Neutrophil infiltration in radiation-induced cardiovascular inflammation

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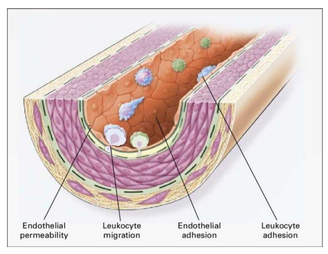
Abstract

Atherosclerosis is aggravated by cardiovascular risk factors that affect endothelial dysfunction and the migration of leukocytes, including the over accumulation of lipids and fluctuations in cytokine levels. To study the infiltration of leukocytes in radiation-aggravated atherosclerosis, we tested Ldlr-/- mice and C57BL/6j mice after exposure to 0.5 or 1 Gy radiation over 16 weeks. We found that radiation exposure induced atherosclerosis development in Ldlr-/- mice, as demonstrated by increased lipid-laden plaque size, reactive oxygen species levels, and levels of the pro-inflammatory cytokines. Total plasma cholesterol, triglyceride, and LDL cholesterol levels were also increased by radiation exposure, along with cardiovascular risk. We also showed dose-dependent increases in neutrophils and monocytes that coincided with a reduction in lymphocytes in the spleens of Ldlr-/- mice. We concluded that chronic radiation exposure increased the production of pro-inflammatory mediators, which was associated with the migration of neutrophils and inflammatory monocytes into sites of atherosclerosis. Therefore, our data suggest that the accumulation of neutrophils and inflammatory monocytes with the reduction of lymphocytes, contribute to aggravated atherosclerosis in Ldlr-/- mice under prolonged exposure to radiation.

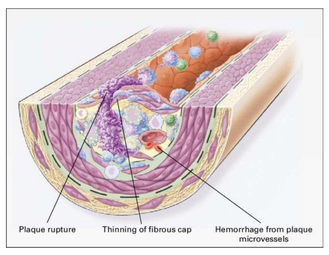
Background

Atherosclerosis is aggravated by cardiovascular risk factors that affect endothelial dysfunction and the migration of leukocytes, including the over-accumulation of lipids and fluctuations in cytokine levels. Radiation exposure can increase the risk of the development and progression of atherosclerosis. Radiation-induced vascular damage can enhance immune cell infiltration into tissues, which is accompanied by imbalanced production of pro- and anti-inflammatory cytokines, increased levels of reactive oxygen species (ROS), and dysregulation of lipid metabolic pathways (Wei et al. 2019). Prolonged exposure to radiation was found to be associated with various markers of inflammation, such as elevated leukocyte counts in the blood. We explored the effect of prolonged radiation exposure on atherosclerosis development and changes in leukocytes in wild-type C57BL/6j and LDL receptor-deficient (Ldlr-/-) mice.



Endothelial Dysfunction in Atherosclerosis. The earliest changes that precede the formation of lesions of atherosclerosis take place in the endothelium.

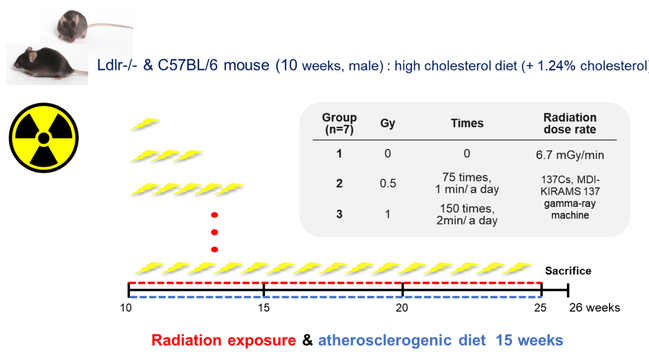
Current Pharmaceutical Design 2012, 18(28):4266-88



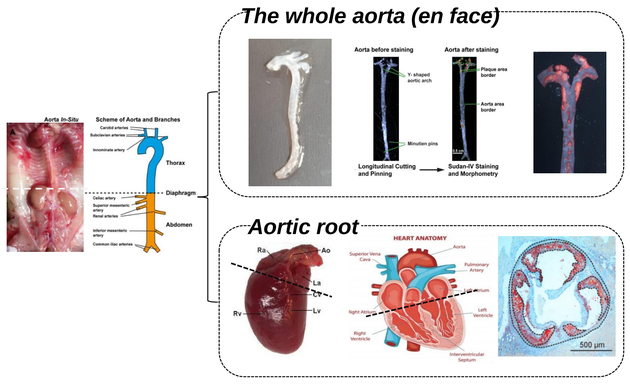
Fibrous Plaques in Atherosclerosis. Rupture of the fibrous cap can rapidly lead to thrombosis and usually occurs at sites of thinning of the fibrous cap that covers the advanced lesion.

