INFECTION AND IMMUNITY, Feb. 1978, p. 533-539
0019-9567/78/0019-0533$02.00/0

Vol. 19, No. 2
Printed in U.S.A.

Mechanisms of Hemopoietic and Immunological Dysfunction Induced by Lymphocytic Choriomeningitis Virus

S. L. SILBERMAN, R. P. JACOBS,† AND G. A. COLE*
The Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland 21205

Received for publication 12 September 1977

Sublethal irradiation (500 R) of C3H mice is followed by a gradual replacement of radiosensitive cells in their spleens by surviving stem cells originating in bone marrow. This compensatory hemopoiesis was quantitated by counting the numbers of stem cell-derived colonies appearing on spleen surfaces, as well as those which grew in vitro after marrow cells, suspended in soft agar, were overlaid onto syngenic mouse embryo fibroblast feeder layers. Compensatory colony formation, both in vivo and in vitro, was severely depressed when mice were infected with lymphocytic choriomeningitis virus (LCMV) 1 day before irradiation, although the induction of virus-specific cytotoxic T cells in their spleens was unimpaired. Without irradiation, mice, acutely infected with LCMV, showed a dramatic reduction in the numbers of specific antibody-forming cells generated in their spleens after priming with sheep erythrocytes during week 1 post-infection, yet the ability of their marrow cells to form colonies in vitro remained normal. Therefore, the basis of immunodepression is distinct from that of defective hemopoiesis since the latter is apparent only when LCMV infection is accompanied by irradiation. However, as discussed, both phenomena may be related to alterations induced within the splenic environment by LCMV.

Primary lymphocytic choriomeningitis virus (LCMV) infections of immunocompetent mice have been shown to cause a transient depression of immune responsiveness to a variety of antigenic stimuli, although infected animals manifest a potent virus-specific immune response and subsequent viral clearance. This phenomenon was first reported by Mims and Wainwright (15) who showed that primary antibody responses to sheep erythrocytes (SRBC), human serum albumin, and ovalbumin were depressed in adult mice given these antigens during the first 2 weeks after infection with the viscerotropic WE_3 strain of LCMV. Quantitation of SRBC-specific bone marrow-derived (B) lymphocytes indicated that the depressed antibody levels seen in infected mice were the result of fewer numbers of antibody-producing cells. In contrast to the effects of acute infection, adult virus-carrier mice, who were chronically viremic as a result of congenital infection, did not significantly differ from normal mice in their ability to produce antibody. These findings were extended by Bro-Jørgensen and colleagues (5, 6) who, in addition, proposed that humoral responses to thymus-derived (T) lymphocyte-dependent antigens were selectively affected.

† Present address: The George Washington University Medical Center, Washington, DC 20037.

Evidence for LCMV-induced depression of cellular immunity was presented by Lehmann-Grube et al. (13) and Güttler et al. (9) who showed, respectively, that rejection of skin allografts was delayed and that splenic cytotoxic T cell (CTC) responses to alloantigens were markedly reduced in mice acutely infected with viscerotropic (Traub) strain of LCMV. The latter situation did not occur in virus-carrier mice.

A further indication of an immune system defect caused by LCMV came from the studies of Jacobs and Cole (10) who found that splenic lymphocytes, obtained from mice shortly after infection with the neuroadapted E-350 strain of virus, proliferated poorly, or not at all, when cultured with either T or B cell mitogens. This proliferative defect persisted for about 1 week and could be corrected by addition of macrophages from normal mice. On the other hand, splenic lymphocytes from established virus-carrier mice gave normal proliferative responses.

Another striking characteristic of LCMV-infected mice, first observed by Bro-Jørgensen and Volkert (4, 5), is an impairment in hemopoiesis, as revealed by the heightened sensitivity of such animals to X irradiation. In seeking an explanation for this observation, these investigators found that the normal reconstitutive response of spleen-seeking bone marrow cells following irradiation failed to occur in adult mice infected

1 week previously with LCMV, but that this response was only slightly depressed in virus-carrier mice. Since LCMV itself is not normally cytopathic for murine cells, it was postulated that the observed hemopoietic defect stemmed from a virus-induced interference with hemopoietic stem cell (HSC) function.

The primary aims of this study were: (i) to establish whether the failure of compensatory splenic hemopoiesis to occur in irradiated mice acutely infected with LCMV could be ascribed either to an effect of virus on the proliferative capacity of bone marrow stem cells, or to a splenic environment rendered incapable of supporting their proliferation; (ii) to determine whether hemopoietic dysfunction and immunodepression might have a common basis.

