

Adoptive T-cell transfer combined with a single low dose of total body irradiation eradicates breast tumors

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The major objectives of tumor vaccination are to induce the regression of established tumors and to favor the establishment of long-lasting tumor-specific immunity, capable of protecting the host from relapse. Immunotherapeutic strategies such as the administration of tumor-associated antigenic peptides offer one means to boost preexisting antitumor CD8⁺ T cell immunity. Our recent work reveals that established breast tumors are rejected and tumor recurrence prevented when low-dose irradiation is combined with the adoptive transfer of Mammaglobin A epitope-specific CD8⁺ T cells.

Mammaglobin A (Mam-A) is a clinically relevant breast cancer-associated antigen that is overexpressed in primary human breast tumors.¹ The expression of Mam-A is similar on breast tumors of increasing grade and frequently persists in metastatic cells.^{1,2} Previous work by others and more recently by our own group examined the effect of full-length Mam-A vaccination toward the generation of tumor-specific CD8⁺ T cells. In these studies, despite initial tumor regression, long-term protection from tumor relapse was not achieved. We hypothesized that this was due to the inability of this vaccine regimen to generate and maintain an effective antitumor memory CD8⁺ T-cell response. Ideally, vaccination would induce a heterogeneous antitumor CD8⁺ T-cell population consisting of both central memory T (T_{CM}) and effector memory T (T_{EM}) cells.

To explore whether Mam-A epitope-specific DNA vaccination would generate a diverse spectrum of Mam-A-specific CD8⁺ T cells varying in both phenotype and functional attributes, we vaccinated HLA-A2⁺ transgenic mice with DNA encoding for the immunodominant epitopes of Mam-A (Mam-A2.1, A2.2, A2.4

and A2.6).² The resulting Mam-A epitope-specific CD8⁺ T cells could then be adoptively transferred into Mam-A-expressing tumor-bearing SCID-beige mice. Phenotypic analysis revealed that vaccination with Mam-A epitope-encoding DNA can induce a heterogeneous population of Mam-A-specific CD8⁺ T cells consisting of both T_{CM} and T_{EM} cells, regardless of the specific Mam-A epitope tested. Furthermore, upon adoptive transfer into tumor-bearing mice, Mam-A2 specific CD8⁺ T cells manifested further heterogeneity with one Mam-A epitope (Mam-A2.4) developing a prevalent T_{EM} cell phenotype, expressing low levels of both CD62L and CD27 while maintaining high levels of CD127. Nonetheless, as it is the case with numerous therapeutic tumor vaccines tested,^{3,4} we found that our one-time adoptive transfer strategy failed to overcome the poor natural T-cell response to Mam-A-expressing breast tumors, demonstrating that even the most functional Mam-A-specific CD8⁺ T-cell population (specific for Mam-A2.4) is unable to sustain long-term tumor-specific immunity. Thus, we incorporated a single low dose (as opposed to higher doses or multiple

low doses) of total body irradiation (TBI) into our protocol, to assess whether or not this was capable of augmenting the antitumor Mam-A-specific CD8⁺ T-cell response. SCID-beige mice bearing large established Mam-A-expressing tumors were treated with low-dose TBI (2.5 Gy) immediately prior to receiving splenocytes from Mam-A2.4 vaccinated HLA-A2⁺ transgenic mice. Although TBI alone resulted in initial tumor regression these tumors quickly began growing back to their original size. This said, the combination of TBI and the adoptive transfer of Mam-A-specific CD8⁺ T cells not only led to persistent tumor regression, but also prevented tumor re-growth. These results emphasize the need for combination therapies to induce an effective antitumor immune response that can prevent tumor escape.

