Hypercholesterolemia Exacerbates Virus-Induced Immunopathologic Liver Disease Via Suppression of Antiviral Cytotoxic T Cell Responses

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Hypercholesterolemia Exacerbates Virus-Induced Immunopathologic Liver Disease Via Suppression of Antiviral Cytotoxic T Cell Responses

Burkhard Ludewig, Martin Jäggi, Tilman Dumrese, Karin Brduscha-Riem, Bernhard Odermatt, Hans Hengartner, and Rolf M. Zinkernagel

The immune system has to be optimally balanced to be highly effective against infections with cytopathic microbial pathogens and must guarantee efficient destruction of cells infected with noncytopathic agents while leaving the integrity of noninfected cells largely unaltered. We describe here the effects of genetically induced hypercholesterolemia on cellular immunity in apolipoprotein E (ApoE<sup>−/−</sup>) and low density lipoprotein receptor-deficient (LDLR<sup>−/−</sup>) mice during infection with the hepatotropic lymphocytic choriomeningitis virus WE strain. In both ApoE<sup>−/−</sup> and LDLR<sup>−/−</sup> mice hypercholesterolemia aggravated virus-induced immunopathologic liver disease. ApoE<sup>−/−</sup> mice exhibited a higher susceptibility to virus-induced immunopathology than LDLR<sup>−/−</sup> mice and usually succumbed to immunopathologic disease when infected with high doses of virus. Initial virus spread was not influenced by the hypercholesterolemia, whereas clearance of the virus from spleen and nonlymphoid organs, including liver, was delayed. Activation of antiviral CTL, measured by ex vivo cytotoxicity and IFN-γ production, and recruitment of specific CTL into blood and liver were impaired in hypercholesterolemic mice, indicating that hypercholesterolemia had a significant suppressive effect on cellular immunity. Taken together, these data provide evidence that hypercholesterolemia suppresses antiviral immune responses, thereby changing the host-virus balance, and can increase susceptibility to acute or chronic and potentially lethal virus-induced immunopathologic disease. These findings impinge on our understanding of hypercholesterolemia as a disease parameter and may explain aspects of the frequent association of persistent pathogens with hypercholesterolemia-induced diseases, such as atherosclerosis. The Journal of Immunology, 2001, 166: 3369–3376.

Hypercholesterolemia is recognized as one of the main risk factors for atherosclerosis (1), with the sequence of cholesterol accumulation in the arterial wall, local inflammatory responses leading to recruitment and activation of macrophages and T cells, and, finally, development of fibrotic lesions involving proliferation of smooth muscle cells (2). In addition, cholesterol metabolism impacts at various points on the responsiveness of the immune system. For example, chronic hypercholesterolemia predisposes the microvasculature to intense leukocyte-endothelial cell adhesion in response to inflammatory stimuli (3). Furthermore, modified LDL increases macrophage chemotaxis (4) and may stimulate T cells in atheromatous lesions (5). However, high lipoprotein levels in plasma diminish systemic cytokine responses (6, 7). Furthermore, high plasma cholesterol levels can result in impaired antibacterial immune responses, as shown by the failure of genetically hypercholesterolemic apolipoprotein E (ApoE)<sup>−/−</sup> mice to rapidly clear Listeria monocytogenes (8) or Klebsiella pneumoniae infection (9). Similarly, hypercholesterolemic mice lacking the low density lipoprotein receptor (LDLR<sup>−/−</sup>) are highly susceptible to disseminated Candida albicans infection (10). Thus, beside local stimulatory effects in vascular inflammatory responses, hypercholesterolemia may also exert negative effects on general immune responsiveness.

