Clin. exp. Immunol. (1980) **40**, 373–382.

The effect of exogenous interferon: acceleration of autoimmune and renal diseases in (NZB/W) F_1 mice

C. ADAM, Y. THOUA, P. RONCO, P. VERROUST, M. TOVEY* &
L. MOREL-MAROGER *Unité de Recherches de Néphrologie Normale et Pathologique de l'INSERM U 64, Hôpital Tenon, Paris and * Institut de Recherches sur le Cancer, Villejuif, France*

(*Accepted for publication 23 November 1979*)

SUMMARY

To determine the effect of prolonged administration of exogenous interferon on the autoimmune disease of NZB/W female mice, forty-four NZB/W mice received three injections per week of $6{\cdot}4 \times 10^4$ interferon units from birth to 33 weeks, forty mice were injected with mock interferon and forty-eight mice were left untreated. In mice injected with interferon growth was delayed, survival was decreased, and the severity of the glomerulonephritis was enhanced. Interferon increased the titre of serum anti-ssDNA and anti-soluble nucleoprotein antibodies, but had no effect on the circulating immune complex load. These results suggest that interferon in some way affects local deposition of immune complexes within the glomerulus.

INTRODUCTION

Considerable work has been devoted to the study of the glomerulonephritis (GN) of New Zealand Black/New Zealand White (NZB/W) mice which is considered to be a prototype of immune complex (IC) mediated GN (Lambert & Dixon, 1968). DNA has been clearly identified as one of the antigens responsible for antigen–antibody complex formation, although vertically transmitted C type oncornaviruses have been potentially implicated in the pathogenesis of the disease of NZB and NZB/W mice (Mellors *et al.*, 1971). Indeed, xenotropic C type viruses are expressed in the two strains and viral antigens such as the viral envelope glycoprotein gp 70 have been found in the circulation at very high levels and deposited in the glomeruli (Imamura *et al.*, 1977). Although Datta *et al.* (1978a) and Andrews *et al.* (1978) showed that the development of autoimmunity did not require the expression of infectious xenotropic virus or the expression of the viral glycoprotein gp 70 (Datta *et al.*, 1978b), complexes of gp 70 and anti-gp 70 have been found only in the sera of mice with systemic lupus-like disease and appeared with the onset of renal disease (Izui *et al.*, 1979). This could suggest that C type viruses are not necessarily required for the triggering of autoimmunity but may be very important in the pathogenesis as a promoting factor of autoimmune disease and particularly of the GN.

Based on this knowledge and on the fact that interferon can inhibit *in vitro* the replication of C type viruses (Billiau, Sobis & De Somer, 1973; Friedman *et al.*, 1975), it seemed justified to study the effect of interferon as a potential therapeutic agent on the disease of NZB/W mice. Attempts had been made previously in this direction using various interferon inducers such as Statolon (Lambert & Dixon, 1970), poly I:C (polyinosinic–polycytidylic acid) (Steinberg, Baron & Talal, 1969;

Correspondence: Dr Christiane Adam, Unité de l'INSERM U 64, Hôpital Tenon, 4 rue de la Chine, 75970 Paris Cedex 20, France.

0099-9104/80/0500-0373$02.00

Carpenter *et al.*, 1970) and Tilorone (Walker, 1977; Walker & Anver, 1978). The effect of treatment on the autoantibody level was variable, depending on the inducer used. These experiments are somewhat difficult to interpret since Statolon and poly I:C contained RNA (or RNA-like) substances which could by themselves stimulate the production of anti-nucleotide antibodies. It is, however, interesting to note that Tilorone and poly I:C produced dramatic acceleration of mortality probably by increasing the severity of glomerulonephritis. This suggested to us that interferon itself could play a role in the progression of GN. Such a possibility appeared worth considering since recently it has been shown that exogenous interferon injected in newborn mice could induce glomerulonephritis (Gresser *et al.*, 1976; Morel-Maroger *et al.*, 1978). Although the effect observed could be demonstrated only in neonates, it is possible that in adult mice carrying a high level of circulating IC, interferon might also aggravate renal disease. Since interferon is considered for many clinical applications and sometimes in high dosages, we felt, therefore, that it would be of interest to study the effect of potent interferon preparation on the development of NZB/W GN.

