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Leukotriene B_4 Protects Latently Infected Mice against Murine Cytomegalovirus Reactivation following Allogeneic Transplantation[1]

Jean Gosselin,[2]*‡ Pierre Borgeat,‡ and Louis Flamand†‡

Human CMV is often associated with transplant rejection and opportunistic infections such as pneumonia in immunosuppressed patients. Current anti-CMV therapies, although effective, show relatively high toxicity, which seriously limits their long-term use. In this study, we provide evidence that leukotriene B_4 (LTB_4) plays an important role in the fight against murine CMV (MCMV) infection in vivo. Intravenous administration of 50 and 500 ng/kg/day of LTB_4 to mice infected with a lethal dose of MCMV significantly increases their survival (50 and 70%, respectively), compared with the placebo-treated group (10% of survival). In mice infected with a sublethal dose of MCMV and treated daily with 50 ng/kg/day of LTB_4, the salivary gland viral loads were found to be reduced by 66% compared with the control group. Furthermore, using an allogeneic bone marrow transplantation mouse model, the frequency of MCMV reactivation from latently infected mice was much lower (38%) in LTB_4 (500 ng/kg)-treated mice than in the placebo-treated group (78%). Finally, in experiments using 5-lipoxygenase-deficient mice, MCMV viral loads in salivary glands were found to be higher in animals unable to produce leukotrienes than in the control groups, supporting a role of endogenous 5-lipoxygenase products, possibly LTB_4, in host defense against CMV infection. ***The Journal of Immunology,*** **2005, 174: 1587–1593.**

As seen with other herpesvirus infections, primary CMV infection is followed by persistent or recurrent infection caused by the reactivation of latent virus. The diseases treated are congenital infections, mononucleosis-like syndrome in normal host, and mild-to-severe infections in immunosuppressed individuals (Ref. 1; reviewed in Ref. 2). The populations at high risk for CMV infections are those undergoing allogeneic transplantation. The ubiquity of this virus, its propensity to reactivation when host defenses are compromised, and its ability to disseminate to several organs are characteristics that explain its frequent occurrence in the transplanted population (reviewed in Ref. 3). The frequency and the severity of CMV infections in transplant patients are variable and depend on the nature of the transplant, the immune status of the recipient, and the duration of the immunosuppressive therapy. The CMV infection, particularly when associated with pneumonitis, is an important cause of morbidity and mortality after bone marrow transplantation (4).

Currently available antiviral agents with proven efficacy against CMV include Cytovene (ganciclovir) and Foscavir (foscarnet) (5, 6). These agents inhibit the synthesis of metabolically active virus and are not effective against nonreplicating or latent virus. Intravenous ganciclovir has been used successfully to treat solid-organ transplant recipients with CMV infection. Because of its renal toxicity, the dosage needs to be decreased in patients with renal disorders, and maintenance therapy is seriously limited (7, 8). Emergence of ganciclovir-resistant strains of CMV following repeated use of ganciclovir is well documented (9, 10). Current CMV treatments are therefore not optimal, and new approaches are needed to prevent CMV infection in organ and bone marrow transplant recipients.

Leukotriene B_4 (LTB_4)[3] is a polyunsaturated fatty acid derived from the oxygenation of arachidonic acid. Leukotrienes represent a large family of lipidic molecules whose precursor, arachidonic acid, is the substrate of many biologically active molecules such as prostaglandins and thromboxanes (11), some of which have been used in the clinic for many years. The major sources of LTB_4 are neutrophils and macrophages (12, 13). Important biologic properties of LTB_4 are its ability to stimulate phagocyte locomotion and chemotaxis (14) and, to a lesser degree, degranulation and superoxide anion production. There are previous studies in animal models supporting the role of LTB_4 in host defense against bacterial infection. In animals lacking the gene coding for the 5-lipoxygenase (5-LO), a markedly increased susceptibility to bacterial pneumonia was observed (15, 16). Furthermore, it was shown that this susceptibility to infection is the consequence of the inability of lung macrophages to produce LTB_4 and to ingest and kill pathogens. Another group has shown that administration of LTB_4 to mice enhances bacterial clearance (17). Interesting observations made in AIDS patients also support the role of LTB_4 in host defense. It was reported that neutrophils and alveolar macrophages from HIV-infected individuals produced significantly less LTB_4 than the cells of healthy subjects, and that such a defect was the cause of reduced antimicrobial activity in the phagocytes (18–20).

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[3] Abbreviations used in this paper: LTB_4, leukotriene B_4; 5-LO, 5-lipoxygenase; MCMV, murine CMV; MEF, murine embryonic fibroblast; FLAP, 5-LO-activating protein; BMT, bone marrow transplant.

