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EFFECT OF ^{89}Sr ON IMMUNITY AND AUTOIMMUNITY IN NZB/NZW F_1 MICE[1]

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NZB/NZW mice develop an autoimmune disease resembling lupus erythematosus. They also develop high levels of natural killing. We have examined the effects of ^{89}Sr on immunity and autoimmunity in female NZB/NZW mice using repeated injections of ^{89}Sr in amounts sufficient to maintain suppression of natural killing (total 270 μCi).

In sham-treated NZB/NZW mice, serum antibodies to native DNA (nDNA) rose rapidly at 5 months, whereas antibodies to nDNA in ^{89}Sr-treated mice did not increase significantly. Sham-treated mice also developed hypergammaglobulinemia, due to a progressive rise in IgG2a, but this increase was aborted in ^{89}Sr-treated mice. The reduction in serum immunoglobulin by ^{89}Sr, however, did not fully account for reduced binding of nDNA; serum from sham-treated mice at 8 months bound 16.3 ± 2.3 ng nDNA/mg serum immunoglobulin, whereas serum from ^{89}Sr-treated mice bound only 9.0 ± 0.98 ng nDNA/mg serum immunoglobulin. ^{89}Sr-treated NZB/NZW mice had less renal disease than controls, as assessed by deposition of glomerular immunoglobulin and by proteinuria.

When tested at age 20 weeks, ^{89}Sr-treated mice (total 120 μCi) had a normal immune response to sheep red cells, and their spleen cells responded maximally to phytohemagglutinin and to concanavalin A. The spleen cell response to lipopolysaccharide was elevated. Splenic morphology (at 24 weeks) was not altered by ^{89}Sr.

Despite a reduction in autoimmunity, ^{89}Sr-treated mice died somewhat more rapidly than sham-treated mice due in large part to the appearance of poorly differentiated sarcomata. These findings are consistent with the hypothesis that natural killer cells play a role in the acceleration of autoimmunity in NZB/NZW mice.

NZB/NZW[3] F_1 mice spontaneously develop an autoimmune disease that resembles systemic lupus erythematosus (1, 2). Female mice develop disease earlier than males. At 4 to 5 months, female NZB/NZW mice develop rising titers of antibodies to DNA, RNA, and thymocyte antigens. Immune complex glomerulonephritis ensues, and most of the mice die at 9 to 10 months.

Before the development of clinical autoimmunity, NZB/NZW mice demonstrate abnormalities in immune regulation. At the B cell level, these include an increase in the ratio of surface IgM to surface IgD (3). Humoral immunity matures early, and the humoral immune response to some antigens may be excessive (4, 5). At the T cell level, NZB/NZW mice appear relatively deficient in the ability to generate adequate T suppressor cells and T suppressor factors, a defect that develops after 4 weeks of age (6–9). In some studies, however, New Zealand mice have been capable of generating suppressor cells but incapable of responding to them (10, 11). At 4 weeks, NZB/NZW mice also lose the ability to become tolerant to soluble γ-globulin, a defect that must involve both B and T cell tolerance (12–14). Thus, although defects in the hematopoietic and lymphoid system can be detected in neonatal NZB/NZW mice, it appears that defects in T cell suppression and immune tolerance are not evident until after 4 weeks.

Four weeks is the age at which natural killing rapidly rises in mice, as does the related cell function, genetic resistance to bone marrow transplantation (GR) (15–17). NZB/NZW mice develop high levels of both natural killing and GR (18–20). This led us to consider that the rise of natural killing might play a role in altering immunity in NZB/NZW mice.

NK cells are mononuclear cells, probably lymphocytes, that rapidly kill certain tumor targets and transformed cell lines *in vitro* without prior sensitization to the target (21, 22). Recently, it has been shown that natural killer (NK) cells are also capable of killing certain normal thymocytes, suggesting that NK cells may be immunoregulatory cells (23, 24).

A relation between NK cells and the immune system is also suggested by treatment of mice with radioactive strontium (^{89}Sr). ^{89}Sr is a powerful β-emitter that localizes to bone, where it intensely irradiates the marrow (25). Under defined protocols, this leads to virtual obliteration of the cellular marrow with relatively little effect on other hematopoietic or lymphoid organs (25, 26). Hematopoiesis, and to a lesser extent, myelopoiesis are assumed by the spleen, and neither B nor T lymphocyte function is overtly impaired (26, 27). ^{89}Sr does, however, reduce certain cell functions, including natural killing and GR

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[3] Abbreviations used in this paper: NZB/NZW, New Zealand Black/New Zealand White F_1 hybrid; nDNA, native deoxyribonucleic acid; NK cells, natural killer cells; GR, genetic resistance to bone marrow transplantation; EDTA, ethylenediamine tetraacidic acid; HEPES, *N*-2-hydroxyethyl piperazine-*N'*-2-ethanesulfonic acid; Ig^+, immunoglobulin-positive (cells).

