FAILURE IN GENERATING HEMOPOIETIC STEM CELLS IS THE PRIMARY CAUSE OF DEATH FROM CYTOMEGALOVIRUS DISEASE IN THE IMMUNOCOMPROMISED HOST

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Cytomegalovirus (CMV) disease with a high incidence of mortality is a serious complication in leukemia patients who receive γ irradiation to eradicate leukemic cells and continued immunosuppressive treatment after bone marrow transplantation to depress graft-vs.-host disease (1). The infection of the transplant recipient can be primary, transferred with the transplant as infectious virus or in the form of latently infected cells, or can result from reactivation of latent virus genome harbored in tissues of the recipient. In either case, the immunocompromised state is decisive for the spread of virus and the manifestation of disease with destruction of target tissues. Recently, it became evident that manifestations of CMV disease are frequent and often represent the final reason for mortality in patients with AIDS (2–4). Interstitial pneumonia is assumed to be the cause of death from human CMV disease, although other organs are also severely affected.

Experimental evidence for the cause of death can be attained only in an animal model. For studying CMV pathogenesis and protective immunity, we use the infection of mice with murine CMV (MCMV)[1] as a model system (5–9). Under the experimental conditions chosen, the immunocompetent host can control the infection, while the immunodepleted host succumbs to viral disease manifested by histopathology in various organs including the lungs, where interstitial pneumonia could be demonstrated (9). Protection against MCMV disease is mediated by virus-specific T lymphocytes of the $CD8^+$ subset (9–12). Among the multitude of proteins specified by MCMV, the nonstructural immediate-early protein pp89 (13, 14) encoded by gene *ieI* (15, 16) was identified as an immunodominant antigen recognized by CTL (17–22) and responsible for the induction of protective immunity (22a).

The definition of the most critical target tissues for viral infection is essential for selective antiviral therapy. We had observed previously that lethally infected

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[1] *Abbreviations used in this paper:* GM, granulocyte/monocyte; MCMV, murine CMV.

mice that survived after adoptive immunocytotherapy established a long-lasting persistent infection, some individuals of which showed considerable virus replication in the lungs 2 mo after lymphocyte transfer without signs of morbidity (11). This finding already raised doubt of whether interstitial pneumonia alone is responsible for mortality. We present evidence that failure in hemopoiesis is the primary cause of death from CMV disease in the murine model system.

Materials and Methods

Dual-parameter Light Scatter Analysis of Bone Marrow Cells. Suspensions of bone marrow cells were obtained by flushing PBS supplemented with 5% FCS through the shafts of the femori and tibiae. The pooled cells yielded from two femori and two tibiae were taken as representative of the bone marrow. Suspensions were passed through nylon mesh to remove clumps, and were enriched for mononuclear bone marrow cells by removing most of the erythrocytes and granulocytes during a density gradient centrifugation (30 min at 800 *g*) using Lymphoprep (Nyegaard, Oslo, Norway) for separation. In normal BALB/c mice (8-wk-old females, MHC haplotype H-2^d), the average yield was 5×10^7 bone marrow cells. At day 14 after irradiation (6 Gy) and infection, the average yield of bone marrow cells was reduced to 2×10^5.

The dual-parameter light scatter analysis was performed on FACS IV (Becton Dickinson FACS Systems, Sunnyvale, CA) measuring 50,000 cells per analysis with a flow rate of 2,000 cells/s. Right angle (90°) light scatter and forward (3°) light scatter, representing granularity and size, respectively, were expressed on scales of 64 channels. Three-dimensional images of light scatter profiles were obtained by connecting the cell frequency peaks of all net points in the 64 × 64 channel matrix normalized to the peak of the highest cell frequency (computer program provided by M. Blaurock, Medical University Clinic, Tübingen, Federal Republic of Germany).

Bone Marrow Transplantation and Adoptive T Lymphocyte Transfer. To serve as recipients of bone marrow or of effector T lymphocytes, 8-wk-old female BALB/c mice were subjected to total-body γ irradiation with a single radiation dose of 6 Gy by using a cesium-137 source, delivering a dose rate of 1.325 Gy/min. After irradiation, mice were kept under laminar flow. For intraplantar infection, 10^5 plaque-forming units of MCMV (strain Smith, VR-194; American Type Culture Collection, Rockville, MD) were injected into the left hind footpad 2 h before cell transfer. Morbidity caused by irradiation alone was indicated by hunching, wasting, and a bleeder symptom resulting from thrombocytopenia. Superimposed intraplantar infection caused hemorrhages spreading from the site of infection and necroses in internal organs including the lungs, spleen, liver, and adrenal glands (9, 12).

