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Th17 cells have stem cell-like features and promote long-term immunity

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Memory T cells are one of the most effective components of anti-tumor immunity. However, limited studies on cancer patients have not addressed the phenotypic, genetic and functional heterogeneity of memory T-cell subsets in the human cancer environments. Human IL-17$^+$CD4$^+$ (Th17) cells are confined to memory T-cell compartment with CD45RO$^+$CD62L$^-$CCR7$^-$ phenotype and are enriched in CD49$^+$CCR6$^+$ population. Th17 cells do not express PD-1, FoxP3, KLRG-1, CD57 and IL-10, making them unlikely candidates for being functionally exhausted PD-1$^+$ T cells or suppressive Foxp3$^+$ or IL-10$^+$ T cells or senescent CD28$^-$CD57$^+$KLRG-1$^+$ T cells. However, Th17 cells express high levels of CD95 and moderate levels of CD27. Th17 cells phenotypically resemble terminally differentiated memory T cells. Interestingly, Th17 cells possess polyfunctional cytokine profile, and have stem cell-like features. Th17 stemness may be partially controlled by signaling pathways of hypoxia inducible factor HIF1α, Notch and Bcl. The stem cell-like character of Th17 cells is an important decisive factor for Th17 cell biology.

Keywords: Th17 cell, memory T cell, survival, apoptosis, stem cell, tumor immunity, IL-17, HIF, Notch, Bcl2

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Polyfunctional Cytokine Profile, but not Surface Phenotype Determines Th17 Functionality

Memory T cells are long-lived cells with a heightened capacity to respond to subsequent insults with the same pathogen. One useful model put forward delineates memory T cells into two subsets based on their expression of CCR7 and CD62L.[1,2] Based on this model, central memory T cells generally express both CCR7 and CD62L which are essential for lymphocytes to traverse high endothelial venules and to enter lymph nodes, whereas effector memory T cells express neither. These characteristics have led to the proposal that central memory T cells predominantly reside in the lymph nodes, blood and spleen, whereas effector memory T cells predominate in non-lymphoid tissues (such as the gut, lung and liver and tumor). In terms of function, freshly isolated effector memory T cells, but not central memory T cells, express high levels of IFNγ, and perforin and granzyme B molecules, which are necessary for lytic activity. However, recent studies have demonstrated that phenotypic and functional heterogeneity exists within memory T-cell populations, and the central vs. effector memory division is much less clear cut in humans. Several reports describe cells with apparent memory phenotypes in cancer patients.[3,4] One function of these cells is to produce effector molecules, such as IFNγ and granzyme B, and was inferred by RNA analysis without detailed genomic and functional analysis.[3,4] Some functional evidence for the induction of memory T cells in patients comes from a humanized model of breast cancer.[5,6] In these studies, a significant proportion of bone marrow cells were memory T cells (CD45RA$^-$), with the majority of these expressing low levels of CD62L. It has also been shown that TAA-specific CD8$^+$ T cells can be established from tumor associated memory T cells in patients with cancer. In vitro experiments demonstrated that these cells respond to tumor antigens, and adoptive transfer of these cells into NOD/SCID mice implanted with autologous tumors led to homing to the tumor tissue and inhibited tumor growth.[7-9] However, most of these studies focus on

CD8+ memory T cells. Human tumor associated CD4+ memory T cells are poorly understood.

Human tumor environmental Th17 cells are confined to memory T-cell compartments with CD45RO+CD62L−CCR7− phenotype and are enriched in CD49+CCR6+ population.[10,11] Th17 cells do not express PD-1, FoxP3, KLRG-1, CD57 and IL-10. Furthermore, Th17 cells express high levels of CD95 and lower levels of CD27. Thus, Th17 cells phenotypically resemble terminally differentiated memory T cells. This phenotype is universally observed in the human microenvironments of cancer, autoimmune lesions and organ transplantation.[12] Recent mouse data supports the notion that Th17 cells have a phenotype of terminally differentiated memory T cells.[13] Human Th17 cells express polyfunctional cytokine profile including IL-2, IFNγ, TNFα and GM-CSF (**Fig. 1**). The synergy between different cytokines derived from Th17 cells is mechanistically important for Th17-medaited effector function. For example, IL-17 and IFNγ synergistically induce β-defensine expression to promote psoriatic progression[11] and stimulate type-I> chemokine production[10] to enhance effector T-cell and NK-cell tumor trafficking (**Fig. 1**). Thus, polyfunctional cytokine profile, but not surface phenotype, determines Th17 cell functionality (**Fig. 1**).[14]