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To investigate the potential role of LTB_4 in viral infection, studies were performed using mice infected with the murine CMV (MCMV). MCMV infection in mice represents a valid surrogate model to human CMV infection and proves useful for evaluating compounds with antiviral activity due to the similarity of its gene products and the organs that are targeted during infection. MCMV is similar to human CMV with respect to the development of acute and chronic infections, latency establishment, and reactivation following immunosuppressive therapy initiation.

In this study, we report that exogenous LTB_4 significantly reduces the severity of acute MCMV infection in mice and prevents viral reactivation in immunosuppressed mice following allogeneic transplantation; furthermore, we provide evidence in support of a role of endogenous 5-LO products in host defense against MCMV infection in vivo.

Materials and Methods

Virus propagation and titration

The MCMV (Smith strain) was purchased from the American Type Culture Collection and propagated in 6-wk-old BALB/c mice. Briefly, mice were infected by i.p. injection with 10^3 PFU of MCMV in a volume of 0.1 ml. Twenty-one days after infection, mice were sacrificed, and the salivary glands were harvested, pooled, and homogenized in 10 ml of DMEM for 45 s using a tissue tearor apparatus (Fisher Scientific) at a setting of 5. Homogenates were centrifuged at 3000 $\times$ *g* for 20 min. Clarified supernatants were collected, pooled, aliquoted, and stored frozen at −150°C until used.

MCMV titer was determined by plaque assay on murine embryonic fibroblasts (MEF). MEF cell suspensions were obtained following overnight tryptic digestion (4°C) of 15-day-old BALB/c embryos. The MEF cells were counted, aliquoted (1×10^7 cells/vial), and stored frozen at −150°C until needed. For MCMV titration experiments, MEF were seeded in 12-well tissue culture plates (5×10^4 cells/well) 1 day before the titration assay. MCMV samples to be assayed were serially diluted in DMEM medium and added (0.5 ml) in duplicate onto fibroblasts for 1 h at 37°C. Nonadsorbed viruses were removed, and the cell layers were covered with culture medium containing 1.5% methylcellulose and 2% FBS. After 4 days, the medium was removed, and the cells were fixed with 5% formaldehyde in saline and stained with violet crystal. After saline washes, the cultures were examined by microscopy, and the number of plaques was determined.

Drugs

LTB_4 (Cascade Biochem) was obtained as an ethanolic solution of the acid form and stored at −80°C. The dosing solutions were prepared by dilution of the ethanolic LTB_4 solution in a 0.45% w/v NaCl solution containing 0.25% w/v dextrose and 0.01% w/v BSA (fraction V; ICN), no more than 1 h before injection. The solutions were warmed to 20°C before injection to prevent a shock to the animal. The following drugs were used: ganciclovir, cyclosporin A (McKessen), FK-506 (Omega), cyclophosphamide (Sigma-Aldrich), and MK-886, an inhibitor of 5-LO-activating protein (FLAP) (Biomol Research Lab).

Survival challenge

Adult female BALB/c mice (6–8 wk old) were infected ($n = 15$/group) by i.v. administration of a lethal dose of MCMV (12,000 PFU). Starting on the day of infection and every day thereafter, mice received a placebo or varying quantity of LTB_4 (50 or 500 ng/kg) by i.v. administration. Survival of mice was recorded every day for each group until day 9 postinfection, at which time all surviving animals were sacrificed. Body weight loss and body temperature were also evaluated on a daily basis.

Infection of mice with MCMV and viral load determination

Adult female BALB/c mice were infected ($n = 10$/group) by i.v. injection in the tail vein with a sublethal dose (500 PFU) of MCMV in a final volume of 0.1 ml of DMEM and treated with exogenous LTB_4. Mice were treated daily, starting on the third day of infection, with a placebo or varying quantities of LTB_4 (0.5–500 ng/kg). On day 12 postinfection, the salivary glands of mice were harvested and stored frozen (−80°C). To evaluate the role of endogenous LTB_4 in MCMV-infected animals, two different experiments were performed. First, 5-LO knockout mice ($5\text{-LO}^{-/-}$) (The Jackson Laboratory) that were deficient for leukotriene synthesis, as well as their wild-type controls ($5\text{-LO}^{+/+}$) were infected with MCMV and studied for their ability to control infection. Second, MCMV viral loads were studied in MK-886-treated BALB/c mice and compared with that of placebo-treated group. The viral loads were determined after homogenization of the organs by standard plaque assay titration on MEF cells as described above.