For bone marrow transplantation, bone marrow cells from normal syngeneic donors (8–12-wk-old female BALB/c mice) were isolated as described above except that granulocytes and erythrocytes were not removed. Mature T lymphocytes contained in the bone marrow cell suspension were eliminated by treatment with mAb anti-Lyt-2.2 (anti-CD8) and mAb anti-L3T4 (anti-CD4) as described previously (9). The purified bone marrow cells were infused intravenously. Syngeneic antiviral effector T lymphocytes were generated and separated into $CD4^+$ and $CD8^+$ subpopulations as detailed in a previous report (11), and were infused accordingly.

Assays for the Determination of Hemopoietic Stem Cells and Progenitors. For the determination of hemopoietic stem cells (CFU-S), bone marrow cells were isolated without depletion of cell populations, and graded numbers were transferred intravenously into irradiated (7.5 Gy), noninfected recipients. The radiation dose of 7.5 Gy was chosen to exclude the formation of endogenous spleen colonies in the recipients. This condition was fulfilled (see Fig. 3 *C*). At day 14 after transfer, the spleens of six recipients per bone marrow cell dose were fixed in Bouin's solution, and surface colonies, each representing the progeny of a single stem cell, were counted (23, 24). Only late colonies present beyond day 10 after transfer are derived from multipotential, self-generating stem cells, whereas transient early colonies visible at day 7 or 8 (CFU-S-II) contain terminally dif-

ferentiating erythroid cells (25, 26). According to Metcalf (27) the term CFU-S-I is used for stem cells that form late colonies. The number of CFU-S-I per donor bone marrow was calculated from the number of spleen colonies counted in the linear portion of the titration (see Fig. 3 *C*) by multiplication with the yield of bone marrow cells. The data were not corrected for the fraction of stem cells that reach the spleen after transfer. For bone marrow cells derived from noninfected mice, this fraction has been found to be 0.17 (24).

For the quantitation of the progenitors of the granulocyte/monocyte lineage, the CFU-GM, mononuclear bone marrow cells were enriched by Lymphoprep separation, and 5×10^4 cells were dispensed in 1 ml of semi-solid Iscove's Modified Dulbecco's Medium (074-2200; Gibco Laboratories, Grand Island, NY) containing 0.9% wt/vol methylcellulose, 20% FCS, and 5% WEHI 3B-conditioned medium as a source of multipotential CSF (28). Granulocyte/monocyte colonies were counted after 8 d of incubation (5% CO_2, 37°C, humidified atmosphere).

Determination of Virus Titers in Tissues. Infectious MCMV in lungs, spleen, and adrenal glands was quantitated 14 d after infection by a plaque assay as described before (9). The detection limit was 100 plaque-forming units of MCMV per organ homogenate. Sets of virus titers are regarded as significantly different for $P < \alpha = 0.05$ (one-sided), where P denotes the observed probability value, and α denotes the selected significance level (Wilcoxon-Mann-Whitney exact rank sum test).

In Vivo Assay for the Detection of Infected Bone Marrow Cells. For an in vivo limiting dilution analysis of infected cells, bone marrow cells were isolated from irradiated (6 Gy), infected mice without depletion of cell populations, and were separated from free virus by centrifugation through an FCS cushion. Graded numbers were transferred intravenously into irradiated (6 Gy) recipients. At day 14 after cell transfer, inoculation of the lungs was scored positive or negative by measuring the virus titer. The frequency (f) of infected cells in the transferred cell population was estimated by the Maximum Likelihood method (29).

By transfer of limiting numbers (8–0.5 in two-fold dilutions) of infected mouse embryo fibroblasts, a cell type that is permissive for productive MCMV infection, this in vivo assay was found to be sensitive enough to detect a single productively infected cell ($f = 1/1.2$, $P = 0.24$).

Results

MCMV Infection Prevents Autoreconstitution of Bone Marrow. Sublethal total-body γ irradiation with a radiation dose of 6 Gy depresses the immune defense of BALB/c mice to an extent that infection with MCMV leads to lethal disease (9). This radiation dose does not eliminate all bone marrow stem cells and, therefore, the bone marrow should be replenished by hemopoiesis.

To test whether or not hemopoiesis is affected during murine CMV disease, the autoreconstitution of bone marrow was monitored in irradiated vs. irradiated and infected animals by means of dual-parameter light scatter analysis of bone marrow cells. The assay is explained in Fig. 1 with an analysis of normal bone marrow serving as a standard for comparison.

