Acta virol. **25** : 283—294, 1981

ACCELERATED APPEARANCE OF AN ASCITIC TUMOUR AFTER LONG-TERM APPLICATION OF INTERFERON TO NZB/W MICE

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Received April 30, 1981

Summary. — In two similarly designed experiments female NZB/W mice treated with mouse L-cell interferon developed an ascitic tumour when the treatment was begun at 3.5 months of age. But no tumour appeared within a 6 months' observation period when 6 months old mice were used. A transient rise of anti-DNA antibodies preceded the tumour appearance in mice. The ascitic tumour was sensitive to antimetabolites and insensitive, or marginally sensitive, to alkylating agents or interferon. The tumour cells were adapted to growth in suspension in vitro. They caused tumour growth in various inbred and random-bred mouse lines. Attempts to transfer the tumour to Swiss mice with cell-free preparations were unsuccessful.

Key words: interferon; NZB/W mice; ascitic tumour

Introduction

NZB/W hybrid mice carry both xenotropic and ecotropic murine leukaemia viruses (C particles) and their superinfection with various viruses often aggravates their autoimmune diseases (Levy 1975; Tonietti *et al.*, 1980). These findings suggest a causative role of leukaemia viruses in the immunological disorder. Another possible aetiological factor implicated in the development of the NZB/W disease is an inborn immune defect which involve the thymus-regulated cell-mediated immunity. According to this hypothesis, the loss of suppression exerted by thymus leads to an accelerated and enhanced production of autoantibodies (Steinberg, 1975).

Taken together, such considerations justify attempts to modify the course of the NZB/W disease by antiviral as well as immunosuppressive substances (Lambert and Dixon, 1968; Walker *et al.*, 1978; Walker and Anwer, 1978; Heremans *et al.*, 1978). Since the replication of C-type viruses has been shown to be susceptible to the antiviral effect of interferon (IFN) and IFN effectively

suppressed the immune response in mice (Billiau *et al.*, 1973; Johnson and Baron, 1976). The exploration of its effect on the NZB/W disease seems to be of both theoretical and practical interest.

Materials and Methods

Experimental animals. The hybrid NZB/W mice were obtained from the Institute of Sera and Vaccires, Prague. They originated from the Breeding Farm of the University of Vienna, Austria (NZB), and the MRC Laboratory Animal Centre, Carshalton, United Kingdom (NZW), respectively. They were 3-3.5 months old in the first 2 series of experiments and 6.5 months old in the third series. The average weight of mice was 29 $\pm$ 2.5 g in the 1st and 2nd series and 32 $\pm$ 2.9 g in the third series. C57B1 and DBA/2-1 inbred mice as well as HA (Swiss) random-bred mice were grown in the Institute of Experimental Oncology, Slovak Academy of Sciences, Bratislava. Swiss random-bred mice from the Breeding Farm of the Slovak Academy of Sciences were also used.

Interferon (IFN) was prepared in LG cells (a subline of L-929 mouse fibroblast cells) using Newcastle disease virus (NDV) strain B1 as inducer. The crude interferon preparation was precipitated with Zn-acetate and subsequently thoroughly (7 days) dialyzed according Fuchsberger *et al.* (1975). The antiviral potency of the preparation, when titrated by the cytopathic effect inhibition method in L-929 cells (Lackovič and Borecký, 1955) contained approximately 1.5×10^4 IFN units pre ml corrected for an internal standard. Specific activity of the preparation was 5×10^3 units per mg of protein.

Anti-interferon (anti-IFN) serum was obtained by long-term immunization of sheep with L-cell interferon. Anti-IFN globulin and normal sheep serum globulin were prepared by ammonium sulfate precipitation (Fuchsberger and Borecký, 1978). The anti-IFN potency of the preparation and of sera from IFN-treated mice was assayed in LG cells as described by Fuchsberger and Borecký (1978). The titre of anti-IFN serum was 4000 anti-IFN units per ml. when tested for the neutralizing capacity of 16 IFN units.

