EFFECT OF LOW DOSE GAMMA IRRADIATION ON THE DIFFERENTIATION AND MATURATION OF MONOCYTE DERIVED DENDRITIC CELLS

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INTRODUCTION
Earlier studies have shown that radiation during low orbit space flight had an adverse effect on the immune system; however, this effect was transient as the responses were normalized within two weeks of return to earth. There are numerous reports of the effects of radiation on the immune system but only anecdotal accounts of the potential effects of radiation encountered during protracted space travel on the immune system. Space or solar radiation is composed of photons, electrons, protons, neutrons, and heavy metal ions. Gridley and co-workers have shown that the effects of low dose (LD) proton irradiation and LD γ-irradiation on the immune system are similar. Dendritic cells (DC) are the most potent antigen presenting cells known and play a pivotal role in both innate and adaptive immunity. There are two subsets of DC, the myeloid derived (MDC) and the plasmacytoid (PDC) dendritic cells, that are essential for all immune function. To investigate the effect of low dose gamma (γ)-irradiation on the MDC, peripheral blood monocytes (PBM) were irradiated with LD (5 Gray) γ-irradiation to determine its effects on the differentiation and maturation of MDC, and their ability to present antigen. We hypothesized that protracted space flight will interfere with the differentiation and maturation of MDC, thereby severely impairing the immune system, and seriously attenuating immune surveillance mechanisms unless appropriate countermeasures are taken.

MATERIALS AND METHODS
We examined the effect of γ-irradiation (cumulative 5 or 15 Gy) on the differentiation, maturation, and activation of MDC. CD14+ PBM were isolated by magnetic bead separation and cultured in vitro with GM-CSF and IL-4 for 6 days to permit the differentiation of monocytes into immature DC that express the costimulatory molecules, CD80 and CD86. Immature DC (iDC) were activated with a cocktail of proinflammatory cytokines to mature (mDC) and to acquire the ability to present antigen to T cells in lymph nodes. CD14+ PBM was divided into 5 groups (G1-G5). G1 was not irradiated; G2 received 5 Gy of γ-irradiation on day (d) 1; G3 received 5 Gy of γ-irradiation on d1, d2, and d3, for a total of 15 Gy; G4 received 5 Gy of γ-irradiation on d1, d2, and d7, for a total of 15 Gy; G5 received 5 Gy of γ-irradiation on d7, prior to activation. All cultures were harvested on d8; the number of DC determined and the expressions of CD11c and HLA-DR (MDC markers); CD80 and CD86 (costimulatory markers), and CD83 (maturation marker) were assessed by flow cytometry.

RESULTS
Expression of co-stimulatory markers, CD80 and CD86, were down-regulated on MDC that were differentiated from all irradiated cultures, irrespective of the time of irradiation or the level of exposure. Moreover, γ-irradiation down-regulated the expression of the maturation marker, CD83. However, irradiation of PBM on d1 or on 3 consecutive days (d1-d3) augmented the number of MDC recovered, compared with non-irradiated controls. By contrast, cultures that were irradiated on d7 after differentiation of PBM into MDC had occurred did not increase the MDC number.

CONCLUSION
1. Repeated exposure of PBM to γ-irradiation on three consecutive days in the early phase of differentiation increased the number of MDC.
2. γ-irradiation at the late phases of PBM differentiating into MDC did not have an effect on the number of CD11c+ DC.
3. γ-irradiation down-regulated the expression of co-stimulatory makers, CD80 and CD86, that are necessary for signal transduction.
4. γ-irradiation down-regulated the expression of maturation marker, CD83.

Further studies are in progress to determine the mechanism(s) responsible for these observations.