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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,

followed by defining the CD3 negative population in the parental gate. CD14+ cells were obtained from the CD3 negative cell population and OLR1 expression was identified on CD14+ and CD14 – cells. The data were obtained as percentage of OLR1 positive cells in the CD14+ cell subset and visualized for each donor according to treatment group. Statistical analysis was performed using one way ANOVA test to investigate the statistical significance of the difference between the treatment groups. The test results are provided for 6 individual donors.

Results

IL-7δ5 protein is expressed in healthy and transformed human tissue

The mono-specific anti-human IL-/δ5 antibody was used to identify IL-7δ5 protein production in healthy human tissue (Figure 1). We examined the presence of IL-7δ5 in tissue sections from 10 different anatomical sites representing skin, mucous membranes, glandular organs, reproductive organs and the nervous system (Table 1); as well as in human atherosclerosis aorta and matched, normal aorta (supplementary Figure S3). IL-7δ5 reactive cells are present in all tissues sections mainly associated with epithelial cells.

A functional IL-7R signalling complex has been described to be expressed on tumor cells[17] and IL-7c, as well as IL-7δ5 have been proposed to promote tumor cell proliferation.[15,18,19] This prompted us to explore IL-7δ5, protein production in tumor tissue as compared to the corresponding healthy tissue (Figure 2). IL-7δ5 protein expression was found to be stronger in tumor lesions as compared to healthy tissue. IL-7δ5–positive cells were not only confined to tumor cells, yet also to cells within the tumor stroma, including monocytes (Table 2).