MATERIALS AND METHODS

Mice. Male 6- to 8-week-old C3H/HeJ mice (H-2^k) were obtained from Jackson Laboratories, Bar Harbor, Me. Male 6- to 8-week-old Swiss mice were obtained from Flow Laboratories, Dublin, Va.

Irradiation. C3H/HeJ mice received whole body sublethal gamma irradiation at a dose rate of 134 rads/min by a Gammacell 40 Caesium 137 irradiation unit (Atomic Energy of Canada, Ltd., Ottawa, Canada). All irradiated mice received 500 rads (3.4 min).

Virus. The neuroadapted E-350 strain of LCMV was used in all experiments. The virus was maintained by brain passage in 6- to 8-week-old Swiss mice and stored at −70°C as a 20% (wt/vol) clarified brain suspension. C3H/HeJ mice were always infected by intraperitoneal (i.p.) inoculation of 10^4 intracerebral (i.c.) mean lethal doses (LD_{50}) of LCMV in 0.5 ml of saline. The virus content of bone marrow and spleen from infected, irradiated mice was titrated by i.c. inoculation of serially diluted frozen-thawed cell suspensions into 6- to 8-week-old Swiss mice. Titers were expressed as the $\log_{10}$ i.c. LD_{50} calculated by the method of Kärber (12).

Preparation of spleen and bone marrow cells. Mice were killed by cervical dislocation. Spleens were removed and placed in cold Hanks balanced salt solution (HBSS; Grand Island Biological Co., Grand Island, N.Y.), teased through 60-gauge wire mesh screens, and aspirated through a 20-gauge hypodermic needle. Cells were first centrifuged for 1 min at 50 × *g* to remove clumps and then for 10 min at 200 × *g*. The cell pellets were treated with 0.83% (wt/vol) ammonium chloride for 2 min to lyse erythrocytes, and the remaining mononuclear cells were washed and suspended in cold minimal essential medium (MEM; GIBCO) containing 10% fetal calf serum (FCS; GIBCO), 2 mM glutamine, and antibiotics.

Bone marrow cells were obtained by irrigation of femurs with HBSS using a 26-gauge hypodermic needle. Cell suspensions were centrifuged for 10 min at 200 × *g* and suspended in MEM + 10% FCS.

Cell counts were made in a hemocytometer after dilution in 0.1% trypan blue in normal saline. Viability was determined by dye exclusion, and viable cells were adjusted to the appropriate concentration.

In vivo splenic CFU assay. A modification of the method used by Curry and Trentin (8) was used. Eight days after sublethal irradiation, spleens were removed and placed in Bouin solution until hemopoietic foci became visible (15 to 30 min). The total number of surface colonies (colony-forming units; CFU) on each spleen was counted macroscopically and expressed as the mean ± 1 standard deviation of five replicate spleens.

In vitro bone marrow HSC assay. The technique of cloning HSC in a double-agar culture was derived from procedures outlined by Bradley and Metcalf (1) and Pluznik and Sachs (17). C3H primary embryo cell cultures, maintained in MEM + 10% FCS, were passaged between four and eight times in 75-cm^2 plastic tissue culture flasks (Falcon Plastics, Oxnard, Calif.) before use. Cells were trypsinized with 0.5% trypsin-ethylenediaminetetraacetic acid in HBSS, suspended in MEM containing 10% FCS, and counted, and 2.0-ml fractions containing 5 × 10^4 cells were added to each well (35 by 17 mm) of plastic six-well tissue culture trays (Bellco, Vineland, N.J.). Cultures were incubated overnight at 37°C in air containing 5% CO_2. The medium was then removed, and 2 ml of a medium-agar mixture warmed to 42°C was applied. The final composition of the mixture was 0.5% agar (Difco Laboratories, Detroit, Mich.), MEM containing 10% FCS, 2.2% (wt/vol) $NaHCO_3$, and supplemented with MEM Eagle nonessential amino acids, MEM Eagle vitamin concentrate, 200 mM glutamine, and antibiotics. This preparation was allowed to harden at room temperature and comprised the "feeder" layer.

