Antimicrobial Activity of Various Immunomodulators: Independence from Normal Levels of Circulating Monocytes and Natural Killer Cells

P. S. MORAHAN,1* W. L. DEMPSEY,1 A. VOLKMAN,2 AND J. CONNOR1

Department of Microbiology and Immunology, Medical College of Pennsylvania, Philadelphia, Pennsylvania 19129,1 and Department of Pathology, East Carolina University School of Medicine, Greenville, North Carolina 278342

Received 12 August 1985/Accepted 26 September 1985

The effects of 89Sr treatment on the natural host resistance of CD-1 mice and the enhancement of resistance by immunomodulators to infection with Listeria monocytogenes or herpes simplex virus type 2 (HSV-2) were determined. In the CD-1 mouse, single-dose treatment with 89Sr caused a profound decrease in the number of circulating monocytes (Mo), lymphocytes, and polymorphonuclear leukocytes (PMN) within 1 week. There was also marked functional impairment of the Mo inflammatory response, as well as markedly decreased spontaneous and activatable cytotoxicity by splenic natural killer (NK) cells. Despite this profound cellular suppression, there was no significant change in natural resistance of CD-1 mice to L. monocytogenes or HSV-2 infection. Furthermore, prophylactic treatment of mice with the biologic immunomodulator Corynebacterium parvum or the synthetic immunomodulators maleic anhydride-divinyl ether or anhydride in liposomes resulted in comparable enhancement of resistance in 89Sr-treated and normal mice. These data indicate that natural and immunomodulator-enhanced resistance of CD-1 mice to microbial infections do not depend on normal levels of Mo, PMN, or NK cells. The resistance enhancement may rely on activated tissue macrophages (MΦ). In contrast to the early changes in circulating leukocytes, the resident peritoneal cell populations were not markedly altered until after day 30. There then was a distinct decline in lymphocytes and a gradual decline in MΦ; the change in MΦ was apparently due to the lack of an age-related increase in the peritoneal MΦ population in 89Sr-treated mice in comparison with a slight increase in resident MΦ in normal mice. After CD-1 mice were treated with 89Sr, the number of PMN and the function of NK cells generally recovered by about day 50 and was followed by partial recovery of circulating Mo, unless a second dose of 89Sr was administered.

This study was designed to assess the contributions of natural killer (NK) cells, monocyte (Mo) macrophages (MΦ), and tissue MΦ to natural and immunomodulator-enhanced antimicrobial resistance. Immunomodulators often activate both mononuclear phagocytes and NK cells; thus, it is difficult to assess their relative contributions to enhanced resistance (21, 25). A variety of cell depletion methods have been used, such as treatment of mice with radiation, agents toxic for macrophages, or antisera specific for NK cells (7, 16, 21). These methods have been useful, but all suffer from some nonspecificity, short depletion period, or both. We have been investigating injection of the bone-seeking isotope 89Sr into mice, which results in concentrated irradiation and subsequent destruction of bone marrow cells (1, 6, 9). An experimental system is thereby provided which appears to offer a more selective means and longer period of suppression in which to study the origins, maturational requirements, and interrelationships of defined bone marrow cell populations. Through the use of 89Sr, it has been demonstrated that the generation of NK cells is an autonomous function of the bone marrow (8) and that a normal bone marrow microenvironment is required for the maturation of NK cells into effective cytotoxic cells (10, 17). 89Sr also appears to be an effective means for investigating MΦ heterogeneity, because resident alveolar and peritoneal MΦ populations are sustained for at least 1 month after 89Sr treatment, while Mo levels in blood fall profoundly within 72 h (26, 28, 30). The simultaneous effects of 89Sr treatment on the mononuclear phagocyte and NK cell populations, however, has not been established, because various 89Sr regimens and mouse strains have been used in these studies directed at Mo or NK cells separately. In this paper we report longitudinal studies that follow changes in both nonspecific effector cell populations.

