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Citation for final published version:

Jones, Gareth, Hill, David, Cardus Figueras, Ana and Jones, Simon ORCID: <https://orcid.org/0000-0001-7297-9711> 2018. IL-27 - a double agent in the IL-6 family. *Clinical and Experimental Immunology* 193 (1) , pp. 37-46. 10.1111/cei.13116 file

Publishers page: <http://dx.doi.org/10.1111/cei.13116>
<<http://dx.doi.org/10.1111/cei.13116>>

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IL-27 – a double agent in the IL-6 family

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Abstract–

The cytokine interleukin (IL)-6 is a major therapeutic target for the treatment of various inflammatory and autoimmune diseases. While IL-6 receives considerable attention in studies of innate and adaptive immunity, the IL-6-related family member IL-27 is increasingly recognized for its effects on cellular proliferation, differentiation and leukocyte effector functions. Both cytokines activate responses in myeloid and stromal tissue cells where they direct the transition from innate to adaptive immunity. However, they are frequently identified as lymphokines that control responses in T cells and B cells. In this regard, IL-27 often opposes the action of IL-6. Here, we will review the role of IL-6 and IL-27 in inflammation, with a particular focus on inflammatory arthritis, and discuss their importance in the diagnosis, stratification and treatment of autoimmune disease.

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The IL-6 family of cytokines –

All members of the interleukin (IL)-6 family share a common 130kDa glycoprotein signal-transducing receptor (gp130, CD130). In this regard, receptors for IL-11, oncostatin-M (OSM), ciliary neurotrophic factor (CNTF), cardiotrophin-1 (CT-1), leukemia inhibitory factor (LIF) and the cardiotrophin-like cytokine (CLC) all utilize gp130 to transmit cytokine responses. These IL-6-related cytokines are structurally related and each contain 4 long α -helical chains, which are arranged in an up-up-down-down topography [1]. In contrast, the IL-6-related cytokine IL-27 is a heterodimeric cytokine consisting of two independent subunits termed IL-27p28 (also known as IL-30) and EBI3 [2]. IL-27 therefore resembles IL-12 (which comprises IL-12p40 and IL-12p35), and the related IL-23 (IL-23p19 and IL-12p40) and IL-35 (IL-12p40 and EBI3) inflammatory cytokines (**Figure-1**) [3]. However, IL-27 also shares several characteristics common to the IL-6 cytokine family. First, the receptor complex for IL-27 contains the IL-27 receptor- α (IL-27R α , also known as WSX-1 and TCCR) subunit together with gp130 [4]. While gp130 is universally expressed in all tissues and organs, IL-27R α is mostly restricted to lymphocytes, monocytes and osteoclasts [1, 3]. The cognate α -subunit of the IL-6 receptor (IL-6R, CD126) also shows a similarly restricted pattern of expression and is found on hepatocytes, leukocyte subsets and megakaryocytes [5]. Second, EBI3 shares close sequence identity with IL-6R [3, 6]. In this regard, the soluble IL-6R when bound to IL-6 resembles a heterodimeric cytokine reminiscent of IL-12, IL-23, IL-27 and IL-35 [5-7]. Third, IL-6 and IL-27 receptor activation leads to signaling through the latent transcription factors Signal Transducer and Activator of Transcription-1 (STAT1) and STAT3 [8]. Interleukin-27 is however the only member of the IL-6-related cytokine family to predominantly signal via STAT1 instead of STAT3 [1, 3]. Consequently IL-6 and IL-27 elicit both common and distinct biological outcomes, and IL-27 can often limit IL-6/STAT3-driven events.

The inflammatory significance of IL-6 and IL-27 –

Based on the biological properties of IL-6, this cytokine was originally named interferon β 2, hepatocyte stimulating factor, cytotoxic T cell differentiation factor, B cell differentiation factor and B cell stimulatory factor-2. These broad definitions identify IL-6 as a lymphokine and activator of the acute phase response [5]. However, clinical experience with the blocking anti-IL-6R monoclonal antibody tocilizumab has helped unearth roles for IL-6 in the control of lipid, glucose and iron metabolism, regulation of the neuroendocrine system, and changes in psychological wellbeing that include pain, fatigue, mood and depression (**Figure-2**) [5]. Thus, IL-6 often displays features of a hormone. In contrast, IL-27 is primarily associated with the control of innate and adaptive immunity to infection [3, 9]. Interleukin-27 was first recognized as a pro-inflammatory cytokine due to its ability to support the development of interferon (IFN)- γ secreting T-helper cells. For example, IL-27 promotes expression of IFN- γ , the transcriptional master regulator T-bet, STAT1 and IL-12R β 2 [2, 8, 10-12]. These activities