To be effective in the defense against pathogens, the immune system has to be maximally effective against cytopathic infections, but may be only optimally balanced against poorly or noncytopathic agents. For example, cells infected with noncytopathic virus should be destroyed rapidly enough to prevent excessive immunopathology and to keep damage of noninfected cells at a minimum. Therefore, factors altering the equilibrium between the spread of poorly or noncytopathic pathogens and the immune response may favor acute or chronic immunopathologic disease. A well-studied model of virus-induced immunopathology is the infection with the lymphocytic choriomeningitis virus (LCMV) (11, 12). Immunopathologic disease in acute LCMV infection is primarily mediated by CTL, which may cause the classical choriomeningitis after intracerebral infection when meningeal cells become targets for the antiviral immune response (13), hepatitis after infection with hepatotropic strains (14), or immunosuppression when APCs in the lymphoid tissues are destroyed (15). The importance of CTL in LCMV-induced immunopathology also has been demonstrated in transgenic mice expressing the LCMV glycoprotein in the islets of Langerhans (16, 17) where contact-dependent, perforin-mediated lysis of viral Ag-expressing cells is crucial to mediate the immunopathologic response (18). The extent of LCMV-induced immunopathologic disease depends on various host and virus parameters, such as viral tropism (14, 19), genetic background (19, 20), and immunocompetence of the host (21).
Thus, LCMV infection offers an experimental system to thoroughly investigate the influence of additional potential disease parameters such as hypercholesterolemia. We used here ApoE<sup>−/−</sup> and LDLR<sup>−/−</sup> mice to determine whether these factors alter virus-host equilibrium and enhance or prevent immunopathologic disease. Mice were infected with the hepatotropic LCMV WE strain, which can lead to substantial impairment of antiviral cellular immune responses, leading to delayed viral clearance.

Materials and Methods

Mice
C57BL/6 mice were obtained from the Institut für Labor tierkunde (University of Zurich, Zurich, Switzerland). ApoE<sup>−/−</sup> mice (24) and LDLR<sup>−/−</sup> mice (25), both on a C57BL/6 background, were obtained from The Jackson Laboratory (Bar Harbor, ME). Animals were fed normal rodent chow (ND, Provini Kliba, Kaiserslautern, Switzerland), or high cholesterol diet (HCD, ND supplement with 1.25% cholesterol, 8% fat, Provini Kliba). All animals were kept under specific pathogen-free conditions. Experiments were conducted with age-matched (6–8 wk) and sex-matched animals.

Viruses, cell lines, and peptides
LCMV-WE was originally obtained from Dr. F. Lehmann-Grube (Hamburg, Germany) and was propagated on L929 cells. EL-4 (H-2<sup>b</sup>), a thymoma cell line, was used as the target cell. LCMV-GP peptides KAVYN (GP33) and FQPQNGQFI (NP396) were purchased from NeoSystem Laboratoire (Strasbourg, France).

Cytotoxicity assay
For detection of primary ex vivo cytotoxicity, effector cell suspensions were prepared from spleen or liver of infected mice on day 7 or 9 after infection. EL-4 cells were pulsed with LCMV GP33 or NP396 (10<sup>6</sup> M, 1.5 h at 37°C) and used in a standard 5-h <sup>3</sup>HCl release assay. Unlabeled EL-4 cells served as controls. The supernatant of the cytotoxicity cultures was counted in a Cobra II Gamma Counter (Canberra Packard, Downers Grove, IL). Spontaneous release was always <20%.

Construction of tetrameric MHC class I-peptide complexes
MHC class I (H2-D<sup>b</sup>) tetramers complexed with defined viral epitopes (22, 23), were used and activation and peripheral recruitment of virus-specific CTL. In addition, antiviral T cell effector function was followed by cytotoxicity and cytokine production assays. The results show that hypercholesterolemia may lead to a substantial impairment of antiviral cellular immune responses, leading to delayed viral clearance from spleen and nonlymphoid organs. As a consequence of the disturbed virus-host equilibrium, mice developed severe immunopathologic disease.

