Clin. exp. Immunol. (1976) **26**, 133–136.

Macrophage clearance function and immune complex disease in New Zealand Black/White F_1 hybrid mice

A. G. MORGAN & M. W. STEWARD *Department of Immunology, Institute of Child Health, London and Laboratory of Immunochemistry, Division of Immunology, Kennedy Institute of Rheumatology, London*

(*Received* 5 *April* 1976)

SUMMARY

Macrophage clearance function in NZB, NZW, NZB/W, Ajax and B10D2 new mice was assessed by measurement of the rate of clearance (K_{PVP}) of intravenously-injected ^{125}I-labelled polyvinylpyrrolidone (PVP). There were significant strain and age-related variations in K_{PVP}. In particular there was a marked fall in K_{PVP} in NZB/W mice with increasing age. This fall was most apparent in female NZB/W and preceded the age at which renal disease usually develops in these animals. We suggest that ineffective macrophage function and production of low affinity antibody contribute to the early development of immune complex glomerulonephritis in these mice.

INTRODUCTION

New Zealand Black/White hybrid (NZB/W) mice have been extensively studied for the interesting range of immunological phenomena which they display and for the similarity between the disease which they spontaneously develop and the human disease systemic lupus erythematosus (SLE) (Helyer & Howie, 1963; Lambert & Dixon, 1968). In both species the aspect of the disease which causes death is a proliferative glomerulonephritis associated with immune complex deposition. Because of the possibility that genetically determined heterogeneity of antibody affinity (Soothill & Steward, 1971), associated with a defect of macrophage clearance of ^{125}I-labelled polyvinylpyrrolidone (PVP) (Morgan & Soothill, 1975a), might predispose to immune complex disease we have investigated these phenomena in NZB/W and other strains of mice. NZB/W mice make antibody of low relative affinity and also show an age-related fall in this qualitative aspect of the antibody response (Petty & Steward, 1972; Steward, Katz & West, 1975). Here we report measurements of macrophage clearance function in NZB/W mice, the NZB and NZW parental strains, and two strains previously shown to have low (B10D2 new) and high (Ajax) PVP clearance (Morgan & Soothill, 1975a).

MATERIALS AND METHODS

Mice. Male and virgin female mice of the two inbred strains (Ajax and B10D2 new) and three New Zealand strains (NZB, NZW, and their F_1 hybrid offspring NZB/W) were studied. Ajax mice were bred at the Institute of Child Health and the other mice at the Kennedy Institute of Rheumatology. Separate groups of four to twelve mice were studied at ages between 6 and 36 weeks.

Measurement of macrophage clearance function. The clearance of an intravenous dose of 25 μg ^{125}I-labelled polyvinylpyrrolidone (PVP; Radiochemical Centre, Amersham) was measured by the method of Morgan & Soothill (1975b). Results have been expressed as the exponential rate constant K_{PVP} (hr^{-1}); the clearance half-time is of the order of 24 hr.

RESULTS

In both male and female Ajax mice, K_{PVP} tended to decrease with age (Fig. 1) although the fall in females

Present address and correspondence: Dr A. G. Morgan, Medical Unit, The London Hospital, London E1 1BB.

was only small and in males K_{PVP} rose again after 20 weeks. The difference between the sexes at 6 weeks is not statistically significant, but at 14 and 20 weeks males have significantly lower values than females ($P<0{\cdot}02$, Mann Whitney U test) and at 36 weeks significantly higher values ($P<0{\cdot}02$). At all ages K_{PVP} was much lower in B10D2 new mice than in Ajax mice. Progressive decrease of K_{PVP} with age was also more definite, but there was no significant sex difference.

