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One Clever Macrophage Checkpoint

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Macrophages are a key component of the tumor microenvironment (TME). Blocking Clever-1, a molecule expressed in M2-like macrophages, unleashed macrophage and T-cell–mediated antitumor immunity. Myeloid checkpoints including Clever-1 hold promise as alternative or complementary immunotherapy strategies.

See related article by Viitala et al., p. 3289

In this issue of *Clinical Cancer Research*, Viitala and colleagues (1) provide evidence that targeting Clever-1, a molecule expressed in M2-polarized macrophages and in tumor-associated macrophages (TAM), results in antitumor activity in transplanted mouse tumor models by unleashing a protective CD8 T-cell response. Targeting Clever-1 synergized with anti–PD-1 checkpoint blockade therapy in resistant cancers. The tumor microenvironment (TME) plays an essential role in the initiation and progression of cancer. TAMs are an important component of the TME and engage in complex bidirectional interactions with cancer cells, stroma, and immunocompetent cells (2). Myeloid cells can exert dual functions in the control of tumor growth and progression. Classically activated M1 macrophages can mediate extracellular killing of tumor cells or elicit tumor tissue destructive reactions. Moreover, myeloid cells recognize antibody-coated tumor cells and mediate antibody-dependent cellular cytotoxicity or phagocytosis. In progressing cancers, TAM are diverse and some have a phenotype similar to alternative activated M2 macrophages and behave as corrupted policemen orchestrating a protumor TME. Indeed, M2 or M2-like macrophages promote angiogenesis and tissue remodeling, which sustain tumor growth and dissemination and suppress effective adaptive immunity.

Myeloid cells are endowed with a complex and diverse armamentarium of immunosuppressive mediators, including triggers of checkpoint blockade in T cells and NK cells (2). Accordingly, evidence suggests that under many clinical instances myeloid cells are a major determinant of resistance to checkpoint blockade immunotherapy.

As generally true for immunocompetent cells, macrophages, and neutrophils are under strict control by negative regulators. Viitala and colleagues (1) report that Clever-1, also known as Stabilin-1, serves as a checkpoint in TAMs and genetic or mAb-mediated inactivation of this molecule unleashes a protective immune response against a variety of transplanted tumor cell lines (lung, breast, lymphoma, and colon). Targeting Clever-1 was associated with a change of TAM metabolic state with increased glycolysis and mTOR activity. In addition, there was a switch in the TAM phenotype toward an M1-like profile that resulted in the activation of a protective CD8-mediated response. Interestingly, Clever-1 targeting resulted in decreased PD-L1 expression both in TAMs and cancer cells, indicating that it can block this mechanism of immunotherapeutic resistance (Fig. 1). In accordance, anti–Clever-1 had additive or synergistic activity with anti–PD-1 treatment only in the immunogenic tumor models with higher PD-L1 expression (breast and colon), but not in the poorly immunogenic one (lung).

Clever-1 belongs to the broad family of scavenger receptors, a heterogeneous group of molecules originally identified by their ability to recognize and remove modified lipoproteins and also involved in pathogen recognition and clearance. Clever-1 mediates the uptake and targeting for degradation of endogenous proteins like oxidized low-density lipoproteins and the extracellular matrix glycoprotein, secreted protein acidic and rich in cysteine (SPARC). It has a restricted pattern of expression being present on endothelial cells and in subpopulations of macrophages. It is induced in macrophages by signals that induce an M2 or M2-like polarization with immunosuppressive function. In this article, Clever-1 was expressed in a subset of TAM (~30%) and its blocking resulted in antitumor activity. TAMs are diverse as now revealed by single-cell analysis. It will be important to dissect the expression of Clever-1 and other novel molecules associated with an M2-like, immunosuppressive phenotype (e.g. ref. 3) in different human tumors and assess their prognostic significance. Interestingly some of the molecules expressed by M2 macrophages, like scavenger receptors, not only mediate their prototypical functions, such as scavenging, but also activate signaling pathways required for the polarized immunosuppressive phenotype and can represent selective targets for cancer therapy. Compared with pan macrophage blocking strategies such as mAb or kinase inhibitors targeting the CSF1 receptor that have given limited results in clinical trials, molecules such as Clever-1 have the conceptual advantage of being aimed at a subset of TAM, thus leaving the antitumor side of the macrophage balance unaffected.

This study raises a number of questions in terms of fundamental mechanisms and translation. The actual ligands in the TME triggering the checkpoint function of Clever-1 and intracellular signaling remain undefined. The Clever-1 ligand, SPARC, is an interesting candidate. It is a prototypic matricellular protein

