

# Tumor conditioned media from colorectal cancer patients inhibits dendritic cell maturation

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**Keywords:** dendritic cells, tumour microenvironment, VEGF, CCL2, CXCL1, CXCL5

**Abbreviations:** DC, dendritic cell; VEGF, vascular endothelial growth factor; TCM, tumour conditioned media; NCM, normal conditioned media

Tumors inhibit dendritic cell maturation and function in order to evade host immunity. We showed that conditioned media from tumor explant tissue, taken from metastatic colorectal cancer patients, significantly inhibits maturation of dendritic cells.

Tumors are poorly immunogenic and are capable of evading the immune system. Dendritic cells (DCs) are professional antigen presenting cells. Immature DCs detect and capture antigens and subsequently undergo a maturation process, which enables them to present the antigens to other immune cells such as T-cells. In addition, DCs secrete cytokines to promote the appropriate immune response to the pathogen/tumor.<sup>1</sup>

To elucidate the effect of the tumor microenvironment on DC function, we used a human colorectal cancer explant model.<sup>2</sup> Tumour tissue and adjacent normal tissue were collected from colorectal cancer patients undergoing surgery in St. Vincent's University Hospital, Dublin. We generated tumor conditioned media (TCM) and normal conditioned media (NCM) by culturing the tumor and adjacent normal tissue for 72 hours. Monocyte derived DCs obtained from healthy controls were treated with the TCM of 21 colorectal cancer patients in the absence or presence of LPS. Levels of maturation markers expressed by DCs were analyzed by flow cytometry and cytokine secretion was measured by ELISA. We found that TCM significantly reduced the LPS-induced upregulation of

CD86, CD83, HLA-DR and CD54. In addition, TCM reduced IL-12p70 and increased IL-10 secretions induced by LPS (Fig. 1): a phenotype associated with tolerance. In contrast, NCM did not significantly affect DC maturation, indicating that the normal adjacent mucosa has functional immunity.

While other studies have shown that conditioned media from single cell cultures have similar inhibitory effects,<sup>3-5</sup> this is the first study to use a 3D explant model which mimics the complex structure and composition of a tumor, including the stroma. A big advantage of this model is that it more closely reflects the inflammatory milieu of the tumor than cancer cell lines, and therefore would be a good model for screening potential new drugs and biomarkers.

We screened the TCM to determine which factors present mediated DC inhibition. VEGF, a growth factor that promotes angiogenesis and has been reported to inhibit DC maturation and function<sup>4,6</sup> was present at high levels in the TCM. In addition, we found high levels of CCL2 (MCP-1), CXCL1 (GRO $\alpha$ ) and CXCL5 (ENA-78) were secreted by all tumors. The main function of CCL2 is to attract monocytes and macrophages, while

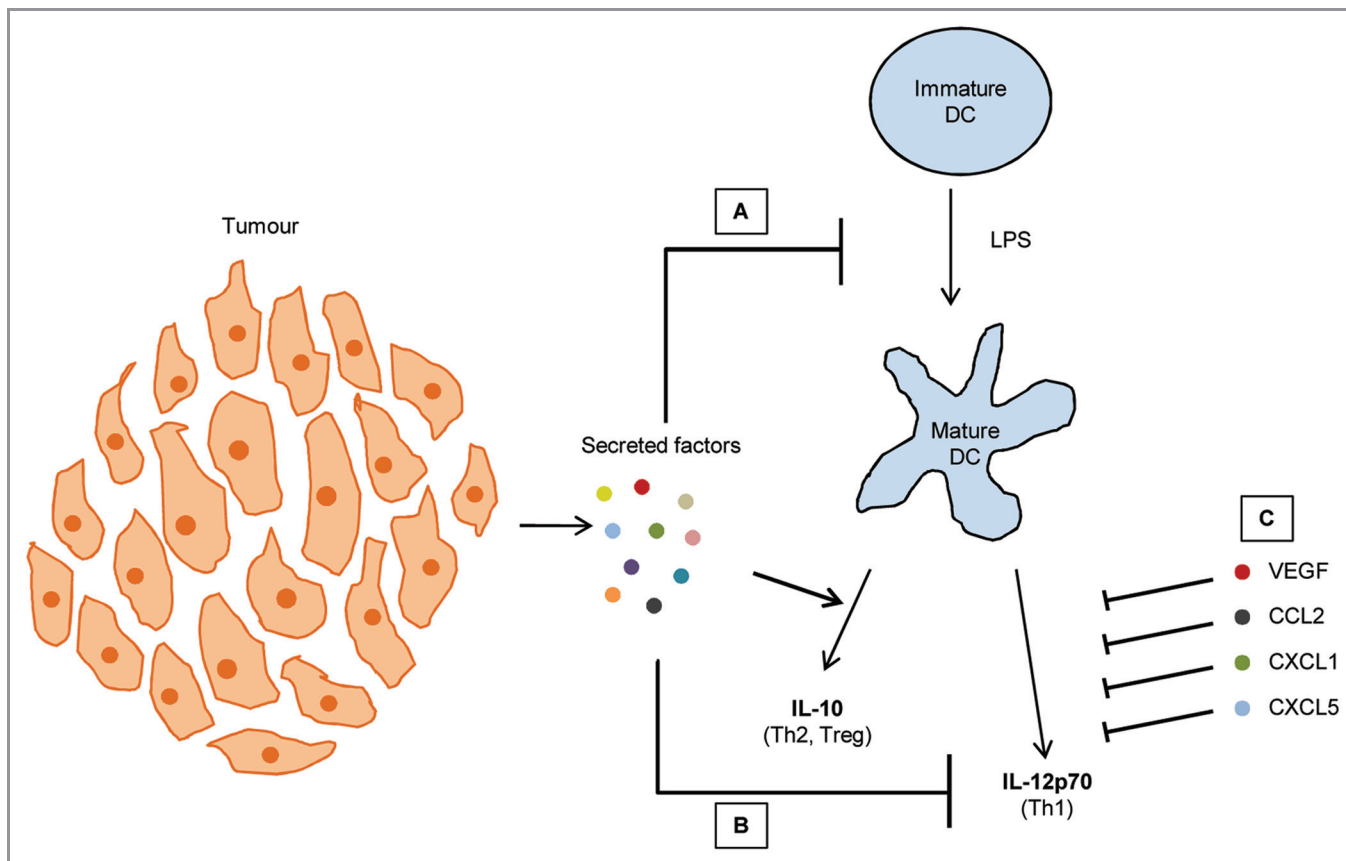
CXCL1 and CXCL5 attract and activate neutrophils. While all of these chemokines can promote tumor growth, proliferation and angiogenesis,<sup>7,8</sup> how they affect DCs has not been investigated. Therefore we exposed monocyte derived DCs obtained from healthy donors to recombinant VEGF (which acts as our positive control for DC inhibition) CCL2, CXCL1 and CXCL5 prior to stimulation with LPS. VEGF and all 3 chemokines reduced LPS-induced secretion of IL-12p70 (Fig. 1), thereby reducing the possibility of a potent Th1 response. VEGF inhibited LPS-induced expression of CD54 and CD80, while CXCL1 inhibited HLA-DR expression, indicating that they, to some extent, affect DC maturation.