2 d after irradiation, the bone marrow was found to be almost depleted of nucleated cells. Autoreconstitution was visible at day 6 as an emerging peak of cells that assumed the shape of normal bone marrow by day 14 (Fig. 2, *left column*). After irradiation and infection (Fig. 2, *right column*), hemopoiesis initially generated new bone marrow cells, but this autoreconstitution discontinued around day 6 with the final result that infected animals were practically devoid of bone marrow cells by day 10 after infection. It should be recollected that day 6 and 14 after infection have been defined before as set-points in murine CMV

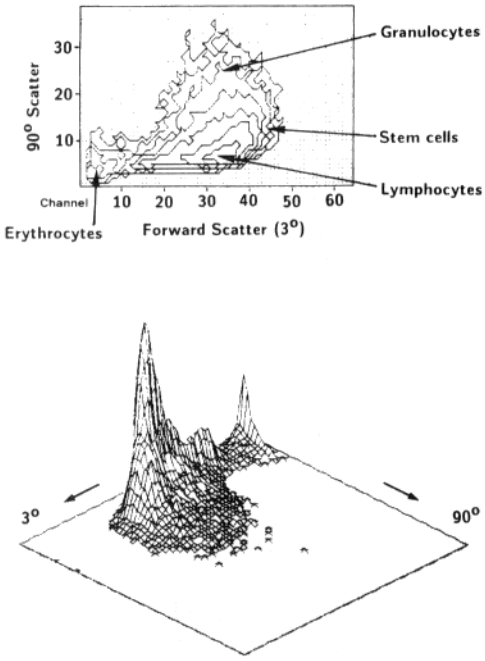

FIGURE 1. Dual-parameter light scatter analysis of normal bone marrow cells. *(Top)* Two-dimensional display of right-angle (90°) vs. forward (3°) light scatter. The contour lines represent levels of 2, 4, 8, 16, 32, 64, and 128 cells with a total of 50,000 Lymphoprep-purified bone marrow cells analyzed. The arrows point to areas in the contour plot where the indicated cell populations are known to be located. *(Bottom)* Three-dimensional image of the same analysis showing the cell frequencies in the third dimension.

disease after irradiation. By day 6, virus has colonized target tissues, such as the lungs, and at day 14 mortality is at its summit (9). Thus, inhibition of hemopoiesis becomes effective at the time when infection is established in host tissues, and mortality coincides with a state of almost complete bone marrow depletion.

MCMV Infection Interferes with the Earliest Step in Hemopoiesis. So far, the data have shown that infection inhibits autoreconstitution already at an early stage of disease, at a time when virus multiplication in permissive tissues has just begun (9). If the depletion of bone marrow observed after day 6 after infection is caused by direct infection of many bone marrow cells, this would indicate a particular tropism of MCMV for bone marrow. The number of infected cells detectable in the bone marrow during the critical time period between day 4 and 8 after infection was, however, very low. This result applied to productively infected cells enumerated by the in vivo titration of bone marrow cells in irradiated recipients (Table I) as well as to the total number of infected cells estimated by dot blot hybridization using cloned genomic fragments of MCMV DNA (not shown). Thus, neither direct elimination of many cells by productive, cytolytic infection nor direct functional inactivation of many cells by nonproductive or latent infection can account for the observed depletion of bone marrow. Rather, it has to be proposed that infection has an effect upon the generation of bone marrow cells from a few stem cells or progenitors.

This idea was supported by quantifying two types of hemopoietic cells (Fig. 3). After irradiation alone, the number of CFU-GM, the progenitors of monocytes and granulocytes, exponentially increased with time indicating successful autoreconstitution (Fig. 3 *A*, *closed circles*), while after irradiation and infection the number of CFU-GM remained stationary on a low level (Fig. 3 *A*, *open cir-*

