Macrophages have the right tools to synthesize all the free cholesterol they need. If macrophages also could degrade cholesterol when they have too much, the concept of in vivo reverse cholesterol transport (RCT) would be irrelevant. Fortunately for those of us who do research in this area, the ability of macrophages to closely regulate their cellular cholesterol levels is accomplished not by cellular degradation, but by multiple complex cholesterol sensing regulatory pathways including enzyme systems for synthesizing it, esterifying it, and actively transporting it out of the cell. RTC can be defined as the transport of cholesterol from peripheral cells such as macrophages to circulating plasma lipoproteins that subsequently deliver the cholesterol to the liver for biliary excretion.1 Active transport of cholesterol out of cells to lipid-poor or lipid-free apolipoprotein AI (apoAI) is accomplished by the LXR-inducible ATP binding cassette transporter, ABCA1.2 This transporter exports both cellular cholesterol and phospholipid. Whereas ABCA1 exports cholesterol and phospholipid to lipid-free apolipoproteins, another more recently described LXR-inducible transporter, ABCG1 actively exports cellular cholesterol to high density lipoproteins (HDL) and other extracellular phospholipid-containing acceptors.3 Recently, it has been suggested that ABCA1 and ABCG1 work in concert to export cellular phospholipid and cholesterol. ABCA1 generates extracellular nascent or discoidal HDL, which then becomes an acceptor for ABCG1-mediated additional cholesterol efflux.4 Although this idea is logically sound, the relevant contributions of ABCA1 and ABCG1 to macrophage cholesterol export are still not completely understood.

Irradiation and bone marrow transplantation eliminates all dividing cells in the body including rapidly dividing bone marrow cells.5 Reconstitution of the irradiated mice is accomplished by i.v. injection of bone marrow cells extracted from nonirradiated donor mice. After reconstitution, successful replacement of bone marrow cells is documented, and the animals are fed a high fat diet to increase plasma cholesterol levels. Macrophage participation is revealed because monocyte-derived macrophages are the major bone marrow–derived cell to enter the intima, accumulate cholesterol, secrete chemokines and cytokines, and thereby contribute to formation of early fatty streak lesions.

Ruud Out and colleagues in Leiden, The Netherlands,6 report that a macrophage ABCG1 deficiency leads to a moderate increase in atherosclerosis in hyperlipidemic LDLr−/− mice. However, 2 additional laboratories including Angel Baldan and colleagues from UCLA7 and Mollie Ranalletta and colleagues from Columbia University8 independently observe that ABCG1-deficient bone marrow–derived cells in LDLr−/− mice leads to decreased atherosclerosis. What can account for such disparate results? Perhaps a simple explanation will be found. It is also possible that a complex polygenic disease like atherosclerosis requires more sophisticated experimental analyses than those we are currently performing. To begin to reconcile these differences, one needs to understand what factors influence lesion development in LDLr−/− mice that have undergone lethal total body irradiation and bone marrow reconstitution. Multiple factors certainly play a role, including the degree of hypercholesterolemia achieved by the consumption of proatherogenic diets, genetic background strain differences, and sex differences.9 Other differences include the amount of stress or environment the various animals experience as well as the time of recovery from irradiation. Each of these factors could play a role in the outcomes reported by different laboratories.

A Brief Comparison of the Three Studies Could Shed Light on This Conundrum

The Table highlights key experimental details of the studies. The Out et al study,6 which reports an increased disease severity in ABCG1-deficient macrophage bone marrow chimeras, used donors obtained from Deltagen, Inc (San Carlos, Calif). Unfortunately, neither the genetic background nor the sex of these donor mice is indicated. Male donor mice from Deltagen are also used in the Ranalletta et al study8 and the Baldan et al study.7 These Deltagen donor mice were backcrossed at least 5 times to a C57BL/6 background. These latter 2 studies also report that macrophage ABCG1 deficiency gives rise to a less severe disease phenotype. After irradiation, all LDLr−/− recipient mice are fed chow diets for 4 to 8 weeks. Plasma cholesterol levels of all the mice while they were consuming the chow diet are comparable,
true rates of disease progression are probably not linear, so severity at a single anatomic site at 2 points in time. However, whereas they differ after the recipients are fed the atherogenic lipidemia and the genetic background differences. Yet an important problem associated with comparisons of murine atherosclerosis studies is that atherosclerosis can progress at different rates even at the same anatomic site. To clarify, let’s assume that the hatched line in the Figure represents lesion areas in LDLr−/− chimeras that receive ABCG1−/− bone marrow and the solid line represents control chimeras that receive wild-type bone marrow. If atherosclerosis is quantified at time A, one would conclude that macrophage ABCG1−/− deficiency is proatherogenic. However, if atherosclerosis is measured at time B, no differences would be observed. If disease is quantified at time C, major differences would be observed, and one would conclude that LDLr−/− chimeras that receive ABCG1−/− bone marrow have less disease. Unfortunately, most atherosclerosis studies involve measurements of disease burden at only a single point in time. To their benefit, the studies by Out et al and Ranalletta et al involve measurements of disease severity at a single anatomic site at 2 points in time. However, true rates of disease progression are probably not linear, so even 2 points are insufficient to measure a true rate. If the effect on atherosclerosis of a single mutation such as ABCG1-deficiency in macrophages is to be understood, the extended course of disease initiation and progression must be known. Current methods of assessing lesion burden make such analyses technically arduous and very expensive. Thus, new methodologies will be needed in mouse models of atherosclerosis to permit accurate assessments of the rates of disease progression and macrophage infiltration at a single anatomic site.

