slanDCs in carcinoma-draining lymph nodes

William Vermi1,2,*, Silvia Lonardi1, Mattia Bugatti1, Federica Calzetti1, Alessandra Micheletti1, and Marco A Cassatella3,*

1Department of Molecular and Translational Medicine; Section of Pathology; University of Brescia; Brescia, Italy; 2Department of Pathology and Immunology; Washington University School of Medicine; Saint Louis, MO USA; 3Department of Pathology and Diagnostics; Section of General Pathology; University of Verona; Verona, Italy

Keywords: carcinoma, slanDCs, tumor-draining lymph nodes

Dendritic cells (DCs) are known to initiate adaptive immune responses against malignant cells. However, the role in anticancer immunosurveillance of 6-sulfo LacNAc-expressing DCs (slanDCs), a distinct population of circulating and tissue-resident pro-inflammatory DCs, is unclear. We have recently demonstrated the involvement of slanDCs in nodal immune responses against carcinoma cells.

By establishing a sophisticated crosstalk with (pre)malignant cells, the immune system significantly influences the growth of developing neoplasms, a process referred to as cancer immunoediting.1 As a result of this interaction, cancer cells can be completely eradicated, persist in an immune-mediated latency or outgrow as variants with low immunogenicity.

Innate immune cells including dendritic cells (DCs) critically regulate anticancer immune responses, hence profoundly influencing the outcome of the cancer cell/immune system interaction. Human CD303+ plasmacytoid DCs and CD11c+ myeloid DCs (mDCs) are commonly found in the tumor microenvironment. mDCs are subdivided into 2 major subsets, including CD1c+ (BDCA1+) DCs and CD141+ (BDCA3+)/CLEC9A+ DCs.2,3 Recently, a new subset of mDCs has been identified and designated “slanDCs,” based on selective expression of the 6-sulfo LacNac (slan) residue on selectin P ligand (SELPLG, also known as M-DC8).4 Because slanDCs exhibit a CD16+HLA-DR+CD14+ surface phenotype, they are also assigned to the non-classical CD14+CD16+ monocyte population.5 slanDCs are more abundant than other DCs in the peripheral blood and exert robust pro-inflammatory effects. They indeed produce higher amounts of tumor necrosis factor α (TNFα) and mature interleukin-12 (IL-12p70) than other mDCs or classical CD14+ monocytes upon stimulation with Toll-like receptor (TLR) agonists.4,6 Moreover, slanDCs efficiently present antigens to naive T cells and favor the maturation of purified CD8+ T cells into alloantigen-specific cytotoxic cells.7 Recent studies have documented the accumulation of slanDCs in peripheral tissues in the course of chronic inflammatory disorders. However, the occurrence and functional relevance of these DCs in the tumor microenvironment was completely unexplored until recently.

We therefore set out to test the hypothesis that slanDCs represent a new component of the human innate immune response to cancer.8 We mapped slanDCs in normal human tissues, finding that they are abundant in mucosal-associated lymphoid tissues, particularly in the tonsils and sub-epithelial dome region of Peyer’s patches. We then screened a large set of primary carcinomas from different anatomical sites and metastatic tumor-draining lymph nodes (M-TDLNs), establishing that slanDCs preferentially home to M-TDLNs but are mostly absent from M-TDLNs, as demonstrated by their close proximity to metastatic deposits. In addition, the homing of slanDCs to TDLNs represents an early event upon cancer cell colonization, as demonstrated by the analysis of sentinel lymph nodes draining breast carcinomas. Notably, slanDCs are distinct from Tie2-expressing monocytes, a subset of CD16+ monocytes that infiltrates primary carcinomas and exerts potent pro-angiogenic activity.9

Although we could not define the precise functional role of slanDCs in M-TDLNs, our in situ analysis clearly indicates that slanDCs are in the close proximity of and efficiently engulf dead cancer cells. Moreover, the slanDCs found within M-TDLNs are activated to limited extent (as revealed by the expression of TNFα and co-stimulatory molecules), although they do interact with CD56+ natural killer (NK) cells, CD66b+ neutrophils, and CD4+ T cells. These findings suggest that tumor-associated antigens derived from carcinoma cells infiltrating M-TDLNs might be directly presented to T cells by local slanDCs, revealing a previously unrecognized role for activated slanDCs in the regulation of nodal immune responses to disseminated cancer cells. In addition, the interaction of slanDCs with NK cells and neutrophils might amplify interferon γ (IFNγ) responses in TDLNs. Functional studies performed on freshly
purified cells indicate that the contingent of circulating slanDCs in colon carcinoma patients, including individuals with advanced stage disease, remains intact and competent in terms of viability, TNFα and IL-12p70 production, induction of T-cell proliferation, and migratory capacity. These findings indicate that unlike other DCs, circulating slanDCs are not developmentally or functionally hijacked by growing tumors and thus represent a relevant source of cytokines and antigen presentation. A number of issues regarding the role of slanDCs in anticancer immunity remains unclear. The clinical significance of slanDC infiltration in M-TDLNs is unknown, a point that needs to be clarified by ad hoc observational studies. Mechanistically, it is still unclear why slanDCs do not infiltrate primary neoplasms and why their accumulation in M-TDLNs is limited to a fraction of carcinoma cases. The absence of slanDCs from primary tumors implies that these cells colonize TDLNs via the bloodstream, through high endothelial venules (HEVs), rather than via lymphatics (Fig. 1). Previous studies indicate that migration of circulating slanDCs is guided by a set of chemotactic receptors including chemokine (C-X-C motif) receptor 4 (CXCR4), chemokine (C-X3-C motif) receptor 1 (CX3CR1), and complement component 5a receptor 1 (C5AR1). Since the ligands of these receptors are abundantly expressed by primary carcinomas, which do not contain slanDCs, we hypothesize that the recruitment of tumor-associated slanDCs requires a more complex system that is in place within M-TDLNs. The accumulation of slanDCs in M-TDLNs specifically requires deposits of malignant cells, suggesting that tumor-derived factors might cooperate in maintaining slanDCs localized and viable in M-TDLNs. Elucidating the precise molecular processes that facilitate the homing of slanDCs to M-TDLNs, as well as the local interaction of slanDCs with malignant cells, will indicate ways to exogenously promote their recruitment to other metastatic sites in patient with advanced stage disease.

