**CCR5 in cancer immunotherapy**

More than an “attractive” receptor for T cells

Alicia González-Martín, Emilia Mira and Santos Mañes*

Department of Immunology and Oncology; Centro Nacional de Biotecnología CNB/CSIC; Campus Cantoblanco; Madrid, Spain

*Current address: The Scripps Research Institute; Department of Immunology and Microbial Science; La Jolla, CA USA

**Key words:** CCR5, chemokines, cancer, cross-priming, T cell, activation, CD4 help, immunotherapy, antigen-presenting cell, maturation, TLR

**Abbreviations:** APC, antigen-presenting cells; CpG-ODN, CpG oligodeoxynucleotides; LN, lymph node; MMTV, mouse mammary tumor virus

Despite intensive study, the role of CCR5 in cancer remains elusive. We showed that CCR5 expression by both CD4+ and CD8+ T cells is necessary to boost anti-tumor responses by optimizing helper-dependent CD8+ T cell priming. Our findings could have implications for cancer treatment in patients with defective CCR5 expression.

Chemokines and their receptors were classically established as key molecules that control immunity by orchestrating leukocyte trafficking into and from lymphoid organs and tissues. The CCR7/CCL19–21 pair regulates naïve T cell encounter with mature antigen-presenting cells (APC) in lymph nodes (LN); the CXCR5-CXCL13 system controls lymphocyte migration into and within lymphoid tissues, and the so-called inflammatory chemokines guide activated leukocytes to peripheral tissues, enabling pathogen eradication and tissue repair. Given the relevance of chemokines in immune and inflammatory responses, their association to cancer is not surprising, as they both promote and restrict tumor onset and/or progression. CCR5, the receptor for the chemokines CCL3 (MIP1α), CCL4 (MIP1β) and CCL5 (RANTES), exemplifies this paradox; whereas some reports suggest that CCR5 activation fosters tumor growth, angiogenesis, metastasis and immune evasion, studies in mouse and humans justify the use of CCR5 agonists as adjuvants to bolster anti-tumor immune responses.

Indeed, CCR5 ligands can recruit both effector and immunosuppressive cells to tumors. Based on our results in a number of transplanted and spontaneous neoplasia models in mice, we propose that CCR5 is central to maximization of the immune response to tumors.

Since its discovery in 1996 as a HIV-1 coreceptor, CCR5 has been implicated in immune-associated processes that include allograft rejection, autoimmunity and clearance of viral infections. CCR5 function in these pathologies might be not limited to chemotraction of specific leukocyte subtypes. Evidence indicates that CCR5 is a regulatory molecule in T cell activation, where it acts as a costimulatory receptor for CD4+ lymphocytes in a migration-independent manner. CCR5 also participates in helper-dependent CD8+ T cell activation, although its role is not well defined; whereas CCR5 was proposed to steer APC to CCL5-producing CD4+ T cells for in situ CD40L-mediated APC licensing, an intravital two-photon study suggested that CCR5 is required to guide “preactivated” naïve CD8+ cells to CCL3- and CCL4-secreting DC-CCL4+ T cell complexes. In both studies, CCR5 expression in CD4+ lymphocytes was apparently dispensable for helper-dependent CD8+ T cell activation.

What is the role of CCR5 in CD4+ helper function in the context of anti-tumor responses? We compared the effectiveness of tumor rejection after adoptive transfer of tumor-specific, CCR5-expressing or -deficient CD4+ and CD8+ T cells into tumor-bearing mice. Potent CD8+ T cell responses require CD4+ T cell help and, accordingly, we observed effective tumor rejection when recipients were co-transferred with CD4+ and CD8+ T cells. We found that CCR5 expression on CD8+ T cells was necessary for their efficient activation and migration to the tumor site and for tumor killing; importantly, CCR5 must also be expressed by CD4+ T lymphocytes to achieve maximal CD8+ T cell effector function. We found a CCR5-dependent enhancement of CCL3, CCL4 and CCL5 secretion by CD4+-APC complexes, which correlated with increased CD8+ T cell priming.

