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Human Th17 cells in patients with cancer
Friends or foe?

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The role of interleukin-17 (IL-17)-secreting CD4+ T (Th17) cells in cancer is under intense investigation. We have demonstrated that CCR4+CCR6+ Th17 cells not only are increased in the peripheral blood of patients affected by hepatocellular carcinoma, but also suppress CD8+ T-cell functions in vitro. These results suggest that Th17 cells may exert immunosuppressive functions in hepatocellular carcinoma.

Human CD4+ T cells can be subdivided into different helper subsets, namely Th1, Th2, Th9, Th17 and regulatory T cells (**Fig. 1**). Interleukin-17 (IL-17)-secreting CD4+ T (Th17) encompass a distinct lineage of pro-inflammatory T helper cells, which has been initially described to play a major role in autoimmune diseases.[1] However, the role of Th17 cells in cancer is not clear. Th17 cells have been shown to actively enhance or inhibit the progression of tumors.[2] It is believed that different factors influence these conflicting results including, but not limited to, the type of cancer, the source of tissue examined and the biological focus of the study, such as immune responses or angiogenesis, as summarized in a recent review.[3]

Our laboratory has been interested in the immunotherapy of hepatocellular carcinoma (HCC) for more than a decade. We have shown that fully functional tumor-specific T cells can be found in patients affected by HCC.[4] However, in many cases their antitumor efficiency is limited by immunosuppressive cells, which can be found at increased frequencies in HCC patients. CD4+FOXP3+ regulatory T cells (Tregs) and CD14+HLA-DR$^{low/neg}$ myeloid derived suppressor cells (MDSCs) represent typical immunosuppressive cells.[5] We have demonstrated that MDSCs facilitate the accumulation of FOXP3+ Tregs in vitro. In contrast CD14+HLA-DR+ monocytes stimulate the differentiation of Th17 cells.[6] Based on these results and on data published by others demonstrating that the number of tumor-infiltrating Th17 cells inversely correlates with disease outcome,[7] we have decided to examine Th17 cells in more detail.

We first examined Th17 cells in the peripheral blood from HCC patients and found them at higher frequencies than in healthy controls or patients with other liver diseases. However, the Th17 cell count was still too low to isolate a number of cells that would suffice for functional in vitro studies. Therefore, we generated Th17 cells in vitro, starting from naïve CD4+ T cells isolated from healthy donors, and sorted them using a bispecific antibody that binds to CD45 and IL-17. This allowed us to obtain a cell purity higher than 95%. In vitro generated Th17 cells suppressed the proliferation of CD3/CD28-stimulated CD8+ T cells as well as their release of interferon γ (IFNγ).

Th17 cells consist of two sub-populations, which express IL-17 alone or in combination with IFNγ. It has been shown that IL-17+IFNγ+ Th17 cells express both CCR4 and CCR6.[8] Therefore, we decided to study this sub-population of Th17 cells in more detail. Unexpectedly, we noticed that only CCR4+CCR6+ Th17 cells but not CCR4−CCR6+ human Th17 cells suppress CD8+ T-cell responses. Additional experiments revealed that CCR4+CCR6+ Th17 also inhibit cytokine release by antigen-specific T cells.

Based on these observations, we examined Th17 cell sub-populations in the peripheral blood of patients affected by HCC. While there was no difference in the frequency of CCR4−CCR6+IL-17+ CD4+ T cells, we observed a clear increase in the frequency of CCR4+CCR6+IL-17+ CD4+ T cells in the peripheral blood of HCC patients as compared with that of healthy donors.

While our study clearly demonstrates the immunosuppressive activity of human CCR4+CCR6+ Th17 cells in vitro, future studies are needed to address the potential relevance of these findings in vivo. In this context, it should be noted that a recent study by Ghiringhelli's group provided evidence that in vitro generated murine Th17 cells express CD39 and CD73, allowing for the release of immunosuppressive adenosine and hence for the suppression of both CD4+ and CD8+ T-cell functions.[9] Our preliminary experiments using human Th17 cells did not provide evidence for such a mechanism. Instead, we observed that the immunosuppressive function of Th17 cells can be partially abrogated by interventions aimed at blocking transforming growth factor β (TGFβ) signaling.

Future studies are needed to understand the biology of CCR4+CCR6+ Th17 cells. In particular, how CCR4+CCR6+ Th17 cells are regulated in vivo, what causes the expression of CCR4 and what leads to the

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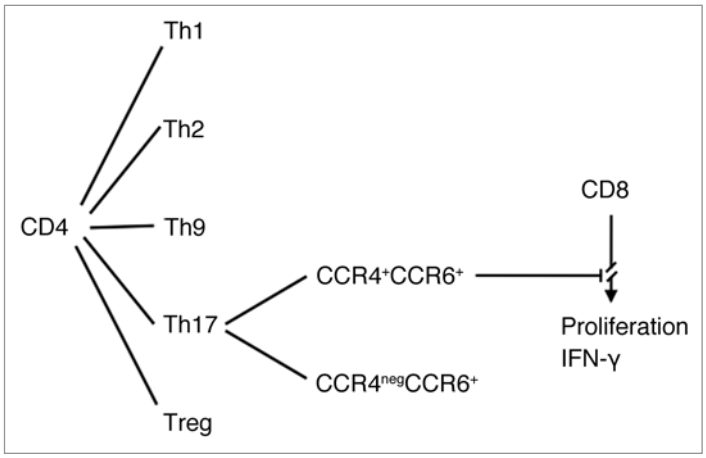

Figure 1. Subsets of CD4+ T helper lymphocytes.

accumulation of this cell type needs to be further investigated. Our study shows that the classification of human helper T cells in 3–4 subsets might constitute an oversimplification, and only genetic studies will help to precisely determine the T helper cell subtypes.

The most important question remains unanswered: do Th17 cells support tumor growth or do they elicit antitumor immune responses? We propose that including Th17 cells in immune monitoring protocols might provide further insights into this issue. Ultimately, this will help in understanding the precise role of Th17 cell sub-populations in cancer immunity.

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