closely resemble those of IL-12. However, subsequent studies have shown that IL-27 is a negative regulator of IL-2 and can restrict development of immune responses (**Figure-3**) [13-18]. For example, IL-27 is required for the development of T-bet⁺, CXCR3⁺ Treg populations following Th1-mediated inflammation using *Toxoplasma gondii* challenged IL-27R-deficient mice [14]. In this regard the anti-inflammatory properties of type-I interferons are largely attributed to the upregulation of IL-27 and the subsequent promotion of IL-10 [19-21]. Consistent with these observations, prominent immunosuppressive roles were discovered for IL-27 through investigations in mouse models of chronic infection and autoimmunity [22-26]. In the absence of a regulatory IL-27 signal, IL-27R-deficient mice developed profound or lethal T cell-mediated pathology [13, 16, 27, 28]. In this context IL-27 often antagonises the actions of IL-6. While IL-6 supports the development and expansion of T helper cell responses [5], IL-27 has emerged as an inhibitor of Th17 activities and in a model of helminth infection limits Th2 responses through inhibiting GATA3 expression (**Figure-3**) [8, 18, 29]. While additional investigations are required to fully explore the wider biological functions of IL-27, emerging data also highlight potential roles for IL-27 in the control of pain [30], myeloid cell activation [31-37] and stromal tissue responses (**Figure-2**) [38-40]. Thus, IL-6 and IL-27 contribute to inflammation and the regulation of both innate and adaptive immune responses.

Although the receptor complex for IL-27 signaling remains fixed, composed of IL-27R α and gp130, the signaling mechanisms employed by IL-6 are highly complex (**Figure-1**), and it is often challenging to understand how IL-6 receptor signaling can elicit a diverse array of biological responses. Three very distinct forms of IL-6 receptor signaling have now been proposed. These are termed classical IL-6 receptor signaling, IL-6 trans-signaling, which is reliant on the presence of a soluble form of IL-6R (sIL-6R), and a newly reported mechanism called IL-6 trans-presentation [5, 41, 42]. In contrast, IL-27 uses a classical IL-27 receptor system based on the cellular expression of IL-27R α and gp130. However, the IL-27p28 subunit of IL-27 has also been reported to antagonize IL-6-mediated T cell responses, and can potentially bind the IL-6R (**Figure-1**) [18, 43, 44]. So why does IL-6 adopt these different forms of signaling? Here, it is important to note that IL-6 contributions to both the regulation of immune homeostasis and inflammatory responses are relevant to infection, trauma or injury [5]. During health, classical IL-6 receptor signaling promotes the maintenance of normal physiology. For example, IL-6 controls various metabolic processes and tissue renewal or regeneration [5]. In contrast, IL-6 trans-signaling is more widely associated with the regulation of inflammatory processes relevant to disease [5, 41]. This distinction is not however black and white. In this regard, classical IL-6 receptor signaling controls both the acute phase response and the generation of certain effector CD4 T cell populations [5]. Similarly, IL-6 trans-signaling has been linked to processes including hematopoiesis and the sleep REM cycle [45, 46]. The newly described IL-6 trans-presentation mode of cell activation is a juxtacrine

mechanism of IL-6 signaling that promotes the engagement of dendritic cells with T cells [42]. While further work is required to establish the precise biological significance of IL-6 trans-presentation, this mode of cell activation may control immunological processes in tissues that rely on resident immune cells to mount an appropriate response to antigen challenge. These locations may include sites with immune privilege such the brain or eye.

In the accompanying sections, we will consider the roles of IL-6 and IL-27 in the progression of inflammatory disease and will focus on their involvement in rheumatoid arthritis.