Results

Hypercholesterolemia aggravates virus-induced immunopathologic liver disease
Infection of C57BL/6 mice with low doses (2 × 10<sup>2</sup> PFU) of LCMV WE induces a mild inflammation in the liver without measurable increase of liver enzymes in serum, whereas infection with high doses (>10<sup>5</sup> PFU) leads to a strong, but transient, increase in liver enzymes in serum (14). Infection of ApoE<sup>−/−</sup> mice with 200 PFU of LCMV (low dose) elicited an increase in liver enzymes in serum (14). Infection of ApoE<sup>−/−</sup> and LDLR<sup>−/−</sup> mice were infected i.v. with the hepatotropic LCMV strain WE. Virus titers in spleen, kidney, liver, and lung were determined at the indicated time points in an LCMV infectious focus assay as previously described (26). Values of virus titers in the various organs are expressed as log<sub>10</sub> PFU per gram. Statistical analysis was performed using Prism 2.01 software (GraphPad Software, Berkeley, CA).

Immunohistology

Freshly removed organs were immersed in HBSS and snap-frozen in liquid nitrogen. Tissue sections of 5-μm thickness were cut in a cryostat and fixed in acetone for 10 min. Sections were incubated with anti-mouse CD8<sup>+</sup> cells mAb (YT169.4-2) (27) or rat anti-LCMV-NP mAb (VL-4) (26). Alkaline phosphatase-labeled, species-specific goat Abs (Tago, Burlingame, CA) were used as secondary reagents. The substrate for the red color reaction was AB-I phosphate/New Fuchsin. Sections were counterstained with hemalum.

Assay of serum TNF

TNF concentrations were determined by solid phase ELISA (BioSource, Camarillo, CA) according to the manufacturer’s instructions. Samples were stored at −20°C and analyzed in a single assay.

Determination of serum enzyme concentrations

Assays for serum concentrations of alanine aminotransferase (ALT), aspartate aminotransferase, and total cholesterol in plasma were performed at the Department of Clinical Chemistry, University Hospital Zurich, using photometric assays on a Hitachi 747 autoanalyzer (Tokyo, Japan).

Infection of C57BL/6 mice with low doses (2 × 10<sup>2</sup> PFU) of LCMV WE induces a mild inflammation in the liver without measurable increase of liver enzymes in serum, whereas infection with high doses (>10<sup>5</sup> PFU) leads to a strong, but transient, increase in liver enzymes in serum (14). Infection of ApoE<sup>−/−</sup> mice with 200 PFU of LCMV (low dose) elicited an increase in liver enzymes compared with levels in C57BL/6 control mice (Fig. 1A). The increased release of liver enzymes was diet-independent, since ApoE<sup>−/−</sup> mice fed a normal chow diet (ND) and those fed a high cholesterol diet (HCD) showed comparable elevations of liver enzymes in serum despite dramatic differences in plasma cholesterol values (Fig. 1A). After high dose infection (2 × 10<sup>5</sup> PFU), both hypercholesterolemic ApoE<sup>−/−</sup> and C57BL/6 mice developed fulminant hepatitis (Fig. 1B). ApoE<sup>−/−</sup> mice fed HCD and infected with a high dose died before day 12 (Fig. 1B). Statistical analysis of the data revealed that liver enzyme values were significantly elevated on day 8 after low dose infection (Fig. 1C), whereas the values on day 8 after high dose infection were not significantly different (not shown). Cholesterol levels on day 8 after high dose infection were not significantly different (not shown). Cholesterol levels on day 12 were elevated after low dose infection in ApoE<sup>−/−</sup> mice (Fig. 1A) and in ApoE<sup>−/−</sup> and C57BL/6 mice infected with a high dose of LCMV (Fig. 1B). Prolongation of HCD exacerbated the liver disease after LCMV infection and caused >50% mortality after 6 wk of HCD infection.
Impaired virus clearance in hypercholesterolemic mice

In essence, there are two possible scenarios to explain the above findings. First, nonspecific resistance and specific immune responses in hypercholesterolemic mice might be increased, leading to a more vigorous antiviral response with more “bystander” damage. In particular, TNF, which has been shown to be up-regulated after infection of hypercholesterolemic mice with bacteria (8, 9) or C. albicans (10), may mediate such pathological effects. To address this first possibility, serum TNF values were determined after infection of hypercholesterolemic mice with bacteria (8, 9) or C. albicans (10), TNF concentrations in serum of LCMV-infected normo- and hypercholesterolemic mice were below the limits of detection (<5 pg/ml; data not shown). This suggested that excessive TNF production in hypercholesterolemic mice is unlikely to contribute importantly to the exacerbated virus-induced immunopathology.