MATERIALS AND METHODS

Animals. NZB and NZW mice were obtained initially from the CNRS (Orleans, France) and the MRC (Carshalton Farms, Surrey, England). NZB and NZW colonies were developed by brother–sister mating in our own laboratory. NZB female and NZW male cross and the reciprocal mating were both performed until a sufficient number of NZB/W females was obtained. A total ot 132 NZB/W females were studied. All mice were weaned at 21 days and then fed a standard laboratory diet.

Interferon. Mouse interferon was prepared from C-243 cells induced with Newcastle disease virus. The methods of production, concentration and assay of the interferon preparations have been described previously (Tovey, Begon-Lours & Gresser, 1974). The mock interferon was prepared in an identical manner to the interferon with the exception that the virus inducer was omitted.

Experimental plan. There were three experimental groups: (1) forty-four mice received three injections per week of $6{\cdot}4 \times 10^4$ interferon units alternatively by subcutaneous or intraperitoneal route from birth to 33 weeks; (2) mock preparation was injected according to the same schedule in forty mice and (3) forty-eight mice were kept untreated.

Mice were examined daily for survival and gross clinical abnormalities. Every 6 weeks, from the age of 9 weeks, they were weighed and tested for proteinuria on freshly expressed urine, using tetrabromophenol paper (Albustix). At the same time, 0·6 ml of blood was taken by retro-orbital bleeding under ether anaesthesia. All samples were allowed to clot for 1 hr at 37°C. Serum was collected by centrifugation and stored at −70°C for determination of antibodies to single-stranded DNA (ssDNA) and soluble nucleoprotein (SNP) as well as circulating immune complexes.

Antibodies to ssDNA and to SNP. Antibodies to ssDNA and to SNP were measured by a solid-phase radioimmunoassay (Adam *et al.*, 1979). Polystyrene tubes or microwells were coated with 1 ml of a 15-μg/ml solution of SNP prepared according to Tan (1967) or 240 μl of a 1-μg/ml solution of heat-denatured calf thymus DNA (Worthington). Suitable dilutions of mouse sera in borate buffer containing 1‰ v/v tween 20 were incubated with the coated material. Antibodies bound to the coat were revealed using a rabbit anti-mouse IgG followed by a further incubation with ^{125}I-labelled sheep anti-rabbit IgG antiserum. Results were expressed in units/ml by reference to a pool of positive sera from old NZB/W female mice, which was arbitrarily considered to contain 200 units/ml. Sera from normal Swiss mice were used as negative controls.

Circulating immune complexes. CIC were measured using the Raji cell assay as described by Theofilopoulos, Wilson & Dixon (1976), the results being expressed in μg equivalent of heat-aggregated mouse γ-globulin, by reference to a standard curve obtained by adding increasing amounts of mouse aggregated IgG to normal mouse serum.

C3 and IgG. C3 was measured by rocket immunoelectrophoresis using a rabbit anti-mouse C3 antisera (Capell Laboratories). IgG levels were measured by radial immunodiffusion using a rabbit

anti-mouse IgG antiserum. C3 and IgG levels were expressed as a percentage of a reference pooled normal mouse serum.

Renal histology and immunofluorescence. Renal fragments were divided in two pieces for light microscopy and immunofluorescence. Renal tissue for light microscopy was fixed in alcoholic Bouin and embedded in paraffin. Three- to four-micron sections were obtained and stained with haematoxylin and eosin, Mallory's trichrome and periodic acid Schiff stains. The following lesions were assessed independently by two of us for each fragment and given a score from 0 to 3. The glomerular lesions considered as active were: cellular proliferation, mesangial thickening, thickening of the capillary loops in relation to wireloop lesions, nuclear debris, polymorphonuclear cells, extracapillary proliferation, necrosis and thrombosis. The score for active lesions was obtained by adding the elementary lesions. Theoretically the maximum is 27. In parallel the degree of sclerosis was estimated from + to + + +.