Viable bone marrow cells at a concentration of 1 × 10^6 ml were warmed to 42°C, and 0.1-ml samples were added to tubes containing 0.9 ml of a soft medium-agar mixture (final concentrations as above, except 0.3% agar). After quickly agitating, the contents of each tube were poured over the feeder layers in corresponding wells.

Following incubation for 12 days in a 37°C humidified atmosphere containing 5% CO_2, HSC colonies were counted through an inverted microscope at ×100 magnification. Counts were expressed as the mean number of colonies per 10^5 plated bone marrow cells from two replicate wells.

Antibody-forming cell plaque assay. C3H mice received 0.2 ml of a 2% suspension of washed SRBC 4 days before assay. Spleens were prepared as described above and suspended in cold HBSS (2 ml per spleen).

Plaque assays were performed in double-slide chambers, as described by Cunningham and Szenberg (7). Mixtures of spleen cells, SRBC, and adsorbed guinea pig complement plus SRBC were made at room temperature and applied to the chambers. Direct (immunoglobulin M) plaques were counted after 30 min of incubation at 37°C. Plaque-forming cells (PFC) were expressed as the mean of four replicate spleens or as the mean per 10^6 cells.

[^{3}H]TdR incorporation assay for bone marrow-stimulating activity of serum. Bone marrow cells were adjusted to a final concentration of 2 × 10^5 cells per ml in RPMI 1640 (GIBCO) containing 10% heat-inactivated FCS. Cells were then divided into 0.2-ml volumes in sterile, flat-bottomed 96-well microtiter plates (Bellco).

Mouse sera to be tested for stimulating activity were obtained by orbital bleeding and stored at −70°C. For each serum each of four wells received 0.1 ml of either medium, normal mouse serum, or test serum. After 14 h of incubation in a 37°C humidified atmosphere containing 5% CO_2, 1 μCi of [^{3}H]thymidine ([^{3}H]TdR, 6.7 mCi/mM, New England Nuclear Corp., Boston, Mass.) in 0.02 ml of HBSS was placed into each well, and the plates were further incubated for 4 h. The cells were then collected on glass-fiber filters (Reeve Angel, Clifton, N.J.) and washed with isotonic saline, using a semiautomatic microculture harvester (Biomedical Research Institute, Rockville, Md.). The filters were dried, and the amount of [^{3}H]TdR incorporated by the cells retained on the filters was determined by liquid scintillation spectroscopy. Results were expressed as the arithmetic means of total counts per minute from each quadruplicate culture.

Cytotoxicity assay. The method is essentially that described in a previous report (11). Target cells were prepared from semiconfluent monolayers of mouse fibroblasts (L929 cells; H-2^k) grown in 75-cm^2 tissue culture flasks (Falcon), which were infected with LCMV at a multiplicity of about 10. Five days later, infected and control uninfected cells were trypsinized, washed, and incubated for 1 h with ^{51}Cr. After washing to remove extracellular label, they were seeded into wells of 96-well microtiter plates (Bellco) in 0.1-ml portions containing 2×10^4 viable cells. Each spleen cell preparation to be assayed was added as 0.1-ml samples to each of three wells of both infected and uninfected targets at a multiplicity of 40. After incubation for 18 h, 0.1 ml of culture fluid was removed from each well and counted in a gamma scintillation spectrometer. The isotope released from triplicate cultures was expressed as the arithmetic mean of counts per minute ± standard deviation, and virus-specific lysis was calculated as described previously (11).

RESULTS

Depression of irradiation-induced CFU by LCMV. In initial experiments it was necessary to determine whether our laboratory strain of LCMV could interfere with bone marrow repopulation of spleen that normally follows sublethal irradiation, and also what interval between infection and irradiation would be optimal for demonstrating such an effect. C3H mice were infected at varying intervals before, and after, irradiation; spleens were removed 8 days after irradiation, and CFU were enumerated. As shown in Fig. 1, control mice given only irradiation exhibited 20 ± 2 colonies per spleen. In contrast, LCMV caused a significant reduction of CFU when given 1 to 3 days before or after irradiation, this effect being most pronounced (5% of controls) 1 day prior to, or 1 day following, irradiation. Mice in which infection was initiated 7 or 5 days before irradiation had numbers of splenic CFU that were the same as those of control spleens. Based on these findings, in subsequent experiments LCMV was inoculated 1 day before irradiation to maximize virus-induced depression of compensatory hemopoesis.

