Genes Within the MHC Region Have a Dramatic Influence on Radiation-Enhanced Atherosclerosis in Mice

Weibin Shi, MD, PhD; Zhimin Zhang, MD; Mei-Hua Chen; John F. Angle, MD; Alan H. Matsumoto, MD

Background—C3H/HeJ (C3H) mice develop much smaller atherosclerotic lesions than C57BL/6 (B6) mice when deficient in apolipoprotein E (apoE−/−) or fed an atherogenic diet. The 2 strains differ in H2 haplotypes, with B6 having H2b and C3H having H2k. C3.SW-H2b/SnJ (C3.SW) is a congenic strain of C3H/HeJ in which H2k is replaced with H2b.

Methods and Results—We performed bone marrow transplantation and found that atherosclerosis-resistant C3.SW.apoE−/− mice reconstituted with bone marrow from either C3.SW.apoE−/− or B6.apoE−/− mice after lethal irradiation had significantly larger atherosclerotic lesions than B6.apoE−/− mice receiving identical treatments and much larger lesions than C3H.apoE−/− mice reconstituted with syngeneic bone marrow. For syngeneic transplantation, C3.SW.apoE−/− mice exhibited a 21-fold increase in lesion size over C3H.apoE−/− mice (152 800±21 937 versus 7060±2290 μm²/section) and a near 4-fold increase over B6.apoE−/− mice (40 529±4675 μm²/section). C3.SW.apoE−/− mice reconstituted with syngeneic bone marrow exhibited enhanced lesion formation relative to those reconstituted with B6 marrow (152 800±21 937 versus 107 000±9374 μm²/section; P=0.067). Sublethal irradiation led to a 6-fold increase of lesion size in C3.SW.apoE−/− mice (9795±2804 versus 1550±607 μm²/section; P=0.008). Wild-type C3.SW mice reconstituted with apoE+/+ or apoE−/− bone marrow had significantly larger atherosclerotic lesions than C3H mice receiving identical treatments on an atherogenic diet.

Conclusions—These results indicate that gene(s) within the H2 region have a dramatic impact on radiation-enhanced atherosclerosis, and their effect is conveyed partially through bone marrow–derived cells. (Circ Cardiovasc Genet. 2010;3:409-413.)

Key Words: atherosclerosis ■ radiation ■ major histocompatibility complex ■ H2 haplotype ■ mice

Radiotherapy is the most commonly used nonsurgical modality for the treatment of malignant tumors, and more than half of long-term cancer survivors have had such treatment.1 Radiotherapy has also been used to treat proliferating, nonmalignant diseases, such as severe hyperthyroidism, skeletal degenerative diseases, pigmented villonodular synovitis, keloid scar growth, and heterotopic ossification.2 However, patients receiving radiotherapy have an increased risk of developing atherosclerotic cardiovascular disease.3 There is accumulating evidence supporting radiation as a risk factor for atherosclerosis independent of such risk factors as hyperlipidemia, age, diabetes, hypertension, smoking, and lack of exercise. In humans, irradiation of the carotid arteries leads to atherosclerosis or accelerates progression of atherosclerosis.4–6 Experimental animal studies also show that irradiation increases atherosclerotic lesion size of high-fat fed C57BL/6 (B6) mice7 and apolipoprotein E–deficient (apoE−/−) mice8,9 and promotes monocyte transmigration into the subendothelial space and transformation into foam cells in hypercholesterolemic rabbits.10 However, it is not yet understood how radiation exacerbates the atherosclerotic condition.

