^{56}FE-RADIATION-INDUCED ALTERATIONS IN CIRCULATING LEUKOCYTE POPULATIONS IN THE APOE MOUSE ATHEROSCLEROSIS MODEL ARE TEMPORARY

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ABSTRACT. Radiation is associated with an increased risk of heart disease and stroke, likely due in part to vascular inflammation. One model used to understand this is the apoE mouse, where gamma irradiation accelerates development of atherosclerosis. Less is known, though, about the effects of high linear energy transfer (LET) radiation, such as ^{56}Fe, likely to be encountered by astronauts in deep space.

Radiation, however, also affects leukocyte numbers. For example, whole-body ^{56}Fe irradiation has been shown to decrease circulating B-cells and T-cells, but whether this was due to radiation of the thymus, of the bone marrow, or both was not determined.

We irradiated ApoE mice with ^{56}Fe focused to the aorta and carotids to determine how irradiation of the thymus with ^{56}Fe affects circulating lymphocyte number, and ultimately to determine the effect of iron ion irradiation on development of atherosclerosis. We found that only T-cells were affected at 13 weeks post-irradiation, but even these recovered at 40 weeks, suggesting that effects on the immune system are limited and temporary. Analysis of atherosclerosis development is pending sacrifice and histological analysis of irradiated mice.

1. INTRODUCTION. Radiation causes inflammation, and chronic, low-level vascular inflammation is a risk factor for atherosclerosis. Consistent with this, exposure to radiation from a variety of sources is associated with increased risk of heart disease and stroke. In the apoE mouse, gamma irradiation focused to the aortic arch and carotid arteries has been shown to accelerate development of atherosclerosis [1]. Radiation encountered in deep-space, however, will include heavy ions, such as ^{56}Fe, which can have biological effects very different from those of gamma rays. With the hypothesis that the underlying mechanism for acceleration of atherogenesis is enhanced vascular inflammation directly caused by the radiation, we have begun a series of apoE mouse experiments to determine the effects of ^{56}Fe on radiation-induced atherogenesis.

A potential complicating factor, however, is that radiation can have substantial effects on the immune system, including negative effects on lymphoid organs, which could complicate interpretation of results. Previous studies have demonstrated that whole-body ^{56}Fe irradiation results in decreased thymus size in as little as 4 days [2]. In addition, whole-body ^{56}Fe irradiation of mice has long-lasting effects on circulating lymphocyte numbers, with decreases in both B-cells and T-cells for at least 16 weeks [2]. Whether these changes are due to irradiation of the thymus, radiation of the bone marrow, or both cannot be determined from whole-body irradiation experiments. When radiation is focused on the aorta and carotids in the apoE mouse atherosclerosis model, the thymus is included in the target area; most of the bone marrow is excluded. To determine whether leukocyte populations in general, or T-cell number in particular, would be affected by radiation to the chest and neck, we measured relative blood leukocyte populations at 13 and 40 weeks following irradiation.

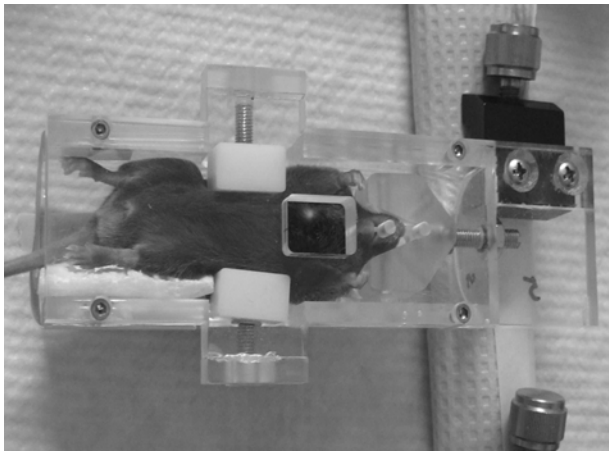

Fig. 1. Mouse holder and area of irradiation. ApoE mice were anesthetized by intraperitoneal injection of 10 mg/kg ketamine to immobilize them and loaded into custom-designed chambers for placement in the ion beam. By use of a collimator (not shown), radiation was limited to an area of 1 X 2 cm., slightly smaller than the cut-out over the chest in the holder. This area included the aortic arch and part of the carotid arteries, as well as the thymus.