Effects of long-term LTB_4 administration on allogeneic stem cell engraftment

Adult (6–8 wk old) female BALB/c mice ($H2\text{-}K^d$) were treated twice (days −5 and −3 relative to transplant) with cyclophosphamide (60 mg/kg). On day 0, mice were irradiated (600 rad) and reconstituted by i.v. injection with 10^7 allogeneic bone marrow cells (10^6 $CD34^+$ equivalent) obtained from the femurs of C57BL/6 mice ($H2\text{-}K^b$). Transplanted mice were immunosuppressed by daily administration of cyclosporin A (5 mg/kg) starting on the day before reconstitution up to day 59. From days 60 to 100, the immunosuppressive pharmacology was switched to FK-506 (90 μg/kg). Starting on the fifth day posttransplant, mice ($n = 10$/group) received a placebo or LTB_4 (50–500 ng/kg) by i.v. administration, three times a week. On day 100 postengraftment, mice were sacrificed by CO_2 asphyxiation. Bone marrow cell suspensions were obtained by flushing the marrow of the femur of each mouse with 2 ml of culture medium and analyzed for the presence of donor cells. In brief, one million bone marrow cells were incubated with a fluorescein-labeled isotype control mAb or with a fluorescein-labeled anti-$H2\text{-}K^{b+}$ Ab. After a 1-h incubation at 4°C, the cells were washed twice with 10 ml of saline and resuspended in 0.5 ml of 1% paraformadehyde. The presence of progenitor stem cell originating from the donor mouse was determined using a combination of a fluorescein-labeled anti-$H2\text{-}K^{b+}$ Ab and a PE-labeled anti-CD34 mAb. The percentage of positive cells was determined after counting 10,000 events using a flow cytometer (Epics XL; Coulter).

Effects of LTB_4 administration on MCMV reactivation following allogeneic splenocyte transplantation

Adult (6–8 wk old) female BALB/c mice ($H2\text{-}K^d$) were treated twice with cyclophosphamide (150 mg/kg) and injected i.p. with 10^8 splenocytes from latently CMV-infected C57BL/6 ($H2\text{-}K^b$) mice. Latency was established by infecting the mice by i.p. injection with 10^4 PFU of MCMV 2 mo prior. Latency was confirmed by the absence of active CMV infection in spleen cells. BALB/c mice carrying the allogeneic splenocytes were divided into four groups of 10 mice and treated by i.v. injection, three times a week, with a placebo or LTB_4 (5, 50, and 500 ng/kg). Twenty-one days following splenocyte injection, mice were sacrificed, and the salivary glands were collected and analyzed for CMV viral loads by standard plaque assay on MEF as described above.

Effects of LTB_4 administration on CMV reactivation following allogeneic bone marrow transplantation

Adult (6–8 wk old) female BALB/c ($H2\text{-}K^d$) and C57BL/6 ($H2\text{-}K^b$) were infected with MCMV (10^4 PFU) by i.p. injection. Six months later, when MCMV infection was fully latent, BALB/c mice were treated twice (days −4 and −2) with cyclophosphamide (60 mg/kg) and irradiated with 600 rad on day 0. Irradiated mice were reconstituted (day 0) by i.v. injection of 10^7 allogeneic bone marrow cells (10^6 $CD34^+$ equivalent) obtained from the femurs of C57BL/6 mice. Transplanted mice were maintained under immunosuppressive therapy by daily administration of FK-506 (90 μg/kg) starting on the day following reconstitution up to day +35. Starting on day +21, mice received a daily i.v. injection of LTB_4 (50 and 500 ng/kg) (for a total of 14 days). On day +36, BALB/c transplanted mice were sacrificed, and the MCMV viral load in salivary glands was determined as described above.

Results

LTB_4 increases survival of mice infected with a lethal dose of MCMV

First, we evaluated whether LTB_4 administration could protect mice against a lethal MCMV infection. Mice were infected with a lethal dose of MCMV and treated by i.v. injection of LTB_4. Animal survival was monitored daily. In general, mice started showing signs of infection on the second day, and mortality was observed between days 5 and 7. By the eight or ninth day postinfection, surviving mice had recovered, and the protocols were ended. Results obtained clearly show that doses of 50 and 500 ng/kg/day of LTB_4 had significant protective effects on CMV-

