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Institutionen för Medicin

Proinflammatory and Antigen-Specific CD4+ T cells in Rheumatoid Arthritis

AKADEMISK AVHANDLING

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av

Jennifer Pieper

Huvudhandledare:

Docent Vivianne Malmström
Karolinska Institutet
Institutionen för Medicin
Enheten för reumatologi

Bihandledare:

Professor Ronald van Vollenhoven
Karolinska Institutet
Institutionen för Medicin
Enheten för klinisk behandlingsforskning,
inflammationssjukdomar

Fakultetsopponent:

Professor Frits Koning
Leiden University Medical Center
Department of Immunohematology and Blood
Transfusion

Betygsnämnd:

Docent Inger Gjertsson
Göteborgs Universitet
Institutionen för Medicin
Avdelningen för reumatologi och
inflammationsforskning

Doktor Liv Eidsmo
Karolinska Institutet
Institutionen för Medicin
Enheten för dermatologi och venereologi

Professor Markus Maeurer
Karolinska Institutet
Institutionen för Laboratoriemedicin
Avdelningen för Terapeutisk Immunologi

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ABSTRACT

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease primarily affecting peripheral joints. In this thesis work we aimed to increase the knowledge about proinflammatory and antigen-specific CD4⁺ T cell subsets involved in RA pathogenesis and I also investigated the effect of T cell directed therapy (abatacept) on CD4⁺ T cell subsets.

Abatacept (CTLA4-Ig) is a biologic therapy that blocks T cell co-stimulation by interfering with the binding of CD28 to CD80/86. Treatment of abatacept leads to reduced T_H1 and T_H17 cytokine production in ACPA-positive RA patients and diminished frequencies of several T_{reg} subsets. This was also confirmed by in vitro studies where abatacept was added in vitro to cell cultures.

Approximately 60% of RA patients have antibodies against citrullinated proteins (ACPA) and their presence is associated to HLA-DRB1*04, one of the strongest genetic risk factors associated with RA. It is believed that T cells recognizing citrullinated epitopes presented by HLA-DRB1*04 alleles may drive the development of ACPA and disease. We have used MHC class II tetramer technology in order to identify and enumerate autoantigen-specific T cells in blood and synovial fluid of RA patients. Several citrullinated epitopes of CILP, a-enolase, fibrinogen and vimentin were identified. The frequency of citrulline-specific T cells was higher in the blood of RA patients compared to HLA-matched healthy controls and the citrulline-specific T cells in RA patients were of a memory T_H1 phenotype. Furthermore, we enumerated and characterized a-enolase native and citrulline-specific T cells in blood and synovial fluid of HLA-DRB1*04:01 RA patients. Higher frequencies of citrullinated a-enolase specific T cells were present in synovial fluid compared to blood and T cells recognizing the citrullinated variant of a-enolase were also more often of a memory phenotype (i.e. had encountered their cognate antigen in vivo) than those recognizing the native a-enolase epitope. Some T cells showed cross-reactivity between the two investigated epitopes. HLA-DRB1*04:01-IE transgenic mice were used to substantiate our findings.

Another potentially contributing T cell subset to RA pathology is an expanded T cell subset that lacks the co-stimulatory molecule CD28, often referred to as CD4⁺CD28null T cells, which are present in approximately 1/3 of RA patients. These are proinflammatory cells and their frequency in blood can be up to 50 % of all CD4⁺ T cells, but they are infrequent in synovial fluid. CD28null T cells are different than conventional CD4⁺ T cells in several aspects, but we demonstrate that even within the CD28null subset there are differences due to their localization. We compared cells from blood and synovial fluid. CD28null cells from synovial fluid expressed more CXCR3 and CCR6 than those from the circulation and CD28null cells from synovial fluid were able to produce IL-17 even though they displayed a hypomethylated IFNG promoter. During my thesis studies, several novel HLA-DRB1*04:01 restricted citrullinated T cell epitopes have been identified. The auto-reactive T cells did not overlap with the CD28null phenotype. We have demonstrated the proof of principle that auto-reactive T cells can be identified by MHC class II tetramer technology in an assay not dependent on in vitro stimulation.