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Revisiting the immunologist's little secret to improve cancer immunotherapy

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Abstract

Synergy between intracellular and extracellular sensing mechanisms of the innate immune system improve adaptive immune responses to cancer vaccines and clearance of tumors

We now think of the immune system as a collaboration between innate and adaptive arms, in which the latter was originally characterized by specificity, diversity and memory, whereas the former lacked these three key elements and consisted of phagocytic or lytic cells or molecules without apparent antigen specificity or memory. Indeed, a feature of the adaptive immune system and its receptors that has set it apart from many other physiologic systems is its exquisite ability to detect and distinguish among many small variations in the structure of antigen molecules, even ones that never occurred in nature. Thus, much interest was focused on the mechanisms of this very finely specific recognition, both at the structural level of the receptors on T and B lymphocytes, and at the genetic level to account for the generation of their diversity. Dissecting this diversity required immunization with soluble proteins and peptides, and this in turn required the use of adjuvants. The need for such adjuvants, termed “the immunologist’s dirty little secret” by Charlie Janeway (1), was accepted but not fully understood. However, this requirement and other findings led to more recent focus on novel receptor molecules of the innate immune system that alerted the adaptive immune system to the presence of danger, such as bacteria and viruses that might be pathogens. These were termed Pattern Recognition Receptors (PRRs) and their ligands displayed Pathogen-Associated Molecular Patterns (PAMPs) (2) or later also Danger-Associated Molecular Patterns (DAMPs) (3). These receptors not only activate the innate immune mechanisms, including phagocytosis and killing of intracellular bacteria and production of antiviral molecules like interferons, but also induce production of cytokines and maturation of antigen-presenting dendritic cells (DCs) that in turn activate the adaptive immune system.

The first such PRRs discovered were found on the surface of cells, especially those of the myeloid lineage like macrophages and DCs, or inside cellular vesicular structures such as endosomes, but outside the cytosol. Because of their homology with the Toll molecule of the fruit fly, these were called Toll-like receptors (TLRs). About 10–12 of these are now identified in humans and mice, detecting molecules that distinguish bacteria and viruses from mammalian cells, such as viral double or single-stranded RNA, or bacterial flagellin, cell wall/membrane components, or DNA (which is richer in CpG sequences than mammalian DNA) (2, 4). More recently, another class of PRRs was found in the cytosol of cells, divided structurally into two major families, the NOD-like receptors (NLRs) and the

RIG-like helicases (RLHs) (5). These detect the presence of viral molecules like double-stranded RNA or bacterial molecules like flagellin in the cytosol of the cell, and signal the presence of such intracellular parasites. Some of these intracellular receptors have or interact with adaptor molecules having segments such as caspase recruitment domains (CARDs) that activate caspase-1, which in turn cleaves precursor molecules and thereby triggers maturation of IL-1 β and IL-18, two pro-inflammatory cytokines. In particular, the NLRs form large intracytoplasmic complexes called inflammasomes that mediate the production of such a pro-inflammatory response. In addition to their direct activity, IL-1 and IL-18 through their receptors lead to activation of the same adaptor molecule, MyD88, that transduces the activity of the majority of TLRs, so the MyD88 pathway can be considered downstream of NLR inflammasomes as well as of TLRs (5).

The discovery of PRRs provided mechanisms explaining the activity of immune adjuvants. Besides a depot function provided by some adjuvants, their mechanism of action is now understood to include the activation of such PRRs to alert the immune system to pathogens, activate antigen-presenting DCs, and thereby trigger an adaptive immune response. For example, the mycobacteria in complete Freund's adjuvant provide bacterial DNA with CpG sequences that trigger TLR9, as well as possibly other TLR ligands. The adjuvants containing endotoxin-like bacterial or synthetic molecules trigger TLR4. Even alum, the most widely used adjuvant and the only one used in licensed vaccines in the US, which consists of inorganic aluminum salts that form precipitates, has now been found to act at least in part by activating the NALP3 inflammasome and caspase-1. Thus, the need for activation of intra- or extracellular PRRs appears to explain "the immunologist's dirty little secret" to enhance immunogenicity.