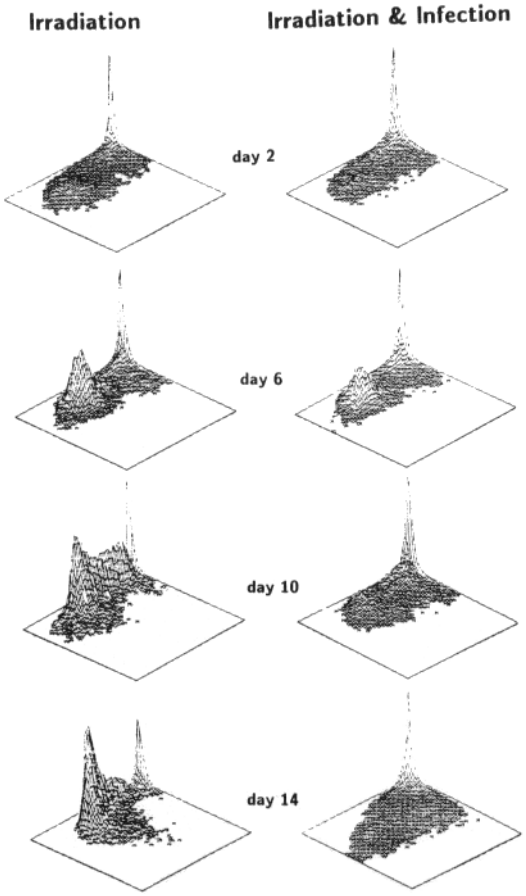

FIGURE 2. CMV infection prevents autoreconstitution of bone marrow in the irradiated host. *(Left column)* Kinetics of bone marrow autoreconstitution after total-body γ irradiation with a radiation dose of 6 Gy, visualized by dual-parameter light scatter analysis of bone marrow cells. *(Right column)* Kinetics of bone marrow autoreconstitution after irradiation and intraplantar infection with MCMV. Five individuals per group and per time point were analyzed and ranked according to increasing progress in bone marrow autoreconstitution. For the sake of brevity, the bone marrow profiles are depicted only for the animals with the median (No. 3) rank.

cles). This finding explains the granulocytopenia observed as one symptom of CMV disease in the mouse (30). It has to be considered, however, that a block in only one differentiation lineage cannot account for the observed almost complete failure in autoreconstitution (Fig. 2). Since all types of progenitors arise from a multipotential stem cell pool, it was reasonable to ask whether the stag-

TABLE I

Frequency of Infected Cells in the Bone Marrow

Days after infection	Proportion of infected recipients*			Frequency
	0.5×10^5 BMC	2×10^5 BMC	8×10^5 BMC	
4	ND	0/12	0/12	—‡
8	0/14	1/14	8/14	1/1,150,000
11	7/14	11/14	14/14	1/100,000

* At the indicated times after infection, bone marrow cells (BMC) were collected from irradiated, infected mice and pooled. For the enumeration of infected cells, graded numbers of BMC were transfused intravenously into irradiated recipient mice. The fractions represent the proportion of recipients that scored positive for infection of the lungs.

‡ No frequency estimate possible.

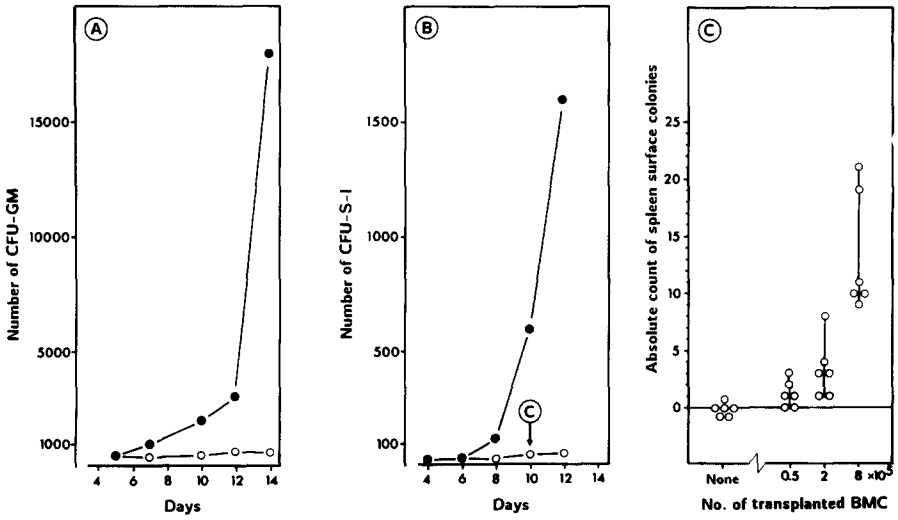

FIGURE 3. Failure in the generation of hemopoietic progenitors and stem cells. (*A* and *B*) Kinetics of the generation of CFU-GM (*A*) and CFU-S-I (*B*) during autoreconstitution after irradiation alone *(closed circles)* or after irradiation and infection *(open circles)*. Data represent the mean numbers of colonies from three individuals tested separately (*A*) or the average numbers of colonies determined for bone marrow cells pooled from an adequate number of donor mice (*B*), both calculated per bone marrow. (*C*) The determination of the CFU-S-I contained at day 10 after infection in the bone marrow of irradiated, infected mice *(arrow in B)* is resolved to greater detail. The absolute count of spleen surface colonies is depicted as a function of the number of transplanted bone marrow cells (BMC). The open circles represent measurements in six individual recipients.