Equally important, disease burden also needs to be measured at multiple sites. Disease can progress at different rates at different anatomic sites. Assessments of disease burden at 2 different sites need not always lead to the same conclu-

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<tr>
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<tbody>
<tr>
<td>Donor mice</td>
<td>Deltagen ABCG1−/− bkg.</td>
<td>At least 5 backcrosses to BL/6</td>
<td>Deltagen ABCG1−/−, 5 backcrosses to BL/6</td>
</tr>
<tr>
<td>Recipients</td>
<td>LDLr−/− BL/6</td>
<td>LDLr−/− BL/6 and apoE−/− BL/6</td>
<td>LDLr−/− BL/6</td>
</tr>
<tr>
<td>BMT recovery</td>
<td>8 wks</td>
<td>4 wks</td>
<td>5 wks</td>
</tr>
<tr>
<td>Plasma chol (chow)</td>
<td>230–270 mg/dl</td>
<td>N/A</td>
<td>167–205 mg/dl</td>
</tr>
<tr>
<td>HFD</td>
<td>15% fat, 0.25% chol</td>
<td>21% fat, 1.25% chol</td>
<td>21.2% fat, 0.2% chol</td>
</tr>
<tr>
<td>Plasma chol (HFD)</td>
<td>511–676 mg/dl at 6 wks</td>
<td>&gt; 1,000 mg/dl at 16 wks</td>
<td>829–911 mg/dl at 7 wks</td>
</tr>
<tr>
<td>Heart sinus lesion</td>
<td>50 000 in +/+ vs 500,000 in +/+ vs</td>
<td>84 000 in +/+ vs</td>
<td>1172–1182 mg/dl at 11 wks</td>
</tr>
<tr>
<td>Area (µm²)</td>
<td>70,000 in −/− at 6 wks, 300,000 in −/− at 7 wks, 125 000 in +/+ vs</td>
<td>78 000 in −/− at 7 wks, 238 000 in +/+ vs</td>
<td></td>
</tr>
<tr>
<td>En face lesion area (%)</td>
<td>ND</td>
<td>13% in +/+ vs 9% in −/−</td>
<td>ND</td>
</tr>
</tbody>
</table>

Abbreviations: HFD, high fat diet; BL/6, C57BL/6; N/A, not available; +/+, ABCG1+/+; −/−, ABCG1−/−; ND, not done; chol, cholesterol.
sion.5,11 Two of the studies provided only information on lesion burden within the aortic root of the heart and did not take into consideration other anatomic sites which could provide additional information regarding the extent of disease. In contrast, Baldan et al7 also measured atherosclerosis in the en face aortas.

Because different results are obtained in these studies, different mechanistic explanations for the outcomes are provided by each group. Because they observed that the absence of ABCG1 in macrophages results in increased disease, Out et al6 logically conclude that the increased disease severity is likely a direct effect of impaired cholesterol efflux in the lesion macrophages. For the other studies, which found that macrophage ABCG1 deficiency results in decreased disease, alternate explanations are necessary. Baldan et al7 suggest that increased apoptosis resulting from increased free cholesterol in the lesion macrophages leads to smaller lesion areas. Direct evidence for an increased susceptibility of ABCG1-deficient macrophages to oxidation-induced apoptosis is indeed demonstrated by this group. A second explanation is provided in the Ranalletta et al report.8 These researchers suggest that an increase in apoE production could be responsible for the decreased atherosclerosis in the ABCG1 defective bone marrow chimeras. Because apoE protects against atherosclerosis, support for this finding is provided by documenting an increase in serum levels of apoE in the bone marrow chimeras that received ABCA1-deficient bone marrow. Interestingly, the Baldan group7 also performs bone marrow transplantations of apoE-deficient animals with ABCG1-deficient bone marrow. Both the ABCG1−/− and wild-type donor macrophages express apoE in the LDLR−/− recipients, which very efficiently reduces plasma cholesterol levels even when the mice are consuming a high fat diet. Nevertheless, a deficiency of macrophage ABCG1 in apoE−/− mice also results in less severe disease. This suggests that apoE production may not be the only factor to account for the observed decreased atherosclerosis in the ABCG1-deficient bone marrow chimeras. Unfortunately, the Baldan studies7 do not report differences in plasma apoE, and the Ranalletta studies8 do not report on apoptosis of lesion macrophages. Additional study of apoE-deficient mice reconstituted with bone marrow from double mutant ABCG1- and apoE-deficient mice will be needed to resolve this issue.

We will not stop studying murine atherosclerosis in the near future, and we will not stop using bone marrow transplantation to reveal the effect of macrophage-expressed genes. Therefore, accurate assessments of “rates” of disease initiation and progression at multiple anatomic sites will be our next real technical challenge.

Disclosures

None.

References

Is Two out of Three Enough for ABCG1?
Linda K. Curtiss

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