Published in final edited form as:
Immunotherapy. 2012 December ; 4(12): 1795–1798. doi:10.2217/imt.12.126.

Taming dendritic cells with TIM-3: Another immunosuppressive strategy by tumors

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Abstract

The identification of TIM-3 expression on tumor associated dendritic cells (TADCs) provides insight into another aspect of tumor-mediated immunosuppression. The role of TIM-3 has been well characterized on tumor-infiltrating T cells, however its role on TADCs was not previously known. The current paper demonstrated that TIM-3 was predominantly expressed by TADCs and its interaction with the nuclear protein HMGB1 suppressed nucleic acid mediated activation of an effective antitumor immune response. The authors were able to show that TIM-3 interaction with HMGB1 prevented the localization of nucleic acids into endosomal vesicles. Furthermore, chemotherapy was found to be more effective in anti-TIM-3 mAb treated mice or mice depleted of all DCs which indicated that significant role played by TADCs inhibiting tumor regression. Taken together, these findings identify TIM-3 as a potential target for inducing antitumor immunity in conjunction with DNA vaccines and/or immunogenic chemotherapy in clinical settings.

Dendritic cells (DCs) play a key role in the induction of immune responses, with their wide array of pattern recognition receptors such as toll-like receptors (TLRs) and sensors that detect cytosolic nucleic acids of various pathogens [1]. The dynamic interplay between DCs and T cells aid in shaping the resulting degree and specificity of the downstream immune responses. In the context of tumor immunity, the induction of a Th1 response is preferred which requires innate cells to secrete pro-inflammatory cytokines such as Type-I interferons [2–4]. However within the tumor microenvironment, immune suppressive factors such as VEGF and IL-10 impede the induction of Th1 immunity by manipulating the functionality and phenotype of many immune cells including DCs [2].

T cell immunoglobulin and mucin domain-3 (TIM-3) is a type I membrane receptor whose expression was initially detected on ‘terminally differentiated’ Th1 cells but not on Th2 cells [5]. Since then, the expression of TIM-3 has been detected on exhausted, virally infected Th1 cells [6–8] and exhausted tumor infiltrating T cells [9]. When TIM-3 is expressed on terminally differentiated Th1 cells, interaction with its ligand galectin-9 triggers TIM-3 to

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Financial & competing interests disclosure
The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.
Writing assistance was not utilized in the production of the manuscript.

impede Th1 responses [10]. However, TIM-3 expression on innate cells has been previously shown to promote inflammation and synergize with TLRs upon LPS activation to lead to TNF-α production in TIM-3$^+$ DCs [10]. Despite this knowledge, the role of TIM-3 on DCs in the tumor microenvironment is not as well defined.

Thus it becomes important to investigate not only how DCs are 'shut down' in the tumor microenvironment and promote tumor progression, but also how to reverse this effect and lead to more robust anti-tumor immune responses.

Summary of methods & results

Chiba *et al.* demonstrate the role of TIM-3 expression on DCs within the tumor microenvironment and its implications on the induction of effective antitumor immune responses. Following challenge with 3LL Lewis lung carcinoma or MC38 colorectal adenocarcinoma cells, the expression of TIM-3 was assessed on different immune cells including conventional and plasmacytoid DCs (pDCs), CD8$^+$ T cells, and CD11b$^+$Gr1$^+$ myeloid cells found within the tumors, lymph nodes and spleen. They report that only tumor associated DCs (TADCs) and infiltrating CD8$^+$T cells expressed high levels of TIM-3, albeit with differing expression kinetics, whereas TIM-3 expression on immune cells outside of the tumor microenvironment was minimal. Tumor-derived factors VEGF, IL-10 and arginase I contributed to the high TIM-3 expression on DCs. Cancer patients with advanced disease also were found to have TIM3$^+$TADCs.

