CD4+ T cells in close proximity to Hodgkin-Reed Sternberg cells are antigen experienced, polyclonal and display an exhausted phenotype

J. Veldman[1], J. Rodrigues Plaça[1, 2], L. Chong[3], M. M. Terpstra[4], M. Mastik[1], L. van Kempen[1], T. Aoki[3, 5], C. Steidl[3, 5], A. van den Berg[1], L. Visser[1], A. Diepstra[1]

[1] *University Medical Center Groningen, University of Groningen, Department of Pathology and Medical Biology, Groningen, Netherlands*
[2] *Ribeirão Preto Medical School, University of São Paulo, Department of Genetics, Ribeirão Preto, Brazil*
[3] *British Columbia Cancer, Centre for Lymphoid Cancer, Vancouver, British Columbia, Canada*
[4] *University Medical Center Groningen, University of Groningen, Department of Genetics, Groningen, Netherlands*
[5] *University of British Columbia, Department of Pathology and Laboratory Medicine, Vancouver, British Columbia, Canada*

Introduction

Little is known about the characteristics of the CD4+ T cells residing in close proximity to the tumor cells in classical Hodgkin lymphoma (cHL). One remarkable observation is that these T cells lack expression of the activation marker CD26 [1].

Methods

In this study we characterized sorted CD4+CD26- and CD4+CD26+ T cell subsets from 19 cHL lymph node derived cell suspensions by RNA sequencing and T cell receptor variable gene segment usage. All 19 cHL patients were positive for human leukocyte antigen (HLA) class II on the tumor cells and 14 were of the nodular sclerosis (NS) histological subtype, 2 of the mixed cellularity (MC) subtype, 2 of the lymphocyte-rich (LR) subtype and 1 was not otherwise specified (NOS). In addition, co-expression of genes of interest was investigated at the single cell level using previously generated single-cell RNA sequencing (scRNA-seq) data [2] and at the protein level by immunohistochemistry.

Results

Gene set variation analysis showed an enrichment of memory Treg, Th17 and T follicular helper cell gene signatures in CD4+CD26- T cells, while naïve and Th1/17 gene cell signatures were enriched in CD4+CD26+ T cells. Although CD4+CD26- T cells displayed an antigen experienced phenotype, the T cell receptor variable gene segment usage was polyclonal and did not differ from CD4+CD26+ T cells, indicating lack of clonal expansion. Differential gene expression analysis revealed a significant enrichment of 100 genes in CD4+CD26- T cells. Seven genes (TOX, TOX2, CXCL13, CTTN, PDCD1, CD200 and NFIA) with a moderate to high expression level were chosen for subsequent co-expression analysis using scRNA-seq data. This revealed that the majority of CD4+CD26- T cells expressed TOX2 either alone or in combination with any of the other selected genes. Protein expression of TOX and TOX2, transcription factors crucial for the acquisition of an exhausted T cell phenotype, was accentuated in T cells that physically interact with tumor cells. More than 50% of these rosetting T cells were positive for TOX in 63% and TOX2 in 79% of cHL cases.

Conclusion

In conclusion, CD4+ T cells residing in close proximity to cHL tumor cells are polyclonal, antigen experienced and exhausted. We propose that TOX and TOX2, transcription factors known to induce expression of a variety of immune checkpoints, are attractive therapeutic targets for cHL.

Affix

References

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