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(28, 29). At the same time, although humoral and cellular immunity are grossly sustained after ^{89}Sr (26), a rise in immune suppressor cells can be demonstrated under certain circumstances (27, 30, see *Discussion*). In light of evidence that NK cells kill a subpopulation of immature thymocytes, it is tempting to speculate that the suppressor cells that can be demonstrated in ^{89}Sr-treated mice may be "natural" targets for natural killing. That is, when natural killing is reduced by ^{89}Sr, the suppressor cell targets increase.

On the basis of this hypothesis, we considered that the high levels of natural killing in NZB/NZW mice might contribute to a loss of immune suppression. In favor of this possibility were past experiments in which agents that would be expected to increase natural killing were shown to exacerbate disease in NZB/NZW mice. There are a number of agents that stimulate NK cells, and it appears that all of them may do so by stimulating interferon (31-33). Interferon and agents that stimulate endogenous interferon also lead to an increase in autoimmunity and to early death in NZB/NZW mice (34-37). It is not known, however, whether this is mediated by a rise in natural killing, and there have been no studies regarding the consequences of depleting NK cells in NZB/NZW mice. We therefore suppressed natural killing in female NZB/NZW mice by the administration of ^{89}Sr, beginning at 4 weeks, and examined the effect on immunity and autoimmunity. The results are consistent with the hypothesis that high levels of marrow-dependent cells, such as NK cells, contribute to the maturation and acceleration of autoimmunity.

MATERIALS AND METHODS

Mice. NZB/NZW mice were bred in our facilities from our colonies of NZB and NZW mice (originally derived from NIH stock). ^{89}Sr-treated mice and control mice were maintained in disposable plastic cages in a room shielded by Plexiglass®. Cages were replaced daily two times after ^{89}Sr, and then twice weekly, at which time mortality was recorded.

^{89}Strontium. ^{89}Sr (Amersham, Elkhart, Ind.), specific activity 20 to 80 μCi/mg, was administered i.p. in a volume of 0.2 ml of sterile saline. Each mouse received 20 μCi at 4 weeks, and 50 μCi at 8, 16, 20, 24, and 30 weeks for a total of 270 μCi.

Experimental design. To examine the course of anti-DNA antibodies and mortality in NZB/NZW mice treated with ^{89}Sr, 20 female mice were treated with ^{89}Sr, and 20 with saline only, beginning at 4 weeks of age. One ^{89}Sr-treated mouse was accidentally killed, and one control mouse disappeared, leaving 19 in each group. A second group of ^{89}Sr-treated mice and controls was maintained in the same colony. This group was used for determining natural killing, renal and splenic histopathology, response to sheep red cells, and response to mitogens.

Natural killing by spleen cells. Natural killing was assessed against YAC-1, a Moloney virus-induced T cell lymphoma that we carry *in vitro* in RPMI 1640 supplemented with 10% fetal calf serum (FCS). Spleen cells were harvested in RPMI plus 5% FCS. The red cells were lysed by incubation on ice in 0.155 M NH_4Cl with 0.1 mM EDTA (see Abbreviations) in potassium bicarbonate buffer (pH 7.4) for 5 min. The cells were then washed three times and brought to the desired concentration in RPMI with 20% FCS, 25 mM HEPES (see Abbreviations) buffer (GIBCO, Grand Island, N. Y.) plus 2 mM glutamine, 50 μg/ml penicillin G, and 100 μg/ml streptomycin. The YAC-1 target was labeled by incubation with ^{51}Cr (New England Nuclear, Boston, Mass.) for 1 hr, washed three times, and resuspended to 4×10^5 live cells/ml by using the supplemented medium described above.

