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14. ABSTRACT The standard of care has produced marginal extension of remission rates in ovarian cancer. Based on preliminary observations that epigenetic regulators like Trichostatin A (TSA) can reduce ovarian tumor burden in a syngeneic murine model system, we tested the notion that TSA treatment leads to dendritic cell maturation that promotes effector CD8+ T cell over CD4+ T regulatory cell generation. At 10 mg/kg/day for 5 times on alternate days, TSA was able to control ovarian tumor growth, however the tumor growth resumed by day 40. Surprisingly, 1-MT; a known inhibitor of indole-amine 2,3-dioxygenase (IDO) was unable to cause ovarian tumor growth inhibition, which was attributed to its inability to block IDO expression in the ovarian tumor microenvironment. However, TSA mediated ovarian tumor control required MHC Class 1 dependent CD8+ T cells. These studies have identified the role of CD8+ T cells in promoting ovarian tumor immunity and delineated the ability of TSA mediated epigenetic alteration to enhance CD8+ T cell mediated ovarian tumor control. Ongoing studies are focused on testing new generation of IDO inhibitors for ovarian cancer.					
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1. **INTRODUCTION:** The use of traditional modalities to treat ovarian cancer has not changed the rate of recurrence and/or the remission period. Emerging evidence indicates that adaptive immunity mediated by T cells can play an important role in controlling ovarian tumor growth, whereby inducing interest in developing approaches to augment T cell responses in ovarian cancer patients to extend remission. The fact that the relative frequency of CD4⁺ T regulatory cells and effector CD8⁺ T cells in ovarian cancer patients and in murine models is indicative of tumor outcomes, behooves developing targeted strategies to cause skewing of T cell responses. In preliminary experiments, we have noted the ability of the epigenetic modifier trichostatin A (TSA) to extend survival of ovarian tumor bearing mice. Based on this observation we hypothesized that TSA caused alteration of dendritic cell maturation whereby leading to decreased CD4⁺ T regulatory cell generation and enhanced effector CD8⁺ T cells, leading to enhanced ovarian tumor control and perhaps durable immunity.

2. **BODY:**

Aim 1: To test whether TSA treatment reverses the generation of CD4⁺ T regulatory cells in MOSEC tumor bearing hosts by reducing IDO expressing immature CD11b⁺ DC's.

Specific Tasks: In the syngeneic murine ovarian tumor (MOSEC) model, to determine the impact of TSA treatment on DC maturation and T reg. generation,

1. Groups of 10 age matched B6 mice bearing MOSEC tumor will be administered TSA (10 mg/kg/day X 5 times on alternate days) by intraperitoneal injection on day 10 post-tumor challenge.
2. The cells harvested from the tumor draining LN's, spleen and the tumor site starting on day 2 after the final TSA administration will be subjected to phenotypic analysis by staining followed by flow cytometry and functional evaluation for regulatory capacity in a standard in vitro MLR reaction. The ability of MOSEC induced immature DC's to promote T reg. generation, we will perform To determine whether tumor induced immature DC's generate T reg. cells in vivo, we will use 1-MT (1-methyl tryptophan)(20 microg/day x 50 days in drinking water) treatment in vivo starting on day 10 of tumor challenge.

Results: In the year (May 2008-Oct 2009), we established that:

1. The administration of HDACi (Trichostatin A) to ovarian tumor bearing mice to significantly reduced the frequency of tumor induced CD4⁺/CD25⁺ cells that are FoxP3 positive regulatory cells in our syngeneic murine tumor model (Figure 1, TSA versus PBS). However, the TSA mediated inhibition of Treg. generation was transient and by day 60 re-emergence of Treg. cells was noted.

Discussion of Results: The ability of TSA to block induction of T regulatory CD4⁺ T cells in MOSEC ovarian tumor bearing animals is encouraging and confirms the précis for our study, which is that epigenetic modification can be used to alter host T cell immune responses. However, the lack of persistence in efficacy indicates that the epigenetic modifier may only transiently reduce the DC maturation status and that continued administration and/or careful dose response of TSA needs to be performed and evaluated to achieve durable responses. This may not be feasible as TSA does not specifically affect DC and T cells and its off target effects may result in overt toxicity.

2. Surprisingly, the inhibition of IDO by 1-MT administration in this ovarian tumor model system failed to inhibit Treg. generation (Figure 1; PBS versus 1-MT).

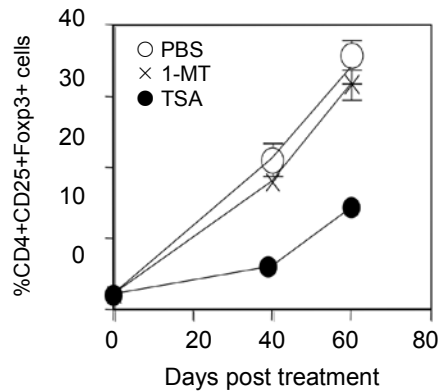


Figure 1: HDACi but not IDO blockade reduces ovarian tumor induced T regulatory cells. Cohorts of three mice bearing ovarian tumor (MOSEC) were treated with either PBS (control), or Trichostatin A (TSA, 10 mg/kg/day X 5) or 1-Methyl tryptophan (1-MT, 10 microg/day x 50 days). The draining lymph nodes from the mice were harvested and evaluated by flow cytometry for the frequency of CD4+ T regulatory cells (CD4+/CD25+/FoxP3+) on indicated days. The error bars represent SD of mean values obtained from 3 mice/group and a representative graph from 3 experiments is shown.