Treatment of NZB/W mice. Three series of experiments were performed. In the 1st series, the mice were divided into 3 groups. The first group of 20 female and 6 male mice received 5 times weekly 5000 units of IFN intraperitoneally (i.p.). The second group of 20 female and 6 male mice was injected twice weekly with 250 units of anti-IFN serum. The third group of 20 mice was not injected and served as control. The injected volume was 0.3 ml per mouse. In the 2nd series only females were used and two additional groups of NZB/W mice were included as control: 16 mice received twice weekly 0.3 ml phosphate buffered saline (PBS) i.p. and another group of 20 mice was given twice weekly normal sheep globulin. In the 3rd series of experiments, two groups of female NZB/W mice were used. A group of 10 mice served as a control and received PBS twice weekly. Ten mice in the second group were injected 5 times weekly i.p. with 5000 units of IFN as in the first two series of experiments. After finishing these 3 series of experiments (6 months each), additional control tests were performed in NZB/W mice with $ZnCl_2$ and mock interferon preparations. The rationale of these control experiments was the possibility that the tumour-inducing agents are either traces of $ZnCl_2$ remaining in IFN preparations (after, precipitation and dialysis of IFN) or proteins that were coprecipitated with IFN and were not removed from the preparation.

Autoantibodies against native calf thymus DNA and synthetic polyinosinic-polycytidylic acid (Miles Laboratories, U.S.A.) were titrated by the method described by Poverennyi *et al.* (1975) using ^{125}I-labelled poly I : C and ^{3}H-labelled DNA as antigens. This assay quantitated the per cent of antigen bound to 5 μl (^{3}H-labelled DNA) or 10 μl ^{125}I-labelled poly I : C) of mouse sera. By this method, control rabbit anti-poly I : C sera gave values of 62.8 — 63.5 %, whereas with normal serum the values of binding were 4.3 — 4.6 %. Sera from patients with systemic lupus erythematosus were used as control anti-DNA sera.

Sensitivity of the tumour cells to cytostatics. The following cytostatics were tested: Cyclophosphamide (Jenafarm, Rudolstadt, G.D.R.) — 20, 30 and 40 mg per kg body-weight per day; Methotrexate (Lederle, U.S.A.) — 2 and 20 per kg body-weight per day; Vincristine (Lilly, U.S.A.) — 0.5 mg per kg body-weight per day, and 5-fluorouracil (Medexport, U.S.S.R.) — 25 mg per kg body-weight per day. IFN was used in a dose of 5000 units per mouse.

Karyological analysis. The chromosome preparations were analyzed according to Rothfels and Siminovitch (1958).

Histology. For light microscopy, paraffin sections from liver, spleen, lungs, kidneys, omentum maius and perirenal fat tissue were prepared and stained with haematoxlyin and eosin.

Electron microscopy. Cells obtained from ascitic fluid of mice were pelleted by centrifugation, fixed with 2.5 % glutaraldehyde in 0.2 mol/l Na-cacodylate buffer for 30 min at 4 °C and post-fixed with 1 % OsO_4 in 0.1 mol/l phosphate buffer pH 7.2 for 1 hr at room temperature. After dehydration, the samples were embedded in Araldit. Thin sections were stained with 2 % uranyl acetate and lead citrate and examined in a Philips EM 300 electron microscope at 80 kV. The pellet of viral particles was negatively stained with 2 % phosphotungstic acid, pH 7.0.

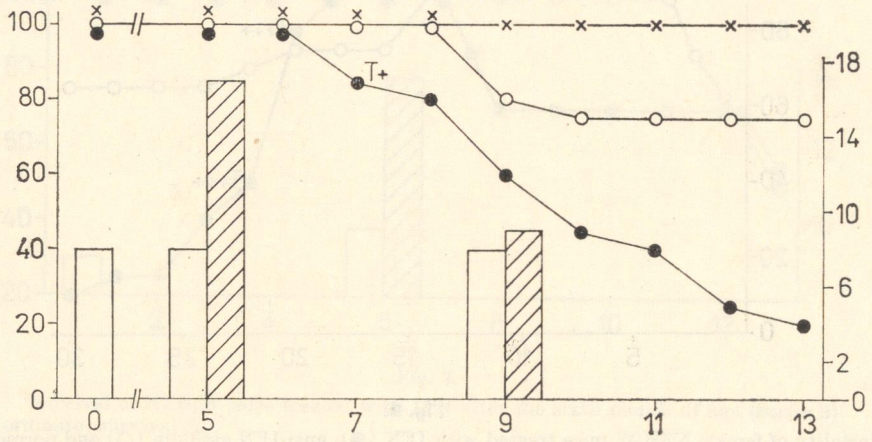

Fig. 1.

Anti-DNA antibodies and cumulative mortality of IFN-treated NZB/W female mice (Series 1)
Left ordinate: survival %
× — control untreated mice
○ — IFN-treated male mice
● — IFN-treated female mice
Right ordinate: anti-DNA antibodies in % of binding
Empty columns — untreated female mice; shaded columns — IFN-treated female mice
Abscissa: weeks after onset of IFN treamtnet (IFN treatment was continued for 10 weeks)
T+: appearance of the first tumour.