The assay was carried out in round-bottom microtiter plates (Linbro Chemicals, New Haven, Conn.). Effector cells in 150λ were added to 50λ of target cells with the effectors at varying concentrations to give effector:target ratios of 80:1, 40:1, 20:1, and 10:1. Triplicate cultures were performed for each concentration. Cultures were incubated at 37°C in 5% CO_2 for 5 hr, then centrifuged at 500 × G for 10 min. The supernatant (100λ) from each well was aspirated and diluted with 1 ml water. Released ^{51}Cr in the supernatant was determined in a Packard gamma scintillation spectrometer. Maximum release was determined by incubation of labeled target cells in 15% saponin, which regularly releases 95 to 100% of radioactivity. Spontaneous release was determined by using unlabeled target cells as effectors and was always less than 20% of maximum release. Percent cytotoxicity was determined by:

$$\left[\frac{\text{CPM effectors} - \text{CPM spontaneous release}}{\text{CPM maximum release} - \text{CPM spontaneous release}}\right] \times 100$$

We find that cytotoxicity in this assay is increased or unchanged if effectors are: a) pretreated with conventional titers of anti-Thy-1.2 plus complement (C); b) depleted of cells adherent to plastic; or c) depleted of B lymphocytes on nylon wool columns.

Antibodies to native DNA (anti-DNA). For the measurement of serum anti-DNA antibodies, mice were bled every 4 weeks from the retro-orbital sinus. The serum was heat inactivated, and binding of native DNA was assessed by the cellulose ester filter radioimmunoassay, as previously described (38). For this assay, ^{3}H-DNA, specific activity 0.35 μCi/μg (17.5 μCi/OD_{260} units) from *Escherichia coli* was purchased from New England Nuclear. The DNA was diluted to 10^5 CPM/ml in borate buffer. Ten microliters (1000 CPM) were then incubated with 10 μl of serum and 80 μl of borate-buffered saline for 30 min at 37°C, followed by overnight incubation at 4°C. The antigen-antibody complexes were collected on cellulose ester filters and washed twice with 10 ml of borate-buffered saline. The filters were placed in counting vials and covered with 10 ml of Liquifluor-toluene scintillation medium for determination of radioactivity in a Packard liquid scintillation counter (Packard Instruments, Downers Grove, Ill.). The results are expressed as nanograms of DNA bound/10 μl of serum and nanograms DNA bound/mg serum immunoglobulin.

Quantitation of serum immunoglobulin. Levels of serum immunoglobulin were determined on a pool of equal aliquots from individual mice in each group. Duplicate samples from each pool were plated in quantitative immunodiffusion plates (Meloy Laboratories, Springfield, Va.) containing monospecific antiserum to IgM, IgG1, IgG2a, or IgG2b. After incubation at room temperature for 18 hr, the diameter of the precipitin ring was measured with a magnifying comparator and compared to dilutions of standards.

Renal histopathology and immunofluorescence. At age 24 weeks, mice were sacrificed by cervical dislocation and their kidneys were removed for examination by light microscopy and by immunofluorescence for the presence of immune complex deposits. Examinations were performed on coded specimens. Light microscope examination was performed on 4-μm paraffin sections stained with hematoxylin and eosin. For quantitation of glomerular immunoglobulin by direct immunofluorescence, cryostat sections 5-μm thick were cut and picked up on Formolgelatin-coated slides, air-dried for 30 min, and washed twice for 15 min in phosphate-buffered saline (PBS) at pH 7.2. Staining with fluorescein isothiocyanate-conjugated rabbit polyvalent anti-mouse immunoglobulin was carried out as previously described (38). Coded sections were examined by using

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a Wild fluorescence microscope (Wild Heerbrugg Instruments, Inc., Farmingdale, N. Y.). The brightness and extent of glomerular immunoglobulin immunofluorescence was graded on a scale of 0 to 5 by two observers who compared specimens to sections from standard specimens used in our laboratory.

Proteinuria. Urine was obtained from mice by gently stroking the abdomen, and each sample was tested for proteinuria by reaction with Albustix® (Ames Division of Miles Laboratories, Elkhart, Ind.). Increasing proteinuria was scored by color change from 0 to 4+.

Response to sheep red cells. ^{89}Sr-treated and control mice, age 20 weeks (total 120 μCi ^{89}Sr, now 4 weeks after last dose), were immunized i.p. with 2×10^8 washed sheep red blood cells. Seven days later, the mice were boosted with the same number of cells, and 5 days later the spleen cells were collected from individual mice for the measurement of direct and indirect plaque-forming cells (PFC) to sheep red cells by means of the Cunningham modification of the Jerne-plaque assay (39). Background levels of PFC were determined by injection of saline instead of sheep cells.

