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# Isoprinosine delays the early appearance of autoimmunity in NZB/NZW $F_1$ mice treated with interferon

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## SUMMARY

NZB/NZW $F_1$ hybrid mice treated for long periods with type $\beta$ interferon developed early symptoms of autoimmune disease. In these animals the level of anti-dsDNA antibody begins to increase at 4–6 months while untreated NZB/NZW mice do not display similar levels until 12 months. The concomitant administration of isoprinosine and interferon delays the early appearance of autoimmune disorders. In interferon-treated NZB/NZW mice the cytotoxic activity of natural killer lymphocytes is maintained at high levels until the age of 5 months. Nevertheless, the natural killer activity is even stronger and detected until at least 7 months in NZB/NZW mice receiving a single dose of interferon 16 hr prior to the test. Lymphoblastoid ascitic tumours appeared early (2–3 months) during interferon treatment in all groups of NZB/NZW mice. However, in the presence of isoprinosine only a few animals developed tumours. Thus, isoprinosine seems to protect NZB/NZW mice both from early autoimmune disorders due to interferon and from early tumour development.

## INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune multisystem and multifactorial disease in a number of species including man (Huston & Steinberg, 1979). The best-characterized abnormalities are immunological: excessive B cell function with formation of large amounts of autoantibodies and T cell abnormalities which include defects in T cell regulatory functions (Talal, 1976; Huston & Steinberg, 1979).

Among murine models of the disease, NZB mice are characterized mainly by autoimmune haemolytic anaemia while their NZB/NZW $F_1$ (NZB/W) hybrids predominantly develop antinuclear and anti-dsDNA antibodies, as well as severe immune complex glomerulonephritis (Lambert & Dixon, 1968).

Interferon, well known as a potent antiviral and anti-tumour agent, has been found to exert modulatory effects on certain immune phenomena (Gisler, Lindahl & Gresser, 1974; DeMaeyer, DeMaeyer-Guignard & Vandeputte, 1975). It has also been reported that treatment with several interferon inducers, such as poly I:C acid (Steinberg, Baron & Talal, 1969) or tilorone (Walker, 1977), reduces survival time of NZB/W mice by accelerating the autoimmune disease. However, these authors did not incriminate interferon (IFN) as the aggravating agent. Subsequently, the

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adverse effect of IFN (type $\beta$), directly administered for long-term periods to NZB mice, was reported (Heremans *et al.*, 1978; Sergiescu *et al.*, 1979). Moreover, Heremans *et al.* (1978) described severe renal immune complex glomerulonephritis appearing in NZB/W mice in the course of treatment with crude IFN. Similar results were obtained with type $\gamma$ IFN (Engleman *et al.*, 1979).

Therefore, it seemed interesting to study in some detail the aggravating effect of IFN on NZB/W $F_1$ hybrid mice, particularly as these animals display a wide variety of immunological parameters susceptible to examination (Steinberg & Reinetsen, 1977). Furthermore, the use of isoprinosine, an immunostimulant which was shown to potentiate the antiviral and anti-tumour action of IFN (Chany & Cerutti, 1977; Cerutti, Chany & Schlumberger, 1979), might afford a new approach to the study of the IFN-induced aggravation of autoimmunity.

Several reports have described the age-dependent decline of certain T cell functions in NZB/W mice and its possible association with autoimmunity (Steinberg & Reinetsen, 1977). Thus, another purpose was the study of the *in vitro* response of lymphocytes to the polyclonal mitogens, as well as the *in vivo* response of cytotoxic natural killer lymphocytes (Senik *et al.* 1979) after long-term administration of IFN to NZB/W mice.

## MATERIALS AND METHODS

*Animals.* NZB/NZW $F_1$ (NZB/W) hybrid mice were received from the breeding colonies of the CNRS (Orléans). Both male and female mice were used in this study. C57Bl/6 mice were used as interferon-treated controls of NZB/W mice; BALB/c mice were controls in the natural killer (NK) cytotoxicity test; Swiss mice were used for B/W tumour grafts. These last three species of mice were from our breeding colony (U 43 INSERM, Paris).

*Interferon.* Crude mouse (type $\beta$) interferon (IFN) was prepared and titrated on L929 cells (Flow) according to techniques previously described (Rousset, 1974). Its specific activity was $3 \times 10^4$/mg of protein. IFN was administered intraperitoneally at doses of $0{\cdot}5–1 \times 10^5$ international units (iu). The same techniques were applied for mock preparations except that normal allantoic fluid was used instead of the IFN inducer virus (Newcastle disease virus). The protein concentration in mock preparations was similar to that of the IFN preparation used.