Discussion of results: The failure of 1-MT mediated IDO blockade to inhibit the ovarian tumor induced CD4+/CD25+/FoxP3+ regulatory T cells could be due to several factors including the possibility that ovarian tumor induced T regulatory cells utilize other molecular pathways such as arginase and/or STAT3, which should be tested. It could also be envisioned that the 1-MT was ineffective at blocking IDO expression and/or activity, which has been recently reported () and new second-generation IDO blocking reagent are undergoing pre-clinical evaluation and should be tested in our ovarian tumor model system.

Aim 2: To test whether TSA/1-MT administration enhances dendritic cell cross-primed antigen presentation to host CD8 T cells.

Specific Tasks: In groups of MOSEC bearing B6 mice, some animals will be treated with TSA/1-MT and cells obtained (four days after TSA treatment) from the draining lymph nodes, spleen and the tumor site (peritoneal cavity) will be evaluated for WT1 antigen specificity and effector phenotype by flow cytometry.

In some B6 animals, the bone-marrow (BM) will be engineered by irradiation and congenic BM replacement such that they lack MHC class I expression (B6 Kb/Db -/-), and these animals will be challenged by the MOSEC tumor and treated with TSA along with control animals to determine the contributions of BM cross-primed WT1 specific CD8 T cell responses after TSA treatment of MOSEC tumor bearing mice.

Results: This aim was not pursued since 1-MT failed to block CD4+ T regulatory cell induction by ovarian tumor (Figure 1). Thus disproving our hypothesis that TSA mediated inhibition of T regulatory cell induction was due to BM-APC generated IDO expression.

Discussion of the Results: The lack of 1-MT effects on induction of CD4+ T regulatory cells could be due to IDO not being involved in Treg. generation by ovarian tumor. It is also possible that 1-MT fails to block IDO in our experimental model. These possibilities urge us to consider new IDO inhibitors in our future experiments and if they also fail to control induction of T regulatory cells then we can conclude that ovarian tumor induced regulatory T cells do not use the IDO pathway. Another approach to directly test the role of IDO expression in DC mediated T

regulatory cell generation is using IDO deficient mice to generate DC and employ the bone marrow chimera strategy to test the notion.

Aim 3: To determine whether TSA treatment enhances CD8 T cell mediated control of MOSEC tumor growth and produce durable ovarian tumor immunity.

Specific Tasks:

1. Groups of MOSEC tumor bearing B6 mice will be depleted of CD8 T cells prior to TSA treatment and the MOSEC tumor growth and survival will be compared to control groups.
2. The CD8 T cells derived from TSA treated MOSEC bearing mice will be adoptively transferred to non-TSA treated MOSEC bearing animals and their tumor growth and survival will be monitored.
3. The durability of TSA induced WT1 specific CD8 T cell responses and their role in generating immunity to MOSEC, will be tested by determining the number of WT1 specific CD8 T cells detected in MOSEC bearing mice up to 100 days (post-TSA treatment) by flow cytometry. MOSEC bearing and TSA treated animals that are determined to be tumor free and survive till day 150, will re-challenged with MOSEC; via subcutaneous injection and the tumor volume will be measured every 5 days to demonstrate durable tumor immunity.
4. To demonstrate the role of CD8 T cells in durable protection against MOSEC, we will deplete tumor free mice of CD8 T cells by antibody administration prior to tumor re-challenge as well as perform adoptive transfer of CD8 T cells from TSA treated tumor free mice and test protection against MOSEC tumor in the recipients.

Results: The administration of the HDACi (TSA) in ovarian tumor bearing normal B6 mice results in increased survival due to poor tumor growth. This augmented survival required MHC class 1 dependent CD8+ T cell responses, as in B6 mice that are deficient for MHC Class 1 (H-2Kb/Db -/-) the TSA mediated protection against ovarian tumor was lost (Figure 2, (WT) TSA versus (MHC 1^{-/-}) TSA). However, in mice that were BM chimera for MHC Class I deficiency we did not observe the loss in TSA efficacy, suggesting that presence of mature CD8+ T cells was sufficient to produce TSA mediated ovarian tumor control.

