

# Antagonizing the innate pattern recognition receptor CD204 to improve dendritic cell-targeted cancer immunotherapy

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Extensive studies have established a role of scavenger receptor CD204 in pattern recognition and ligand uptake. Strikingly, we recently revealed a previously unrecognized feature of CD204 action in attenuating T-cell activation and antitumor immunity. Blocking its activity in dendritic cells represents a promising approach to the improvement of cancer immunotherapy.

CD204, also called scavenger receptor A, is a phagocytic pattern-recognition receptors (PRRs) expressed primarily on macrophages and dendritic cells (DCs) that are sentinels in the body. Extensive studies in the past two decades have demonstrated that CD204 binds to a broad range of polyanionic ligands (e.g., modified lipid proteins, pathogen-derived molecules), and is involved in atherosclerosis as well as innate defense against pathogens. However, the regulatory role of CD204 in an adaptive T cell response and tumor immunity was not appreciated until the recent identification of heat shock protein (HSP)-binding receptors on antigen-presenting cells (e.g., DCs). It was believed that the highly efficient immune stimulating activities of HSP-based vaccines required interactions with their specific receptors.<sup>1</sup> Given that these putative receptors, including CD204, contributed to the binding and uptake of HSPs, at least under culture conditions, we investigated the impact of CD204 on HSP vaccine-generated antitumor immunity using CD204 knockout mice. Strikingly, we discovered that genetic ablation of CD204 profoundly enhanced tumor protective immune responses induced by HSP-based vaccines.<sup>2</sup> Surprisingly, the

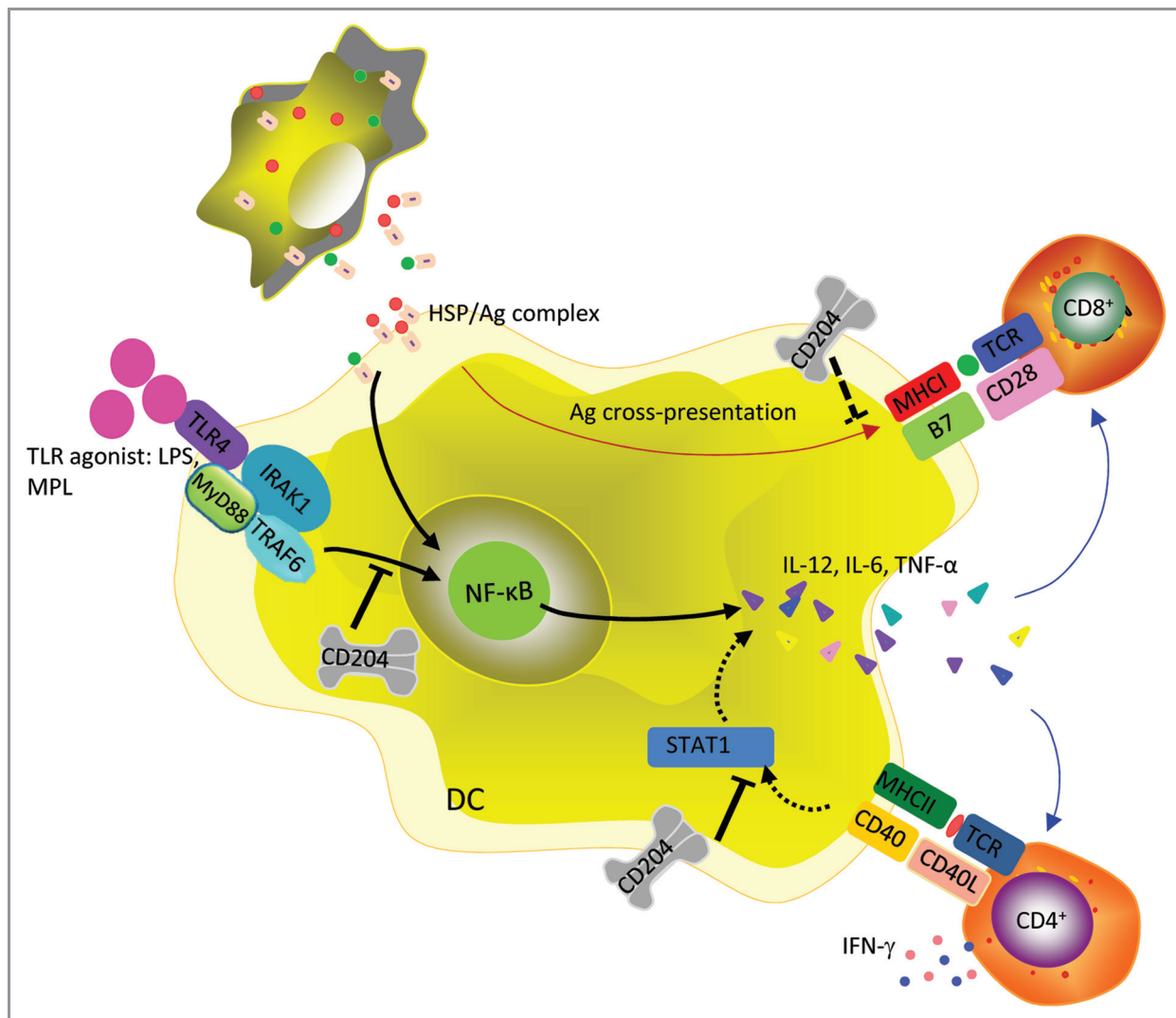
lack of CD204 also restored the immune recognition or immunogenicity of several poorly immunogenic mouse tumors, which depended on CD8<sup>+</sup> T cells and phagocytic cells. CD204 ablation-enhanced antitumor immunity was also similarly seen following vaccination designed to engage the TLR4 signaling pathways by using monophosphoryl lipid A (MPL),<sup>3</sup> a TLR4 agonist used extensively in clinical trials. These findings indicate that CD204 is capable of dampening the immunostimulatory adjuvant activities derived from damage-associated molecular patterns (DAMPs, e.g., HSPs) or pathogen-associated molecular patterns (PAMPs, e.g., LPS). Moreover, the observations of a robust cytotoxic T cell response in the presence of CD204 also raise a question regarding the classic phagocytic property of this molecule in antigen uptake and subsequent T cell priming. Despite that antigen sampling is essential for the transfer and presentation of antigenic information, the possibility remains to be determined as to whether the CD204-mediated antigen capture or HSP binding induces tolerance *in vivo*.