*Chemicals.* Isoprinosine (ISO), supplied by Delalande Laboratories (Courbevoie, France), has been described elsewhere (Ginsberg & Luebben, 1971). It was dissolved in phosphate-buffered saline (PBS) and given intraperitoneally at a dose of 1 mg/g of mouse weight, in 0·5 ml, 3 hr prior to interferon (Chany & Cerutti, 1977).

*Experimental procedure.* Four randomized groups of 15 NZB/W mice, aged 2 months, were treated i.p. at 48-hr intervals with the following preparations: (1) PBS + IFN; (2) ISO + IFN; (3) PBS + mock; and (4) ISO + mock. C57Bl/6 mice were inoculated in parallel with the same IFN and mock preparations. Mice were bled periodically from the orbital plexus and by cardiac puncture at killing. The mice belonging to all NZB/W groups and to C57Bl/6 controls were killed beginning from months 6–12; serum, kidneys, lymph nodes, and spleen were removed for immunological tests.

*Grafting of tumours.* The *in vivo* passages of B/W tumour cells were made by grafting $10^6$ cells i.p. to Swiss mice. Ascitic cells from tumour-bearing animals were passaged at 10-day intervals.

*Cells and media.* YAC-1 lymphoma cells were used as target cells in NK cytotoxicity tests (Kiessling *et al.*, 1976). B/W-1–3 cell lines were isolated from peritoneal ascitic cells of IFN-treated mice which had developed anasarca. Both cell lines were cultivated with RPMI 1640 medium, 10% foetal calf serum (FCS) and $0{\cdot}3 \times 10^{-5}$ M 2-mercaptoethanol. They were passaged at 48-hr intervals. SC1 mouse (Hartley & Rowe, 1975) and Mink $S^+L^-$ cells (Peebles, 1975) were cultivated in RPMI 1629 medium with 10% FCS.

*Virus isolation.* Ecotropic MuLV isolation from the B/W tumour cells was performed by co-cultivation with SC1 cells (Hartley & Rowe, 1975). The virus was detected by a direct XC test on infected SC1 cells. Xenotropic MuLV isolation was attempted by co-cultivating the B/W cells with Mink $S^+L^-$ cells (Peebles, 1975) and by performing several blind passages.

*Serological tests*

*Anti-nuclear antibodies (ANA).* ANA were detected by the technique described by Mach *et al.*

(1979) using smears of rat liver-free nuclei as substrate. The acetone-fixed smears were first incubated with dilutions of mouse serum (1/10 to 1/1,000), then with fluorescein isothiocyanate (FITC) conjugated goat antiserum to mouse IgG (Meloy Laboratories, Maryland). Results were considered positive if fluorescence was observed in the presence of dilutions of mouse sera higher than 1/10.

*Anti-DNA levels.* Anti-dsDNA antibody was assessed by the Farr technique using an Amersham kit. DNA was labelled with $^{125}I$. Results are expressed in international units of DNA bound to 1 ml of mouse serum. Levels higher than 25 iu were considered positive.

*Immune complex detection in kidneys.* Kidney cryostat sections were incubated with the same fluorescent antiserum as described above. The presence of fluorescent IgG deposits was considered as a positive result.

*Characterization of B/W ascitic cells*

*Tests for lymphocyte markers.* Tests for intracytoplasmic IgG were made on smears of B/W cells, using an FITC-conjugated anti-mouse IgG rabbit serum (Institut Pasteur, Paris).

The presence of Thy 1.2 antigen on the cell surface of B/W cells was analysed by direct microcytotoxicity and by absorbtion of an anti-Thy 1.2 serum prepared in C3H mice (Boyer *et al.*, 1980).

*Sensitivity of B/W cells to interferon.* The antiviral effect of IFN in B/W cells was tested with encephalomyocarditis (EMC) virus using conventional methods. The growth-inhibitory action of IFN was assayed by labelling B/W cells with $^{3}H$-thymidine 48 hr after the onset of the experiment (Bourgeade & Chany, 1979).

*Electron microscopy.* Kidneys were harvested at killing, fixed with 5% glutaraldehyde, postfixed with 0·1% osmium tetraoxide, dehydrated with increasing concentrations of ethanol, and embedded into epon 812. Ultrathin sections were contrasted with uranyl acetate and lead citrate. Observations were made in a transmission electron microscope (Elmiskop 101 Siemens). At least 20 glomeruli were examined and the average size of the basement membrane (BM) was computed. BM larger than 250 nm were considered pathological. The lesions were classified as mild for BW = 250 to 400 nm and severe for BM > 400 nm.

