The University of Wisconsin - Madison

CMP

Cellular and Molecular Pathology

Melba Marie Tejera

Program of the Thesis Defense Seminar for the

Degree of Doctor of Philosophy

in Cellular and Molecular Pathology

"Role of FoxO1and IκBα in memory T cell differentiation"

Thursday, July 24, 2014
11:00 A.m.
Room 2255 School of Veterinary medicine

Research conducted in the lab of
Suresh Marulasiddappa, PhD
Department of Pathobiological sciences

Melba Marie Tejera's Thesis Abstract

"Role of FoxO1and IκBα in memory T cell differentiation"

Melba Marie Tejera
Under the supervision of
Professor Suresh Marulasiddappa, PhD
at the University of Wisconsin-Madison

It is well established that T cells play a crucial role in defense against viral, intracellular bacterial and protozoon infections. Therefore, vaccines against these agents need to elicit potent T cell memory. However, the mechanisms that regulate the establishment and maintenance of T cell memory are poorly understood and identifying and understanding the mechanisms that govern the differentiation of memory T cells is fundamentally important for the development of effective T cell-based vaccines against viral infections of humans and animals. Typically acute viral infections are known to induce durable and often life-long immunity to re-infection. Therefore, for rational design of vaccines, it is important to understand the mechanisms underlying T cell memory to acute viral infections. Acute viral infections in humans and mice elicit strong CD8 T cell response, which is characterized by massive expansion of virus-specific effector T cells. Following viral clearance, ~90% of the virus-specific effector cells undergo apoptosis and the remainder of the cells differentiate into long-lived memory T cells. Upon reinfection, memory T cells display: accelerated responses, differentiate into effector cells, and clear the infection expeditiously, which is the basis of vaccination. A critical question in the field of T cell memory is, what governs the apoptosis of the majority of effector cells and survival of memory T cells? Recent work has shown that the population of effector cells present at the peak of the CD8 T cell response is comprised of two subsets: the terminally differentiated short-lived effector cells (SLECs) that are slated for deletion and the memory precursor effector cells (MPECs) that differentiate into long-lived memory CD8 T cells (CD4 T_{H1} and T_{FH}- MPEC and SLEC type subsets have also been elucidated). The work in the Suresh lab is interested in deciphering the molecular mechanisms underlying the differentiation of SLECs and MPECs, and the subsequent differentiation of MPECs into long-lived memory CD8 and CD4 T cells. Specifically, we are interested in examining how the PI3K/Akt/FoxO1 signaling pathway and the IκBα/NF-κB nuclear export pathway integrate signals emanating from multiple cell surface receptors to modulate the downstream substrates and control the differentiation of memory CD8 and CD4 T cells.

Publications

- David J. Gasper, **Melba Marie Tejera**, M. Suresh. CD4 T-Cell Memory Generation and Maintenance. Critical Reviews in Immunology.34 (2):121–146 (2014) PMCID: PMC4062920
- **Tejera MM**, Kim EH, Sullivan JA, Plisch EH, Suresh M. FoxO1 controls effector-to-memory transition and maintenance of functional CD8 T cell memory. *J Immunol.* 2013 Jul 1; 191 1):187-99. PMCID: PMC3691324
- Kim EH, Sullivan JA, Plisch EH, **Tejera MM**, Jatzek A, Choi KY, and Suresh M. Signal integration by Akt regulates CD8 T cell effector and memory differentiation. *J Immunol.* 2012 May 1; 188(9): 4305-14. PMCID: PMC3331885
- Jatzek A, **Tejera MM**, Plisch EH, Fero ML and Suresh, M. T Cell Intrinsic and Extrinsic Mechanisms of p27Kip1 in the Regulation of CD8 T Cell Memory. *Immunol Cell Biol*. 2013 Feb; 91 (2):120-9. PMCID: PMC3570738
- Jatzek A, **Tejera MM**, Sullivan JA, Plisch EH, and Suresh, M. p27Kip1 Negatively Regulates the Magnitude and Persistence of CD4 T Cell Memory. *J. Immunol* 2012 Dec 1; 189 (11):5119-28. PMCID: PMC3504176