Although young NZB/W mice of both sexes had K_{PVP} values very similar to those of young Ajax mice, they showed by contrast a marked and progressive decrease of K_{PVP} with age which occurred earlier in

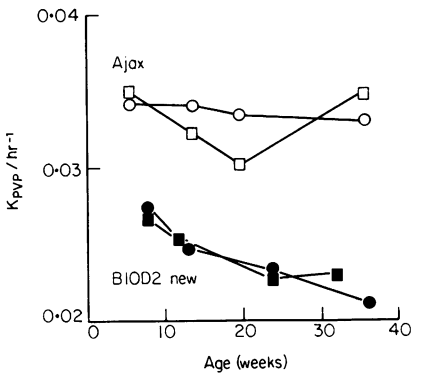

FIG. 1

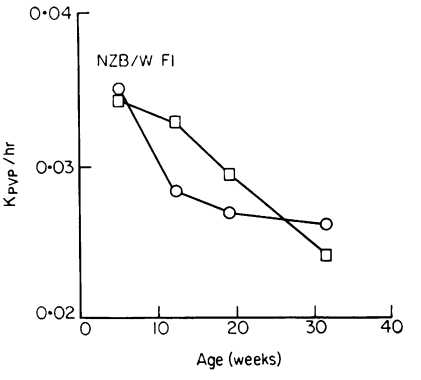

FIG. 2

FIG. 1. PVP clearance (K_{PVP}) by male (□) and female (○) Ajax mice, and male (■) and female (●) B10D2 new mice. Each point represents the mean value for a group of between five and eight mice.

FIG. 2. PVP clearance (K_{PVP}) by male (□) and female (○) NZB/W F1 mice. Each point represents the mean value for a group of between four and twelve mice.

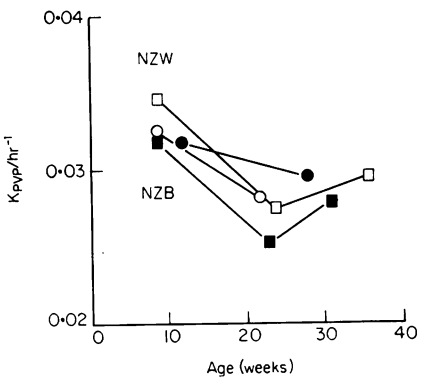

FIG. 3. PVP clearance (K_{PVP}) by male (□) and female (○) NZW, and male (■) and female (●) NZB mice. Each point represents the mean value for a group of between four and eight mice.

females than in males (Fig. 2). The sex difference at 13 and 20 weeks is statistically significant ($P<0{\cdot}05$) but that for 32-week-old mice is not. The oldest NZB/W mice had K_{PVP} values very similar to B10D2 new mice whereas at no age did values in Ajax mice fall to this level.

K_{PVP} values in young NZB and NZW mice were similar to those of young NZB/W mice and in older mice there was a decrease with age, but it was not as marked as in NZB/W females and there was a small rise after 20 weeks (Fig. 3). Values in male and female NZW mice are not significantly different; K_{PVP} in male NZB mice of 23 weeks of age is probably lower than in females although this could not be tested formally because groups of equal age were not available.

DISCUSSION

The most prominent features of the disease of NZB/W mice are the development of anti-DNA antibodies and immune complex glomerulonephritis. The prevalence of each increases with age, and the nephritis is more severe and occurs at a younger age in females than in males (Howie & Helyer, 1968). Nephritis also occurs in old NZB mice and to a much lesser extent in NZW mice (Hahn & Shulman, 1969). The affinity of the antibody response to human serum transferrin is lower in young NZB/W, NZB and NZW mice than in almost all other strains (Petty & Steward, 1972 in preparation) and falls even lower with increasing age. Spontaneously-produced anti-DNA antibody detected in the circulation of NZB/W mice is of low avidity, particularly in the females and also decreases with age (Steward, Katz & West, 1975).

Macrophage clearance function in mice is usually found to remain stable (DiCarlo, Haynes & Phillips, 1963; Perkins, 1971) or to decrease slightly with increasing age (Benacerraf *et al.*, 1957). The data from the present experiments show that Ajax and B10D2 new mice are typical in this respect and that there is little difference in macrophage clearance function between males and females. In all three types of New Zealand mice the decline in clearance rate with age was marked. This was particularly noticeable in female NZB/W mice in which the decrease of K_{PVP} occurred earlier than in males and well before the age of 20 weeks when evidence of nephritis is first found (Howie & Helyer, 1968). The observation that old NZB/W mice clear pre-formed immune complexes of BSA and anti-BSA more slowly than do young animals (Mannik, Haakenstadt & Arend, 1974) is consistent with our findings. On the other hand the report that carbon clearance is increased in old NZB mice (Morton & Siegel, 1970) is not. This apparent contradiction may arise because of differences in the way in which the RES clears particles of different physico-chemical properties. PVP clearance may be a better indicator of the physiological function of the RES than colloidal carbon, perhaps because it is closer in size to immunogenic microaggregates of proteins (Morgan & Soothill, 1975a).