B/W tumour cells obtained either by peritoneal puncture from tumour-bearing Swiss mice grafted with B/W cells, or from *in vitro* cultures of B/W-1 cells, were pelleted, fixed with 3% glutaraldehyde and further processed as described above.

*Lymphoblastic transformation test (LTT).* The LTT was performed on 3-day lymphocyte cultures derived from lymph nodes and spleens of NZB/W, C57Bl/6 and BALB/c mice in the presence of the polyclonal mitogens of T lymphocytes – phytohaemagglutinin M (PHA), pokeweed mitogen (PWM) and concanavalin A (Con A). Mitogen doses, culture conditions and the assessment of stimulation were performed according to techniques previously described (Kahan *et al.*, 1976).

*Natural killer (NK) cytotoxicity assay.* Continuously treated NZB/W mice, as well as animals which had received a single dose of IFN 16 hr prior to the test, were killed and spleen cells were harvested. The NK activity of splenic lymphocytes was assayed according to Senik *et al.* (1979) in a 4-hr $^{51}Cr$-release test with $^{51}Cr$-labelled YAC-1 cells as target cells. Cytolytic units were defined as the number of effector cells needed to produce cytotoxicity of 10% above the baseline controls (Herberman *et al.*, 1979).

*Statistical processing of the data.* The chi-square test with Yates' modification was applied to estimate the significance of differences in mortality values. Wilcoxon's test of the range was used to compare serological data.

## RESULTS

*Isoprinosine prevents early development of ascitic tumours in interferon-treated and control NZB/W mice*

During IFN treatment, an unexpected outbreak of ascitic tumours was observed, which occurred

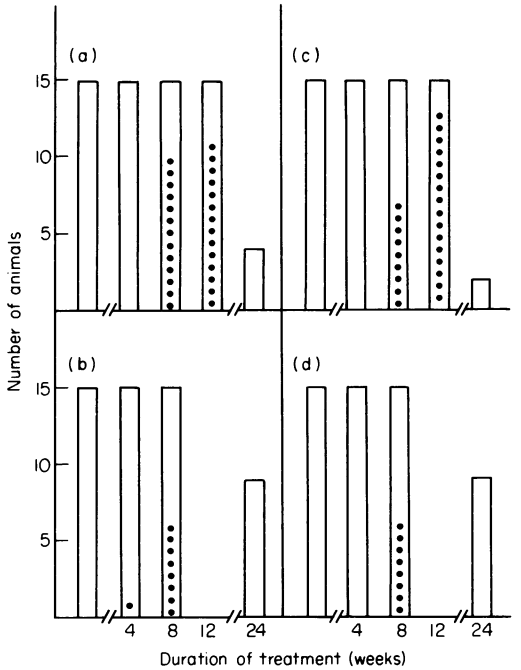


**Fig. 1.** Development of ascitic tumours in NZB/W mice treated with interferon and isoprinosine. Four groups of NZB/W mice were treated i.p. at 48-hr intervals with either $10^5$ iu of IFN (a & b) or mock preparations (c & d). In addition, half of the animals were treated with 1 $\mu$g/g mouse weight of ISO (b & d). The treatment started at 2 months of age and continued throughout the period of tumour development. The number of survivors (last column) among the ISO-treated mice (b & d) is significantly larger than in groups (a) and (c) ($P < 0{\cdot}001$). Points within the columns indicate mice bearing ascitic tumours.

mainly in IFN- and mock-treated animals, while leaving the isoprinosine-treated groups almost intact. As can be seen in Fig. 1, the tumours appeared early (8–12 weeks) after the onset of the treatment. The animals died after 5–10 days with generalized neoplasia.

Since in both ISO-treated groups only a few tumour-bearing mice were lost, the treatment and follow-up of the remaining animals could be continued to the end of the observation period (12 months). These mice did not develop tumours until they were killed at the age of 12 months when small nodules were noticed in their lymph nodes and spleen.

The mortality due to early neoplasia in the ISO-treated groups was less than that in the equivalent non-ISO-treated groups. Mortality: for ISO-IFN < IFN, $\chi^2 = 4{\cdot}88$ ($P \leqslant 0{\cdot}05$); for ISO-mock < mock, $\chi^2 = 9{\cdot}18$ ($P \leqslant 0{\cdot}01$); for ISO-treated/non-treated groups, $\chi^2 = 25{\cdot}20$ ($P < 0{\cdot}001$).

*Cytological, immunological and virological characteristics of ascitic tumour cells isolated from NZB/W mice*

Ascitic tumour cells obtained from peritoneal washings of IFN-treated NZB/W mice were maintained *in vivo* by passage in Swiss mice. They were also adapted to *in vitro* conditions, yielding three cell lines (B/W-1–3). Cells from both *in vivo* and *in vitro* lines displayed similar lymphoblastic morphology under electron microscopy.