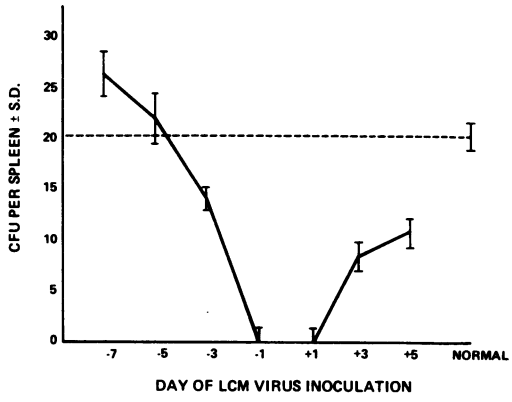

FIG. 1. *Depression of irradiation-induced splenic CFU in C3H mice by LCMV given on the days indicated before (−) or after (+) 500 R irradiation.*

Lack of an effect by LCMV on HSC colony formation in vitro. To determine whether the observed depression of endogenous repopulation of spleens from infected irradiated mice was due to a virus-induced proliferative defect localized within the bone marrow compartment, 10^5 viable marrow cells from normal mice, and mice infected with LCMV 3 and 4 days previously, where plated on feeder layers (see above). In addition, normal marrow was incubated with infectious or heat-inactivated LCMV, before plating, to determine whether or not an inhibition of proliferative capacity could be attributed to a direct action by virus. After a 30-min adsorptive period at an approximate multiplicity of 5 infectious particles per cell, the cell-virus mixtures were plated in the usual manner. The data shown in Table 1 illustrate two major points: (i) without irradiation, no significant differences in bone marrow-derived HSC were demonstrable between normal and infected mice, and (ii) HSC formation in vitro by normal bone marrow was not affected by the initial presence of LCMV in amounts exceeding those detectable in bone marrow of infected irradiated mice (see below).

Titration of LCMV in spleen and bone marrow post-irradiation. Since the presence of infectious LCMV in spleen, and possibly bone marrow, has been presumed to be causally related to depression of both immune responsiveness and compensatory hemopoiesis, the levels of virus in these tissues were quantitated. C3H mice were inoculated i.p. with LCMV 1 day before sublethal irradiation, and, at 1, 3, and 5 days thereafter, uniform samples of spleen and marrow cells from five mice were pooled, frozen at −70°C, thawed, and then titrated i.c. in Swiss mice. Significant levels of LCMV were present

TABLE 1. *Bone marrow HSC formation in vitro: lack of an effect by LCMV*

Marrow donor	HSC colonies[a]		
	Expt 1	Expt 2	Expt 3
Normal	60	58	50
LCMV day 3[b]	55	54	52
LCMV day 4		55	50
Normal + inactivated LCMV[c]	60	71	
Normal + infectious LCMV[c]	58	69	

[a] Mean number of colonies per 10^5 bone marrow cells in each of two wells. Variation between wells of any given pair was ≷15%.

[b] Marrow obtained 3 and 4 days postinfection.

[c] Normal marrow incubated for 30 min with either inactivated or infectious LCMV before culture.

in spleen cells at all sampling times and in marrow at 3 and 5 days post-irradiation. Thus, the interval between infection and irradiation that resulted in maximal depression of compensatory splenic repopulation by bone marrow cells was more readily associated with virus in spleen rather than in bone marrow (Table 2).

LCMV-induced depression of SRBC-specific B cells. Although depressed primary antibody responses to SRBC have been thoroughly documented in mice acutely infected with viscerotropic strains of LCMV, it has been reported that a similar depression failed to occur using the neuroadapted E-350 strain (15). In view of this strain's ability to virtually ablate splenic CFU formation in irradiated mice (Fig. 1), its effect on SRBC-specific B-cell induction was reexamined to determine whether the phenomenon of antibody depression was merely a function of virus strain rather than a possible manifestation of the same mechanism involved in hemopoietic dysfunction.

Groups of five C3H mice were inoculated i.p. with LCMV 3, 5, and 7 days before, and on the same day as, an intravenous primary dose of SRBC was given. Four days after priming, individual spleens were assayed for numbers of direct (immunoglobulin M) PFC. Included as controls were spleens from mice given only SRBC. Because LCMV infections cause a marked splenomegaly, PFC were expressed as the number per 10^6 spleen cells and per whole spleen.

