Proceedings of the National Academy of Sciences
Vol. 68, No. 1, pp. 124–126, January 1971

Radiation Sensitivity of New Zealand Black Mice and the Development of Autoimmune Disease and Neoplasia

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Communicated by Karl Paul Link, October 16, 1970

ABSTRACT **Young New Zealand Black (NZB) mice manifested extremely high resistance to the lethal effects of acute exposures to ionizing radiation, with a dose necessary to kill 50% of the animals within 30 days, $LD_{50(30)}$, of 964 roentgens (R) at 30 days of age and of 856 R for 90-day-old mice. In contrast, Coombs' positive 9-month-old NZB mice (with low primary immune response) were highly susceptible ($LD_{50(30)}$ = 543 R), possibly because of anemia-stimulated erythropoiesis leading to a depletion of stem cells. The radiation resistance of young NZB mice, combined with previous observations of their immunologic hyper-responsiveness, support the concept that NZB mice possess an unusually large pool of hematopoietic stem cells, an abnormality which may predispose them to the development of autoimmune disease and neoplasia.**

Studies from this laboratory (1–4) have demonstrated hyperresponsiveness of young adult New Zealand Black (NZB) mice to immunization with sheep erythrocytes and have culminated in the speculation that this mouse strain possesses a relative abundance of hematopoietic stem cells. In mice of several strains, survival after acute exposures of up to 1000–1500 R largely depends on the number of stem cells present at the time of irradiation (5–7). With this in mind we have tested our hypothesis of enhanced stem cell numbers by determining the susceptibilities of NZB mice of various ages to acute doses of ionizing radiation.

MATERIALS AND METHODS

Animals

The NZB mice employed represented the 4th–6th generations raised in this laboratory from breeding pairs, generations 57 and 58, obtained originally from W. H. Hall, Otago University Medical School, Dunedin, New Zealand. At the time of x-irradiation, equal numbers of male and female mice were selected for each of the following groups: 1-month-old (27–32 days, which are Coomb's negative), 3-month-old (88–94 days which are also Coombs' negative) and 9-month-old (9–9.5 months, Coombs' positive). In an effort to have a homogeneous 9-month-old group, we selected animals with an intense anti-globulin reaction in the test of Norins and Holmes (8) for Coombs' positivity. Mice were housed 5–6 per cage and maintained on Purina laboratory chow and tap water *ad libitum*, with conventional conditions of husbandry both before and after irradiation.

Additional experiments were performed with 3-week-old (17–23 days) animals weaned one day before irradiation and with nursling 2-week-old (11–14 days) NZB mice. For the latter study, babies were removed from mothers just before irradiation, litters were pooled, and individuals were toe-marked for identification and apportioned into different x-irradiation groups. After irradiation, one baby from each of six treatment groups was returned to each mother. Cages were checked daily during the entire experimental period and deaths were recorded.

Abbreviation: NZB, New Zealand Black.

Irradiation

Irradiations were performed with an x-ray unit (G.E. Maxitron) operated at 300 kV (peak) and 20 mA with added filtration, yielding a half-value layer equivalent to 2.0 mm Cu. Exposure rates were measured with Victoreen ionization chambers inserted in mouse phantoms, and averaged 28–29 R/min. Animals were exposed in individual plastic containers mounted around the periphery of a turntable which was rotated during exposure.

The exposure necessary to kill 50% of the animals (LD_{50}) was calculated by computer using an Oregon State University Radiation Center program based on probit analysis (9) as modified for computer by Aitchison and Brown (10). The LD_{50} values were determined from the maximum likelihood regression of the normal equivalent deviate (N.E.D. = probit minus 5) of the percentage mortality on the natural logarithm of the exposure in roentgens. The maximum likelihood method is an iterative procedure that determines the line of best fit to transformed data by applying weights that decrease toward the extremes of the distribution (11).

RESULTS AND DISCUSSION

Radiation sensitivity increased with age for 1-, 3-, and 9-month-old NZB mice, and dose-related deaths for the 1- and 3-month-old animals showed characteristically steep sigmoidal curves (Fig. 1). The erratic 30-day death response of the 9-month-old animals might reflect a heterogeneous population with regard to autoimmune disease development, in spite of efforts to minimize this by prior selection of animals based on the intensity of their Coombs' reactions. Seven-day survivals for 9-month-old NZB mice were likewise observed to be poor ($LD_{50(7)}$ = 699 R) and irregularly related to intermediate irradiation exposures (Fig. 1). These early deaths could reflect bone marrow injury of animals undergoing chronic hematopoietic stress, although gastrointestinal sensitivity, which is reported (12) to become prominent with age in such strains as C57Bl, may also be responsible. Since deaths of untreated NZB mice in our colony ordinarily do not occur as

early as 9–11 months, we made no correction for natural mortality in this age group.

