The ontogeny of tumor-associated macrophages: a new understanding of cancer-elicited inflammation

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Clinical and experimental models have identified macrophages as potential targets for cancer therapy, however, the nature of macrophage differentiation and function in the context of malignant disease remain largely uncharacterized. This commentary provides the author’s perspective on the recently published article “The cellular and molecular origin of tumor-associated macrophages,” which demonstrated that tumor growth elicits a specific macrophage differentiation pathway.

Macrophages are functionally diverse innate immune cells with important roles in tissue homeostasis and immunity to pathogens.1 These tissue-resident cells originate from both embryonic precursors and hematopoietic stem cell-derived monocytes. During steady state, most systemic macrophages are embryo-derived, whereas monocytes give rise to macrophage populations in a limited number of tissues, including the intestine,2 dermis,3 heart,4 and mammary gland.5 However, during inflammatory conditions such as bacterial infection and in response to other forms of physiological stress, monocytes are the major source of influx expanding the macrophage pool.6 As macrophages are found in the majority of tissues, it comes as no surprise that these pleiotropic cells contribute to a wide variety of organ-specific and systemic disease pathologies, including cancer. Macrophages are found in all solid tumors and studies of both human and mouse malignancies have found a positive correlation between macrophage density and tumor growth.7,8 Genetic studies in mice, for example, have shown that rather than stimulating effective anticancer immune responses, macrophages instead support tumor development by promoting angiogenesis and local invasion. Thus, macrophages are now recognized as important therapeutic targets in the treatment of cancer.

With the recent success of tumor immunotherapy in only a subset of cancer patients, an improved understanding of the immune regulatory mechanisms at play during carcinogenesis, including those elicited by macrophages, is urgently needed. Tumor progression is characterized by an accumulation of macrophages, both in experimental and clinical settings, yet the precise origin and function of these cells have remained largely unknown. However, our recent study15 demonstrated that tumor development triggers a unique innate immune response characterized by the differentiation of inflammatory monocytes into tumor-associated macrophages (TAMs) that further proliferate within growing tumors. Furthermore, using a mammary carcinoma mouse model we found that Notch signaling via the key transcriptional regulator, recombination signal binding protein for immunoglobulin kappa J region (Rbpj), is required for TAM terminal differentiation and uncovered a possible mechanism by which TAMs promote tumor growth via their modulation of tumor-elicited adaptive immune responses.

Past work aiming to elucidate TAM biology used a limited set of markers to identify and characterize these cells, however, it is now appreciated that macrophage identity is complex and the choice of markers to identify different myeloid populations is instrumental to reveal both their ontogeny and function. Specifically, work by the Immunological Genome Project has demonstrated great macrophage phenotypic diversity depending on anatomical location.9 This led us to perform a comprehensive transcriptome analysis to clarify the identity of myeloid cells in mammary tumors. Not only did we compare the tumor-associated population with other known myeloid populations, but we also compared tumor-associated cells with their counterparts during steady-state. We found that a very specific population, identified by high MHC Class II expression and low CD11b expression made up the bona fide TAM population and was transcriptionally very different from the tissue-resident “mammary tissue macrophage” (MTM) population. This highlights the importance of precise analysis of individual rather than bulk myeloid populations.

Macrophage polarization and function during disease is under active investigation. Current dogma suggests that macrophages present in growing tumors are alternatively activated or “M2”-polarized...
via a number of tumor-associated factors. In contrast, our study demonstrated that tumor growth does not inherently promote M2 polarization of macrophages. In fact, the cells comprising the dominant macrophage population in mouse mammary tumors do not express most of the conventional M2 markers. Additionally, and perhaps more importantly, we found that M2-polarized macrophages do not, by definition, promote tumor growth. The (C-C) chemokine receptor 2 deficient (Ccr2−/−) genetic model, which depletes MTMs selectively without significantly affecting the TAM population, allowed us to evaluate tumor growth in the absence of the M2-polarized MTM population. Surprisingly, tumor growth was unaltered in these mice and the cytotoxic T-cell response remained unchanged, unlike the delay in tumor growth and loss of T-cell exhaustion observed in TAM-deficient mice. These findings suggest that tissue type must be carefully considered before falling under the assumption that all TAMs are phenotypically akin to M2.

In addition to characterizing the identity and phenotype of TAMs, our work determined that TAMs differentiate from inflammatory monocytes. The (C-C) chemokine receptor 2 deficient (Ccr2−/−) genetic model, which depletes MTMs selectively without significantly affecting the TAM population, allowed us to evaluate tumor growth in the absence of the M2-polarized MTM population. Surprisingly, tumor growth was unaltered in these mice and the cytotoxic T-cell response remained unchanged, unlike the delay in tumor growth and loss of T-cell exhaustion observed in TAM-deficient mice. These findings suggest that tissue type must be carefully considered before falling under the assumption that all TAMs are phenotypically akin to M2.

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In addition to characterizing the identity and phenotype of TAMs, our work determined that TAMs differentiate from inflammatory monocytes. This finding led us to ask, do tumors promote monocyte recruitment and differentiation via similar molecular mechanisms as occurs in response to other inflammatory conditions, such as infection, or is TAM differentiation a specific tumor-elicited response? Our data suggest the latter, that TAM differentiation occurs through a unique inflammatory response distinct from both homeostatic and infection-triggered monocyte differentiation pathways (Fig. 1). Furthermore, we propose that in the case of mouse mammary carcinoma, monocyte recruitment itself is not augmented by the tumor microenvironment, but rather a tumor-specific differentiation pathway is activated. Tumor development limits MTM differentiation and instead favors the aberrant differentiation of monocytes into TAMs in part via the Notch signaling pathway.

It is now appreciated that macrophages have the ability to proliferate and maintain their populations independently from blood-borne precursors in a number of different settings. However, the extent of this self-maintenance during tumor growth had not previously been explored. Importantly, we found that TAMs exhibit potent proliferative capacity upon their differentiation from inflammatory monocytes, which renders their accumulation in tumors less dependent on monocytic precursors compared to MTMs. These findings suggest that proliferation may be a major obstacle for therapeutic targeting of macrophages. The extent to which TAMs in human patients are capable of self-renewal remains to be explored. Our findings also suggest that TAMs promote tumor growth through their modulation of the adaptive immune response. However, it is known that macrophages play diverse homeostatic functions in many different tissues. Therefore, it is important to investigate other functions TAMs may have in the tumor microenvironment in addition to the immunomodulatory roles principally examined so far.

In summary, our study demonstrated that TAM differentiation is a specific tumor-elicited response distinct from other known homeostatic and infection-induced differentiation pathways of monocyte-derived macrophages. This work raises important questions including, to what extent do inflammatory monocytes contribute to macrophage populations in other murine cancer models, as well as in human tumors? Furthermore, are there general differentiation pathways in monocytes that are activated by tumor development? It is important to note that in our mouse model lacking terminally-differentiated TAMs due to a loss of Notch signaling, monocytes are still able to begin the process of specification into TAMs. Therefore, there are undefined signals driving the initial differentiation step preceding Notch activation. The answers to these questions will help further determine the relevance and feasibility of
targeting macrophages for cancer immunotherapy.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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