The results in Table 3 show that the E-350 strain of LCMV caused a reduction of PFC per 10^6 spleen cells that was most pronounced (10% of controls) when infection preceded SRBC priming by 5 to 7 days. In a similar study by others (15), E-350 infection did not suppress the immune response to SRBC given 10 days after virus. However, in that study circulating antibody (rather than PFC) was measured 7 days after i.p. priming, C57Bl mice were used, and virus was given in the footpad.

Suppression of irradiation-induced in vitro proliferation of bone marrow by LCMV. The experimental results presented thus far indicated that although LCMV infection initiated within 1 day of sublethal irradiation prevented compensatory endogenous splenic repopulation, this phenomenon was not correlated with any reduction in the in vitro proliferative capacity of bone marrow, either from virus-infected mice or that from normal mice cultured in the presence of virus.

We next examined the in vitro proliferative capacity of marrow from irradiated mice and from mice infected with LCMV before irradiation. Bone marrow cells were obtained from groups of five C3H mice irradiated 1, 3, 5, and 7 days previously and also from matching groups infected with LCMV 1 day before irradiation. Controls included marrow taken from nonirradiated mice, either uninfected or infected with LCMV 3 days previously. The cells from each group were pooled and plated in the usual fashion, and after 12 days, HSC colonies were counted.

As shown in Table 4, bone marrow from nonirradiated mice gave the expected numbers of colonies, virus infection alone having no depres-

TABLE 2. *Infectivity of spleen and bone marrow from LCMV-infected irradiated C3H mice*[a]

Days after irradiation	Titer per 10^7 cells[b]:	
	Spleen	Marrow
1	3.3	Undetectable
3	3.1	1.5
5	2.7	1.5

[a] LCMV given i.p. 1 day before irradiation (500 R).

[b] Log_{10} i.c. LD_{50} of pooled cells from 5 spleens or 10 femurs.

TABLE 3. *Depression of SRBC-specific PFC by preceding LCMV infection*

No. of days SRBC were preceded by LCMV	Cells/spleen $\times 10^6$	PFC/spleen $\times 10^2$ ± SD	PFC/10^6 cells ± SD
Control[a]	22.0	206 ± 32 (100.0)[b]	938 ± 66 (100.0)
7	88.5	85 ± 27 (41.3)	96 ± 30 (10.0)
5	90.0	71 ± 13 (34.5)	78 ± 14 (8.3)
3	60.5	196 ± 21 (95.2)	316 ± 44 (33.8)
0	21.5	149 ± 30 (72.3)	584 ± 15 (62.3)

[a] Control mice received SRBC only (day zero).

[b] Numbers in parentheses express PFC as a percentage of control values; SD, Standard deviation.

TABLE 4. *Suppression of irradiation-induced in vitro proliferation of bone marrow HSC by LCMV*

LCMV 1 day before irradiation	Days after irradiation (500 R)	HSC colonies[a]	
		Expt 1	Expt 2
− Control[b]	Nonirradiated	69	64
+ Control		76	61
−	1	5	7
+		13	18
−	3	151	120
+		19	32
−	5	62	79
+		23	41
−	7	15	25
+		31	40

[a] Mean number of colonies per 10^5 bone marrow cells in each of two wells. Variation between wells of any given pair was ≤15%.

[b] Controls were marrow from nonirradiated mice either uninfected (−) or infected 3 days previously (+).

sive effect. One day post-irradiation with or without virus inoculation, colony-forming potentials were markedly depressed. However, marrow taken 3 days post-irradiation showed an impressive enhancement in proliferative capacity as evidenced by about a 20-fold increase in colony numbers (as compared with day 1 post-irradiation). Equally impressive was the failure of this compensatory proliferation to occur in marrow from matched irradiated mice given LCMV. At 5 and 7 days, marrow from irradiated mice, with or without virus introduction, yielded colony numbers that, in general, indicated a gradual return of normal function.

