

Diverse appearance of human CD8⁺ T cells: pro-inflammatory or regulatory

Contribution to YSC Davos 2008

Thematic topic: Natural sciences

Kerstin Siegmund^{1, 2}, Beate Rückert¹, Nadia Ouaked¹, Simone Bürgler¹, Cezmi A. Akdis¹ and Carsten B. Schmidt-Weber^{1, 3}

¹ Swiss Institute of Allergy and Asthma Research (SIAF), Davos, Switzerland

² siegmund@siaf.uzh.ch

³ Allergy and Clinical Immunology, National Heart & Lung Institute, Imperial College London, London, United Kingdom

Key words: immune regulation/tolerance; T cell immunology; CD8⁺ T cells

INTRODUCTION

The human immune system defends our body against pathogens, like bacteria, fungi and viruses, while being tolerant to the body's own constituents and harmless environmental antigens. T cells as part of the adaptive immunity are of particular importance in this context. T cells are divided by the expression of their co-receptors into CD4⁺ and CD8⁺ T cell subsets. Historically, these subsets were named, due to their originally described function, helper T (T_H) cells and cytotoxic T cells (T_C) for the CD4⁺ and CD8⁺ T cells, respectively. The main function of CD4⁺ T cells was thought to help other immune cells like B cells and macrophages, whereas CD8⁺ T cells kill virus-infected cells.

Among the CD4⁺ and CD8⁺ T cell subsets, several specialized T cell populations exist to fulfill the diverse tasks of the immune system. In the mid 1980s, Robert Coffman and Tim Mosmann introduced the T_H1/T_H2 hypothesis, which explained how effector T cell populations account for the distinct outcomes of immune reactions (Coffman 2006). Recently, another effector T cell subset was added to the picture: the T_H17 cells. They comprise a new T cell lineage distinct from T_H1 and T_H2 cells. The different T cell subsets are characterized first and foremost by the cytokines they produce. Thus, T_H1 cells secrete mainly IFN- γ , whereas T_H2 cells secrete IL-4, IL-5 and IL-13. T_H17 cells are characterized by the expression of pro-inflammatory cytokines of the IL-17 family. While these subpopulations are well described for CD4⁺ T cells, much less is known about CD8⁺ T cell subpopulations. However, T_C1, T_C2 and T_C17 seem to behave similar than their T_H counterparts.

Beside these effector cell subsets regulatory T cells (Tregs) exist. T cells with regulatory capacity have been shown to belong to the CD4⁺ as well as the CD8⁺ T cell subset. Although the first suppressive T cells described in the 1970s were claimed to belong to the CD8⁺ subset (Green 1983), not much is known about CD8⁺ Treg development and function. In contrast to effector T cells, Tregs express mainly anti-inflammatory cytokines like IL-10 and TGF- β and suppress the proliferation and the activation of other immune cells.

The development of the distinct T cell lineages depends in particular on the lineage-specific transcription factors T-bet, GATA3, ROR γ t and FOXP3 for T_HC1, T_HC2, T_HC17 and Tregs, respectively.

In this project, we aimed to characterize distinct CD8⁺ T cell populations especially FOXP3-expressing CD8⁺ regulatory T cells.

MATERIAL & METHODS

Isolation of T cell subsets from human blood and T cell cultures

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinised whole blood or from buffy coats by centrifugation on a density cushion. Thereafter, naive (CD45RO⁻) CD4⁺, as well as CD8⁺ T cells, were obtained from the PBMCs by using antibody-labelled magnetic beads and magnetic separation system according to the manufacturer's instructions (Dyna and Miltenyi Biotec). For differentiation cultures, the sorted T cells were stimulated polyclonally with soluble anti-CD3 and anti-CD28. The T cells were either cultured under non-polarizing conditions or polarized towards T_{H/C}1, T_{H/C}2 and Tregs by addition of the following neutralizing antibodies and cytokines to the cultures: T_{H/C}1 (IL-12/anti-IL-4); T_{H/C}2 (IL-4/anti-IL-12); non-polarizing (anti-IL-4/anti-IL-12) and Treg (anti-IL-12/anti-IL-4/TGF-β). For co-culture experiments T cells were cultured as described above. At day 6, the cells were washed once and then rested in complete RPMI supplemented with IL-2. At day 10 of culture, the cells were harvested and co-cultured with freshly isolated CFSE-labeled CD4⁺ T cells and irradiated CD4/CD8-depleted PBMCs as APCs from the same donor in the presence of anti-CD3 antibody. The labeling with CFSE (5-carboxyfluorescein diacetate succinimidyl ester) enabled the monitoring of cell divisions by flow cytometry. At day 5 of co-culture, cells were analyzed for proliferation. To exclude dead cells, propidium iodide was added.