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Mouse strains B6 and C3H/HeJ (C3H) exhibit marked differences in their susceptibility to atherosclerosis. C3H mice develop much smaller atherosclerotic lesions than B6 mice when fed an atherogenic diet or deficient in apolipoprotein E (apoE−/−).11–13 Atherogenesis is a complicated process involving interactions among arterial wall cells, bone marrow–derived blood cells, and lipids. A recent study by our group defined the arterial wall as a major source of the difference in atherosclerosis susceptibility because B6 aortic grafts developed significantly larger atherosclerotic lesions than C3H grafts when Anastomosed between divided infrarenal aorta of identical F1 mice derived from the 2 strains.14 However, because the 2 parental strains exhibited much larger differences in atherosclerotic lesion sizes in nontransplanted aortas than in transplanted aortas, other components may also contribute. In the present study, bone marrow transplantation was undertaken to define the contribution of bone marrow–derived cells to atherosclerosis susceptibility. The findings excluded a significant role in this regard but

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revealed a huge impact of genes within the H2 region on the progression of radiation-enhanced atherosclerosis.

**Materials and Methods**

**Mice**

C3H/HeJ (C3H), C3.SW-H2b/SnJ (C3.SW), and B6.apoE<sup>−/−</sup> mice were purchased from the Jackson Laboratory, Bar Harbor, Maine. C3H.apoE<sup>−/−</sup> mice and C3.SW.apoE<sup>−/−</sup> mice were generated in our laboratory. The creation of C3H.apoE<sup>−/−</sup> mice was previously reported.15 C3.SW.apoE<sup>−/−</sup> mice were generated by initially crossing female C3H.apoE<sup>−/−</sup> mice with male C3.SW mice, followed by 3 generations of sequential back-cross mating to C3.SW mice and 1 generation of intercross mating with selection for the H2<sup>b</sup> and apoE null alleles. The presence of the H2<sup>b</sup> allele was determined by PCR using microsatellite markers D17Mit147 (36.35 Mb), D17Mit125 (36.63 Mb), and D17Mit148 (37.47 Mb), as reported by Xie et al.16 All procedures were carried out in accordance with current National Institutes of Health guidelines and approved by our Institutional Animal Care and Use Committee.

**Irradiation and Bone Marrow Transplantation**

Mice were irradiated from a 137Cs source (JL Shepherd Mark I 137Cs Irradiator) with a single dose of 0, 600, or 1100 rads. The bone marrow transplantation procedure was performed as we previously described.17 In brief, 5-week-old female recipient mice were lethally irradiated with a single dose of 1100 rads and then reconstituted with 10<sup>7</sup> bone marrow cells harvested from the femurs and tibias of donor male mice. To evaluate the effect of radiation alone on atherosclerosis, 5-week-old female C3.SW.apoE<sup>−/−</sup> mice were irradiated with a sublethal dose of 600 rads. ApoE<sup>−/−</sup> mice were fed a rodent chow diet throughout the entire experiment period. Wild-type mice were switched from a chow to an atherogenic diet containing 15% fat, 1.25% cholesterol, and 0.5% cholic acid (TD 90221, Harlan Teklad, Inc.) 4 weeks after transplantation and fed the diet for 12 weeks, as previously reported.13,17–19

**Plasma Lipid Measurements**

Mice were fasted overnight before blood was collected through retroorbital puncture under isoflurane inhalation. Plasma total cholesterol, HDL cholesterol, and triglyceride levels were measured with enzymatic assays as we previously described.20

**Aortic Lesion Analysis**

The method for quantification of atherosclerotic lesions in the aorta was as we previously reported.21 The lesion areas of 5 sections with the largest readings were averaged for each mouse and this average was used for statistical analysis.

**Statistical Analysis**

Data are expressed as means±SEM, with “n” indicating the number of mice. The Student t test was used to test for differences between 2 groups in lesion size or plasma lipid levels. All t tests were performed under the assumption of unequal variances. Differences were considered statistically significant at P<0.05.

