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Dendritic cell inhibition correlates with survival of colorectal cancer patients on bevacizumab treatment

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Abbreviations: BCM, bevacizumab-conditioned media; DC, dendritic cell; TCM, tumor-conditioned media

We demonstrated that dendritic cell (DC) inhibition by tumor-conditioned media in the presence or absence of bevacizumab correlates with colorectal cancer patient survival. Monitoring the influence of the tumor microenvironment on infiltrating immune cells may offer an avenue for the discovery of biomarkers to guide the use of bevacizumab.

Colorectal cancer (CRC) is a common cancer with a high mortality rate. While the 5 year survival rate for metastatic CRC patients is only 11%,[1] this has improved with the introduction of new targeted therapies including bevacizumab (Avastin), a humanized monoclonal antibody targeting the vascular endothelial growth factor (VEGF). However, a response rate of 50% or less has been reported, and currently there are no biomarkers that can predict the response to bevacizumab.[2]

Dendritic cells (DCs) are professional antigen-presenting cells capable of activating naive T cells. DCs are present in tissues in an immature state and can undergo a functional maturation process in response to inflammatory mediators such as Toll-like receptor (TLR) agonists. Increased expression of several cell surface markers, including CD54, CD80, CD86, CD83 and HLA-DR, on DCs is associated with DC maturation and T-cell activation.[3] DCs that secrete high levels of biologically active interleukin-12 (IL-12p70) induce optimal antitumor immunity, as they have an increased capacity to enhance natural killer (NK) cell activity, skew the immune response to a Th1 profile and prime tumor antigen-specific $CD8^+$ T cells.[4,5] DCs also express CD1d, which presents lipid antigens and specifically activates natural killer T (NKT) cells, including invariant NKT (iNKT) cells.[6]

In our study,[7] we wished to determine if DC infiltration into the tumor tissue of CRC patients receiving bevacizumab correlates with patient survival. We found significantly increased numbers of $CD11c^+$ cells in tumor lesions and in the adjacent tissues, as compared with age-matched healthy control tissue (85 CRC patients; 23 healthy controls). However, there was no significant difference in the infiltration of DCs in the tumor tissues and the adjacent normal tissues of CRC patients. This may indicate that even though the adjacent tissue appears histologically normal, there may be a genomic instability field effect that can influence the response of cells to damage signals.[8] We also found that the presence of $CD11c^+$ cells in the tumor did not correlate with survival of CRC patients that received bevacizumab treatment. While activated DCs are known to promote immunity, immature DCs induce tolerance. Therefore, the maturation status of DCs may be more important for the activation of a therapeutic immune response than the number of tumor-infiltrating DCs.

We previously demonstrated that the tumor microenvironment can suppress DC maturation and IL-12p70 secretion in response to lipopolysaccharide (LPS).[9] We therefore tested whether the immunosuppressive activity of tumors would have any influence on the response to bevacizumab or patient survival. To this aim, we measured the degree of suppression by tumor-conditioned media (TCM) on LPS-induced DC activation, in the absence or presence of bevacizumab.

Conditioned media were prepared by culturing ex vivo tumor tissues from 22 patients in the absence (TCM) or in presence of bevacizumab (bevacizumab-conditioned media, BCM) for 72 h. Monocyte-derived DCs were subsequently cultured in the presence of TCM and BCM. We showed that both TCM and BCM significantly inhibit the LPS-induced expression of several maturation markers, including CD80, CD86, HLA-DR, CD83, CD54 and CD1d. In addition, TCM and BCM significantly induced the production of IL-10 while reducing that of IL-12p70. These results point to a DC phenotype associated with immune tolerance. We observed no significant difference between the effect of TCM and BCM on DC maturation and

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cytokine secretion. We have previously shown that the neutralisation of VEGF in the TCM did not reverse its immunosuppressive effect.[9] This implies that VEGF is not the main immunosuppressive component of TCM. Furthermore, the fact that BCM (in which VEGF levels are reduced due to the presence of bevacizumab) do not significantly differ from TCM with regards to their effects on DCs indicates that other immunosuppressive factors secreted by the tumor are not altered by bevacizumab.

Interestingly, we found that the ability of the tumor microenvironment to suppress DC maturation correlates with clinical outcome. Specifically, we demonstrated that inhibition of the LPS-induced expression of CD1d and CD83 by TCM and the inhibition of CD1d, CD83 and IL-12p70 by BCM (**Fig. 1**) significantly correlates with the survival of CRC patients receiving bevacizumab. In particular: patients with high levels of immunosuppression had poorer prognosis than patients with low levels of immunosuppression.

CD1d is expressed by DCs and presents lipid antigens to NKT and iNKT cells. Our data suggests that, in addition to DCs, NKT and iNKT cells may play an important role in the immune response to CRC and survival following bevacizumab treatment. We have also shown that patients whose TCM did not effectively suppress the expression of CD83 by DCs exposed to LPS have a better prognosis compared with patients with robust CD83 inhibition. As CD83 is expressed by mature DCs, our data confirms the importance of DC maturation for functional immunity against colorectal tumors. Finally, we have shown that the inhibition of IL-12p70 secretion by BCM inversely correlates with patient survival. IL-12p70 is a pro-inflammatory cytokine that is vital for the induction of antitumor Th1 responses. Our data suggests that IL-12p70 secretion from DCs is important for the survival of metastatic CRC patients on bevacizumab treatment.

