Chapter 3

IL-7 in Rheumatoid Arthritis: Pathogenesis, Biomarker and Rationale for Anti-IL-7 Therapy

Frederique Ponchel1,* Ph.D., Agata Burska1, Ph.D., Effie Myrthianou2, Ph.D., and George Goulielmos2, Asst. Prof.

1Leeds Institute of Rheumatic and Musculoskeletal Medicine & Leeds Musculoskeletal Biomedical Research Unit, The University of Leeds, Leeds, UK
2Molecular Medicine and Human Genetics Section, Department of Medicine, University of Crete, Heraklion, Greece

Abstract

Rheumatoid arthritis (RA) is a chronic disease primarily affecting the joints and producing marked inflammatory changes in the synovial membrane and adjacent structures. Although any joint can be affected the disease normally affects the hands, feet, knees and wrists. Disease activity and the rate of progression to involve new joints are variable. The pain and disability associated represent a real burden both for the patients and society and the disease is also associated with premature mortality [1, 2].

There is currently no cure for RA. Historically, treatments which deplete T-cells led to reduction of symptoms supporting the notion of a T cell mediated disease. Today, the pathology is thought to be the product of a series of complex cellular interactions where synovial T-cells orchestrate disease through their interaction with fibroblasts, B-cells, dendritic cells, and macrophages. Modern treatment options include the use of “biological” drugs, which block cytokines such as TNF alpha, IL-1, IL-6, limit co-stimulation between B and T-cells or deplete B-cells. Although their use bring great benefit, a wide range of adverse events have been reported (including infections, cancer, vasculitis, lupus-like and multiple sclerosis, liver disease, hematologic abnormalities (such as aplastic anemia, lymphoma) and aseptic meningitis). In addition resistance to these agents or loss of response, relapse on cessation of treatment, side effects occurs in more than 40% of patients. The cost of these therapies is also major pitfall.

* Corresponding author: Dr. Frederique Ponchel, Email: mmefp@leeds.ac.uk.
The complex cellular interactions in the synovium between stromal cells, T-cells and their subsequent effect on B-cells, macrophages and endothelial cells provide a large panel of alternative targets, both cellular and molecular, for therapeutic intervention in RA. Inhibiting cytokines signalling has proven successful and we (and others) hypothesise that IL-7 may be an appropriate therapeutic target in RA as well as in several other autoimmune diseases. The main rational for targeting IL-7 is that it is (i) over expressed specifically at the disease site, however only in active disease, (ii) not capable of exiting the joint (due to its retention and presentation by extra-cellular matrix) (iii) produced by local resident cells with no mobility (i.e. fibroblasts). In addition, low levels of circulating IL-7 in RA patients suggest that efficient inhibition of IL-7 signalling may only take place in the joint. The role of IL-7 in the disease itself is slowly being dissected and will be reviewed to support this statement.

Abbreviation List

ACPA- anti-citrullinated-peptide antibody
AP1- activator protein 1
ATK- Protein Kinase B
Bcl2- B-cell lymphoma 2
BM-Bone marrow
CD3, 4, 28, 127- cluster of differentiation 3, 4, 28 and 127
CDC25- Cell division cycle 25 phosphatase
CIA- collagen induced arthritis
DAS44- Disease activity score 44 MTX- methotrexate
DCs-dendritic cells
DM1- Diabetes mellitus type 1
DMARD- Disease-modifying anti-rheumatic drugs
EAE- experimental autoimmune encephalomyelitis
ELISA- enzyme-linked immunosorbent assay
GATA1,3 - transcription factors binding "GATA" sequence of DNA
GCs-germinal centres
HLA- Human leukocyte antigens
IFN-gamma- Interferon gamma
IHC-Immunohistochemistry
IL-1,4,5,6,7,11,12,17,23- interleukins 1,4,5,6,7,11,12,17 and 23
IL-12R- Interleukin 12 receptor
IL-7R- Interleukin 7 receptor (membrane-bound form)
IMID- Immune mediated inflammatory diseases
IRF1,2,3,7- Interferon regulatory factor 1,2,3 and 7
JAK3- Janus kinase 3
JIA-Juvenile Idiopatic Arthritis
KGF- keratinocyte growth factor
LN-lymph nodes
MBP- myelin basic protein
MHC- Major Histocompatibility Complex
MMP9- Matrix metalloproteinase 9
Introduction

Interleukin 7 (IL-7) has emerged as an important factor in the development of autoimmune diseases when a possible role in enhancing reactivity to self-antigens was proposed while levels of IL-7 were increased in response to lymphopenia and may predispose to the development of autoimmunity [3]. Animal models and human studies provided further evidence that IL-7 is involved in perpetuating autoimmune inflammation.

Immune mediated inflammatory diseases (IMID) represent a vast number of disorders of which Rheumatoid Arthritis (RA) is the most prominent affecting approximately 1% of the population. RA is a chronic disease primarily affecting the joints and producing marked inflammatory changes in the synovial membrane and adjacent structures resulting in severe disability and reduced life expectancy [1, 2]. Although any joint can be affected the disease normally affects the hands, feet, knees and wrists. Disease activity and the rate of progression to involve new joints are variable. For some, the disease is mild with little or no progression but for many the disease is progressive with the involvement of new joints within months. The pain and the disability associated with the disease can affect an individual’s ability to carry out everyday tasks. The disease may not be confined to the joints and surrounding
tissues but become systemic, involving extra-articular tissues throughout the body including the skin, blood vessels, heart, lungs and muscles. Many of those with RA also suffer from anaemia either as a consequence of the disease itself or following gastrointestinal bleeding as a side effect of drugs, especially non-steroidal anti-inflammatory agents used for analgesia. The long-term prognosis for sufferers is poor with severe disability reported in approximately 80% of patients after 20 years.

The exact pathogenesis of RA remains uncertain, however, autoimmune processes are known to play a role as evidenced by Major Histocompatibility Complex (MHC) linkage [4, 5], autoantibody production (with novel specificity recently identified) [6] and lymphocyte infiltration in synovial tissue [7, 8]. These features supported the hypothesis of a T-cell driven disease which was developed in the late 80s [9-11] and following the demonstration that the main genetic risk associated with RA was associated with T-cells related genes, recently regained considerable interest [12] considering the interplay between T-cell activation and immune suppression (naturally occurring regulatory T-cells (Treg)). In addition, immune dysfunctions related to IL-7 are present in RA patients [13-15], some identified by our group [16-19].