However, LPS-induced IL-10, IL-1 $\beta$ , TNF $\alpha$ , IL-8 and IL-6 secretion was not affected. Neither did CCL2, CXCL1, CXCL5 or VEGF affect DC migration, T-cell proliferation or IFN $\gamma$  production from T-cells. These results show that these chemokines alone are not the only factors in the TCM that inhibit DCs; however they contribute to the immunosuppressive environment by reducing IL-12p70 secretion from DCs. Furthermore, these chemokines may work synergistically to inhibit DC maturation and function. To

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Submitted: 02/01/12; Accepted: 02/02/12

<http://dx.doi.org/10.4161/onci.19570>



**Figure 1.** The tumor microenvironment inhibits dendritic cell maturation and function in response to LPS. Tumors produce a large array of soluble factors, which inhibit LPS induced dendritic cell (DC) maturation (A). In addition, they affect DC function by increasing IL-10 and decreasing IL-12p70 secretion (B). VEGF, CCL2, CXCL1 and CXCL5, which are secreted at high levels by the tumor, affect DC function by inhibiting LPS induced IL-12p70 secretion (C).

test this we combined the cytokines, we found that CXCL1 and VEGF had an additive effect on the LPS-induced inhibition of IL-12p70 secretion from DCs. However, when CXCL1 and VEGF were neutralised in the TCM, either alone or together, the inhibitory effect of the TCM was not reversed. These results show that it is very likely that it is not a single agent but multiple factors present in the TCM affect DC maturation and function.

Timing of the exposure of DCs to tumor derived cytokines is important. While we did not study the effects of CCL2, CXCL1, CXCL5 and VEGF on DC differentiation, other studies have shown that the presence of VEGF, IL-6,

G-CSF or prostanoids during the differentiation stage significantly inhibit DC function.<sup>3-5</sup> Moreover, neutralisation of IL-6 or G-CSF in conditioned media of the pancreatic cancer cell line BxPC-3 significantly reversed its inhibitory effects.<sup>5</sup> Inhibition of COX1/2 in primary colon tumor cells supernatants, which results in reduced prostanoid production, also significantly reversed the inhibitory effects of the conditioned media;<sup>3</sup> however neither induced a complete reversion. These results also illustrate that multiple factors secreted by the tumor are responsible for inhibiting DC differentiation in addition to DC maturation and function.

In summary, we show that the TCM components CCL2, CXCL1, CXCL5 and VEGF play a role in modulating the inflammatory response through inhibition of IL-12p70 secretion by DCs, a novel strategy employed by the tumor to evade the immune response. In addition, CXCL1 and VEGF act together in the inhibition of IL-12p70 secretion from DCs. Even though CCL2, CXCL1, CXCL5 and VEGF are abundantly present in the TCM, they may not be the most biologically active in affecting DC maturation and function. It is probable that the action of the entire tumor microenvironment is required to elicit such a significant effect.

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