Response to mitogens. Again at age 20 weeks, ^{89}Sr and control mice were sacrified for determination of the response of spleen cells to mitogens as follows: lipopolysaccharide (LPS, *S. typhus*, Difco, Detroit, Mich.) 2, 10, and 50 μg/ml; concanavalin A (Con A, Miles Laboratories) 2, 10, and 50 μg/ml; and phytohemagglutinin (PHA-P, Burroughs Wellcome, Triangle Park, N. J.) 0.2, 1.0, and 5 μg/ml. Spleen cells were washed and incubated in RPMI 1640 plus 5% horse serum at a concentration of 2.5×10^5 cells/well, in a volume of 0.2 ml in round-bottom microtiter plates (Linbro), with triplicate cultures for each mitogen concentration. (Stimulation was carried out in 5% horse serum because this reduces the high background normally seen on incubation of NZB/NZW spleen cells in FCS.) ^{3}H-thymidine (1 μCi in 10λ) was added after 40 hr. At 48 hr, the cells were collected on filters by using an automated cell harvester (Otto Hiller, Madison, Wis.) and washed with 5% trichloroacetic acid followed by methanol. The filters were placed in Liquifluor-toluene, and retained counts were determined in a Packard scintillation spectrophotometer.

Splenic histopathology. At age 24 weeks, five ^{89}Sr-treated mice (total 170 μCi, 4 weeks since last dose) and five control mice were sacrificed for examination of splenic histopathology. Light microscopy was as described for renal histopathology. Immunoglobulin-positive (Ig$^+$) cells were identified by using fluorescein isothiocyanate-conjugated rabbit polyvalent anti-mouse immunoglobulin, and T cells were identified by staining for nuclear esterase, as we have previously described (40).

RESULTS

Natural killing. The administration of 20 μCi ^{89}Sr at 4 weeks suppressed natural killing at 8 weeks (Fig. 1). An additional 50 μCi at 8 weeks maintained suppression at 12 weeks, but at 16 weeks there was near recovery. Therefore, 50 μCi doses were continued at 4-week intervals for another four doses, with sustained suppression of natural killing. In control mice, natural killing peaked at about 2 months and then gradually declined.

Effect of ^{89}Sr on anti-nDNA. As demonstrated in Figure 2, untreated NZB/NZW female mice develop a progressive rise in antibodies to native DNA (nDNA) beginning at about 4 to 5 months. This rise did not occur in ^{89}Sr-treated mice.

Effect of ^{89}Sr on serum immunoglobulin. Untreated NZB/NZW mice developed a progressive increase in immunoglobulin levels, similar to the hypergammaglobulinemia seen in patients with lupus erythematosus. As shown in Figure 3, this increase was largely due to a rise in IgG2a, the major serum immunoglobulin subclass. ^{89}Sr-treated mice instead showed a decline in IgG2a between 5 months and 8 months. The other IgG subclasses were not reduced. Throughout the study, IgM was higher in ^{89}Sr-treated mice than in controls, suggesting that levels of immature B cells were either normal or increased.

From these results, it can be seen that the reduced levels of anti-nDNA in ^{89}Sr-treated mice may in part reflect a reduction in total serum IgG. However, at 8 months, serum from ^{89}Sr-treated mice still bound significantly less nDNA/mg immunoglobulin than controls (9.0 ± 0.98 ng/mg Ig for ^{89}Sr-treated mice *vs* 16.3 ± 2.3 ng/mg Ig for controls).

Effect of ^{89}Sr on renal disease. At 24 weeks, when levels of anti-nDNA were just beginning to rise in control mice, there was also significant pathology and deposition of immunoglobulin in the renal glomeruli. Figure 4 shows that ^{89}Sr-treated mice had significantly less immunoglobulin in their glomeruli when judged by scoring of histologic sections. This difference

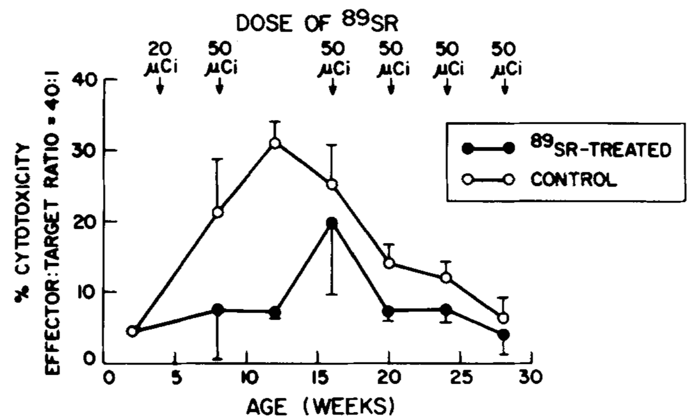

Figure 1. Natural killing by spleen cells from female NZB/NZW mice at an effector to target ratio of 40:1, showing suppression of natural killing in ^{89}Sr-treated mice compared with control mice. Similar results were obtained at ratios of 80:1 and 20:1.