1. Evidence for Genetic Association in RA with IL-7 Axis

a. Genetic Studies in Autoimmune Diseases

The IL-7/IL-7R pathway has recently been implicated in large-scale genetic studies. IL-7R is one of the novel putative autoimmune susceptibility loci that was recently associated with multiple autoimmune diseases. Particularly, polymorphisms in IL7R (alpha subunit) gene, encoding the specific subunit of the IL7R, were found to be associated with an increased risk of developing multiple sclerosis [20, 21], sarcoidosis [22], ulcerative colitis [23], rheumatoid arthritis [24], type 1 diabetes [25] and primary biliary cirrhosis [26].

All individual nucleotide polymorphisms (SNPs) in the IL-7R locus, which have been associated with autoimmunity, are in strong linkage disequilibrium with rs6897932, a functional SNP located in exon 6 of IL-7R gene. This is a non-synonymous SNP (corresponding to a rs6897932*C or rs6897932*T genotype) affecting whether amino acid 244 is transcribed as a threonine or an isoleucine, thus. The rs6897932 SNP has a functional effect on protein expression by influencing the amount of soluble (sIL-7R) and membrane-bound (IL-7R) isoforms of the receptor, through a disruption of an exonic splicing silencer.

The SNP rs6897932 of the IL-7R gene was first associated with susceptibility to multiple sclerosis (MS) independently confirmed in various studies [20, 21, 27-32]. Although, it is worth noting that in a study conducted in Norway no association between the SNP rs6897932 and MS was found [33]. The rs6897932 SNP was suggested to explain only 0.2% of the variance in the risk of development of MS [34]. A genome-wide analysis offered evidence of Diabetes type 1 (DM1) association with the rs6897932 [25, 35] as well as showed evidence of DM1 association with rs3194051 SNP, another SNP in the IL-7R [25]. The frequency of TT-rs6897932 genotype was significantly reduced in the young patients and was confirmed to have protective effect [36]. In an analysis of 27 new ulcerative colitis risk loci, an increased susceptibility to the disease was found with the rs3194051 SNP of the IL-7R gene only but
not to rs6897932 [23]. Finally, the same “C” allele of rs6897932 SNP was very recently associated with an increased risk for SLE [37]. Therefore, a modest influence on disease susceptibility appeared in most diseases studies so far, the overall data suggesting a possible role of the IL-7R polymorphism in the development of human autoimmune diseases.

In RA more specifically, an initial tendency for association of the rs6897932 SNP of IL7R gene was reported [24] followed by a few studies which did not achieve statistical significance at the corrected p-value threshold probably due to an inadequate power. Plant et al failed to detect any association of the same SNP with RA [38] but, Hinks et al [39] found a weak trend toward association of the same SNP with Juvenile Idiopathic Arthritis (JIA) probably because of the very low power (18%) of the study. In a study combining RA and JIA patients no allelic association was found between these diseases and any of the 13 SNPs analysed; however, an association between the diseases and the TT genotype of rs6897932 SNP appeared [40]. In various European RA patient’s populations (Table 1, data kindly provided by our collaborators Prof Javier Martín Ibañez, Dr María Teruel from Spain, Prof Rene Toes, Dr FinaKurreeman from the Netherlands and Prof Jane Worthington, Dr Stephen Eyre from the UK) showed again low levels of significance for this particular SNP. Data form a Cretan population (provided by Dr Effie Myrthianou and Prof George Goulielmos (Greece)) also did not show particular association. Data in RA therefore appear quite negative across the range despite the initial association. An interesting observation was however made when refining the analysis to anti-citrullinated-peptide antibody (ACPA) negative disease in patients from the Netherlands (Table 1) although this was not verified in other populations.

Interestingly, the association detected in a few studies reporting association is RA patients was opposite to that seen in MS, associating the “C” allele (not the “T”) with disease. If confirmed, these studies would substantiate diverging roles for the IL-7/IL-7RA axis in RA pathogenesis compared to other autoimmune disease.

b. Exploring the Functional Significance of the IL-7R-alpha rs6897932 Polymorphism

Numerous studies in several autoimmune diseases demonstrated an association between the IL-7R gene polymorphism and the development of the disease. However, the precise mechanism by which the SNP leads to altered risk has not been elucidated so far. A possible role of the rs6897932 SNP in the pathogenesis of MS has been suggested. This SNP may be involved in the alternative splicing of exon 6, which subsequently may have potential consequences for the function of the receptor [41]. SNP rs6897932 changes a threonine to isoleucine at amino acid position #244 and the disease-associated allele leads to decreased inclusion of exon 6 [20]. This exon codes for a transmembrane domain of the receptor [42]. When exon 6 is included splicing produces a membrane-bound isoform (IL-7R). A soluble isoform of the receptor (sIL-7R) results from alternative splicing where exon 6 is lacking [43, 44]. Both isoforms are able to bind IL-7 with high affinity [45, 46]. The presence of the SNP therefore results in a modified IL-7R/ sIL7R ratio [20, 28, 47]. As a consequence, IL-7 levels were significantly decreased in MS patients compared with healthy individuals, while levels of the soluble sIL-7R were increased in patients with the C (risk) allele of the rs6897932 SNP [31, 48]. Moreover, the higher mRNA levels of both IL-7R and IL-7 detected in the cerebrospinal fluid of patients with MS compared to those found in non-inflammatory neurological diseases, emphasizes the putative involvement of IL-7/IL-7R in the pathogenesis of MS [27].
<table>
<thead>
<tr>
<th>Population</th>
<th>group</th>
<th>MAF (controls)</th>
<th>n Cases</th>
<th>n Controls</th>
<th>effect estimate/ OR</th>
<th>95% CI</th>
<th>p-value</th>
<th>regression/association</th>
<th>method</th>
</tr>
</thead>
<tbody>
<tr>
<td>UK* [156]</td>
<td>RA</td>
<td>0.29</td>
<td>3943</td>
<td>3505</td>
<td>0.91</td>
<td>0.84-0.97</td>
<td>0.007</td>
<td>association</td>
<td>GWA</td>
</tr>
<tr>
<td>Northern Ireland [40]</td>
<td>RA+JIA</td>
<td>0.264</td>
<td>532</td>
<td>368</td>
<td>1.19</td>
<td>0.96-1.46</td>
<td>0.110</td>
<td>association</td>
<td>GWA</td>
</tr>
<tr>
<td>UK [39]</td>
<td>JIA</td>
<td>0.29</td>
<td>943</td>
<td>3505</td>
<td>0.90</td>
<td>0.80-1.01</td>
<td>0.060</td>
<td>association</td>
<td>GWA</td>
</tr>
<tr>
<td>Spanish</td>
<td>total</td>
<td>0.2597</td>
<td>838</td>
<td>1940</td>
<td>1.039</td>
<td>0.91-1.19</td>
<td>0.572</td>
<td>logistic (additive)</td>
<td>immunochip</td>
</tr>
<tr>
<td></td>
<td>ACPA+</td>
<td></td>
<td></td>
<td></td>
<td>1.097</td>
<td>0.92-1.29</td>
<td>0.2783</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ACPA-</td>
<td></td>
<td></td>
<td></td>
<td>1.037</td>
<td>0.83-1.28</td>
<td>0.7436</td>
<td></td>
<td></td>
</tr>
<tr>
<td>English</td>
<td>ACPA+</td>
<td>0.2719</td>
<td>2406</td>
<td>8430</td>
<td>0.9991</td>
<td>0.92-1.074</td>
<td>0.9803</td>
<td>logistic regression</td>
<td>immunochip</td>
</tr>
<tr>
<td></td>
<td>ACPA-</td>
<td></td>
<td>1000</td>
<td>8430</td>
<td>0.966</td>
<td>0.86-1.075</td>
<td>0.5248</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dutch</td>
<td>total</td>
<td>0.2654</td>
<td>648</td>
<td>1085</td>
<td>1.146</td>
<td>0.98-1.33</td>
<td>0.083</td>
<td>logistic (additive)</td>
<td>immunochip</td>
</tr>
<tr>
<td></td>
<td>ACPA+</td>
<td></td>
<td>332</td>
<td>1085</td>
<td>0.9904</td>
<td>0.81-1.21</td>
<td>0.923</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ACPA-</td>
<td></td>
<td>330</td>
<td>1085</td>
<td>1.275</td>
<td>1.04-1.56</td>
<td>0.016</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Greek (Crete)</td>
<td>total</td>
<td>0.18</td>
<td>600</td>
<td>600</td>
<td>1.028</td>
<td>0.83-1.26</td>
<td>0.79</td>
<td>association</td>
<td>TaqMann</td>
</tr>
</tbody>
</table>