Immunological tests were unable to identify the main surface markers of B or T cells on B/W-1 or on *in vivo*-passaged tumour cells. Also, the search for natural killer cytotoxicity in splenic cells from mice inoculated with tumour cells was negative. B/W-1 cells were sensitive to interferon. The antiviral and cellular effect of type $\beta$ IFN was found to be similar in these cells and in L929 reference cells. When examined for the presence of retroviruses, an ecotropic XC plaque-forming MuLV was isolated by co-cultivating B/W-1 cells with SC1 cells. However, no xenotropic MuLV could be

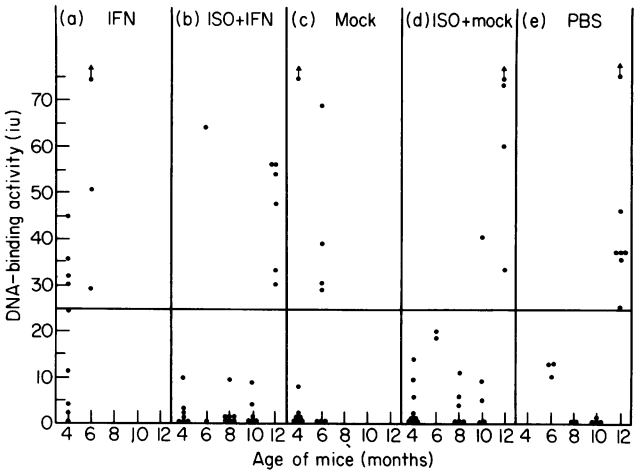


**Fig. 2.** Development of dsDNA-binding antibodies in IFN-treated and control NZB/W mice. The mice were treated as described in Fig. 1. Results are considered positive when higher than 25 iu. Levels of anti-DNA antibodies at 4 months are significantly higher in group (a) (IFN) than in (c) (mock) and (b) (ISO+IFN), $P=0{\cdot}01$. The comparison between (c) (mock) and (d) (ISO+mock) is not significant. Arrow indicates values higher than 75 iu.

obtained from B/W-1 cells after co-culture with Mink $S^+L^-$ cells, followed by several blind passages.

*Accelerating effect of interferon on the development of autoimmunity in NZB/W mice*

The incidence of ANA as well as of antibodies to dsDNA was periodically scored in the sera of NZB/W mice receiving continuous treatment either with IFN or mock preparations. The terminal assessment of these parameters and of renal disease was made after 4–10 months of treatment.

Since a large proportion of the NZB/W mice treated only with IFN or mock died with tumours, these groups could be followed up for only 4 months, whereas animals receiving ISO in addition to IFN and mock could be studied for 9–10 months.

In Fig. 2, the development of antibodies to dsDNA is shown. In contrast to PBS-treated NZB/W mice, where DNA-binding antibodies appeared at 11–12 months of age, in the IFN-treated group, the antibody levels increased at 4–6 months of age (2–4 months of treatment). After administration of mock preparations, no such early increase was observed. If compared by Wilcoxon's test, the values at 4 months are significantly different ($W_8^9=44{\cdot}5$; $P=0{\cdot}01$).

The early appearance of ANA and DNA-binding antibodies in the sera of IFN-treated NZB/W mice was associated with signs of renal disease, i.e. renal fluorescence (deposits of IgG) and involvement of the basement membrane. As may be seen in Table 1, there was agreement in most cases between the results of the three parameters of autoimmunity tested.

*Isoprinosine delays the development of autoantibodies in IFN-treated NZB/W mice*

In both groups of mice treated with ISO, in addition to IFN or mock, DNA-binding antibodies did not appear before the age of 11–12 months, i.e. the same as observed in control PBS-treated animals. As shown in Table 1, the incidence of ANA, as well as that of renal lesions, is in accordance with this observation. When the incidence of DNA-binding autoantibodies in the IFN and ISO+IFN-treated groups is compared at the age of 6 months, the difference is significant ($\chi^2=5{\cdot}62$, $P\leqslant 0{\cdot}05$).

