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Requirement for Neutralizing Antibodies to Control Bone Marrow Transplantation-Associated Persistent Viral Infection and to Reduce Immunopathology[1]

Karl S. Lang,[2,3] Mike Recher,[2] Alexander A. Navarini,[2] Stefan Freigang, Nicola L. Harris, Maries van den Broek, Bernhard Odermatt, Hans Hengartner, and Rolf M. Zinkernagel

Bone marrow transplantation (BMT) is commonly used in the treatment of leukemia, however its therapeutic application is partly limited by the high incidence of associated opportunistic infections. We modeled this clinical situation by infecting mice that underwent BMT with lymphocytic choriomeningitis virus (LCMV) and investigated the potential of immunotherapeutic strategies to counter such infections. All mice that received BMT survived LCMV infection and developed a virus carrier status. Immunotherapy by adoptive transfer of naive splenocytes protected against low (200 PFU), but not high (2×10^6 PFU), doses of LCMV. Attempts to control infection of high viral titers using strongly elevated frequencies of activated LCMV-specific T cells failed to control virus and resulted in immunopathology and death. In contrast, virus neutralizing Abs combined with naive splenocytes were able to efficiently control high-dose LCMV infection without associated side effects. Thus, cell transfer combined with neutralizing Abs represented the most effective means of controlling BMT-associated opportunistic viral infection in our in vivo model. These data underscore the in vivo efficacy and immunopathological "safety" of neutralizing antibodies. *The Journal of Immunology,* **2005, 175: 5524–5531.**

Bone marrow transplantation (BMT)[4] is currently used as a therapy for leukemia (1–4) and severe genetic disorders such as Wiskott-Aldrich syndrome (5, 6). In addition, it demonstrates some potential in the treatment of some aggressive autoimmune diseases (7, 8). However, associated with BMT are several side effects including alloreactivity, pancytopenia, and a high risk of opportunistic infection by viruses, fungi, and parasites (9). For instance, infections with varizella-zoster virus occur in up to 40% of bone marrow (BM) recipients and, if left untreated, result in a mortality rate of >30% (10). Fifteen percent of BMT patients develop CMV pneumonia, which results in lethality for up to 50% of cases (11), whereas ∼6% (12) of BMT patients suffer from aspergillosis, for which mortality rates approach 100% (13–16). Notable but less frequent infections noted in BMT patients include *Toxoplasma gondii*, *Pneumocystis carinii*, and several bacterial and viral species including *Streptococcus pneumoniae*, *Listeria* monocytogenes, respiratory syncytial virus, and human herpes virus 6 (9).

Pharmacotherapy is commonly used in the treatment of BM recipients; however, if given late, it is ineffective against invasive aspergillosis or CMV and can result in the emergence of drug-resistant variants (14, 17–21). Immunotherapy has been proposed recently as an alternative and effective means of pathogen control after BMT. Transfer of viral-specific CD8$^+$ T cells has been used to treat patients suffering from infection with CMV (22–24) or EBV (25, 26), and Ab transfer was demonstrated to abrogate aspergillosis in an experimental model of BMT (27).

To analyze the potential and limitations of cellular and humoral adoptive immunotherapy associated with BMT, we examined mouse infection with lymphocytic choriomeningitis virus (LCMV) immediately after BMT. Although LCMV infection is not a noteworthy complication after BMT in humans, it represents the well-characterized prototypic poorly cytopathic virus that is controlled in a CD8$^+$ T-cell-dependent manner, persists lifelong similar to human CMV (28–30), and has been reported to reactivate in organs during organ transplantation (31). Furthermore, LCMV neutralizing Abs are induced rarely after single infection and therefore are thought to play a minor role at least in early virus control (32–34). In our model, we irradiated recipient C57BL/6 mice lethally, transferred syngeneic BM, and infected mice with different doses of LCMV. In parallel, we treated recipient mice with naive or specifically activated T cells or with neutralizing Abs alone or in combination (Table I).

Here, we show that cellular immunotherapy with supplemented protective Abs is the most effective means to protect BMT recipients from opportunistic, clinically persistent, and immunopathology causing viral infection.

Materials and Methods

Mice and viruses

LCMV strain WE was originally obtained from F. Lehmann-Grube (Heinrich Pette Institute, Hamburg, Germany) and propagated on L929 cells. Viral titers were measured using a plaque-forming assay (35). Splenocytes from C57BL/6 mice, LCMV-gp61/I-A^b-specific TCR-transgenic mice (SMARTA) (36), and LCMV-gp33/H-2D^b-specific TCR-transgenic mice (318) (37) were used for adoptive transfer. All mice used in this study were maintained on the C57BL/6 background and housed under specific pathogen-free conditions.

Table I. *Summary of transferred immune components*

	LCMV-Specific CD4$^+$ T Cells (total number)	LCMV-Specific CD8$^+$ T Cells (total number)	LCMV Neutralizing Ab (titer)
	0^a	0^a	<1:10
Naive splenocytes	10	10	<1:10
Naive TCR-tg 318 splenocytes	10	5×10^5	<1:10
Activated TCR-tg 318 splenocytes	2×10^5	5×10^5	<1:10
Naive TCR-tg SMARTA splenocytes	1×10^6	0	<1:10
Naive TCR-tg 318/SMARTA splenocytes	1×10^6	5×10^5	<1:10
Hyperimmune serum	0	0	1:400
Hyperimmune serum + naive splenocytes	10	10	1:400

Immunotherapy of BMT recipients. Values represent the estimated numbers of LCMV-specific cells in BMT recipients after transfer of splenocytes (per mouse) or Ab titers in the serum of recipient mice after transfusion of hyperimmune serum.

^{a} BM from naive C57BL/6 mice contained 0.40 ± 0.10% naive CD8$^+$ and 0.57 ± 0.06% naive CD4$^+$ T cells (in accordance with Ref. 51). We transferred probably one LCMV-specific CD8 T cell and one specific CD4 T cell within the BM in one of three mice.

