Role of dendritic cells in the regulation of antitumor immunity

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Abbreviations: DC, dendritic cell; HMGB1, high mobility group box 1; ICAM1, intercellular adhesion molecule 1; LFA1, lymphocyte function-associated antigen 1; NRPI, neuropilin 1; PD1, programmed death 1; PD-L1, PD1 ligand 1; TIM3, T-cell immunoglobulin mucin 3

Introduction

Accumulating evidence indicated that spontaneous antitumor immune responses exert a strong impact on the clinical outcome of malignant diseases, confirming the concept of cancer immunosurveillance. Moreover, the infiltration of tumors by immune cells in response to radio- or chemotherapy has been associated with a favorable prognosis, suggesting that these conventional therapeutic regimens may not only affect tumor growth in a cell-intrinsic manner, but also activate anticancer immunity, at least in some settings.1

Large clinical studies have revealed the prognostic impact of tumor infiltration by T lymphocytes for colorectal cancer, 2,3 ovarian carcinoma, 4,5 and metastatic melanoma 6 patients. In addition, a few studies have linked immune system-related genes to the response to chemotherapy.7,8 In patients as well as in murine models, there is ample evidence for a critical role of T<sub>H1</sub> and cytotoxic responses in tumor rejection, 9,10 although innate immunity, in particular as mediated by natural killer (NK) cells, is also clearly involved in this process.11,12

Collectively, these observations highlight a critical role for T lymphocytes in antitumor immune responses. An important issue in this context is the contribution of DCs to T-cell priming, which presumably occurs in tumor-draining lymph nodes, as well as to the functional differentiation of intratumoral T lymphocytes.

Here, we review a few recent reports illustrating how DCs may affect anticancer immunity and the efficacy of chemotherapy. Some observations suggest indeed that intratumoral DCs may dysregulate tumor-specific immune responses.

HMGB1, a Prototypic Tumor-Derived Immunostimulatory Factor

To identify the Toll-like receptors (TLRs) that might control the immune response to dying tumor cells, Apetoh et al. inoculated oxaliplatin-treated EG7 cells into the footpads of either wild-type or TLR-deficient hosts and monitored interferon (IFN)<sub>γ</sub> production upon re-stimulation in vitro with the model antigen ovalbumin (OVA).13 IFN<sub>γ</sub> was produced by lymph node cells in WT mice as well as in animals individually lacking several TLRs but not in Tlr4<sup>−/−</sup> mice. Moreover, DC depletion abrogated the priming of T lymphocytes against dying tumor cells and high mobility group box 1 (HMGB1) was shown to constitute the principal damage-associated molecule to activate TLR4. These data identified one TLR ligand as a critical factor in death-derived immunoadjuvant effect of chemotherapy. HMGB1 is a non-histone chromatin-binding nuclear protein that interacts with TLR4, advanced glycosylation end product receptor (AGER) and probably other receptors, hence modulating inflammatory responses. These results illustrate the contribution of the immune system to the efficacy of chemotherapy, although this depends on the nature of the cytotoxic agent, tumor type,14 the host immunocompetence. In addition, this work highlights the critical role of TLR4<sup>+</sup> dendritic cells as sensors and efficient cross-presenters of tumor-associated antigens (TAAs) derived from dying cancer cells (Fig. 1).

