BRIEF REPORT

The Development of Lymphomas in Mice with Autoimmune Disorders Treated with Azathioprine

By T. P. Casey

NZB MICE develop autoimmune hemolytic anemia after 3 months of age and NZB × NZW F₁ hybrids develop lupus nephritis after 6 months of age, the disorders being analogous to autoimmune diseases in humans.¹ ²

Investigation was undertaken into the immunosuppressant effect of azathioprine on these disorders in mice. It is the purpose of this paper to report that the administration of the immunosuppressant drug, azathioprine, to a group of NZB mice and to a group of NZB × NZW hybrids was associated with a high incidence of malignant lymphoid tumours, particularly involving the thymus.

METHODS

A dose of 0.3 ml. of an alkaline solution of 3 mg. of azathioprine was injected into the thigh. For 30 g. mice this approximates 100 mg./kg./dose. Control mice of a similar age and sex were given injections of the alkaline solvent.

8 NZB male mice, aged 2 months, were given 3 doses of azathioprine per week for 1 month, 2 doses a week for 1 week and then 1 dose a week for a total treatment period of 6 months. Surviving animals were then electively killed when 8 months old.

12 NZB × NZW F₁ female hybrid mice, aged 3 months, were given 3 doses per week for 3 weeks, 2 doses a week for 1 week and then 1 dose a week for a total treatment period of 10 months, when the 3 surviving animals were electively killed when 13 months old.

RESULTS

Immunosuppression

There was no clear-cut difference in the autoimmune hemolytic anemia of the treated NZB mice, compared with the controls. On the other hand, there was clear evidence of modification of the renal lesions in the treated NZB × NZW hybrids. The 12 untreated NZB × NZW controls all died of uremia and had severe renal lesions on histological examination. In contrast, none of the treated animals developed uremia and in all of them the kidneys showed only minor histological evidence of lupus nephritis.

Malignant tumors

Six of the eight treated NZB male mice developed malignant thymomas.
These were large tumors, often showing extensive local infiltration and constricting the heart. In 4 of the 6 there were tumor deposits in other organs, particularly kidney, spleen, liver and lung, in varying combinations. No tumors were seen in 6 control animals.

Seven of the 12 treated NZB × NZW female hybrids developed malignant thymomas. All were locally invasive and in 6 of the 7 there was tumor in distant sites, especially kidney, spleen, liver and lung. In 3 of these 6 mice there was frank leukemia. No tumors were seen in 12 control animals.

Histologically in the earliest tumors there was widespread involvement of the thymic cortex and, to a lesser extent, of the medulla. The tumor mainly comprised large neoplastic lymphoreticular cells whose hyperchromatic nuclei often contained prominent nucleoli. Mitotic figures were numerous. Cytoplasm was usually scanty and showed varying degrees of pyroninophilia. Some of the largest cells had more vesicular nuclei and plentiful cytoplasm which often contained nuclear debris. The overall pattern was that of the "starry-sky" appearance seen in some lymphoblastic neoplasms. Similar histological appearances were seen in the tumor in distant sites.

The first animals with malignant thymomas to die during the experiment did so after 3 months of therapy in the NZB animals and 4 months in the NZB × NZW group.

**DISCUSSION**

NZB and NZB × NZW mice show widespread and often extensive lymphoreticular proliferation, particular interest having centered on the thymic abnormalities. In small selected groups of NZB mice the incidence of lymphomas, including thymomas, has been surprisingly high, up to 25 per cent being quoted. Bielschowsky and Bielschowsky noted 5 lymphomas in 137 mice while in a survey of 250 NZB animals it was stated that the incidence of lymphomas in the NZB strain was 2 per cent. The marked lymphoid proliferation that occurs in NZB mice and the difficulties of distinguishing this proliferation from neoplasia have been discussed. The incidence of malignant lymphoid tumors in untreated NZB × NZW hybrids is not remarkable.