Interstrain differences in macrophage activity as measured by PVP clearance have been shown to correlate with differences of antibody affinity (Morgan & Soothill, 1975a). Of the strains of mice used in the present studies Ajax mice have higher K_{PVP} values and make higher affinity antibody than B10D2 new mice (Morgan & Soothill, 1975a; Soothill & Steward, 1971). Furthermore, B10D2 new are more susceptible to immune complex glomerulonephritis following neonatal infection with lymphocytic choriomeningitis virus than are Ajax mice (Oldstone & Dixon, 1969), perhaps because of a low affinity antibody response which is poor at immune elimination of antigen (Alpers, Steward & Soothill, 1972). The evidence that macrophage blockade can reduce antibody affinity and that macrophage stimulants can increase it (Soothill & Steward, 1971; Passwell, Steward & Soothill, 1974) suggests that these cells play an important role in determining antibody affinity. We therefore suggest that defective macrophage function may be a factor in the development of glomerulonephritis in NZB/W mice once they have been stimulated (perhaps by virus infection; Croker *et al.*, 1974) to produce antibody against nuclear components. As macrophage function declines with age so the affinity of the antibody response to DNA antigens may fall, allowing antigen to accumulate in the circulation with the formation of soluble complexes. An alternative explanation is that the decline of antibody affinity is independent of changes in macrophage phagocytosis and that glomerulonephritis only occurs when the capacity of the RES becomes reduced to a level at which circulating complexes can no longer be removed. Under these circumstances the decline in serum anti-DNA activity which occurs at about the time of the development of renal disease (Steward, Katz & West, 1975) might be a secondary effect of deposition of high affinity antibody in tissues in combination with antigen. It is possible that the fall in K_{PVP} is also a secondary effect of immune complex disease because of saturation of the RES with immune complexes. However we think these last two possibilities unlikely since the decline of K_{PVP} and the production of low affinity antibody to both DNA and unrelated antigens are characteristics of the parental New Zealand strains in which immune complex glomerulonephritis is a much less prominent phenomenon.

Circulating DNA-like material has been demonstrated in the sera of patients with SLE (Tan *et al.*, 1966) but in NZB/W mice with anti-DNA antibody, injected nucleic acids are cleared very rapidly

(Chused, Steinberg & Talal, 1972). This is surprising in view of the prolonged circulation of antigen in other models of chronic immune complex disease (Dixon, Feldman & Vasquez, 1961; Pincus, Haberkern & Christian, 1968) and highlights the important question of the precise nature of the nucleic acid antigen (or antigens) involved in immune-complex formation in NZB/W mice.

We are grateful to the Arthritis and Rheumatism Council and the Medical Research Council for providing financial support.

REFERENCES

ALPERS, J.H., STEWARD, M.W. & SOOTHILL, J.F. (1972) Differences in immune elimination in inbred mice. The role of low affinity antibody. *Clin. exp. Immunol.* **12**, 121.

BENACERRAF, B., BIOZZI, G., HALPERN, B.N. & STIFFEL, C. (1957) Physiology of phagocytosis of particles by the R.E.S. *Physiopathology of the Reticulo-endothelial System* (ed. by B. N. Halpern, B. Bennacerraf and J. F. Delafresnaye), p. 52. Blackwell Scientific Publications, Oxford.

CHUSED, T.M., STEINBERG, A.D. & TALAL, N. (1972) The clearance and localisation of nucleic acids by New Zealand and normal mice. *Clin. exp. Immunol.* **12**, 465.