The significance of genetic polymorphisms linked with IL-6 and IL-27 –

Several lines of genetic evidence support a role for IL-6 and IL-27 in autoimmunity, cancer and infection. Genome-wide association studies and analyses of genetic polymorphisms have identified several susceptibility loci relevant to IL-6 and IL-27 that predict a predisposition for autoimmune disease. For example, a single nucleotide polymorphism proximal to the *IL6* (rs1800795) transcriptional start site is associated with an increased incidence of coronary heart disease, idiopathic juvenile arthritis and other inflammatory conditions [47-50]. Equally, genetic variants associated with *IL6st* (gp130; rs10940495) and *IL6R* (rs2228145) are common to patients with cardiovascular disease and rheumatoid arthritis [47-50]. Several polymorphisms linked with *IL27* (encoding IL-27p28; rs153109, rs181206, rs17855750) also display risk susceptibilities with asthma, certain cancers, metabolic disorders and some viral infections [51-53]. For example, rs153109 is linked to more severe forms of rheumatoid arthritis [54]. While additional functional genetic studies are required to determine the biological relevance of these genetic variants, several contribute to changes in cytokine or cytokine receptor expression. For example, mutations within *IL6R* (rs2228145) and *IL6* (rs1800795) contribute to elevations in circulating sIL-6R or IL-6, which reflected an altered risk of cardiovascular disease, enhanced susceptibility to insulin resistance, obesity, and other inflammatory complications [47-50, 55-57]. Comparable studies of IL-27 related polymorphisms require further investigation.

The therapeutic opportunities afforded by IL-6 and IL-27 –

The success of interleukin-6 inhibitors in rheumatoid arthritis and related conditions illustrates the prominent role this cytokine plays in the underlining pathology. There are now several biological drugs that target the cytokine itself (e.g., clazakizumab, olokizumab, vobarilizumab, sirukumab), the IL-6R (e.g., tocilizumab, sarilumab), or the soluble form of IL-6R (e.g., olamkicept) [5]. Some of these are now approved for the treatment of rheumatoid arthritis, systemic juvenile arthritis, polyarticular juvenile idiopathic arthritis, giant cell arteritis, and Castleman's disease. In addition, Janus kinase inhibitors (e.g., tofacitinib, baracitinib, ruxolitinib) also impact IL-6 receptor signaling as part of their mode of action [5,

41]. While these drugs are well tolerated and offer clinical benefit, the development of sirukumab was recently stopped following a negative review from the FDA. However, the anti-inflammatory properties of IL-27 suggests that an IL-27 supplementation intervention may offer an alternate therapeutic strategy. For example, studies in experimental models of inflammatory arthritis, and *ex vivo* culture systems show that IL-27 inhibits the expansion of IL-17 secreting CD4 T cells (Th17 cells), restricts the development of ectopic lymphoid aggregates, and can reduce the severity of joint damage and bone erosion [58-60]. No clinical trials have however been conducted to explore this approach in more detail. A similar strategy was previously adopted to test the anti-arthritic properties of recombinant IL-11. However clinical trials with recombinant IL-11 in rheumatoid arthritis failed to reach clinical endpoint and were suspended [61]. The biology of IL-27, and its particular influence on adaptive immune responses (**Figure-3**), means that IL-27 may be a more attractive intervention therapy.

In rheumatoid arthritis, synovial IL-6 and IL-27 levels correspond with differences in disease activity [58, 62-64]. For example, IL-6 expression correlates with poor disease prognosis including elevated acute phase activity, fatigue and increased cardiovascular risk. In contrast, synovial IL-27 levels correspond with a reduction in IL-17 and IL-6, and the Th17 chemoattractant CCL20 [62]. Thus, elevated levels of synovial IL-27 in inflamed rheumatoid arthritis joints may reflect an effort to counteract a persistent adaptive immune response. These findings are also reflected by studies in mice. Histological assessments of joint synovitis revealed that local IL-27-treatment resulted in suppressed leukocyte infiltration, synovial hyperplasia, cartilage and bone erosion, vascularization, and IL-6 and IL-17 expression in inflamed joints [59]. Systemic administration of IL-27 during collagen-induced arthritis also reduced type II collagen-specific antibody titers and serum levels of IL-17 and IL-6 [58]. Analysis of the peripheral immune CD4 T cell response also revealed that IL-27 inhibited the generation of IL-17-producing collagen-specific T cells, but promoted an increase in IL-10 secreting CD4 T cells and suppressive regulatory T (Treg) cells that regulate the expression of cytotoxic T-lymphocyte antigen 4 (CTLA-4) and programmed cell death protein 1 (PD-1) [65]. Interestingly, mice lacking IL-12p35 also develop a mild form of antigen-induced arthritis. This was attributed to an increased production of IL-27 and IL-10, and the expansion of Treg cells [66]. In this regard, IL-12p35 acts to suppress the action of IL-27 during inflammatory arthritis, and blockade of IL-27 activity was shown to restore the severity of synovitis [66]. The above studies highlight the therapeutic potential of IL-27 for the treatment of inflammatory arthritis and other diseases associated with autoimmune T cell-mediated pathology.