Suppression of an irradiation-induced bone marrow-stimulating serum factor by LCMV. The results presented in the preceding section suggested that the depressed splenic repopulation occurring in irradiated LCMV-infected mice was related to the failure of their bone marrow cells to undergo the compensatory in vitro proliferative response shown by marrow from uninfected animals 3 days post-irradiation. We next explored the possibility that this virus-induced defect was extrinsic to the marrow compartment by seeing whether LCMV suppressed the production of a hypothetical, irradiation-induced humoral factor capable of stimulating marrow cells in vitro. Serum pools were collected from groups of five C3H mice at 1, 3, and 5 days post-irradiation and, at these same times, from matched groups inoculated with LCMV 1 day before irradiation. In a similar experiment, the radiomimetic drug cyclophosphamide (CY) was given i.p. (300 mg/kg of body weight) in lieu of irradiation. When added to cultures of normal marrow cells (final concentration, 10%), sera obtained from uninfected mice 3 days after treatment with either irradiation or CY caused a pronounced stimulation of deoxyribonucleic acid synthesis (and presumably proliferation) as measured by [^{3}H]TdR incorporation (Table 5). Sera obtained before or after this time stimulated poorly or not at all (data not shown). Interestingly, marrow taken from mice infected with LCMV 3 days previously was stimulated as well as normal marrow by 3-day post-irradiation serum. Sera obtained from mice 3 days after irradiation or CY treatment, preceded by LCMV infection, also caused enhanced [^{3}H]TdR uptake but to a lesser degree, suggesting that production of the extrinsic stimulating factor was suppressed as a consequence of infection.

LCMV-specific CTC induction after irradiation. Previous studies done in our laboratory have shown that, during the first 2 weeks after primary LCMV (E-350) infections, virus-specific splenic CTC are induced that mediate tissue pathology and virus clearance (11). With the notion that CTC induction might be another factor related to the depression of both splenic SRBC-specific PFC and CFU responses, spleens of infected C3H mice, with or without radiation exposure, were assayed for virus-specific cytolytic activity in vitro. Groups of five mice were inoculated with LCMV 5, 7, 9, and 11 days before assay. Lytic activity in pooled spleens from these groups was compared with that in spleens of identical groups, which, in addition, were irradiated 1 day after LCMV inoculation. The results are presented in Fig. 2 and show that irradiation had essentially no effect on the generation of effector lymphocytes having virus-specific lytic potential. Noteworthy was the fact

TABLE 5. *Suppression of an irradiation-induced bone marrow-stimulating serum factor by LCMV*

Serum donor[a]	[^{3}H]TdR incorporation by bone marrow cells (cpm × 10^3)[b]	
	Normal	LCMV
No serum	8.4 ± 0.6	9.1 ± 0.8
Normal	6.9 ± 0.6	9.8 ± 1.1
Irradiated	26.5 ± 5.8	27.0 ± 2.5
LCMV irradiated	13.6 ± 2.3	
CY	27.5 ± 2.5	
LCMV-CY	14.1 ± 1.8	

[a] Serum pools from normal C3H mice or those given irradiation or CY ± LCMV 1 day previously.

[b] Mean counts per minute ± 1 standard deviation of triplicate cultures of marrow cells from normal C3H mice or those given LCMV 3 days previously after incubation for 18 h in 10% serum.

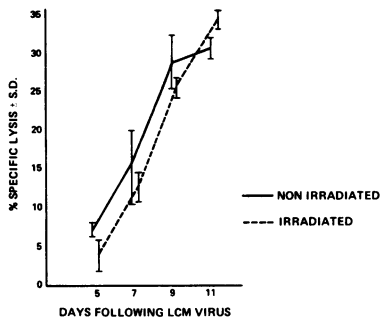

FIG. 2. *Generation of virus-specific CTC in LCMV-infected C3H mice ± irradiation 1 day previously. (Effector:target ratio = 40:1).*

that spleens of irradiated mice were about 80% smaller than those from nonirradiated mice, indicating that in the former lytic activity per spleen was actually greater. In other experiments (data not presented), it was shown that these effector lymphocytes were sensitive to anti-θ serum and complement, thus establishing their T cell lineage.

The mechanism by which the microenvironment of the spleen may be disadvantageously altered by resident CTC is discussed below.

DISCUSSION

Previously published studies have shown that acute, abortive LCMV infections of immunocompetent mice are followed by two striking, albeit transient, phenomena. Prominent during the first 2 weeks postinfection, they consist of a severe immune hyporesponsiveness involving both humoral (5, 6, 15) and cellular effector mechanisms (9, 13) and a defect in hemopoiesis that is revealed when infection is combined with irradiation (3-5). These effects have been observed when using viscerotropic strains of LCMV, which produce an extensive infection of lymphoid tissues (14) where low levels of virus may persist for many weeks before finally being eliminated (19). Although our investigations utilized a neuroadapted strain of LCMV that has a relatively restricted capacity to replicate in lymphoid tissues and which is cleared by about 1 week postinfection (11), we were able to demonstrate both phenomena.