Renal tissue for immunofluorescence was snap-frozen in liquid nitrogen and stored at −70°C. Four-micron cryostat sections were stained with a rabbit anti-mouse γ-globulin antiserum conjugated with fluorescein isothyocyanate. IgG glomerular deposits were graded in the following manner: 0 = no deposits; I = focal and segmental deposits localized to the mesangial areas; II = diffuse mesangial deposits; III = diffuse deposits both in the mesangium and along the capillary loops; IV = confluent granular deposits localized exclusively along the glomerular basement membrane (GBM); V = presence of massive deposits of IgG invading the whole glomerular tufts.

Statistical analysis. The relation between clinical, immunological and histological parameters were compared for the three groups using a Student *t*-test. The distribution of the renal lesions by light or immunofluorescence microscopy and the survival curve were evaluated by a ranking test (Mann–Whitney).

RESULTS

Growth

From birth to 27 weeks of age, all animals gained weight, but all injected mice were lighter than the non-injected controls. However, the mice treated with interferon were significantly lighter than the mice injected with mock interferon during the first 21 weeks of the study ($P < 0{\cdot}01$; Table 1). The occurrence of nephrotic syndrome with peripheral oedema and ascitis after 27 weeks rendered the interpretation of the weight curve difficult.

Survival

As shown in Fig. 1, mice injected with interferon died significantly earlier than mice untreated or receiving mock interferon. The first deaths occurred at 146 days as compared to 172 days in the two other groups. Fifty per cent of the mice treated with interferon had died at day 208 as compared to 20% of the controls treated with mock interferon and 30% of the untreated mice. The mean survival was 223 days in the mice injected with interferon (146–329), 280 days in the mice injected with the mock preparation (173–518), and 240 days in the untreated animals (171–351).

Table 1. Evolution of the body weight in mice treated with interferon and control populations

Age (weeks)	9	15	21	27
Untreated	30·1 ± 0·4*	34·5 ± 0·5	38·3 ± 0·5	37·2 ± 1·1
Interferon	26·7 ± 0·3†	30·8 ± 0·3†	33·6 ± 0·4†	35·6 ± 0·6 (n.s.)
Mock interferon	28·1 ± 0·4‡	33·0 ± 0·4‡	34·9 ± 0·4‡	36·6 ± 0·6 (n.s.)

* Weight in grams, mean ± s.e.m.

† *P* value interferon group/untreated group, $P \leqslant 0{\cdot}001$.

‡ *P* value interferon group/mock interferon group, $P \leqslant 0{\cdot}01$.

n.s. = Not significant.

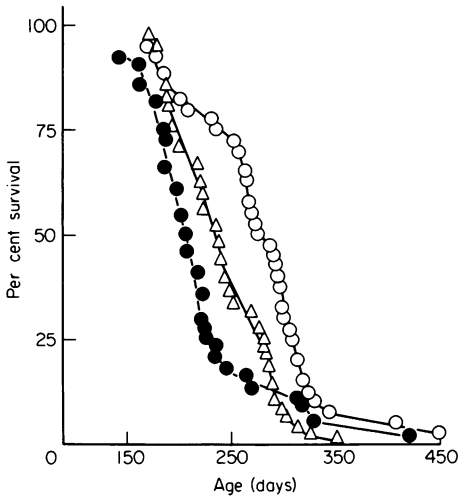

Fig. 1. Survival curves of NZB/W mice injected with interferon (●—●), or mock interferon (○—○), or kept untreated (△—△).

