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Tumor immunity times out: TIM-3 and HMGB1

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Abstract

The characteristics of the tumor microenvironment vary widely. New work shows that after tumor-associated expression of the receptor TIM-3 by dendritic cells, TIM-3 inhibits the antitumor efficacy of DNA vaccines and chemotherapy by binding to the damage-associated molecular pattern molecule, HMGB1.

In their seminal paper 60 years ago describing the chemical structure of DNA, Watson and Crick closed by stating that “It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.”¹ What did escape their attention, however, was the protean role of DNA expressed outside the nucleus, where it serves as perhaps the most potent natural adjuvant. Particularly in the disordered tumor microenvironment, associated with unscheduled cell death and the release of DNA, the means to balance the twin goals of limiting further damage and promoting repair is mediated by the sensing of DNA by inflammatory cells. In this issue of *Nature Immunology*, Chiba *et al.* find that interaction of the receptor TIM-3 with the chromatin-associated damage-associated molecular pattern molecule, HMGB1, in tumor-associated dendritic cells (TADCs) is critical for evasion of the immune system by tumor cells in response to DNA-containing vaccines and chemotherapy² (Fig. 1a). Thus, examining this previously unknown pathway seems particularly promising for understanding how the response to DNA is limited³.

TIM-3 was first identified as a receptor specifically expressed by T helper type 1 cells³ that binds ligands, including galectin-9 and exposed, cell-surface phosphatidylserine. When bound to Tim-3, galectin-9 generates an inhibitory signal that results in the apoptosis of T helper type 1 cells (Fig. 1b). TIM-3 is also expressed by natural killer cells⁴ and naive dendritic cells (DCs), acting in synergy with Toll-like receptor (TLR) signaling to induce inflammation by activating the transcription factor NF- κ B and enhancing the secretion of proinflammatory cytokines such as TNF. TIM-3 expressed on monocytes and macrophages promotes the phagocytosis of apoptotic cells through interaction with phosphatidylserine, which enhances antigen cross-presentation. Chiba *et al.* find high expression of TIM-3 by TADCs (Fig. 1a), as well as much earlier expression of TIM-3 by TADCs than by CD8⁺ T cells, in mouse models of subcutaneous tumors². Furthermore, they find that TADCs in tumor samples from patients and differentiated DCs from human peripheral blood

monocytes also express TIM-3. Critically, tumor cells as well as tumor-derived immunoregulatory factors (such as IL-10 and VEGF) promote TIM-3 expression on immature bone marrow-derived DCs², which suggests that immunosuppressive factors in the tumor microenvironment induce DCs to express TIM-3.

DCs are professional antigen-presenting cells and constitute several subsets with distinct phenotypes and crucial roles in promoting both immune tolerance and immunity. Immature DCs, which are actively phagocytic, lose the ability to take up antigen when they mature after the recognition of pathogen-associated and damage-associated molecular patterns released from stressed cells. Those molecular patterns interact with pattern-recognition receptors such as TLRs, RLRs, NLRs and RAGE. DCs are essential targets in efforts to generate therapeutic immunity to cancer; they interact with natural killer cells to promote their further activation and immunity to tumors through the release of HMGB1 (ref. 5). DCs can capture tumor antigens released from tumor cells, either alive or dying, and cross-present those antigens to T cells in tumor-draining lymph nodes. This results in the generation of tumor-specific T lymphocytes that contribute to tumor rejection. Chiba *et al.* find that DC-expressed TIM-3 suppresses responses to various nucleic acids (such as ligands for TLR3, TLR7 and TLR9, and cytosolic sensors of DNA and RNA), as measured by the production of cytokines (IFN- β 1 and IL-12) and activation of the transcription factors IRF3 and NF- κ B *in vivo* and *in vitro*². However, TIM-3 has no influence on the TLR2- or TLR4-mediated production of cytokines by bone marrow-derived DCs. Thus, TIM-3 has a crucial and previously unknown role in regulation of nucleic acid-mediated innate immune responses.

HMGB1 is a DNA-binding protein that acts as a chromatin factor with roles in genome stability and the promotion of transcription in the nucleus. In addition to its nuclear role, HMGB1 is translocated to the cytosol and is released into the extracellular space after cell stress and is also actively secreted, with roles in inflammation, immune responses, DC differentiation, the migration of inflammatory cells and mesangioblasts, the regulation of autophagy and mitochondrial 'quality control'. Chiba *et al.* demonstrate that the regulation of nucleic acid-mediated innate immune responses by TIM-3 is dependent on HMGB1 but not on galectin-9 or phosphatidylserine². HMGB1 consists of DNA-binding A and B boxes, as well as an acidic carboxyl terminus. TIM-3 actively competes with nucleic acids to bind the A box of HMGB1. As a receptor for HMGB1, TIM-3 impairs the HMGB1-mediated recruitment of nucleic acids into endosomes, a key step in the sensing of DNA by the innate immune system. Published studies have shown that HMGB proteins (HMGB1–HMGB3) function as universal sentinels for nucleic acid-mediated innate immune responses⁶, and HMGB1 and its receptor RAGE are required for DNA-mediated activation of TLR9. The interactions among TIM-3, RAGE and other HMGB1 receptors (such as TLR2, TLR4 and CD24), autophagy^{7,8} and internalization pathways remain unknown.