In general agreement with the results of Bro-Jørgensen and Volkert (6), a marked depression of compensatory splenic hemopoiesis was seen when LCMV infection was initiated within a 3-day period before or after irradiation. This was reflected by a reduction in the number of spleen surface CFU visualized 8 days post-irradiation (Fig. 1). Splenic CFU reduction could not be attributed simply to an inhibitory effect of LCMV on bone marrow function, since neither normal marrow cells to which virus was added, nor those from acutely infected mice given no irradiation showed any abnormality in their ability to form HSC colonies in vitro (Table 1). It appeared, therefore, that a demonstrable hemopoietic lesion could be revealed only when LCMV infection was combined with irradiation.

In comparing HSC colony formation by bone marrow from irradiated mice with that of marrow from mice infected with LCMV 1 day before irradiation, an impressive difference was seen. Whereas marrow, obtained from uninfected mice 3 days post-irradiation, showed a compensatory burst in proliferative activity, marrow from infected irradiated animals remained depressed (Table 4). Since proliferation of bone marrow-derived HSC within the spleen would be required for the development of splenic CFU, these findings alone could account for concomitant defect in splenic hemopoiesis (Fig. 1). However, additional observations suggested that in animals receiving irradiation after LCMV infection, changes localized to the spleen may have contributed to this defect. Such mice showed a depression of a bone marrow-stimulating factor that appeared in the serum of uninfected mice 3 days after irradiation (Table 5). Although the source of this factor is not necessarily exclusive to spleen, its ability to stimulate deoxyribonucleic acid synthesis in cultured marrow cells suggests that it may have a similar effect within the splenic environment. Mice irradiated after infection were also able to generate a virus-specific CTC response that was essentially indentical to that of mice given virus alone (Fig. 2). CTC are induced only in acutely infected immunocompetent mice, and they are responsible for the destruction of virus-infected cells, which in turn eventuates in virus clearance (11). The period when both virus and the earliest induced CTC coexist in the spleen is between 3 and 7 days after primary infection with E-350 strain of LCMV (11). Therefore, during this period, cell types within the spleen that are engaged in hemopoiesis induced by irradiation could be infected with virus and then lysed by resident CTC. Hemopoiesis involves cell proliferation, and it is well established that LCMV has a selective affinity for dividing cells (16).

It should be noted that CTC are not likely to have been a major factor contributing to the depressed splenic CFU responses of animals irradiated before LCMV infection. Under these conditions, the induction of CTC would be delayed or prevented because their precursors are radiosensitive before being triggered by antigens (18).

On the other hand, it is quite possible that

the depressed PFC responses to SRBC seen in animals given only LCMV occurs by CTC-mediated lysis of one or more of the splenic cell types participating in immune induction. Among these, macrophages are likely targets of CTC since they become infected and then dysfunctional during week 1 after LCMV inoculation (10).

A recent study by Bro-Jørgensen and Knudtzon (3) has shown that irradiation is not required to reveal depression of bone marrow and splenic hemopoiesis in mice acutely infected with viscerotropic LCMV. On the basis of an observed temporal relationship between virus-induced circulating interferon and hemopoietic dysfunction, these investigators suggested that interferon may suppress hemopoiesis. It is unlikely that such a mechanism is relevant to the findings presented here since the E-350 strain of LCMV induces little or no interferon in the blood or spleen at any time after i.p. inoculation (G. A. Cole, unpublished observations). Furthermore, in the absence of irradiation, we were unable to demonstrate any evidence of HSC depression (Table 1). These contrasting results are probably best explained by differences in the biological properties between viscerotropic and neuroadapted strains of LCMV.

We suggest that the transient depression in immunological reactivity after acute LCMV infection is a consequence of ongoing virus-specific CTC generation. Whether proliferating cells are directly lysed within lymphoid tissues after becoming infected or are non-specifically suppressed by local factors produced as a consequence of virus-induced T-cell activation remains to be determined. Depression of HSC is a separable phenomenon and is likely the result of several mechanisms, depending on conditions created by a particular experimental protocol. Our studies indicate that the most prominent of these mechanisms is a direct inhibitory effect of LCMV on a subpopulation of radioresistant bone marrow HSC.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant NS-11286 from the National Institute of Neurological and Communicative Disorders and Stroke.