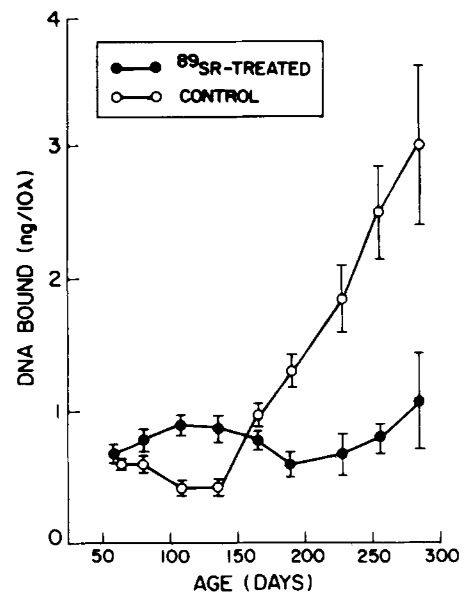

Figure 2. Anti-nDNA antibodies in the serum of female NZB/NZW mice, showing reduced levels of antibody in ^{89}Sr-treated mice compared with control mice.

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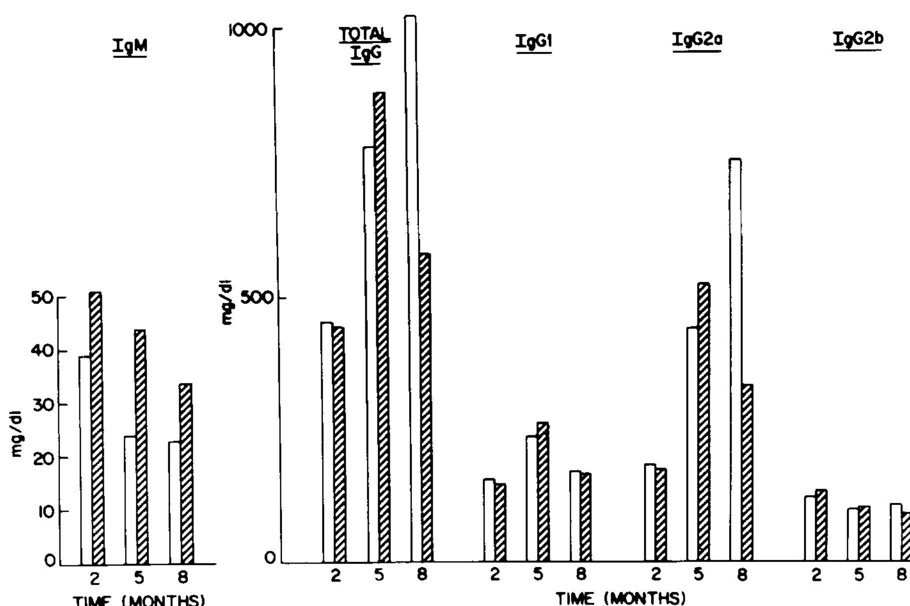

Figure 3. Serum immunoglobulin levels in female NZB/NZW mice at 2, 5, and 8 months. Note the progressive increase in IgG2a with age in control mice (*open bars*). In ^{89}Sr-treated mice (*hatched bars*), IgG2a rises but then falls, whereas other IgG subclasses do not differ from controls. IgM is higher in ^{89}Sr-treated mice than in controls at each age.

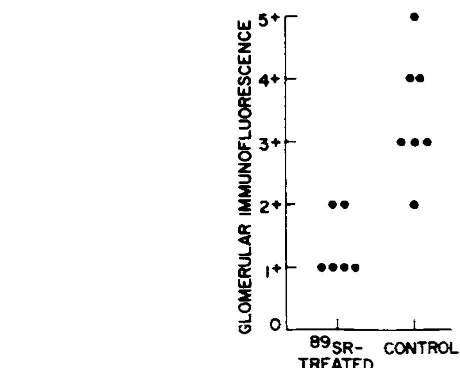

Figure 4. Deposition of immunoglobulin in the kidneys of female NZB/NZW mice at 24 weeks. Glomeruli from ^{89}Sr-treated mice have significantly less immunoglobulin than controls ($p < 0.01$ by Wilcoxen rank test).