While such PRRs are often adequate to allow the innate immune system to recognize the presence of pathogens, they fall down when it comes to immunosurveillance of tumor cells, which are host mammalian cells that do not express such PAMPs (although molecules like ATP released from dying cells can activate inflammasomes (5)). Even though tumors often contain mutated proteins or proteins not expressed in adult tissues, which could serve as potential tumor antigens, and even though immunosurveillance has now been well documented to occur and to lead to immuno-editing that can result in tumor escape from such surveillance (6)), a remaining problem with tumor immunotherapy is making tumor cells or their antigens that resemble self sufficiently immunogenic. Thus, adjuvants containing PAMPs such as TLR ligands have been used to improve the efficacy of cancer vaccines. In pathogens, TLR ligands often occur in combinations, and accordingly, researchers have looked for possible synergies between different TLRs and found that certain combinations of TLR ligands are more effective than either alone (7–9). Indeed, such synergies can lead not only to quantitative increases in immune response, but also improvements in quality of immune response, such as T-cell avidity, important for clearance of viruses and cancers (8, 10). In the current issue, Garaude et al (11) report a different type of synergy, in which a single bacterial molecule, flagellin, serves to activate two different classes of PRRs, the cell surface TLR5 and an intracytoplasmic NLR, the NLRC4/NAIP5 inflammasome (also known as the IPAF/NAIP inflammasome). While activation of TLR5 alone is sufficient to trigger inflammatory cells to clear co-injected tumor cells in mice by innate mechanisms, the combination of both TLR and NLR signals was found necessary to

induce effective CD4⁺ and CD8⁺ T cell responses to provide long-term immunity against the parent tumor and to cause rejection of established tumors. Disruption of flagellin recognition by either TLR5 or NLRC4/NAIP5 by point mutations or use of knock-out mice was sufficient to abrogate protection, demonstrating that both functions were necessary for T cell activation and tumor rejection.

Further, because tumor antigens are often unique to individual cancers, it would be desirable to be able to use whole tumor cells as vaccines, so that any tumor antigen present could potentially induce a protective response. Because, as mentioned, the tumor cells notoriously lack PAMPs, they are generally not sufficiently immunogenic to serve as therapeutic cancer vaccines. Garaude et al (11) show that just mixing tumor cells with recombinant protein flagellin, while sufficient to induce innate cell recruitment and clearance of injected live tumor cells in the vicinity, is not adequate when mixed with irradiated tumor cells as a vaccine to induce an adaptive CD4⁺ or CD8⁺ T cell response or to lead to subsequent tumor rejection. In contrast, expression of flagellin by retroviral transduction of tumor cells, either alone or as a fusion protein with a tumor antigen, was required to induce an adaptive immune response and tumor rejection. This may be in part because, as the authors had previously shown, DCs segregate particles containing TLR ligands from particles that do not, such as apoptotic tumor cells, into different phagocytic vesicles (12). Only the vesicles that contain TLR ligands were able to process antigen for presentation to class II MHC-restricted CD4⁺ T cells because the TLR trigger was found necessary to induce cleavage of the CLIP peptide that protects the class II MHC molecule from loading antigenic peptides. Thus, only peptides from the phagocytosed particles containing TLR ligands were loaded onto class II MHC molecules that were transported to the cell surface to activate CD4⁺ T cells, whereas peptides from particles lacking TLR ligands, even taken up by the same DCs, were not presented (12). Further, TLR induction of MyD88 signaling was necessary for relocation to early endosomes of the transporter of antigenic peptides (TAP) necessary for loading of class I MHC molecules to present peptides to CD8⁺ T cells (13). Thus, both types of T cell responses required association between the PAMP and the tumor antigen to achieve effective immunogenicity. Importantly, however, Garaude et al (11) show that expression of flagellin in the tumor, even without linkage as a fusion protein to a defined tumor antigen, is sufficient to induce a strong CD4⁺ and CD8⁺ T cell response to such irradiated cells and cause elimination of the parental tumor that itself lacks flagellin expression. This approach then simultaneously solves two key problems for cancer vaccines and immunotherapy, the need to make tumor cells or their antigens more immunogenic, and the need to induce responses to tumor cells without having to identify and purify or clone specific tumor antigens.

The problem remains how to translate this novel strategy from murine to human tumors and patients. Flagellin is one of the few TLR ligands that is a protein that can be expressed by recombinant DNA technology, lending itself to expression in tumor cells or DCs. The studies of Garaude et al (11) used established murine tumor cell lines that could be retrovirally transduced to express flagellin or a flagellin-tumor antigen fusion protein. In human patients, tumor cells are not always readily available, and when they are, are often difficult to grow in culture to transduce them. For that reason, this approach in its original form may be limited to tumors that can be grown in culture, at least transiently. The use of