Notably, Chiba *et al.* show that DC-expressed TIM-3 diminishes the antitumor efficacy of DNA, as blockade of TIM-3, deletion of TIM-3 or conditional deletion of CD11c⁺ DCs enhances the antitumor potential of a plasmid DNA adjuvant in a model of melanoma subcutaneous tumors². Unexpectedly, they demonstrate that type I interferon and IL-12 are responsible for the synergistic antitumor effects of DNA and blockade of TIM-3, but CD8⁺ T cells or the response to specific antigen are not; other studies, however, indicate that CD8⁺ T cells are required for the antitumor immunity mediated by the blockade of TIM-3 (ref. 2). Individual experimental tumor models might also account for the following or the unique aspects of DNA-dependent responses observed by Chiba *et al.*². In addition, IL-12 also induces expression of TIM-3 and causes T cell exhaustion in lymphoma patients by a mechanism independent of type II interferon (IFN- γ)⁹. The main sources of type I interferon and IL-12 in the tumor micro-environment are plasmacytoid DCs, natural killer cells, T

cells, macrophages and stromal cells, which suggests that elucidating the interactions among different types of cells of the immune response is essential for understanding the full function of TIM-3 in tumor immunity and that other molecules, including PD-1, are important¹⁰.

Chiba *et al.* extend the relevance of their studies to chemotherapy in mouse tumor models². Anticancer chemotherapies are particularly efficient when they elicit immunogenic cell death with the release of damage-associated molecular patterns, including HMGB1, ATP and DNA. Chiba *et al.* show that antibody to TIM-3 enhances the antitumor effects of the chemotherapeutic drug CDDP in a subcutaneous colon tumor model². *In vitro*, treatment with antibody to TIM-3 results in more production of IFN- β 1 and IL-12 induced by dying cancer cells; these cytokines are dependent on damage-associated molecular patterns (such as DNA and HMGB1) and the kinase TBK1. These results suggest that it might be beneficial to couple treatments with agents that block the expression or activity of TIM-3.

Several issues remain unresolved about the role of TIM-3 in tumor responses. First, the function of TIM-3 in other tumor-infiltrating non-DC cells, such as macrophages, T cells and stroma, is unclear. The crosstalk among TIM-3⁺ and TIM-3⁻ DC subsets and cells of the immune response in tumor microenvironments remains to be explored. Further studies of the origins, inducers and functional specialization of TIM-3-expressing DCs in the tumor microenvironment could help elucidate the balance between the promotion and inhibition of antitumor immune responses. Additionally, the nature of the DNA, whether it contains CpG motifs that 'preferentially' bind HMGB1 or whether it is oxidized or in some other format remain important issues.

Second, how can ligation of TIM-3 on different cell types mediate such different effects? TIM-3 includes an amino-terminal immunoglobulin variable domain followed by a mucin domain, a transmembrane domain and a cytoplasmic tail. Galectin-9 binds to TIM-3 through amino-linked carbohydrates, whereas HMGB1 and phosphatidylserine bind to TIM-3 through a metal ion– dependent ligand-binding site in the FG loop of the immunoglobulin variable domain. The binding of ligands to the extracellular site of this receptor leads to conformational changes in the cytoplasmic site, which activates alternative signaling pathways. Post-translational modification such as phosphorylation can affect the function of TIM-3 in T cells and possibly also in DCs.

Third, what are the roles of autophagy and phagocytosis in the regulation of the HMGB1–TIM-3 signaling pathway? Dying tumor cells mobilize at least three types of signals when interacting with DCs and other phagocytes, including signals that indicate 'find me', 'eat me' or 'do not eat me'. Reactive oxygen species are emerging as regulators of 'eat-me' processes such as autophagy (a lysosomal degradation pathway) and phagocytosis (a process of killing cells or microbes). HMGB1 is well positioned at the crossroads of autophagy¹¹, phagocytosis and oxidative stress. Reduced, nonoxidized HMGB1 promotes autophagy, inflammation and survival, whereas oxidized HMGB1 promotes apoptosis and immune tolerance. Autophagy not only regulates pathogen-associated innate and adaptive immunity but also immunity mediated by the death of tumor cells¹¹. Such findings, including the identification of scaffolding molecules such as PKR that are important in HMGB1 secretion¹², may help guide studies of the molecular mechanism by which HMGB1–TIM-3 signaling regulates escape from the immune response.

Finally, these studies have focused on the role of DC-expressed TIM-3 in nucleic acid–mediated antitumor immune responses. Because the DNA or RNA of viruses or bacteria contributes to 15–20% of all human cancers, the potential interactions of redox variants of HMGB1 (ref. 13), DNA and TIM-3 in the tumorigenesis of virus-associated human cancer

need to be studied. The targeting of TIM-3 in immunological diseases (such as psoriasis and arthritis)¹⁴ or human cancer¹⁵ needs further study as a means of enhancing tumor-specific immunological memory and limiting the side effects elicited by the present cancer therapies.

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Figure 1.

TIM-3 serves as a major regulator of immunity. **(a)** TIM-3 suppresses nucleic acid-mediated antitumor immune responses and promotes cancer immune escape by directly binding HMGB1. Blocking TIM-3 through the use of antibody to TIM-3 enhances the antitumor efficacy of DNA vaccines and a nonimmunogenic chemotherapeutic agent by acting on DCs in the tumor microenvironment (TADCs). mAb, monoclonal antibody; IFN, interferon. **(b)** Expression and function of TIM-3 in various cells of the immune system. T_H1, T helper type 1; NK, natural killer.