The few studies using 89Sr administration to probe the antimicrobial functions of bone marrow-dependent cells have revealed striking differences, depending on the microbial infection and the genetic background of the mice used (2, 3, 13, 15, 20, 27). The results indicate that natural resistance to some, but not all, microbial infections may involve bone marrow-dependent or -derived cells. The mechanisms, however, are still uncertain. Still less is known about the effect of 89Sr treatment on the ability of immunomodulators to enhance nonspecific resistance or to reverse 89Sr-induced suppression of resistance. We have shown recently that the immunomodulator C. parvum exhibited comparable enhancement of resistance to encephalomyocarditis virus infection in normal CD-1 mice and in mice treated with a single dose of 89Sr (20). Resistance appeared to be associated with activation of peritoneal MΦ, but the question of C. parvum activation of NK cells in 89Sr-treated mice was not investigated. We now present data on the effect of 89Sr in CD-1 mice on resistance enhancement by additional immunomodulators against infection with Listeria monocytogenes and herpes simplex virus type 2 (HSV-2) and relate these data to long-term kinetic changes in the Mo-, MΦ-, and NK-nonspecific effector cell populations.

* Corresponding author.
MATERIALS AND METHODS

Animals. Female CD-1 mice were purchased from Charles River Laboratories, Inc., Kingston, Mass. Mice were certified to be free of the common apparent viral infections and were monitored periodically for seroconversion to Sendai and mouse hepatitis viruses (Biocon, Inc., Rockville, Md.). For experiments with $^88$Sr, mice were generally maintained on acidified water (Cold Spring Products, Cold Spring Harbor, N.Y.) in cages under fiber glass filter tops in vertical laminar-flow units (Lab Products, Maywood, N.J.) and were kept on low-dust bedding (Alpha Dri; Buckshire Feed, Lansdale, Pa.). Mice were housed in animal facilities that have been approved by the U.S. Department of Agriculture and were monitored by institutional animal care committees.

Strontium. Radioactive $^89$Sr with specific activity greater than 100 mCi/g of Sr was purchased from Amersham Corp., Arlington Heights, Ill. For a few experiments, $^88$Sr with a specific activity of greater than 6,000 Ci/g of Sr was purchased from Oak Ridge National Laboratory, Oak Ridge, Tenn. Mice were inoculated intravenously or intraperitoneally (i.p.) with $^89$Sr (4 μCi/g of body weight); no significant differences were apparent with respect to the route of administration. Control groups consisted of mice that were injected with either 0.87% physiological saline or nonradioactive $^89$Sr at the same concentration (in milligrams per kilogram) as the carrier $^88$Sr in the radioactive $^88$Sr. No significant differences with respect to the substance injected were apparent between the two control groups. Experiments were performed at both The Medical College of Pennsylvania and East Carolina University School of Medicine; analysis of variance revealed no significant differences between the data from the two sites, and experimental data therefore were treated as being from one source. The regimens of $^89$Sr administration are referred to as (i) single-dose $^89$Sr administration, or (ii) double-dose $^89$Sr administration, which indicates that a second injection of $^89$Sr was made approximately 1 month after the first injection. This prevented any recovery of bone marrow due to reduced radioactivity of the $^89$Sr, which has a half-life of 50.4 days.

Analysis of peripheral blood cells and peritoneal cells. Experimental groups generally consisted of five to seven mice. Individual mice were anesthetized with ether, and blood was obtained from the retroorbital plexus or the tail vein. Leukocyte counts were obtained with a cell counter (ZM or D; Coulter Electronics, Hileah, Fla.). Leukocytes were prepared for differential counts by removing erythrocytes in heparinized 3% dextran (blood-dextran 1:3, by volume) and washing the layer of concentrated leukocytes by centrifugation at 200 $\times$ g. Cyto centrifuge slides (Cytospin; Shandon-Southern, Sewickley, Pa.) were prepared from the leukocyte concentrates and stained with Diff-Quik stain (Fisher Scientific Co., Pittsburgh, Pa.) or May-Gruenwald Giemsa stain; at least 200 cells were counted to establish a differential count.

After mice had been exsanguinated, peritoneal cells (PC) were obtained by lavage with 5 ml of heparinized (2 U/ml) phosphate-buffered saline as described previously (30). Total and differential cell counts were prepared as described above for leukocyte counts.

Total and absolute counts of nucleated cell classes were determined by multiplying the percentage of each cell class found on the differential counts by the concentration of total leukocytes or PC in the individual mouse. To compare the effect of treatment of mice with $^89$Sr or immunomodulators, the value for each treatment group was calculated as a percentage of the average value for the control group determined on the same day. Comparisons between the treatment groups and control groups were analyzed by a two-way analysis of variance.