The second explanation is that impairment of virus-specific immune responses in hypercholesterolemic mice may cause an imbalance between virus control vs immunopathologic damage. We therefore followed the initial viral spread and determined the clearance of LCMV from spleen and nonlymphoid tissues. Initial virus distribution (day 4 postinfection) was not affected by the hypercholesterolemia, and comparable levels of infectious virus were found in spleen (Table I) and other organs (liver, lung, and kidney; data not shown). On day 7 postinfection, however, viral loads were slightly elevated in spleen and liver of ApoE−/− and LDLR−/−/2 mice compared with those in C57BL/6 controls. Increasing the hypercholesterolemia by HCD further impaired the clearance of the virus, particularly in ApoE−/− mice (Table I).

FIGURE 1. Kinetics, virus dose, and cholesterol dependence of the changes in liver enzyme concentrations in serum of LCMV-WE-infected ApoE−/− and control mice. Mice were infected i.v. with 200 PFU of LCMV (low dose; A) or 2 × 10⁵ PFU of LCMV (high dose; B) on day 0 and bled at the indicated time points, and liver enzyme levels in serum and plasma cholesterol concentrations were determined. Mice were fed ND or HCD from the day of infection. Values are the mean ± SD of three to five mice per group. C, Statistical analysis (Mann-Whitney test) of cumulated day 8 values from two independent experiments revealed significant differences in liver enzyme release between ApoE−/− and control C57BL/6 mice. D, Influence of long term HCD on LCMV-induced immunopathologic liver disease. ApoE−/− and C57BL/6 mice were fed HCD for 3 or 6 wk and then infected with 200 PFU of LCMV. Data points represent ALT values on day 8 postinfection of single mice; mean values are indicated by the horizontal bars. Five of nine 6-wk HCD-fed ApoE−/− mice died between days 7 and 8 postinfection. ALT values for uninfected mice after 6 wk of HCD: ApoE−/−, 51 ± 21; C57BL/6, 36 ± 5.

FIGURE 2. Survival of ApoE−/− and control mice after LCMV infection. ApoE−/− (n = 10; dotted line) or C57BL/6 mice (n = 10; solid line) infected i.v. with 2 × 10⁵ PFU of LCMV or ApoE−/− mice infected with 200 PFU of LCMV (n = 8; dashed line) were fed HCD, and survival was monitored for 20 days. Data are pooled results from two independent experiments.
levels on virus clearance from liver and spleen, ApoE

Kinetics and virus dose dependence of liver enzymes in

To test the effect of long-lasting elevated plasma cholesterol levels on virus clearance from liver and spleen, ApoE/−/− and C57BL/6 mice were fed HCD for 6 wk and then infected with 200 PFU of LCMV. The presence of viral Ag in liver and spleen was assessed on day 9 postinfection using a sensitive immunohistochemical method and correlated with antiviral CTL activity in spleen. After long term HCD feeding, large numbers of CTL were found in livers of C57BL/6 mice (Fig. 4A), and virus was cleared from liver (Fig. 4B) and spleen (not shown). CTL activity in spleen (Fig. 4C) was comparable to that in C57BL/6 mice fed ND (not shown). CTL infiltration in livers of ApoE/−/− mice fed HCD from the day of infection (Fig. 4D) was high, whereas ApoE/−/− mice fed HCD for 6 wk showed a strong decrease in liver-infiltrating CTL (Fig. 4G). Furthermore, the failure of both short term (Fig. 4E) and long term (Fig. 4H) HCD-fed ApoE/−/− mice to completely clear LCMV Ag from the liver correlated well with a progressive loss of CTL activity in spleens after short term (Fig. 4F) and long term (Fig. 4I) HCD. These findings suggest that the hypercholesterolemia in ApoE/−/− and LDLR/−/− mice had a negative impact on the virus-host balance, leading to delayed clearance of the virus.