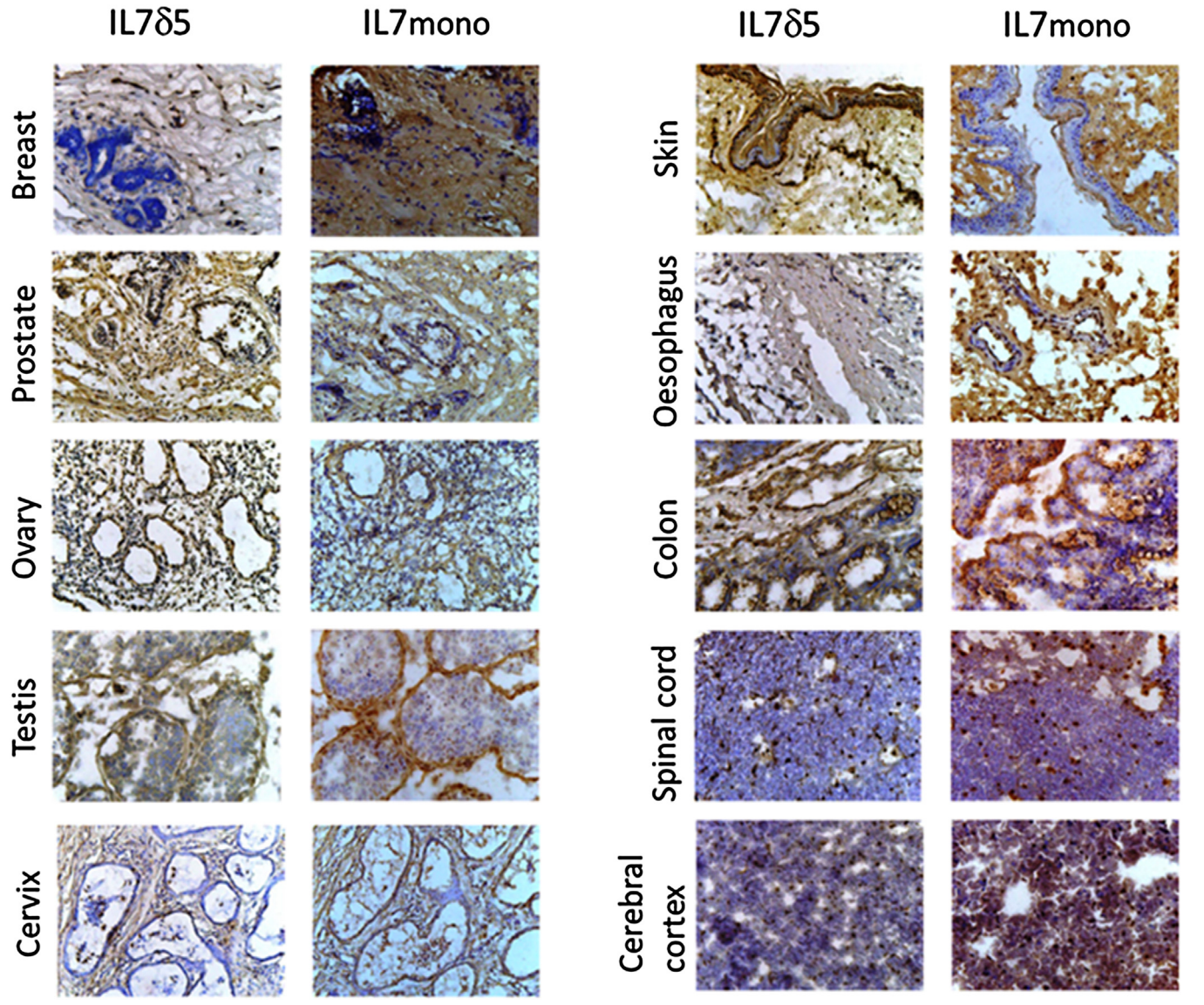

Figure 1. The monospecific antibody against IL-7δ5 enabled identification of the IL-7 δ5 protein expression in human tissues. The immunohistochemistry staining shows representative tissue sections from human healthy tissues stained for IL-7 δ5 using the rabbit anti-hIL-7δ5 antibody. A control monoclonal antibody ('IL-7mono') capable of recognizing all IL-7 transcript variants was used as a positive control. The IL-7δ5 staining reaction is positive in all tissue sections but with distinct patterns among different tissues. In general, the IL-7δ5 staining is largely associated to epithelial cells in skin and the gastrointestinal tract, it can also be observed scattered among cells in the sections from spinal cord and cerebral cortex.

Table 1
Compilation of IL-7δ5 staining characteristics in tissue sections.

No.	Tissue	expression	IL-7δ5 staining pattern
1	Breast	++	associated mostly with glandular structures
2	Prostate	++	associated with glandular epithelial cells
3	Ovary	++	epithelial cells
4	Testis	+	associated with vascular epithelial layer
5	Spinal cord	+	present in a few scattered cells
6	Skin	+++	the subcutaneous epithelial cells are strongly positive; scattered expression in connective tissue
7	Oesophagus	+	few positive cells
8	Colon	+++	strong positive staining associated with epithelial cells
9	Cervix	+	Imbibition of cells with IL-7δ5 reactive material
10	Cerebral cortex	+	present in a few cells scattered in CNS tissue

Summary of IL-7δ5 staining (Figure 1), graded from weak (+) to strong (+++) IL-7δ5 reactive material based on number of positive cells in respective section.