LITERATURE CITED

1. **Bradley, T. R., and D. Metcalf.** 1966. The growth of mouse bone marrow cells in vitro. Aust. J. Exp. Biol. Med. Sci. **44**:287–300.
2. **Bro-Jørgensen, K., F. Güttler, P. N. Jørgensen, and M. Volkert.** 1975. T lymphocyte function as the principal target of lymphocytic choriomeningitis virus-induced immunosuppression. Infect. Immun. **11**:622–699.
3. **Bro-Jørgensen, K., and S. Knudtzon.** 1977. Changes in hemopoiesis during the course of acute LCM virus infection in mice. Blood **49**:47–57.
4. **Bro-Jørgensen, K., and M. Volkert.** 1972. Haemopoietic defects in mice infected with lymphocytic choriomeningitis virus. 1. The enhanced X-ray sensitivity of virus infected mice. Acta Pathol. Microbiol. Scand. Sect. B **80**:845–852.
5. **Bro-Jørgensen, K., and M. Volkert.** 1972. Haemopoietic defects in mice infected with lymphocytic choriomeningitis virus. 2. The viral effect upon the function of colony-forming stem cells. Acta Pathol. Microbiol. Scand. Sect. B **80**:853–862.
6. **Bro-Jørgensen, K., and M. Volkert.** 1974. Defects in the immune system of mice infected with lymphocytic choriomeningitis virus. Infect. Immun. **9**:605–614.
7. **Cunningham, A. J., and A. Szenberg.** 1968. Further improvements in the plaque technique for detecting single antibody-forming cells. Immunology **14**:599–600.
8. **Curry, J. L., and J. J. Trentin.** 1967. Hemopoietic spleen colony studies. I. Growth and differentiation. Dev. Biol. **15**:395–413.
9. **Güttler, F., K. Bro-Jørgensen, and P. N. Jørgensen.** 1975. Transient impaired cell-mediated tumor immunity after acute infection with lymphocytic choriomeningitis virus. Scand. J. Immunol. **4**:327–336.
10. **Jacobs, R. P., and G. A. Cole.** 1976. Lymphocytic choriomeningitis virus-induced immunosuppression: a virus-induced macrophage defect. J. Immunol. **117**:1004–1009.
11. **Johnson, E. D., and G. A. Cole.** 1975. Functional heterogeneity of lymphocytic choriomeningitis virus-specific T lymphocytes. I. Identification of the effector and memory subsets. J. Exp. Med. **141**:866–881.
12. **Kärber, G.** 1931. Beitrag zur Kollectiven Behandlung pharmakologischer Reihenversuche. Arch. Exp. Pathol. Pharmakol. **162**:480–483.
13. **Lehmann-Grube, F., I. Niemeyer, and J. Löhler.** 1972. Lymphocytic choriomeningitis of the mouse. IV. Depression of the allograft reaction. Med. Microbiol. Immunol. **158**:16–25.
14. **Mims, C. A., and F. A. Tosolini.** 1969. Pathogenesis of lesions in lymphoid tissue of mice infected with lymphocytic choriomeningitis (LCM) virus. Br. J. Exp. Pathol. **50**:584–592.
15. **Mims, C. A., and S. Wainwright.** 1968. The immunodepressive action of lymphocytic choriomeningitis virus in mice. J. Immunol. **101**:717–724.
16. **Monjan, A. A., G. A. Cole, D. H. Gilden, and N. Nathanson.** 1973. Pathogenesis of cerebellar hypoplasia produced by lymphocytic choriomeningitis virus infection of neonatal rats. I. Evolution of disease following infection at 4 days of age. J. Neuropathol. Exp. Neurol. **32**:110–124.
17. **Pluznik, D. H., and L. Sachs.** 1966. The induction of clones of normal mast cells by a substance from conditioned medium. Exp. Cell Res. **43**:553–563.
18. **Taniguchi, K., K. Nomoto, M. Sato, Y. Yano, and K. Takeya.** 1976. Analysis of the developmental stage of specific and nonspecific cytotoxicity. I. Separation of the developmental steps of specific cytotoxicity by susceptibility to irradiation. Cell. Immunol. **25**:228–236.
19. **Volkert, M., and C. Lundstedt.** 1967. The provocation of latent lymphocytic choriomeningitis virus infections in mice by treatment with antilymphocytic serum. J. Exp. Med. **127**:327–339.