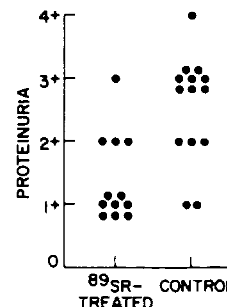

Figure 5. Proteinuria in female NZB/NZW mice at 8 months. ^{89}Sr-treated mice have significantly less proteinuria than controls ($p < 0.01$ by Wilcoxen rank test).

was also reflected in the degree of proteinuria at 8 months, as shown in Figure 5; ^{89}Sr-treated mice had significantly less proteinuria than control mice.

Effect of ^{89}Sr on humoral immunity. ^{89}Sr-treated mice and control mice were tested for immunity to sheep red cells at 20 weeks, after a total of 120 μCi of ^{89}Sr (last dose 4 weeks before). Although the study involved continuation of ^{89}Sr beyond this age (another 150 μCi total), mice were tested at this age because it is the age when autoimmunity begins to accelerate, but the mice are not overtly ill. As shown in Table I, ^{89}Sr-treated mice had an increased number of PFC to sheep red cells/10^6 spleen cells when compared to controls. Of note, were increased levels of direct (IgM) PFC in ^{89}Sr-treated mice. The total number of PFC/spleen, however, was the same in both groups.

Effect of ^{89}Sr on response to mitogens. Spleen cells from ^{89}Sr-treated and control mice were tested for their response to mitogens at 20 weeks, for the reasons cited above. As shown in Figure 6, the response to LPS by cells from ^{89}Sr-treated mice was significantly higher than the response by controls at LPS concentrations of 2 μg/ml and 10 μg/ml ($p = 0.021$ and 0.004, respectively). At 50 μg/ml, the response by cells from ^{89}Sr-treated mice was again higher, but the difference was not significant ($p = 0.105$). These results again suggest that levels of splenic B cells are normal or increased in ^{89}Sr-treated mice.

TABLE I

Response to sheep red cells by female NZB/NZW mice at 20 weeks (splenic plaque-forming cells)

	PFC/10^6 Cells		PFC/Spleen	
	IgM	IgG	IgM	IgG
^{89}Sr-treated mice	127 ± 34	3,128 ± 1,256	15,719 ± 7,828	345,816 ± 78,899
Control mice	89 ± 52	2,782 ± 1,323	11,545 ± 6,451	356,271 ± 109,634

Maximum stimulation by the T cell mitogens PHA and Con A was not significantly altered by ^{89}Sr. At high concentrations of these mitogens, however, the response by cells from ^{89}Sr-treated mice fell. The possibility that this fall reflects the induction of a suppressor cell is being investigated.

Effect of ^{89}Sr on splenic morphology. At age 24 weeks, no difference could be determined between ^{89}Sr-treated and control mice with regard to splenic morphology or the content of Ig+ or T cells. Specifically, ^{89}Sr-treated mice still had germinal centers, with the classic distribution of Ig+ and T cells.

Effect of ^{89}Sr on survival. Despite a reduction in autoimmune disease, ^{89}Sr-treated mice died slightly more rapidly than control mice. This early death was in a large measure accounted for by the development of grossly evident tumors, often in the spine, with paralysis of the rear legs (Fig. 7). From this cohort, histology of only one tumor was obtained, showing a poorly differentiated sarcoma (kindly performed by Dr. Tom Gind-

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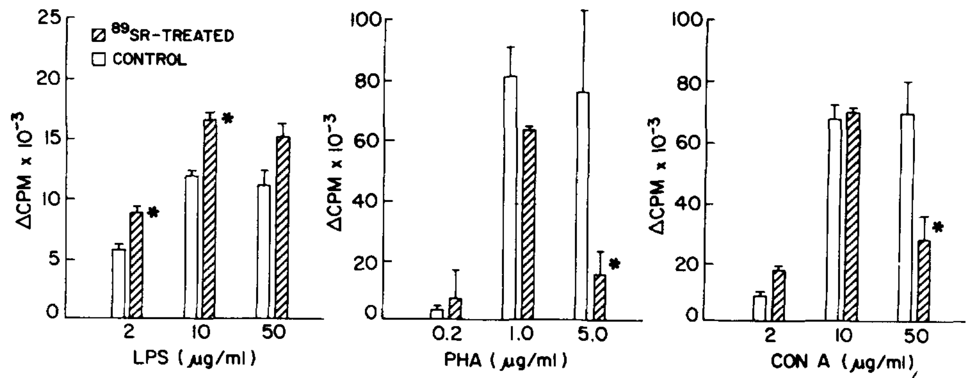

Figure 6. Response to mitogens by spleen cells from female NZB/NZW mice at 20 weeks. *Open bars,* control mice; *hatched bars,* ^{89}Sr-treated mice. Asterisks indicate significant differences (see text). Less than 500 cpm were recovered from unstimulated cells.