The ability of TBI to augment the functional attributes of adoptively transferred tumor-specific CD8⁺ T cells has been shown to result from the removal of host immunosuppressive cells, cytokine sinks and/or from the release of Toll-like receptor (TLR) agonists that can activate the innate immune system.⁵⁻⁷ In our tumor model,

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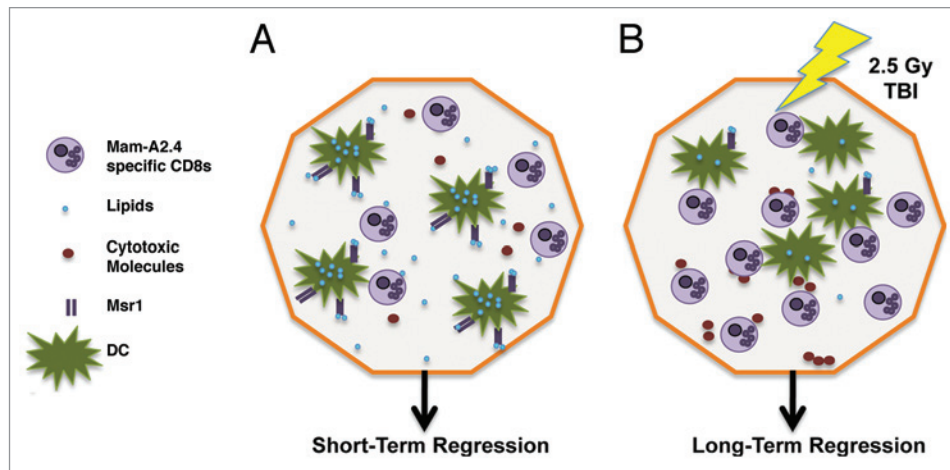


Figure 1. Mechanism through which low-dose total body irradiation (TBI) may stimulate long-term tumor regression and prevent relapse. **(A)** When mamoglobin A (Mam-A)-specific CD8⁺ T cells are adoptively transferred into tumor-bearing SCID-beige mice, they encounter dendritic cells (DCs) that are loaded with lipids and unable to efficiently present tumor-associated antigens. This results in short-term tumor regression but eventual relapse. **(B)** When tumor-bearing mice are treated with low dose TBI immediately prior to the transfer of Mam-A2.4-specific CD8⁺ T cells, tumor-resident DCs downregulate macrophage scavenger receptor 1 (Msr1) expression resulting in the accumulation of lower amounts of lipids. In turn, this leads to improved antigen presentation to Mam-A2.4-specific CD8⁺ T cells and hence long-term tumor regression and relapse prevention.

TBI presumably was not acting through the removal of cytokine sinks (which are predominantly constituted by NK and T cells) or FOXP3⁺ regulatory T cells (Tregs), as we used SCID-beige mice, which lack B, T and NK cells, as recipients. Therefore, in an effort to understand the mechanism by which TBI provides the necessary cues for transferred Mam-A2.4-specific CD8⁺ T cells to induce a productive antitumor response, we explored the possible effects of TBI on tumor-resident dendritic cells (DCs). The premise for this was a recent report showing that tumor-resident DCs taken from tumor-bearing mice or individuals were functionally defective due to an increase lipid uptake.⁸ Consistent with these findings, we found that DCs isolated from non-irradiated tumor-bearing mice exhibited a more than 2-fold increase in BODIPY (a lipophilic fluorescent dye that

estimates the amount of intracellular lipids) staining as compared with DCs isolated from irradiated tumor-bearing mice. Additionally, we evaluated the expression of the macrophage scavenger receptor 1 (Msr1), which has previously been shown to be upregulated on tumor-resident DCs and to be responsible for the increased lipid uptake of DCs in tumor-bearing hosts.⁸ In our model, TBI promoted the downregulation of Msr1 by tumor-resident DCs. This decrease in turn affected the ability of tumor resident DCs to take up lipids, thereby potentially increasing the functional capacity of adoptively transferred Mam-A epitope-specific CD8⁺ T cells to induce tumor regression and prevent relapse. It is likely that the accumulation of lipids by tumor-resident DCs is due to the presence of tumor-derived factors. However, the nature of such factors

remains unclear and warrants further investigation.

While we cannot rule out the possibility that TBI is also acting to increase TLR signaling and/or inducing microbial translocation, we propose a new mechanism of action for TBI in our model of breast cancer that may or may not be acting synergistically with the potential increase in TLR signaling. Collectively, our findings point to a mechanism by which low-dose TBI promotes the downregulation of Msr1, resulting in the inhibition of lipid uptake by tumor-resident DCs, in turn enabling these cells to present tumor antigens more efficiently to adoptively transferred Mam-A epitope-specific CD8⁺ T cells (Fig. 1).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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