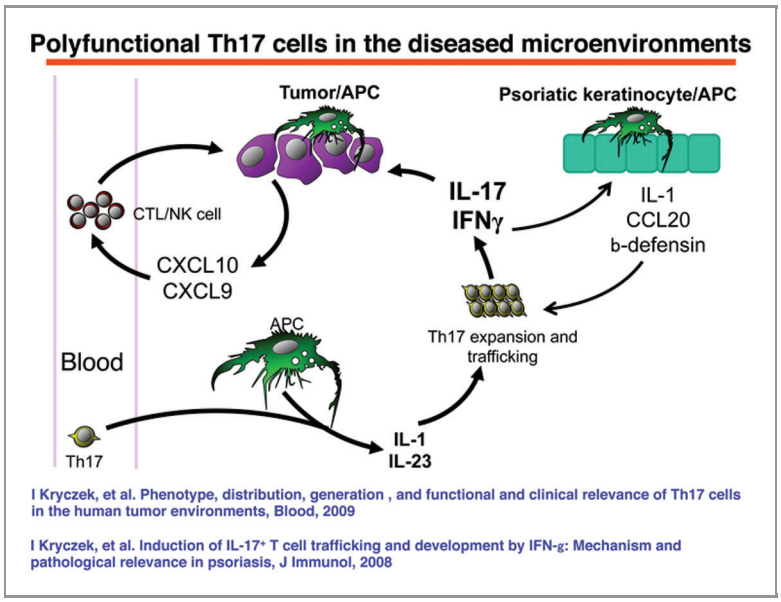

Figure 1. Polyfunctional Th17 cells in the diseased microenvironments. Th17 cells from blood and peripheral tissues are recruited into the microenvironments of tumor and autoimmune lesions. Myeloid antigen-presenting cells (APCs) secrete IL-1 and IL-23, which results in Th17 cell expansion. Th17 cells express polyfunctional cytokines including IL-17 and IFNγ. Th17 cell-derived IL-17, IFNγ, along with Th1-derived IFNγ, stimulates expression of CXCL9 and CXCL10 in the tumor environment. These chemokines recruit T cells and NK cells into the local environment, where they execute antitumor responses. Or, Th17 derived IL-17 and IFNγ induce keratinocytes and APCs to secrete β-defensin 2 and CCL20 in the psoriatic environment, which further increase the recruitment of Th17 cells into the autoimmune lesion and promote keratinocyte proliferation, and psoriasis.

Genetic Pattern but not Surface Phenotype Determines the Fate of Memory Th17 Cells

It is thought that terminally differentiated memory T cells may have a short half-life with senescent and exhausted phenotype, and provide limited protective anti-tumor immunity. Given the terminally-differentiated phenotype of Th17 cells, it is assumed that mouse Th17 cells may be short-lived T cells.[15] Interestingly, we recently demonstrated that human Th17 cells express high levels of multiple stem cells associated genes including HIF1α, Notch, Bcl2, OCT4, and Nanog. These cells are long-lived with high self-renewal capacity, are resistant to apoptosis induced by TCR engagement or chemotherapy, and mediate/promote long-term anti-tumor immunity (**Fig. 2**).[12] Similar observations were made in mouse studies.[13] Furthermore, we have demonstrated that HIF1α/Notch/Bcl-2 is a key signaling pathway controlling Th17 cell survival and apoptosis pattern (**Fig. 2**).[12] Therefore, genetic pattern, but not surface phenotype, determines the fate of memory Th17 cells.

The self-renewal, expansion and multi-lineage developmental potential define the unique properties attributed to stem cells. The capacity to continuously generate effector memory T cells will replenish the effector memory T-cell pool, and help maintain a constant repertoire of memory T cells for a human lifetime, despite the finite lifespan of individual effector cells and reduced thymus function.[1,2,16] Based on the genetic and functional observations, but not the surface phenotype, our data provide evidence supporting the "stem cell-like CD4+ memory T cell" concept in humans,[17] and indicate that human Th17 cells have stem cell-like properties (**Fig. 2**). This concept is supported by recent studies on Th17 cells in tumor bearing mouse model.[13]