This said, it has recently been demonstrated that DCs may inhibit pattern recognition receptor (PRR)-mediated innate immune responses in the tumor microenvironment. In particular, Chiba et al. elegantly identified the receptor T-cell immunoglobulin mucin 3 (TIM3) as a key factor that prevents the immunological response of DCs to nucleic acids.15 The authors first noticed...
that CD11c<sup>high</sup> conventional DCs expressed much higher levels of TIM3 when they infiltrate subcutaneous Lewis lung tumors or MC38 colorectal adenocarcinomas than when they are found in autologous normal lymphoid organs. They identified vascular endothelial growth factor (VEGF), interleukin (IL)-10 and arginase I as soluble factors that upregulate TIM3 expression on DCs. Moreover, TIM3 was shown to interfere with the response of DCs to immunostimulatory nucleic acids, as TIM3 prevented the nucleic acid-triggered production of Type I IFN and IL-12 by DCs (Fig. 1). TIM3 and nucleic acids compete for binding HMGB1, resulting in the inhibition of the HMGB1-dependent transfer of nucleic acids to endosomes. Based on results from previous studies, Chiba et al. propose that HMGB1 may promote the access of nucleic acids to endosomal vesicles, thereby inducing innate responses. Of note, a similar mechanism may occur in cancer patients, as high expression levels of TIM3 have been detected on tumor-associated DCs isolated from patients bearing advanced non-small cell lung carcinoma (NSCLC), gastric adenocarcinoma or neuroendocrine tumors.  

In the same study, TIM3 appeared to be expressed on T cells at late time points, and it had previously been demonstrated that inhibiting TIM3- and programmed death 1 (PD1)-transduced signals reverse the exhaustion of tumor-specific CD8<sup>+</sup> T cells isolated from melanoma patients in vitro. Most of these cells express indeed TIM3 and PD1 and are dysfunctional in terms of cytokine production. In a model of murine colon carcinoma (based on CT26 cells), the combined administration of anti-TIM3 and anti-PD1 ligand 1 (PD-L1) antibodies resulted in delayed tumor growth, with 50% of the mice exhibiting complete tumor regression and long-term survival. Thus, TIM3 may impair the function of several types of immune cells in the tumor microenvironment and may constitute a promising target to enhance spontaneous tumor-specific immune responses.

**Maturation of DCs**

One of the hallmarks of DCs is their progressive acquisition of specialized functions. Immature DCs efficiently take up and process antigens (usually in peripheral tissues), whereas mature DCs become fully competent to prime naïve T cells (mainly in lymphoid organs). The maturation of DCs is induced by microbial components (so-called pathogen-associated molecular patterns, PAMPs) or pro-inflammatory factors, eventually resulting in the elicitation of immune responses against non-self, microbial
antigens. Of note, immature DCs can induce a state of unresponsiveness in T lymphocytes (anergy) via poorly defined mechanisms, hence preventing or dampening antitumor T-cell responses in vivo. In addition, immature DCs appear to interact preferentially with regulatory T cells, as compared with naïve helper T cells, hence mediating to a “default immunosuppression.” Indeed, CD4+CD25+ cells preferentially interact with immature DCs as compared with their CD4+CD25− counterparts, correlating with a comparatively higher expression of the adhesive molecule neuropilin 1 (NRP1).20 Thus, immature DCs may directly or indirectly dampen helper T-cell functions.

Several reports suggest that tumor-infiltrating DCs are functionally impaired. Early evidence in support of this notion was provided in 2002 by Vicari and colleagues, who observed that DCs infiltrating transplantable tumors and hepatocarcinomas developing in X/myc transgenic mice essentially displayed an immature phenotype.21 Moreover, these immature DCs were refractory to lipopolysaccharide (LPS) plus IFNγ plus anti-CD40 antibody stimulation, but could be re-activated by CpG oligonucleotides (a TLR9 agonist) coupled to anti-IL-10 receptor (IL-10R) antibodies. This combination induced the secretion of IL-12 by a large proportion of DCs infiltrating CCL21-expressing C26–6CK colon carcinoma and exerted therapeutic antigens. These conjugates, however, were not productive as T cells were unable to lyse TAA-expressing target cells. Thus, tumor-associated DCs appear to be incompetent to sustain cytotoxic T lymphocyte (CTL) activity. The administration of IL-2, CpG or imiquimod (a TLR7 agonist) in vitro partially restored the capacity of tumor-infiltrating DCs to stimulate CTLs.