*Interferon treatment of NZB/W mice has no effect on the response of their lymphocytes to mitogens*

The possible action of IFN on cellular response was investigated by comparing the reactivity of

**Table 1.** Effect of interferon and isoprinosine treatment on the development of ANA, dsDNA-binding antibodies and renal lesions in NZB/W mice

| | | | Incidence of:* | | | | |
|---|---|---|---|---|---|---|---|
| | | | | | Renal lesions | | |
| | | | | | | Electron microscopy | |
| Age (months) | Duration of treatment (months) | Treatment | ANA | Anti-dsDNA antibodies | Fluorescence | Mild | Severe |
| 6 | 4 | IFN | 8/10 | 7/9 | 4/4 | 1/2 | 1/2 |
| | | Mock | 7/12 | 5/12 | 1/3 | 2/2 | |
| | | ISO+IFN | 3/9 | 1/8† | 1/2 | 1/2 | 1/2 |
| | | ISO+mock | 4/11 | 0/10 | 1/1 | 1/2 | 1/2 |
| 12 | 10 | ISO+IFN | 5/5 | 4/6 | 1/1 | | 5/5 |
| | | ISO+mock | 5/5 | 4/6 | 1/1 | | 2/2 |

* No. of positive animals/total no. of mice tested. For positive test criteria, see Materials and Methods section.

† Significantly less than in 6-month-old NZB/W mice treated with IFN alone ($P \leqslant 0{\cdot}05$).

splenic and lymph node cells from IFN-treated and control NZB/W mice after stimulation with polyclonal mitogens.

It was noticed that splenic and particularly lymph node lymphocytes from normal or treated B/W mice responded regularly to PHA and to PWM. No significant differences could be recorded between these results and those obtained with IFN and ISO + IFN-treated mice.

*Natural killer (NK) activity in NZB/W mice continuously treated with interferon*

NK activity is stimulated in several strains of mice by the injection of interferon 1–24 hr prior to killing. It was interesting to know if continuous treatment of NZB/W mice would lead to the same result. BALB/c mice which had received IFN 16 hr before the test represented the positive control in all assays.

It may be seen in Table 2 that in NZB/W mice continuously treated with IFN (every 48 hr) the NK response was maintained at high levels up to 5 months of age (3 months of treatment) and dropped thereafter to the level of control animals. However, NK activity in continuously treated NZB/W mice seemed to be less strong than in animals receiving a single dose of IFN 16 hr prior to the test (Fig. 3).

All groups of mice tested after 10 months had a low NK response. No NK activity was found in mice developing ascites tumours.

## DISCUSSION

Several authors recently reported that IFN could exert an adverse effect on NZB and NZB/W $F_1$ mice (Heremans *et al.*, 1979; Sergiescu *et al.*, 1979; Engleman *et al.*, 1979). This effect could be either the consequence of some impurities present in the crude IFN preparations (Heremans *et al.*, 1978) or due to the modulatory action of IFN on immunological phenomena (Gresser, 1977; Strannegard *et al.*, 1978).

The above-described experiments seem to confirm previous observations since NZB/W mice develop autoantibodies (mainly anti-dsDNA) after only 4 months of IFN treatment at the age of 6 months.

It is well documented that autoimmune disease has a more rapid progression in female than in

**Table 2.** NK activity of spleen cells from interferon-treated NZB/W mice

| Mice | | | | |
|---|---|---|---|---|
| Strain | Treatment | Age (months) | *n* | Median per cent cytotoxicity* (range) |
| NZB/W | IFN continuous† | 5 | 3 | 26 (19–38) |
| | | 6–7 | 3 | 13 (9–19) |
| | | 11–12 | 10 | 11·5 (6–23) |
| | Control | 6–7 | 3 | 10 (9–18) |
| | | 11–12 | 6 | 11·5 (4–18) |
| BALB/c | IFN‡ single dose | 5 | 13 | 21 (12–42) |
| | Control | 5 | 3 | 12 (6–14) |

* Specific cytotoxicity against $^{51}$Cr-labelled YAC-1 cells. Median and range (in parentheses) of values obtained at effector/target ratio of 100/1 in mice assayed individually.

† For continuous treatment, mice were injected with $10^5$ iu every 48 hr starting at age 2 months. The animals were killed after 3–5 or 9–10 months of treatment.

‡ BALB/c mice injected with IFN 16 hr prior to the test were positive controls in each experiment.

