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IL-7δ5 protein is expressed in human tissues and induces expression of the oxidized low density lipoprotein receptor 1 (OLR1) in CD14+ monocytes

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ABSTRACT

Objectives: The 6-exon-spanning 'canonical' Interleukin-7 (IL-7c) is a non-redundant cytokine in human T-cell homeostasis that undergoes extensive alternative pre-mRNA splicing. The IL-7 gene variant lacking, exon 5 (IL-7δ5), exhibits agonistic effects as compared to IL-7c. We studied in this report for the first time the protein expression of IL-7δ5 variant in tissues and its role in monocyte activation.
Methods: We visualized the expression of IL-7δ5 protein by immunohistochemistry in both healthy and malignant (human) tissues and investigated the impact of IL-7δ5 stimulation on CD14+ monocytes using gene expression analysis and flow cytometry.
Results: IL-7δ5 is largely expressed by human epithelial cells, yet also by stromal cells in malignant lesions. Gene expression analysis in CD14+ monocytes, induced by the 6-exon spanning IL-7 or IL-7δ5 showed similar changes resulting in a pro-inflammatory phenotype and increased expression of genes involved in lipid metabolism. IL7δ5 was superior in inducing upregulation of the oxidised low density lipoprotein receptor (OLR), measured by flow cytometry, in CD14+ cells.
Conclusion: IL-7δ5, produced from non-transformed and transformed cells, may contribute to chronic inflammatory responses and development of 'foamy' cells by increased OLR1 expression that mediates increased oxLDL uptake.

Introduction

Interleukin-7 (IL-7) is a well-studied, non-redundant cytokine in T-cell biology.[1–4] The role of IL-7 in shaping immune compartments is increasingly being understood, particularly in the case of innate lymphoid cells and during formation of the secondary lymphoid structures.[5–7] As observed in most eukaryote species, the human IL-7 gene is known to undergo alternative splicing at the pre-mRNA stage. The alternative pre-mRNA splicing of the IL-7 gene produces six 'in frame' mRNA species generated by exon skipping of exons 3, 4 and 5, or their respective combinations. IL-7δ5 is one of the alternatively spliced IL-7 variants which lacks the fifth exon in the canonical IL-7 m-RNA (IL-7c).[8,9] IL-7δ5 is the only IL-7 gene variant that interacts with the IL-7 receptor (IL-7R, CD127) and has been shown to act as an 'IL-7 super-agonist' defined by signalling events.[8]

IL-7c protein expression *in situ*, i.e in organs or tissues, is less understood in humans. Most data concerning IL-7 protein have been obtained by the examination of human thymic cells, bone marrow stromal cells[10] intestinal epithelial cells,[11] skin or liver tissue.[12] A far better understanding of IL-7 protein expression can be obtained in murine models,[13] but these studies cannot take into account the extent and diversity of human IL-7 variants as the pre m-RNA splicing events are different for humans and mice IL-7: IL-7 does not undergo alternative splicing in mice similarly as the

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human IL-7 gene. Although more recent reports indicated that IL-7 variants can impact on neural cell differentiation and tumour cell migration,[14,15] the biology of IL-7 variants and the cells producing IL-7 variants have not been studied in detail up to now.

Previous studies addressed the question whether IL-7δ5 variants are present at the transcript level.[8,9] In this study, we studied IL-7δ5 protein expression in healthy and diseased human tissue by immunohistochemistry and explored the transcriptomic changes induced by IL-7c or the IL-7δ5 in CD14+ monocytes.

Material and methods

Reagents and samples.

IL-7c and IL-7δ5 were produced in a CHO cell expression system and provided by Cytheris Inc. (Cytheris Inc., Paris, France). The isoform specific reagent, a polyclonal antibody (monospecific) directed against the IL-7δ5 protein, recognizes a unique junction peptide on the IL-7δ5 surface. The antibody was produced inrabbits (Ethical approval no dnr. A 52-11, animal ethics committee Stockholm), followed by affinity-purification using the immunizing peptide. The specificity has been confirmed to detect exclusively the IL-7δ5 protein (Rane et al., submitted for publication). Human buffy coat samples from healthy blood donors were obtained from the Karolinska Blood Component Unit (approved by local ethical committee Dnr.2010- 760-31) and PBMC separation was carried out by a ficoll gradient to obtain peripheral blood mononuclear cells (PBMC) for gene expression and flow cytometry analysis.

IL-7δ5 protein detection by Immunohistochemistry was performed using commercially available Human FDA/CE Standard Normal Frozen Tissue Arrays, Human Cancer/Normal Frozen Tissue Arrays and Frozen Single Tissue Section Slides from Human Arteriosclerosis Aorta and Matched Non-arteriosclerosis Aorta purchased from Biocat Inc. (Heidelberg, Germany).