nation in CFU-GM generation could be traced back to a depletion of stem cells or to a failure in the differentiation of stem cells to lineage progenitors. The self-generating, multipotential blast colony-forming cells CFU-S-I represent the earliest stem cells for which an assay is available, namely the formation of clonally derived cell colonies in the spleen of lethally irradiated bone marrow recipients (23–27). It was found that infection prevents the proliferation already of the CFU-S-I (Fig. 3 *B*). It is worth noting that a low number of CFU-S-I could be isolated from the bone marrow at all tested times after infection (Fig. 3 *B, open circles*). To show convincingly that these CFU-S-I were derived from the infected donor and not by autoreconstitution in the recipients used for detection, the determination at day 10 (Fig. 3 *B, arrow*) is resolved to greater detail by depicting the absolute count of spleen surface colonies as a function of the number of transferred donor bone marrow cells (Fig. 3 *C*). Thus, although in the donor bone marrow the number of CFU-S-I remained stationary, these CFU-S-I could proliferate and form colonies when translocated to recipient spleens. This fact argues against eradication of CFU-S-I by direct infection. Altogether, the findings allow the conclusion that MCMV infection interferes with the earliest detectable step in hemopoiesis, the generation of CFU-S-I.

Postulates for Proving a Proposed Vital Importance of Bone Marrow Autoreconstitution. An obvious question to be answered is whether the observed virus-mediated failure in hemopoiesis plays an essential part in lethal disease. If one

is to propose that not the histopathology observed in important tissues, e.g., interstitial pneumonia, hepatitis, and adrenalitis, but the depletion of bone marrow, is the primary cause of death, two conditions must be fulfilled for confirmation. First, as it is known that adoptive transfer of virus-specific $CD8^+$ T lymphocytes limits virus multiplication, prevents tissue destruction, and mediates survival (9–12), these cells must also restore the autoreconstitution by defeating the antihemopoietic effect of infection. Second, bone marrow transplantation should mediate survival notwithstanding continued virus multiplication in other tissues.

Adoptive Transfer of $CD8^+$ T Lymphocytes Restores Autoreconstitution of Bone Marrow. $CD8^+$ T lymphocytes, but not $CD4^+$ T lymphocytes, prevent histopathology by an antiviral function (11). However, this fact did not allow predictions concerning the roles of T lymphocyte subsets in preventing the failure in hemopoiesis. $CD8^+$ T lymphocytes could control virus multiplication by their antiviral function, while $CD4^+$ T lymphocytes could restore hemopoiesis by producing hemopoietic lymphokines. This possibility had to be tested (Fig. 4).

Short-term lines of virus-specific T lymphocytes propagated in IL-2 without feeder cells were depleted of either subset, and graded numbers of $CD8^+$ or $CD4^+$ T lymphocytes were transferred at the day of infection. The purity of the transferred T lymphocyte subsets was controlled by cytofluorometric analysis as documented in a previous report (11). In addition, the absence of hemopoietic stem cells in both preparations was ascertained by the absence of spleen colonies after transfer into lethally irradiated (7.5 Gy), noninfected recipients (not shown). The bone marrow of the sublethally irradiated (6 Gy), infected recipients was analyzed at day 14 after infection and T lymphocyte transfer (Fig. 4). Already, after transfer of only 10^4 $CD8^+$ T lymphocytes, an effect was visible, and the degree of autoreconstitution improved with increasing doses of protec-