BMT and adoptive transfer

Mice were lethally irradiated (950 rad) on day −1. On day 0, 8×10^6 BM cells, generated by flushing the femur and tibia of naive C57BL/6 mice, were injected i.v. BM was not T cell depleted and would therefore lead to minimal transfer of LCMV-specific T cells (Table I; Ref. 51). Recipient and donor mice were age and sex matched. For immunotherapy, mice were also given injections of 10^7 of the indicated splenocytes on day 0 or 200 µl of hyperimmune serum (titer, 1:4000). Activated LCMV-specific T cells were generated in vivo by infected mice 8 days before with 2×10^6 PFU of recombinant vaccinia expressing the LCMV glycoprotein (vaccG2) (38). For adoptive transfer of CD8-depleted cells, donor mice were given injections of 3 mg of anti-CD8 Ab (YTS 169.8) on days −3 and −1. Hyperimmune serum was generated in mice infected with 10^5 PFU of LCMV-WE, boosted by subsequent viral infection on days 30 and 60, and bled 20–40 days after the last infection.

FACS analysis

FACS analysis was performed as described previously (39). Briefly, 10^6 splenocytes or BM cells were stained using PE-labeled LCMV-WE GP33 tetramer (GP33-Db) (39) for 15 min at 37°C, followed by staining with anti-CD8-APC, anti-Vα2-FITC (for detection of transgenic LCMV-specific CD4$^+$ T cells), or anti CD4-PercP (BD Pharmingen) for 30 min at 4°C. Intracellular cytokine staining was performed using 10^6 splenocytes previously incubated for 6 h at 37°C in RPMI 1640 containing 10% FCS, antibiotics, and 25 U/ml IL-2 and stimulated with gp33 (10^{-7} M), gp61 (10^{-6} M), or left untreated. Brefeldin A (Sigma-Aldrich) was added (final concentration, 5 µg/ml) after 1 h of incubation. At the end of the stimulation period, splenocytes were surface stained with anti-CD4 FITC, anti CD4-PE, anti-CD8-PercP, or anti-CD8-PE (BD Pharmingen) for 30 min at 4°C. Cells were fixed and permeabilized using 4% paraformaldehyde in PBS containing 0.1% saponin for 10 min at room temperature, stained with anti-IFN-γ-APC (BD Pharmingen), and analyzed using a FACSCalibur and Cell Quest software.

Histology

Histological analysis was performed on tissue fixed in 4% formalin or snap frozen and stained with eosin G and thiazine dye (Diff-Quik; Dade Behring) or with goat mAbs against murine CD4 (YTS 191) or CD8 (YTS 169), respectively. Goat mAbs were detected using alkaline phosphatase-labeled donkey anti-goat Abs (Jackson ImmunoResearch Laboratories) and alkaline phosphatase visualized using naphthol AS-BI (6-bromo-2-hydroxy-3-naphtholic acid 2-methoxy anilide) phosphate and new fuchsin as a substrate. The presence of alkaline phosphatase activity yielded a red reaction product.

Statistical analysis

All data are expressed as mean ± SEM. Unless mentioned otherwise, the different treatment groups involved the use of six animals in at least two independent experiments. Analysis of organ titers and intracellular cytokine stainings were performed within groups of two to six animals.

Results

### LCMV infection of BM recipients results in persistent LCMV viremia

Specific pathogen-free C57BL/6 mice were lethally irradiated (950 rad), treated the next day with 8×10^6 syngenic BM cells, and infected with 200 PFU (low-dose) or 2×10^6 PFU (high-dose) of LCMV-WE. After low-dose LCMV-WE infection, the virus could not be detected in the blood before day 10; thereafter, blood viral titers rose steadily, reaching a peak of 1×10^5 PFU/ml on day 20 and remaining at this level for the remainder of the 50-day experimental period (Fig. 1A). Infection with a high dose of LCMV-WE resulted in the quicker appearance of blood viral titers (day 5). However, the maximal virus blood titer was similar to that observed after low-dose infection, and this level was maintained throughout the remainder of the experiment (Fig. 1A). Viral titers from the liver, lung, spleen, and kidney were analyzed at day 50 after infection, and all organs from mice infected with low or high doses of LCMV-WE were found to contain between 1×10^7 and 1×10^8 PFU of virus, indicating that all mice had reached a virus-carrier status (Fig. 1B) (28, 40). Of note, all carrier mice survived the 50-day period, probably due to the specific pathogen-free conditions used (data not shown) (40).

Virus-infected BM recipients were also examined for the presence of LCMV-specific CD8$^+$ T cells. In normal C57BL/6 mice, tetramer staining using the LCMV-glycoprotein-derived epitope (gp33) revealed the presence of significant numbers of virus-specific CD8$^+$ T cells 50 days after infection with low or high doses of LCMV-WE (Fig. 1, D and F). In contrast, mice that underwent BMT revealed absence of tetramer-positive CD8$^+$ T cells (Fig. 1, C and E). To rule out the possibility that these cells had down-regulated the TCR, we stimulated splenocytes in vitro with gp33 and examined IFN-γ production. This assay confirmed our observation that no LCMV-specific CD8$^+$ T cells were present in mice having previously undergone BMT (data not shown). We consider it likely that the absence of LCMV-specific CD8$^+$ T cells in BM recipients results from negative selection of these cells in the thymus, or from peripheral exhaustion induced by persisting virus (41).

