The tumor immune microenvironment is dynamic and the abundance and phenotype of tumor-infiltrating immune cells has prognostic significance in patients affected by a number of distinct malignancies.1 In many neoplasms, the immunosuppressive nature of the tumor microenvironment prevents development of a productive anticancer immune response. In line with this notion, the inhibition of specific immunosuppressive pathways within the tumor microenvironment, such as those mediated by programmed cell death 1 (PD-1) and its ligand PD-L1 provide durable clinical benefits to some patients. However, multiple immunosuppressive mechanisms (mediated by various immune cells) operate within the tumor microenvironment and may confer resistance to immunotherapeutic regimens. An alternative approach relies on therapeutic interventions that stimulate tumor-infiltrating immunosuppressive cell populations to adopt a pro-inflammatory phenotype or directly kill malignant cells. Such a repolarization of the tumor microenvironment, which can result in robust anticancer immune responses in in vivo models, may be successfully employed in patients who are relatively insensitive to immune checkpoint inhibitors.

Both Th1 and Th2 cytokines influence the antitumor functions of macrophages. We have recently shown that interferon γ (IFNγ) licenses the antineoplastic functions of CD40 ligand (CD40L)-stimulated macrophages more efficiently than interleukin (IL)-4 and IL-13. The presence of a Th1 and Th2 skewed tumor microenvironment may therefore influence therapeutic responses to CD40 agonists, agents that are showing promise in preliminary clinical trials.

Tumor-associated macrophages (TAMs) constitute a major component of the immune tumor infiltrate. These functionally plastic cells can exist in a spectrum of different phenotypes. M1 and M2 macrophages represent two extremes of such a functional polarization.2 Pro-inflammatory M1 macrophages have direct tumoricidal activity and normally promote antitumor Th1 immune responses. In contrast, M2 macrophages support tumor growth and metastasis, not only by stimulating angiogenesis and the invasive potential of malignant cells, but also by suppressing antitumor Th1 immune responses. The polarization of TAMs is determined by signals from the tumor microenvironment including hypoxia as well as malignant cell- or infiltrating T cell-derived cytokines. While TAMs generally exhibit an M2-like phenotype, the polarization state of macrophages can vary between tumor types as well as in different areas of the same neoplastic lesion.

CD40, a tumor necrosis factor α receptor superfamily member, is widely expressed by cells of the immune system including B cells, dendritic cells (DCs) and macrophages. CD40 agonists inhibit tumor growth in animal models, and promote clinical responses in patients with advanced solid tumors.3 CD40 stimulates antitumor immunity by enhancing the ability of antigen-presenting cells (APCs) to cross-present tumor-associated antigens to CD8+ T cells,4 and by activating TAMs.5,6 The acquisition of tumoricidal activity by TAMs in response to CD40 signaling has been reported to require interferon γ (IFNγ),7 suggesting that a Th1-skewed tumor microenvironment is necessary for CD40-driven antitumor activity. However, human neoplasms are often characterized by a Th2-skewed, rather than Th1-skewed, microenvironment,1 and the influence of Th2 cytokines on human macrophages remains unclear.

We set out to investigate how Th1 and Th2 cytokines might alter the functional consequences of CD40 activation in macrophages. To this aim, we compared the phenotype of human macrophages differentiated with colony stimulating factor 1 (CSF1, best known as M-CSF) and activated by recombinant CD40 ligand (CD40L) in the presence of either the Th1 cytokine IFNγ or the Th2 cytokines IL-4 and IL-13.8 In the absence of these cytokines, CD40-activated macrophages have
a very limited ability to kill tumor cells and produce high levels of the immunosuppressive cytokine IL-10. In line with previous reports, we observed that the activation of CD40 in the presence of IFNγ switched macrophages from producing IL-10 to producing the TH1 cytokine IL-12p70. Also, we found that these macrophages are able to skew allogeneic CD4⁺ T cells toward a TH1 profile and are endowed with an improved ability to kill neoplastic cells. Conversely, CD40 signaling in the presence of IL-4 and IL-13 resulted in a population of activated macrophages with characteristics intermediate between M1 and M2. These cells produce both IL-12p70 and IL-10 and cause TH1 T-cell skewing in co-culture with allogeneic CD4⁺ T cells, yet do not display enhanced tumoricidal activity compared with CD40L stimulation in the absence of TH1 or TH2 cytokines.

Our findings demonstrate that the presence of TH1 or TH2 cytokines determine how macrophages respond to CD40 ligation (Fig. 1). This implies that the tumor microenvironment determines the functional outcome of treatment with CD40 agonists. A TH1-dominated tumor microenvironment may optimally license the antitumor functions of CD40-activated macrophages, including their ability to directly kill malignant cells and the acquisition of a pro-inflammatory phenotype. However, the ability of CD40 signaling to repolarize macrophages from an M2-like to an M1-like phenotype even in the presence of IL-4 and IL-13 suggests that CD40 agonists could provide clinical benefits even when the tumor microenvironment is skewed toward a TH2 profile.

In rodent tumor models, CD40-activating antibodies have been reported to synergize with T-cell-activating immunotherapeutic interventions. For example, in TH1-skewed renal cell carcinomas, the antitumor activity of CD40 combined with IL-2 or IL-15 correlates with the repolarization of TAMs to an M1-like phenotype and the conversion of the tumor microenvironment towards one with a TH1 profile including enhanced CD8⁺ T-cell infiltration. These data indicate that T-cell activation influences the extent to which CD40 agonists are able to license the antitumor functions of TAMs. Thus, our findings highlight the importance of the tumor microenvironment in determining the antitumor activity of CD40 agonists and suggest a possible mechanism for the reported synergy between CD40 agonists and T-cell-activating interventions in animal tumor models.

Disclosure of Potential Conflicts of Interest

Nadia Luheshi, Gareth Davies, and James Legg are employees of MedImmune Ltd, a wholly owned subsidiary of AstraZeneca Ltd and are salaried and own stock and/or options in AstraZeneca.

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