Determination of ectoenzyme phenotypes. Pools of PC were plated at concentrations to give approximately $2 \times 10^6$ in 35-mm-diameter plastic petri dishes. Mφ were allowed to adhere for 2 h at 37°C, and the nonadherent cells were removed by vigorous washing. The Mφ were then lysed in 0.05% Triton X-100, and the cell lysates were frozen at $-20°C$ and assayed for protein, 5'-nucleotidase, alkaline phosphodiesterase I, and leucine aminopeptidase ectoenzyme activities as described previously (22). All enzyme activities are expressed as nanomoles of product formed per minute per milligram protein at 37°C.

NK activity. NK cell activity of spleen cells from individual mice was determined against $^{51}$Cr-labeled Yac-1 cells in a 4-h chromium release assay at an effector-to-target cell ratio of 200:1. To establish whether NK cells were able to respond to interferon with enhanced NK cell activity, spleen cells were preincubated with $10^4$ U of murine alpha and beta interferon (specific activity, $5 \times 10^7$ U/mg of protein; courtesy of D. M. Murasko) for 1 h prior to the addition of Yac-1 cells. The percent cytoxicity was calculated by the following formula: [(cpm experimental − cpm spontaneous)/(cpm maximum − cpm spontaneous)] $\times$ 100. The data for each group were analyzed by a one-way analysis of variance.

Mφ-activating agents. Mice were injected i.p. with 0.5 ml of aged Brewer thioglycolate (TG) broth (10%) 5 days prior to harvest of PC. C. parvum (Burroughs Wellcome Co., Research Triangle Park, N.C.) was injected into mice i.p. at 35 mg/kg 7 days prior to the harvest of PC or the infection of mice. Maleic anhydride-divinyl ether (MVE-2; courtesy of D. Breslow, Hercules, Inc., Wilmington, Del.) was dissolved in phosphate-buffered saline, to provide a final inoculation concentration of 50 mg/kg i.p. and was inoculated into mice 1 day prior to microbial infection (18). Avridine (CP20,961; courtesy of A. R. Kraska, Pfizer Inc., Groton, Conn.) was obtained as a preparation in dimyristole phosphatidyl choline liposomes. The drug was inoculated i.p. at 50 mg/kg 1 day prior to microbial infection (18).

Microbial infections. A pool of the MS strain of HSV-2 was prepared in HEP2 cells as described previously, and titrated for FFU on Vero cells (19). A pool of L. monocytogenes 29303, serotype 4b, was grown in Tryplicase (BBL Microbiology Systems, Cockeysville, Md.) soy broth as described previously and titrated for CFU on blood agar plates (18). All pools were dispensed into small fractions and frozen at $-70°C$. For the antimicrobial protection experiments, groups of six to eight mice were treated with active agents or placebo and infected with 10-fold dilutions of the microorganism. Generally, four to five dilutions were used. Mortality was monitored daily for the percent mortality, and survival distribution at different microbial challenge levels was calculated. The 50% lethal dose ($LD_{50}$) was also calculated. Mortality at a given dilution was analyzed by the chi-square test with the Yates correction factor, and the survival distribution was analyzed by the Mann Whitney U test.

RESULTS

Effects of $^89$Sr on the long-term kinetics of effector cell populations. The effects of both single- and double-dose administration of $^89$Sr (see above) were followed for 72 days.