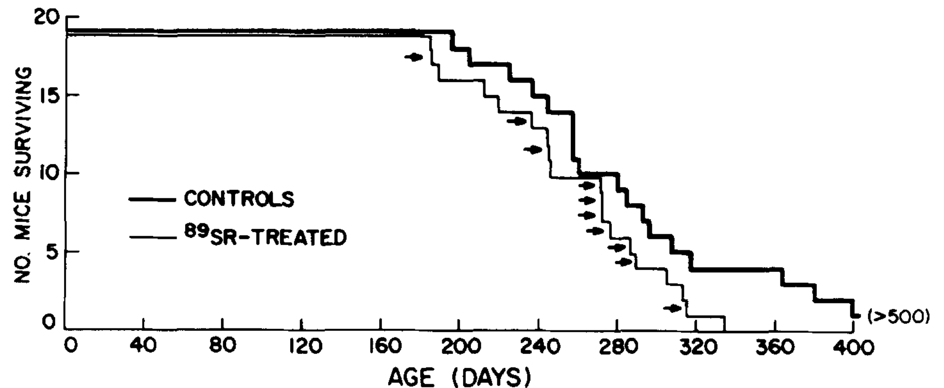

Figure 7. Survival of female NZB/NZW mice, showing controls (*heavy line*) and ^{89}Sr-treated mice (*thin line*). Arrows indicate mice that died with grossly evident tumors. All of the mice with tumors were in the ^{89}Sr-treated group.

hart). However, in other NZB/NZW mice that we have treated with ^{89}Sr, osteosarcomas have predominated.

DISCUSSION

Our studies demonstrate that treatment of female NZB/NZW mice with ^{89}Sr, using amounts sufficient to reduce NK cells, leads to a reduction in autoimmune disease. This was shown by a reduction in anti-nDNA antibody and a reduction in immune-complex glomerulonephritis, as assessed by histology, by deposition of glomerular immunoglobulin, and by proteinuria.

This reduction in humoral autoimmunity occurred despite evidence for persistence of B cell numbers and B cell function. First, only IgG2a was reduced by ^{89}Sr; IgG1 and IgG2b were unchanged, whereas IgM levels were moderately increased. Second, the mitogenic response to LPS, a B cell mitogen, was significantly increased in ^{89}Sr-treated mice. Third, the response to immunization with sheep red cells was not reduced by ^{89}Sr. Fourth, spleen cell morphology in ^{89}Sr-treated mice revealed normal germinal centers and normal numbers of immunoglobulin-bearing cells.

The majority of these tests of B cell function were carried out at 5 months, about halfway through the protocol for treatment with ^{89}Sr. We chose this point for extensive examination of mice because female NZB/NZW mice soon thereafter become clinically ill with a lupus-like syndrome, and we wished to avoid the influence of changes in disease activity. It is possible, however, that the continued administration of ^{89}Sr beyond this age led directly to B cell inactivation and consequent reduction in autoimmunity. Against this possibility are previous studies demonstrating that ^{89}Sr in total dosage up to 200 μCi has no discernible effect on B cell number or function (27, 29). Moreover, in our study, serum levels of IgM in ^{89}Sr-treated mice remained elevated at 8 months, and levels of IgG1 and IgG2b were unchanged.