**Results**

We previously reported marked differences between B6.apoE<sup>−/−</sup> and C3H.apoE<sup>−/−</sup> mice in atherosclerotic lesion formation when fed a chow and Western diet.13 To determine whether cells of hematopoietic origin were a source of the difference in atherosclerosis susceptibility, we constructed C3.SW.apoE<sup>−/−</sup> mice, which carry the same H2<sup>b</sup> haplotype as B6.apoE<sup>−/−</sup> mice, and performed reciprocal bone marrow transplantation between the 2 strains. After transplantation, mice continued on the chow diet. Atherosclerotic lesions in the aortic root were measured 16 weeks after transplantation. Unexpectedly, lethally irradiated C3.SW.apoE<sup>−/−</sup> mice reconstituted with bone marrow from either B6.apoE<sup>−/−</sup> or C3.SW.apoE<sup>−/−</sup> mice had significantly larger atherosclerotic lesions than B6.apoE<sup>−/−</sup> recipients reconstituted with bone marrow from the same donors (P<0.0001; Figure 1). For syngeneic marrow transplant recipients, C3.SW.apoE<sup>−/−</sup> mice had a lesion size of 152,800±21 937 μm²/section (n=20), which was nearly 4-fold larger than the lesion size of 40 529±4675 μm²/section in B6.apoE<sup>−/−</sup> mice (n=14; P=0.00059). C3.SW.apoE<sup>−/−</sup> mice reconstituted with syngeneic bone marrow exhibited a 50% increase in lesion size over those reconstituted with bone marrow from B6.apoE<sup>−/−</sup> recipients reconstituted with bone marrow from C3.SW.apoE<sup>−/−</sup> mice (152 800±21 937 versus 107 000±9374 μm²/section; n=20 and 23, respectively), although the difference did not reach statistical significance (P=0.067). In contrast, B6.apoE<sup>−/−</sup> mice reconstituted with bone marrow from C3.SW.apoE<sup>−/−</sup> mice had a lesion size comparable to those reconstituted with syngeneic bone marrow (42 188±5378 versus 40 529±4675 μm²/section; n=16 and 14, respectively; P=0.82).

C3.SW.apoE<sup>−/−</sup> mice had a significantly higher total cholesterol level than B6.apoE<sup>−/−</sup> mice (404.2±9.0 versus 352.0±23.2 mg/dL; P=0.009), although their HDL cholesterol (28.7±2.5 mg/dL; P=0.38) were comparable (Figure 2). The higher non-HDL

![Figure 1. Atherosclerotic lesions in the aortic root of female B6.apoE<sup>−/−</sup> and C3.SW.apoE<sup>−/−</sup> mice transplanted after lethal irradiation with donor bone marrow from either B6.apoE<sup>−/−</sup> or C3.SW.apoE<sup>−/−</sup> mice. Four groups of experiments were performed: C3.SW.apoE<sup>−/−</sup>→B6.apoE<sup>−/−</sup> (n=16), B6.apoE<sup>−/−</sup>→B6.apoE<sup>−/−</sup> (n=14), B6.apoE<sup>−/−</sup>→C3.SW.apoE<sup>−/−</sup> (n=23), and C3.SW.apoE<sup>−/−</sup>→C3.SW.apoE<sup>−/−</sup> (n=20). After transplantation, mice were fed a chow diet for 16 weeks. Values are expressed as means±SEM. P<0.05 versus B6.apoE<sup>−/−</sup> recipients.](http://circgenetics.ahajournals.org/doi/10.1161/CIRCGENETICS.110.000067)

![Figure 2. Plasma cholesterol and triglyceride levels in female B6.apoE<sup>−/−</sup> (n=14) and C3.SW.apoE<sup>−/−</sup> mice (n=20) reconstituted with syngeneic bone marrow. Blood was collected from overnight-fasted mice at 16 weeks after transplantation. Values are means±SEM. P<0.05 versus B6.apoE<sup>−/−</sup> mice.](http://circgenetics.ahajournals.org/doi/10.1161/CIRCGENETICS.110.000067)
cholesterol level could be partially responsible for the increased lesion formation in C3.SW.aopoE<sup>−/−</sup> mice.