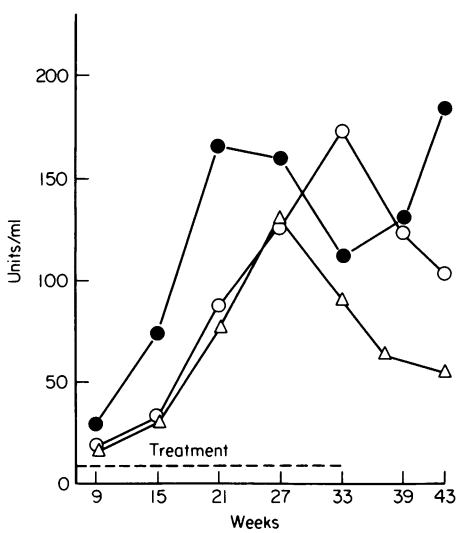

Fig. 2. Anti-ssDNA antibodies in NZB/W mice injected with interferon (●—●), or mock interferon (○—○), or kept untreated (△—△).

Table 2. Anti-SNP antibodies in mice treated with interferon and control populations

Age (weeks)	9	15	21	27	33	39	43
Untreated	13*	34	35	65	67	75	154
Interferon	35	67	90	143	158	272	563
Mock interferon	21	136	48	73	194	172	82

* Mean level in units/ml.

Serological data

Antibodies to ssDNA (Fig. 2). As early as 9 weeks mice injected with interferon had a higher titre of anti-ssDNA antibodies (mean 28·6 units/ml, $P<0·05$) than mice injected with the mock preparation (mean 17·7 units/ml), untreated mice (15·2 units/ml), or Swiss mice (12·7 units/ml). At 15 and 21 weeks this phenomenon was more conspicuous ($P<0·001$). There was no difference between the two control populations during the whole experiment. After 27 weeks, there was no significant difference between the three populations.

Antibodies to SNP. As shown in Table 2 mice injected with interferon or mock interferon had higher levels of anti-SNP antibodies than the mice left untreated. The mice injected with interferon differed significantly from the untreated animals during the 33 weeks of the experiment ($P<0·01$). Mice receiving mock interferon did not differ from the untreated population at 21 and 27 weeks. Mice injected with interferon had significant higher levels of anti-SNP antibodies ($P<0·01$) than the mice receiving mock interferon except at 15 weeks where the highest levels were seen in this latter population.

Circulating immune complexes (Fig. 3). Elevated levels of CIC were detected in the three populations as early as 9 weeks. Mice injected with interferon or the mock preparation had higher levels of CIC than the non-injected controls ($P<0·01$). There were no significant differences between the two groups injected except at 33 weeks where high levels were found in the mice receiving mock interferon ($P=0·02$).

IgG. Mice receiving interferon or mock preparation had a higher level of IgG as compared to the

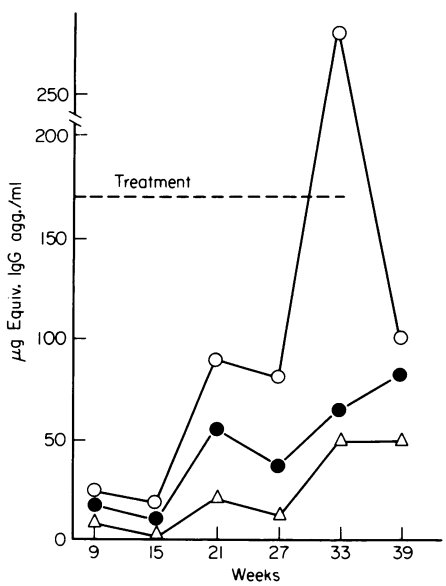

Fig. 3. Circulating IC levels in NZB/W mice injected with interferon (●—●), or mock interferon (o—o), or kept untreated (▵—▵).

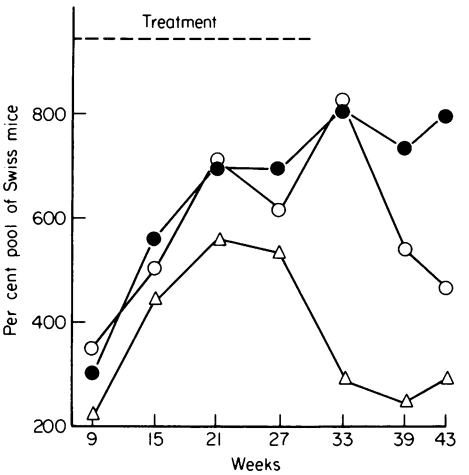

Fig. 4. Serum IgG levels in NZB/W mice injected with interferon (●—●), or mock interferon (o—o), or kept untreated (▵—▵).

non-injected population. There was no significant difference in the IgG level between the mice injected with interferon and the mice injected with the mock preparation (Fig. 4).