Altered antiviral CTL responses in hypercholesterolemic mice

To evaluate the antiviral immune response in hypercholesterolemic mice more thoroughly, we first used MHC class I tetramers to detect and enumerate virus-specific CTL in blood, spleen, and liver. Cells were stained with H2-Db (GP33) tetramers, and the percentage of CD8 lymphocytes positive for GP33 tetramers was calculated. In livers of ApoE/−/− mice, 7.1 ± 0.4% of the CD8 T cells were specific for GP33 on day 7 postinfection (Fig. 5A). Values for LDLR/−/− (Fig. 5B) and C57BL/6 control mice (Fig. 5C) were always higher. Statistical analysis of all mice tested revealed that ApoE/−/− mice fed either ND or HCD suffered from significantly impaired activation of virus-specific CTL in the spleen and reduced recruitment of antiviral CTL into blood and liver (Fig. 5D). CTL activation in LDLR/−/− mice after LCMV infection was only slightly reduced, and alterations were significant only for blood values after HCD feeding (Fig. 5D). Examination of the cytotoxicity of liver-infiltrating CTL by comparison of the E:T cell ratios for the 33% lysis revealed a 3- to 5-fold reduction of the relative CTL activity in ApoE/−/− mice compared with that in control C57BL/6 mice that may be partially due to the differences in the frequencies of GP33- or NP396-specific CTL in the cytotoxicity assay. Liver-infiltrating CTL in LDLR/−/− mice were less affected (Fig. 5E). No clear effect of the diet on relative CTL activity within one strain of mice was observed (compare left and right columns in Fig. 5E).

IFN-γ is important for the control of LCMV infection (29, 30) and may contribute to the elimination of LCMV from hepatocytes by noncytolytic mechanisms (31). We therefore assessed the production of IFN-γ in virus-specific CTL in spleen of HCD fed ApoE/−/− (Fig. 6, A and B), LDLR/−/− (Fig. 6, C and D), and C57BL/6 control mice (Fig. 6, E and F) on day 7 after infection with LCMV. Freshly isolated splenic lymphocytes from C57BL/6

Table I. Virus titers in organs of ApoE/−/−, LDLR/−/−, and C57BL/6 mice after LCMV infection*

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ApoE/−/−, LDLR/−/−, or C57BL/6 mice fed either ND or HCD were infected with 200 PFU LCMV, and virus titers in spleen, liver, lung, and kidney were determined 4 and 7 days later. Values represent mean ± SEM of four to five mice per group.

* Statistically significant differences (p < 0.05, Student’s t test) between hypercholesterolemic ApoE/−/− or LDLR/−/− and C57BL/6 mice.
mice produced significant amounts of IFN-γ after 6 h of restimulation in vitro with GP33 (Fig. 6E) or NP396 (Fig. 6F). IFN-γ production of virus-specific CD8+ T cells from LDLR−/− was reduced (Fig. 6, E and D). In ApoE−/− mice, again, the impairment of antiviral CTL responses was most severe (Fig. 6, A and B). Taken together, the activation of virus-specific CTL was severely affected in hypercholesterolemic mice, suggesting that viral clearance from spleens and nonlymphoid organs in hypercholesterolemic mice was impaired because hypercholesterolemia-induced immunosuppression inhibited the generation of a sufficient antiviral CTL response.

**Impaired antiviral memory responses in hypercholesterolemic mice**

The maintenance of high precursor frequencies and efficient reactivation of CTL are important to confer antiviral protection after re-encounter with virus (32, 33). Maximal expansion of virus-specific CTL after LCMV infection is reached around day 8, followed by a continuous decrease until day 30, when a stable memory population with elevated precursor frequencies is established (34, 35). To determine whether the hypercholesterolemia-induced reduction of immune responsiveness also affected LCMV-specific memory responses, mice infected 30 days previously with LCMV were challenged with a high dose of LCMV, and MHC class I tetramers were used to visualize Ag-specific CD8+ T cells (Fig. 7). Expansion of GP33- and NP396-specific CTL in spleen on day 4 after LCMV challenge infection was reduced in ApoE−/− (Fig. 7, A and B) and LDLR−/− mice (Fig. 7, C and D) compared with that in C57BL/6 control mice (Fig. 7, E and F). Thus, antiviral cellular immunity in ApoE−/− and LDLR−/− mice was impaired in both acute and memory anti-LCMV responses.

