Radiation-induced immunosuppressive macrophages limit CD8 T-cell mediated tumor killing

1. **Keaton I. Jones,**
2. **Jiske Tiersma,**
3. **Jon Buzzelli,**
4. **Alex Gordon-Weeks,**
5. **Arseniy Yuzhalin,**
6. **Jaehong Im, and**
7. **Ruth J. Muschel**

1. *University of Oxford, Oxford, United Kingdom.*

Abstract

Emerging pre-clinical data suggests that radiation both stimulates and suppresses tumor immunity. The role of tumor associated macrophages (TAMs) has been extensively investigated in the non-irradiated tumor microenvironment. We aimed to investigate the role of macrophages in the immune response to radiation. Single dose 10Gy irradiation to tumors generated from colorectal (MC38) and pancreatic (KPC) cell lines induced Colony Stimulating Factor (CSF-1). Coincident with the elevation in CSF1, significant increases of CD11b+ F480+ macrophages within the tumor microenvironment peaked five days following irradiation returning to baseline during tumor regrowth. These TAMs were skewed toward an immunosuppressive phenotpye ($CD206^{hi}$ $iNOS^{lo}$) compared to those from controls. Furthermore, the TAMs from irradiated tumors were more effective in suppression of CD8 T cell expansion in ex vivo assays.

As a result of these data, we investigated the effect of anti-Colony Stimulating Factor (αCSF-1), which reduced macrophages in both control and irradiated tumors. αCSF had no effect on the growth of control tumors, yet led to a significant enhancement of tumor growth delay after radiation. TAMs sorted from irradiated tumors after aCSF treatment had reduced inflammatory gene expression and increased expression of some genes associated with immune suppression (Arg-1, IL-10, CCL2). Following aCSF, there was a significant increase in CD8 T lymphocytes with greater expression of cytotoxic markers. Radiation is also immune-stimulatory to tumors consistent with enhanced recognition of tumor cell antigens by T cells isolated from irradiated tumors. CD8 depletion however abrogated the growth delay from aCSF further documenting the immunosuppressive effect of the macrophages within irradiated tumors. aCSF1 was given to mice bearing two tumors, one of which was irradiated. An increased growth delay was only observed in the irradiated tumours. These data document the immunosuppressive nature of macrophages generated in irradiated tumors. TAMs expressed high levels of PD-L1 and aCSF treatment resulted in a reduction in tumor PD-L1. As a result we determined the effect of checkpoint inhibition using anti-PD-L1 in combination with aCSF following 10Gy irradiation. Whilst combination treatment did not augment outcomes in mice bearing MC38 tumors, there was complete tumor regression in a minority of mice bearing KPC tumors. These results suggest that infiltrating macrophages limit the adaptive immune response initiated following of radiation. Whilst it has been demonstrated that tumor cell PD-L1 expression plays an important role in checkpoint blockade, our data suggests macrophages are an important additional source. This is of particular importance in the context of radiation which can elicit a potent immune response but in which the TAMs from irradiated tumors also suppress this in part via altered PD-L1 expression.