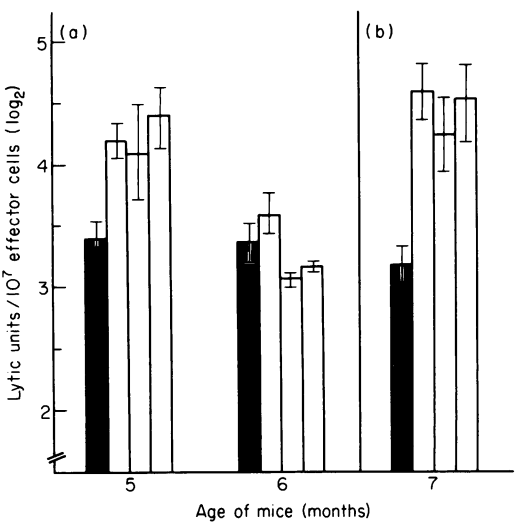


**Fig. 3.** Comparison of spleen NK cell activity in NZB/W mice after long-term administration of interferon, or a single dose. (a) Spleen cells of mice continuously treated for 3–4 months with IFN ($10^5$ iu). (b) Seven-month-old mice receiving a single dose of IFN ($10^5$ iu) 16 hr prior to the test. (■) Splenic cells from control (PBS-treated) NZB/W mice. Splenic lymphocytes were tested for their ability to lyse YAC-1 cells labelled with $^{51}$Cr. The results are expressed as $log_2$ number of lytic units/$10^7$ effector cells. Bars are arithmetic means (±s.d.) of four parallel determinations in individual mice.

male NZB/W mice (Huston & Steinberg, 1979). However, it seems from these experiments that the aggravating effect of IFN is not exerted preferentially on female animals (data not shown).

Conversely, NZB/W mice treated with ISO (in addition to IFN or mock preparations) do not develop anti-DNA antibodies until 11–12 months, i.e. at a similar time to that at which PBS-treated control animals develop anti-DNA antibodies. This protective action of ISO appears to antagonize the effect of IFN in mice simultaneously treated with both preparations. This is in contrast with the potentiating effect of ISO on the antiviral and anti-tumour effect of IFN observed in other inbred mice (Chany & Cerutti, 1977; Cerutti *et al.*, 1979). Another immunostimulant, *Corynebacterium parvum*, was shown to exert an enhancing effect on autoantibody development in NZB mice (Halpern & Frey, 1969). On the contrary, ISO might control the IFN-induced aggravation of immune disorders. The target site and the mechanism of action of both agents are still unknown.

Renal fluorescence due to IgG deposits and/or capillary lesions were detected in some IFN-treated NZB/W mice in parallel with serological modifications (Table 1). However, it should be mentioned that IFN has been shown to induce renal lesions in other species of mice also (Swiss). In these experiments, IFN was either inoculated at birth in strong concentrations or was of endogenous origin (Gresser *et al.*, 1976, 1978).

It has been reported that ageing NZB/W mice have a reduced number of recirculating T cells, impaired T function and proliferation in response to T cell mitogens (Steinberg & Reinetsen, 1977). In our study the response to mitogens of splenic and lymph node lymphocytes was not significantly different between NZB/W cells and control BALB/c cells, or between IFN-treated and other groups of NZB mice. Thus, it may be assumed that IFN does not activate the development of autoimmunity by the inhibition of T cells functional in the lymphoblast transformation test. Alternatively, the target of IFN might be a B cell (Huston & Steinberg, 1979). However, the study of subpopulations of T and B lymphocytes in IFN-treated NZB/W mice may reveal more subtle modifications which have not been looked for in this study.

The administration of IFN is followed, after 1–24 hr, by cytolytic NK activity directed against different target cells with a peak at 16 hr (Senik *et al.*, 1979). The NK effect is age-dependent, being highest in young-adult animals (Haller *et al.*, 1979). It is also known that certain species of mice are better responders than others (Haller *et al.*, 1979). As seen above, NZB/W mice are among the good responders, at least until 7 months. However, no data have been published about NK activity in mice after continuous treatment with IFN. The present study suggests that long-term treatment with IFN can maintain a good level of NK activity in NZB/W mice. However, it seems that the NK response decreases after 5–6 months of age (3–4 months of treatment), while 7-month-old control NZB/W mice still respond well to a single dose of IFN (Fig. 3). Additional experiments are currently in progress, using a greater number of animals, in an attempt to determine the role of the different factors (i.e. age of mice, duration of IFN treatment, or the IFN dose) in the kinetics of the NK response in NZB/W mice. These findings could be of interest since NK activity is frequently viewed as a potentially important anti-tumour mechanism (Haller *et al.*, 1979).

In addition to the production of autoantibodies, NZB and NZB/W mice are predisposed to lymphoid neoplasia (Steinberg & Reinetsen, 1977). Tumours appear after month 12 in 9% of untreated animals, whereas in NZB/W mice treated with cyclophosphamide, tumour development is more frequent and begins significantly earlier. It is also preceded by the disappearance of autoantibodies (Walker & Anver, 1979). In our study tumours appeared 4–8 weeks after the onset of the treatment with IFN or mock, when the animals were only aged 4–5 months (Fig. 1). At that time autoantibodies were present in most of the animals tested (data not shown).