Immunotherapy with naive splenocytes prevents the development of an LCMV-carrier status in a CD8$^+$ T cell-dependent manner

The absence of LCMV-specific CD8$^+$ T cells in BM recipients lead us to investigate whether immunotherapy with naive C57BL/6 splenocytes would be able to control viral spread. After BMT, we

FIGURE 1. LCMV infection during BMT causes persistent LCMV viremia. Mice were lethally irradiated (950 rad) on day -1. On day 0, 8×10^6 BM cells were injected and mice were infected with LCMV-WE at 200 PFU (●) or 2×10^6 PFU (■). *A*, Blood viral titers ($n = 6$). *B*, Virus titers in organs at day 50 after infection ($n = 3$). *C–F*, Representative tetramer stain at day 50 from splenocytes of mice that received BMT and LCMV infection at 200 PFU (*C*) or 2×10^6 PFU (*E*). Mice that did not receive BMT and were infected with 200 PFU (*D*) or 2×10^6 PFU (*F*) served as positive control. Uninfected C57BL/6 mice served as negative control ($0.22 \pm 0.06\%$). Values are given as mean $\pm$ SEM derived from staining three individual mice per treatment group.
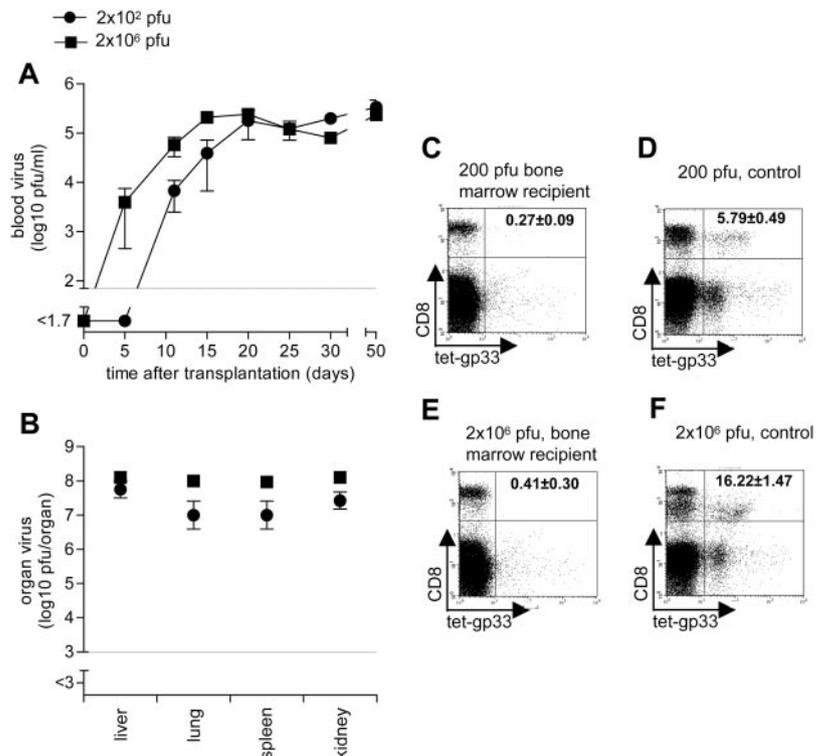
treated mice with 10^7 naive C57BL/6 splenocytes on day 0. After infection with a low dose of LCMV-WE (day 0), only one from a total of six mice receiving naive splenocytes demonstrated virus titers in blood, and virus was cleared in this mouse by day 15 (Fig. 2*A*). No virus was detected in any of the examined organs from these mice (Fig. 2*B*). Both virus-specific CD8$^+$ and CD4$^+$ T cells were found to be present and functional (Fig. 2, *D–F*). Control of viral infection was clearly CD8$^+$ T cell dependent, because adoptive transfer of CD8-depleted splenocytes failed to mediate viral clearance (Fig. 2*A*). Mice that received naive splenocytes and were infected with a high dose of LCMV-WE were unable to control viral spread (Fig. 2, *A* and *B*), despite the presence of functional virus-specific CD8$^+$ T cells (Fig. 2, *G–I*). All mice receiving a low dose of LCMV-WE and five of six mice receiving a high dose of LCMV-WE survived immunotherapy with naive splenocytes (Fig. 2*C*), and immunopathology was limited (data not shown). The latter result probably reflected at least partial exhaustion of virus-specific CD8$^+$ T cells (41).

Immunotherapy with virus-specific CD8$^+$ T cells results in immunopathology

To analyze whether increased precursor frequencies of virus-specific CD8$^+$ T cells could provide improved protection against LCMV infection, we treated BMT recipients with 10^7 splenocytes from naive or previously immunized 318 mice in which 50% of CD8$^+$ T cells carry a transgenic TCR specific for LCMV-GP$_{33-41}$ (gp33) (37). Naive splenocytes from 318 TCR-transgenic mice were able to inhibit viral spread after infection with a low dose of LCMV-WE, with very low or no titers of virus in any of the organs tested (Fig. 3*B*). Both virus-specific CD8$^+$ and CD4$^+$ T cells were found to be present and functional (Fig. 3, *D–F*), and all mice survived the immunotherapy (Fig. 3*C*). In contrast, these mice were not able to control viral spread after infection with a high dose of LCMV-WE (Fig. 3*A*), and the majority of mice had died by day 17 after infection (Fig. 3*C*).

Immunotherapy with activated splenocytes from previously immunized 318 TCR-transgenic mice (with LCMV GP-recombinant vaccinia virus, vaccG2) was also unable to protect BM recipients against infection with a high dose of LCMV-WE, with all mice exhibiting initially high blood viral titers (Fig. 3*A*) and half of the mice dying by day 10 (Fig. 3*C*). The surviving mice were found to eventually clear the virus (Fig. 3, *A* and *B*) and displayed functional CD4 and CD8 T cells in the spleen (Fig. 3, *G–I*). Mice that died exhibited extensive CD8$^+$ T cell infiltration of various organs, including the BM (Fig. 4*A* and data not shown), and demonstrated severely reduced numbers of hemopoietic precursor cells in the BM (Fig. 4*B*). These mice also displayed reduced hemoglobin (6.5 ± 1.6 g/dl; $n = 3$) compared with mice not receiving immunotherapy (13.6 ± 1.7 g/dl; $n = 3$). Together, these data indicated that the transfer of high numbers of LCMV-specific CD8$^+$ T cells prevented T cell exhaustion and promoted rapid T cell expansion and viral clearance; however, such treatment carried a high risk of inducing immunopathology and early death.