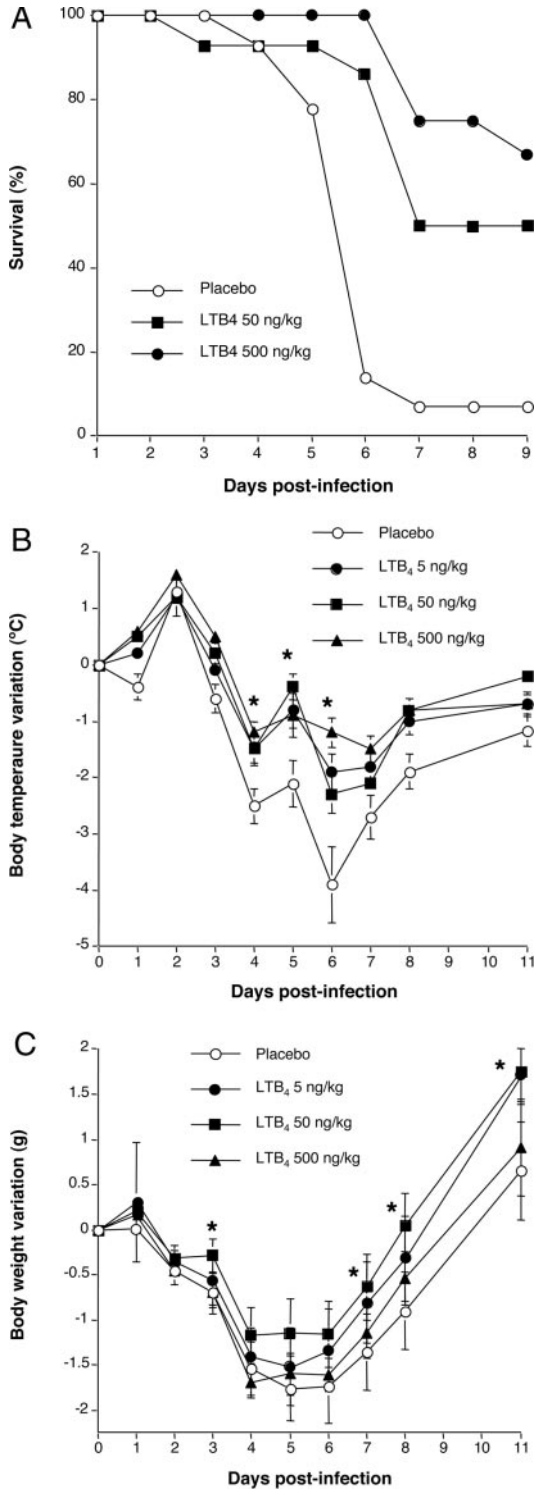

FIGURE 1. Effects of LTB_4 administration on survival of mice infected with a lethal dose of MCMV. *A*, Adult BALB/c mice were infected (n = 15/group) by i.v. injection with a lethal dose of MCMV. On the day of infection and every day thereafter, mice were injected i.v. with a placebo or LTB_4 (50 and 500 ng/kg/mouse). Mice survival was monitored daily until day 9, at which time the protocol was ended. Results presented are representative of four independent studies. On day 6 postinfection and every subsequent day, percentages of survival in mice treated with LTB_4 were significantly higher ($p < 0.0003$, using a χ^2 analysis) compared with the placebo-treated group. *B*, Mice (n = 15/group) were infected with a sublethal dose of MCMV and treated daily with a placebo or LTB_4 (5–500 ng/kg) starting on the day of infection. Body temperature was measured daily using a small animal rectal thermometer. Results are expressed as mean body temperature variations (°C) relative to day 0 ± SEM and are representative of three independent experiments using unpaired two-tailed Student's t test. (*, $p < 0.05$). *C*, Mice (n = 15/group) were infected and treated as described above, and body weight was measured daily. Results, expressed as mean body weight variations (grams) relative to day 0 ± SEM, are representative of three independent experiments (*, $p < 0.03$), using unpaired two-tailed Student's t test.

induced mortality (Fig. 1*A*). Typically, when administration of LTB_4 (50 and 500 ng/kg/day) started on the day of infection and everyday thereafter, more than twice as many mice survived the lethal viral challenge compared with the placebo-treated group. In addition, body weight and temperature of infected mice were also monitored daily in placebo and LTB_4-treated mice. During acute MCMV infection, mice typically develop a fever (38–39°C) that lasts 2 days followed with a reduction in body temperature. Our results (Fig. 1*B*) indicate that the placebo-treated group experienced greater body temperature reduction compared with the LTB_4 (5–500 ng/kg)-treated mice, with the largest variations observed on day 6 postinfection. Statistically significant differences were observed on days 4, 5, 6, and 8 postinfection. In terms of body weight, infected mice started losing weight on the second day after infection and continued to do so up to the sixth day postinfection. All three groups treated with LTB_4 (5–500 ng/kg) experienced less severe weight losses than the placebo-treated group (Fig. 1*C*). Statistically significant differences ($p < 0.03$) were recorded between the placebo and LTB_4 (50 ng/kg) group on days 7, 8, and 11 postinfection. Body weight and temperature data are therefore in accordance with the increased survival of mice treated with LTB_4.

LTB_4 reduces the viral load in salivary glands of MCMV-infected mice

Detection of viral particles in organs and body fluids is by far the best criteria to evaluate the spread of the infection and the efficacy of a drug. Because MCMV is known to replicate heavily in salivary glands of mice (21), viral loads were measured in this organ. Mice were infected with a sublethal dose of MCMV and treated daily with a placebo or LTB_4 by i.v. injection starting on the third day of infection. On day 12, the viral loads were determined in salivary glands of mice by standard plaque assay titration on MEF cells. Results indicate that a dose of 50 ng/kg/day appeared optimal in reducing CMV viral load (66% reduction) in the salivary glands of infected mice (Fig. 2). Although less pronounced (30% reduction), a dose of 5 ng/kg/day of LTB_4 also caused a reduction in CMV viral load.