When compared with C3H.aopoE<sup>−/−</sup> recipients (n=12) reconstituted with syngeneic bone marrow, C3.SW.aopoE<sup>−/−</sup>→C3.SW.aopoE<sup>−/−</sup> mice exhibited a 21-fold increase in lesion size (152 800 ± 21 937 versus 7060 ± 2290 μm<sup>2</sup>/section; n=5 and 9, respectively; P=0.017) (Figure 4). However, when compared with those C3.SW.aopoE<sup>−/−</sup> mice lethally irradiated and reconstituted with syngeneic bone marrow, these mice had much smaller aortic lesions (9795 ± 2804 versus 1558 ± 607 μm<sup>2</sup>/section; n=9 and 15, respectively; P=0.50). When compared with C3H.aopoE<sup>−/−</sup>→C3H.aopoE<sup>−/−</sup> mice (1422 ± 242 μm<sup>2</sup>/section, n=15), C3.SW.aopoE<sup>−/−</sup>→C3.SW.aopoE<sup>−/−</sup> mice exhibited a 5-fold increase in aortic lesion size. C3H.SW.aopoE<sup>−/−</sup>→C3H.SW.aopoE<sup>−/−</sup> mice exhibited a 6-fold increase in lesion size compared with C3H.aapoE<sup>−/−</sup>→C3H.aapoE<sup>−/−</sup> mice (1537 ± 383 μm<sup>2</sup>/section, n=14).

Wild-type C3.SW mice were totally resistant to atherosclerosis, with no atherosclerotic lesions in the aortic root after 12 weeks on the atherogenic diet containing 15% fat, 1.25% cholesterol, and 0.5% sodium cholate (Figure 6). In contrast, lethally irradiated C3.SW mice reconstituted with bone marrow from either C3.SW or C3.SW.aopoE<sup>−/−</sup> mice had atherosclerotic lesions on the atherogenic diet. The difference in lesion size between mice reconstituted with apoE<sup>−/−</sup> bone marrow and those reconstituted with apoE<sup>−/−</sup> bone marrow was not statistically significant (9183 ± 2603 versus 7033 ± 1717 μm<sup>2</sup>/section; n=9 and 15, respectively; P=0.95). When compared with C3H.aapoE<sup>−/−</sup>→C3H.aapoE<sup>−/−</sup> mice (1422 ± 242 μm<sup>2</sup>/section, n=15), C3.SW.aapoE<sup>−/−</sup>→C3.SW.aapoE<sup>−/−</sup> mice exhibited a 5-fold increase in aortic lesion size. C3H.SW.aapoE<sup>−/−</sup>→C3H.SW.aapoE<sup>−/−</sup> mice exhibited a 6-fold increase in lesion size compared with C3H.aapoE<sup>−/−</sup>→C3H.aapoE<sup>−/−</sup> mice (1537 ± 383 μm<sup>2</sup>/section, n=14).

Figure 3. Comparison between female C3.SW.aopoE<sup>−/−</sup> (n=20) and C3H.aapoE<sup>−/−</sup> mice (n=12) reconstituted with syngeneic bone marrow in atherosclerotic lesion size at 16 weeks after transplantation. Values are expressed as means ± SEM. *P<0.05 versus C3H.aapoE<sup>−/−</sup> mice.

Figure 4. Atherosclerotic lesion size in the aortic root of female C3.SW.aapoE<sup>−/−</sup> mice with (n=10) or without irradiation (n=5). Mice were irradiated with a single dose of 0 or 600 rads. After irradiation, mice were fed the chow diet for 16 weeks. Values are means ± SEM. *P<0.05 versus nonirradiated mice.

Figure 5. Plasma cholesterol and triglyceride levels in female C3.SW.aapoE<sup>−/−</sup> mice with (n=9) or without irradiation (n=10). Values are means ± SEM. Radiation with a single dose of 600 rads had no significant influence on plasma lipid levels of the mice.