C3. There was a progressive decrease in C3 levels in all three groups (Fig. 5). However, no significant difference could be demonstrated at any time.

Haematocrit and white blood count. The haematocrit was measured at 27 weeks. Although it was lower in the animals injected with interferon, there was no significant difference between the three groups of animals—41·6, 45·7 and 44·5% for the interferon, mock interferon-injected and untreated populations respectively. The white blood cell count was significantly higher in the mice injected with interferon (mean 3·325/mm^3; $P < 0{\cdot}02$) than in the two control populations (2,647/mm^3 in the mice injected with the mock interferon, 2,415/mm^3 in the untreated mice).

Renal disease

Proteinuria. Proteinuria was first detectable at 15 weeks in all three groups of mice and increased progressively. Mice injected with the mock preparation had the lowest excretion (Fig. 6).

Renal lesions (Fig. 7). When renal biopsy was performed at 27 weeks, mice treated with interferon had a more severe glomerulonephritis than the other two groups. Indeed, whereas 60%

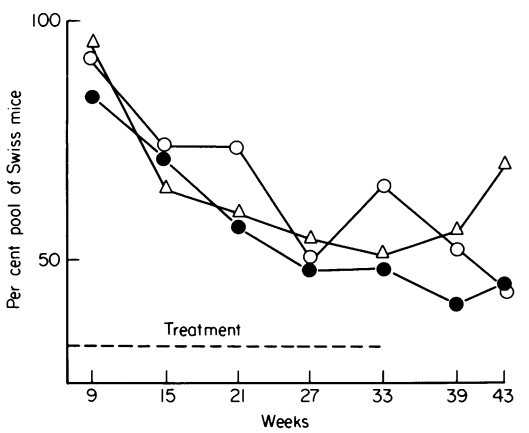

Fig. 5. Serum C3 levels in NZB/W mice injected with interferon (●—●), or mock interferon (o—o), or kept untreated (▵—▵).

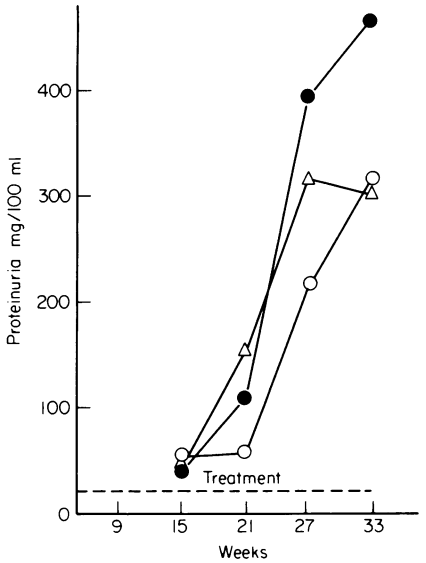

Fig. 6. Proteinuria in NZB/W mice injected with interferon (●—●), or mock interferon (o—o), or kept untreated (△—△).