Fig. 2 depicts cumulative deaths with time for all mice dying in each of the age groups represented in Fig. 1. Most irradiation deaths among 9-month-old mice occurred between days 5 and 7, and on days 7–12 and 11–13 for 3- and 1-month-old mice respectively. Thus, the incidence of early deaths and high 30-day radiation sensitivity among these mice appeared to be directly related.

In Fig. 3 are plotted $LD_{50(30)}$ values with 95% confidence limits as calculated by computer analysis for these animals and for additional groups of 2- and 3-week-old NZB mice. To more readily visualize the differences with age between the radiation sensitivity of NZB mice and that of a number of nonautoimmune strains, curves derived from data reported by other investigators for C57Bl (13), SAS/4 (14), and CAF1 (15) mice are also presented.

The most striking characteristic of the NZB response was a peak radiation resistance at 1 month of age, an observation in stark contrast to previous demonstrations (13–15) of minimum or low resistance to irradiation by 30-day-old mice of other strains. The lower resistance of 3-week-old weanling NZB mice (Fig. 3) may, however, correspond to this minimum, which has been attributed (16) to high sensitivity of both gastrointestinal and hematopoietic systems. Our results suggest that by the age of 30 days the NZB mice had a high intestinal resistance to the damaging effects of x-irradiation as well as a superior capacity for hematopoietic recovery. The early appearance of high radiation resistance in NZB mice provides an interesting corollary to the observations of Evans *et al.* (17) and Playfair (18) that suggest an early maturation of the immune system for this mouse strain, and tends to

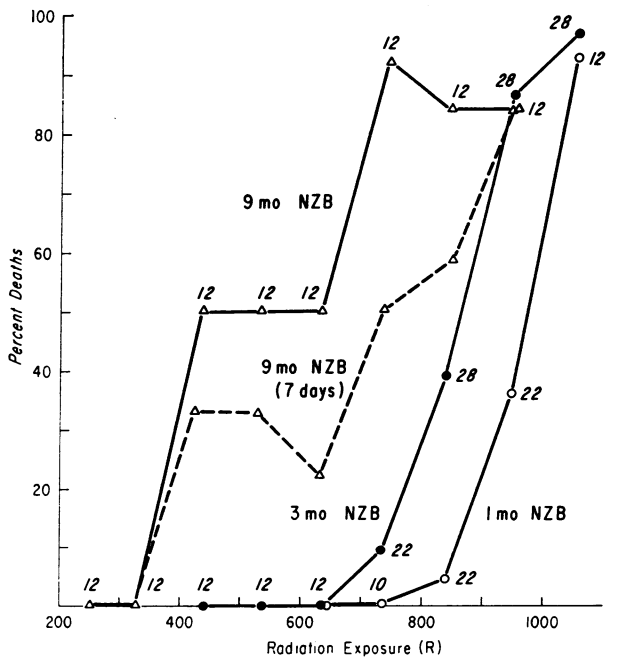

Fig. 1. Percentage of NZB mice of different ages dying within a period of 30 days after different exposures to x-irradiation. The total number of mice studied at each radiation exposure is recorded beside the corresponding experimental point. Deaths occurring within 7 days after irradiation of 9-month-old mice are also included (- - -) in the figure.

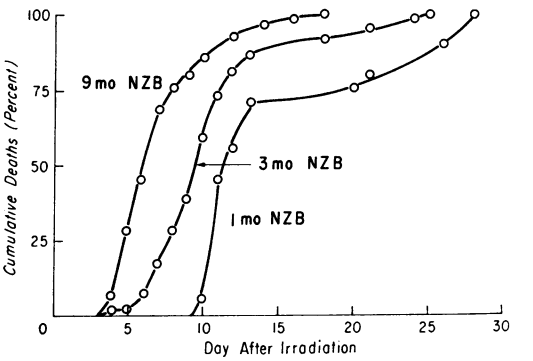

Fig. 2. Pattern of cumulative deaths for all NZB mice in each of the age groups of Fig. 1 which died within 30 days after irradiation.

support the concept of a possible relationship between primary immune capacity and the size of the stem cell pool (2).