* Another SNP in the IL-7 was also showing association.
In RA, levels of IL-7 are also reduced however, in absence of clear association with the rs6897932 SNP, an alternative mechanism may be responsible for this reduction. As such, increased expression of the sIL-7R was associated with direct stimulation of pro-inflammatory cytokine on the IL-7R gene promoter [49, 50].

2. Regulation of IL-7 Expression

The regulation of IL-7 expression is not fully elucidated and is likely to be cell-type specific. Loops in cytokine networks of regulation are probably involved. To date, one transcriptional mechanism has been well characterised but post-transcriptional regulation may also be relevant. INF-gamma is a known positive regulator of IL-7 [51] whereas TGF-beta1 is a negative regulator [52]. Both the mouse and human IL-7 promoters have been sequenced and present large region of high homology. They are unusual promoters, lacking definitive initiation signals (such as TATA box). Transcriptional initiation is nevertheless highly regulated and different regions of the promoter are used to nucleate a transcriptional machinery complex (transcriptional initiators) [51, 53]. These are either used for spontaneous expression or in response to IFN-gamma. Response to INF-gamma, TGF-beta1 and other stimuli use transcription factors such as IRF1,2,3,7; SMAD3,4 or NFAT, AP1, GATA1,3. TGF-beta1 and IL-7 actually share a close reciprocal relationship where one is capable of down-regulating the other at the mRNA level [52, 54]. We exemplified this close relationship at the protein levels following therapeutic lymphodepletion in cancer patients and importantly showed that it is lost in RA [55]. Further regulation has been established in response to TNF-alpha [56] and by the keratinocyte growth factor (KGF) [57] or IL-6 [58].

3. IL-7 and RA

a. IL-7 Expression in the Joint

IL-7 is highly expressed in the joints of RA patients [15, 59, 60] in contrast to the circulation where levels are reduced compared to health [61, 62]. IL-7 was consistently detected in the synovial fluid and tissue of RA patients in higher amounts compare to a non-inflammatory form of arthritis, osteoarthritis (OA) [15, 59]. In the RA synovial membrane IL-7 expression was also reported in the late 1990s as an activator of cells now identified as osteoclasts [56, 59, 63, 64]. IL-7 is known to be expressed by stromal cells (bone marrow (BM), thymus, soft tissue), epithelial cells (liver, gut), endothelial cells, fibroblasts, smooth muscle cells and keratinocytes but not by immune cells with the exception of dendritic cells (DCs) however, only following activation [65-70]. Accordingly, its’ expression was not detected in synovial immune cells (T, B or macrophages) but mRNA was found in RA chondrocytes, but not in OA [63, 71]. Immunohistochemistry (IHC) analysis associated IL-7 expression with one of the OMERACT biomarkers [15], an antibody clone which reacts with several cell types (macrophages and fibroblasts) [72] and is a surrogate measure of disease activity [73]. We also showed that the expression of IL-7 in the synovium of patients was directly related to a measure of local inflammation (Arthroscopic VAS) [60] and was lowered
when they went into remission [60]. Expression was however difficult to analyse due to heterogeneity of the patterns. Some tissues showed diffuse, “patchy” expression with scattered positive single cells and other showed more consistent staining around blood vessels or associated with lymphocyte aggregates and more complex tissue architecture (Figure 1). The expression of IL-7 in RA tissue was therefore associated with fibroblasts and endothelial cells but was also largely extracellular [74].