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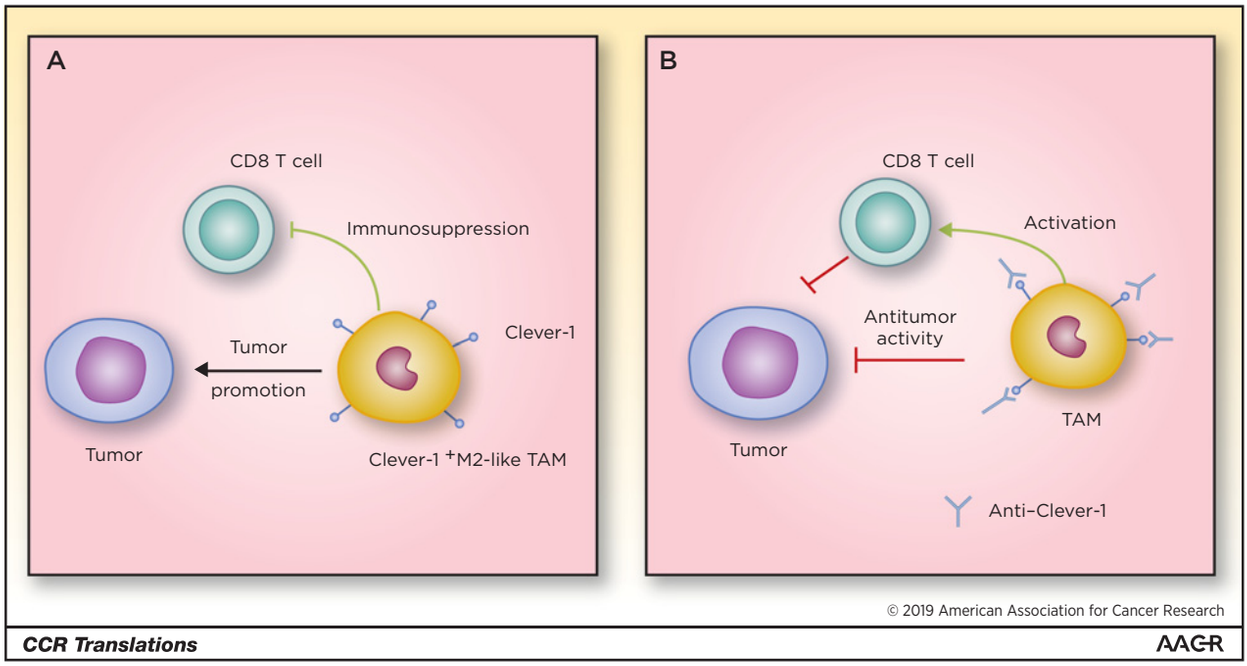

Figure 1.

Clever-1 as a macrophage checkpoint. Targeting Clever-1 on TAM unleashed their antitumor potential and resulted in $CD8^+$ T-cell activity against various transplanted murine tumors. Macrophages in the TME are a heterogeneous population. A subpopulation of M2-like TAM-expressing Clever-1 inhibited adaptive immunity and promoted cancer (**A**). Genetic or mAb-mediated blocking of Clever-1 unleashed the antitumor activity of TAM and reactivated $CD8^+$ T cells (**B**).

known to regulate the deposition and remodeling of extracellular matrix produced at high levels in many types of cancers and associated with malignant progression, promoting cell proliferation and migration. However, it has to be noticed that Clever 1 has not been shown to induce major transcriptional changes in macrophages and being a constitutive internalizing receptor could exert its function modulating endosomal trafficking of other molecules like mTOR.

Clever-1 is also expressed by some endothelia. Although genetic deficiency and mAb targeting was not associated with vascular pathology, selective deletion of Clever-1 in myeloid cells achieved better results. It can be possible that Clever-1 expressed by other cells, presumably endothelial cells, could affect tumor growth and dissemination and delivering of anti–Clever-1 mAb to macrophages would appear conceptually desirable. Moreover, it will be important to provide a framework for rational combination with T-cell checkpoint blockade, chemotherapy, or antitumor mAb, as indicated by the recent promising results of anti-CD47 combined with anti-CD20 in non-Hodgkin lymphoma (4).

The CD47–SIRPα axis has long been known to serve as a checkpoint ("don't eat me") in myeloid cells. Anti-CD47 in concert with anti-CD20 has recently been reported to have remarkable antitumor activity. Interestingly, targeting of other innate immunity checkpoint molecules, such as the receptors LILRB2, CD163, CD200, has shown promising antitumoral activity by promoting macrophage polarization toward antitumor phenotypes and potentiating or unleashing phagocytosis. New targets are also emerging from the better knowledge of the molecules regulating phagocytosis, like the secreted molecule calreticulin that promotes the recognition and removal by macrophages of unwanted cells. In the same general vein of targeting tumor-promoting inflammatory cells and mediators, blocking IL1 has been associated with protection against carcinogenesis in humans (5). Thus, myeloid checkpoint blockade and targeting tumor-promoting inflammation hold promise to complement current immunotherapy strategies.

Disclosure of Potential Conflicts of Interest

A. Mantovani reports receiving commercial research grants from Novartis, is a consultant/advisory board member for Novartis, Roche, Ventana, Pierre Fabre, Verily, AbbVie, Compugen, Macrophage Therapeutics, AstraZeneca, Biovelocita, BG Fund, Third Rock, and Verseau, is an inventor of patents related to PTX3 and other innate immunity molecules, and receives royalties for reagents related to innate immunity. No potential conflicts of interest were disclosed by the other author.

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