Results

Appearance of tumours

After continuous administration of 25000 units per week of mouse IFN to mice, unexpectedly an ascitic tumour was detected in a female mouse 7.5 weeks after the onset of the treatment in the 1st series of experiments (Fig. 1). Further similar tumours appeared in females of the IFN-treated group in the subsequent weeks. The tumour was diagnosed by enlarged soft abdomen, an unusual increase of the cell number in the peritoneal washing, and a weight arrest. The number of cells in the peritoneal exudate reached $80-150 \times 10^6$ cells per ml. The occurrence of tumours enhanced the mortality of IFN-treated

mice. No tumours were detected in male mice and their mortality, in comparison with the uninjected mice, was only slightly enhanced. After 13 weeks of IFN treatment, 4 of 6 mice in the male group were still alive. A sudden rise of anti-DNA antibodies was observed in the IFN-treated group immediately before tumour appearance. Unfortunately, after 8.5 weeks of treatment

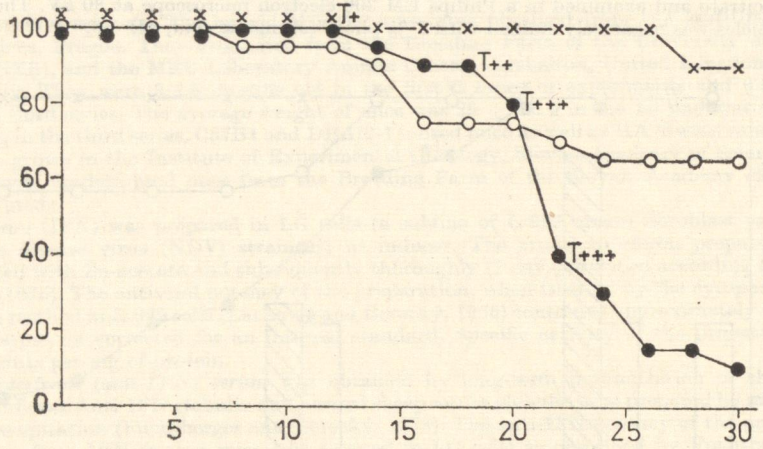

Fig. 2.

Mortality of female NZB/W mice treated with IFN (●), anti-IFN globulin (○) and normal globulin (×) (Series 2)

Ordinate: survival %

Abscissa: weeks after onset of IFN treatment (IFN treamtnet was continued for 25 weeks)

T+, T++, T+++: appearance of 1, 2 or 3 tumour-carrying mice, respectively

a similar type of tumour was also found in the group of NZB/W mice given anti-IFN globulin in weekly 500 anti-IFN units per mouse (not shown). The time of appearance of this tumour suggested the possibility of a transfer from the IFN-treated mice. No tumours were detected in the control group during an observation period of 6 months.

The finding of the ascitic type tumour in the anti-IFN globulin-treated group obscured the interpretation of the causative mechanism and made a second series of more carefully controlled experiments necessary. In this, only female mice were employed and further control groups were included. The ascitic tumour appeared again in the IFN-treated group but not in the anti-IFN globulin-treated group thus supporting the suspicion of a contamination in the anti-IFN globulin group in the first series of experiments. In the 2nd series, the tumours appeared later, i. e. after 12 weeks of treatment of the mice with IFN. The last mouse in this group died 30 weeks after the onset of experiment (Fig. 2). The suspicion of contamination with tumour cells in the anti-IFN globulin group was supported by the finding that the tumour is easily transferable to various mouse strains (see below).

In the 3 series of experiments on 6.5 months old female mice, the IFN treatment did not lead to tumour appearance and all mice survived the observation period of 6 months. However, their mortality increased during administration of a second series of IFN injections (Fig. 3). In two additional control tests on a mock IFN preparation and $ZnCl_2$ (0.4—40 μg per mouse per day) no tumours appeared within 6 months. However, an ascitic tumour appeared in one saline-treated mice after 9 months.