NK Cells. The present data revealed that the CD-1 mouse
strain has a low spontaneous NK cell activity, with an average cytotoxicity of 11.8% ± 1.3 (standard error of the mean) at an effector-to-target cell ratio of 200:1 and that treatment with \(^{89}\text{Sr}\) reduced this activity to negligible levels within 1 week (Fig. 1). Equally rapid reduction occurred with \(^{89}\text{Sr}\) with a specific activity in the range of 70 to 120 mCi/g or 6,000 Ci/g. In other mouse strains (e.g., C57B1/6) with high spontaneous NK cell activity, both higher specific activity of \(^{89}\text{Sr}\) and longer treatment times appear to be necessary for NK cell depression (unpublished data). There was partial recovery of NK cell activity in CD-1 mice by about 50 days, which was aborted when mice received a second injection of \(^{89}\text{Sr}\) at 30 days. Treatment with \(^{89}\text{Sr}\) did not significantly decrease spontaneous natural cytotoxic cell activity against EL-4 target cells for at least 30 days after treatment with \(^{89}\text{Sr}\) (data not shown). Treatment with interferon did not restore NK activity throughout the entire experimental period (Fig. 1). Within 1 week after treatment with \(^{89}\text{Sr}\), NK cells failed to show the expected normal increase to about 20% cytotoxicity in response to treatment with interferon in vitro. Treatment in vivo with an interferon inducer, poly(rI)-poly(rC), also failed to enhance NK activity in \(^{89}\text{Sr}\)-treated mice (data not shown).

Leukocytes. Circulating leukocytes, especially Mo and polymorphonuclear leukocytes (PMN), are significantly depressed within 1 week after \(^{89}\text{Sr}\) administration to CD-1 mice (30). The present long-term kinetics data revealed that the first cells to recover were PMN, which reached normal levels by about 50 days, although this apparent recovery was not sustained at all time points thereafter (Fig. 2). Mo recovered more gradually, with the population approaching near normal levels by about day 60. Recovery of both populations was prevented by a second administration of \(^{89}\text{Sr}\).

FIG. 2. Kinetics of effects of single (1×, \(^{89}\text{Sr} \#1\)) or double (2×, \(^{89}\text{Sr} \#1\) and \#2) treatment of CD-1 mice with \(^{89}\text{Sr}\) (specific activity, 70 to 6 \times 10^6 mCi/g) on total leukocytes (WBC) and the circulating Mo, Ly, and PMN populations. Values are expressed as the percentage of the controls measured on the same day. The asterisks indicate values significantly different (P < 0.05) than those from the control group on the day of observation.

FIG. 1. Effect of single (1×, \(^{89}\text{Sr} \#1\)) and double (2×, \(^{89}\text{Sr} \#1\) and \#2) treatment of CD-1 mice with \(^{89}\text{Sr}\) (specific activity, 70 to 120 mCi/g) on spontaneous NK cell activity and on enhancement of NK cell activity by treatment with interferon (IFN) in vitro. Values are expressed as the percentage of spontaneous NK cell activity in control CD-1 mice, which was 11.8% ± 1.3 (standard error of the mean) at a 200:1 spleen cell-to-target cell ratio. The asterisks indicate values significantly different (P < 0.05) than those from the control group on the day of observation.
Between days 63 and 72 after treatment with $^{89}$Sr, the resident peritoneal Mφ population was significantly depressed to approximately 50% of the levels in age-matched controls, in the face of rising Mo counts (Fig. 2). The difference in Mo between control and $^{89}$Sr-treated mice may be due to an increase with age in the number of resident peritoneal Mφ in normal mice, from 4.4 x $10^6$ cells in 6-week-old mice to 6.7 x $10^6$ cells in 14-week-old mice. In $^{89}$Sr-treated mice there was no increase in resident peritoneal Mφ, which remained at 3.5 x $10^6$ cells at 6 weeks of age and 4.0 x $10^6$ cells at 14 weeks of age. The long-term changes in Ly and Mo populations were not markedly affected by a second injection of $^{89}$Sr, despite an additional depressive effect on circulating Mo.

Functional status of leukocytes and PC. The ability of mice to mount an inflammatory exudate and the resulting peritoneal Mφ ectoenzyme phenotypes were measured at various periods after administration of $^{89}$Sr. During the first 30 days after single-dose administration of $^{89}$Sr, CD-1 mice responded poorly or not at all with a Mo exudate to inflammatory stimuli (Table 1 [20, 30]). The ability to mount a Mo-Mφ-rich exudate to the thioglycollate stimulus was regained by 57 to 58 days after administration of $^{89}$Sr, but it was again suppressed for at least 39 days following a second $^{89}$Sr injection (Table 1). Exudation of PMN may be regulated differently from that of Mo-Mφ exudation. After either single- or double-dose $^{89}$Sr treatment, a significant number of elicited PMN were found in the peritoneal cavity 7 days following $C.\ parvum$ treatment, despite the low circulating PMN levels (Fig. 2). $C.\ parvum$ injection increased PMN from 0.05 x $10^6$ to 2.4 x $10^6$ in normal age-matched controls ($P < 0.05$) and from 0.04 x $10^6$ to 1.0 x $10^6$ in mice treated with double doses of $^{89}$Sr ($P < 0.05$).