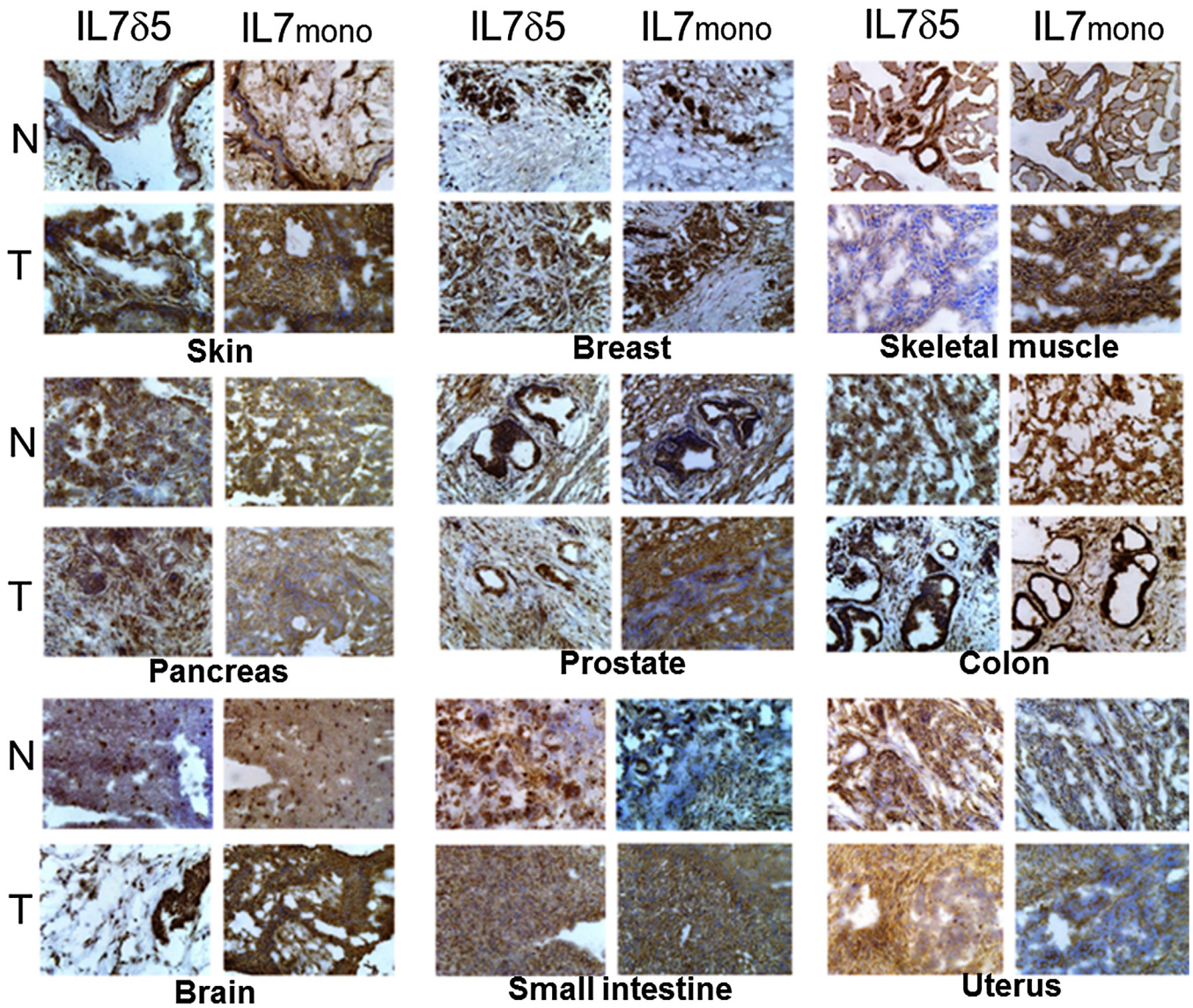

Figure 2. The IL-7δ5 protein was visualized in tumor tissue sections (T) along with corresponding normal tissues (N) using immunohistochemistry on tissue sections of skin, pancreas, brain, breast, prostate, small intestine, skeletal muscle, colon and uterus. IL-7δ5 protein distribution is associated with epithelial cells in healthy tissue. Sections from corresponding tumor tissues stained positive for cells distributed in tumor lesions.

IL-7δ5 is produced in CD14+ cells

Next, we investigated the presence and relative abundance of IL-7δ5 mRNA by calculating the percent abundance of IL-7δ5 transcript among all IL-7 alternatively spliced transcripts amplified in an end-point PCR (Supplementary Figure 1) in CD14+ monocytes. We observed the presence of four different alternatively spliced IL-7 transcripts in monocytes (IL-7c, IL-7δ5, IL-7δ4, IL-7δ4,5), whereas six IL-7 alternatively spliced variants (IL-7c, IL-7δ5, IL-7δ4, IL-7δ4,5, IL-7δ3,4, and IL-7δ3,4,5) were measurable in

Table 2
Compilation of IL-7δ5 staining in healthy tissue and malignant lesions.