High CD40L expression in CD4+ cells was CCR5-dependent, and led to enhanced upregulation of CD80, CD86 and MHC-II in APC. We propose that CCR5 and its ligands regulate communication between CD4+, APC and CD8+ cells in the draining LN, not only by steering encounter of...
suggest intricate crosstalk circuits for the combinatorial control of CCR5 responses by TLR.

In summary, our results, together with other studies recently published, implicate CCR5 and its ligands as key elements in maximizing T cell-mediated responses, of potential importance in tumor elimination and pathogen clearance.

Deciphering how the inflammatory environment affects CCR5 function, determining the role of CCR5 in acute vs. chronic immune responses, and the planning of clinical trials to determine the therapeutic response of cancer patients with polymorphisms in CCR5 and/or CCR5 ligand genes, are challenges that, if resolved, could have considerable impact on the design and/or improvement of cancer therapies.

Acknowledgments
We thank lab members for discussion and C. Mark for editorial assistance. We would like to dedicate this work to the memory of our collaborator Joseph Lustgarten. Our apologies to colleagues who were not cited due to space restrictions. This work was supported in part by the Spanish Ministry of Science and...
Innovation (SAF2011-24453), the Carlos III Health Institute (RIER Network, RD08/0075), and the Comunidad de Madrid (IMMUNOTHERCAN; S2011/BMD-2326).

References
1. Viola A, Contento RL, Molon B. T cells and their partners: The chemokine dating agency. Trends Immunol 2006; 27:421-7; PMID:16860609; DOI:10.1016/j.it.2006.07.004.
2. Lapteva N, Huang XF. CCL5 as an adjuvant for cancer immunotherapy. Expert Opin Biol Ther 2010; 10:725-33; PMID:20233026; DOI:10.1517/14712591003657128.
3. Conforti R, Ma Y, Morel Y, Paturel C, Terme M, Viaud S, et al. Opposing effects of toll-like receptor (TLR3) signaling in tumors can be therapeutically uncoupled to optimize the anticancer efficacy of TLR3 ligands. Cancer Res 2010; 70:490-500; PMID:20688181; DOI:10.1158/0008-5472.CAN-09-1890.
4. González-Martín A, Gómez L, Lungarten J, Mira E, Mañes S. Maximal T cell-mediated antitumor responses rely upon CCR5 expression in both CD4+ and CD8+ T cells. Cancer Res 2011; 71:5455-66; PMID:21715565; DOI:10.1158/0008-5472.CAN-11-1687.
5. Molon B, Gri G, Bette C, Gómez-Mouriño C, Lanzavecchia A, Martinez AC, et al. T cell costimulation by chemokine receptors. Nat Immunol 2005; 6:465-71; PMID:15821758; DOI:10.1038/ni1191.
6. Nesheth YC, Martinez DG, Totaya S, Scarlett UK, Cubillos-Ruiz JR, Rutkowski MR, et al. CD4+ T cells elicit host immune responses to MHC class II ovarian cancer through CCL5 secretion and CD40-mediated licensing of dendritic cells. J Immunol 2010; 184:5654-62; PMID:20400704; DOI:10.4049/jimmunol.0903247.
7. Castellino F, Huang AY, Altan-Bonnet G, Stoll S, Schein C, Germain RN. Chemokines enhance immunity by guiding naive CD8+ T cells to sites of CD4+ T cell-dendritic cell interaction. Nature 2006; 440:890-5; PMID:16614374; DOI:10.1038/nature04651.
8. Melief CJ. Cancer immunotherapy by dendritic cells. Immunity 2008; 29:372-83; PMID:18799145; DOI:10.1016/j.immuni.2008.08.004.
9. Lungarten J, Domínguez AL, Caudros C. The CD8+ T cell repertoire against Her-2/neu antigens in neu transgenic mice is of low avidity with antitumor activity. Eur J Immunol 2004; 34:752-61; PMID:14991605; DOI:10.1002/eji.200324427.
10. Crawford A, Angelosanto JM, Nadwodny KL, Blackburn SD, Wherry EJ. A role for the chemokine RANTES in regulating CD8+ T cell responses during chronic viral infection. PLoS Pathog 2011; 7:1002098; PMID:21814510; DOI:10.1371/journal.ppat.1002098.