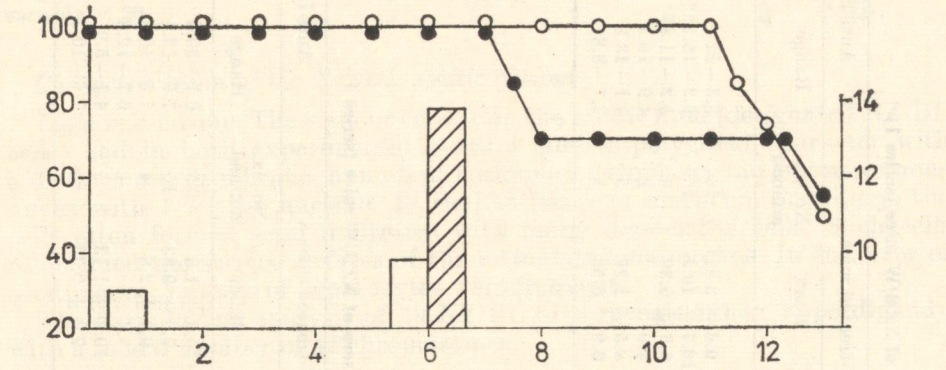

Fig. 3.

Survival of NZB/W mice treated with IFN after the sixth month of age (Series 3)
Left ordinate: survival %

○ — untreated mice
● — IFN-treated mice

Right ordinate: anti-DNA antibody in % of binding

empty columns — untreated mice
shaded columns — IFN-treated mice

Abscissa: months after onset of IFN treatment (IFN treatment was applied during the first 5 months, then discontinued for 2 months, and renewed during the 7th—9th month).

The tumour-bearing mice in the 1st and 2nd series of experiments died usually 1-3 weeks after detection of the ascitic abdomen. Females in all treated groups showed a weight loss when compared with males. As shown on Figs 1 and 3, IFN treatment of mice was accompanied by a presumably transient increase in the level of anti-DNA antibodies in their sera. Eight of 15 mice in the IFN-treated group had anti-DNA values of over 15 %. In other groups such values were exceptional. Interestingly, the level of anti-poly I : C antibodies remained, on the average, under the 15 % level (Tables 1 and 2). The raise of anti-DNA antibodies seemed to coincide also with an increased clearance of IFN from the sera of IFN-treated mice (Fig. 4). No significant increase in the level of anti-IFN antibodies could be detected during treament in this mice. The spleens and livers of tumour-bearing mice were often but not regularly enlarged.

Table 1. Binding of ^{3}H-DNA by sera of NZB/W mice (series 1)

Treatment (weeks)	Sex	Control mice			IFN-treated mice			Anti-IFN treated mice		
		Range	Mean	No. examined	Range	Mean	No. examined	Range	Mean	No. examined
—	F	6.0—10.6	8.2 ± 1.2	15	5.7—20.3	9.6 ± 3.8	15	5.3—22.2	9.5 ± 4.35	15
5	F	5.7—13.5	8.5 ± 2.08	15	7.6—43.7*	16.7 ± 10.5	15	6.2—16.2**	9.3 ± 2.25	15
9	F	5.9—7.7	6.7 ± 1.3	3	6.0—7.0	6.6 ± 0.39	4	5.2—11.8	6.9 ± 2.15	4
13	F	6.6—9.0	7.6 ± 1.75	5	4.1—5.7	4.9	1	4.9—10.5	6.8 ± 1.76	6
—	M	4.9—7	6.1 ± 0.75	5	6.5—12.5	8.5 ± 2.27	5	4.1—13.8	1.1 ± 3.3	5
13	M	4.6—9.9	6.6 ± 1.5	5	6.4—7.8	6.9 ± 0.58	5	7.7—8.8	8.2 ± 0.43	4

Each serum sample was assayed twice. Values in per cent.
* Eight samples with values over 15 %.
**One sample with value over 15 %.

Table 2. Binding of ^{125}I-poly I: poly C by sera of NZB/W mice (series 1)

Treatment (weeks)	Sex	Control mice			IFN-treated mice			Anti-IFN-treated mice		
		Range	Mean	No. examined	Range	Mean	No. examined	Range	Mean	No. examined
—	F	9.6—26.9	12.4 ± 4.84	15	7.5—13.6	10.9 ± 1.8	15	6.7—30.9	10.6 ± 6.44	15
5	F	4.1—11.3	6.4 ± 2.25	15	6.2—9.0	7.3 ± 0.83	15	7.9—13.1	9.8 ± 2.66	15
9	F	6.0— 9.8	8.0 ± 1.65	3	6.6—13.9	9.6 ± 2.8	4	7.5—10.3	9.0 ± 1.28	4
13	F	8.5—17.5	10 ± 3.4	5	6.9—7.9	7.4	1	6.7—12.3	01.4 ± 2.22	6
—	M	6.3—12.5	9.7 ± 2.35	5	9.0—11.5	10.3 ± 1.13	5	4.1—5.9	5.4 ± 0.69	5
13	M	5.9—9.8	7.4 ± 1.4	5	6.4—7.3	7.0 ± 0.35	8	7.6—10.3	8.7 ± 1.15	4

Each serum sample was assayed twice. Values in per cent.