**Discussion**

In the course of an antiviral immune response a well-balanced equilibrium between virus spread and antiviral effector mechanisms is usually established. The major finding of this study is that hypercholesterolemia can disrupt this equilibrium and enhance severe immunopathology after infection with a hepatropic noncytopathic virus. Although the role of hypercholesterolemia in the pathogenesis of atherosclerosis has been studied extensively in ApoE−/− and LDLR−/− mice (36–39), a thorough analysis of T cell reactivity in these mice in response to a viral infection has not been previously described. This is particularly important since viral infections (40, 41) and antiviral immune reactions in the vascular wall (42, 43) are thought to crucially contribute to vascular immunopathology. Our studies documenting the impairment of antiviral T cell immunity in genetically hypercholesterolemic mice impinge on our understanding of hypercholesterolemia as a cofactor in immunopathologic disease. In view of the fact that atherosclerosis can be defined at least partially as an immunopathologic vascular disease (43), our findings may explain the mechanism of how particular infectious agents may participate in establishment and maintenance of atherosclerotic disease.

This study extends and complements previous studies on the susceptibility of hypercholesterolemic mice to infectious pathogens. Successful immune responses against fast replicating, cytopathic infectious agents depend mainly on innate immune mechanisms, such as type I IFN (29), or complement (44, 45). The high susceptibility of LDLR−/− mice to generalized Candidiasis (10) and of ApoE−/− mice to L. monocytogenes (8) and Klebsiella infection (9) suggests that hypercholesterolemia leads to an impairment of innate immune responses. In noncytopathic LCMV infection, innate immune responses contribute to limit the initial spread of the virus and therefore limit or prevent immunopathologic disease or exhaustion (46). However, in the present study we could not detect differences in the initial spread of LCMV in hypercholesterolemic ApoE−/− or LDLR−/− mice vs wild-type controls, suggesting that innate control of LCMV was not affected significantly by the defect in cholesterol metabolism. Furthermore, we could not detect massive TNF production as had been observed in the bacterial (8, 9) or fungal infections (10) of hypercholesterolemic mice, supporting the idea that LCMV may trigger TNF production only to a limited extent.
Viruses or other micro-organisms with a low cytopathicity often establish persisting infections in varying host-pathogen balances that permit the survival of both host and pathogen. However, the immunopathologic consequences of the immune response, for example against LCMV, critically depend on both virus distribution and kinetics of the T cell response. The wider the virus spreads and the longer it persists, the more serious are the pathological consequences of the antiviral immune response, unless in an extreme situation, T cells are exhausted (47). Furthermore, defects in cellular immunity, such as perforin deficiency, favor LCMV persistence and may lead to increased immunopathologic disease generally and in the bone marrow (48). The data of this study indicate that the anti-LCMV response in hypercholesterolemic mice may be too weak to eliminate the virus efficiently from infected hepatocytes and other peripheral tissues, but is sufficiently strong to elicit substantial immunopathology. Since virus replication