Materials & Methods

Experiment design



Atherosclerotic lesion analysis



Lipid profile in mouse plasma

After fasting for 12 h, the mice were sacrificed, and blood was collected by cardiac puncture into tubes containing sodium citrate. Plasma was separated by centrifugation at 1,500 × g for 20 min at RT. Plasma levels of total cholesterol (TCHO), total triglyceride (TG) and high-density lipoprotein cholesterol (HDL-c)were measured with a commercial assay kit using Fuji Dry-Chem 4000i (Tokyo, Japan). Levels of low-density lipoprotein cholesterol (LDL-c; [TCHO – HDL-c + (TG/5)]) were calculated using the Friedewald formula. Further, we calculated atherogenic indices, including the Atherogenic Index of Plasma (AIP; Log10 [TG/HDL-c]), Castelli Risk Index-I (CRI-I; [TCHO/HDL-c]), Castelli Risk Index-II (CRI-II; [LDL-c/HDL-c]), and Atherogenic Coefficient (AC; [(TCHO-HDL-c)/HDL-c]), for use as predictors of the risk of atherosclerosis

Cell surface marker staining and flow cytometry

For staining, 200 μL of the cell suspension was incubated for 20 min in the dark at 4°C with antibodies against cell type-specific makers. All events were first gated on CD45 to identify hematopoietic cells, and then gated on CD11b to identify neutrophils and monocytes. The events were further sub-gated on markers for neutrophils (CD11b+Ly6G+). Furthermore, the populations of lymphocytes (CD45+CD3+) in splenocytes were analyzed using the BD FACSCanto II flow cytometer (BD Biosciences, San Jose, CA, USA) and FlowJo v7.6.5 64-bit software. All antibodies for flow cytometric analysis were purchased from Biolegend (San Diego, CA, USA).

Statistical analysis

Values are presented as the mean ± standard deviation (SD) unless otherwise indicated, and were compared using the Student's t-test, or with one-way ANOVA followed by Dunnett's multiple comparison test. Values of $P < 0.05$ were considered statistically significant.