Paraffin embedded sections were cut from RA synovial biopsies, dewaxed (Access Super solution Menarini diagnostics), then stained using standard protocols (X-Cell plus staining kit, Menarini diagnostics), blocking solution, HRP reagent and 3, 3'-Diaminobenzidine (DAB) and counterstained in haematoxylin. The primary antibody anti- IL7 was a mouse monoclonal (R&D labs MAB207 1: 200 dilution) a) and b) isotype control. c) Low magnification view of a biopsy with staining surrounding lymphocyte aggregates). Higher magnification of IL-7 positive region surrounding lymphocyte d,e,f,i,h) blood vessel, g) blood vessel endothelium staining, j,k) positive and negative lining layer staining.
Figure 2. Transgenic IL-7 mice.
Thanks to a gift from Dr Daniela Finke, we were able to examine mouse legs from a transgenic IL-7/CITTA mouse (TG), which has been shown to have elevated basal levels of IL-7 in all tissues. Histology (H&E) staining of the paw showed inflammatory infiltrate in TG mice (right) and an outgrowth of the synovial membrane compared to the wild type (WT) littermates. In the TG mice cartilage surface damage was also observed. Upon dissection, clear phenotypic differences were observed in the femur and tibia of the TG mice compared to the WT. Following further and boiling, the femur and tibia of the TG mice lacked a smooth cortical bone and were spongy in nature. X-ray analysis demonstrated disorganised bone architecture within the femur and proximal tibia of TG mice. X-rays of the TG mice also proved a narrowing of the joint cavity (red arrow). TG mice had a similar bone length but a significantly greater bone diameter in the femur and tibia than WT mice. Osteoblast and osteoclast activity was analysed using alkaline phosphatase and tartrate resistant acid phosphatase (TRAP) methods. Histological analysis verified that the bone architecture was disorganised, with little compact cortical bone and the bone marrow cavity was exposed to the surrounding muscle and large numbers of osteoblasts and osteoclasts throughout the bone architecture. Larger numbers of osteoclasts were noted in TG bones compared to WT littermates Fast Green/Safranin ‘O’ staining was used to examine cartilage. Articular surface was damaged and lower Safranin ‘O’ staining, indicative of lower proteoglycan content, was evident in cartilage of TG mice compared to WT littermates. Fast Green/Safranin ‘O’ staining also confirmed that the bone architecture was disorganised with little compact cortical bone and trabecular bone invading the marrow cavity in the TG mice.

Figure 3. Synovial IL-7 mRNA expression.

Synovial biopsy were digested and cell grown in tissue culture for 3 passages (157). Cell cultures were then stimulated with IFN-gamma or TNF-alpha for 8 hours. RNA was extracted and IL-7 mRNA measured using qPCR (55) as previously described (158). The direct relationship between IL-7 mRNA expression and local levels of inflammation measure during arthroscopic knee inspection (VAS) is displayed (black diamonds, n=9, rho=0.937 p<0.0001) (55). Stimulation with IFN-gamma (open diamonds) or TNF-alpha (open triangles) increased the levels of IL-7 expression to its maximum however no more than what in vivo exposure to high levels of inflammation would have produced.

Using a transgenic IL-7 mice model whereby IL-7 expression is increased in all but particularly in stromal cells (generous gift of Prof Danielle Finke, Zurich, Switzerland) we
investigated the effect of increased IL-7 expressing in joints (Figure 2). Histology analysis revealed infiltration in the transgenic mice, absent in the WT, suggesting that the mere presence of more IL-7 in the synovial membrane was sufficient to create an environment allowing tissue infiltration by lymphocytes.

The regulation of IL-7 expression in the synovial tissue however remains elusive. Primary cells from RA biopsy expressed more IL-7 mRNA in RA compared with OA [59]. Synovial fibroblasts grown from RA biopsies spontaneously produce detectable amounts of IL-7 at both the transcriptional and protein levels [59]. In synovial fibroblast cultures from RA patients this effect was directly dependent on in vivo exposure to local levels of inflammation (measured during arthroscopic joint inspection) at the time of tissues resection [55] importantly demonstrating an effect maintained over 3 passage in culture.

Expression in expanded synovial fibroblasts was significantly increased by stimulation with different cytokines such as TNF and IL-1 [59]. We confirmed these data and further showed that maximum stimulation was achieved in all cultures independently of the degree of previous activation of IL-7 expression in relation with inflammation (Figure 3). In contrast, TGF-beta-1 and 3 had lost their inhibiting activity in these synovial fibroblast cultures compared to BM cells (data not shown).

b. IL-7 in the Circulation

The discrepancy between low systemic but high synovial expression has been difficult to explain and has brought confusion. Therefore the idea that RA can co-exist as a systemic and a synovial disease is attractive [75]. The origin of circulating IL-7 remains to be fully elucidated. Several tissues including the BM, lymph node, skin, gut and liver are all capable of producing IL-7. Under normal conditions, IL-7 was thought to be released from stromal cells in lymphoid organs, although this was not formally proven. Furthermore, IL-7 expressed in tissue is presented at the surface of stromal cells by extracellular matrix protein (fibronectin and heparan sulphate) [76-78] and signal in a cell-to-cell fashion. These potential tissue origins are therefore unlikely to be the source of circulating IL-7 with the exception of the liver which is capable of producing large amount of protein when needed. Recent data demonstrated that indirect TLR-ligand activation of an unknown cell intermediate, promoted IL-7 production from hepatocytes using IFN-gamma as mediator [79]. Our own experiments [55] confirmed that primary BM stromal cells and hepatocyte cultures, liver and colon cell lines, synovial fibroblast from RA and OA patients but not skin fibroblasts all spontaneously produced IL-7 and released additional amounts (3–30 fold) when stimulated with IFN-gamma, but only synovial fibroblast when stimulated with TNF-alpha and IL-1beta.

Following lymphodepletion, a prolonged CD4+T-cell lymphopenia is observed in RA patients [80-82]. We showed that the thymus in RA patients has a similar reserve to that of disease controls (solid tissue cancer patients and RA patients in clinical remission) although, thymic rebound response to lymphopenia is delayed in RA by several months or does not occur [61]. This was associated with a reduced amount of BM expression of IL-7 available to support progenitors and by extension low thymic IL-7 and absence of de novo mature T-cell generation. Furthermore we showed an absence of circulating IL-7 rebound in response to lymphopenia and no homeostatic proliferation of T-cells, notably CD4+T-cells. The mechanism that controls IL-7 levels in the circulation remain unknown but several hypotheses
have been proposed to explain how IL-7 in turn controls T-cell homeostasis. IL-7 controls a key-molecular point between two pathways: survival (JAK3/STAT5/Bcl2) and cell cycle progression (p38/CDC25) as well as provides energy to sustain these critical cellular activities (Pi3K/ATK/metabolism) [83]. In the absence of external control over the levels of circulating IL-7, the rate of IL-7 consumption by T-cells determines its levels [84]. IL-7 and T-cells are in equilibrium most of the time. When T-cells are depleted (due to disease or therapy) they will encounter abundant IL-7 leading to homeostatic proliferation. When T-cells proliferate (following activation), overconsumption of IL-7 will reduce its circulating levels and T-cells will die. The lack of rebound in circulating IL-7 in RA [61] does not support such a mechanism in this disease. Altruism has been proposed as an alternative hypothesis: some T-cells having satisfied their IL-7 need to survive and proliferate will abstain from consuming IL-7 by down-regulating their expression of CD127/IL-7R in turn allowing other T-cells to acquire IL-7 signalling [85, 86]. No changes in levels of IL-7R expression on T-cells (detected by flow-cytometry) were found following therapeutic lymphodepletion (F Ponchel unpublished observations) to support this hypothesis. We proposed a possible role for TGF-beta as a negative regulator of IL-7 [55]; however we have not been able to verify this proposition in RA patients. The most promising hypothesis may however be that levels of circulating IL-7 are regulated through a decoy sIL-7R. Circulating sIL-7R is expressed as an active regulation of alternative splicing of the IL-7 mRNA rather than through cell surface shedding [45]. The affinity of IL-7 is equivalent for both forms of receptor. Inflammatory cytokines such as IL-17 and TNF-alpha synergise to increase the expression of IL-7 as well as IL-7R in synovial fibroblast (personal communication from Prof Pierre Miossec, gene expression microarray). Neutralization of IL-7 by its sIL-7R may therefore represent an important level of regulation. A similar role for the sIL-2R has long been recognised in RA [87, 88] and investigation need to be performed as it was reported at the American College of Rheumatology annual conference in 2011 that sIL-7R levels are high in SLE [49], and furthermore, that inflammation triggers the expression of the sIL-7R by fibroblasts [89]. Available ELISA for the IL-7 protein recognize full length IL-7 (as well as 2 shorter forms of the protein [90]) but are not able to make the difference between free IL-7 and IL-7/sIL-7R complexes. An ELISA for the sIL-7R has now been developed (Human sIL-7R ELISA Kit, CUSABIO) and it need to be used to investigate if this is indeed the mechanism by which inflammation may exert some control over IL-7 levels in the circulation.