No	Tissue	Healthy	Tumor
A	Skin	subcutaneous epithelial cells	diffused expression throughout the tumor mass
B	Pancreas	positive staining in few cells scattered in pancreatic tissue	sporadic and does not seem to be associated with tumor cells
C	Brain	appear to be scattered in the brain tissue	mostly associated with tumor mass
D	Breast	epithelial cells of the mammary gland	associated with tumor cells
E	Prostate	present in epithelial layer and few cells positive in surrounding tissue	glandular structure is stained positive but sporadic expression in surrounding tissue
F	Small Intestine	low/sporadic expression diffused in the section	low but consistent expression diffused in the section
I	Skeletal muscle	muscle fiber as well as vasculature is positively stained	seems absent
J	Colon	positive staining reaction for IL-7δ5 protein in epithelial cells	mostly associated with epithelial tumor cells
K	Uterus	associated with smooth muscle cells	low level of expression throughout the tumor

whole blood and PBMCs. The relative distribution of IL-7δ5 transcript constituted almost 15% of the total IL-7 transcript in CD14+ monocytes, yet represented less than 3% in whole blood or PBMCs suggesting that CD14+ monocytes may contribute IL-7δ5 production in peripheral blood cells.

Effect of IL-7δ5 on gene expression in CD14+ monocytes

As monocytes also express IL-7R on the cell surface,[20,21] we investigated the effect of IL-7 or IL-7δ5 on monocytes by gene expression profiling. The statistical analysis by significance of microarray test (SAM) identified 81 protein coding transcripts which were significantly upregulated at 10% false discovery rate (FDR) in IL-7c *versus* unstimulated cells and 19 upregulated genes for IL-7δ5-stimulated *versus* unstimulated CD14+ cells. Interestingly, we did not identify significant downregulation of coding genes at 10% FDR with either IL-7c or IL-7δ5 treatment.

Differentially expressed genes were clustered using the pathway analysis and ontology tool to visualize physiological processes (Figure 3). Both IL-7c and IL-7δ5 treatment resulted in upregulation of genes involved in metabolic processes, cell communication and cellular response elements to external stimuli. The genes clusters with significant enrichment based on functional grouping at less than 5% FDR formed 7 clusters (supplementary table S1) with genes involved in G1/S phase transition, response to stress and upregulation of immune response. The surface receptor transcripts among the upregulated genes indicated a pro-inflammatory phenotype, particularly through the upregulation of the Oxidised Low density Lipoprotein Receptor-1 (OLR-1) gene (Figure 3).

Cytokine and chemokine production in response to IL-7δ5 stimulation was assessed by a 26 plex Luminex based multiplex cytokine panel (Supplementary Figure 2). We did not observe statistically relevant differences in cytokine levels

