Retinoic acid on stage in antitumor immunity

Yanxia Guo and Randolph J. Noelle

1Department of Microbiology and Immunology; The Geisel School of Medicine; Dartmouth University; Lebanon, NH USA;
2Medical Research Council Centre of Transplantation; Guy’s Hospital; King’s College London; London, UK

Keywords: retinoic acid, tumor microenvironment, CD8+ T cell, survival, anti-tumor immunity

The morphogenetic role of retinoic acid (RA) in regulating immunity is underscored by our recent studies that show increased regional concentrations of RA and commensurate RA signaling in CD8+ T cells within the tumor microenvironment. This study demonstrated for the first time that intrinsic RA signaling is required for tumor associated antigen (TAA)-specific CD8+ T-cell survival and hence for tumor surveillance.

The active form of Vitamin A (VA), retinoic acid (RA), in an established morphogen during embryogenesis.1 The fact that RA tissue concentrations are dynamically regulated in the periphery including in the tumor microenvironment is novel. To date, no studies have focused on the role of intrinsic RA signaling in the regulation of antitumor CD8+ T-cell responses.

We have recently shown a striking 5-fold increase in the regional concentrations of RA at B16.OVA melanoma sites as compared with the naïve surrounding skin, concomitant with induction of regional RA signaling, as visualized in tumor-bearing RARE-Luc mice.2 The source of elevated all-trans RA (ATRA) was identified in the hematopoietic compartment, in particular various dendritic cell (DC) subsets, rather than in tumor cells, by ALDEFLOUR staining. The measurement of RA signaling by imaging tumor-bearing bone marrow chimera mice confirmed a tumor-induced RA signaling in the hematopoietic compartment of the host. Specifically, we measured CD8+ T-cell responses to RA in situ, both by quantifying RA signaling-induced luciferase activity in endogenous CD8+ T cells and by imaging adoptively transferred antigen-specific (OTI × RARE-Luc) CD8+ T cells. The source of RA (DCs) and the specific tumor-induced RA signaling in CD8+ T cells strongly suggest that RA signaling is required for antitumor CD8+ T-cell immunity. Such a conclusion is supported by the impaired immune surveillance in mice in which T cells were rendered unresponsive to RA by genetic manipulations.

To define the functional role of RA in T-cell immune surveillance, our studies were performed in transgenic mice in which RA signaling was conditionally blocked through the overexpression of a dominant negative RA receptor α (dnRARα), specifically in T cells. We reasoned that this would be the most effective method for blocking RA signaling and mimic VA deficiency in humans, as it constitutively suppresses the expression of all RA-responsive genes. To unequivocally define a role for RA signaling to CD8+ T cells in tumor immune surveillance, all studies were done in CD4+ T-cell-depleted mice. In this model, we found that RA signaling in CD8+ T cells is required for the priming of CD8+ T cells against tumor associated-antigens (TAs). As such, RA signaling-deficient CD8+ T cells failed to accumulate and control the tumor growth. Such deficient accumulation of TAA-specific CD8+ T cells was observed in both tumor-draining lymph nodes (TDLNs) and primary tumor sites. Our findings were surprising because DCs (the producers of RA at the tumor site) have been regarded as tolerizing antigen-presenting cells (APCs), promoting tumor escape instead of surveillance.3 Recent studies have shown that tumor-infiltrating DCs are indeed pro-inflammatory during the early phases of tumor growth in an inducible ovarian tumor model, supporting the notion that RA producers at least initially may promote antitumor immunity rather than tolerance.4 Nevertheless, at late phases of tumor growth, immunosuppressive molecules such as transforming growth factor β (TGFβ)5 and interleukin-10 (IL-10)6 can be produced by tumor cells, immunosuppressive myeloid-derived suppressor cells (MDSCs) and immature/tolerizing DCs. In this scenario, RA may promote the development of regulatory T cells (Tregs) through synergy with other immunosuppressive molecules. Undoubtedly, more studies are required to address the question whether RA signaling is required for the differentiation, stability and function of Tregs in the tumor microenvironment.

Consistent with our findings, a defective accumulation/expansion of antigen-specific CD8+ T cells has also been observed in a robust anti-CD40 antibody and Toll-like receptor (TLR) agonist vaccination-driven CD8+ T-cell response. Unexpectedly, real-time tracking of endogenous antigen-specific CD8+ T cells in vivo revealed defective survival but not proliferation of RA signaling-deficient CD8+ T-cell precursors per se. Our unpublished results also suggest that defective memory CD8+ T-cell responses develop in the absence of RA signaling, probably reflecting a combinatorial
In summary, our study demonstrates that RA plays an important morphogenic role within the tumor microenvironment. However, many questions regarding the regulation of antitumor immunity by RA remain to be addressed. For instance, it is still unresolved what tumor-derived factors induce the regional production of RA within the tumor microenvironment. In addition, a possible role for RA deficiency in primary expansion and memory differentiation. These findings suggest that cell-intrinsic RA signaling is required for the development of both primary and memory CD8+ T-cell responses, indicating the potential application of RA as an adjuvant for cancer vaccines to boost systemic antitumor immunity beyond the gut-tropic effect, as demonstrated in another recent study.

References
1. Yashiro K, Zhao X, Uehara M, Yamashita K, Nishijima M, Nishino J, et al. Regulation of retinoic acid distribution is required for proximodistal patterning and outgrowth of the developing mouse limb. Dev Cell 2004; 6:411-22; PMID:15030763; http://dx.doi.org/10.1016/S1534-5807(04)00062-0.
2. Guo Y, Pino-Lagos K, Ahonen CA, Bennett KA, Wang J, Napoli JL, et al. A Retinoic Acid–Rich Tumor Microenvironment Provides Clonal Survival Cues for Tumor-Specific CD8+ T Cells. Cancer Res 2012; 72:5230-9; PMID:22902413; http://dx.doi.org/10.1158/0008-5472.CAN-12-1727.
3. Norian LA, Rodriguez PC, O’Mara LA, Zabaleta J, Ochoa AC, Cella M, et al. Tumor-infiltrating regulatory dendritic cells inhibit CD8+ T cell function via L-arginine metabolism. Cancer Res 2009; 69:3086-94; PMID:19293186; http://dx.doi.org/10.1158/0008-5472.CAN-08-2826.
4. Scarlett UK, Rutkowski 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.
5. Yang L. TGFbeta, a potent regulator of tumor microenvironment and host immune response, implication for therapy. Curr Mol Med 2010; 10:574-80; PMID:20455854; http://dx.doi.org/10.2174/156652410791317039.
6. Vicari AP, Chiodoni C, Vaure C, Att-Yahia S, Dercamp C, Matsos F, et al. Reversal of tumor-induced dendritic cell paralysis by CpG immunostimulatory oligonucleotide and anti-interleukin 10 receptor antibody. J Exp Med 2002; 196:541-9; PMID:12186045; http://dx.doi.org/10.1084/jem.20020732.