Figure 6. Atherosclerotic lesion size in the aortic root of wild-type female C3.SW or C3H mice. Mice were lethally irradiated and transplanted with bone marrow from apoE<sup>−/−</sup> or apoE<sup>−/−</sup> mice. Four weeks after transplantation, mice were started on an atherogenic diet and maintained on the diet for 12 weeks. One group of C3.SW mice did not receive irradiation and transplantation but were challenged with the atherogenic diet. Values are means ± SEM. *P<0.05 versus C3H mice. C3.SW (n=9); wild-type C3.SW mice receiving no irradiation and no transplantation; C3.SW→C3S.W (n=9); lethally irradiated wild-type C3.SW mice were reconstituted with bone marrow from wild-type C3.SW mice; C3.aapoE<sup>−/−</sup>→C3.SW (n=15); lethally irradiated wild-type C3.SW mice reconstituted with bone marrow from C3.SW.aapoE<sup>−/−</sup> mice; C3H→C3H (n=15); lethally irradiated wild-type C3H/HeJ mice reconstituted with bone marrow from wild-type C3H/HeJ mice; C3H.aapoE<sup>−/−</sup>→C3H (n=14): lethally irradiated wild-type C3H/HeJ mice were reconstituted with marrow from C3H.aapoE<sup>−/−</sup> mice.
Discussion

Although radiation is known to accelerate the development of atherosclerosis, the influence of genetic factors on this effect has not been reported. This study has shown clearly that the H2b haplotype is a major genetic determinant of radiation-enhanced atherosclerosis in C3.SW mice.

We previously demonstrated that the arterial wall was a major source of the marked difference between B6.apoE<sup>−/−</sup> and C3H.apoE<sup>−/−</sup> mice in atherosclerosis susceptibility, and now we have evaluated the role played by bone marrow–derived cells through bone marrow transplantation. Monocyte-derived foam cells are the principal cellular component of atherosclerotic lesions, and these cells also produce cytokines and growth factors that may affect the development and progression of atherosclerosis. Because the H2 haplotype of C3H.apoE<sup>−/−</sup> mice is H2<sup>b</sup> and that of B6.apoE<sup>−/−</sup> mice is H2<sup>h</sup>, we constructed C3.SW.apoE<sup>−/−</sup> mice, which have the same H2<sup>h</sup> haplotype as B6.apoE<sup>−/−</sup> mice, for transplantation. C3.SW.apoE<sup>−/−</sup> and C3H.apoE<sup>−/−</sup> mice are genetically identical except for the H2 region. The present finding that B6.apoE<sup>−/−</sup> mice reconstituted with bone marrow of C3.SW.apoE<sup>−/−</sup> mice had similar lesion size as those reconstituted with syngeneic bone marrow indicates that macrophages or other marrow–derived cells contribute little to the difference in atherosclerosis susceptibility. Nevertheless, the finding that C3.SW.apoE<sup>−/−</sup> mice reconstituted with bone marrow of B6.apoE<sup>−/−</sup> mice had smaller aortic lesions than those reconstituted with syngeneic bone marrow suggests that B6 marrow–derived cells actually protect against atherosclerosis.

The huge impact of H2<sup>b</sup> haplotype on the development of atherosclerosis in C3.SW.apoE<sup>−/−</sup> mice after exposure to radiation was not expected. H2, which was named for its role in histocompatibility more than 60 years ago by Snell, is orthogonal with the human HLA (human leukocyte antigen) or MHC. The MHC region is located on chromosome 17 in the mouse and on the short arm of chromosome 6, 6p21.3 in humans. This region has been shown to be linked to radiation-induced pulmonary fibrosis in the mouse. Because pulmonary fibrosis and atherosclerosis are chronic inflammatory diseases, it is highly likely that both diseases are affected by the same genes causing cytokine–driven inflammation and tissue destruction.