To determine the effect of TIM-3 expression on overall DC function, TIM-3$^+$ BMDCs were isolated, stimulated with various TLR agonists and assayed for IFN-β1, IFN-α4, IL-6 and IL-12 secretion relative to TIM-3$^-$ BMDCs. Cytokine secretion was much lower following stimulation with nucleic acid ligands for TLRs 3, 7 and 9 and for cytosolic RNA sensors. Treatment with a TIM-3 specific mAb or siRNA enhanced the secretion of IFN-β1 *in vitro*. TIM-3$^-$ mouse embryonic fibroblasts (MEFs) transfected with TIM-3 showed decreased cytokine production in response to genomic DNA derived from tumor cells and bacterial and viral pathogens. TADCs isolated from tumor-bearing mice or cancer patients had lower levels of IFN-β1 and IL-12 transcripts and protein levels, both of which increased following TIM-3 mAb treatment.

A combination treatment of nucleic acid adjuvants with TIM-3 mAb led to a significant reduction in B16-F10 melanoma tumor growth and enhanced IFN-β and IL-12 production within the tumors. The antitumor effects of plasmid DNA along with anti-TIM-3 mAb treatment was most effective in CD11c-DTR mice following diphtheria toxin administration which depleted all CD11c$^+$ DCs. Additionally, combination treatment was most effective when TIM-3 deficient BMDCS were adoptively transferred whereas the treatment was ineffective when TIM-3$^+$ BMDCs were transferred. The authors also demonstrated that the antitumor immunity induced by blocking TIM-3 expression is drastically reduced following administration of neutralizing antibodies to IL-12p40 and Type I IFN receptor.

To elucidate the mechanism of TIM-3 regulation on innate immune responses, the authors examined galectin-9 interactions with TIM-3 on TADCs. Galectin-9 mRNA was decreased in bulk tumor tissue, tumor cells, and in TADCs compared to splenic DCs and treating

tumor bearing mice with anti-galectin-9 mAb did not affect tumor growth upon plasmid DNA administration, suggesting that TIM-3 and galectin-9 interaction did not play a key role in TIM-3 regulation of nucleic acid sensing. Furthermore, phosphatidylserine, another TIM-3 ligand, did not affect IFN-β1 expression after treatment of TIM-3^+ MEFs with B-form double-stranded DNA (B-DNA).

However, TIM-3 did bind to the nuclear protein HMGB1, which activates innate immunity nucleic acid sensing. When biotin-labeled nucleic acid was added to HMGB1, its binding to HMGB1was inhibited in the presence of unlabeled TIM-3 suggesting that TIM-3 and nucleic acids competitively bind to HMGB1. Confocal microscopy showed that HMGB1 colocalized with TIM-3^+ DCs and bound with a higher affinity than TIM-3^- DCs. However, anti-TIM-3 mAb inclusion inhibited immunoprecipitation of HMGB1 by TIM-3^+ DCs. Moreover, HMGB1 mRNA increased in tumor tissue compared to other organs, demonstrating that HMGB1was produced in tumor tissue.

Using microscopy, TIM-3 transfected MEFs stimulated with HMGB1, prevented localization of nucleic acids into endosomal vesicles compared to wild type MEFs, and TIM-3^- DCs had greater quantities of nucleic acids in endosomal vesicles than TIM-3^+ DCs. TIM-3^+ DCs stimulated with HMGB1 showed that TIM-3 colocalized with HMGB1 in endosomal vesicles, in which less B-DNA was found. Furthermore, IFN-β1 production was diminished in TIM-3^+ MEFs stimulated with HMGB1 after the addition of B-DNA, and was abrogated by neutralizing HMGB1 mAb when TIM-3^+ DCs were blocked with anti-TIM-3 mAb. These data showed that TIM-3 on DCs prevented transport of nucleic acid into endosomal vesicles by HMGB1.

