *et al.*, 1969) and anti-lymphocyte globulin has a

similar effect in mice previously rendered tolerant to rabbit γ-globulin (Denman, Russell & Denman, 1970). A diminished immune response to sheep erythrocytes has been recorded during malaria infection in mice (Salaman, Wedderburn & Bruce-Chwatt, 1969; Greenwood, Playfair & Torrigiani, 1970) but it seems probable that this period of immunosuppression is only short-lived. However Russell and Hicks (1968) have shown that cyclophosphamide treatment for only 4 weeks at an age of 80–250 days significantly reduces the incidence of fatal renal disease in B/W mice. It is thus possible that even a transitory period of immunosuppression is capable of breaking the complex cycle of events leading to the deposition of immune complexes in the kidneys of mice of this strain.

There is good evidence that the renal disease of B/W mice is due to deposition of DNA–anti-DNA complexes in the kidney (Lambert & Dixon, 1968; McGiven & Ironside, 1968). NZB and B/W mice rendered tolerant to bovine γ-globulin were found to lose tolerance more rapidly than C3H mice (Staples & Talal, 1969) and inability to maintain tolerance to DNA may be one of the factors responsible for the formation of DNA–anti-DNA complexes by B/W mice. Malaria parasites contain nucleoprotein (Turner & McGregor, 1969), large amounts of which are probably released into the circulation when the parasites are destroyed. It is possible that in young B/W mice the release of large amounts of nuclear material into the circulation during a period of immunosuppression enables them to maintain tolerance to nuclear antigens for a prolonged period.

It has been assumed in the preceding discussion that the protective effect of malaria infection on the autoimmune disease of B/W mice has been due to the malaria parasite itself. However, during a few serial passages in experimental animals, the parasite might have become contaminated with a virus and it is possible that the suppression of renal disease that we observed could have been due to a viral contaminant rather than the malaria parasite itself, for a number of viruses are known to have immunosuppressive properties (Salaman, 1970). This could be tested by studying the effect of strains of *P. berghei* obtained from other sources, by using sporozoite induced infections and by the use of *P. berghei* inocula in mice protected with chloroquine. It would be of interest to know whether deliberate infection with one of the known immunosuppressive viruses such as the small spleen virus (Salaman, 1970) would have a protective effect. It would also be of interest to establish whether the protective effect that we have observed is specific to infection with the malaria parasite or whether other protozoal infective agents would have a similar action.

The relevance of these experimental findings in a small group of animals to considerations of human autoimmune disease is uncertain. Although the closest animal model of human systemic lupus erythematosus the autoimmune disease of B/W mice differs from the human disease in a number of features. Similarly *P. berghei* infection differs in many respects from infection with human Plasmodia. However, our findings do support the view that parasitic infection may influence the course of autoimmune disease. It therefore seems possible that repeated parasitic infections, particularly with malaria, may contribute to the apparently low incidence of autoimmune disease in parts of tropical Africa as has been suggested (Greenwood, 1968). Malaria has in the past been used as a treatment for a variety of conditions, including the connective tissue diseases, and beneficial results from malaria therapy are still claimed (Corelli, 1968) although this form of treatment has widely fallen into disrepute. It is possible that elucidation of the mechanism by which malaria infection protects B/W mice from developing fatal autoimmune disease might provide information relevant to the treatment of human autoimmune disease.

ACKNOWLEDGMENTS

This study was supported by grants from the Wellcome Trust, The Medical Research Council and the World Health Organization.

We wish to thank Dr A. M. Denman for his continuous help and encouragement throughout this study and Dr E. M. Herrick and Mrs D. Green for their assistance. We also wish to thank Mr J. Dorling for the electron-micrographs, Mr P. Fiske for the photographs and Dr A. S. Russell for the gift of antiserum to mouse β_1C.

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