C3.SW.apoE<sup>−/−</sup> mice reconstituted with bone marrow from either strain developed much larger atherosclerotic lesions than B6.apoE<sup>−/−</sup> counterparts despite the fact that they share the same H2<sup>b</sup> haplotype. The reason for this is unknown. Previous studies of F<sub>2</sub> mice from B6.apoE<sup>−/−</sup> and C3H.apoE<sup>−/−</sup> mice showed that multiple loci contribute to atherosclerotic lesion formation and alleles derived from C3H may contribute to the susceptibility to atherosclerosis, whereas alleles derived from B6 may contribute to the resistance to atherosclerosis. Thus, it is likely that genes outside the H2 region protect against radiation-enhanced atherosclerosis in B6.apoE<sup>−/−</sup> mice. The present observation that C3.SW.apoE<sup>−/−</sup> mice reconstituted with B6 bone marrow had smaller atherosclerotic lesions than those reconstituted with C3.SW bone marrow supports this speculation. In addition, C3.SW.apoE<sup>−/−</sup> mice had higher non–HDL cholesterol levels than B6.apoE<sup>−/−</sup> mice, which could be partially responsible for the increased lesion formation.

Sublethal irradiation alone resulted in accelerated formation of atherosclerotic lesions in C3.SW.apoE<sup>−/−</sup> mice without leading to significant alterations of plasma lipid levels. However, the effect was much smaller when lesion size was compared with that of mice that had lethal irradiation and bone marrow transplantation. This discrepant result may reflect the dose-dependent effect of radiation on atherosclerosis rather than an effect inherited from the transplantation.

The present finding that wild-type C3.SW mice were totally resistant to diet-induced atherosclerosis is in agreement with previous observations made in C3H/HeJ mice. However, C3.SW and C3H/HeJ mice that received lethal irradiation and bone marrow transplantation had development of atherosclerosis on the high-fat diet. This result is consistent with previous conclusions that irradiation results in accelerated atherosclerosis in wild-type B6 mice or rabbits fed an atherogenic diet. When compared with C3H/HeJ mice receiving syngeneic marrow transplantation, C3.SW mice displayed 5- to 6-fold increases in atherosclerotic lesion size, indicating that genes within the H2 region also promote diet-induced atherosclerosis after irradiation.

ApoE deficiency in bone marrow–derived cells has been shown to markedly increase susceptibility of B6 mice to atherosclerosis in the absence of a significant influence on plasma lipid levels. In contrast, we found that reconstitution with apoE<sup>−/−</sup> bone marrow had little influence on the susceptibility of C3.SW or C3H/HeJ mice to atherosclerosis. This finding is consistent with our previous observation of C3H/HeJ mice. It is also highly unlikely that the absence of apoE in bone marrow–derived cells was responsible for radiation-enhanced atherosclerosis in C3.SW.apoE<sup>−/−</sup> mice.

In summary, we have demonstrated for the first time that the MHC is a major genetic determinant of susceptibility to radiation-induced atherosclerosis in mice. Because the MHC is one of the most clearly defined genetic determinants affecting almost every autoimmune disease and some inflammatory diseases, further investigation of susceptibility genes with this radiation hypersensitivity model may uncover critical common pathways shared by these conditions.

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Disclosures

None.

References

In this investigation, we report an unexpected finding that genes within the major histocompatibility complex (MHC) have a dramatic influence on radiation-enhanced atherosclerosis in mice. The mouse strain C3H/HeJ (C3H) is extremely resistant to atherosclerosis, with development of much smaller lesions than the strain C57BL/6 (B6) when the mouse is deficient in apolipoprotein E (apoE−/−) or fed an atherogenic diet. The 2 inbred strains differ in the MHC haplotype, with C57BL/6 having H2b and C3H having H2k. C3.SW is a congenic strain of C3H/HeJ in which the H2k haplotype is replaced with H2b. The objective of this study was to determine whether the resistance of C3H to radiation-enhanced atherosclerosis is genetically determined and to identify the genes involved.

To address this question, we performed experiments in which patients are at risk for radiation-induced complications could facilitate the development of patient-specific treatment regimens toward maximizing therapeutic efficacy while minimizing the incidence of side effects.
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