Effects of LTB_4 administration of allogeneic stem cell engraftment

The results presented so far highlight a potential usefulness of LTB_4 in the treatment of primary CMV infection. In addition to primary CMV infection, reactivation of latent CMV in transplant patients may also lead to clinical problems. Considering that bone marrow (or organ) transplant patients must receive immunosuppressive therapies, the concomitant usage of immunomodulatory drugs such as LTB_4 to combat viral infections may represent a paradox. First, the next set of experiments was aimed at determining the innocuousness of prolonged i.v. administration of LTB_4 on engraftment of allogeneic bone marrow cells in mice using a standard bone marrow transplant (BMT) protocol. Bone marrow cells from C57BL/6 (H2-K^{b+}) mice were grafted into BALB/c mice. Grafted mice received either a placebo or LTB_4, at 50 or 500 ng/kg/mouse, three times a week (i.v. injection) for 100 days. The results obtained indicate that mice receiving a placebo had an identical survival rate (50%) after 100 days with those receiving LTB_4.

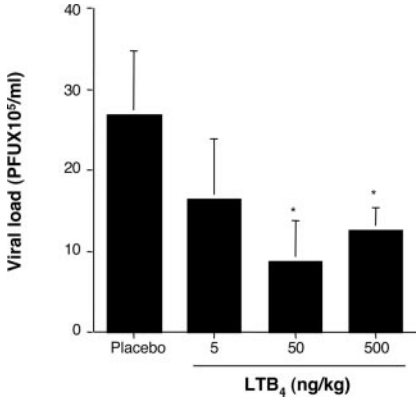

FIGURE 2. Effects of LTB$_4$ administration on MCMV viral loads in salivary glands. Adult BALB/c mice (n = 10/group) were infected with a sublethal dose (500 PFU) of MCMV by i.v. injection. Mice were injected i.v. on a daily basis, starting on the third day postinfection, with a placebo (control) or varying quantities of LTB$_4$ (5–500 ng/kg/mouse). On day 12 postinfection, mice were sacrificed, and viral loads in salivary glands were determined by plaque assays as detailed under *Materials and Methods*. Results, expressed as mean viral loads ± SEM, are representative of four independent experiments (*, $p < 0.05$), using Mann-Whitney U test.

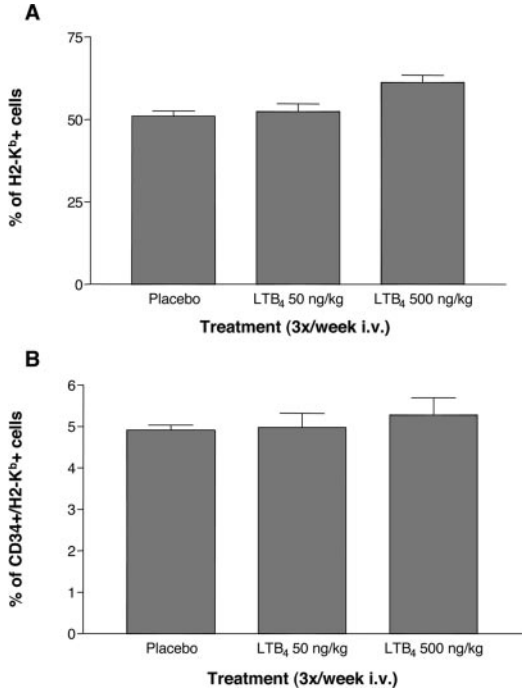

FIGURE 3. Effects of prolonged LTB$_4$ administration on bone marrow donor cell engraftment. Bone marrow cells (10^7) from C57BL/6 mice were grafted into BALB/c (n = 8/group) mice using a standard BMT protocol, as detailed under *Materials and Methods*. From day 5 posttransplant up to day 100, mice were injected i.v. three times a week with a placebo or LTB$_4$ (50 and 500 ng/kg/mouse). On day 100, the surviving mice (n = 4–5/group) were sacrificed, and bone marrow cells were isolated from the femurs and analyzed by flow cytometry for total donor cell (H2-K^{b+}) engraftment (*A*) and donor stem cell (H2-K^{b+}/CD34$^+$) engraftment (*B*). Results are expressed as mean percentage ± SEM of positive cells.

This result suggests that LTB$_4$ administration does not promote graft-vs-host disease. Such results are in accordance with in vitro experiments demonstrating that LTB$_4$ does not interfere with cyclosporin A or FK-506 inhibition of human T lymphocyte proliferation and *IL-2* gene activation (data not shown). In addition, flow cytometric analysis of bone marrow cells from transplanted mice indicates that LTB$_4$ administration had no negative impact on donor cell (H-2K^{b+}) implantation and marrow regeneration (Fig. 3*A*). A chimerism between cells from recipient and donor mice was observed with ~50% of the cells originating from the donor mouse. Donor cells present within the marrow of recipient mice were further analyzed relative to the composition of hemopoietic bone marrow progenitors (CD34$^+$/H2-K^{b+}). The analyses performed indicate that LTB$_4$ administration for 100 days, had no negative impact on donor stem cell survival and engraftment (Fig. 3*B*).