Result

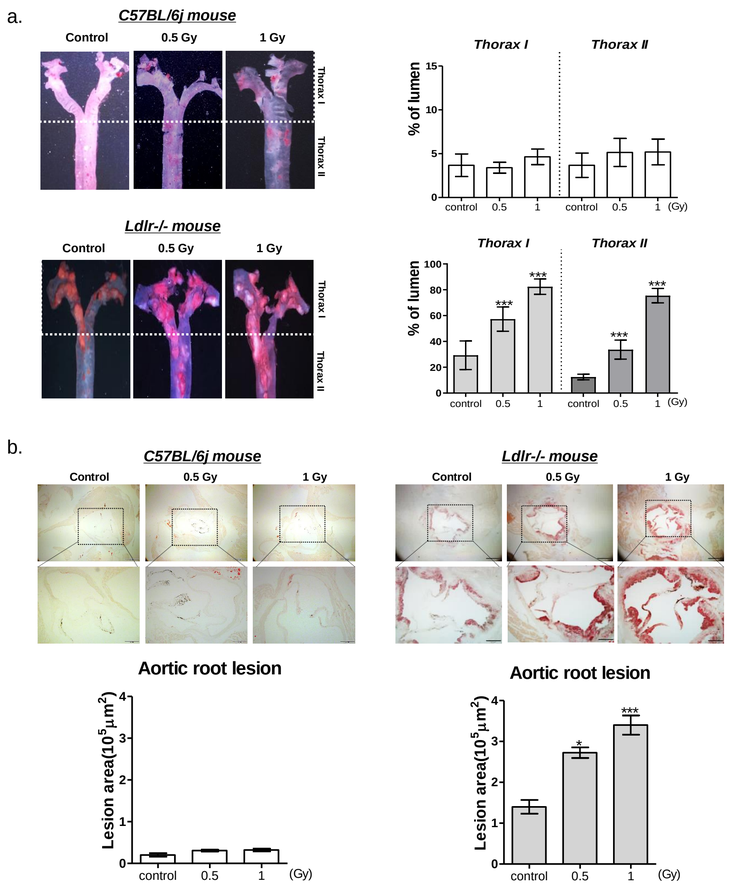


Figure 1. Atherosclerotic lesion progression in western diet-fed C57BL/6j and Ldlr-/- mice exposed to radiation. (a) Representative images of *en face* aortic plaque analysis, and quantitative results after Oil Red O staining of plaques. (b) Representative images of aortic root at 40X (upper panels) and 100× magnification (lower panels), with quantitative analysis after Oil Red O staining shown below. The data are presented as mean ± SD (n = 7). *P < 0.05, ***P < 0.001 vs. the unirradiated control.

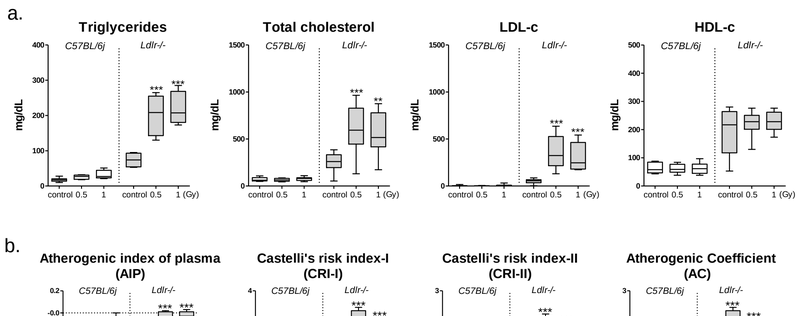


Figure 2. Alterations in lipid and lipoprotein profiles and the risk of atherosclerosis in western diet-fed C57BL/6j and Ldlr-/- mice exposed to radiation. (a) Plasma levels of triglycerides, total cholesterol, LDL cholesterol (LDL-c) and HDL cholesterol (HDL-c) were measured on the day of sacrifice. (b) Atherogenic indices. The data are presented as mean ± SD (n = 7). **P < 0.01, ***P < 0.001 vs. the unirradiated control.

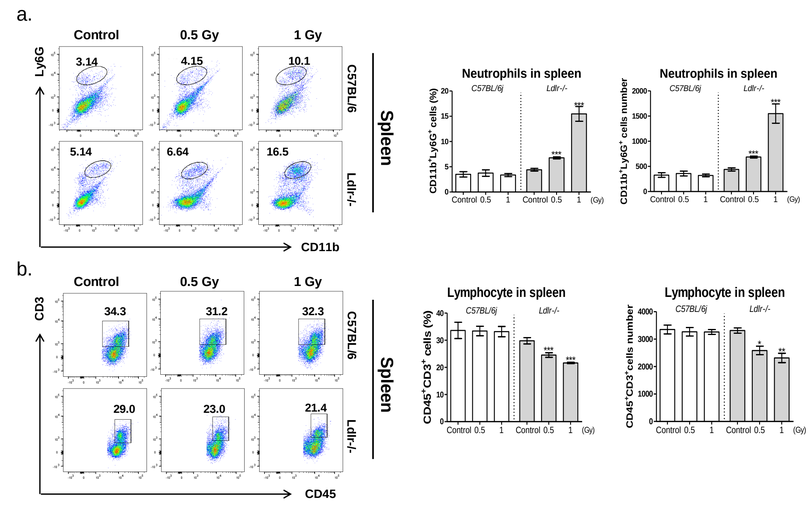


Figure 3. Changes in CD11bLy6G neutrophils and in CD3 lymphocytes in spleens of irradiated mice. (a) and (b) show representative dot plots of the gating strategy used to identify CD11b and Ly6G cells in the spleens of C57BL/6j and Ldlr-/- mice exposed to 0, 0.5, or 1 Gy radiation. The dot plots show the flow cytometric analyses of cell frequencies in spleens of irradiated mice. The data are presented as mean ± SD (n = 7). *P < 0.05, **P < 0.01, ***P < 0.001 vs. unirradiated control.