c. IL-7 Effect on T-Cells

The ability of IL-7 to affect synovial T-cells in RA was examined a long time ago and compared to the effect of IL-7 on circulating T-cells [59]. Purified synovial macrophages and T-cell did not spontaneously released IL-7 in contrast to fibroblasts. The proliferation of synovial tissue T-cells from RA patients was stimulated by IL-7 however less than by IL-15 another cytokine of the same family. CD4+ T-cells and macrophages isolated from SF were hyper-responsive to IL-7 when compared with peripheral blood cells [15] but IL7-stimulated lymphocyte responses were not inhibited by TNF-alpha blockade [91]. The cell-cell contact dependent activation of T-cells by macrophages was also enhanced by IL-7, resulting in IL-7 driven expression of TNF-alpha from such co-cultures [91]. IL7 and TNF-alpha levels in RA synovial fluid and synovial tissue were therefore directly correlated.
Considering the role of IL-7 driving the Th1/Th2 balance [92-94] it was hypothesized that IL-7 may affect such balance in the RA synovium [14]. Naïve circulating CD4+ T-cells stimulated by CD3/CD28 in the presence of IL-7 spontaneously produced twice as much IFN-gamma but little more IL-4. Stimulation under Th2 polarisation conditions (in the presence of IL-4) did reduced slightly the production of IFN-gamma but Th2 polarisation in the presence of IL-7 abolished the Th1 bias [14]. Synovial T-cells stimulated with IL-7 produced twice as much TNF-alpha and IL-4 but 3 time more IFN-gamma showing clear Th1 engagement and little effect on Th2 [13]. The activity of IL-7 was mediated by induction of the IL-12R expression for Th1 polarisation (IFN-gamma) but not for Th2 or the pro-inflammatory activation of T-cells (TNF-alpha).

Prolonged and profound CD4+T-cell lymphopenia is a hallmark of RA patients treated with different lymphocyte-depleting therapy [95-97] and we showed that poor reconstitution result from a lack of IL-7 mediated homeostatic proliferation as well as poor progenitor support and thymic rebound [61]. The response of RA patients circulating CD4+ T-cells to IL-7 was not different to that of healthy control; however it is the absence of IL-7 rebound itself that appears to be the main limiting factor in the BM and thymus for the generation of new T-cells and in the circulation for the lack of homeostatic proliferation.

Taking advantage of a remission RA clinic where 50% of patients achieving clinical remission also recovered normal levels of circulating IL-7 [61], we examined the fine-tuning role that IL-7 exerts on T-cells in the circulation (activation and regulation) and compared it with exogenously provided IL-7 mimicking the situation in the joint [98]. Reduced levels of circulating IL-7 [61] probably underlie the dysfunctions associated with circulating T-cells in RA as evidenced by the direct relationship between circulating levels of IL-7 and T-cell responses to stimuli such PHA, CD3/CD28 as well as recall antigen [60] and may provide a mechanism for some of the anergic characteristics of T-cells in the disease. Similarly, synovial regulatory T-cell were affected by the presence of IL-7 in RA [99] and suppression in the presence of IL-7 was shown to be abolished [100-102]. We confirmed these findings in RA [98]. In contrast the effect of additional stimulation provided by IL-7 (like in the joint) had the potential to modify T-cells’ role towards sustaining the vicious circle, enhancing proliferation and responsiveness to stimulation, altogether contributing to perpetuating inflammation [98].

d. IL-7 and Cellular Networking

Analysis at the disease sites (synovium) may actually provide additional information. The cellular composition of rheumatoid synovial membrane is relatively consistent amongst patients including resident cells, fibroblasts, macrophages and endothelial cells. The inflammatory cell infiltrate consists mostly of T and B-cells, dendritic cells (DCs) and plasma cells. Any direct effect of IL-7 on B-cells is unlikely as human B-cells do not express the IL-7R which was confirmed on RA synovial B-cells [103]. Monocytes in RA were shown to express high levels of IL-7R [104] possibly closing a loop between TNF and IL-7 co-activation with fibroblasts in relation with chronic inflammation.
A synovial biopsy with clear TA including GCL structure (a) (H&E) was stained with anti IL-7 (e and f) and lineage marker (as described in figure 1) using (b) anti CD3 (rabbit monoclonal clone SP7 Abcam ab16669, 1:200) (c) anti CD20 (M-20 Goat polyclonal Santa Cruz sc-7735, 1:200) and (d) anti CD68 (Rat monoclonal Abcam ab53444, 1:100).

The tissue architecture (TA) of the synovial membrane in RA is complex and sometimes highly organized. A diffuse infiltration in which T cells, B cells and macrophages are scattered among resident fibroblasts with no higher level of organization is observed in a third of tissues [8]. In the remaining patients, B and T cells organize themselves into defined structures: lymphoid aggregates formed around blood vessels, or structures showing clear features of ectopic formation of lymphoid tissue germinal centres (GCs) (Figure 4), with separated T- and B -cell zones, [105, 106]. Colocalisation of IL-7 with T-cells but
interestingly also with B-cells was observed in the RA synovium [74]. Therefore, the hypothesis that IL-7 can orchestrate the synovial cellular network leading to chronic inflammation and joint destruction was put forward [60]. IL-7 stimulation of DC in vitro results in their DC1 polarisation and IL-7 also induce the expression of IL-12R on T-cells [92]. Whether synovial IL-7 can activate local DC in RA remains to be investigated.