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Abstract Continued

FoxO1 is a member of the forkhead box subgroup 'O' transcription factor family. FoxO1 is preferentially expressed in lymphoid cells and functions by regulating the expression of genes that are involved in promoting cellular quiescence and apoptosis. Recently, studies have shown FoxO1 might play a crucial role in the maintenance of T cell homeostasis, but its role in CD8 T cell memory remains unknown. To elucidate the potential role for FoxO1 in regulating CD8 T cell memory to acute viral infections, we utilized the well-characterized mouse model of infection with lymphocytic choriomeningitis virus (LCMV). Infection of mice with LCMV stimulates a potent CD8 T cell response and virus is eliminated by day 8-10 post-infection; LCMV-immune mice develop life-long CD8 T cell memory-dependent protective immunity to re-infection. To understand the role of FoxO1 in CD8 T cell memory, we developed mice that are conditionally deficient for FoxO1 in T cells (FOXO1-/-). In chapter three, I show that during the mouse T cell response to LCMV, FoxO1 controls the effector-to-memory transition of CD8 T cells. I also show that FoxO1 plays a non-redundant role in maintaining the functionality of CD8 T cell memory and secondary responses. Also, I find that FoxO1 regulates the function of effector and memory CD4 T cells. In my studies using bone marrow chimeras I show qualitatively that, T-cell intrinsic FoxO1 is not required for clonal expansion, but it is necessary for the survival of memory CD8 T cells under competitive conditions. Furthermore, by the use of microarrary, I was able to elucidate that the deficiency of FoxO1 dysregulated the transcriptome of memory CD8 T cells.

These studies lead us to further assess the temporal requirement for FoxO1 in memory CD8 T cell differentiation and protective immunity. In chapter four, to address whether FoxO1 regulates the maintenance of memory CD8 T cells by CD8 T cell-intrinsic bone marrow chimera model, of the inducible Cre-mediated mice. Using this model, I show that FoxO1 is required for the transcription of several target genes that regulate the differentiation of CD8 T cell memory at all the phases of a CD8 T cell response to LCMV. Together, these findings have implications for the development of novel strategies to modulate FoxO1 activity in T cells, hence enhancing vaccine-induced CD8 T cell memory.

In un-activated, resting T cells, NF- κB is sequestered in the cytoplasm by inhibitor IκBs. Upon T cell activation, specific serine residues on the IκBs are phosphorylated, signaling them for ubiquitination, which in turn targets IκBs for proteasome-mediated degradation. When the NF-κB dimer is released from IκB, it translocates into the nucleus and activates the transcription of target genes, which include those involved in inflammatory, immune, and acute phase responses. In chapter five I investigated the requirement for IκBα nuclear export on effector and memory CD8 T cell differentiation in response to an acute LCMV infection. By mutating the nuclear export sequence of IκBα in mice ($Nfkbia^{NES/NES}$ mice), I show that in response to a viral infection, there is a significantly reduced overall activation of CD8 T cells as well as a reduced number of virus-specific CD8 T cells during the expansion phase. However, they have greater proliferation.

Abstract Continued

Surprisingly, upon assessing the differentiation of the effector subset, I found that the lack of IκBα nuclear export reduced the capability of Nfkbia$^{NES/NES}$ mice to populate a CD8 T cell effector pool and there was also a reduced amount of CD8 T cells in Nfkbia$^{NES/NES}$ mice capable of producing IFN-γ. Upon assessing memory CD8 T cells, we found that only the MPEC and SLEC subset differentiation and IFN-γ production was affected by the loss of IκBα's ability to export out of the nucleus.

It was previously shown that developmentally, T cells from naive Nfkbia$^{NES/NES}$ mice were unperturbed and had no observable defects from *in vitro* proliferation assays, but CD4 T cells were found to be more "memory-like". In chapter six, I further investigated the requirement of IκBα nuclear export of NF-κB in the differentiation CD4 T cells during an acute LCMV infection. Collectively, data presented illuminate a role for IκBα nuclear export of NF-κB as a key regulator in the differentiation of effector and memory subsets of T_{H1} and T_{FH} CD4 T cells by significantly reducing the overall accumulation of CD4 T cells, reducing the activated status of CD4 T cells, and increasing the accumulation of LCMV-specific CD4 T cells-and by regulating the homeostatic balance of key transcription factors of these subsets. These results are expected to have significant implications in the development of vaccines that induce enduring immunity of T_{H1} and T_{FH} CD4 T cells.