CROKER, B.P., DELVILLANO, B.C., JENSEN, F.C., LERNER, R.A. & DIXON, F.J. (1974) Immunopathogenicity and oncogenicity of murine leukaemia viruses. I. Induction of immunologic disease and lymphoma in (Balb/c × NZB) F1 mice by Scripps leukaemia virus. *J. exp. Med.* **140**, 1028.

DICARLO, F.J., HAYNES, L.J. & PHILLIPS, G.E. (1963) Effect of *Mycobacterium phlei* upon reticulo-endothelial system of mice of different ages. *Proc. Soc. exp. Biol. (N.Y.)*, **112**, 651.

DIXON, F.J., FELDMAN, J.D. & VASQUEZ, J.J. (1961) Experimental glomerulonephritis. The pathogenesis of a laboratory model resembling the spectrum of human glomerulonephritis. *J. exp. Med.* **113**, 889.

HAHN, B.A. & SHULMAN, L.E. (1969) Autoantibodies and nephritis in the white strain (NZW) of New Zealand mice. *Arthr. and Rheum.* **12**, 335.

HELYER, B.J. & HOWIE, J.B. (1963) Renal disease associated with positive lupus erythematosus tests in a cross-bred strain of mice. *Nature (Lond.)*, **197**, 197.

HOWIE, J.B. & HELYER, B.J. (1968) The immunology and pathology of NZB mice. *Advanc. Immunol.* **9**, 215.

LAMBERT, P.H. & DIXON, F.J. (1968) Pathogenesis of the glomerulonephritis of NZB/W mice. *J. exp. Med.* **127**, 507.

MANNIK, M., HAAKENSTAD, A.O. & AREND, W.P. (1974) The fate and detection of circulating immune complexes. *Progress in Immunology II*, Vol. 5 (ed. by L. Brent & J. Holborow) p. 91. North Holland Publishing Co., Amsterdam.

MORGAN, A.G. & SOOTHILL, J.F. (1975a) Relationship between macrophage clearance of PVP and affinity of antiprotein antibody response in inbred mouse strains. *Nature (Lond.)*, **254**, 711.

MORGAN, A.G. & SOOTHILL, J.F. (1975b) Measurement of the clearance function of macrophages with ^{125}I-labelled polyvinylpyrrolidone. *Clin. exp. Immunol.* **20**, 489.

MORTON, J.I. & SIEGEL, B.V. (1970) Reticulo-endothelial activity of New Zealand Black mice. *Proc. Soc. exp. Biol. (N.Y.)*, **133**, 1055.

OLDSTONE, M.B.A. & DIXON, F.J. (1969) Pathogenesis of chronic disease associated with persistent lymphocytic choriomeningitis viral infection. I. Relationship of antibody production to disease in neonatally infected mice. *J. exp. Med.* **129**, 483.

PASSWELL, J.H., STEWARD, M.W. & SOOTHILL, J.F. (1974) Inter-mouse strain differences in macrophage function and its relationship to antibody responses. *Clin. exp. Immunol.* **17**, 159.

PERKINS, E.H. (1971) Phagocytic activity of aged mice. *J. reticuloend. Soc.* **9**, 642.

PETTY, R.E. & STEWARD, M.W. (1972) Relative affinity of antiprotein antibodies in New Zealand mice. *Clin. exp. Immunol.* **12**, 343.

PINCUS, T., HABERKERN, R.N. & CHRISTIAN, C.L. (1968) Experimental chronic glomerulitis. *J. exp. Med.* **127**, 819.

SOOTHILL, J.F. & STEWARD, M.W. (1971) The immunopathological significance of the heterogeneity antibody affinity. *Clin. exp. Immunol.* **9**, 193.

STEWARD, M.W., KATZ, F.E. & WEST, N.J. (1975) The role of low affinity antibody in immune complex disease: the quality of anti-DNA antibodies in NZB/W F1 hybrid mice. *Clin. exp. Immunol.* **21**, 121.

TAN, E.M., SCHUR, P.H., CARR, R.I. & KUNKEL, H.G. (1966) Deoxyribonucleic acid (DNA) and antibodies to DNA in the serum of patients with systemic lupus erythematosus. *J. clin. Invest.* **45**, 1732.