Accumulating evidence indicates that the stage of maturation of tumor-infiltrating DCs may be of prognostic value. Immunohistochemistry- and immunofluorescence microscopy-based analyses of DCs infiltrating 20 lung cancer specimens from randomly selected patients indicated that the presence of PD-L1+ lung carcinoma cells as well as the presence of immature DCs correlates with poor patient prognosis.23 The authors suggest that lung carcinoma cells as well as the presence of immature DCs are engaged in non-productive interactions with T cells. These tumor-associated DCs appear to be incompetent to sustain cytotoxic T lymphocyte (CTL) activity. The administration of IL-2, CpG or imiquimod (a TLR7 agonist) in vitro partially restored the capacity of tumor-infiltrating DCs to stimulate CTLs.
lymphocytes as well as with the amount of mature DCs within primary lesions.38

Plasmacytoid DCs

In addition to their maturation status, the precise nature of tumor-infiltrating DCs also influences tumor-specific immune responses. Thus, the presence of plasmacytoid DCs (pDCs) in the ovarian cancer epithelium was associated with an early relapse.39 This report confirmed initial results on 33 ovarian cancer patients showing that pDCs infiltrating primary lesions, but not ascitic tumors, was an independent negative prognostic factor.40 The deleterious prognostic effect of tumor-associated pDCs in ovarian cancer could be related to an altered IFNα production caused by tumor-derived soluble factors such as tumor necrosis factor α (TNFα) and transforming growth factor β (TGFβ) (Fig. 2). Along similar lines, several studies have correlated high levels of tumor-infiltrating pDCs with poor prognosis in breast carcinoma,41 multiple myeloma42 and melanoma43 patients.

Functional Changes in DCs

The analysis of ovarian cancer progression revealed unexpected changes in DCs that turned out to accelerate tumor expansion. To recapitulate in rodents the immune infiltrates of human tumors, Scarlett et al. generated a p53-dependent, inducible metastatic ovarian carcinoma in C57Bl/6 mice.44 These authors used the ablation of p53 and the constitutive activation of K-Ras (two oncogenes that are dysregulated in a majority of cancer patients) to induce palpable abdominal tumors exhibiting 100% penetrance and short latencies. In this model, a homogenous population of DCs resembling the cells that infiltrate human ovarian carcinomas was found in neoplastic lesions as well as in tumor-draining lymph nodes. Interestingly, the tumors developing in p53/K-Ras double transgenic mice remained under control for about 28 d, became palpable after approximately 35 d and then grew very aggressively. This growth coincided with a change in their inflammatory infiltrate: DCs isolated from advanced tumors expressed high levels of tolerogenic PD-L1 and displayed immunosuppressive arginase I activity. Consequently, tumor-specific T cells became progressively less responsive and neoplastic cells escaped the immune control. Of note, the depletion of DCs accelerated tumor progression when performed 7 d after tumor induction, whereas it retarded tumor growth in 100% of mice if performed at the beginning of the immunoevasive phase. These data highlight a critical role for tumor-associated DCs during the equilibrium phase and indicate that tumor-derived mediators including prostaglandin E2 (PGE2) and TGF-β may convert immunocompetent DCs into immunosuppressive APCs. These findings are in line with previous results demonstrating that the DCs that infiltrate ovarian cancers growing in C57Bl/6 mice acquire the expression of both PD-L1 and PD1.24

Concluding Remarks

Collectively, these observations demonstrate that tumor-infiltrating DCs may constitute an important aspect of the dysregulation of antitumor immunity and critically contribute to immune

Figure 2. Tumor-infiltrating plasmacytoid dendritic cells (pDC) correlate with poor prognosis. The secretion of tumor necrosis factor α (TNFα) and transforming growth factor β (TGFβ) in the tumor microenvironment may inhibit the production of interferon α (IFNα) by plasmacytoid dendritic cells, negatively affecting anticancer immunity.
evasion. The identification of the mechanisms whereby malignant cells subvert DC function may help to define interventions that reinforce spontaneous antitumor immune responses by interfering with the immunosuppressive tumor microenvironment and may provide clues to select the best cell population for DC-based anticaner immunotherapy.