The question may arise whether IFN could have a tumour-inducing role in immunologically abnormal animals. Indeed, Ryd *et al.* (1979) have demonstrated that in CBA and C57Bl/6J inbred mice, the same dose of IFN that depressed the growth of ascitic tumours enhanced the growth of the corresponding solid tumours. However, in our study incidence of tumours in NZB/W mice which had received mock preparations was the same as in the IFN-treated group (Fig. 1). The search for tumour-inducing impurities present in both IFN and mock preparations is indicated. So far, no proof is available that such impurities exist, and the origin of NZB/W tumours is still undetermined.

In most cases the tumours developing in NZB/W mice are lymphomas: reticulum cell sarcoma or lymphoblastic tumours or variants thereof (Datta *et al.*, 1978) In our experiments, almost all

tumours were ascitic. Morphologically they were lymphoblastic in nature. Nevertheless, the search for antibody-forming B cells and for the main T cell marker (Thy 1·2) was negative. Therefore, as there is a high proportion of null cells in NZB/W mice (Michalsky, McCoombs & Talal, 1979), an attempt was made to determine if NZB/W tumours originated from NK cells. A similar finding was made in SJL/J mice by Fitzgerald & Ponzio (1979). The absence of NK response in tumour-bearing Swiss mice grafted with NZB/W cells did not support this assumption.

The presence of ecotropic MuLV in NZB/W cells was not unexpected since NZB/W mice express both ecotropic and xenotropic viruses (Levy *et al.*, 1975; Stephenson *et al.*, 1975).

The anti-tumour effect of combined treatment with IFN and ISO was described by Cerutti *et al.* (1979). In the present experiments, ISO given alone displayed a significant anti-tumour action. This was also recorded in mock + ISO-treated mice (Fig. 1). However, ISO did not prevent the appearance of small tumour nodules, as detected in some NZB/W mice when they were killed at 12 months.

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## REFERENCES

BOURGEADE, M.F. & CHANY, C. (1979) Effect of sodium butyrate on the antiviral and anticellular action of interferon in normal and MSV-transformed cells. *Int. J. Cancer*, **24**, 314.

BOYER, B., GISSELBRECHT, S., DEBRE, P., MCKENZIE, J. & LEVY, J.P. (1980) Genetic control of sensitivity to Moloney leukemia viruses in mice. IV. Phenotypic heterogeneity of the leukemic cells. *J. Immunol.* **125.** (In press.)

CERUTTI, I., CHANY, C. & SCHLUMBERGER, J.F. (1979) Isoprinosine increases the antitumor action of interferon. *J. Immunopharmacol.* **1**, 59.

CHANY, C. & CERUTTI, J. (1977) Enhancement of antiviral protection against encephalomyocarditis virus by a combination of isoprinosine and interferon. *Arch. Virol.* **55**, 225.

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

DEMAEYER, E., DEMAEYER-GUIGNARD, J. & VANDEPUTTE, M. (1975) Inhibition by interferon of delayed-type hypersensitivity in the mouse. *Proc. Natl. Acad. Sci. USA*, **72**, 1953.

ENGLEMAN, E.G., BANTING, G., MERIGAN, T. & MCDEVITT, O. (1979) Type II interferon accelerates glomerulonephritis and death in NZB/NZW mice. *Arthritis Rheum.* **22**, 606.

FITZGERALD, K.L. & PONZIO, N.M. (1979) Natural killer cell activity in reticulum cell sarcomas (RCS) of SJL/J mice. *Cell. Immunol.* **43**, 185.

GINSBERG, T. & LUEBBEN, G.E. (1971) Metabolic studies of inosine in combination with *N-N'*-dimethyl-1-aminopropan-2-ol. *Fed. Proc.* **30**, 388 (abstract No. 1095).

GISLER, R.H., LINDAHL, P. & GRESSER, I. (1974) Effects of interferon on antibody synthesis *in vitro*. *J. Immunol.* **113**, 438.

GRESSER, I. (1977) On the varied biological effects of interferon. *Cell Immunol.* **34**, 400.

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

GRESSER, I., MOREL-MAROGER, L., VERROUST, P., RIVIÈRE, 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.

HALLER, O., KIESSLING, R., GIDLUND, M. & WIGZELL, H. (1979) Natural killer cells to leukemia: *in vitro* and *in vivo* studies. In *Tumor-Associated Antigens and their Specific Immune Responses* (ed. by F. Serafico and R. Arnon), p. 151, Academic Press, New York.