Immunotherapy with virus-specific CD4$^+$ T cells resulting in immunopathology

To determine the ability of increased precursor frequencies of virus-specific CD4$^+$ T cells to mediate viral clearance in BM recipients, we also treated BM-transplanted mice with 10^7 splenocytes from SMARTA mice, in which <95% of CD4$^+$ T cells carry a transgenic TCR specific for the LCMV-glycoprotein-derived epitope gp61 and which exhibit severely reduced numbers of CD8$^+$ T cells (36). These mice were unable to control infection with either low or high doses of LCMV-WE (Fig. 5*A*), and all mice died before day 20 (Fig. 5*B*). FACS analysis indicated the presence of large numbers of functional LCMV-specific TCR-transgenic CD4$^+$ T cells in the spleen and the BM of these mice (Fig. 5, *D* and *F*). Histological analysis
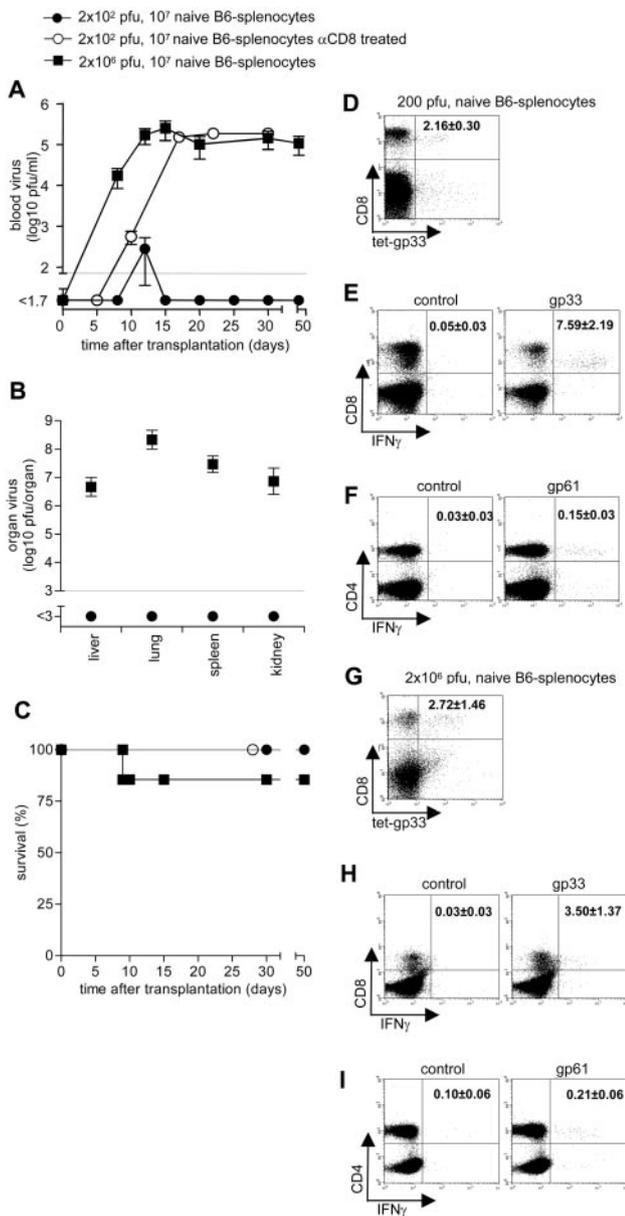
FIGURE 2. Adoptive transfer of naive splenocytes prevents development of a LCMV-carrier status in a CD8^{+} T cell-dependent manner. Mice were subjected to BMT as detailed in Fig. 1. Mice were also treated with 10^7 naive C57BL/6 splenocytes and infected with LCMV-WE at 200 PFU (circles) or 2×10^6 PFU (squares). *A*, Blood viral titers ($n = 6$). An additional group of mice received CD8^{+} T cell-depleted splenocytes and were infected with 200 PFU of LCMV-WE ($\bigcirc$; $n = 4$). *B*, Virus titers in organs at day 50 after infection ($n = 3–5$). *C*, Survival curves ($n = 6$). *D–F*, On day 50, splenocytes from BMT mice treated with naive C57BL/6 splenocytes and infected with 200 PFU of LCMV-WE were stained for gp33-specific T cells by tetramer (*D*) and stained for intracellular IFN-γ after restimulation with the CD8 T cell epitope gp33 (*E*) and the CD4 T cell epitope gp61 (*F*). Values are given as mean $\pm$ SEM derived from