In summary, we have shown that TCM and BCM inhibit the LPS-induced maturation and functional activation of DCs. However, there was no significant difference between TCM and BCM, which indicates that factors secreted by the

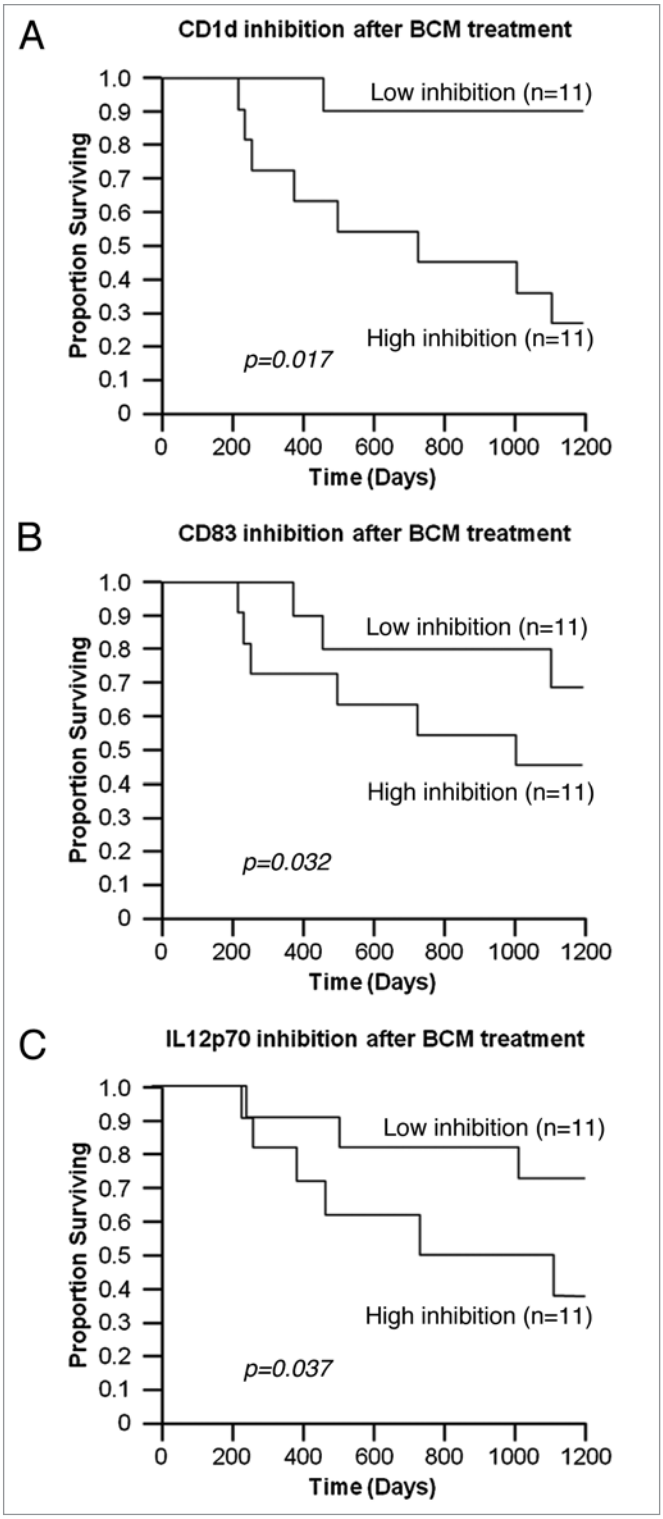

Figure 1. Inhibition of LPS-induced CD1d, CD83 and IL-12p70 secretion by DCs treated with BCM correlates with colorectal cancer patient survival. Monocyte derived dendritic cells (DCs) were treated with bevacizumab-conditioned media (BCM) for 4 h before lipopolysaccharide (LPS) was added for additional 18 h. Inhibition of CD1d (**A**), CD83 (**B**) and IL-12p70 (**C**) by BCM correlated with poor survival of colorectal cancer patients. Kaplan-Meier curves of high vs. low inhibition are shown. Statistical significance was calculated using the Cox proportional hazards model. (Adapted from Michielsen et al.[7]).

tumor other than VEGF are capable of modulating DCs in situ. Importantly, the inhibition of LPS-induced upregulation of CD1d and CD83 expression by TCM and CD1d, CD83 and IL-12p70 expression/secretion by BCM significantly correlated with poor clinical outcomes in CRC patients receiving bevacizumab. Overall, our study shows that a deeper understanding of the tumor microenvironment and the interaction between the cancer cells and infiltrating immune cells may potentially yield not only new prognostic/predictive biomarkers but also new therapeutic targets for the battle against CRC.

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