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

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*J Immunol* 2001; 166:3369-3376; doi: 10.4049/jimmunol.166.5.3369

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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>-deficient (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 CTL-mediated liver cell damage in a dose-, age-, and MHC-dependent fashion (14, 20). Using MHC class I tetramers complexed with defined viral epitopes (22, 23), we followed 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.

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, Provin Klub, Kaisergarten, Switzerland), or high cholesterol diet (HCD, ND supplement with 1.25% cholesterol, 8% fat, Provin Klub). All animals were kept under specific pathogen-free conditions. Experiments were conducted with age-matched (6–8 wk) and sex-matched animals. The animal protection law of the Kanton of Zurich (Zurich, Switzerland) limits the number of mice to be used in experiments, particularly if disease is severe and potentially lethal. Therefore, experiments generally were repeated twice with groups of three to five mice.

**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-WE GP peptides KAVYNGAQAT (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>3</sup> M, 1.5 h at 37°C) and used in a standard 5-h 51Cr 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 (H-2<sup>d</sup>) tetramers complexed with GP33 or NP396 were produced as previously described (22). Briefly, H-2<sup>d</sup> and human β2-microglobulin molecules were recombinantly expressed in Escherichia coli (the plasmids were provided by John Altman, Emory University, Atlanta, GA). Biotinylated H-2<sup>d</sup> peptide complexes were purified using an Aekta Explorer 10 chromatography system (Pharmacia, Uppsala, Sweden) and tetramerized by addition of streptavidin-PE (Molecular Probes, Eugene, OR).

**Intracellular cytokine staining**

To determine the number of IFN-γ-producing cells, single-cell suspensions were prepared from liver or spleen by gently pressing the organs through a stainless steel grid. Liver cell suspensions were underlaid with Ficoll separating solution (Biochrom KG, Berlin, Germany) and spun at 2500 rpm for 20 min. The cells from the interface were washed twice and resuspended in PBS/0.2% FCS/0.5 mM EDTA (FACS buffer). Lymphocytes (10<sup>6</sup>) from spleen or liver were left untreated, stimulated with LCMV peptides (10<sup>−2</sup> M), or treated with PMA (10 ng/ml); Sigma, St. Louis, MO) and ionomycin (500 ng/ml; Sigma) for 6 h at 37°C. Brefeldin A (12.5 ng/ml; Sigma) was added for the duration of the culture to facilitate intracellular accumulation of the cytokine. Cells were first stained with PE-labeled anti-CD8 (PharMingen, San Diego, CA) at 4°C, followed by fixation with 4% paraformaldehyde in PBS for 20 min. Cells were permeabilized using saponin buffer (PBS/2% FCS, 5 mM EDTA, and 0.1% saponin) and stained with anti-IFN-γ-PE (PharMingen).

**Flow cytometry and tetramer staining**

On day 7 postinfection with 200 PFU of LCMV, single-cell suspensions were prepared from liver or spleen, and aliquots of 5 × 10<sup>5</sup> cells or three drops of blood were stained using 50 μl of a solution containing tetrameric class I-peptide complexes at 37°C for 10 min, followed by staining with anti-CD8-PE (PharMingen) at 4°C for 20 min. Erythrocytes in blood samples were lysed with FACS lysis solution (Becton Dickinson, Mountain View, CA), and cells were analyzed on a FACScan flow cytometer (Becton Dickinson) after gating on viable lymphocytes.

**Viral titers**

C57BL/6, ApoE<sup>−/−</sup>, or 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 (YTS169.4.2) (27) or rat anti-LCMV-VP 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 AS-BI phosphate/New Fuchsian. 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).

**Results**

Hypercholesterolemia exacerbates 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>3</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>3</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
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 by the virus, particularly in ApoE−/− mice (10), may mediate such pathological effects. To address this first possibility, serum TNF values were determined after infection with 2 × 10^5 PFU of LCMV in ApoE−/−, LDLR−/−, and C57BL/6 control mice fed either ND or HCD. In contrast to the enhanced TNF production in hypercholesterolemic mice after infection 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−/− 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).
levels on virus clearance from liver and spleen, ApoE

FIGURE 3.

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

<table>
<thead>
<tr>
<th>Mouse Strain</th>
<th>Diet</th>
<th>LCMV Titer (log_{10} PFU per gram organ)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 4</td>
</tr>
<tr>
<td>ApoE/−/−</td>
<td>ND</td>
<td>Spleen</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.9 ± 0.1</td>
</tr>
<tr>
<td>ApoE/−/−</td>
<td>HCD</td>
<td>7.3 ± 0.5</td>
</tr>
<tr>
<td>LDLR/−/−</td>
<td>ND</td>
<td>7.4 ± 0.1</td>
</tr>
<tr>
<td>LDLR/−/−</td>
<td>HCD</td>
<td>7.1 ± 0.2</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>ND</td>
<td>7.3 ± 0.6</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>HCD</td>
<td>7.2 ± 0.4</td>
</tr>
</tbody>
</table>

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.

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-D^b (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

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.
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 induction 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...
seemed little affected by the hypercholesterolemia, effects on T cell responsiveness in the induction and/or effector phase are suggested. It is possible that alterations in membrane cholesterol composition may influence T cell reactivity, as suggested by early in vitro studies by Cerottini and colleagues (49). Similar processes may also influence immune responses against noncytopathic and ubiquitous pathogens frequently associated with hypercholesterolemia. Indeed, Chlamydia pneumoniae is efficiently controlled in normocholesterolemic and immunocompetent C57BL/6 mice, but spreads more widely and infects newly formed atheromatous lesions in hypercholesterolemic ApoE−/− mice (50). Similar findings have been reported for LDLR−/− mice, where the pathogenic, atherosclerosis enhancing effect of C. pneumoniae became apparent only after nine mo on a high cholesterol diet (51). Furthermore, it is striking that CMV induces immunopathologic vascular disease exclusively in immunocompromised hosts; e.g. only irradiated mice (52) or rats (53) develop severe arterial inflammation after infection with murine or rat CMV, respectively. In addition, mice lacking the IFN-γ receptor are more susceptible to infection with murine CMV (54) or gammaherpesvirus 68 (55) and develop progressive chronic arterial inflammation. It is therefore likely that the frequent association of human CMV infection with atherosclerotic disease (56, 57) is due at least in part to immunosuppression. Long-lasting hypercholesterolemia might, as shown in this report, mediate such defects in cellular immunity and therefore favor development of immunopathologic disease. Importantly, infection with herpesviruses (52, 58) and acute LCMV infection, as shown here, can further alter cholesterol metabolism. Thus, self-perpetuating immunopathologic disease circuits may develop when chronic hypercholesterolemia-mediated immunosuppression impairs the usually well-balanced host-pathogen equilibrium.

It will be important to further elucidate the mechanisms underlying 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.

Acknowledgments
We thank Kathy McCoy for helpful discussions and critical reading of the manuscript, Lenka Vlk and Anne Henzelin for expert technical assistance, and Norbert Wey and Ida Schmieder for excellent photographic work.

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