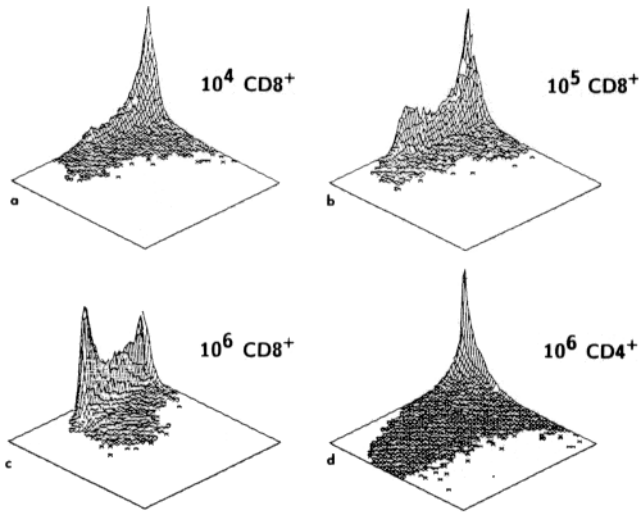

FIGURE 4. Effect of T lymphocyte transfer on bone marrow autoreconstitution. Antiviral T lymphocytes isolated from draining popliteal lymph nodes of immunocompetent donors 8 d after intraplantar infection with MCMV were propagated in vitro for a further 8 d with rIL-2 and were then depleted of either subset (11). Graded numbers of purified $CD8^+$ or $CD4^+$ T lymphocytes were transferred intravenously into irradiated, infected recipients. After 14 d, the success of bone marrow autoreconstitution was controlled by dual-parameter light scatter analysis. Of five individuals tested per group, the bone marrow profile of the one with the median virus titer in the lungs (see Fig. 5) is depicted.

tive lymphocytes, leading to almost normal bone marrow when 10^6 $CD8^+$ T lymphocytes were transferred (Fig. 4 *a–c*). $CD4^+$ T lymphocytes had no effect, not even at the highest cell number tested (Fig. 4 *d*), as was the case also with nonspecific T lymphocytes activated by IL-2 in vitro (not included in Fig. 4). In conclusion, the same T lymphocyte subset that controls virus multiplication and mediates survival also facilitates bone marrow autoreconstitution.

Syngeneic Bone Marrow Transplantation Has No Effect on the Virus Multiplication in Host Tissues. For the same individuals whose bone marrow autoreconstitution was studied (Fig. 4), virus titers in the lungs, spleen, and adrenal glands are depicted in Fig. 5. It is apparent that low virus titers in tissues correspond with successful autoreconstitution of bone marrow.

One reasonable explanation for this observation is that a direct antiviral function of $CD8^+$ T lymphocytes is responsible for both effects. So far, however, the data were also consistent with the interpretation that the only direct in vivo function of $CD8^+$ T lymphocytes was to restore hemopoiesis, and that newly arising bone marrow cells did control infection. This idea was formally disproved by the demonstration that syngeneic bone marrow cells did not exert an antiviral effect within the test period (Fig. 5; compare groups *A* and *D*, $p \geq 0.1$ in all tissues). In conclusion, bone marrow transplantation does not affect the replication of MCMV in host tissues, and the control of virus replication is mediated by a direct antiviral function of $CD8^+$ T lymphocytes.

Transfer of $CD8^+$ T Lymphocytes and Syngeneic Bone Marrow Transplantation Both Mediate Survival. The second prediction of the working hypothesis was that bone marrow cells alone should prevent death, despite their inability to limit MCMV multiplication in host tissues.

Irradiated, infected mice died of MCMV disease without exception between day 10 and 20 after infection (Fig. 6, *open squares*). Mortality set in when the

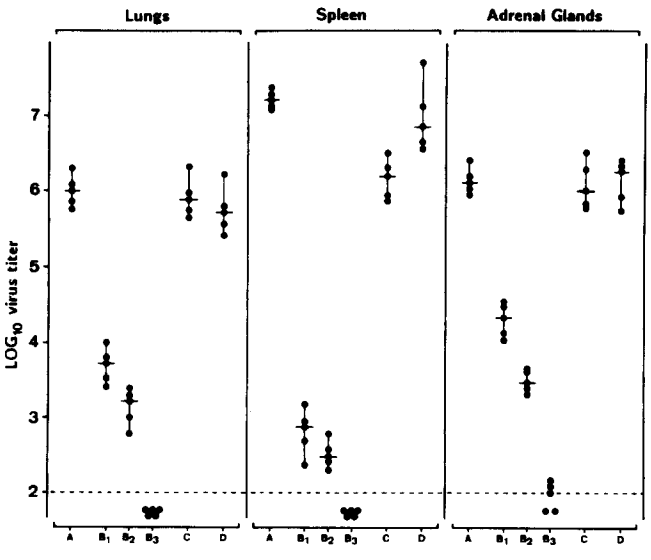

FIGURE 5. Antiviral efficacy of T lymphocyte subsets and bone marrow cells. Virus titers in tissues were measured at day 14 after infection in five recipients per group. Individual titers are signified by closed circles, and median values are marked by horizontal bars. The dashed line represents the detection level. (*A*) No transfer of lymphocytes. (B_1, B_2, B_3) Transfer of 10^4, 10^5, and 10^6 $CD8^+$ T lymphocytes, respectively. (*C*) Transfer of 10^6 $CD4^+$ T lymphocytes. (*D*) Transfer of 10^6 bone marrow cells depleted of T lymphocytes. The individuals in group B_1, B_2, B_3, and *C* with the median virus titers in the lungs are those whose bone marrow profiles are shown in Fig. 4 *a*, *b*, *c*, and *d*, respectively.