Although we clarified that the circulating counterpart of slanDCs is still competent in cancer patients, we could only speculate on the residual function of these cells in the M-TDLN microenvironment. It is likely that their tight interactions with cancer cells profoundly dictate their transcriptional program, and might lead to the acquisition of an immunosuppressive phenotype. A better understanding of this issue will be obtained by purifying slanDCs from M-TDLNs. Elucidating the precise role of slanDCs in oncogenesis and tumor progression will greatly benefit from the identification and characterization of their murine counterparts.

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

References
1. Schreiber RD, Old LJ, Smyth MJ. Cancer immunoeediting: integrating immunity's roles in cancer suppression and promotion. Science 2011; 331:1565-70; PMID:21436444; http://dx.doi.org/10.1126/science.1203486
2. Dzionek A, Fuchs A, Schmidt P, Cremer S, Zytk M, Milenyi S, Buck DW, Schmitz J. BDCA-2, BDCA-3, and BDCA-4: three markers for distinct subsets of dendritic cells in human peripheral blood. J Immunol 2000; 165:6037-46; PMID:11086035
3. Ziegler-Heitbrock L, Ancuta P, Crowe S, Dalod M, Grau V, Hart DN, Leenent PJ, Liu YJ, MacPherson G, Randolph GJ, et al. Nomenclature of monocytes and dendritic cells in blood. Blood 2010; 116:e74-80; PMID:20628149; http://dx.doi.org/10.1182/blood-2010-02-258558
4. Schäkel K, Kannagi R, Knipe B, Goto Y, Mitsuoka G, Zwiener J, Soruri A, von Kiertzell M, Rieber E. 6-Sulfo LaNac, a novel carbohydrate modification of PSGL-1, defines an inflammatory type of human TDLNs. Cell Immunol 2002; 17:289-301; PMID:12354382; http://dx.doi.org/10.1016/S0090-7662(02)00393-X
5. Schäkel K, von Kiertzell M, Hänsel A, Ebling A, Schulze L, Haase M, Semmler C, Sfarati M, Barclay AN, Randolph GJ, et al. Human 6-sulfo LaNac-expressing dendritic cells are principal producers of early interleukin-12 and are controlled by cytotoxic T lymphocytes. Immunity 2006; 24:767-77; PMID:16782032; http://dx.doi.org/10.1016/j.immuni.2006.03.020
6. Hänsel A, Gütter C, Ingwersen J, Starke J, Schmitz M, Bachmann M, Meurer M, Rieber EP, Schäkel K. Human slan (6-sulfo LaNac) dendritic cells are inflammatory dermal dendritic cells in psoriasis and drive strong TH17/TH1 T-cell responses. J Allergy Clin Immunol 2011; 127:787-93; PMID:21377044; http://dx.doi.org/10.1016/j.jaci.2010.12.009
7. Schäkel K, Mayer E, Federle C, Schmitz M, Riehmuller G, Rieber EP. A novel dendritic cell population in human blood: one-step immunomagnetic isolation by a specific mAb (M-DC8) and in vitro priming of cytotoxic T lymphocytes. Eur J Immunol 1998; 28:4084-93; PMID:9862344; http://dx.doi.org/10.1002/(SICI)1521-4141(199812)28:12<4084::AID-JEMM4084-3.0.CO;2-4
8. Vermi W, Michelelli A, Lonardi S, Costantini C, Calzetti F, Nascimbeni R, Bugatti M, Codazzi M, Pinter PC, Schäkel K, et al. slanDCs selectively accumulate in carcinoma-draining lymph nodes and mar-ginate metastatic cells. Nat Commun 2014; 5:3029; PMID:24398631; http://dx.doi.org/10.1038/ncomms4029

9. Venneri MA, De Palma M, Ponzoni M, Pucci F, Sicielzo C, Zonari E, Mazzieri R, Doglioni C, Naldini L. Identification of proangiogenic TIE2-expressing monocytes (TEMs) in human peripheral blood and cancer. Blood 2007; 109:5276-85; PMID:17327411; http://dx.doi.org/10.1182/blood-2006-10-053504

10. Costantini C, Calzetti F, Perbellini O, Michelelli A, Scarponi C, Lonardi S, Pelleter M, Schakel K, Pizzolo G, Facchetti F, et al. Human neutrophils interact with both 6-sulfo LacNAc+ DC and NK cells to amplify NK-derived IFN-gamma: role of CD18, ICAM-1, and ICAM-3. Blood 2011; 117:1677-86; PMID:21098395; http://dx.doi.org/10.1182/blood-2010-06-287243