It is important to point out that "stem-like memory T cells" may encompass the capability to both self-renew and to generate more differentiated, memory T-cell populations. This concept was initially stemmed from mouse studies. Mouse central memory T cells are arrested at a pre-differentiation stage by transcriptional inhibitors and retain replicative potential and long-term production of effector T cells after a second antigenic challenge.[2] The capacity to continuously generate effector memory T cells will replenish the effector memory T-cell pool, and help maintain a constant repertoire of memory T cells for a human lifetime, despite the finite lifespan of individual effector cells.[1] This notion has recently received certain experimental support. In a mouse model of graft-vs.-host disease, $CD44^{low}CD62L^{high}$ memory CD8+ T cells express high cell surface levels of stem cell antigen-1 (Sca-1), B-cell lymphoma protein-2 (Bcl-2) and common IL-2 and IL-15 receptor β chain (CD122).[18] Because these cells showed robust

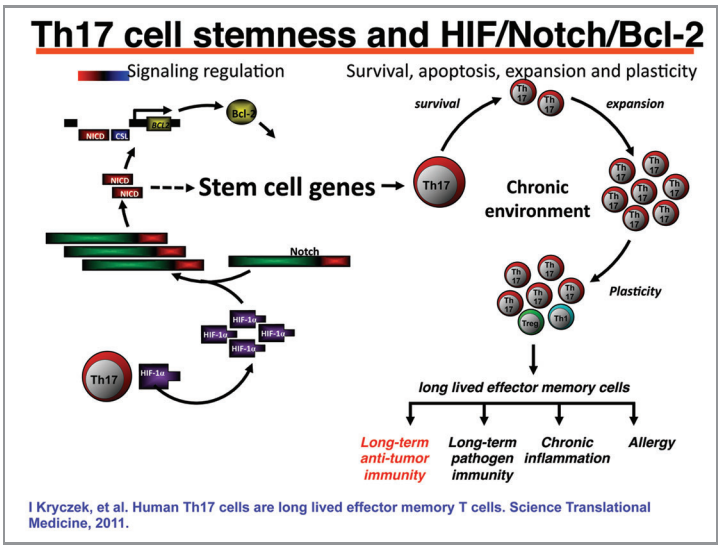

Figure 2. Th17 stemness, HIF/Notch/Bcl2 signaling and T cell immunity. Human Th17 cells are resistant to apoptosis and long-lived memory T cells. Th17 cells express high levels of HIF-1α, Notch signaling molecules and Bcl2, and multiple stem cell core genes. HIF/Notch/Bcl2 signaling pathway controls Th17 cell survival, and promote Th17 cell-mediated T cell immunity. Th17 cells are polyfunctional and highly plastic in the chronic inflammatory environment, and would be converted into regulatory T cell-type and Th-1 type effector cells. It remains unknown if HIF/Notch/Bcl2 signaling pathway regulates Th17 cell plasticity.

self-renewal and the multipotent capacity to generate central memory and effector memory T cells, it is thought that these cells have "stem cell" characters. More recent work has shown that by blocking T-cell differentiation, Wnt signaling promoted the generation of this memory stem cell population in mice[19] and in human.[20] Mouse studies also indicate that Th17 cells possess stem cell properties, are long-lived and mediate potent tumor immunity.[13] We have further revealed the molecular mechanisms by which HIF1α/Notch/Bcl-2 signaling pathways are important for maintaining Th17 cell stemness (**Fig. 2**). Interestingly, a recent report in mouse system also supports the concept that HIF1α signaling pathway is crucial for controlling Th17 cell biology.[21]

Conclusion

The existence of "memory stem T cells or stem-like memory T cells" or the concept of "memory stem T cells" is arguable. Nonetheless, our understanding of memory T cells in human tumor microenvironment lags behind the much more comprehensive analyses of these cells in infectious disease models. This deficiency significantly tempers our efforts toward understanding basic human memory T-cell biology, establishing and evaluating immune therapeutic regimens and tumor vaccines in treating patients with cancer. It is essential to conduct comprehensive phenotypic, genetic and epigenetic, and functional research on the nature of memory T cells in the human tumor microenvironment.

In clinical settings, although clinical efficacy needs to be improved, adoptive T-cell therapy indicates that tumor associated memory T cells (or tumor draining lymph node T cells) can be isolated and expanded by a variable of methods, and these T cells can induce tumor regression in patients with cancer.[22,23] Altogether, current information indicates that memory T cells, including Th17 cells, could be one of the most effective components of anti-tumor immunity. However, limited studies on cancer patients have not addressed the phenotypic and functional heterogeneity of memory T cells in the human cancer environment, in which genetic and epigenetic factors, but not surface phenotype, influence and/or determine the fate of these memory T cells. Thus, mechanistic, clinical and comparative analyses on human memory T-cell heterogeneity will pave the way for manipulating these cells for clinical benefit.

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