When the results of the two experiments are combined it can be stated that 13 out of 20 (65 per cent) of the NZB and NZB × NZW mice treated with azathioprine developed malignant thymic and lymphoid tumors, while none was seen in 18 untreated control animals. Applying the strict criterion of a frankly infiltrative lesion for diagnosis, the present report of the occurrence of malignant lymphoid tumors in such a high percentage of azathioprine treated NZB mice and their NZB × NZW hybrids, compared with the controls, must be regarded as significant. It indicates that azathioprine had a carcinogenic effect. An apparently wide distribution of malignant cells in the thymus, while its architecture is still clearly defined, raises the question of a wide field of origin of the malignant cells.

The development of lymphoid neoplasia reported here after administration of the antimitotic drug azathioprine has occurred in mice prone to autoimmune disorders. It is well known that secondary autoimmune disorders may follow...
x-ray or alkylating drug therapy of malignant lymphomas in man. The reverse occurrence of an autoimmune disorder in man going on to a malignant lymphoma is less often seen but is well recognized.5,11 The carcinogenic effect was seen in NZB male mice which develop autoimmune hemolytic anemia and in NZB × NZW females which develop lupus nephritis. Thus the design of the experiment makes it unlikely that the neoplastic effect is closely related to the sex of the mouse or to the particular type of autoimmune disease. Some of the treated NZB × NZW female mice survived to 13 months of age because of the therapy. It could be considered that the effective immunosuppression of otherwise fatal lupus nephritis in the NZB × NZW hybrids allowed these mice to survive to an age when their lymphoproliferative disorder progressed naturally to a malignant one. However similar neoplasms in the NZB mice at an age when this strain usually remains well point strongly against this interpretation.

Explanation of the present results as an inherent susceptibility to neoplasia because of genetic or immunological abnormality has been rendered unlikely by the demonstration of a similar carcinogenic effect from 6-mercaptopurine given to young C57BL mice where 29 per cent of the treated mice developed malignant lymphomas affecting the thymus, compared with less than 2 per cent of the controls.12

The nature and action of the drug itself may be responsible. An interference with natural defences against tumor development, against tumor spread or against viruses can be envisaged. Whether the initial large dosage, the prolonged smaller dosage or the combination of the two are responsible warrants further study. It is possible that differences will be found in the effects of the range of immunosuppressant agents available. Lymphoid tumors were not a feature of long-term treatment of NZB × NZW mice using cyclophosphamide.13 It should be pointed out that no initial intensive dose was used in the cyclophosphamide experiments.

The present results appear to bear a close resemblance to the effects of radiation in inducing thymomas, leukemia and lymphomas in both high and low leukemic incidence mouse strains.14 Radiation is usually regarded as inducing a change in the relationship between host and the radiation leukemia virus. A similar potentiating effect on radiation leukemia virus has been documented experimentally with urethane and mentioned with thio-TEPA.14 The findings of Doell et al.12 with 6-mercaptopurine and the present report with the closely related drug azathioprine indicate that certain immunosuppressant drugs can cause malignant lymphoid tumors when given to young mice. The relevance of a carcinogenic effect in mice to clinical usage of the drugs in man is not clear. Caution is needed with antimitotic therapy, especially in conditions which are not already neoplastic. Other drugs require investigation in similar and different strains of mice at different ages. Whether the tumors are transmissible as cells or as cell-free filtrates requires further study. If a virus can be shown to cause the lymphoid neoplasia, the possibility exists that the same virus or a related one is responsible for the lymphoid proliferation of the autoimmune disorders to which these mice are subject.
DEVELOPMENT OF LYMPHOMAS IN MICE

SUMMARY
Administration of azathioprine causes suppression of lupus nephritis in NZB × NZW F1 hybrids. Malignant lymphoid neoplasms, particularly affecting the thymus, occurred in 7 out of the 12 mice treated. Similar neoplasms developed in 6 out of 8 young NZB mice treated with azathioprine. No such tumors occurred in the control animals.

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SUMMARIO IN INTERLINGUA
Le administration de azathiopnina causa suppression de nephritis a lupus in hybridas NZB × NZW F1. Maligne neoplasmas lymphoide, afficiente in particular le thymo, occurreva in 7 del 12 muses tractate. Simile neoplasmas se disveloppava in 6 de 8 juvene muses NZB tractate con azathioprina. Nulle tal tumores occurreva in le animales de controlo.

REFERENCES
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