2. RESULTS. ApoE mice were irradiated at Brookhaven National Laboratory with 600 MeV ^{56}Fe at 2 and 5 Gray (Gy) with the beam restricted to an area

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that included the aortic arch, part of the carotid arteries, and, unavoidably, the thymus (Fig. 1). We were unable to detect differences in circulating levels of monoctyes, granulocytic cells, and B-cells, as compared to controls (data not shown). Circulating levels of T-cells, however, were reduced at 13 weeks post-irradiation (Fig. 2).

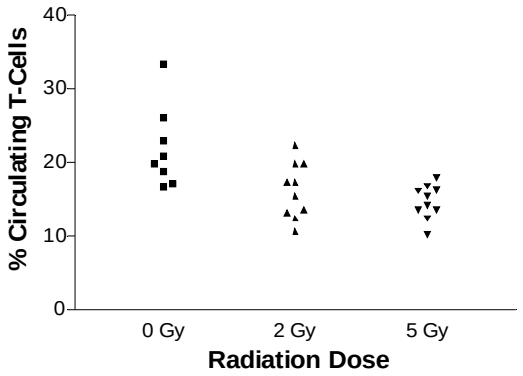

Fig. 2: Irradiation with ^{56}Fe decreases percentage of circulating T-cells at 13 weeks. Blood samples were collected for flow cytometry at 13 weeks. Each point represents one mouse. At least 8 mice were included in each group. T-cell percentage of total circulating leukocytes was reduced by both 2Gy and 5Gy ^{56}Fe ($p < 0.01$ by one-way ANOVA). .

Circulating T-cell percentages were determined for a second group of mice at the 40 weeks post-irradiation time point (Fig. 3). By 40 weeks, the effect of prior irradiation had disappeared, indicating that depression of circulating T-cells is a temporary effect from which the mouse is able to recover.

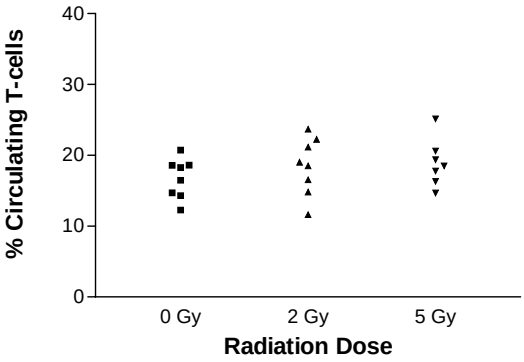

Fig. 3: T-cell percentages at 40 weeks post-irradiation. Blood samples were collected and analyzed for flow cytometry as in Figure 2. No effect of prior irradiation on circulating T-cells, as compared to 0 Gy controls, is detected at 40 weeks.

3. DISCUSSION. Our results demonstrate that ^{56}Fe radiation selectively alters circulating T-cell levels, even though there is little effect on other leukocytes. Since the thymus was in the field of irradiation, and since ^{56}Fe irradiation is known to cause shrinkage of the thymus [2], we suggest that the relative deficit in T-cells is due to damage to the thymus. At 40 weeks, however, the percentage of circulating T-cells is normal compared to controls. This may be due to the fact that the importance of the thymus in T-cell generation declines as the mouse matures, due to natural, age-related involution of the thymus even in the un-irradiated mice. In any case, at that point the irradiated mice are indistinguishable from the controls with respect to circulating leukocytes. Aside from the temporary effect on T-cells, we did not detect any defect in the immune system of irradiated mice as compared to un-irradiated controls either at 13 weeks or at 40 weeks. This does not rule out effects that may have been missed by our limited analysis. The mice remained healthy, however, and showed no evidence of either increased susceptibility to infection or adverse autoimmune effects.

This study has addressed the concern that, since irradiation of the aortic arch and carotid arteries to assess the atherogenic potential of ^{56}Fe irradiation necessarily includes the thymus in the target area, effects on the immune system might overwhelm effects on the vascular endothelium. We have shown that although T-cell levels are changed, levels normalize as the mouse ages, to resemble un-irradiated mice by 40 weeks. Of course, since T-cell levels are depressed for a substantial time period, this may yet contribute, either positively or negatively, to any potential mechanism of acceleration of atherosclerosis. Analysis of atherosclerosis development is pending sacrifice and histological analysis of irradiated mice.

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