staining three individual mice per treatment group. *G–I*, On day 50, splenocytes from BMT mice treated with naive C57BL/6 splenocytes and infected with 2×10^6 PFU of LCMV-WE were stained for gp33-specific T cells by tetramer (*G*) and stained for intracellular IFN-γ after restimulation with the CD8 T cell epitope gp33 (*H*) and the CD4 T cell epitope gp61 (*I*). Values are given as mean $\pm$ SEM derived from staining three individual mice per treatment group.
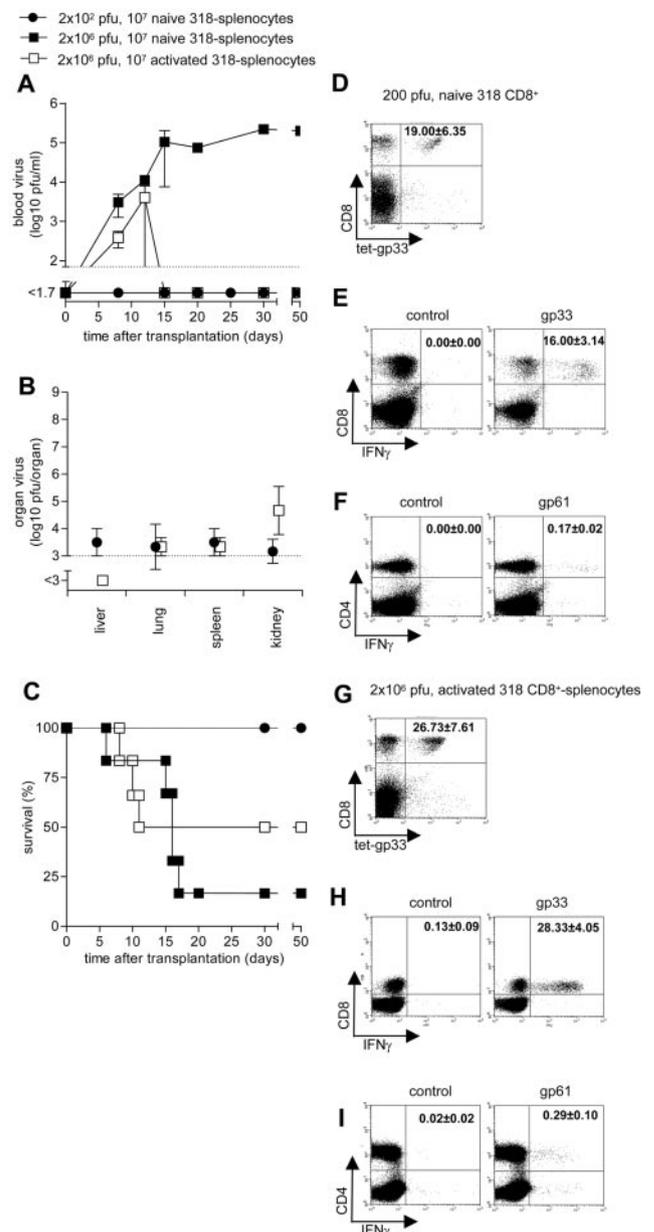
FIGURE 3. Virus-specific CD8^{+} T cells mediate immunopathology. Mice were subjected to BMT as detailed in Fig. 1. Mice were also given injections of 10^7 naive (filled symbols) or activated (open symbols) splenocytes from 318 TCR-transgenic mice and infected with LCMV-WE at 200 PFU (circles) or 2×10^6 PFU (squares). *A*, Blood viral titers ($n = 6$). *B*, Virus titers in organs at day 50 after infection ($n = 3$). *C*, Survival curves ($n = 6$). *D–F*, On day 50, splenocytes from BMT mice treated with naive 318 TCR-transgenic splenocytes and infected with 200 PFU of LCMV-WE were stained for gp33-specific T cells by tetramer (*D*) and stained for intracellular IFN-γ after restimulation with the CD8 T cell epitope gp33 (*E*) and the CD4 T cell epitope gp61 (*F*). Values are given as mean $\pm$ SEM derived from staining three individual mice per treatment group. *G–I*, On day 50, splenocytes from BMT mice treated with activated 318 TCR-transgenic splenocytes and infected with 2×10^6 PFU of LCMV-WE were stained for gp33-specific T cells by tetramer (*G*) and stained for intracellular IFN-γ after restimulation with the CD8 T cell epitope gp33 (*H*) and the CD4 T cell epitope gp61 (*I*). Values are given as mean $\pm$ SEM derived from staining three individual mice per treatment group.