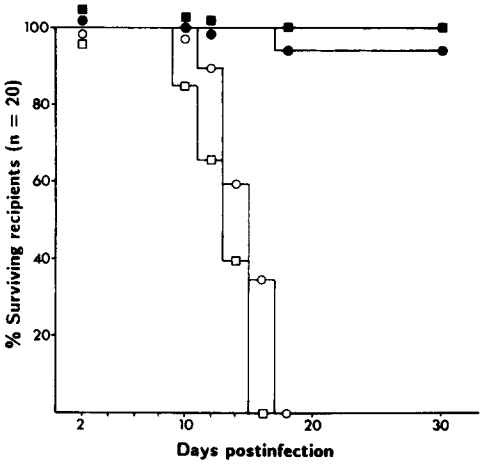

FIGURE 6. Antiviral $CD8^+$ T lymphocytes and bone marrow cells both protect against lethal CMV disease. Mortality in 20 irradiated (6 Gy), infected recipients per group ($n = 20$) was monitored for 30 d after no transfer of cells *(open squares)*, transfer of 10^6 bone marrow cells depleted of T lymphocytes *(closed squares)*, transfer of 10^6 $CD4^+$ T lymphocytes *(open circles)*, and transfer of 10^6 $CD8^+$ T lymphocytes *(closed circles)*.

bone marrow was depleted of cells and when virus multiplication caused necroses in organs that are of vital importance, e.g., the lungs, liver, and adrenal glands. Adoptive transfer of $CD4^+$ T lymphocytes did not cure either symptom (Figs. 4 and 5) and, accordingly, all recipients died of disease (Fig. 6, *open circles*). Adoptive transfer of $CD8^+$ T lymphocytes cured both symptoms. Accordingly, recipients survived (Fig. 6, *closed circles*), and it was not possible to judge whether bone marrow autoreconstitution or prevention of histopathology was more important. The decision came from the transfer of bone marrow cells. Although bone marrow cells did not limit virus multiplication in tissues, all recipients survived (Fig. 6, *closed squares*).

In three repeated experiments, which were all performed with 20 animals per group, the mortality was always 100% after no transfer of cells or after transfer of $CD4^+$ T lymphocytes. After transfer of bone marrow cells, the survival rate was 90–100%, whereas after transfer of $CD8^+$ T lymphocytes, the survival rate ranged from 45 to 95%. This range reflects the variance in the survival rate observed after 6 Gy irradiation without infection (not shown). It has been discussed in a previous report (11) that $CD8^+$ T lymphocytes cannot remedy radiation sickness but can only prevent the adverse effects of additional infection.

In essence, mice that did not possess bone marrow cells during a critical phase of disease around day 10 after infection died (Fig. 6, *open symbols*), while bone marrow reconstituted mice survived *(closed symbols)*, regardless of whether or not virus multiplication in tissues was controlled by antiviral effector cells. We therefore conclude that aplasia resulting from the virus-induced failure in hemopoiesis is the primary cause of death from CMV disease in the immunocompromised murine host.

Discussion

Recent findings have indicated a similarity in the pathogenesis of murine and human CMV also with regard to an effect on hemopoiesis. During MCMV disease, granulocytopenia and thrombocytopenia were observed (30, 31) and, like-

wise, a failure in thrombocytopoiesis has also been diagnosed in patients with opportunistic human CMV infection (32). It has been reported that human early hemopoietic cells can be infected with human CMV in vitro (33). The spleen colony-forming assay that defines the multipotential stem cell CFU-S-I is not feasible in humans, and consequently, nothing is known of the human equivalent of CFU-S-I. At this point of investigation, only a model system can provide further insight into the causes of malfunction.

Our findings demonstrate for MCMV disease and predict for human CMV disease that CMV infection interferes with the generation already of the earliest detectable stem cell in hemopoiesis, the CFU-S-I.

It is of interest to know the mechanism by which CMV inhibits the generation of CFU-S-I as well as the mechanism by which $CD8^+$ T lymphocytes prevent that inhibition. A final answer to these questions is beyond the scope of our present contribution, but apparent possibilities might be discussed.