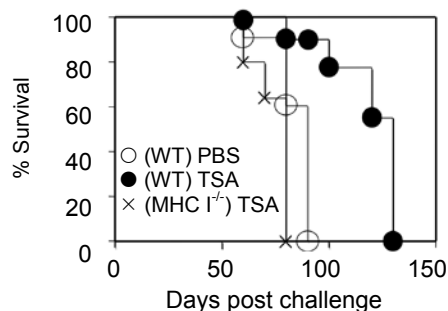


Figure 2: HDACi promotes CD8+ T cell mediated survival of ovarian tumor bearing hosts. Cohorts of 15 mice bearing ovarian tumor (MOSEC) were treated with either PBS (control), or Trichostatin A (TSA, 10 mg/kg/day X 5). One cohort of 15 mice MHC Class 1^{-/-} (no CD8+ T cells) was treated with TSA. The animals with considerable ovarian tumor burden (as determined by IACUC protocol of RPCI) were sacrificed and the remaining percentage of animals is plotted.

Discussion of Results:

The observations demonstrate that MHC Class restricted CD8+ T cells are critical for TSA mediated protection from ovarian tumor growth. To the best of our knowledge this is the first evidence indicating an immune based impact of epigenetic modifiers on tumor growth. These findings needs further study to exploit the immune mechanisms for epigenetic targeted cancer

therapy. Strikingly, bone marrow chimeras lacking MHC Class 1 failed to show loss of TSA efficacy, suggesting that the cross-primed presentation by BM-DC may not be a critical pathway that regulates TSA mediated augmented ovarian tumor immunity. This is in contradiction to our hypothesis, but it lends support to a new notion implying a direct role for TSA and MHC Class 1 mediated ovarian tumor control. This is a concept under investigation.

OVERALL PROJECT SUMMARY: The current standard of care has produced marginal extension of remission rates in ovarian cancer. Based on preliminary observations that epigenetic regulators like Trichostatin A (TSA) can reduce ovarian tumor burden in a syngeneic murine model system, we tested the notion that TSA treatment leads to dendritic cell maturation that promotes effector CD8⁺ T cell over CD4⁺ T regulatory cell generation. When TSA was administered at 10 mg/kg/day for 5 times on alternate days, the ovarian tumor growth was restricted, however the tumor growth resumed by day 40. Surprisingly, 1-MT; a known inhibitor of indole-amine 2,3-dioxygenase (IDO) was unable to cause ovarian tumor growth inhibition, which can be attributed to its inability to block IDO expression in the ovarian tumor microenvironment. However, TSA mediated ovarian tumor control required MHC Class 1 dependent CD8⁺ T cells. These studies have identified the role of CD8⁺ T cells in promoting ovarian tumor immunity and delineated the ability of TSA mediated epigenetic alteration to enhance CD8⁺ T cell mediated ovarian tumor control. Ongoing studies are focused on testing new generation of IDO inhibitors for ovarian cancer.

The objective of the proposed study was to test our hypothesis that HDACi (TSA) treatment of MOSEC-ovarian tumor bearing hosts promotes dendritic cell differentiation to favor generation of WT1 specific effector CD8⁺ over regulatory CD4⁺ T cells for durable tumor immunity. Three specific aims were proposed.

There were no publications or meeting abstracts resulting from this project.

The personnel receiving pay from the research effort:

1. Rajesh Rao, Pre-doctoral Trainee (Year 2)
2. Qingsheng Li, Post-Doc (Year 2)

3. **KEY RESEARCH ACCOMPLISHMENTS:**

1. Identified the ability of epigenetic modifiers like TSA to regulate CD8⁺ T cell mediated ovarian tumor immunity.
2. Established that 1-MT failed to block regulatory CD4⁺ T cell induction in ovarian cancer.
3. Determined the transient efficacy of TSA in affecting ovarian tumor growth.

4. **REPORTABLE OUTCOMES:**

1. The ability of TSA to enhance survival of ovarian tumor bearing host demonstrates the potential use for clinical benefit. However, comprehensive studies directed at determining the dose and duration of TSA administration for optimal/durable benefits in the pre-clinical murine models are required to generate protocols for Phase 1 clinical trials.
2. The fact that 1-MT failed to alter CD4⁺ T cell numbers in ovarian tumor bearing mice has profound implications. A straight forward conclusion would be that IDO expression by DC's does not induce CD4⁺ T regulatory cells, this is unlikely to be cause as IDO has been

implicated in ovarian tumor immunity and tolerance. Thus leading us to conclude that 1-MT was unable to block IDO expression by DC's in the ovarian tumor setting, this possibility must be tested and the availability of new second/third generation IDO inhibitors certainly encourages these investigations.

3. The fact that TSA treatment leads to a MHC Class 1 dependent ovarian tumor control unravels an unrealized and described role for epigenetic modifiers in cancer treatment, which may be mediated via affecting host immunity. This has profound implications for the application of TSA and other epigenetic modifiers in immune therapy of not only ovarian cancer, but several other challenges including breast/brain/prostate cancers, chronic inflammation and infections.

5. CONCLUSION:

1. The epigenetic modifier TSA enhances survival of ovarian tumor bearing host demonstrating its potential use for clinical benefit.
2. The use of 1-MT failed to block regulatory CD4+ T cell numbers in ovarian tumor bearing mice.
3. The TSA treatment leads to a MHC Class 1 dependent ovarian tumor control , which has profound implications for the application of TSA and other epigenetic modifiers in immune therapy.

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7. APPENDICES: N/A