also revealed that large numbers of CD4^{+} T cells were infiltrating the liver and lung (Fig. 4*A*). Thus, immunotherapy with high numbers of LCMV-specific CD4^{+} T cells did not mediate

viral clearance but caused severe immunopathology and early death. Functional gp33-specific CD8^{+} T cells could be detected in the spleen and BM (Fig. 5, *C* and *E*) and were likely to
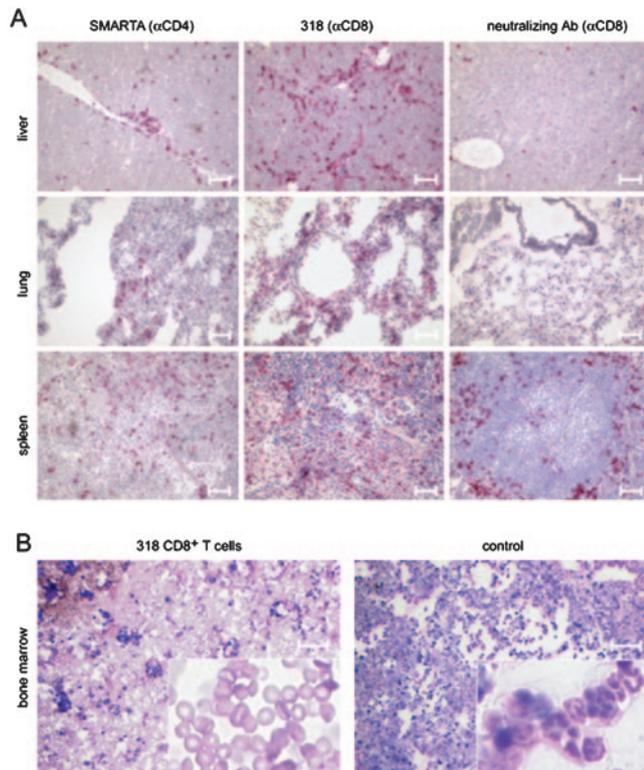
FIGURE 4. Histological analysis of immunopathology. Mice were subjected to BMT as detailed in Fig. 1 and infected with 2×10^6 PFU of LCMV-WE. *A*, Histological analysis of various organs from mice also given injections of naive splenocytes from SMARTA TCR-transgenic mice (*left panels*; day 10), naive splenocytes from 318 TCR-transgenic mice (*middle panels*; day 15), or hyperimmune serum (*right panels*; day 50). Sections were stained with Abs directed against murine CD8 or CD4, as indicated. The dark red color represents positive staining. Mice subjected to BMT and LCMV infection, but not receiving immunotherapy, did not display any immunopathology (data not shown). One representative slide of three is shown. Scale bars, 50 μm. *B*, One (of three) representative BM smear of BMT mice infected with 2×10^6 PFU of LCMV-WE 15 days before (*right*) or of LCMV-infected BMT mice also given injections of naive splenocytes from 318 TCR-transgenic mice (*left*) stained with eosin G and thiazine dye. Scale bars, 50 μm. One section (50 μm long) is shown in higher magnification (*insets*).
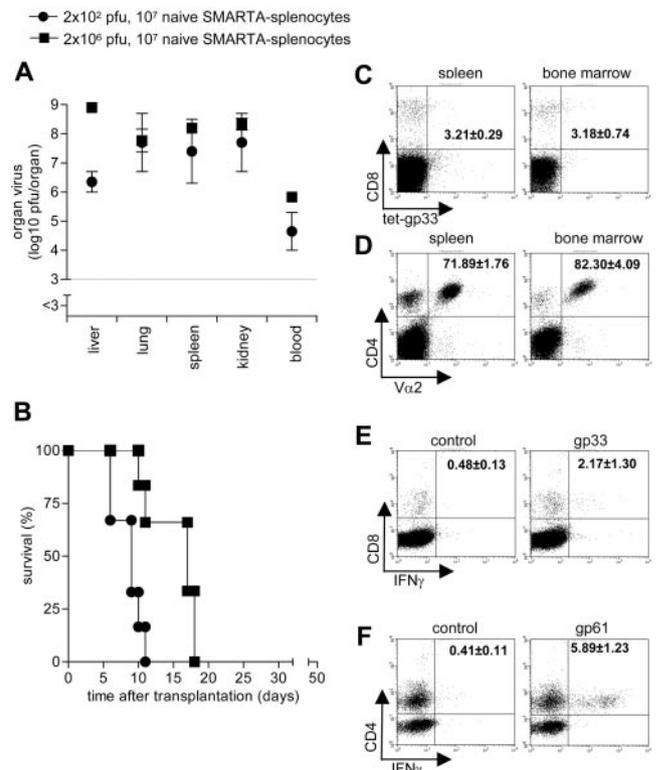

FIGURE 5. Virus-specific CD4$^+$ T cells mediated immunopathology. Mice were subjected to BMT as detailed in Fig. 1. Mice were also given injections of 10^7 naive splenocytes from SMARTA TCR-transgenic mice and infected with LCMV-WE at 200 PFU ($\bullet$) or 2×10^6PFU ($\blacksquare$). *A*, Viral titers from liver, lung, spleen, kidney, and blood (given in PFU/organ or PFU/ml blood; $n = 2$–6) at day 10 after infection. *B*, Survival curves ($n = 6$). *C–F*, On day 50, splenocytes and BM from BMT mice treated with naive SMARTA TCR-transgenic splenocytes and infected with 200 PFU of LCMV-WE were stained for gp33-specific CD8 T cells by tetramer (*C*) and transgenic gp61-specific CD4 T cells by anti-Vα2 Ab (*D*). Splenocytes were stained for intracellular IFN-γ after restimulation with the CD8 T cell epitope gp33 (*E*) or the CD4 T cell epitope gp61 (*F*). Values are given as mean $\pm$ SEM derived from staining three individual mice per treatment group.

represent endogenous cells because SMARTA mice contained low numbers of CD8$^+$ T cells (36).

We also investigated the effect of immunotherapy with increased precursor frequencies of both CD4$^+$ and CD8$^+$ T cells specific for LCMV. Mice that underwent BMT were treated with splenocytes from SMARTA and 318 mice and infected with a high dose of LCMV-WE. The virus was not cleared, and death occurred within 20 days (data not shown).

Immunotherapy with neutralizing Abs results in viral clearance

Cellular immunotherapy was effective at clearing low-dose viral infections of BM recipients but could not protect BM recipients from high-dose viral infection. We therefore analyzed the ability of neutralizing antibodies, present in hyperimmune serum, to protect against persistent infection and immunopathological disease. Administration of as little as 200 μl of hyperimmune serum (titer, 1:4000) inhibited viral spread in all BM-transplanted mice infected with low-dose LCMV (Fig. 6*A*). Moreover, all organs analyzed on day 50 were free of detectable virus (Fig. 6*B*), all mice survived (Fig. 5*C*), and LCMV-specific CD4$^+$ and CD8$^+$ T cells were

present and functional (Fig. 6, *D–F*). These cells presumably arose from donor BM, and their presence indicated that administration of hyperimmune serum protected the endogenous T cell response from rapid exhaustion.