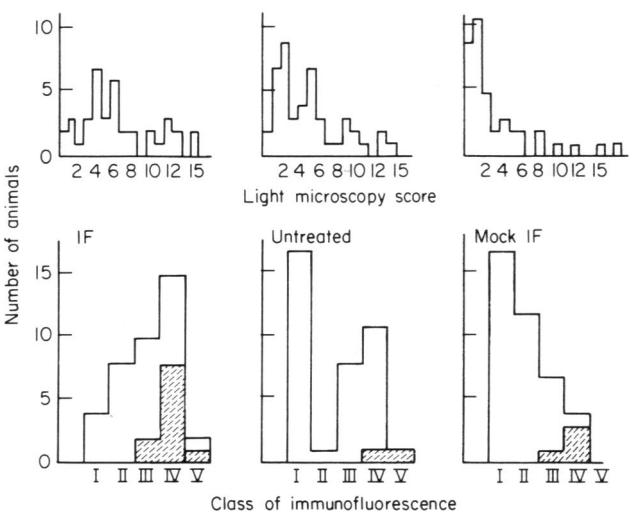

Fig. 7. Renal histology and immunofluorescence in NZB/W mice injected with interferon, mock interferon, or left untreated. The upper part of the figure represents the distribution of light microscopy scores in each of the three populations. The lower part represents the distribution of the glomerular IgG deposits by immunofluorescence. The open bar represents data obtained by renal biopsy at 27 weeks, the hatched area represents data obtained by immunofluorescence on postmortem examination.

(47/78) of the mice untreated or receiving mock interferon had deposits confined to the mesangium (classes 0–II), 70% (27/39) of the mice receiving interferon had extensive glomerular deposits (classes III–V). By light microscopy the activity and sclerosis indices were 6·5 and 0·6 for the mice receiving interferon as compared to 4 and 0·4 for the control populations. Furthermore, at the time of biopsy, eleven of forty-four mice injected with interferon had died with extensive glomerular lesions compared with six of eighty-eight in the two control groups.

The enhancement of glomerular lesions by interferon was statistically significant when immunofluorescence classes and light microscopy indices were compared with those of either untreated mice, or mice receiving mock interferon. There was, however, no significant difference between the latter two groups.

DISCUSSION

The experiments clearly show that prolonged administration of interferon decreases survival of NZB/W mice and increases the severity of nephritis and autoimmune manifestations. Indeed, in mice treated with interferon there was a delay in growth, and death occurred prematurely from day 146. Renal disease was also conspicuous, with active lesions and heavy deposits of IgG, and occurred earlier than in the control mice. The data therefore confirm, on a large number of mice, results which had been suggested previously by experiments using either interferon inducers such as poly I:C (Steinberg *et al.*, 1969; Carpenter *et al.*, 1970), Statolon (Lambert & Dixon, 1970), Tilorone (Walker, 1977; Walker & Anver, 1978) and recently interferon itself (Heremans *et al.*, 1978). These observations are in contrast with those obtained using other anti-viral agents such as Methisazone (Gabriel, 1971), and Ribavirin (Klassen *et al.*, 1977, 1979) which have a beneficial effect on survival, renal disease and autoimmune manifestations.

The mechanism whereby such an acceleration of renal disease could occur is difficult to determine, but is probably related to some of the numerous biological effects of interferon apart from its anti-viral activity (Gresser, 1977). Our observations suggest that interferon can increase the titres of anti-ssDNA and anti-SNP antibodies as early as 9 weeks. One could therefore speculate that DNA–anti-DNA immune complexes are formed earlier and in greater amounts, therefore favouring immune complex deposition. Such a possibility, however, is not consistent with the fact that some of the mice receiving the mock interferon preparation also have at some time markedly increased titres of antibody, as well as the highest titres of circulating immune complexes, in spite of the fact that their glomerulonephritis is considerably less severe. This suggests, therefore, that the serological parameters of autoimmunity are not the only determinants of the severity of renal disease. This situation is somewhat similar to our findings with lymphocytic choriomeningitis virus (LCM) induced glomerulonephritis in Swiss mice (Gresser *et al.*, 1978). In this model we have been able to show that neutralization of interferon considerably decreased the severity of glomerulonephritis whereas the immune complex load remained unchanged (Ronco *et al.*, 1979). In both instances it can be suggested that interferon could act in some way to favour glomerular localization of IC. It is possible that interferon activates systemic mechanisms leading to IC localization, at present unknown in the mouse, but analogous to the IgE-mediated system described in the rabbit by Benveniste, Henson & Cochrane (1972). Alternatively it can be suggested that interferon produces local alteration of the GBM favouring IC localization. Previous experiments have shown that exogenous interferon or interferon produced during neonatal infection with LCM virus could induce striking lesions of the GBM before immune deposits could be detected in the glomerular capillary loops (Gresser *et al.*, 1976; Morel-Maroger *et al.*, 1978; Woodrow *et al.*, 1979). Although it is unlikely that these lesions by themselves are responsible for immune complex trapping, this observation clearly shows that interferon can act locally on the glomerular capillary wall, possibly by altering the function of the glomerular cells which synthesize the GBM. It is then possible that a disturbed function of the glomerular capillary wall favours immune complex localization.

