



**Karolinska
Institutet**

Institutionen för mikrobiologi, tumör- och cellbiologi

BIOLOGY OF INTERLEUKIN-7 VARIANT ISOFORMS

AKADEMISK AVHANDLING

som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i 4U (SOLEN), Alfred Nobels Alle 8, Karolinska Institutet, Huddinge.

Måndag den 2 Juni, 2014, kl 09.00

av

Lalit Rane

M.Sc.

Huvudhandledare:

Professor Markus Maeurer
Karolinska Institutet
Institutionen för laboratoriemedicin

Bihandledare:

Professor Mattias Löhr
Karolinska Institutet
Institutionen för klinisk vetenskap,
intervention och teknik

Professor Jan Hillert
Karolinska Institutet
Institutionen för klinisk neurovetenskap

Fakultetsopponent:

Professor Steffen Stenger
Universitetet i Ulm
Institutet för medicinsk mikrobiologi
och hygien

Betygsnämnd:

Professor Rolf Kiessling
Karolinska Institutet
Institutionen för onkologi-patologi

Profesor Francesca Chiodi
Karolinska Institutet
Institutionen för mikrobiologi, tumör- och
cellbiologi

Professor Carmen Fernández
Stockholms universitet
Institutionen för molekylär biovetenskap

Stockholm 2014

ABSTRACT

The interleukin-7 (IL-7) gene produces nine alternatively spliced mRNA variants. Until now, the clinical and biological significance of alternatively spliced IL-7 products has not been well defined. In this thesis, we investigated IL-7 gene alternative splicing in association with chronic inflammation and infection. This thesis also investigated the biology of alternatively spliced IL-7 variants in T-cell subsets and CD14⁺ monocytes. We identified for the first time the alternatively spliced IL-7 δ 5 variant protein among IL-7 protein variants in healthy and diseased human tissue.

In paper I, we investigated the extent and impact of IL-7 pre-mRNA alternative splicing in Peripheral Blood Mononuclear Cells (PBMCs) from 16 patients with multiple sclerosis and 15 healthy individuals by transcript specific amplification of interleukin-7 and the interleukin-7 receptor gene. The relative abundance of the gene transcripts was correlated to health and disease (MS) status and also with the IL-7R SNP rs_6897932, which has been shown to affect alternative splicing of the IL-7R gene. Flow cytometric analysis of PBMC samples was carried out to enumerate abundance of immune cell subsets in healthy and diseased individuals along with the enumeration of IL-7R molecules on single cells to identify correlation with specific IL-7 alternative splicing patterns.

In paper II, we investigated IL-7 and IL-7R alternative splicing in a rhesus macaque model. The study aimed to identify the extent of IL-7 and IL-7R pre mRNA alternative splicing and to gauge its impact in chronic inflammation i.e in *M. tuberculosis* (MTB) infection in a vaccination setting defined by clinical endpoints (improved survival after challenge with virulent MTB). This enabled to study the IL-7 protein directly in lung tissue. Fifteen rhesus macaques were divided in BCG vaccine groups and controls, followed by a challenge with a virulent MTB strain. PBMC samples before the vaccination, as well as before and after the virulent MTB challenge, along with tissues (lung with and without lesions, pulmonary hilus lymph nodes and spleen) from macaques which succumbed to MTB infection were analysed for IL-7 and IL-7R pre-mRNA alternative splicing. Measurement of IL-7, IL-17 and TGF- β at the site of infection (lung) was performed by immunohistochemistry and correlated with survival.

In paper III, we elaborated on the data produced in the first 2 reports and investigated the effect of IL-7 δ 5 variant on T-cells. A mono-specific (affinity-purified) polyclonal antibody recognising exclusively IL-7 δ 5 (and no other IL-7 isoforms) was developed using a junction peptide forming the unique junction of the IL-7 δ 5 protein. The binding affinity and off-rate of IL-7 and IL-7 δ 5 (using a soluble IL-7 receptor, sIL-7R) was examined by surface plasmon resonance on a biacore platform in order to study mechanistic properties of IL-7 δ 5 and IL-7R interaction. Sorted CD4⁺ and CD8⁺ T-cell subsets were stimulated with IL-7 δ 5 or IL-7c (along with unstimulated controls) and changes in the transcriptome were studied using a microarray gene expression platform. The effect of IL-7 δ 5 and IL-7c on T-cell subsets was investigated based on the phosphorylation of signalling transducers and BCL-2 induction using flow cytometry.

In paper IV, we investigated the effects of the IL-7c versus the IL-7 δ 5 spliced variant in regard to CD14 monocyte functions. Sorted CD14⁺ cells from healthy donors were stimulated with IL-7c or IL-7 δ 5 (along with unstimulated controls in triplicates) and changes in gene expression levels after 72 hours were measured by an RNA microarray platform; cell culture supernatants were examined for differential production of immune mediator cytokines and chemokines by a luminex assay. In order to investigate IL-7 δ 5 protein expression, we elaborated on findings in paper III and performed immunohistochemistry to identify IL-7 δ 5 protein production in association with healthy and corresponding transformed tissues. We were able to show that IL-7 δ 5 can be identified in both, human healthy and diseased tissue; IL-7 δ 5 can be produced by epithelial cells as well as by CD68⁺ tissue residing macrophages.