Hyperimmune serum could also prevent early viral spread after high-dose LCMV infection (Fig. 6*A*). However, by day 50 after infection, viral titers were high in the blood (Fig. 6*A*) and other analyzed organs (*B*), and no functional LCMV-specific T cells were detected (data not shown). These mice showed little apparent immunopathology, and five of six mice survived (Figs. 4*A* and 6*C*). In an attempt to improve the immunotherapeutic treatment of BM recipients receiving high-dose viral infection, we treated mice with both hyperimmune serum and naive splenocytes. This treatment was successful in that no virus was detected in the blood (Fig. 6*A*) or any of the organs analyzed on day 50 (Fig. 6*B*). Moreover, all mice survived the immunotherapy (Fig. 6*C*), and the spleen was found to contain significant numbers of functional LCMV-specific CD4$^+$ and CD8$^+$ T cells (Fig. 6, *G–I*). This result demonstrated that a combination of transferred cellular and humoral immunity controlled a 10^4-fold higher viral load than either component alone.
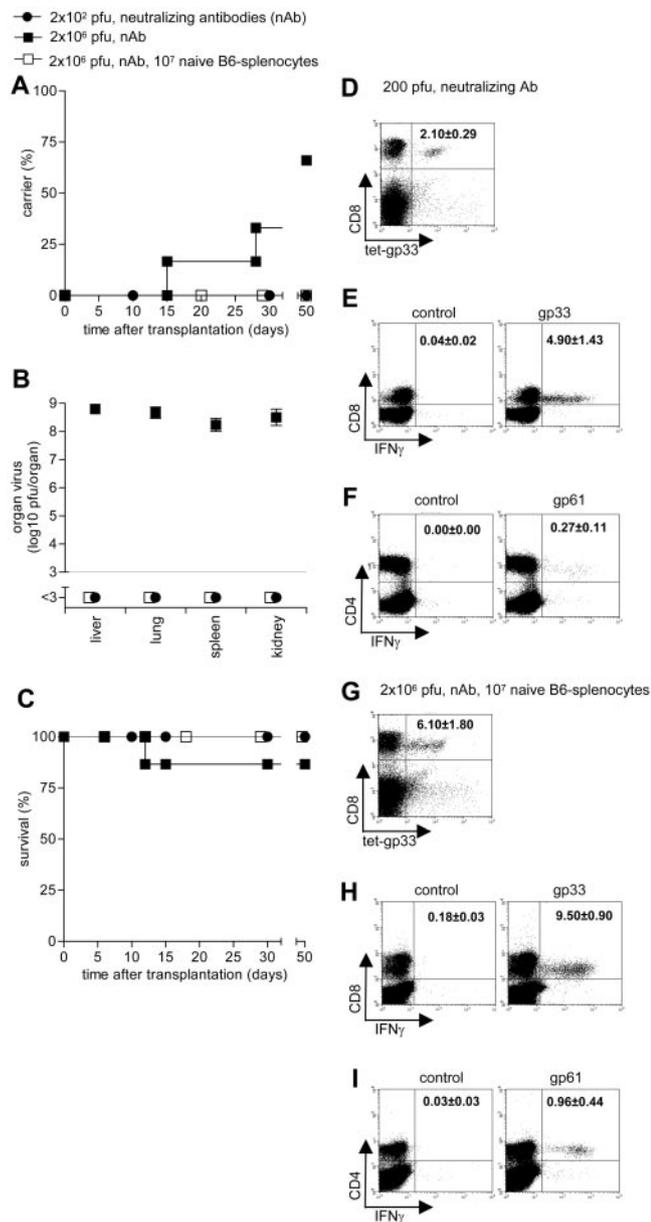
ameliorate the outcome of immunopathology. Indeed, immunopathology was absent if mice were treated with both immunopathology causing 318 CD8^{+} T cells and hyperimmune serum on the day of infection (Fig. 7A). Virus was cleared until day 14 (Fig. 7B) but was still able to activate virus-specific CD8^{+} T cells, because they showed high frequencies of tet-gp33^{+} cells in the blood (Fig. 7C). One-half of the gp33-specific CD8^{+} T cells contained an IL7-Rα^{high}-expressing memory population (Fig. 7D), whereas the others still were effector T cells (IL7-Rα^{low}) (42). Nearly all T cells from mice that were not treated with hyperimmune serum showed an effector phenotype (IL-7Rα^{low}) that is in agreement with high viral load and immunopathology (43). Because hyperimmune serum, given at the time of infection, could inhibit immunopathology, we wondered whether hyperimmune serum could even reduce ongoing immunopathology. Therefore, we supplied BM-transplanted mice with 10^7 318 splenocytes. Seven days later, one of eight mice died and the other mice showed clinical signs of immunopathology. At that time, we treated three mice with hyperimmune serum. Although hyperimmune serum slightly reduced viral load (Fig. 7B), mice did not show a reduction in clinical signs, and all mice died between days 10 and 15 (Fig. 7A). Blood lymphocytes displayed high frequencies of tet-gp33^{+} T cells all expressing low levels of IL-7Rα (Fig. 7, C and D).

Thus, hyperimmune serum was able to prevent immunopathology; however, if given late, it had no benefit on survival.

FIGURE 6. Hyperimmune serum prevented viral spread and CD8^{+} T cell exhaustion without causing immunopathology. Mice were subjected to BMT as detailed in Fig. 1. All mice were given injections of hyperimmune serum containing neutralizing Abs and infected with LCMV-WE at 200 PFU (circles) or 2×10^6 PFU (squares). A third group of mice also received naive C57BL/6 splenocytes (○). *A*, Percentage of carrier mice determined by a viral load of $>10^4$ PFU/ml blood ($n = 6$). *B*, Virus titers in organs at day 50 after infection ($n = 3$). *C*, Survival curves ($n = 6$). *D–F*, On day 50, splenocytes from BMT mice treated with hyperimmune serum and infected with 200 PFU of LCMV-WE were stained for gp33-specific T cells by tetramer (*D*) and stained for intracellular IFN-γ after restimulation with the CD8 T cell epitope gp33 (*E*) and the CD4 T cell epitope gp61 (*F*). Values are given as mean ± SEM derived from staining three individual mice per treatment group. *G–I*, On day 50, splenocytes from BMT mice treated with hyperimmune serum and naive C57BL/6 splenocytes and infected with 2×10^6 PFU of LCMV-WE were stained for gp33-specific T cells by tetramer (*G*) and stained for intracellular IFN-γ after restimulation with the CD8 T cell epitope gp33 (*H*) and the CD4 T cell epitope gp61 (*I*). Values are given as mean ± SEM derived from staining three individual mice per treatment group.