Professor I. Gresser provided the interferon and mock preparations. We thank Mrs M. Delauche, G. Neuilly, M. Galceran and Mr C. Mouas for technical assistance and Miss M. Bizien for secretarial assistance.

This work was supported by a grant from the Conseil Scientifique de l'Université Pierre et Marie Curie, Paris, by grant no. 77.5.038.5A from INSERM and by grant no. 79.7.1530 from DGRST..

REFERENCES

ADAM, C., VERROUST, P., ROBITAILLE, P. & PONTILLON, F. (1979) A solid phase radioimmunoassay for anti-SNP antibodies. *J. Immunol. Methods,* **27,** 133.

ANDREWS, B., EISENBERG, R., THEOFILOPOULOS, A., IZUI, S., WILSON, C.B., MCCONAHEY, P., MURPHY, E.D., ROTHS, J. & DIXON, F. (1978) Spontaneous murine lupus-like syndrome. Clinical and immunopathological manifestations in several strains. *J. exp. Med.* **148,** 1198.

BENVENISTE, J., HENSON, P.M. & COCHRANE, C.G. (1972) Leukocyte-dependent histamine release from rabbit platelets: the role of IgE, basophils and platelet-activating factor. *J. exp. Med.* **136,** 1356.

BILLIAU, A., SOBIS, H. & DE SOMER, P. (1973) Influence of interferon on virus particle formation in different oncornavirus carrier cell lines. *Int. J. Cancer,* **12,** 646.

CARPENTER, D.F., STEINBERG, A.D., SCHUR, P.H. & TALAL, N. (1970) The pathogenesis of autoimmunity in New Zealand mice. II. Acceleration of glomerulonephritis by Polyinosinic-Polycytidylic acid. *Lab. Invest.* **23,** 628.

DATTA, S.K., MANNY, N., ANDRZEJEWSKI, C., SCHWARTZ, J.A. & SCHWARTZ, R.S. (1978a) Genetic studies of autoimmunity and retrovirus expression in crosses of New Zealand Black mice. I. Xenotropic virus. *J. exp. Med.* **147,** 854.

DATTA, S.K., MCCONAHEY, P.J., MANNY, N., THEOFILOPOULOS, A.N., DIXON, F.J. & SCHWARTZ, R.S. (1978b) Genetic studies of autoimmunity and retrovirus expression in crosses of New Zealand Black mice. II. The viral envelope glycoprotein gp 70. *J. exp. Med.* **147,** 872.

FRIEDMAN, R.M., CHANG, E.H., RANSEUR, J.M. & MYERS, M.W. (1975) Interferon directed inhibition of chronic murine leukemia virus production in cell cultures: lack of effect on intracellular viral markers. *J. Virol.* **16,** 569.

GABRIEL, R. (1971) Methylisatin 3-thiosemicarbazone treatment of NZB/NZW hybrid mice. *Br. J. exp. Pathol.* **52,** 271.

GRESSER, I. (1977) On the varied biologic effects of interferon. *Cell. Immunol.* **34,** 406.

GRESSER, I., MAURY, C., TOVEY, M., MOREL-MAROGER, L. & PONTILLON, F. (1976) Progressive glomerulonephritis in mice treated with interferon preparations at birth. *Nature,* **263,** 420.

GRESSER, I., MOREL-MAROGER, L., VERROUST, P., RIVIERE, Y. & GUILLON, J.C. (1978) Anti-interferon globulin inhibits the development of glomerulonephritis in mice infected at birth with lymphocytic choriomeningitis virus. *Proc. Natl. Acad. Sci. (USA),* **75,** 3413.