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

References

1. Rahir G, Moser M. Tumor microenvironment and lymphocyte infiltration. Cancer Immunol Immunother 2012; 61:751-9; PMID:22488275; http://dx.doi.org/10.1007/s00262-012-1253-1.

2. Galon J, Costes A, Sanchez-Cabo F, Kirilovsky M, Melicke B, Lagorce-Pagès C, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science 2006; 313:1961-4; PMID:17088531; http://dx.doi.org/10.1126/science.1129193.

3. Nusko K, Baha Y, Tanaka N, Shimka K, Hayashi M, Meyerhardt JA, et al. Tumour-infiltrating T-cell subsets, molecular changes in colorectal cancer, and prognostic: cohort study and literature review. J Pathol 2010; 222:59-66; PMID:20397778; http://dx.doi.org/10.1002/path.2774.

4. Sato E, Olson SH, Aham J, Bundy B, Nishikawa H, Qan F, et al. Intraperitoneal CD8+ tumor-infiltrating lymphocytes and a high CD8+/regulatory T cell ratio are associated with favorable prognosis in ovarian cancer. N Engl J Med 2003; 348:203-13; PMID:12529460; http://dx.doi.org/10.1056/NEJMoa021777.

5. Zhang L, Conejo-Garcia JR, Katsaros D, Gimotty PA, Zhang LX, Winer WK, et al. Tumor-infiltrating lymphocytes predict survival of patients with breast carcinoma. Cancer Invest 2009; 27:827-33; PMID:19218377; http://dx.doi.org/10.3109/07357900802098165.

6. Horonyova H, Melchior B, Tomova M, Mergancova J, Urminska H, Ryska A. Tumour-infiltrating lymphocytes predict response to neoadjuvant chemotherapy in patients with breast carcinoma. Cancer Invest 2008; 26:1024-31; PMID:19095260; http://dx.doi.org/10.1080/07357900802098165.

7. Koehl CM, Vermi W, Swann JB, Zerafa N, Rodig SJ, Old LJ, et al. Adaptive immunity maintains occult cancer in an equilibrium state. Nature 2007; 450:903-7; PMID:18026089; http://dx.doi.org/10.1038/nm1630.

8. Tsouli M, Kirovlkova A, Melicke B, Fredsken T, Mauger S, Bindra G, et al. Clinical impact of different classes of infiltrating T cytotoxic and helper cells (Th1, Th2, treg, th17) in patients with colorectal cancer. Cancer Res 2011; 71:1263-71; PMID:21309379; http://dx.doi.org/10.1158/0008-5472.CAN-10-2007.

9. O’Sullivan T, Saddawi-Konefka R, Vermi W, Koehl CM, Arthur C, White JM, et al. Cancer immunodetion by the innate immune system in the absence of adaptive immunity. J Exp Med 2012; 209:1869-82; PMID:22827549; http://dx.doi.org/10.1084/jem.20112738.

10. Pachynski RK, Zabel BA, Kohar HE, Tejeda NM, Monnier J, Swanson CD, et al. The chemotransactivating chemo arrestin suppresses melanoma by recruiting natural killer cell antirumor defenses. J Exp Med 2012; 209:1427-35; PMID:22753924; http://dx.doi.org/10.1083/jem.20111224.

11. Apte RS, Ghiraghele F, Togniere A, Obeid M, Ortiz C, Grillo A, et al. Toll-like receptor 4-dependent contribution of the immune system to antitumor chemotherapy and radiotherapy. Nat Med 2007; 13:1050-9; PMID:17704786; http://dx.doi.org/10.1038/nm1622.

12. Clibiana J, Hau CS, Dornebal CW, Jonkers J, de Visser KE. Chemotherapy response of spontaneous mammary tumors is independent of the adaptive immune system. Nat Med 2012; 18:344-6; author reply 346; PMID:22395903; http://dx.doi.org/10.1038/nm.2576.