allogeneic tumor cell lines is problematic because they will not express the same HLA molecules to present the antigens, although if the apoptotic tumor cells are taken up by DCs and their antigens cross-presented on host HLA molecules, then that strategy could work. In that circumstance, alloantigens may even provide sources of “help” to improve the immune response to the tumor antigens. However, immune responses could be generated only to shared tumor antigens expressed in the allogeneic tumor cells, not to antigens unique to the patient’s own tumor. Another approach, if there are known tumor antigens expressed, would be to produce a fusion protein gene linking flagellin with that known tumor antigen and express it in a vector that could be used for immunization, or in the patient’s autologous PMBC that could be irradiated and used as a vaccine to be phagocytosed and cross-presented by DCs. That these pathways apply in human cancers can be seen in a recent study examining the role of ATP released from dying tumor cells after treatment with anthracycline chemotherapy, which then interacts with the P2RX7 receptor to activate the NLRP3 inflammasome (14). Breast cancer patients with an inherited mutation in this receptor developed metastases sooner than those without the mutation, indicating the ability of inflammasome-mediated innate immunity to trigger a protective immune response. Thus, exploiting the synergy between two innate receptors, the TLRs and the NLRs, should provide a rational strategy to take advantage of our current better understanding of the “immunologist’s dirty little secret” to provoke an immune response to otherwise not-very-immunogenic tumor cells or tumor antigens. We hope thereby to improve the prospects for effective immunotherapy of cancer.

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References

1. Janeway CA, Jr, Approaching the asymptote? Evolution and revolution in immunology. Cold Spring Harb Symp Quant Biol 54 Pt 1, 1 (1989).
2. Janeway CA, Jr, Medzhitov R, Innate immune recognition. Annu Rev Immunol 20, 197 (2002). [PubMed: 11861602]
3. Matzinger P, The danger model: a renewed sense of self Science 296, 301 (2002); [PubMed: 11951032]
4. O’Neill LA, The interleukin-1 receptor/Toll-like receptor superfamily: 10 years of progress. Immunol Rev 226, 10 (2008); [PubMed: 19161412]
5. Martinon F, Mayor A, Tschopp J, The inflammasomes: guardians of the body. Annu Rev Immunol 27, 229 (2009)10.
6. Dunn GP, Old LJ, Schreiber RD, The three Es of cancer immunoediting. Annu Rev Immunol 22, 329 (2004). [PubMed: 15032581]
7. Napolitani G, Rinaldi A, Berton F, Sallusto F, Lanzavecchia A, Selected Toll-like receptor agonist combinations synergistically trigger a T helper type 1-polarizing program in dendritic cells. Nat Immunol 6, 769–76 (2005); [PubMed: 15995707]
8. Zhu Q, Egelston C, Gagnon S, Sui Y, Belyakov IM, Klinman DM, Berzofsky JA, Using 3 TLR ligands as a combination adjuvant induces qualitative changes in T cell responses needed for antiviral protection in mice. J Clin Invest 120, 607 (2010); [PubMed: 20101095]
9. Kwissa M, Kasturi SP, Pulendran B, The science of adjuvants Expert Rev Vaccines 6, 673 (2007); [PubMed: 17931149]

10. Berzofsky JA, Ahlers JD, Belyakov IM, Strategies for designing and optimizing new generation vaccines. *Nature Reviews Immunology* 1, 209 (2001).
11. Garaude J, Kent A, van Rooijen N, Blander JM, Superior tumor immunotherapy by simultaneous targeting of Toll-like and Nod-like receptors. *Science Translational Medicine* In press, (2012).
12. Blander JM, Medzhitov R, Toll-dependent selection of microbial antigens for presentation by dendritic cells *Nature* 440, 808 (2006); [PubMed: 16489357]
13. Burgdorf S, Scholz C, Kautz A, Tampe R, Kurts C, Spatial and mechanistic separation of cross-presentation and endogenous antigen presentation *Nat Immunol* 9, 558 (2008); [PubMed: 18376402]
14. Ghiringhelli F, Apetoh L, Tesniere A, Aymeric L, Ma Y, Ortiz C, Vermaelen K, Panaretakis T, Mignot G, Ullrich E, Perfettini JL, Schlemmer F, Tasdemir E, Uhl M, Genin P, Civas A, Ryffel B, Kanellopoulos J, Tschopp J, Andre F, Lidereau R, McLaughlin NM, Haynes NM, Smyth MJ, Kroemer G, Zitvogel L, Activation of the NLRP3 inflammasome in dendritic cells induces IL-1beta-dependent adaptive immunity against tumors. *Nat Med* 15, 1170 (2009); [PubMed: 19767732]

Fig. 1- Scheme of hypothetical synergistic action of a TLR and NLR to induce immune response to cancer vaccine of irradiated tumor cells expressing flagellin, an agonist for both TLR5 and NLRC4 inflammasomes.

Tumor cells transduced to express flagellin, when irradiated, form apoptotic bodies that are phagocytosed by DCs. In phagosomes, endocytosed TLR5 increases loading of MHC II with tumor antigens by promoting removal of the Ii CLIP peptide, and increases TAP recruitment to promote cross-presentation of tumor antigens on MHC I, based on the references cited. Flagellin also enters the cytosol where it activates the NLRC4 inflammasome to cause

maturation and secretion of cytokines IL-1 β and IL-18. These effects synergize to induce CD4⁺ and CD8⁺ T cells that cause tumor rejection.

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