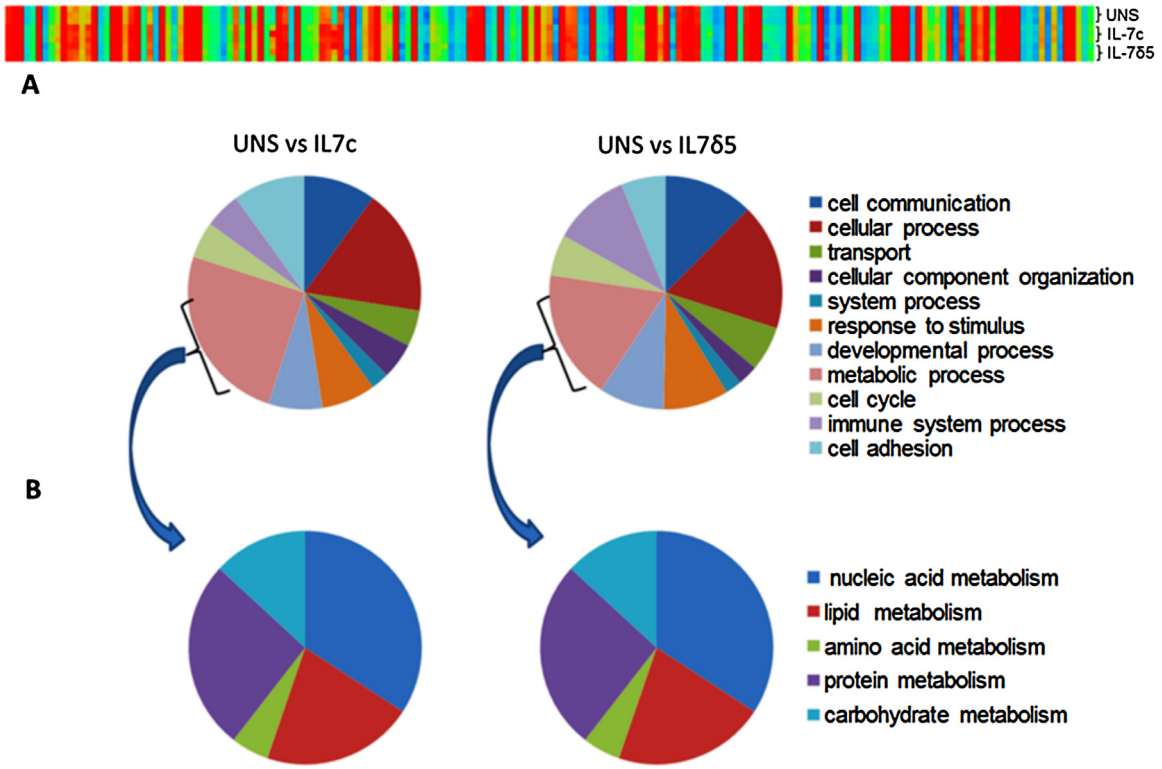

Figure 3. Heat map of differentially regulated genes in human CD14+ cell subset upon IL-7c and IL-7δ5 stimulation (A). The picture represents log10 gene expression values from 0 (blue) to 7.53 (red) in a color gradient manner. The CD14+ cells show differentially regulated genes upon IL-7 and IL-7δ5 treatment. The gene ontology analysis (B) shows genes that are differentially regulated by either IL-7c or IL-7δ5. The largest gene cluster in IL-7c or IL-7δ5 treated CD14+ cells are genes involved in metabolic pathways including lipid metabolism.

between the IL-7 and IL-7δ5 treatment groups. Both IL-7 and IL-7δ5 induced increased GMCSF, IFNγ, IL-10, and IL-1α in supernatant from (unseparated) PBMCs compared with unstimulated control cells; but the increased cytokine production was not present in supernatants from highly purified (>97% purity) CD14+ cells. Similarly, chemokines from IL-7c or IL-7δ5 stimulated PBMC cultures were upregulated defined by increased etoxin, MCP-1, MIP-1a, MIP-1b and IP-10. Analysis of supernatants from CD14+ cells showed increased (MIP1a) production upon IL-7 and IL-7δ5 stimulation.

IL-7δ5 increases OLR1 expression in CD14+ cells

In order to confirm the observation of increased OLR1 gene expression upon Il-7δ5 stimulation, we tested OLR1 protein expression on IL-7 or IL-7δ5-stimulated CD14+ cells. PBMCs obtained from (n = 6) healthy subjects were cultured in IL-7c or IL-δ5 for 72 hours and exhibited an increase in CD14+ cells expressing the OLR1 protein (Figure 4). The frequency of CD14+ OLR1+ cells in unstimulated controls was 0,32% (STDV 0.104) of the total CD14+ population which increased to 3.16% (STDV 3.15) with IL-7c stimulation and 4.34% (STDV 2,87) with IL-7δ5. The change in frequency of CD14+ cells expressing OLR1 ($p = 0.035$) was significant among the treatment groups with the one way ANOVA test. Statistical analysis between the groups using paired T- test revealed that the frequency of CD14+ cells expressing OLR1 is significantly different between unstimulated cells and the IL-7δ5 treatment group ($p = 0.02$). The same was found to be true for the comparison of IL-7c vs IL-7δ5 ($p = 0.05$).