These results clearly show that LTB$_4$ administration does not promote donor graft rejection in bone marrow-allografted mice. In addition, the survival rate at day 100 was identical between all groups, providing reassuring evidence that LTB$_4$ administration to immunosuppressed animals does not compromise bone marrow engraftment nor promotes graft-vs-host-disease.

Effects of LTB$_4$ administration on MCMV reactivation following allogeneic splenocyte or bone marrow transplantation

Having determined that LTB$_4$ does not negatively influence stem cell engraftment, we wanted to test the effects of LTB$_4$ on CMV reactivation. As mentioned above, one common problem associated with allogeneic transplantation is the reactivation of latent CMV following the transplant. In an attempt to mimic these events, 32 adult CMV naive BALB/c mice were treated with cyclophosphamide and injected i.p. with 100 million splenocytes from latently MCMV-infected C57BL/6 (H2k^b) mice. Mice were divided in four groups and treated by i.v. injection with a placebo or LTB$_4$ (5, 50, or 500 ng/kg) three times a week for 3 wk. The salivary glands were taken from each mouse and assayed for CMV viral load by standard plaque assay. The results obtained indicate (Fig. 4*A*) that eight of eight mice that were treated with the placebo had detectable CMV viral load in their salivary glands. Because recipient mice were naive for CMV, the source of infection originates from the reactivation of CMV from latently infected donor spleen cells. In sharp contrast to the placebo-treated group, no active CMV infection could be detected in any of the groups of mice (0 of 24) treated with LTB$_4$. When this experiment was repeated, 33% of the placebo group reactivated MCMV, compared with 16, 0, and 8% for mice treated with 5, 50, and 500 ng/kg of LTB$_4$, respectively (data not shown).

We next proceeded to test the effects of LTB$_4$ administration on CMV reactivation/infection in latently MCMV-infected mice undergoing allogeneic bone marrow transplantation. A protocol nearly identical with those used in human bone marrow transplantation was used. Latently MCMV-infected BALB/c (H2k^d) mice were treated with cyclophosphamide on days −4 and −2, then sublethally irradiated and engrafted with 10^7 bone marrow cells from latently MCMV-infected C57BL/6 (H2k^b) mice. FK-506 (90 μg/kg) was administered daily starting on the first day posttransplant. Mice were divided in three groups and treated i.v. with a placebo or LTB$_4$ (50 and 500 ng/kg) from days 21 to 35 posttransplant. In preliminary experiments, it was established that 1 mo posttransplant, mice had undergone reactivation of latent MCMV. This is somewhat similar to what is observed in humans, where CMV reactivation is most frequent during the second month posttransplant. On day 36 posttransplant, mice were sacrificed, and salivary glands were then taken from each mouse and assayed for MCMV viral load by standard plaque assay. Results obtained indicate that MCMV reactivation was less frequently observed in LTB$_4$-treated mice (38–50%) than in the placebo-treated group (78%) (Fig. 4*B*).