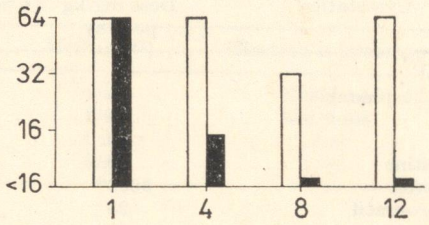

Fig. 4.
Effect of prolonged IFN treatment on IFN levels in the sera of NZB/W mice
Abscissa: weeks of IFN treatment; ordinate: IFN titre (units/ml)
Empty columns: IFN in sera of control mice 20 min after i. p. injections
Black columns: IFN in IFN-treated mice
The anti-IFN titre of sera from IFN--treated mice (samples taken 24 hr after IFN administration) were invariably < 20.

Characterization of the NZ-BL ascitic tumour

Light microscopy. The tumour cells from the ascitic fluid (designated NZ-BL cells) had in both experimental series a similar polygonal character with a distinct cell membrane, abundant eosinophil cytoplasm and leptochromous nuclei with 1-2 large nucleoli. In the fat tissue of omentum or kidney, the cells often formed solid infiltrates with many dissociated cells. Such cells often lined the serous surfaces of the intraabdominal organs. In the liver of ascitic mice, mitoses of hepatocytes were frequent.

The karyological analysis of 50 NZ-BL cells revealed their hyperdiploidy with a modal number of 42 chromosomes:

No. of chromosomes:	40	41	42	43	44
No. of metaphases:	1	9	24	13	3

In all metaphasic cells, one large metaphasic marker chromosome was found.

Electron microscopy of tumour cells revealed the presence of numerous intracisternal A-type particles 90-120 nm in diameter. They were in various stages of maturation that took place in a viromatrix consisting of numerous cisternae of the smooth endoplasmic reticulum with a fibrillar structure. The nuclei were small, elongated and excentrically located. Free and budding immature C (A-type) viral particles were also present. The extracellular immature C (A-type) particles were coated with a unit-membrane structure which enclosed a nucleoid.

Growth characteristics of the NZ-BL cells. The tumour could be easily transplanted to mice and rats. As few as 10 NZ-BL cells from the 3rd i. p. passage of ascitic cells were capable of producing ascitic tumor in HA (Swiss) mice with a 75 % efficiency. A higher number of NZ-BL cells caused tumour appearance regularly with a 100 % efficiency. The survival of mice after inoculation of tumour cells was dose-dependent. Mice injected i. p. with 10^5—10^6 NZ-BL cells died within 3 weeks, while those injected with 10^2—10^4 NZ-BL cells dies after 4-5 weeks. The $C_{57}B1$ and DNA/2-1 inbred mice showed comparable sensitivity to the oncogenic effect of NZ-BL cells. Also, the subcutaneous route proved to be effective in the transfer of the tumour in rats but, as yet, not in hamsters.

We succeeded in adapting the NZ-BL cells to the growth in suspension in vitro. After 20 such in vitro passages at 3-4 days' intervals in Eagle's

Table 3. Sensitivity of the NZ-BL tumour cells to cytostatics in DBA/2-1 mice (series 1)

Cytostatics	Dose mg/kg per day	Treatment on days	Average survival time (days)
Control	—	—	23.1 ± 2.0
Cyclophosphamide	30	1	21.2 ± 2.0
	20	1—4	27.1 ± 5.2
	40	1—4	29.8 ± 3.0
Vincristine	0.5	1	23.5 ± 2.7
Interferon	5000*	1—5	31.0 ± 3.0
5-Fluorouracil	25	1—4	38.7 ± 7.1
Methotrexate	20	1	47.8 ± 21.4
	2	1—4	45.8 ± 17.7
	20	1, 3, 6	64.2 ± 20.9

*5000 units per mouse per dose.

Minimal Essential Medium supplemented with 10 % tryptose phosphate broth and 5 % inactivated calf serum, the tumour-inducing capacity of NZ-BL cells [NZ-BL(Sv) line] in mice was unchanged.

Sensitivity of NZ-BL cells to cytostatics. As shown in Table 3, DBA/2-1 mice injected with 10^5 NZ-BL cells on day 0 died on the evarage after 23 ± 2 days. The survival of such mice was only slightly increased by 5 daily injections of mouse IFN or by cyclophosphamide administered on days 1 to 4 in doses of 20-40 mg/kg/day. Also Vincristine apparently had no effect on the survival of mice. On the other hand, Methotrexate in doses from 2 to 20 mg/kg/day significantly increased the lifespan of DBA/2-1 mice. Also 5-fluorouracil seemed to be effective in this respect. These results indicate that the growth of tumour cells was sensitive to antimetabolites but not to alkylating agents.