Immunohistochemistry

Frozen tissue sections were tested for IL-7δ5 production by the ABC-method using diaminobenzidine substrate (Vector laboratories, Burlingame; CA, USA) and hematoxylene for nuclear counter staining. Briefly, the fresh frozen tissue array slides are thawed in a humidity chamber and tissue sections were permeabilized using saponin (Sigma Aldrich AB, Stockholm, Sweden) for 10 minutes followed by incubation with the rabbit anti–human IL-7δ5 antibody or a mouse anti-human IL-7 monoclonal antibody (clone B-N18, Diaclone inc, Besancon Cedex, France) overnight at 4 °C. Positive immunostaining was detected using a biotinylated secondary swine anti-rabbit F(ab') antibody and goat anti-mouse IgG respectively and developed by addition of a diaminobenzidine substrate (DAB, Vector laboratories, Burlingame, USA). Haematoxylin stain was used to counterstain the nuclei. Stained images were analyzed on a DMR-X microscope to determine the percent positively stained area in the total cell area using the computerized image analysis system Quantimet Q5501W (Lecia Microsystems. Wetzlar, Germany, Qwin 550 program).

IL-7δ5 production in CD14+ monocytes

Among circulating human lymphocyte populations, monocytes themselves are a source of IL-7 protein.[8] To investigate the relative abundance of IL-7δ5 transcript in CD14+ monocytes in comparison with whole blood and PBMCs, heparinized blood was obtained from healthy donors and immediately processed to obtain the PBMC fraction by a ficoll gradient. The CD14+ cells were separated from the PBMC using CD14+ magnetic beads and isolated by MACS LS columns (Miltenyl biotech Inc. Lund, Sweden) according to supplier's instructions. Total RNA was extracted from whole blood, PBMCs and sorted CD14+ monocyte subsets using the Qiagen RNeasy plus RNA extraction kit (Qiagen Inc. Hilden, Germany). 1 ug of total RNA from each extract was converted to cDNA using OligodT cDNA synthesis protocol from RevertAid first strand cDNA synthesis kit (Thermo scientific, Hudson, NH, USA). The measurement of the IL-7 isoform relative distribution was performed by amplifying the complete IL-7 gene sequence by PCR using primers IL-7 forward: 5′ GCAGACCATGTTCCATGTTTC(21), IL-7 reverse: 5′ CAGTGTTCTTTAGTGCCCATCA (22) and analyzed using the Agilent 2100 DNA 1000 capillary electrophoresis kit (Agilent Inc., CA, USA) as described previously.[9]

Microarray gene expression analysis

CD14+ monocytes were separated by magnetic labeling from a freshly harvested human buffy coat obtained from healthy donors. The purity of separation was analyzed by flow cytometry analysis and confirmed to be at least 97%. 2 million CD14+ cells were stimulated with either 100 ng/ml IL-7c CHO or 100 ng/ml IL-7δ5 CHO to ensure optimal stimulation, along with unstimulated control (CD14+) cells (1 million cells/ml serum free AIMV medium; Gibco, Life Technologies, Gaithersburg, MD, USA) to avoid unspecific stimulation caused by fetal bovine serum components.Cells and cell culture supernatants were harvested after 72 hours of stimulation and total RNA was extracted using the Qiagen RNeasy plus RNA extraction kit (Qiagen Inc. Hilden, Germany). Gene expression analysis was performed at Bioinformatics and Expression Analysis (BEA) core facility at Karolinska Institutet using affymetrix platform and the affymetrix human gene ST1.1 kit. Results were normalized by RMAexpress software (http://stat-www.berkeley.edu/users/bolstad/RMAExpress/RMAExpress.html). Normalized data were analyzed by Significance of Microarray (SAM) test using MeV software (Saeed AI, 2013) to identify gene expression changes in IL-7c and IL-7δ5–stimulated cells against unstimulated controls. The genes which were differentially regulated among the treatment were analyzed and clustered according to biological processes represented by them using PANTHER database.[16]

Measurement of chemokines and cytokines

Cell culture supernatants after 72 hours of culture in IL-7c, IL-7δ5 or medium (unstimulated control group) were harvested and analyzed for cytokines and chemokines. The measurement was performed using the Luminex 26 plex panel (Luminex inc. Austin, TX) on a Luminex 100 instrument according to supplier's instruction. Values from unstimulated (CD14+) controls were subtracted to obtain net values for IL-7 and IL-7δ5 mediated cytokine and chemokine production from CD14+ monocytes.

OLR1 expression on CD14+ monocytes

The effect of IL-7 and IL-7δ5 on up regulating OLR1 gene expression on CD14+ monocytes at the protein level, was investigated using flow cytometry. Peripheral blood mononuclear cells from healthy individuals were stimulated by either 100 ng/ml IL-7c or 100 ng/ml IL-7δ5 for 72 hours in serum free AIMV medium. The cells were collected at the end of the stimulation and stained for expression of OLR1 protein using the PerCP anti-human OLR1 antibody (clone 472413, R&D Biosystems, UK) along with anti CD14 APC-H7(clone MφP9, BD Biosciences) and CD3-ECD (clone UCHT1, Beckman Coulter Inc. USA). The flow cytometry analysis was performed on a FACSAria instrument (BD Biosciences, Stockholm, Sweden) and results were analyzed using FlowJo analysis software. The analysis was performed by defining the parental lymph population based on forward and side scatter,