INFECTION AND IMMUNITY, Aug. 2009, p. 3514–3515 Vol. 77, No. 8
0019-9567/09/$08.00+0 doi:10.1128/IAI.00469-09

Letter to the Editor
Is Hypercholesterolemia a Friend or a Foe of Tuberculosis?

Martens and coworkers' work (5) concerning the relationship between hypercholesterolemia and *Mycobacterium tuberculosis* pathogenesis is an important addition to the investigation involving the correlation between (apo)lipoproteins and infections.

In their report, the authors conclude that hypercholesterolemic ApoE knockout ($ApoE^{-/-}$) mice are highly susceptible to tuberculosis (TB) and that this susceptibility depends on the severity of hypercholesterolemia. However, it may be more reasonable for the authors to stress that ApoE deficiency itself, rather than hypercholesterolemia, is a critical risk factor for TB. Low-density lipoprotein (LDL) receptor-deficient ($LDLR^{-/-}$) mice were not tested in their experiment, and the authors did not observe any differences in the survival time between low- and high-cholesterol-diet-fed wild-type mice infected with *M. tuberculosis*. Additionally, it is well known that ApoE plays an important role in host defense and modulation of T-cell activation (3). It has also been repeatedly demonstrated that hypocholesterolemia but not hypercholesterolemia correlates with susceptibility to TB (2, 8, 11).

The authors imply that hypercholesterolemia can have detrimental effects on host defense in a mouse model. However, this is not always true. Hypercholesterolemia induced by either ApoE or LDLR deficiency renders mice more resistant to *Salmonella enterica* serovar Typhimurium infection than control mice (7). Moreover, despite both $LDLR^{-/-}$ and $ApoE^{-/-}$ mice being hypercholesterolemic, $LDLR^{-/-}$ mice, in comparison to control mice, are more resistant to *Klebsiella pneumoniae* infection, whereas $ApoE^{-/-}$ mice, in comparison to control mice, are more susceptible (1, 6). Importantly, much epidemiological and clinical evidence suggests that high cholesterol may be protective against respiratory and gastrointestinal infections (9).

In the Discussion section, the authors also suggest that diabetes increases susceptibility to TB, and roughly half of the diabetics in the United States have high cholesterol. However, previous work published by the same team shows that the initiation of adaptive immunity is impaired in mice with chronic hyperglycemia but not hypercholesterolemia, which results in a higher steady-state burden of *M. tuberculosis* in the lungs (4). Moreover, it has been reported that LDLR but not ApoE deficiency increases diet-induced obesity and diabetes in mice (10).

The interplay between infections and plasma lipoproteins may be highly complex, and it might be necessary to distinguish hypercholesterolemia induced by LDLR deficiency from that induced by ApoE deficiency.

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Authors' Reply

We appreciate Runlin Han's interest in our article "Hypercholesterolemia impairs immunity to tuberculosis" (2) and wholeheartedly agree with his concluding comment that the interplay between plasma lipoproteins and protective immunity against infectious diseases may be complex and merits further investigation. However, we take issue with some of Dr. Han's specific comments regarding the experiments we presented and the conclusions that may be drawn from them. His suggestion that apolipoprotein E (ApoE) deficiency alone might account for the increased TB susceptibility of $ApoE^{-/-}$ mice is not supported by our data. As we reported, $ApoE^{-/-}$ mice fed a low-cholesterol diet resulting in a serum total cholesterol (TC) level of ~300 mg/dl had only a modest ~0.5-$\log_{10}$-higher bacterial lung burden than wild-type mice and exhibited no overt signs of illness up to the 3-month-postinfection time point, when all mice in that study were euthanized. In contrast, $ApoE^{-/-}$ mice fed a high-cholesterol diet (TC, ~2,100 mg/dl) had a bacterial lung burden ~2 $\log_{10}$ higher than that of wild-type mice and 100% mortality 1 month postinfection. Similarly, dramatic differences in the lung leukocyte recruitment and histopathology were observed. Four weeks postinfection, $ApoE^{-/-}$ mice on the high-cholesterol diet had an average of 10 million lung leukocytes and giant abscess-like lesions, while $ApoE^{-/-}$ mice on the low-cholesterol diet had ~2 million lung leukocytes after 12 weeks of TB disease, and their lung histopathology was indistinguishable from that of simultaneously infected wild-type controls. ApoE