Effect of IFN on tumorigenicity and growth of NZ-BL cells. Incubation in vitro of 10000 or NZ-BL cells in the presence of 1000 units of mouse IFN for 3 hr at 37 °C delayed the death of Swiss mice when the inoculum of NZ-BL

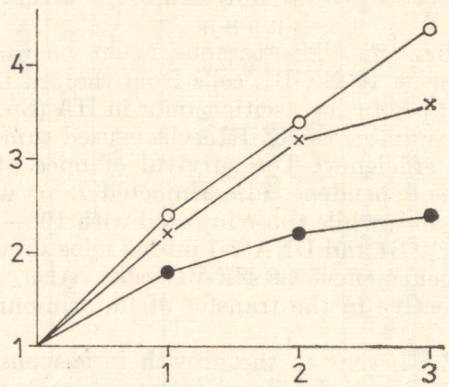

Fig. 5.

Effect of IFN on growth of NZ-BL tumour cells in vitro (Series 1)
Abscissa: days; ordinate: No. of cells per ml × 10^{-5}
× — Control untreated cells
○, ● — Cells treated with 2 (○) or 500 (●) units of IFN

Table 4. Attempts to transfer the NZ-BL tumour with cell-free ascites preparations to Swiss mice (series 1)

Preparation	Tumorigenicity[1])	Mortality[2])	Electron microscopy[3])
Supernate 1500 × g	4/4	4/4	Not done
Filtrate G5[4])	0/5	2/5	Not done
Pellet 1000,000 × g	0/4	0/4	+
Supernate 100,000 × g	0/4	0/4	−

[1]) No. of tumour-bearing/No. of inoculated mice.
[2]) No of dead/No. of inoculated mice as evaluated after 3 weeks.
[3]) + and −: presence and absence of virus-like particles; respectively.
[4]) Filtrate through a sintered glass (G5) filter with an average pore diameter of 0.5 μm.

cells was high (10000 cells per 0.3 ml), but not when it was low (100 cells per ml). The dynamics of multiplication of NZ-BL (Sv) cells treated with two doses of mouse IFN in vitro is shown in Fig. 5.

Interferon production by NZ-BL cells was tested both in the line adapted to growth in vitro [NZ-BL(Sv)] and that passaged in vivo by the i. p. route. NZ-BL (Sv) cells produced IFN when stimulated in vitro with about 256 haemaglutinating units of NDV per ml the IFN titres reached with 1, 2, 4, 6, 8 and 10 × 10^5 NZ-BL(Sv) cells were 64, 128, 256, 512, 512 and 256, respectively, while the IFN titre obtained with the same amounts of non-stimulated cells was invariably < 16. An IFN amount comparable to that in control mice was found in ascitic fluid from the peritoneal cavity of Swiss mice.

Attempts to transfer the NZ-BL tumour to mice by cell-free preparations. As shown in Table 4, attempts to transfer the tumour to newborn and adult Swiss mice with a cell-free filtrate or the pellet obtained after centrifugation of the filtrate at 100 000 × g were unsuccessful, although virus particles were found in the 100 000 × g pellet (Fig. 6, see Plate XLII) and the G-5 filtrate killed 2 of 5 inoculated mice.

Discussion

Various agents such as viruses, DNA or polyinosinic-polycytidylic acid have been shown to accelerate the autoimmune diseases of NZB and NZB/W (hybrid) mice (Steinberg, 1975; Tonietti *et al.*, 1980). The mechanism of acceleration remains unclear since natural ds-RNA, in contradistinction to poly rI : rC, enhanced the survival of these mice in some studies (Kleinschimdt *et al.*, 1968; Rovenský *et al.*, 1975). On the other hand, hydrocortisone, cyclophosphamide and Tilorone have been found to prolong the lifespan of NZB/W mice (Walker *et al.*, 1978; Walker and Anwer, 1978; Gabriel, 1971). Two observations deserve attention in these reports. First, a high incidence of solid neoplasms has accompanied the prolonged survival of NZB/W mice. Second, the effect seems to be age-dependent since, in contrast to older mice. Tilorone had an accelerating effect on the development of the disease in young mice.