There is reason to believe that the effect of ^{89}Sr in NZB/NZW mice may reflect a rise in immune suppression. Merluzzi *et al.* (27) have shown that the *in vitro* response to sheep red cells in ^{89}Sr-treated mice is reduced, and this reduction is due to the appearance of a suppressor cell in the cultures. Similarly, infection of ^{89}Sr-treated C57BL/6 mice with Friend virus leads to cellular suppression of the response to both B and T cell mitogens, whereas untreated mice are resistant to this effect of the virus (26). Finally, ^{89}Sr-sensitive cells (NK cells and the cells responsible for GR) are absent during the first 3 weeks of life in mice, a period during which a cellular suppressor of *in vitro* humoral immunity is present in the spleen (41, 42). This neonatal suppressor cell disappears from the spleen about the time that NK rises (42). In neonatal mice and in ^{89}Sr-treated mice, the splenic suppressor cell is resistant to anti-Thy-1 (or requires very high concentrations of anti-Thy-1 for killing) (26, 42, 43). Moreover, ^{89}Sr will induce the suppressor in nude (athymic) mice (27). These observations suggest that the suppressor cell(s) is not a mature T cell, although it may be an immature T cell or prothymocyte. Neither the ^{89}Sr-induced suppressor nor the neonatal suppressor adheres to nylon wool, suggesting that the suppressor is not a B cell or a macrophage, although the ^{89}Sr-induced suppressor does adhere to Sephadex G10 (44). One interpretation of these observations is that ^{89}Sr-sensitive cells, perhaps NK cells, regulate levels of an immune suppressor cell. When natural killing is reduced, as in neonatal mice or ^{89}Sr-treated mice, the suppressor cell can be detected in the spleen. However, it has been suggested that the reverse may be true, that ^{89}Sr leads to the induction of a suppressor cell that is responsible for the decline in natural killing (45).

It is particularly interesting to note that NK cells have recently been shown to kill a fraction of normal thymocytes and, to a lesser extent, spleen cells and lymph node cells (23, 24). As NK cells rise, the target cells are diminished (24). Moreover, the target is higher in strains with congenitally low levels of natural killing (24). The nature of the target cell is as

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yet unknown, but the evidence suggests that NK cells specifically regulate a subpopulation of lymphocytes. Whether these lymphocytes are potential suppressor cells is unknown.

In NZB/NZW mice, both natural killing and a related cell function, genetic resistance to bone marrow transplantation, are high. These mice have the highest known levels of GR and are among the few strains that sustain high levels of natural killing (18-20). If NK cells do regulate immune suppression, then the high NK cell function in NZB/NZW mice might contribute to their relative lack of immune suppression and consequent autoimmunity. Our studies support this hypothesis, but we have not yet been able to directly demonstrate that ^{89}Sr leads to a restoration of immune suppression in NZB/NZW mice.

It has recently been shown that levels of NK cells are regulated *in vivo* by interferon (31-33). This may explain the high levels of natural killing in NZB/NZW mice, which have persistently high levels of endogenous virus and viral proteins (46-48). It is also of note that patients with systemic lupus erythematosus, Sjögren's syndrome, or rheumatoid arthritis have recently been found to have high levels of circulating interferon (49). From this observation, one might expect that untreated patients with these autoimmune diseases will also have high levels of natural killing, but this has not been extensively tested.

Despite the suppression of autoimmunity in ^{89}Sr-treated NZB/NZW mice, there was no improvement in survival. In part, this was due to the occurrence of malignancy in about one-half of the ^{89}Sr-treated mice. This is probably an underestimate, since not all mice were autopsied. In other experiments, we have found that ^{89}Sr induces malignancy in close to 100% of NZB/NZW mice (unpublished). Many bone-seeking radionuclides, including ^{89}Sr, regularly induce malignancy in mammals, particularly osteosarcoma (50-55). Such isotopes transform normal cells, a process that may involve the activation of oncogenic viruses, though this is not established (56-59). In addition, bone-seeking radionuclides may reduce host defenses against malignancy (60). With regard to a reduction in natural killing, the effect of isotopes other than ^{89}Sr has not yet been reported. It is of note, however, that Bacillus Calmette-Guérin, a stimulus to NK cells (among others), will reduce the incidence of tumors after ^{90}Sr (61, 62). It will be interesting to examine the susceptibility of radiation-induced tumors to natural killing.

Although ^{89}Sr ameliorates autoimmunity, 17β-estradiol, which also reduces natural killing, accelerates autoimmunity (19, 38). Clearly, one (or both) of these agents must have effects on autoimmunity that do not relate to the loss of natural killing. Estradiol seems less selective than ^{89}Sr in that it is thymolytic and induces new bone formation with a consequent loss of bone marrow (63, 64). However, ^{89}Sr may yet prove to have direct effects on the immune system other than its effect on NK cells. A selective and nontoxic means of reducing natural killing *in vivo* would clarify the role of NK cells in immune regulation and could allow a new means for restoration of immune regulation in autoimmunity. In mice, NK cells express a unique cellular antigen that may permit the selective reduction of these cells without the side effects of ^{89}Sr or estradiol (18). We are now examining the effects of antiserum to the NK antigen *in vivo*.

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