13. Blasius AL, Beutler B. Intracellular toll-like receptors. Immunity 2010; 32:305-15; PMID:20436772; http://dx.doi.org/10.1016/j.immuni.2010.03.012.

14. Yanai H, Ban T, Wang Z, Choi MK, Kawamura T, Negishi H, et al. HMGB1 proteins function as universal sentinels for nucleic-acid-mediated innate immune responses. Nature 2009; 462:99-103; PMID:19989303; http://dx.doi.org/10.1038/nature08512.

15. Fourcade J, Sun Z, Benallaoua M, Guillaume P, Luescher IF, Sander C, et al. Upregulation of Tim-3 and the alarmin HMGB1. Nat Immunol 2010; 11:382-92; PMID:20842436; http://dx.doi.org/10.1038/ni.2276.

16. Sasaki K, Apetoh L, Sullivan JM, Blazar BR, Sorensen C, Werb Z, et al. Marginating dendritic cells suppress nucleic acid-mediated innate immune responses through interactions between the receptor for HMGB1 and the alarmin HMGB1. Nat Immunol 2012; 13:832-42; PMID:22842346; http://dx.doi.org/10.1038/ni.2376.

17. Sattler M, Andersen KG, Randow F, Mayr L, Berz M. Neutrophil-1 expression on regulatory T cells enhances their interactions with dendritic cells during antigen recognition. Immunity 2008; 28:402-15; PMID:18328743; http://dx.doi.org/10.1016/j.immu.2008.01.012.

18. Vicari AP, Chiodoni C, Vaure C, An-Yehia S, Dercamp CL, Hartmann L, et al. Tumor-infiltrating CD8+ T cells function as effectors of adaptive immunity and tumor vaccine efficacy and permits effective immunity. J Exp Med 2012; 209:1978-96; PMID:22978683; http://dx.doi.org/10.1084/jem.201202171.

19. Sreekumar P, Karyampudi L, Behroz MA, Erkine CL, Hartmann L, Dong H, et al. Tumor-infiltrating programmed death receptor-1 dendritic cells mediate immune suppression in ovarian cancer. J Immunol 2011; 186:6905-13; PMID:21551565; http://dx.doi.org/10.4049/jimmunol.2010037.

20. Bai A, Higham EM, Wintrup KD, Chen J. Differential requirement for CD70 and CD80/CD86 in dendritic cell-mediated activation of tumor-tolerized CD8 T cells. J Immunol 2012; 189:1708-16; PMID:22978683; http://dx.doi.org/10.4049/jimmunol.20120171.

21. Koebel CM, Swann JB, Zerafa N, Rodig SJ, Old LJ, et al. Adaptive immunity maintains occult cancer in an equilibrium state. Nature 2007; 450:903-7; PMID:18026089; http://dx.doi.org/10.1038/nm1630.

22. Ferrarini M, Henin C, Cosset S, Aït-Yahia S, Dercamp CL, Hartmann L, et al. Tumor-infiltrating CD8+ T cells function as effectors of adaptive immunity and tumor vaccine efficacy and permits effective immunity. J Exp Med 2012; 209:1978-96; PMID:22978683; http://dx.doi.org/10.1084/jem.201202171.

23. Mu CY, Huang J, Chen Y, Chen C, Zhang XG. High expression of PD-L1 in lung cancer may contribute to poor prognosis and tumor cells immune escape through suppressing tumor infiltrating dendritic cells maturation. Med Oncol 2011; 28:682-8; PMID:20373055; http://dx.doi.org/10.1007/s12032-010-9532-x.

24. Kemppki J, Karyampudi L, Behroz MA, Erkine CL, Hartmann L, Dong H, et al. Tumor-infiltrating programmed death receptor-1 dendritic cells mediate immune suppression in ovarian cancer. J Immunol 2011; 186:6905-13; PMID:21551565; http://dx.doi.org/10.4049/jimmunol.2010037.