Both genetic and infectious factors have been implicated in the development of the autoimmune disease (Lambert and Dixon, 1968; Levy, 1975; Tonietti *et al.*, 1980). This justifies the attempts to influence the course of the disease by antiviral and/or immunosuppressive treatments. Since IFN unifies the requirements for such a substance, after encouraging results with Statolon (Lambert and Dixon, 1968; Kleinschimdt *et al.*, 1968) and Methisazon (Gabriel, 1971), several studies have dealt with its effect in NZB or NZB/W mice. Surprisingly, a beneficial effect of IFN could not be demonstrated and, in fact, IFN had both an accelerating effect on the development of the disease and shortened the lifespan of mice (Heremans *et al.*, 1978; Banting *et al.*, 1979; Sergiescu *et al.*, 1979). Again, the mechanism of acceleration remains unclear.

In our study, prolonged IFN treatment of NZB/W hybrid mice was tested in 2 age groups (3 and 6 months, respectively). Whereas the treatment led to the appearance of an ascitic tumour in about 45 % of the younger mice, no tumour could be detected in the older group treated by the same schedule. Only female mice were found to develop the ascitic tumour. The tumour appeared relatively shortly after the onset of IFN treatment (7 and 12 weeks in two series, respectively) and its appearance was accompanied by a rise of anti-DNA antibodies, enhanced clearance of IFN and weight arrest of mice. The role of the sudden and transient increase of anti-ss-DNA antibodies in the appearance of the tumour remains unexplained. It may reflect a reaction to stress because a similar phenomenon was observed in our previous study in which NZB/Swiss hybrid mice were treated with sonicated phage ds-RNA (Pekárek *et al.* 1979). Recently, also Adam *et al.* (1980) observed this phenomenon. On the other hand, the subsequent decrease of anti-DNA antibodies (as found in this study) may simply represent the antibody level of resistant mice and does not necessarily prove their transitory character.

Induction of tumours in mice as a consequence of IFN administration in our first experiment was unexpected. Nevertheless, it was confirmed in a repeated, similarly designed experiment with a new batch of NZB/W mice. The tumour does not seem to possess a high invasivity since metastases were not found in tumour-bearing mice. On the other hand, it leads to an accelerated mortality in mice (Fig. 1 and 2). As could be expected from its presence in IFN-treated mice, the tumour proved only moderately sensitive to cell-growth inhibition by IFN or alkylating agents. However, its growth in mice could be effectively suppressed by antimetabolites such as Methotrexate and 5-fluorouracil. The adaptation of the NZ-BL tumour cells [designated NZ-BL (Sv) cells] to growth in suspension in vitro was successful.

As stressed by Walker *et al.* (1978), NZB/W mice have two characteristics which should predispose them to tumour development. First, they carry C-type viruses and viral superinfection often aggravates the underlying disease (Levy, 1975; etc.). Second, they lose the T-cell mediated protection in older age. Although in both the NZ-BL tumour cells and their cell-free supernates virus-like particles were found by electron microscopy, attempts

to transfer the ascitic tumour to untreated mice by cell-free reparations were, as yet, unsuccessful. The possibility that the tumour was caused by continuous administration of Zn ions remaining in the IFN preparation after its concentration, or by an unknown constituent of the final IFN product seems, at this time, improbable since prolonged treatment of NZB/W mice with $ZnCl_2$ or a mock-IFN preparation (supernate from L cells precipitated by Zn-acetate) in additional control experiments failed to lead to tumour appearance in mice during a 6-month period.

On the other hand, a stress-responsive endocrine (corticosteron and/or sex-hormones) -mediated mechanism (Seifter *et al.*, 1976) as a tumour-activating factor in our experiments cannot be excluded since, after a prolonged period, the tumour appeared even in saline-treated mice. This indicates that IFN accelerates not only the development of the basic autoimmune disease but also the apperance of a spontaneous (?) tumour. Since normal sheep globulin, anti-IFN globulin and mock IFN proved unable to induce tumours in the same time period, this suggests a "specific" role of IFN in the tumour acceleration mechanism that awaits elucidation. The link might be in the findings that a rise of anti-DNA antibodies preceded tumour appearance in sera of the IFN-treated group and that it was associated with an acceleraated clearance of IFN from the serum.

In summary, the results of our study indicate that long-term administration of IFN in predisposed individuals may function as a potential oncogenic factor.

References

Adam, C., Thona, Y., Ronco, P., Verronst, P., Tovey, M., and Morel-Maroger, L. (1980): The effect of exogenous interferon: acceleration of autoimmune and renal diseasesin (NZB/W) F1 mice. *Clin. exp. Immunol.* **40**, 373–382.