Many chemotherapy treatments lead to immunogenic cell death of tumor cells, in which endogenous adjuvants such as HMGB1 and nucleic acids are released. However, cis-diamminedichloroplatinum(II) (CDDP, cisplatin) treated MC38 cells loaded onto TIM-3^+ DCs in the presence of anti-TIM-3 mAb, had increased IFN-β1 mRNA production compared to control Ig administration, and these effects were diminished in the presence of DNase and RNase, indicating that TIM-3 affects immunogenicity in a nucleic acid-dependent manner. Furthermore, in the absence of kinase TBK1 and TNF, production of IFN-β1 mRNA decreased regardless of TIM-3 expression on DCs or the inclusion of anti-TIM-3 mAb, suggesting a key role of TBK1 in nucleic acid-mediated innate immunity. Moreover, *in vivo* experiments showed that tumor size in tumor-bearing mice treated with CDDP alongside anti-TIM-3 mAb decreased compared to control Ig, and depletion of DCs in these CD11c-DTR mice led to enhanced tumor size reduction, suggesting that TIM-3 expression specifically on DCs was leading to decreased tumor regression after immunogenic cell death by chemotherapy treatment.

Discussion & significance

Considering that within the tumor microenvironment, T cells are often exhausted and immune responses are skewed away from Th1 responses, targeting the activation and proliferation of tumor infiltrating T cells has been a common antitumor therapy. However, the findings by Chiba *et al*. demonstrate that tumor-mediated immunosuppression can act at

an earlier stage, where DC-mediated innate immune responses are blocked, thus preventing the downstream anti-tumor adaptive immune response to occur. Whereas the expression of TIM-3 has been studied on T cells in the tumor microenvironment, its expression on TADCs was clarified by Chiba *et al.*, who demonstrated that TIM-3 expression on TADCs interacts with HMGB1 and prevents localization of nucleic acids into endosomal vesicles, thus preventing the TADCs from eliciting an anti-tumor response. The authors further go on to demonstrate that TIM-3 expression specifically on TADCs is leading to tumor progression upon chemotherapy treatment, whereas administration of anti-TIM-3 mAb alongside chemotherapy leads to tumor regression. These results demonstrate the importance of targeted therapies to treat cells, such as DCs, that play a vital role during the earlier stages of immunity.

Future perspective

The current paper by Chiba *et al.* demonstrated the role that TIM-3 expressed on TADCs plays in inhibiting a nucleic acid-dependent innate immune response. However, interaction of TIM-3 on APCs with galectin-9 induces DC maturation and anti-tumor T cell immunity [10, 11]. Therefore this dual role of TIM-3 expression on TADCs needed further investigation. The composition of the tumor microenvironment, such as the ratio between galectin-9 and HMGB1, may play a vital role in determining whether TIM-3 expression on DCs can be beneficial or detrimental to the tumor.

Since DCs bridge the gap between innate and adaptive immune responses, targeting TIM-3 on DCs within the tumor microenvironment may prove more effective than pursuing downstream effector cells. The findings of this paper reveal yet another layer of immune suppression that must be effectively combated to obtain significant antitumor immune responses. In doing so, it demonstrates the potential efficacy of therapeutically targeting TIM-3 in conjunction with DNA vaccination and/or chemotherapy where immunogenic cell death occurs by the release of nucleic acids from dying tumor cells. However, since TIM-3 serves as an inhibitory molecule, its blockade may lead to potential autoimmunity which illustrates the delicate balance that must be achieved between beneficial and potentially harmful immunological responses leading to effective antitumor immunity.

Acknowledgments

This work was supported by NIH grants 1 R01 CA138993-01A1 (PS), 1 F31 CA165632-01 (JMP) and 1 F31 CA165897-01 (EB).

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Executive summary

DCs play an important role in the induction of anti-tumor immune response. DCs can become activated through the detection of nucleic acids released from tumor cells following chemotherapy.

Chiba *et al*. revealed that TIM-3 is expressed on tumor associated DCs and its interaction with the nuclear protein HMGB1 prevented localization of nucleic acids into endosomal vesicles, thus preventing IFN-β production and leading to increased tumor burden after immunogenic chemotherapy treatment. However, administration of a mAb against TIM-3 in these tumor bearing mice led to tumor regression.

These results allow for further examination into the use of anti-TIM-3 mAb in combination with DNA vaccination and/or immunogenic chemotherapy in a clinical setting.