The radiation $LD_{50(30)}$ of 856 R noted here for 3-month-old NZB mice, although lower than that of the 1-month-old animals, was higher than values previously reported for other mouse strains of similar age, including the highly resistant SJL/J strain (19). We do not yet know whether the further diminished resistance observed for 9-month-old (Fig. 3) as compared to 3-month-old NZB mice was the consequence of a gradual increase in sensitivity with time, or if it represented a precipitous event associated with a particular stage of disease development. This depressed capability of Coombs' positive 9-month-old NZB mice to survive irradiation also seemed to parallel the decreased capacity of 9-month-old NZB mice to mount a primary immune response (2–4, 20). Conceivably, both these phenomena might be ascribed to a paucity of stem cells in these anemic animals (2). The high sensitivity of NZB mice of this age to x-irradiation was in marked contrast to the gradual increase in radiation resistance which has been reported for other mice after 1 month of age, where a maximum is reached between 6 and 18 months,

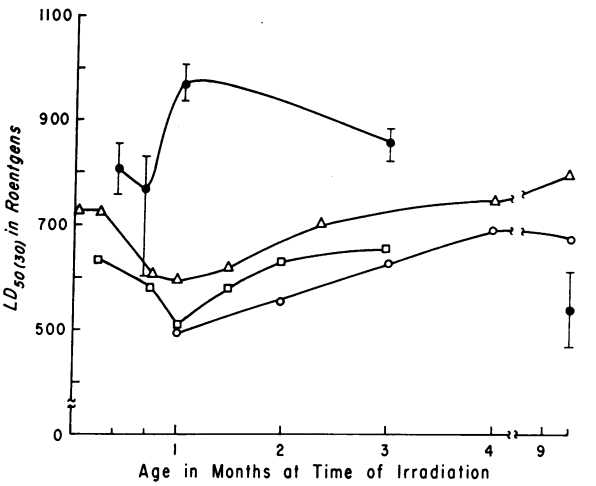

Fig. 3. $LD_{50(30)}$ values for NZB mice (●) of different ages. Upper and lower 95% confidence limits are designated by the vertical bars. Also presented are curves constructed from data reported for three other mouse strains. △, SAS/4 (ref. 14); □, C57Bl (ref. 13); ○, CAF_1 (ref.15).

with diminished resistance appearing only in advanced old age (13–15). In general, the age-associated changes in radiation resistance observed with NZB mice appeared to represent an acceleration and magnification of those changes which in nonautoimmune strains develop gradually.

The mechanism by which the presence of an enlarged pool of hematopoietic stem cells in young NZB mice might predispose them to the development of autoimmune disease has been discussed previously (2) and envisions an enhanced opportunity for the triggering of autoantibody formation as a consequence of increased availability of potentially responsive target cells. It seems feasible that augmented numbers of stem cells could similarly contribute to the generation of reticular neoplasia observed in many of the old NZB mice (21). In this regard, cytologic studies of the type B neoplasm common to the NZB strain have indicated the involvement of a recticular stem cell capable of lymphocytic, plasmacytic, and histiocytic differentiation (22).

On the basis of a high radiation resistance coupled with immunologic hyper-responsiveness, it could be speculated that the genetic defect responsible for the development of autoantibodies and neoplasia in the NZB strain might involve an early failure to adequately restrict the size of the hematopoietic stem cell compartment. The necessity for such a regulatory mechanism has been defined by previous studies concerned with stem cell repopulation kinetics in the irradiated host (23). Further elucidation of the mechanism controlling stem cell numbers would seem of great importance for the comprehension and management of those disease processes deriving from regulatory dysfunction.

We are grateful to Prof. Donald J. Kimeldorf for supervision and use of the x-ray facility at the Oregon State University Radiation Center, Corvallis, and to Dr. Fred A. Hodge for design and execution of the computer analysis of LD_{50} values, and to both for their critical evaluation of this manuscript. Meir Stampfer and Daniel Suher provided valuable technical assistance.

This work was supported by U.S. Atomic Energy Commission Contract RLO-1927-50 and Public Health Service research grant CA 10089 from the National Cancer Institute.

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