Banting, G., Merigan, T., and Mc Bewitt, H. O. (1979): Type II interferon accelerates glomerulonephritis and death in NZB/NZW mice. *Arthritis Rheum.* **22**, 606–607.

Billiau, A., Sobis, H., and De Somer, P. (1973): Influence of interferon on virus particle formation in different oncornavirus carrier cell lines. *Int. J. Cancer* **12**, 646–653.

Fuchsberger, N., and Borecký, L. (1978): Quantitative immunoelectrophoresis of human interferon. A new approach to characterization of interferon preparations. *Acta virol.* **22**, 238–240.

Fuchsberger, N., Hajnická, V., and Borecký, L. (1975): Antiviral and cell-growth inhibitory activities of highly purified L-cell interferon. An analysis of quantitative disproportions. *Acta virol.* **19**, 59–66.

Gabriel, R. (1971): 1-Methylisatin-3-thiosemicarbasone treatment of NZB × NZW hybrid mice. *Brit. J. exp. Pathol.* **52**, 271–275.

Heremans, H., Billiau, A., Colombatte, A., Holgers, J., and De Somer, P. (1978): Interferon treatment of NZB mice: Accelerated progression of autoimmune disease. *Infect. Immun.* **21** 925–930.

Johnson, H. M., and Baron, S. (1976): Regulatory role of interferons in the immune response. *IRCS J. med. Sci.* **4**, 50–52.

Kleinschmidt, W. J., Ellis, L. F., van Frank, R. M., and Murphy, E. B. (1968): Interferon stimulation by a double-stranded RNA of a mycophage in Statolon preparation. *Nature (Lond.)* **220**, 167–168.

Lackovič, V., and Borecký, L. (1965): The reticuloendothelial system and virus infection II. Production of interferon- and antibody-like substances in mouse peritoneal cells infected with myxoviruses in vivo. *Arch. ges. Virusforsch.* **17**, 619–630.

Lambert, P. H., and Dixon, F. J. (1968): Pathogenesis of the glomerulonephritis of NZB/W mice. *J. exp. Med.* **127**, 507—521.

Levy, J. A. (1975): Xenotropic C-type viruses and autoimmune diseases. J. *Rheum.* **2**, 135—149.

Pekárek, J., Rovenský, J., Doskočil, J., Rossman, P., Korčáková, L., Rovenská, E., Lackovič, V., Borecký, L., Švejcar, J., Miština, T., and Žitňan, D. (1979): The effect of native and sonicated double-helical polynucleotides on the course of spontaneous autoimmune disease in NZB/Swiss F2 mice. *Acta virol.* **23**, 481—488.

Poverennyi, A. M., Podgorodnichenko, V. K., Tsyplyakovskay, L. M., and Potopova, N. V. (1975): The study of the reaction between antibodies and DNA by the method of the adsorption of immune complexes on membrane filters (in Russian). *Mol. Biol.* **9**, 828—835.

Rothfels, K. H., and Siminovitsch, L. (1958): An air-drying technique for flattening chromosomes in mammalian cells grown in vitro. *Stain Technol.* **33**, 73—77.

Rovenský, J., Doskočil, J., Pekárek, J., Borecký, L., Žitňan, D., and Švejcar, J. (1975): Prevention of spontaneous autoimmunity to DNA in NZB/Swiss mice by treatment with natural double stranded RNA. *Immunology* **29**, 745—748.

Seifter, E., Chohen, M. H., and Riley, V. (1976): Of stress, vitamin A, and tumors. *Science* **193**, 74—75.

Sergiescu, D., Cerutti, I., Efthymion, E., Kahan, A., and Chany, C. (1979): Adverse effects of interferon treatment on the life span of NZB mice. *Biomedicine* **31**, 48—51.

Steinberg, A. D. (1975): Immune complexes and autoimmune disease, pp. 95—117 In C. Koprowski and H. Koprowski (Eds): *Viruses and Immunity*, Academic Press, New York.

Tonietti, G., Oldstone, M. D., and Dixon, F. J. (1980): The effect of induced chronic viral infections on the immunologic disease of New Zealand Mice. *J. exp. Med.* **132**, 89—109.

Walker, S. E., and Anwer, M. R. (1978): Stimulated autoantibody response and increased longevity in NZB/NZW mice treated with cyclophosphamide and tilorone. *Clin. exp. Immunol.* **33**, 453—462.

Walker, S. E., Anver, M. R., Schechter, S. L., and Bole, G. G. (1978): Prolonged lifespan and high incidence of neoplasms in NZB/NZW mice treated with hydrocortisone sodium succinate. *Kidney Int.* **14**, 151—157.