Discussion

IL-7 pre-mRNA alternative splicing gives rise to 6 different variant proteins.[9] We have previously mapped the IL-7 alternative splicing at the mRNA level in tissues of human origin and noted that the alternative splicing pattern of the IL-7 gene is unique based on the tissue origin.[8] IL-7δ5, in addition to the 6-exonspanning IL-7, appears to be biologically relevant since it has been shown to mediate STAT5 phosphorylation upon T-cell stimulation and to drive maturation of human thymocytes.[8] IL-7δ5 m-RNA was predominantly found in liver, salivary gland, thyroid, stomach, peripheral blood mononuclear cells, in testis as well as in granuloma lesions from individuals with latent TB.[8] More recently, we investigated the role of IL-7δ5 in T-cell activation and were able to i) produce a mono-specific IL-7δ5 reactive antibody and ii) showed that IL-7δ5 is able to induce stronger BCL-2 production in human T-cells as compared to the 6-exon full length IL-7 (Rane and coworkers, submitted). In this report, we investigated the production of IL-7δ5 protein in healthy and transformed human tissue and tested its impact of human CD14+ monocytes.

Most of the data available to date stem from IL-7 gene expression analysis using a reporter murine model[13,22,23] and there is only limited data available on IL-7 protein staining in human tissue.[24]. The inherent differences in pre-mRNA processing of the IL-7 gene in humans, as compared to mice, makes the IL-7 gene expression information from murine models inconclusive if (human) IL-7 gene alternative splicing is to be measured. The canonical (corresponding to full length protein) IL-7 gene in mice