Quantitative analysis of mRNA expression by Real-time RT-PCR

Total RNA was isolated using the RNeasy mini kit from Qiagen according to the manufacturer's protocol. Reverse transcription was performed with RevertAid™ M-MuLV Reverse Transcriptase using random hexamer primers. Gene expression was analyzed by quantitative real-time PCR using iTaq SYBR Supermix with ROX on an 7900HT Fast Real-Time PCR instrument. Primer pairs were evaluated for integrity by analysis of the amplification plot, dissociation curves and efficiency of PCR amplification. Relative quantification and calculation of the range of confidence were performed using the comparative ΔΔCT method. The housekeeping gene EF-1α was used for normalization.

Analysis of the T cell phenotype by flow cytometry

For flow cytometric analyses of surface markers, T cells were incubated at 4°C in the dark for 15 minutes with fluorochrome-labeled antibodies. To detect intracellular cytokines, the cells were polyclonally stimulated. Brefeldin A was added to block the release of cytokines from the cells. Following the surface staining, the cells were fixed, permeabilized and the cytokines were stained with fluorochrome-labeled antibodies.

RESULTS

The expression of the T cell lineage-specific transcription factors T-bet, GATA3, RORγt and FOXP3 was analyzed in CD8⁺ T cells subsets. Therefore, naive CD8⁺ T cells were differentiated under T_C1, T_C2, Treg and non-polarizing conditions and the mRNA expression of the transcription factors was analyzed by real-time PCR at day 4 of culture. As expected, the expression of the T_{H/C}1-specific transcription factor T-bet and the T_{H/C}2-specific transcription factor GATA3 were increased particular under T_C1 and T_C2 conditions, respectively. FOXP3 as well as the T_{H/C}17-specific transcription factor RORγt, were elevated under Treg conditions that contained TGF-β.

Further investigations focused on the expression of the Treg-specific transcription factor FOXP3 in CD8⁺ T cells. Non-stimulated naive CD8⁺ T cells *ex vivo* expressed minuscule amounts of FOXP3. However, during culture, FOXP3 expression increased successively over time. The FOXP3 expression was dependent on the T cell receptor signal strength as well as the concentration of TGF-β in the cultures. TGF-β treatment not only enhanced FOXP3 expression, but led to a decreased expression of pro-inflammatory cytokines.

Induced/forced FOXP3 expression is described to confer regulatory activity. Therefore, the suppressive capacity of the *in vitro* differentiated T cells was analyzed in co-cultures. Differentiated T cells suppressed the proliferation of CD4⁺ T cells in a dose-dependent manner. This was observed for differentiated CD4⁺, as well as CD8⁺ T cell, regardless if TGF- β was present during initial differentiation or not. In contrast, freshly isolated CD8⁺ T cells showed only marginal effects on CD4⁺ T cell proliferation in these assays.

CONCLUSIONS & OUTLOOK

The current study shows that naive CD8⁺ T cells can be driven *in vitro* towards distinct effector T cells. Remarkably, the expression of the Treg-specific transcription factor FOXP3 can also be induced in CD8⁺ T cells during *in vitro* differentiation and confers suppressive activity. The mechanism that these cells use for inhibition of proliferation is currently not known.

We have shown the existence of a small fraction of FOXP3-expressing CD8⁺ T cells *in vivo* in humans. The characterization of this subset, with regard to cytokine expression and surface markers, is ongoing. Further investigations are needed to determine, how FOXP3⁺ CD8⁺ T cells are generated *in vivo* and whether they contribute to immune regulation in humans.

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