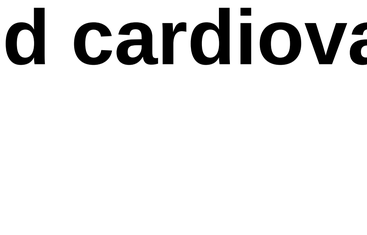


Figure 4. Ratios of neutrophils to lymphocytes in spleens of irradiated C57BL/6j and Ldlr-/- mice. The data are presented as mean ± SD (n = 7). ***P < 0.001 vs. unirradiated control.

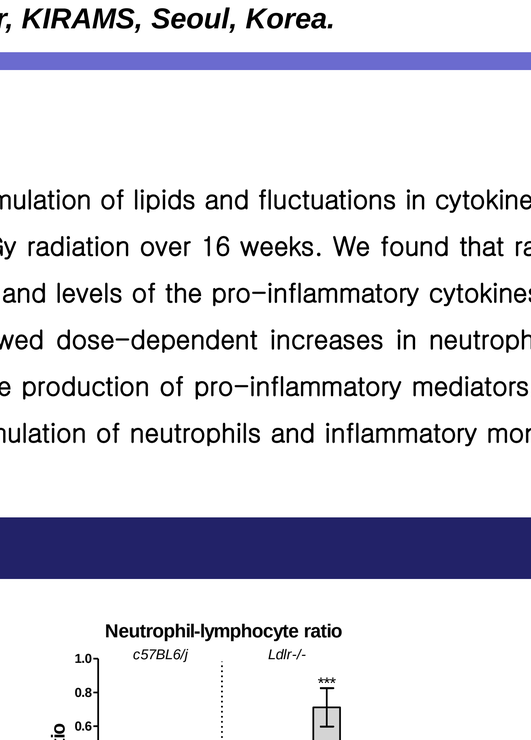


Figure 5. Representative immunofluorescence images of Ly6G (green) and CD3 (red) staining in spleen sections. DAPI staining (blue) was used to determine nuclei number. Scale bar: 50 μm. Below, quantitative analysis of Ly6G and CD3 cells in randomly selected regions of three independent slides. The data are presented as mean ± SD (n = 7). *P < 0.05, **P < 0.01, ***P < 0.001 vs. unirradiated control.

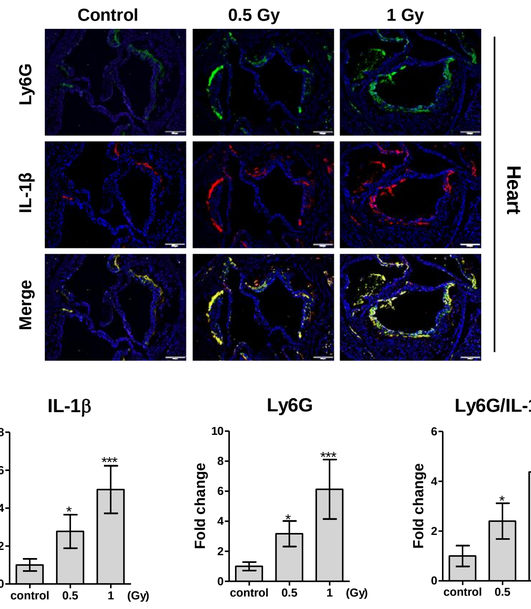


Figure 6. Radiation-induced pro-inflammatory cytokine production in aortic root of Ldlr-/- mice. Immunofluorescence staining of Ly6G (green), IL-1β (red), and Ly6G/IL-1β (yellow) in cells of the aortic root of Ldlr-/- mice. Original magnification: 100×. Below, quantitative analysis of each cell marker was performed in randomly selected regions of three independent slides. The data are presented as mean ± SD (n = 7). *P < 0.05, *** P < 0.001 vs. unirradiated control.

Conclusions

- Our findings in the atherosclerosis-susceptible Ldlr-/- mouse model collectively show that the risk of atherosclerosis is increased by prolonged exposure to 0.5 Gy radiation.
- Chronic radiation-induced neutrophil infiltration into atherogenic tissues, which is mediated by inflammatory mediators, such as IL-1β , Ly6G, is closely associated with the reduction of lymphocytes.
- This suggests that the dynamic profile of inflammatory cells is a potential target to estimate the health risk of radiation exposure in atherosclerotic development, accompanying with the change of immune mediators.

This work was supported by the National Research Foundation of Korea (NRF) grants funded by the Korea government (MSIT), grant number (No.NRF2019R1A2C208741613).

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