“Flagellated” cancer cells propel anti-tumor immunity

Johan Garaude¹ and J. Magarian Blander²

¹Institute for Research in Biotherapy; INSERM U1040; Montpellier, France; ²Department of Medicine; Immunology Institute; The Tisch Cancer Institute; Mount Sinai School of Medicine; New York, NY USA

Keywords: Toll-like receptor, Nod-like receptor, flagellin, pattern recognition receptor, vaccination, immunotherapy, inflammasome, T cell response, adaptive immunity, innate immunity

The use of innate immune receptor agonists in cancer therapies has suffered from many drawbacks. Our recent observations suggest that some of these hurdles can be overcome by introducing flagellin into tumor cells to promote tumor antigen presentation by dendritic cells (DCs) and simultaneously trigger two types of pattern recognition receptors (PRRs).

Pathogens possess a variety of molecular structures [collectively termed pathogen-associated molecular pattern (PAMPs)] that are perceived by the immune system through PPRs, which signal the presence of an infection. Thus, various PAMPs—those for Toll-like receptors (TLRs)—have been included in cancer vaccine compositions with the goal of mobilizing immune immunity against tumor cells in a manner similar to that against a life threatening infection.¹ The rationale was based on the knowledge that PRR engagement induces co-stimulatory molecule expression and secretion of pro-inflammatory cytokines critical for effective immunity.² While developing these strategies, it was neglected that most PAMPs are in fact physically or spatially associated with microbial antigens, and therefore, end up in the same phagocytic compartment of antigen-presenting cells (APCs) where antigens are loaded onto major histocompatibility complex molecules (MHC). A few years ago, Blander and Medzhitov uncovered a phagosome autonomous mechanism of MHC Class II (MHC-II) presentation in DCs whereby TLR signaling via PAMPs present within phagosomes induces the selective loading of antigens within those phagosomes allowing for the selection of microbial antigens for presentation to T cells within a co-stimulatory and inflammatory context.³ This type of “associative recognition”² of antigen with PAMPs thus became an important biological process that could potentially be exploited in designing tumor vaccines. Hence we reasoned that introducing a PAMP within tumor cells would supply the pattern characteristic of pathogens, allowing APCs to mark tumor-associated antigens (TAA) for successful priming of tumor-specific T cells. We forced expression of flagellin, a bacterial protein that triggers TLR5, into various tumor cell lines to ensure its co-delivery into phagosomes that also contain tumor antigens.⁴ This strategy efficiently induced tumor cell clearance by innate immune cells, prevented tumor growth and activated tumor-specific CD8⁺ and CD4⁺ T-cell responses in mice (Fig. 1). When used as an anti-tumor treatment or cancer vaccine, irradiated flagellin-expressing tumor cells efficiently impaired parental tumor growth. In sharp contrast, co-administration of recombinant flagellin with tumor cells did not reproduce these effects underscoring the importance of co-delivery of TLR ligand and antigen to the same antigen processing compartments (Fig. 1). This can be achieved either via direct linkage of the flagellin to a TAA, or more simply via the expression of flagellin within tumor cells such that tumor antigens are physically present with flagellin within the same phagosomal space.⁴ In fact, our studies demonstrated that expression of flagellin-antigen fusion proteins within tumor cells was not necessary for protection against tumor.⁴

One of the mechanisms by which TLR signaling controls antigen presentation by MHC-II is through inducing proteolytic degradation of the invariant chain (Li or CD74), which protects the MHC-II binding groove from premature peptide binding.⁵ This could explain how CD4⁺ T cells are primed by flagellin-expressing tumor cells. However, tumor-specific CD8⁺ T cell cross-priming was also enhanced upon injection of flagellin-expressing cells, but not when flagellin was co-injected suggesting that physical association of TLR ligand and antigens also induces cross-presentation. How TLRs regulate this process is still imperfectly understood.⁵ A recent study uncovered a role for CD74 in MHC-I trafficking from endoplasmic reticulum to endolysosome in cross-presentation.⁶ This raises the possibility that a mechanism similar to the one described for MHC-II mediated CD4⁺ T cell priming may also ensure TLR regulation of CD8⁺ T cell cross-priming.