The role of IL-7 in the formation of lymph nodes (LN) and Peyer’s patch has long been known (for review see [107, 108]) in addition to a critical role in driving T-cell homeostasis in LN [109]. No evidence of a sequential relationship with time or location between these different forms of TA has been reported. Similar structures were identified in several other IMID (Crohn’s disease, multiple sclerosis, gastritis, hepatitis, thyroiditis and Sjögren’s syndrome). Gene expression analysis in RA synovial tissue, confirmed IL-7 signalling to be highly associated with structures resembling GCs [74].

e. IL-7 and Bone in RA

IL-7 is a recognised regulator of bone turnover (for review see [110]). Bone mass results from a complex equilibrium between bone formation (activation of osteoblasts) and resorption (activation of osteoclasts), the later mediated by IL-7 [56, 111]. IL-7 deficient mice present a significant increase in bone mass [112], which is directly related to the absence of IL-7-driven expression of RANK ligand (RANKL) by T-cells [56]. On the other hand, a clear bone mass deficit is associated with over expression of IL-7 and such transgenic IL-7 mice are usually unable to survive very long in addition to other immune phenotype [110, 113]. Using the IL-7 transgenic mice model (see above), we confirmed that bone shape was altered in these animals (Figure 2) associated with evidence of reduced thickness of bone and increased presence of osteoclasts, further associated with cartilage proteoglycan loss. This was also observed in collagen induced arthritis where the intra-peritoneal administration of IL-7 exacerbated arthritis leading to a more severe destructive phenotype [114]. The mechanism by which IL-7 mediate this bone destruction has been related to stromal cells expression of IL-7, enhancing the expression of RANKL by T-cells and inducing the differentiation of CD14 monocytes into multi-nucleated, giant, bone-resorbing, tartrate resistant acid phosphatase (TRAP)-positive cells [115].

4. IL-7 as Biomarker in RA

We previously reviewed existing data published for the detection of IL-7 [60]. It is not one of cytokines known to need particular pre-analytical precautions during blood collection (related to stress, cachexia, diurnal rhythm or diet, delay in possessing, storage temperature (-20 acceptable) and 1 to 2 freeze thaw cycle) that may influence its measurement; although there are several ELISAs and other types of assay (cytometric beads assay or Luminex assay) commercially available. Importantly, levels of IL-7 reported in 17 publications in healthy controls using 5 different kits were quite similar (reviewed in [60]). In RA however, the use of multiplex Luminex beads assay yielded false positive results as this method is sensitive to heterophilic antibody interferences such as RF [116, 117].
a. Diagnostic

We demonstrated [62] that low serum IL-7 can identify patients with very early inflammatory joint symptoms who will progress to RA over the next 2 years (sensitivity 30% and specificity 83%, independently of ACPA status) using a cut-off value of 10 pg/mL. Our data also suggested that IL-7 would be the second best diagnostic biomarker of RA after ACPA however, it was the best one for the sub-group of ACPA(-) patients for which such novel biomarker is of utter most importance.

One of the issues discussed in this original report of the IL-7 diagnostic potential [62] was the need to look into factors that can influence IL-7 measurements in addition to donor variability in both health and disease. Reports of the presence of sIL-7R responsible for reducing circulating levels of IL-7 in HIV-infected patients have recently been published [118, 119]. More work is ongoing to validate IL-7 as a diagnostic biomarker.

b. Prognostic

In patients with recent onset of joint inflammatory symptoms and a confirmed RA diagnosis, disease progression over the next 2 years (evaluated using an increase in DAS44-Ritchie at 1 and 2 year) was associated with ACPA(+) disease and longer symptom duration at baseline [62]. Using a different cut-off from diagnostic (upper quartile of the distribution >17.0 pg/ml), higher IL-7 levels at baseline were associated with low levels of disease activity (DAS<1.6) at 1 year. Using multivariate logistic regression, absence of disease progression was clearly associated with lack of reduction in IL-7 above ACPA-negativity (n=108) and was even more predictive in the ACPA(-) subgroup (n=67). A regression analysis of the development of novel erosion over 2 years, showed that only reduced levels of IL-7 (using a low level cut-off <10 pg/ml at baseline) was predictive.

The effect of TNF-alpha blockade on circulating IL7 levels was studied [91]. Baseline levels were not investigated with respect to prediction of response but IL7 levels were reduced in patients who successfully responded to anti-TNF-alpha treatment and persisted in non-responders. Our own data in early RA treated with methotrexate (MTX) or MTX combined with TNF-alpha blocking agent as 1st line treatment suggests that there is no predictive value for IL-7 at baseline with respect to response to treatment (n=50, p=0.749).

c. Remission and Relapse

In established RA, we have showed that circulating levels of IL-7 that remained low (<10pg/ml) in clinical remission on DMARDs (DAS<2.6), were predictive of relapse over the next 12 months [98]. In patients with early RA achieving remission on DMARDs pilot data suggest that low IL-7 was also associated with relapse over the next 12 month (n=10, p=0.03). The main parameters allowing the prediction of safe discontinuation of anti-TNF drug was actually duration of disease before biologics treatment and T-cell subset phenotyping [120]. Nevertheless, lack of IL-7 recovery in established RA in clinical remission post anti-TNF treatment was again associated with relapse in the next few months (n=21, p=0.05); however in patients treated early, no difference in IL-7 levels were observed.
between those destined to relapse or not (n=14, p=0.533). The absence of IL-7 recovery in clinical remission could indicate low levels of disease activity, however we could not establish any relationship between IL-7 levels and residual disease activity using advanced ultrasound and MR imaging technique [121, 122]. Further associations with smoking and early disease onset were also observed and discussed [98], notably as associations between IL-7 and smoking have been reported but the mechanisms behind these observations remains to be clarified.

5. Rationale for Blocking IL-7 in RA

RA is one of the most common autoimmune diseases and is the main cause of potentially treatable disability in the western world [123, 124]. Uncontrolled inflammation over time imposes a significant patient and health economic burden which is expected to continue to increase due to population ageing, and changes in lifestyle (increased obesity and lack of physical activity) [123]. The worldwide incidence of RA is about 1-2% with the disease being more common in women than men and most often starts between the ages of 30 and 40. There is an increased incidence in those with a family history and an association with HLA-specific alleles. In the UK the RA annual incidence is ~36/100,000 in women and ~14/100,000 in men with a prevalence of 0.8% in the adult population. The current impact of RA in terms of resource usage is considerable, somewhere in the region of £400 million annually for healthcare services, £60 million in laboratory tests, and £40 million in medicines costs. Add to this the costs of the social services, the loss of income and the benefits to be paid, brings the overall annual costs to almost to £1 billion per year.