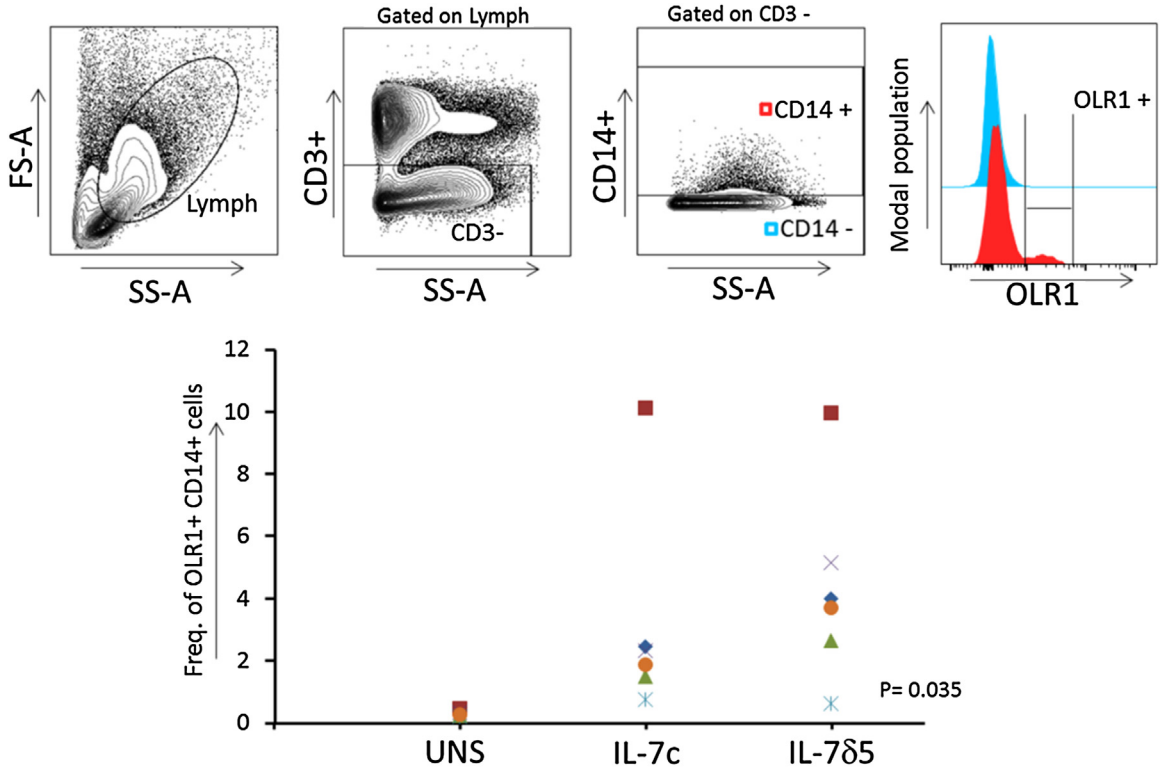

Figure 4. Expression of the oxidized low density lipoprotein receptor was investigated by flow cytometry. The scatterplot shows the gating strategy, cells were visualized using side and forward scatter within the parental lymph gate. CD3+ cells were identified and the CD3- cell population was used to identify CD14+ cells. OLR1 expression on CD14+ cells was investigated for each donor and the frequency of OLR1 expressing CD14+ cells was used to compare the effect of IL-7δ5 treatment. In the scatter plot, each shape and color denotes a donor, the X axis represents the frequency of CD14+ OLR1+ cells in the CD14+ cell subsets; the stimulus is indicated on the Y axis (UNS (unstimulated), IL-7c and IL-7δ5). The frequency of CD14+ OLR1+ cells was obtained for each group and compared using one way ANOVA.

consists of 5 exons where its human counterpart is comprised of 6 exons and produces nine alternatively spliced mRNA species.

Circulating Interleukin-7 protein levels are quite low,[10] suggesting IL-7 *in situ* production and subsequent consumption of IL-7 by IL-7R+ cells. This led to the suggestion that IL-7R+ tumor cells may provide a 'sink' for biologically available IL-7 limiting the availability of IL-7 to T-cells. Studies addressing IL-7 protein expression, using reporter mice, showed IL-7 gene expression in mesenchymal and epithelial cells in lymphoid organs, in the epithelial cells of the intestine, liver skin and lung tissue.[22,23] This corresponds to the limited data available for human tissues, yet these studies can only address the full length IL-7 transcripts in mice (due to the lack of alternative splicing). Protein expression analysis in human tissues has not been able to address the presence of specific IL-7 isoforms due to the lack of specific reagents; this has been remedied here in this report.

The monoclonal antibody used as a control reagent has been shown to recognize several IL-7 isoforms, including the full length, 6 exon IL-7c, as well as IL-7δ5. Thus, we have at this point not a reagent available that would only stain for IL-7c. Using the specific reagent that reacts exclusively with Il-7δ5, we were able to show IL-7δ5 protein expression in most healthy and transformed tissues. Similar to IL-7c (as defined by mRNA length analysis defining isoforms), the IL-7δ5 protein production was associated with cells of epithelial origin in healthy tissue, but appeared to be more diffuse in tumors, which could be due to either increased IL-7δ5 protein production and/or IL-7 producing stromal cells in malignant lesions, including fibroblasts. Not only fibroblasts, but also tissue resident monocytes or monocyte-derived cells are known to express IL-7 and could therefore be sources for local IL-7 production.[25–27]

IL-7δ5 production has been shown to be associated with CD68+ cells in patients with TB, suggesting that *M. tuberculosis*-infected monocytes or monocyte-directed cells could serve as a source for IL-7δ5 production in situ (Rane et al., submitted). This is of particular interest since the receptor for IL-7 (CD127) is known to be expressed by circulating monocytes as well as by dendritic cells. IL-7 stimulation of monocytes has been shown to increase recruitment to endothelial areas in an atherosclerosis murine model.[28]