HEREMANS, H., BILLIAU, A., COLOMBATTI, A., HILGERS, J. & DE SOMER, P. (1978) Interferon treatment of NZB mice: accelerated progression of autoimmune disease. *Infect. Immun.* **21,** 925.

IMAMURA, M., MELLORS, R.C., STRAUD, M. & AUGUST, J.F. (1977) Murine type C viral envelope glycoprotein gp 69/71 and lupus-like glomerulonephritis of New Zealand mice. An immunoperoxidase study. *Am. J. Pathol.* **86,** 375.

IZUI, S., MCCONAHEY, P.J., THEOFILOPOULOS, A.N. & DIXON, F.J. (1979) Association of circulating retroviral gp 70–anti gp 70 immune complexes with murine systemic lupus erythematosus. *J. exp. Med.* **149,** 1099.

KLASSEN, L.W., BUDMAN, D.R., WILLIAMS, G.W., STEINBERG, A.D. & GERBER, N.L. (1977) Ribavirin: efficacy in the treatment of murine autoimmune disease. *Science,* **195,** 787.

KLASSEN, L.W., WILLIAMS, G.W., REINERTSEN, J.L., GERBER, N.L. & STEINBERG, A.D. (1979) Ribavirin treatment in murine autoimmune disease. I. Therapeutic efficacy and effect on the immune response. *Arthritis Rheum.* **22,** 145.

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

LAMBERT, P.H. & DIXON, F.J. (1970) Genesis of antinuclear antibody in NZB/W mice: role of genetic factors and of viral infections. *Clin. exp. Immunol.* **6,** 829.

MELLORS, R.C., TOSHIZAKU, S., TADOA, A., HUEBNER, R.J. & KRAWCZYNSKI, K. (1971) Wild type gross leukemia virus and the pathogenesis of the glomerulonephritis of New Zealand mice. *J. exp. Med.* **133,** 113.

MOREL-MAROGER, L., SLOPER, J.C., VINTER, J., WOODROW, D. & GRESSER, I. (1978) An ultrastructural study of the development of nephritis in mice treated with interferon in the neonatal period. *Lab. Invest.* **39,** 513.

RONCO, P., RIVIERE, Y., VERROUST, P., GUILLON, J.C., GRESSER, I. & MOREL-MAROGER, L. (1979) Further studies on the inhibition of lymphocytic choriomeningitis-induced glomerulonephritis by anti-interferon globulin. I. Circulating immune complexes. (Submitted for publication.)

STEINBERG, A.D., BARON, S. & TALAL, N. (1969) The pathogenesis of autoimmunity in New Zealand mice. I. Induction of antinucleic acid antibodies by polyinosinic–polycytidylic acid. *Proc. Natl. Acad. Sci. (USA),* **63,** 1102.

TAN, E.M. (1967) An immunologic precipitin system between soluble nucleoprotein and serum antibody in systemic lupus erythematosus. *J. clin. Invest.* **46,** 735.

THEOFILOPOULOS, A.N., WILSON, C.B. & DIXON, F.J. (1976) The Raji cell radioimmune assay for detecting immune complexes in human sera. *J. clin. Invest.* **57,** 169.

TOVEY, M.G., BEGON-LOURS, J. & GRESSER, I. (1974) A method for the large scale production of potent interferon preparations. *Proc. Soc. exp. Biol. Med.* **146,** 809.

WALKER, S.E. (1977) Accelerated mortality in young NZB/NZW mice treated with the interferon inducer tilorone hydrochloride. *Clin. Immunol. Immunopathol.* **8,** 204.

WALKER, S.E. & ANVER, M.R. (1978) Stimulated autoantibody response and increased longevity in NZB/NZW mice treated with cyclophosphamide and tilorone. *Clin. exp. Immunol.* **33,** 453.

WOODROW, D., RONCO, P., MOSS, J., RIVIERE, Y., VERROUST, P., GUILLON, J.C., GRESSER, I., SLOPER, J.C. & MOREL-MAROGER, L. (1979) Further studies on the inhibition of lymphocytic choriomeningitis-induced glomerulonephritis by anti-interferon globulin. II. Ultrastructural studies. (Submitted for publication.)