deficiency might have immunological consequences unrelated to cholesterol, but the profound TB susceptibility of ApoE$^{-/-}$ mice that we reported is clearly cholesterol dependent. The question of whether serum cholesterol influences TB susceptibility in wild-type mice remains open. In our study, the high-cholesterol chow was given for only 2 weeks prior to aerosol infection, and the TC of wild-type mice on this diet was one-third lower than that of ApoE$^{-/-}$ mice on the low-cholesterol diet. It remains to be tested whether chronic hypercholesterolemia is deleterious, and in this regard we note that ApoE$^{-/-}$ mice and high-cholesterol diets are commonly used experimentally to accelerate the development of atherosclerosis.

The idea of using low-density-lipoprotein-receptor-deficient (LDL-R$^{-/-}$) mice is a good one, and we have conducted a series of aerosol TB infection studies with LDL-R$^{-/-}$ mice since the publication of our article. Although ApoE$^{-/-}$ and LDL-R$^{-/-}$ mice both model hypercholesterolemia, they have very different distributions of lipoprotein. Very-low-density lipoprotein (VLDL) is particularly elevated in ApoE$^{-/-}$ mice, while LDL-R deficiency results in higher LDL cholesterol levels (5) with the potential for quite different biological consequences of elevated TC in these different mice. It would be ill advised to assume that ApoE$^{-/-}$ and LDL-R$^{-/-}$ mice will have similar TB susceptibility phenotypes. The question of whether LDL, VLDL, and high-density lipoprotein (HDL) cholesterols have unique effects on immunity is interesting and well worth studying. Our results so far indicate that LDL-R$^{-/-}$ mice share some features of TB susceptibility with ApoE$^{-/-}$ mice, but certain other aspects are distinctly different (unpublished data).

Dr. Han cites three papers as evidence that hypocholesterolemia correlates with susceptibility to TB (1, 4, 6), but these three interesting reports are not directly relevant to our study since none of them described investigations of hypercholesterolemia and one of them (Wilson et al. [6]) was a comment on cholesterol in trauma and sepsis patients having nothing to do with TB. The work of Deniz et al. (1) confirmed prior reports linking low TC with pulmonary TB, which is generally assumed to be a consequence rather than a cause of infection. Their work showed that low HDL and LDL cholesterol were associated with TB disease severity, but there was no correlation with VLDL, which raises the interesting question of whether VLDL might have an adverse impact on at least some parameters of immunity while other particles, such as HDL or LDL, might have an opposite effect. Finally, the work of Perez-Guzman et al. (4) demonstrates that a cholesterol-rich diet accelerates sterilization of TB lesions in patients with established disease who were also receiving antimicrobial therapy in comparison with patients randomized to receive a normal diet. Dietary cholesterol did not actually raise serum TC, which was comparably reduced in both patient groups enrolled in the study and increased modestly in both treatment groups during the first 2 weeks of treatment. The mechanism of the dietary cholesterol effect was not evaluated. We are not aware of any epidemiologic study that has specifically looked for an association between preexisting hypercholesterolemia and TB susceptibility.

Dr. Han misrepresents our work on TB and diabetes (3), suggesting that it showed an adverse effect of hyperglycemia but not hypercholesterolemia on TB defense. We did not measure TC or manipulate dietary cholesterol in those experiments, but we did raise in our discussion the question of whether hypercholesterolemia might accelerate the deleterious effect of hyperglycemia on TB defense, as it does for diabetic vascular disease. Whether hyperlipidemia contributes to the TB susceptibility in people with diabetes is unknown and is a question we plan to investigate with animal models and in clinical studies. Regardless of its ultimate connection with human susceptibility, our published study and the model we have developed may provide unique insights into TB pathogenesis and should contribute to understanding the influence of common metabolic disorders on immunity.

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Editor: J. L. Flynn