Optimal management of such chronic condition is therefore a priority. DMARDs remain the cornerstone of management of RA although it is clear that sub-optimal response limits the potential of conventional therapies. The need however to intervene at the earliest opportunity and aim for maximal disease control to minimise the impact of disease is now well-established [125-127]. The development of modern biologic therapies has made the treatment aim of achieving long-term remission an attainable target [128]. However, differences in response to therapy between early and established RA have been observed that remain to be explained. Response to anti-TNF therapies in early RA is qualitatively and quantitatively superior [129], with virtually complete suppression of inflammation in most patients [128-130] compared to classic DMARDs. In comparison, in late RA TNF-alpha blockade produces only partial control over inflammation [131] and it is clear that response is neither complete nor universal [132, 133]. These findings have suggested the existence of a critical therapeutic window during which optimal control of inflammation can be obtained.

RA is a very heterogeneous disease, notably recently highlighted by difference in the genetic contribution to ACPA(+/−) disease [134-136], illustrating two divergent pathogenic models, with different rates of progression [137-139] and response to treatment [138, 140]. Although an improved understanding of RA pathogenesis has identified TNF-alpha and IL-6 as pivotal in driving inflammation, the spectrum of clinical responses to these cytokine-blockade suggest that they may play a particularly important role in the early phases of disease, with the development of a more heterogeneous disease drive and notably independency towards TNF and IL-6 later. The need for alternative treatments for RA
therefore remains high; however the cost of developing new biologicals as well as their side effects and resistance suggest that alternative approaches may be more successful.

We proposed the IL-7/IL-7R signalling axis as a potential candidate for therapeutic intervention for RA [60] in agreement with others [141] and with the general interest for IL-7 as therapeutic target in several other autoimmune diseases. In support of this hypothesis MS autoreactive T-cells against MBP were increased by IL-7 however only in MS patients with active disease [142]. A transgenic mouse model with a mutant form of the IL-6 receptor gp130-subunit (F759) with enhanced signal transduction and activation of STAT-3, spontaneously developed a RA-like joint disease (Table 2) [58]. The mutation was sufficient and necessary only in non-haemtopoietic cells and resulted in specific increased production of IL-7 by stromal cells.

Similarly, administration of IL-7 in the CIA model (at the time of disease development day 21 to 31) exacerbated disease (increased clinical severity and radiological scores) [143, 144] with no major other side effect on T and B-cells. Our own findings in transgenic mice showed that the sole over-expression of IL-7 in stromal cells was sufficient to increased cellular infiltration (Figure 2). In the F759 model, a blocking IL-7 antibody completely abolished the development of arthritis when injected intra-peritoneally into 7-day-old thymectomised neonatal mice every 2 days for 2 weeks [58]. Thymectomy was necessary to overcome the difficulty to maintain enough anti–IL-7 antibodies in vivo over 1 year. However, a cross-over between an IL-7R-KO and the F759 mice showed reduced incidence of arthritis (11% of mice developing disease) over more than 1 year compared to the F795 animals (85%). Following these data a prophylactic protocol using an IL-7R blocking antibody showed reduced severity of arthritis (clinical and radiologic scores) in the CIA model as well as delayed the appearance of diseases by a few days [145]. Similarly, a receptor blocking antibody used in a proteoglycan induced disease in BalbC mice, showed reduced incidence (from 92% to 58%) and less severe disease [144]. Using IL-7R blocking antibody in a therapeutic protocol in the CIA model, more limited effect were observed with mostly reduced severity score [145]. Investigating the mechanism by which IL-7 inhibition may prevent disease progression has not been fully elucidated in RA animal models, however a mild reduction in T-cell number (splenic and thymic cell counts, both CD4 and CD8 as well as both naïve and memory CD4) and no difference in B-cell, monocyte or DC numbers were observed in the CIA model treated with an IL-7R blocking antibody [145]. The anti-collagen antibody titres were not affected but T-cell cytokine secretion was reduced (IFN-gamma, IL-5, IL-17). Local levels of several cytokines (IL-1beta, IL-11, TNF-alpha, IL-6) chemokines (IP10, MCP-5), tissue factors (RANKL, MMP9) and vascular factors (vWF, VCAM-1) were reduced. Furthermore, a prophylactic protocol using IL-7R blockade in the CIA model considerably reduced monocyte recruitment into the joint [104] as well as their differentiation into osteoclast. This also resulted in clear inhibition of bone erosions as well as suppressed vascularisation mediated by a loss of the MIP2 chemokine expression.

Additional insight may be provided by experiments in the non-obese diabetic mice (NOD) [146-148]. Using an IL-7R receptor blockade, disease was reversed in new onset diabetic mice.Pathogenic T-cell were not depleted but specifically suppressed in their expression of IFN-gamma and conversely expressed more receptor Programmed Death-1. Cells from animals treated with the IL-7R antibody were no longer able to transfer the disease suggesting that the IL-7R blockade induced T-cell tolerance. The balance between regulatory and pathogenic T-cells was also altered. These data provide strong evidence that in T-cell dependent (phases of) autoimmune disease, IL-7 signalling inhibition represent an important target for therapy.
Table 2. Animal model testing IL-7/IL-7R signalling inhibition