**FIGURE 5.** CTL activation and recruitment into blood and liver in hypercholesterolemic and control mice after LCMV infection. ApoE−/−, LDLR−/−, or C57BL/6 mice fed either ND or HCD were infected i.v. with 200 PFU of LCMV. Seven days later, GP33-specific CD8+ T cells in liver, spleen, and blood were visualized using MHC class I tetramers. Representative FACS stainings from liver of ND-fed ApoE−/− (A), LDLR−/− (B), and C57BL/6 (C) mice are shown. The mean percentage of CD8 T cells specific for GP33 (±SEM) is indicated in the corresponding upper right quadrant. D, Mean percentages ± SEM of tetramer-GP33-positive CD8+ T cells in spleen, liver, and blood of the indicated mouse strains. Pooled data from two independent experiments are shown. Statistically significant differences (p < 0.05, by Mann-Whitney test) between hypercholesterolemic ApoE−/− or LDLR−/− and C57BL/6 mice are indicated by an asterisk. E, Ex vivo CTL activity of pooled liver-infiltrating lymphocytes on day 7 postinfection. 51Cr-labeled, GP33-pulsed (■), NP396-pulsed (▲), or unpulsed (□) EL4 cells were used as target cells. The dashed line indicates 33% specific lysis.

**FIGURE 6.** IFN-γ production of LCMV-specific CD8+ T cells in hypercholesterolemic ApoE−/− and LDLR−/− mice. ApoE−/− (A and B), LDLR−/− (C and D), or C57BL/6 (E and F) mice fed HCD were infected i.v. with 200 PFU of LCMV. On day 7 post infection, splenocytes and liver-infiltrating lymphocytes were restimulated with LCMV peptides GP33 or NP396 in vitro, and specific IFN-γ-producing CD8 T cells were detected by intracellular cytokine staining. Mean percentages (± SEM) of IFN-γ-producing CD8 T cells in spleen and liver of the indicated mouse strains are shown in the upper right quadrant of the respective histograms. Four mice per group were analyzed. One of two comparable experiments is shown.

**FIGURE 7.** Virus-specific CD8 T cell memory responses in hypercholesterolemic mice. ApoE−/− (A and B), LDLR−/− (C and D), or C57BL/6 (E and F) mice infected 30 days previously with a low dose of LCMV were challenged with 2 × 10⁵ PFU of LCMV. Four days after the challenge infection, the percentage of GP33-specific (A, C, and E) and NP396-specific (B, D, and F) CD8 T cells in spleens were determined by tetramer staining. Mean percentages (± SEM) of specific tetramer-positive CD8 T cells are shown in the upper right quadrant of the respective histograms. Three or four mice per group were analyzed. One of two experiments is shown.
Long-lasting hypercholesterolemia might, as shown in this report, further alter cholesterol metabolism. Thus, self-perpetuating immunopathologic disease circuits may develop when the hypercholesterolemia-mediated defects in innate and adaptive immunity observed in this and previous reports (8–10). An important link between innate and adaptive immunity is provided by macrophages rapidly producing large amounts of effector molecules upon encounter with pathogens (59). Since macrophages are critically involved in cholesterol metabolism (60), it is likely that chronic hypercholesterolemia leads to pre- and/or over-stimulation of macrophages. This could explain the elevated TNF responses of hypercholesterolemic mice in response to bacterial and fungal pathogens (8–10). In LCMV infection, the integrity of the macrophage system and its appropriate activation is of prime importance for efficient control of the pathogen (61). It is therefore possible that metabolic distress due to hypercholesterolemia may cause macrophage alterations and may inhibit Ag presentation leading to impaired induction of specific T cells. Furthermore, hypercholesterolemia may alter the microenvironment between APC and T cells leading to preferential Th2 differentiation (62) and may thereby impair generation of efficient antiviral CTL responses. Alternatively, but not mutually exclusive, cellular membrane characteristics may be altered in hypercholesterolemic mice, leading to changes in the functionality of membrane domains containing glycosphingolipids and cholesterol, called lipid rafts (63). In resting and activated T cells, membrane-protein interactions and TCR signaling critically depend on the integrity of cholesterol-containing lipid rafts (64). It is therefore possible that the observed reduced T cell reactivity in hypercholesterolemic mice is at least in part due to impaired TCR-associated signaling pathways.

In summary, using a well-characterized model of virus-induced immunopathology, we assessed the influence of genetically induced hypercholesterolemia on antiviral CTL responses. Hypercholesterolemia was found to substantially impair antiviral T cell immunity, causing exacerbation of potentially lethal immunopathologic disease.

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