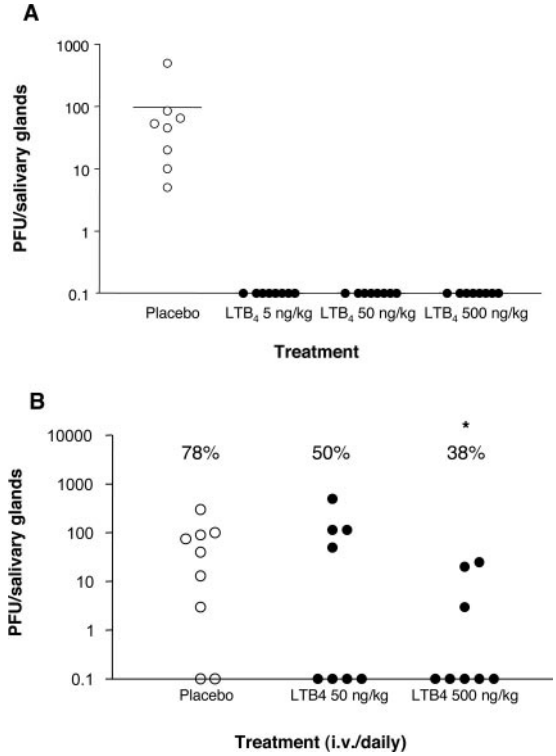

FIGURE 4. Effects of LTB_4 administration on the reactivation/transmission of latent MCMV following allogeneic splenocyte or bone marrow transplantation. *A*, Cyclophosphamide-treated BALB/c mice were injected i.p. with 10^8 splenocytes obtained from latently CMV-infected C57BL/6 mice. BALB/c mice ($n = 8$/group) were injected i.v. three times a week with a placebo or LTB_4 (5, 50, and 500 ng/kg). Twenty-one days following splenocyte injection, mice were sacrificed, and the salivary glands were collected and analyzed for viral loads by standard plaque assays as described under *Materials and Methods*. Results are expressed as MCMV viral loads (PFU) in salivary glands for each mouse. *B*, Latently MCMV-infected BALB/c mice were grafted with 10^7 bone marrow cells from latently MCMV-infected C57BL/6 mice using a standard BMT protocol as detailed under *Materials and Methods*. Three weeks posttransplant, mice were divided into three groups ($n = 8$ or 9/group) and injected i.v. daily for 14 days with a placebo solution or LTB_4 (50 and 500 ng/kg). On day 36 posttransplant, mice were sacrificed, and viral loads in the salivary glands were determined. Results are expressed as MCMV viral loads (PFU) in salivary glands for each mouse. *, Viral loads in treated group were significantly lower compared with the placebo group ($p < 0.05$ using Mann-Whitney *U* test).

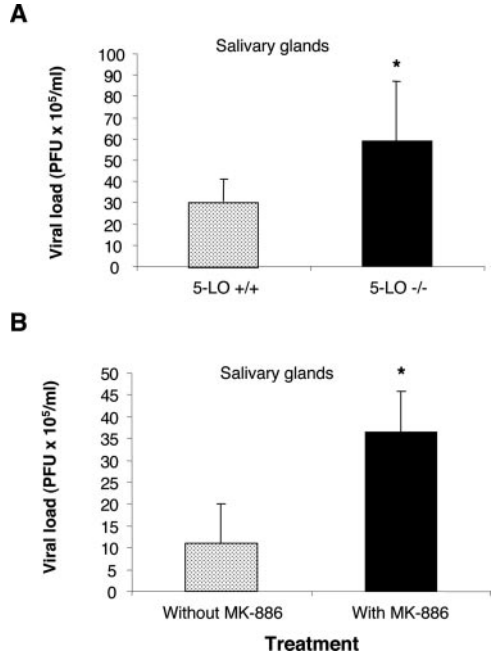

FIGURE 5. Effects of endogenous 5-LO metabolite deficiency and administration of MK-886 on MCMV infection in mice. *A*, Wild-type (5-$LO^{+/+}$) and 5-LO gene knockout (5-$LO^{-/-}$) female mice ($n = 8$/group) were infected with a sublethal dose (500 PFU) of MCMV by i.p. injection. On day 12 postinfection, mice were sacrificed, and viral loads were determined in salivary glands. Results, expressed as the mean viral load ± SEM, are representative of three independent experiments. *B*, Adult BALB/c female mice ($n = 10$/group) were infected with a sublethal dose (500 PFU) of MCMV. One day before the infection and every day thereafter, mice were treated i.p. with 60 μg of MK-886 (an inhibitor of FLAP) (in 100 μl of saline solution) or with a placebo. On day 12 postinfection, viral loads were determined in salivary glands of mice. Results, expressed as the mean viral load ± SEM, are representative of three different experiments. *, Data significantly different from control ($p < 0.05$), using unpaired two-tailed Student's *t* test.

Protective effect of endogenous 5-LO products in MCMV-infected mice

In the experiments presented above, we have shown that administration of exogenous LTB_4 to infected mice enhances resistance to MCMV infection. This suggests that LTB_4 may play an important role in the natural antiviral immunity. To support this hypothesis, we used 5-LO knockout (5-$LO^{-/-}$) mice, which are unable to produce endogenous leukotrienes. Mice were infected with MCMV, and viral load was assayed in salivary glands on day 12 postinfection. As shown in Fig. 5*A*, 5-$LO^{-/-}$ animals have higher viral loads in salivary glands (an increase of 93%), compared with the wild-type (5-$LO^{+/+}$) controls. To further support the role of endogenous 5-LO products as putative mediators of the antiviral defense, another set of experiments was performed. MCMV-infected BALB/c mice were treated daily for 11 days starting on the day of infection with MK-886, an inhibitor of FLAP, a protein shown to be essential to leukotriene biosynthesis. On day 12 postinfection, viral load measurements in the salivary glands indicate that MK-886-treated mice had significantly higher MCMV viral loads (an increase of 233%) (Fig. 5*B*) compared with that of the placebo-treated group.

Discussion

LTB_4 was first recognized as a potent chemoattractant of neutrophils (22, 23). In tissues exposed to LTB_4, circulating neutrophils rapidly adhere to the endothelium and migrate into extravascular space (24). LTB_4, and the cysteinyl leukotrienes, are considered to play an important role in inflammatory diseases. In the present study, we show that LTB_4 has protective effects in mouse models of MCMV infection and reactivation in immunosuppressed animals. Such actions of LTB_4 in host defense against viruses represent a new role for LTB_4.