The distinct CTL responses observed *in vivo* can be attributed, at least partially,

to the CD204-altered immunostimulatory activity of DCs upon TLR4 activation, as evidenced by upregulation of co-stimulatory molecules in DCs from LPS-challenged mice, and elevation of proinflammatory cytokines and chemokines in LPS-stimulated DCs.<sup>3</sup> These results were also mirrored by increased susceptibility of CD204-deficient mice to endotoxic shock.<sup>4</sup> Indeed, our recent work uncovered a molecular basis underlying the CD204-mediated suppression of an inflammatory response in DCs induced by TLR4 ligation.<sup>4</sup> Interestingly, CD204 interferes with LPS-induced recruitment of TRAF6 and its trimerization, as well as TRAF6 ubiquitination, which results in attenuation of the TLR4-MyD88-TRAF6-NFκB signaling cascade. It has been shown that TLR4-NFκB activation is essential for the functions of DCs, and plays a pivotal role in the cross-presentation of tumor cell-associated antigens following cancer therapies.<sup>5</sup> Thus, CD204 inhibition or blockade enhances DC activation in response to inflammatory “danger” signals, such as LPS,<sup>3</sup> HSP<sup>6</sup> or cell lysates,<sup>7</sup> which contributes to an increased CTL response and antitumor immunity.

We have also demonstrated that vaccination with MPL leads to a robust

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**Figure 1.** CD204 acts as an immune regulator inhibiting DC functions and T cell activation. Upon stimulation with adjuvant (e.g., MPL or HSP), intracellular CD204 interferes with the NF- $\kappa$ B signaling pathways in DCs, resulting in reduced functions of DCs in presenting tumor antigens (Ags) and stimulating CD4<sup>+</sup> or CD8<sup>+</sup> T cells. During DC-T cell interaction at a physiologic mode, CD204 also inhibits STAT1 activity induced by CD40 ligation and IFN- $\gamma$  stimulation, therefore limiting the activation of a Th1 response.

Th1 response in the absence of CD204, which is signified by preferential production of IFN $\gamma$ , not IL-4 or IL-17, in antigen-specific CD4<sup>+</sup> T cells. However, CD204 ablation does not influence the induction and regulatory activity of CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup> regulatory T cells (Treg).<sup>8</sup> It is intriguing that CD204 suppresses CD4<sup>+</sup> T cell-stimulating capability of DCs even in the absence of LPS-mediated inflammatory stimulus, suggesting that CD204 may also serve as an intrinsic regulator restricting DC activity.<sup>8</sup> Indeed, CD204 downregulates the activation of Janus kinase 1 (JAK)/signal transducer and activator of transcription (STAT1), mitogen-

activated protein kinase (MAPK) p38 and NF $\kappa$ B signaling in DCs upon CD40 ligation combined with IFN $\gamma$  stimulation, which mimics a physiologic mode of DC-T cell interaction (Fig. 1).

The suppressive activity of CD204 in DC functions makes it an appealing target for cancer immunotherapy. Given the recent approval of the DC vaccine sipuleucel-T (Provenge) by the US Food and Drug Administration for treatment of metastatic prostate cancer, the selective blockade of CD204 activity using genetic, pharmacologic or immunologic strategies are expected to enhance the anticancer potency of DC vaccines or other

DC-targeted immunotherapies. Recently, we have successfully achieved CD204 silencing in DCs using lentivirus-mediated shRNA approach. These genetically modified DCs with reduced CD204 expression exhibited markedly increased capability to immobilize CTLs recognizing tumor-associated antigens, resulting in significantly improved therapeutic efficacy in controlling established melanoma and its metastases. This enhanced vaccine activity was accompanied with greater tumor infiltration by CD8<sup>+</sup> T cells and NK cells, as well as increased intratumoral ratios of T<sub>eff</sub> to T<sub>reg</sub>.<sup>9</sup> Study from other group also reported that administration

of CD204-targeted immunotoxin substantially inhibited the burden of ovarian cancer.<sup>10</sup>

Tumor-associated macrophage and tumor-derived suppressor cells, are prominent in the tumor microenvironment, and are known to engage in an extensive and dynamic crosstalk with other immune cells as well as tumor cells. These tumor-infiltrating myeloid cells can suppress antitumor T cell response and promote tumor progression or invasion. Given the

abundant expression of CD204 on these myeloid cells, it is of importance to determine how this immunosuppressive molecule may potentially influence the tumor microenvironment during tumor progression or in response to cancer therapies.

Collectively, our recent work underscored a previously unrecognized immunoregulatory function of CD204 in an adaptive T cell response and antitumor immunity. Although more studies are

necessary to define the molecular mechanisms by which the CD204 restricts T cell activity, accumulation evidence indicates that CD204 on DCs and possibly other myeloid cells executes regulatory functions beyond pattern recognition or ligand uptake. Elucidating the precise immunologic activities of CD204 should provide new opportunities to develop novel approaches that strategically target this molecule for improving the efficacy of immunotherapy against human cancer.

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