Hyperimmune serum prevents immunopathology

Hyperimmune serum appeared to be of benefit for the clearance of virus. We asked whether this reduction in viral load could also
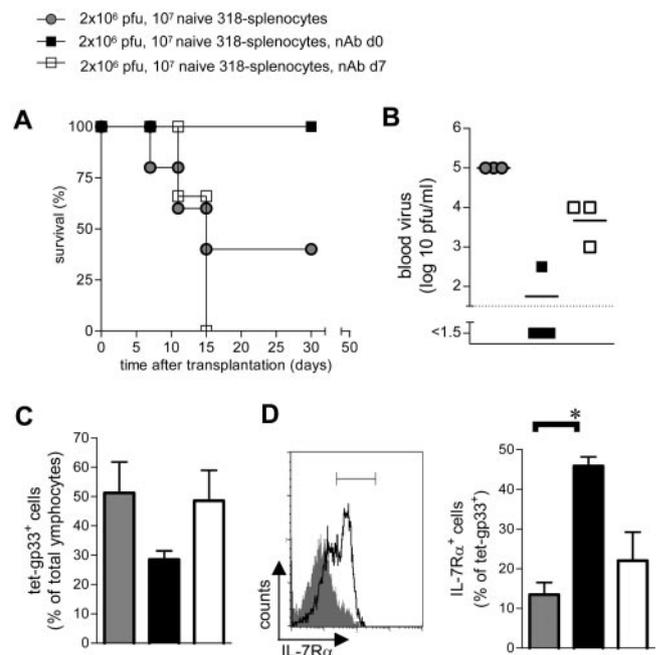

FIGURE 7. Hyperimmune serum prevented immunopathology. Mice were subjected to BMT as detailed in Fig. 1. All mice were also given injections of 10^7 naive splenocytes from 318 TCR-transgenic mice and infected with 2×10^6 PFU of LCMV-WE. One group of mice was also treated with hyperimmune serum on day 0 ($n = 4$; ■, filled bars). Another group of mice was treated with hyperimmune serum on day 7 ($n = 3$; □, open bars). One group of mice was not treated with hyperimmune serum ($n = 5$; gray circles, gray bars). *A*, Survival curves. *B*, Virus titers in blood at day 14 after infection. *C*, Blood lymphocytes were stained for gp33-specific T cells by tetramer. Values are given as mean ± SEM derived from staining three individual mice per treatment group. *D*, CD8^{+} T cells, which were tet-gp33^{+}, were analyzed for expression of IL-7Rα. One representative FACS blot and the summary of three to four mice per treatment group is shown. *, $p < 0.05$.

Discussion

Opportunistic infections frequently complicate the recovery of BMT patients, and to date there is no satisfying mean of protecting such patients. We investigated the potential of immunotherapeutic strategies to prevent or lower the risk of opportunistic viral infection in the well characterized murine LCMV infection model. Although LCMV itself is not an important human pathogen, it behaves partly similar to CMV, which is the most frequent pathogen associated with human BMT. Like CMV, LCMV persists lifelong in infected recipients and induces a strong CD8 T cell response (29, 30), which is crucial for the control of the virus (28).

We showed that the adoptive transfer of CD8$^+$ T cells was able to control viral spread after BMT but eventually resulted in severe immunopathology, presumably caused by the lytic potential of CD8$^+$ T cells and resultant tissue destruction. Adoptive immunotherapy with CD4$^+$ T cells also resulted in immunopathology, although the mechanism by which this occurred is likely to be more complex, possibly involving cytokines such as TNF-α (44). Previous studies have shown that virus-specific CD4$^+$ T cells alone are not able to control LCMV infection (36, 44), and in support of this, we show that adoptive immunotherapy using CD4$^+$ T cells alone was not able to prevent viral spread in BM recipients.

In many cases, T cell immunopathology resulted in death of recipient mice, a phenomenon that probably is mediated at least in part by the immunological destruction of the newly established BM and resultant aplastic anemia (45, 46). Of interest, CMV reactivation after human BMT is often associated with graft failure and is thought to be mediated by virus-induced autoimmunity (47, 48). An additional complication that should be considered in human BMT patients is that immunopathology can be enhanced by graft-vs-host reactions resulting from allogenic BMT.

Instead, our data indicate a potential role for neutralizing Abs in the prevention of pathogenic infections after BMT. Under normal circumstances, neutralizing Ab induction is delayed after infection with LCMV, and were previously thought to play a minor role in initial virus elimination. Nevertheless, treatment of LCMV-infected BM recipient mice with hyperimmune serum was able to reduce early viral spread and to prevent exhaustion of endogenous viral-specific CD8$^+$ T cells, allowing efficient viral clearance. This phenomenon may well apply to other noncytopathic or poorly cytopathic viruses, including perhaps HIV, hepatitis B virus, and hepatitis C virus, and so neutralizing Abs may provide a useful therapy for the treatment of ongoing persistent infections with these viruses (49, 50). We found that immunopathology could be prevented if neutralizing Abs were supplied early; however, if given late, they demonstrated no benefit. From our experiments, we may wish to speculate that those neutralizing Abs should be giving shortly before or early after BMT.

In conclusion, we have completed a thorough comparison of the ability of cellular and humoral immunotherapeutic strategies to control chronic viral infection after BMT. These studies demonstrated that naive splenocytes can prevent an infection with low but not high doses of virus. Increased precursor frequencies of virus-specific T cells resulted in immunopathology and death of BM recipients. The combined use of neutralizing Abs and naive splenocytes inhibited virus spread even after infection with high doses of virus and allowed the development of endogenous virus-specific CD8$^+$ T cells. Together, these data suggest that neutralizing Abs combined with limited numbers of CTLs may represent a powerful tool for limiting opportunistic persistent viral infection in BMT patients.

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