The mechanism(s) of action of LTB_4 in vivo as antiviral is (are) not yet elucidated. LTB_4 may activate several effector functions of lymphocytes and NK cells, two important players of the antiviral immunity. LTB_4 enhances IL-2 synthesis by $CD4^+$ T cells (25) and increases expression of IL-2Rs on $CD8^+$ T cells (26) favoring their proliferation. Similarly, the expression of IL-2Rs is also increased on NK cells by LTB_4, resulting in an increased responsiveness to IL-2 and greater cytotoxic activity. More recently, it was reported that LTB_4 mediates migration of $CD4^+$ and $CD8^+$ T cells out of the lymphoid compartment and their recruitment into peripheral tissues (27, 28). LTB_4 was also found to act on B cells

by enhancing their activation, proliferation, and Ig secretion (29, 30). Thus, these observations substantiate a potential role of LTB_4 as activator of cytotoxic cells, which are known to be involved in the killing of infected cells. In the allogeneic transplantation protocols presented in our study, we show that viral reactivation in latently MCMV-infected recipients was much less frequently observed in animals treated with LTB_4. Latently infected mice have developed immune memory T and B cells. Thus, we speculate that such effector functions may have been potentiated in mice treated with LTB_4, promoting resistance to MCMV reactivation.

Such an activation of lymphocyte effector functions involves Ag processing and the generation of Ag-specific effector cells. This process requires a certain amount of time following infection to be effective and, thus, does not participate to the early response against viral infection. LTB_4 is also known to be a potent activator of phagocytes such as neutrophils, a cell type associated with the nonspecific defense. Activation of neutrophils is an early event of the immune response. Neutrophils are rapidly mobilized, and their number and distribution throughout the body ensures that they are often the first leukocytes to encounter invading organisms. In addition to neutrophil recruitment, LTB_4 stimulates their phagocytic activity, the formation of reactive oxygen species (31), and the release of granular enzymes (32). Although we cannot overlook that an effective Ag-specific response could be involved, the activation of neutrophils is probably the main mechanism by which LTB_4 exerts its antiviral activity within the first days of the infection. For example, in studies using animals infected with a lethal dose of MCMV and treated with LTB_4 (500 ng/kg) on the day of infection and every day thereafter, no mortality was observed within 7 days postinfection (at least 80% mortality was observed in the placebo group), suggesting a rapid activation of the innate immune response. Furthermore, in naive mice injected i.p. with splenocytes from latently MCMV-infected mice and treated with LTB_4, no active MCMV infection has been detected in salivary glands in contrast to the placebo group. In these experiments, we can argue that the viral spread was rapidly controlled by LTB_4-stimulated phagocytes in the peritoneal cavity. This assumption is supported by previous studies showing that alveolar macrophages and neutrophils from mice unable to produce leukotrienes demonstrated a reduced ability to phagocytose and kill bacteria. This impairment (defective phagocytosis) could be overcome by treatment with exogenous LTB_4 (15, 33). In this respect, our experiments using 5-LO knockout mice infected with MCMV show that leukotriene-deficient mice have a higher viral load in salivary glands compared with the wild-type mice. Viral loads were also found to be higher in animals treated with MK-886, a potent FLAP antagonist and inhibitor of leukotriene synthesis, further supporting a critical role of 5-LO products, possibly LTB_4, in host defense against infectious agents.

Another mechanism triggered by LTB_4 that likely contributes to its antiviral activity is the release of antimicrobial proteins called α-defensins (reviewed in Ref. 13). These molecules are polypeptides that can nonspecifically disrupt the membrane of various microorganisms including bacteria, protozoa, and viruses. Epithelial cells and monocytes can produce α-defensins, but the main producers are neutrophils. Recent studies have demonstrated the anti-HIV activity of defensins, thus stimulating the interest for these mediators of the innate immunity (34, 35). In a recent clinical trial (phase I) aiming to evaluate the safety and tolerability of i.v. administered LTB_4 to humans, we observed that LTB_4 was a potent inducer of α-defensin release with a maximal plasma concentration at 2 h post-LTB_4 injection (36). The release of α-defensins by LTB_4-activated neutrophils may then represent another mechanism that contributes to viral clearance. However, the relative antiviral contributions of α-defensins in our murine models of CMV infections remain to be determined considering that it is reported that neutrophils from mice do not contain or secrete such antimicrobial peptides (37).

The present study clearly shows that administration of LTB_4 reduces the viral loads in MCMV-infected mice and prevents MCMV reactivation in latently infected mice following allogeneic bone marrow transplantation. By stimulating leukocyte recruitment and a wide range of effector functions of both the innate and the adaptive immunity, LTB_4 behaves as a potent immunomodulator with a great potential for the prevention and the treatment of viral infections.

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