<table>
<thead>
<tr>
<th>Disease</th>
<th>Animal model</th>
<th>Treatment</th>
<th>Results</th>
<th>ref</th>
</tr>
</thead>
</table>
| RA                             | F759 mutation in the gp130 IL-6R subunit enhanced signal transduction activation of STAT-3 | Anti-IL-7 antibody (M25 hybridoma culture supernatant) injection intraperitoneally into 7-day-old thymectomised neonatal mice | - Spontaneous arthritis by 6-7 months of age  
- Disease is CD4+T-cell dependent  
- Increased IL-7 expression restricted to stromal cells  
- Blocking antibody fully prevent the development of arthritis  
- A cross over between an IL-7R-KO and the F759 mice showed reduced incidence of arthritis | [58].            |
|                               | BalbC proteoglycan induced arthritis                                         | IL-7R blocking Antibody                                                   | - Reduced incidence  
- Less severe disease                                                                                                                                                                                  | EWRR Warsaw 2009 [144] |
|                               | DBA/1J mice CIA                                                               | IL-7 Injection at the time of disease development (day 21 to 31)          | - Exacerbation of disease: increased clinical severity and radiological scores  
- No major side effect on T and B-cell.                                                                                                                                                                   | EWRR Warsaw 2009 [143] |
|                               | DBA/1J mice CIA                                                               | IL-7 R blocking Antibody (M595, Amgen) prophylactic protocol              | - Reduced severity of arthritis (clinical and radiologic scores)  
- Delayed the appearance of diseases by a few days                                                                                                                                                       | [145]             |
|                               | DBA/1J mice CIA                                                               | Blocking Antibody (M595, Amgen) therapeutic protocol                      | - Used when arthritis score > 2  
- Reduced the arthritis score                                                                                                                                                                            | [145]             |
|                               | DBA/1J mice CIA                                                               | Anti-IL-7R antibody (R&D Systems) Therapeutic protocol                    | - Reduced synovial inflammation (40%), joint lining thickness (45%), and erosion (40%)  
- Reduced joint TNF-a  
- Reduced serum levels of MCP-1  
- Reduced synovial fluid mediated monocyte migration                                                                                                                                                  | [104]             |
|                               | Transgenic mice                                                              |                                                                            | - Over-expression of IL-7 in stromal cells  
- Increased cellular infiltration                                                                                                                                                                         | Own unpublished observation |
<table>
<thead>
<tr>
<th>Disease</th>
<th>Animal model</th>
<th>treatment</th>
<th>results</th>
<th>ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>colitis</td>
<td>TCR α–/– mice</td>
<td>anti-IL-7R mAb (A7R34) therapeutic protocol</td>
<td>Selective depletion of IL-7Rα high CD4+ LPLs completely ameliorated established colitis</td>
<td>[153]</td>
</tr>
</tbody>
</table>
|                   | TCRα–/– mice                                   | anti-IL-7R mAb (A7R34) intraperitoneal injection. Prophylactic protocol  | – Inhibits the development of colitis  
– Decrease expansion of memory CD4+ LPLs                                                                                                                                                                      | [149]|
| bacterial-induced | bacterial-induced colitis in Mdr1a–/– mice 9T  | anti-IL-7Ra (M595) therapeutic protocol                                  | Colitis model involving T cells but also innate immune cells (macrophages, DC, and NK cells).  
– Inhibition of colitis was associated with decreases in T-cell (especially reduced pool of naïve T-cells) and non-T-cell population  
– Reduction of inflammatory cytokines and chemokines.                                                                                     | [154]|
| NOD, NOD mice    | anti–IL-7Rα mAb prophylactic and therapeutic protocols |                                                                          | Efficacy in the prevention of diabetes  
– Reverses established DM1 by modulating effector T-cell function  
– Induces durable disease remission in newly established DM1 cases                                                                                                                                  | [146]|
| NOD mice,        | Anti–IL-7Rα (clone A7R34) prophylactic and therapeutic protocols |                                                                          | Prevents and  
– Reverses Autoimmune Diabetes                                                                                                                                                                       | [148]|
| MS/EAE            | MOG immunized mice with EAE                    | Anti–IL-7Rα(SB/14; BD Biosciences) in-house clone 28G9 prophylactic and therapeutic protocols | Treatment before or after onset of paralysis exhibited reduced clinical signs of EAE  
– Reduction in peripheral naïve and activated T cells,  
– Central memory T, regulatory T, B, and natural killer cell populations were largely spared.  
– Treatment markedly reduced lymphocyte infiltration into the central nervous system in mice with EAE.                                                                 | [41] |
<table>
<thead>
<tr>
<th>Disease</th>
<th>Animal model</th>
<th>treatment</th>
<th>results</th>
<th>ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease</td>
<td>Animal model</td>
<td>treatment</td>
<td>results</td>
<td>ref</td>
</tr>
<tr>
<td>Disease</td>
<td>Animal model</td>
<td>treatment</td>
<td>results</td>
<td>ref</td>
</tr>
<tr>
<td>Disease</td>
<td>Animal model</td>
<td>treatment</td>
<td>results</td>
<td>ref</td>
</tr>
<tr>
<td>Disease</td>
<td>Animal model</td>
<td>treatment</td>
<td>results</td>
<td>ref</td>
</tr>
<tr>
<td>Disease</td>
<td>Animal model</td>
<td>treatment</td>
<td>results</td>
<td>ref</td>
</tr>
</tbody>
</table>
Several other models of autoimmune disease were also effectively controlled by anti-IL-7R therapies. In several animal models of autoimmune diseases the use of prophylactic protocols with either anti-IL-7 or receptor-inhibition allowed prevention or complete inhibition of disease development (Table 2) [41, 145, 148-152]. Therapeutic protocols showed important effects as well in many models [41, 104, 145, 146, 148, 150, 153, 154] notably with abrogation of established colitis, MS and diabetes. Therefore, animal studies have clearly demonstrated that IL-7-signalling blockade can prevent the development and ameliorate or reverse established autoimmune diseases.

The previously un-described mechanism of action of IL-7/IL-7R signalling in Th17 cells survival and expansion [150] may provide powerful explanations for the treatment efficacy of IL-7R antagonism in EAE and therapeutic implications for human autoimmune diseases such as multiple sclerosis. The IL-7 signalling blockade offered the selectivity that distinguishes pathogenic Th1 and Th17 cells from Treg and unrelated immune cells. Additional therapeutic advantages of IL-7R antagonism involve its selective effect on survival and expansion of effector Th17 cells and Th17 cell differentiation. In contrast, IL-6 or IL-23 antagonism given as prevention protocol when Th17 cells still undergo differentiation is effective during EAE, whereas the same regimen administered once EAE is established showed no efficacy [155]. IL-7 antagonism mainly targeting committed Th17 cells [150] therefore has unique therapeutic advantages.

Altogether, these data suggest that the best window of opportunity for anti-IL-7 therapy is in the early phases of the disease when T-cells are more likely to be essential however good result may also be obtained in established diseases. In RA they may also indicate that the best time to use anti-IL-7 therapy maybe in preventing progression to RA from very early symptom or even in pre-clinical phases such as ACPA+ arthralgia.

This work has been partly supported by a European Union funded FP7-integrated project Masterswitch No. 223404 and the IMI funded project BeTheCure No 115142-2.

References

IL-7 in Rheumatoid Arthritis


Circulating levels of Interleukin-7 correlate with peripheral T-cell responsiveness. submitted 2013.


Crawley AM, Faucher S, Angel JB. Soluble IL-7R alpha (sCD127) inhibits IL-7 activity and is increased in HIV infection. J. Immunol. 2010;184(9):4679-87.