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33. Eisenbarth SC, Williams A, Colegio OR, Meng H, Strowig T, Rongeaux A, et al. NLRP10 is a NOD-like receptor essential to initiate adaptive immunity by dendritic cells. Nature 2012; 484:510-3; PMID:22538615; http://dx.doi.org/10.1038/nature11012
34. Villablanca EJ, Racosta L, Zhou D, Fontana R, Maggioni D, Negro A, et al. Tumor-mediated liver X receptor-alpha activation inhibits CC chemokine receptor-7 expression on dendritic cells and dampens antitumor responses. Nat Med 2010; 16:98-105; PMID:20037599; http://dx.doi.org/10.1038/nm.2074
35. Moussion C, Girard JP. Dendritic cells control lymphocyte entry to lymph nodes through high endothelial venules. Nature 2011; 479:542-6; PMID:22080953; http://dx.doi.org/10.1038/nature10540
36. Webster B, Ekland EH, Agle LM, Chyou S, Raggieri R, Lu TT. Regulation of lymph node vascular growth by dendritic cells. J Exp Med 2006; 203:1903-13; PMID:16831898; http://dx.doi.org/10.1084/jem.20052272
37. Dieu-Nojean MC, Antoine M, Danel C, Heudes D, Wiedez M, Pauleit V, et al. Long-term survival for patients with non-small-cell lung cancer with intratumoral lymphoid structures. J Clin Oncol 2008; 26:4410-7; PMID:18802153; http://dx.doi.org/10.1200/JCO.2007.15.0284
38. Martini L, Le Gueller S, Filleron T, Lamant L, Meyer N, Rochaix P, et al. High endothelial venules (HEVs) in human melanoma lesions: Major gateways for tumor-infiltrating lymphocytes. Oncoimmunology 2012; 1:829-39; PMID:23162750; http://dx.doi.org/10.4161/onci.20492
39. Labidi-Galy SI, Teilleux I, Goddard-Leon S, Combes JD, Blay JY, Ray-Coquard I, et al. Plasmacytoid dendritic cells infiltrating ovarian cancer are associated with poor prognosis. Oncoimmunology 2012; 1:380-2; PMID:22737622; http://dx.doi.org/10.4161/onci.18801
40. Labidi-Galy SI, Sisirak V, Meeus P, Gobert M, Teilleux I, Bajard A, et al. Quantitative and functional alterations of plasmacytoid dendritic cells contribute to immune tolerance in ovarian cancer. Cancer Res 2011; 71:5423-34; PMID:21697280; http://dx.doi.org/10.1158/0008-5472.CAN-11-0367
41. Sisirak V, Faget J, Gobert M, Goutagny N, Vey N, Teilleux I, et al. Impaired IFN-α production by plasmacytoid dendritic cells favors regulatory T-cell expansion that may contribute to breast cancer progression. Cancer Res 2012; 72:5188-97; PMID:22836755; http://dx.doi.org/10.1158/0008-5472.CAN-11-3468
42. Vermi W, Soncini M, Melocchi L, Sozzani S, Facchetti F. Plasmacytoid dendritic cells and cancer. J Leukoc Biol 2011; 90:681-90; PMID:21730085; http://dx.doi.org/10.1189/jlb.0411190
43. Jensen TO, Schmidt H, Møller HJ, Donskov F, Høyer M, Sjoegren P, et al. Intratumoral neutrophils and plasmacytoid dendritic cells indicate poor prognosis and are associated with pSTAT3 expression in AJCC stage I/II melanoma. Cancer 2012; 118:2476-85; PMID:21953023; http://dx.doi.org/10.1002/cncr.26511
44. Scarlett UK, Rukkowski MR, Rauwerdink AM, Fields J, Escovar-Fadul X, Baird J, et al. Ovarian cancer progression is controlled by phenotypic changes in dendritic cells. J Exp Med 2012; 209:495-506; PMID:22351930; http://dx.doi.org/10.1084/jem.20111413