The results with ectoenzymes in this study reveal that changes in the Mφ cell membrane in CD-1 mice reflect the influence of the particular immunomodulator. After single- or double-dose $^{89}$Sr treatment, whether an exudate occurred or not, the ectoenzyme phenotype of the TGM $\phi$ was typical of the TG type of inflammatory Mφ (Table 1). After two doses of $^{89}$Sr, the ectoenzyme phenotypes of $C.\ parvum$-activated Mφ from control and $^{89}$Sr-treated mice were also both typical of the $C.\ parvum$ type of activated Mφ, with an 83 to 93% reduction in alkaline phosphodiesterase I and a 98% reduction in 5'-nucleotidase ($P < 0.05$; data not shown).

Effects of single-dose $^{89}$Sr treatment on natural and immunomodulator-induced resistance of CD-1 mice. CD-1 mice exhibited an intermediate level of natural resistance to both $L.\ monocytogenes$ and HSV-2 as compared with that of other mouse strains (12, 14). Approximately 10$^6$ CFU of $L.\ monocytogenes$ and 10$^3$ PFU of HSV-2 were required to produce an LD$_{50}$ (Tables 2 through 4). Moreover, despite the profound decrease in circulating Mo and PMN and in NK cell activity during the first 30 days after treatment with $^{89}$Sr, there was little change within this time period between the splenic titer or the LD$_{50}$ for $L.\ monocytogenes$ or HSV-2 in normal CD-1 mice and in mice treated with $^{89}$Sr (Tables 2 through 4).

Mice were treated with $C.\ parvum$ 3 to 5 days after single-dose $^{89}$Sr treatment and infected with $L.\ monocytogenes$ 7 days later. Treatment with $C.\ parvum$, whether in normal mice or $^{89}$Sr-treated mice, significantly enhanced resistance (Table 2). $L.\ monocytogenes$ growth in the spleen was reduced at least 4 log$_{10}$, and there was approximately a 100-fold increase in resistance in both $^{89}$Sr-treated and control mice.

Prophylactic treatment with the synthetic immunomodulators MVE-2 or avridine in liposomes 1 day prior to infection with HSV-2 was also effective in mice treated with a single dose of $^{89}$Sr. At least 100-fold more virus was required to produce an LD$_{50}$ in MVE-2-treated mice as compared with that in untreated mice, whether the mice were treated with $^{89}$Sr or not (Table 3). The median survival time of mice treated with MVE-2 also increased significantly. In $^{89}$Sr-treated mice, NK cell activity was decreased and not activatable with interferon, whether mice were treated with MVE-2 or the saline placebo. Treatment with the lipoidal amine avridine in liposomes was also effective against HSV-2 infection in normal mice and in mice treated with $^{89}$Sr (Table 4). Mortality was significantly reduced, and the median survival time of mice increased comparably in the two groups.

DISCUSSION

Our data show that the CD-1 mouse is uniquely suitable for nonspecific effector cell depletion studies, because with the use of $^{89}$Sr it is possible to achieve an early and profound depression of both blood Mo and NK effector cell populations, without apparent impairment of the resident peritoneal Mφ. The CD-1 mouse thus differs from other mouse strains, in which it is not possible to achieve early depression of both effector cell populations. With the CD-1 mouse, treatment with $^{89}$Sr ranging in specific activity from 70 to 6 x $10^6$ mCi/g appears to produce comparable effects on the effector cell
TABLE 1. Effect of $^{89}$Sr on elicitation of PC exudate with TG broth