Another advantage of choosing flagellin as a PAMP to introduce into tumor cells is that it is sensed by NAIP5 (Neuronal apoptosis inhibitor protein 5) when localized in the cytosol of myeloid cells. In turn, NAIP5 recruits NLRC4...
but how NLRs control T cell priming is still elusive. Work from the groups of L. Zitvogel and G. Kroemer has demonstrated importance of the NLRP3 inflammasome in priming tumor-specific CD8+ T cells via controlling IL-1β secretion in response to chemotherapy induced tumor cell death.10 In light of these findings, it would be important to address whether inflammasome-dependent cytokines are also required for adaptive antitumor immune responses generated by flagellin-expressing cells.

We thus propose two approaches to improve the clinical use of TLR agonists in cancer vaccines. First, promoting associative recognition of tumor antigens with a TLR ligand can easily be achieved once a TAA has been identified or by introduction of flagellin in tumor cells following tumor resection to enhance presentation of several TAAs. Second, TLR ligands may be combined with NLR ligands to evoke inflammasome-dependent immune responses. However, careful assessment of which combinations of TLR and NLR ligands provide the highest application potential in the clinic will be a critical step toward the design of new adjuvant-based cancer immunotherapies.

References
1. Kanzler H, Barrat FJ, Hessel EM, Coffman RL. Therapeutic targeting of innate immunity with Toll-like receptor agonists and antagonists. Nat Med 2007; 13:552-9; PMID:17479101; http://dx.doi.org/10.1038/nm1589.
2. Iwasaki A, Medzhitov R. Regulation of adaptive immunity by the innate immune system. Science 2010; 327:291-5; PMID:20075244; http://dx.doi.org/10.1126/science.1183021.
3. Blander JM, Medzhitov R. Toll-dependent selection of microbial antigens for presentation by dendritic cells. Nature 2006; 440:808-12; PMID:16489357; http://dx.doi.org/10.1038/nature04596.
4. Garaude J, Kent A, van Rooijen N, Blander JM. Simultaneous targeting of toll- and nod-like receptors induces effective tumor-specific immune responses. Sci Transl Med 2012; 4:20; PMID:22352829; http://dx.doi.org/10.1126/scitranslmed.3002868.
5. Watts C, West MA, Zaru R. TLR signaling regulated antigen presentation in dendritic cells. Curr Opin Immunol 2010; 22:124-30; PMID:2083398; http://dx.doi.org/10.1016/j.coi.2009.12.005.
6. Basha G, Omilusik K, Chavez-Steenbock A, Reinicke AT, Lack N, Choi KB, et al. A CD74-dependent MHC class I endolysosomal cross-presentation pathway. Nat Immunol 2012; 13:237-45; PMID:2230692; http://dx.doi.org/10.1038/ni.2225.
7. Koofoed EM, Vance RE. Innate immune recognition of bacterial ligands by NAIPs determines inflammasome specificity. Nature 2011; 477:592-5; PMID:21874021; http://dx.doi.org/10.1038/nature10394.
8. Hu B, Elinav E, Huber S, Booth CJ, Strowig T, Jin C, et al. Inflammation-induced tumorigenesis in the colon is regulated by caspase-1 and NLRC4. Proc Natl Acad Sci USA 2010; 107:21635-40; PMID:21118981; http://dx.doi.org/10.1073/pnas.1016814108.

9. Normand S, Delanoye-Crespin A, Bessenot A, Huot L, Grandjean T, Peyrin-Biroulet L, et al. Nod-like receptor pyrin domain-containing protein 6 (NLRP6) controls epithelial self-renewal and colorectal carcinogenesis upon injury. Proc Natl Acad Sci USA 2011; 108:9601-6; PMID:21593485; http://dx.doi.org/10.1073/pnas.1100981108.

10. Ghiringhelli F, Apetoh L, Tesniere A, Aymeric L, Ma Y, Ortiz C, et al. Activation of the NLRP3 inflammasome in dendritic cells induces IL-1beta-dependent adaptive immunity against tumors. Nat Med 2009; 15:1170-8; PMID:19767732; http://dx.doi.org/10.1038/nm.2028.