First, virus could eliminate CFU-S-I by direct infection. Two observations argue against that mechanism. (*a*) A low but significant number of CFU-S-I could be demonstrated at all tested times after infection in the bone marrow of irradiated, infected hosts. Although the number of infected cells detectable in the bone marrow increased tenfold between day 8 and 11 after infection, the number of residual CFU-S-I did not decrease, suggesting that CFU-S-I did not serve as targets for MCMV. (*b*) Transfer of normal bone marrow cells into infected recipients led to the development of CFU-S-I colonies in spleens (not shown), where at the same time, virus was replicating. Moreover, the reconstitution with bone marrow was successful in irradiated, infected mice as all recipients survived. These findings showed that stem cells are not eradicated in the bone marrow of infected mice and that after adoptive transfer, the remaining CFU-S-I can proliferate at least in the environment of the spleen.

Second, the proliferation of CFU-S-I in the bone marrow could be discontinued by cessation of the production of essential growth-promoting cytokines. To date, the only cytokine supposed to promote the generation of CFU-S-I is the lymphokine IL-3 (synonym: multi-CSF) produced mainly by activated T lymphocytes of the $CD4^+$ subset (28, 34). Our finding that activated $CD4^+$ T lymphocytes did not restore hemopoiesis does not exclude the explanation that a deficiency in IL-3 is responsible for the failure in hemopoiesis, as we do not know whether adoptively transferred $CD4^+$ T lymphocytes secrete IL-3 at the site where it is needed. One should also consider that the generation of CFU-S-I in autoreconstituting bone marrow after sublethal irradiation does certainly not depend upon IL-3 produced by antigen-activated T lymphocytes. The regulation of CFU-S-I proliferation must rather be either autocrine or mediated by a cytokine delivered by a radiation (6 Gy)-resistant stroma cell.

Third, impairment of the stromal microenvironment could account for the failure in stem cell proliferation. The hemopoietic microenvironment of the bone marrow stroma consists of endothelial cells and a network of fibroblasts, macrophages, and adipocytes (for review see reference 35). It is known that stroma cells play a critical role in hemopoiesis by promoting maintenance and self-renewal of CFU-S-I (36), and it has been shown recently that fibroblastoid adventitial reticular cells of the stroma support hemopoiesis (37). Several col-

ony-stimulating cytokines are secreted by stroma-derived cell lines (38–41). In this context it is important to recollect that murine CMV productively infects fibroblasts in vitro (42) and in vivo (9). The observation that CFU-S-I that remained resting in the bone marrow of the infected host could form colonies when translocated by adoptive transfer into the spleen (recapitulate Fig. 3 *B* and *C*) suggests an influence of the hemopoietic microenvironment on the generation of CFU-S-I.

It is known from clinical experience that (*a*) mainly recipients of allogeneic bone marrow, but not recipients of syngeneic bone marrow, develop severe CMV disease, although the incidence of CMV infection is similar in both groups of patients (1), and that (*b*) recovery from CMV infection correlates with the presence of CMV-specific CTL (4). Our finding that an important function of CTL is to prevent the antihemopoietic effect of CMV infection discloses a common protective principle of syngeneic bone marrow transplantation and antiviral cellular immunity, namely the replenishment of bone marrow by reconstitution and autoreconstitution, respectively. Thus, the murine model system offers an explanation for the clinical observations.

We postulate that the high incidence of lethal CMV disease in recipients of allogeneic bone marrow is the result of an ineffective reconstitution by histoincompatible hemopoietic cells. An incomplete or delayed generation of immune effector cells then leads to a failure in the control of disease. The antihemopoietic effect of CMV infection has the same consequence. Thus, a synergism in the effects of histoincompatibility and CMV infection could explain the association between graft-vs.-host disease and fatal CMV disease.

Summary

We have shown in a murine model system for cytomegalovirus (CMV) disease in the immunocompromised host that CMV infection interferes with the earliest detectable step in hemopoiesis, the generation of the stem cell CFU-S-I, and thereby prevents the autoreconstitution of bone marrow after sublethal irradiation. The antihemopoietic effect could not be ascribed to a direct infection of stem cells. The failure in hemopoiesis was prevented by adoptive transfer of antiviral $CD8^+$ T lymphocytes and could be overcome by syngeneic bone marrow transplantation. $CD8^+$ T lymphocytes and bone marrow cells both mediated survival, although only $CD8^+$ T lymphocytes were able to limit virus multiplication in host tissues. We concluded that not the cytopathic effect of virus replication in host tissues, but the failure in hemopoiesis, is the primary cause of death in murine CMV disease.

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