<table>
<thead>
<tr>
<th>Expt. no.</th>
<th>Days after $^{89}$Sr treatment</th>
<th>PC exudate/mouse ($\times 10^6$)</th>
<th>Ectoenzyme SA (nmol/mg of protein per min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$^{89}$Sr</td>
<td>$^{89}$Sr</td>
<td>TG</td>
</tr>
<tr>
<td>1</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>57</td>
<td>29</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>57</td>
<td>29</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>57</td>
<td>−</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>57</td>
<td>29</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>57</td>
<td>−</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>57</td>
<td>−</td>
<td>−</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Mice were inoculated i.p. with 4 $\mu$Ci/g body weight of $^{89}$Sr at the indicated times, and mice were inoculated with Brewer TG broth 4 to 5 days before PC were harvested. Similar results were obtained using high SA (6,000 Ci/g) $^{89}$Sr inoculated intravenously (data not shown). Abbreviations: 5'N, 5'-nucleotidase; APD, alkaline phosphodiesterase 1; LAP, leucine aminopeptidase.

$^b$ Specific activity, 76 mCi/g.

$^c$ Specific activity, 73 and 69 mCi/g.

$^d$ P < 0.05 for the TG group in comparison with the respective resident control group.

$^e$ P < 0.05 for the resident $^{89}$Sr-treated group in comparison with the respective control group.

populations and natural host resistance. Moreover, the alterations in effector cells persist for at least 50 days, unlike those obtained by many other depletion methods (7, 16, 21).

Results of our studies have established that despite profound monocytopenia, granulocytopenia, and depressed NK cell function, the CD-1 mouse expresses relatively normal natural resistance to three diverse microbial infections: encephalomyocarditis virus (20), L. monocytogenes (Table 2), and HSV-2 (Tables 3 and 4). The results of this study have also documented that prophylactic treatment of the CD-1 mouse with various immunomodulators (C. parvum, the synthetic polyanion copolymer MVE-2, and the lipoidal amine avridine) produces comparable activity in normal and Mo- and NK cell-depleted mice. Preliminary data on therapeutic treatment of mice with either purified natural murine alpha and beta interferon or human alpha A/D recombinant interferon have also revealed significantly prolonged survival against encephalomyocarditis virus in normal and $^{89}$Sr-treated mice.

Our data and those obtained in other mouse strains (10) indicate that the immunomodulators do not restore the depressed NK cell activity in the $^{89}$Sr-treated mouse while they produce normal enhancement of some activated MΦ functions (20, 30). Results of previous work by us and others have also clearly established the nonspecific nature of the resistance enhancement, in that protected animals do not develop heightened T- or B-cell-specific antimicrobial immune responses (21). These lines of evidence suggest that immunomodulator-enhanced nonspecific antimicrobial resistance of the $^{89}$Sr-treated CD-1 mouse may be due to direct antiviral action of interferon or immunomodulator-enhanced activity of resident tissue MΦ in target organs. A cautionary note, however, must be added, since there is always the possibility that we did not deplete NK cells and circulating Mo below a minimum threshold needed for resistance. However, we view this as being unlikely. In many experiments, NK cells and circulating or emigrated Mo are not detectable. Furthermore, using a variety of inflammatory stimuli, we have found minimal evidence for the presence of a significant number of circulating monocytes to form an inflammatory influx (26, 30). In support of the concept of local tissue macrophage involvement, histopathologic studies on inflammatory foci (presumably Mo derived) in target organs indicate that there is mild

### TABLE 2. Effect of single-dose $^{89}$Sr treatment on natural resistance to HSV-2, protection produced by MVE-2 and NK cell activity

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Log$<em>{10}$ CFU per LD$</em>{50}$ (ΔLog$_{10}$)</th>
<th>Protection after challenge with ca. 52,000 CFU</th>
<th>% NK cytotoxicity$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{89}$Sr</td>
<td>MVE-2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>−</td>
<td>−</td>
<td>14/18 (78)</td>
<td>6.1 ± 20.4</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>17/17 (100)</td>
<td>2.8 ± 6.2</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>22/22 (29)</td>
<td>2.2 ± 4.2</td>
</tr>
</tbody>
</table>

$^a$ CD-1 female mice were inoculated intravenously with 2 $\mu$Ci/g body weight of $^{89}$Sr (specific activity, ca. 6,000 Ci/g), and were treated i.p. with 35 mg of C. parvum per kg on day 3. Seven days later mice were challenged i.p. with dilutions of L. monocytogenes, mortality was followed, and the CFUs required to produce an LD$_{50}$ were calculated.