IL-7c stimulation of monocytes leads to an pro-inflammatory phenotype.[20] Our investigations (supplementary Figure S2) substantiate this finding with a strong production of MIP1a in both IL-7c or IL-7δ5 stimulated CD14+ cells. The transcriptomic analysis showed that IL-7c and IL-7δ5 induce very similar changes (Figure 3) in CD14+ monocytes with a trend of increased gene transcripts involved in lipid metabolism particularly for IL-7δ5, mainly through upregulation of the oxidized low density lipoprotein receptor 1 (OLR1) molecule, a finding that was corroborated via flow cytometry (Figure 4). OLR1, represents a lectin-like scavenger receptor that is conserved in mammals.[29] OLR1 recognizes several ligands, e.g. oxidized-LDL (ox-LDL), gram-positive and gram-negative bacteria as well as apoptotic cells.[30] Overexpression of OLR1 has been shown in atherosclerotic lesions[31]; an interesting observation is that IL-7δ5, which induces OLR1 expression, is also strongly expressed in atherosclerotic plaques (supplementary Figure S3). OLR-1 expression on monocytes is known to mediate inflammatory changes in CD14+ monocytes, in part by activating NF-κB.[32] Recent studies have linked the expression of the lectin-like ox-LDL receptor 1 (OLR1) to tumorigenesis suggesting that OLR1 may serve as an 'oncogene' by NF-κB activation leading to aberrant cellular proliferation, migration, as well as inhibition of apoptosis and activation of *de novo* lipogenesis.[33]

The observation that IL-7δ5 is strongly expressed in healthy tissue as well as in tumor lesions may have several biological consequences. First, IL-7δ5, elaborated *in situ*, may induce BCL-2 expression and increase survival, not only in immune cells, yet also in cells undergoing malignant transformation. IL-7c has also been shown to overcome tumor-induced CD27-CD28- suppressor cells,[34] the role of IL-7δ5 in rescuing biologically relevant anti-tumor responses has to be determined. Second, IL-7δ5 may contribute to OLR1 expression on tumor cells. The effects may be diverse: Components of oxLDL have been shown to stimulate cellular proliferation, mutagenesis *in vitro*[35] and to induce epithelial mesenchymal transition.[36] In contrast, oxLDL mediated effects, via OLR1, may also be able to arrest cell growth activating p53-dependent apoptosis or initiate autophagy.[37,38]

Since earlier studies showed preferential expression of IL-7δ5 (on the mRNA level) in patients with latent TB,[8] the IL-7δ5 mediated upregulation of OLR1 may be instrumental for anti-*M.tuberculosis* directed immune responses. *M. tuberculosis* has been shown to drive differentiation of macrophages into foam cells, characterized by lipid body biogenesis. These lipids provide nutrients to *M.tuberculosis*, leading to an enhanced ability to survive and replicate in host cells. This pathogen-induced manipulation of the host machinery is responsible for the survival of *M. tuberculosis* inside the macrophage and may therefore be facilitated by IL-7δ5 driven increased expression for the OLR, leading to increased oxLDL uptake. Lipid-laden macrophages are a prominent and consistent feature of granulomatous lesions in animals as well as in humans infected with *M. tuberculosis*[39,40] Lipid-laden macrophages have been shown to support the persistence of non-replicating bacilli and demonstrated to be defective in phagocytic and bactericidal activity.[41] The diminished antimicrobial capacity of infected macrophages may enable *M. tuberculosis* to exploit this unique intracellular microenvironment for survival and replication *in vivo*: loading of guinea pig lung macrophages with OxLDL resulted in enhanced replication of bacilli compared to macrophages loaded with non-oxidized LDL.[42] *M. tuberculosis* mutants, with defects in cholesterol uptake, fail to establish chronic infection in murine models.[43]

To conclude, we showed in this report for the first time that IL-7δ5 is expressed in tumor lesions and that IL-7δ5 leads to OLR1 expression in human monocytes that may enable the increased uptake of oxLDL. Increased oxLDL uptake has been associated with increased *M.tuberculosis* pathogenicity and possibly with malignant transformation, suggesting that IL-7δ5 may indirectly, via OLR1 upregulation, contribute to immune-pathology in infection and cancer.

Competing interests

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijid.2017.03.001.

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