Given the current worldwide challenges of devising better and more effective vaccines to prevent a variety of diseases such as AIDS, influenza, mononucleosis, tuberculosis and malaria, gaining a better understanding of the mechanisms that guide the differentiation and maintenance of memory CD8 and CD4 T cells remains of great importance to researchers. In my dissertation I have defined the requirement for and the temporal necessity for FoxO1 in the maintenance of CD8 T cell memory and identified novel FoxO1-regulated genes that govern the quality and protective ability of memory CD8 T cells. Furthermore, I have identified a novel role of IκBα nuclear export of NF-κB, and potential regulation of key transcription factors in the differentiation of effector and memory CD8 and CD4 T cells during an acute infection. These findings should lay the foundation for exploring the function of FoxO1 and NF-κB target genes in regulating T cell memory.

Presentations

- **Melba Marie Tejera** and M. Suresh. "FOXO1 is required for maintenance of protective CD8 T cell memory." Keystone Symposium. Immunological Mechanisms of Vaccination. December 13-18, 2012, Ottawa, Canada.
- **Melba Marie Tejera** and M. Suresh, "Forkhead Transcription Factor FoxO1 Regulated CD8 T Cell Contraction and Differentiation of Memory Cells." Autumn Immunology Conference, 20-23 November 20-23, 2009. Chicago, Illinois.

Presentations Continued

- **Melba Marie Tejera**, Christine Wendt, Ashley Husebye, and Chad Cummings. "Differential Protein Expression in Chronic Lung Transplant Rejection by Human BALF-BOS and Murine OB-iTRAQ Profiles." Annual Biomedical research conference for minority students. November 7-10, 2007. Austin Texas.
- **Melba Marie Tejera** and M. Suresh. "NF-κB regulates the homeostasis of CD8 and CD4 T cells during a LCMV infection." Virology Training Program Seminar Series. January 30^{h}, 2014
- **Melba Marie Tejera** and M. Suresh. "FOXO1 is required for maintenance of protective CD8 T cell memory." Virology Training Program Seminar Series. November 8th, 2013
- **Melba Marie Tejera** and M. Suresh. "FOXO1 is required for maintenance of protective CD8 T cell memory." Student Seminar Series. November 15^{h}, 2012
- **Melba Marie Tejera** and M. Suresh. "Regulation of Memory CD8 T cells by FoxO1." Student Seminar Series. November 17^{h}, 2011
- **Melba Marie Tejera** and M. Suresh. "Short lived effector memory CD8 T cells live long and prosper: Foxo1 a hidden secret." Pathobiological Sciences Seminar Series. March 9^{h}, 2011
- **Melba Marie Tejera** and M. Suresh. "The Role of Forkhead Transcription Factor Foxo1 in the Regulation of CD8 T Cell Homeostasis During an Acute Viral Infection." Student Seminar Series. Febuary 3^{h}, 2011
- **Melba Marie Tejera** and M. Suresh. "Forkhead Transcription Factor FOXO1 Regulates CD8 T Cell Contraction and Differentiation of Memory CD8 T Cells." Pathobiological Sciences Seminar Series. March 18, 2010
- **Melba Marie Tejera** and M. Suresh. "P27^{kip1} and its role in T-Cell differentiation during an acute viral infection. Student Seminar Series. May 7, 2009

Honors & Awards

2004-2008	Bright Futures Florida Medallion Scholarship
2004-2008	University of South Florida Scholars
2006-2008	Minority Access to Research Careers (MARC) Scholarship
2008- Present	Advanced Opportunity (SciMed GRS) Fellowship
Fall 2009	John H. Wallace Award *travel award*
Fall 2012-Present	UW-Virology Training Program- NIH Scholarship
Fall 2012	Keystone Underrepresented Trainee Scholarship *travel award*