$^b$ Two days after infection, four mice from the groups challenged with 1.2 × $10^9$ CFU were sacrificed, and the growth of L. monocytogenes in the spleen and the spleen weight were measured.

$^c$ P < 0.05 for the C. parvum group in comparison with the respective control group.

$^d$ CD-1 female mice were inoculated intravenously with 2 $\mu$Ci/g body weight of $^{89}$Sr (specific activity, 76 mCi/g), inoculated i.p. 8 days later with 50 mg of MVE-2 or saline per kg, and challenged i.p. 24 h later with serial dilutions of HSV-2; mortality followed for 21 days, and the number of PFU required to produce an LD$_{50}$ was calculated. Specific mortality and median survival time data for the groups inoculated with 5.2 × $10^6$ PFU are also shown.

$^e$ The mean NK cell activity that was measured in spleens from two individual mice each on day 7 and day 9 after treatment with $^{89}$Sr with and without the presence of exogenous interferon (IFN). Activity in the mice treated with MVE-2 was measured on day 9 (1 day after treatment with MVE-2).

$^f$ P < 0.05 for the MVE-2 group in comparison with the respective control group.
inflammation in control animals infected with encephalomyocarditis virus, and there is no apparent inflammation in $^{89}$Sr-treated and infected mice (unpublished data). The extent of possible $^{89}$Sr-induced changes in functions among resident tissue Mφ populations has not been completely established. A distinct alteration after $^{89}$Sr administration occurs in the spleen in which suppressor Mφ can no longer be induced by C. parvum treatment (Y. Shibata and A. Volkman, J. Immunol., in press).

The results of this study are the first indication that there may be long-term effects of $^{89}$Sr administration on the normal regulation of the resident Mφ population. Resident peritoneal Mφ in CD-1 mice were maintained in $^{89}$Sr-treated mice at a level of approximately $4 \times 10^6$ Mφ per mouse between 7 and 70 days after one treatment with $^{89}$Sr, while there was an age-related increase in Mφ (24) in controls. These effects may result from cumulative absorption of low-level radiation in soft tissues (4) or from the regulation of the number of cells in the peritoneal cavity by body size. Mice treated with high specific activities of $^{89}$Sr (6,000 Ci/g) appear to grow more slowly than their controls (unpublished data). A relatively slow turnover of resident Mφ could also result in the changes that were observed late in the experiment. However, the half-time for resident Mφ in the peritoneal cavity appears to be less than 30 days (5, 26, 29), so that the readily detectable and earlier decline in resident Mφ that should have occurred in the precursor pool occurred immediately when they were destroyed by $^{89}$Sr. It is noteworthy that the steepest decline in the resident Mφ was seen at the late intervals, when Mφ levels were returning to normal levels in the single-dose $^{89}$Sr treatment regimen. These observations add additional support for the view that resident Mφ are not immediately derived from blood Mφ and that these populations may be independently regulated. In view of the late changes in the resident peritoneal Mφ population, it would be of interest to determine the effects of double-dose $^{89}$Sr treatment on host resistance and other important Mφ populations in tissues. Our initial results suggest that natural resistance of the CD-1 mouse to L. monocytogenes may be decreased after double-dose $^{89}$Sr treatment, because there was a 93-fold decrease in the number of bacteria required to produce the LD$_{50}$ in the $^{89}$Sr-treated mice. In contrast, there was not a marked decline in the natural resistance that CD-1 mice exhibited to HSV-2; the LD$_{50}$ was $10^{2.9}$ PFU in normal mice and $10^{2.5}$ PFU in $^{89}$Sr-treated mice.

**ACKNOWLEDGMENTS**

We thank Michael Ackermann, Patrick Hwu, George Ericsson, Mary Helen Hackney, Hope Linton, and Cheryl Vance for excellent technical assistance. We thank Elizabeth Newsome for excellent secretarial assistance in preparing the manuscript. We also appreciate the excellent care and concern for the animals expressed by the Animal Care Staffs of the two institutions.

This research was partially supported by grant N0014-82-K-0699 from the Office of Naval Research, Public Health Service grant CA 35961 from the National Cancer Institute, and Public Health Service grant AI 17162 from the National Institute for Allergy and Infectious Disease.

**LITERATURE CITED**