HALPERN, B. & FREY, A. (1969) Déclenchement de l'anémie hemolytique autoimmune chez de jeunes souriceaux NZB par l'administration de *Corynebacterium parvum*. *Ann. Inst. Pasteur*, **117**, 778.

HARTLEY, J.V. & ROWE, W.P. (1975) Clonal cell lines from mouse embryos which lack host range restrictions for murine leukemia viruses. *Virology*, **65**, 128.

HERBERMAN, R.B., HOLDEN, H.T., WEST, W.H., BONNARD, G.D., SANTONI, A., NUNN, M.E., KAY, H.D. & ORTALDO, J.R. (1979) Cytotoxicity against tumors by NK and K cells. In *Tumor-Associated Antigens and their Specific Immune Responses* (ed. by F. Serafico and R. Arnon), Vol. 16, p. 130. Academic Press, London.

HEREMANS, H., BILIAU, A., COLOMBATTI, A., HILGERS, J. & DE SOMMER, P. (1978) Interferon treatment of

NZB mice: accelerated progression of autoimmune disease. *Infect. Immun.* **21,** 925.

HUSTON, D.P. & STEINBERG, A.D. (1979) Animal models of human systemic lupus erythematosus. *Yale J. Biol. Med.* **52,** 289.

KAHAN, A., PERLIK, F., LEGO, A., DELBARRE, F. & GIROUD, J.P. (1976) Adjuvant induced arthritis in four inbred strains of rats. *Agents Actions*, **6,** 219.

KIESSLING, R., PETRANYI, G., KÄRRE, K., JONDAL, M., TRACEY, D. & WIGZELL, H. (1976) Killer cells: a functional comparison between natural, immune T cells and antibody dependent *in vitro* system. *J. exp. Med.* **143,** 772.

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

LEVY, J.A., KAZAN, P., VARNIER, O. & KLEINMAN, H. (1975) Murine xenotropic type C viruses. I. Distribution and further characterization of the virus in NZB mice. *J. Virol.* **16,** 844.

MACH, P.S., PIATIER, D., LEGO, A. & DELBARRE, F. (1979) Interaction between IgM antiglobulins and IgG antinuclear antibodies. Some aspects of D-penicillamine. *Clin. exp. Immunol.* **36,** 311.

MICHALSKY, J.P., MCCOOMBS, C.C. & TALAL, N. (1979) Suppressor cells and immunodeficiency in (NZB × NZW)$F_1$ hybrid mice. *Eur. J. Immunol.* **9,** 440.

PEEBLES, P.V. (1975) An *in vitro* focus-induction assay for xenotropic murine leukemia viruses, feline leukemia virus C and the feline-primate virus RD-114/ccc/M-7. *Virology*, **67,** 288.

ROUSSET, S. (1974) Refractory state of cells to interferon induction. *J. gen. Virol.* **22,** 9.

RYD, W., HAGMAR, B., LUNDGREN, E. & STRANNEGARD, O. (1979) Discrepant effect of interferon on murine syngeneic ascites tumors and their solid metastasizing counterparts. *Int. J. Cancer*, **23,** 397.

SENIK, A., GRESSER, I., MAURY, C., GIDLUND, M., ANDERS, O. & WIGZELL, H. (1979) Enhancement by interferon of natural killer cell activity in mice. *Cell. Immunol.* **44,** 186.

SERGIESCU, D., CERRUTI, I., EFTHYMIOU, E., KAHAN, A. & CHANY, C. (1979) Adverse effects of interferon treatment on the life span of NZB mice. *Biomedicine*, **31,** 48.

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

STEINBERG, A.D. & REINETSEN, J.L. (1977) Lupus in New Zealand mice and dogs. *Bull. Rheum. Dis.* **28,** 940.

STEPHENSON, J.R., REYNOLDS, R.K., TRONICK, S.R. & AARONSON, S.A. (1975) Distribution of three classes of endogenous type C-RNA viruses among inbred strains of mice. *Virology*, **67,** 404.

STRANNEGARD, O., LARSSON, I., LUNDGREN, E. MORNER, H. & PARRSON, H. (1978) Modulation of immune response in newborn and adult mice by interferon. *Infect. Immun.* **20,** 334.

TALAL, N. (1976) Disordered immunologic regulation and autoimmunity (1976). *Transplant. Rev.* **31,** 240.

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. (1979) Accelerated appearance of neoplasms in female NZB/NZW mice treated with high dose cyclophosphamide. *Arthritis Rheum.* **22,** 1338.

*Note added in proof*

Observations in agreement with ours concerning the aggravating effect of IFN on autoimmunity in NZB/NZW mice have been published after this manuscript was submitted (Adam *et al.*, *Clin. exp. Immunol.* **40,** 373).