IL-6 and IL-27 in inflammatory arthritis –

Interleukin-6 is the archetypal member in the IL-6-related cytokine family. While many of these factors elicit similar biological responses *in vitro*, IL-6 often displays an over-riding influence on the same

responses *in vivo*. Experiments in animal models show that IL-6-deficient mice are protected from various forms of disease [5, 41, 67]. For example, the induction of inflammatory arthritis in IL-6 deficiency is associated with an absence of synovial infiltration, synovial hyperplasia and joint damage (reviewed in [67]). This is not true for other cytokines in the IL-6 family. In models of arthritis, IL-11 Receptor- α (IL-11R α)-KO mice and OSM Receptor- β (OSMR β)-KO mice develop disease severity comparable to wild type controls [68]. However, there may be a context dependent caveat to this generalization [69-72]. For example, interleukin-11 regulates many anti-inflammatory outcomes in arthritis, which fuelled the aforementioned clinical trials with recombinant IL-11 [61, 73, 74]. The relationship between IL-6 and IL-27 is however different. In models of inflammation, IL-27 deficiency contributes to a more active pathology that includes the development of a more severe form of synovitis, and enhanced adaptive immune responses that are reflected by an increase in effector CD4 T cell numbers and antibody responses [60]. Thus, IL-6 and IL-27 acting *via* a common signaling receptor subunit elicit contrasting inflammatory outcomes that influence the initiation, maintenance and severity of joint pathology. While these activities primarily pertain to the control of adaptive immunity, both cytokines have influences on stromal tissue responses to inflammation. For example, IL-6 and IL-27 play important roles in bone remodeling, where an imbalance between bone resorption and formation contributes to bone destruction in inflammatory arthritis [75-78]. Here, *Il27ra* deficient mice with experimental arthritis displayed severe synovitis and synovial hyperplasia, and an increased incidence of focal bone erosions [60]. Systemic delivery of IL-27 reversed the development of these inflammatory parameters and inhibited osteoclastogenesis [79]. Notably, the action of IL-27 on inflammation-driven bone destruction can be both direct and indirect. For example, IL-27 abrogates RANKL responsiveness in osteoclast precursors and suppresses signaling downstream of RANK [36]. Interleukin-27 has also been shown to inhibit the production of RANKL in activated CD4⁺ T cells [80]. The impact of IL-27 on bone turnover is however not unexpected, and other IL-6 related cytokines including IL-11, OSM, LIF and CNTF also control aspects of bone homeostasis [81-84]. In summary, both IL-6 and IL-27 contribute to the control of synovitis and associated changes in cartilage and bone erosion.

IL-27 suppresses synovial ectopic lymphoid-like structure development –

Interleukin-6 and IL-27 are both lymphokines that control the survival, proliferation and effector characteristics of T cells and B cells and are thus poised to shape adaptive immune responses within inflamed joints. IL-6 has long stood as a key mediator in the generation of antibody responses and the formation of germinal center reactions [85, 86]. Recent studies have also highlighted the importance of B cell-derived IL-6 in promoting class-switch recombination of autoantibodies and spontaneous germinal center formation that are required for establishing systemic lupus erythematosus in mice [87].

While IL-27 has also been shown to drive the secretion of IL-21 in T follicular helper cells and support germinal center function [88], the IL-27p28 subunit can counteract IL-6-driven antibody responses and inhibit germinal center development [43]. Consistent with these roles, overexpression of IL-6 and the IL-6R in mice results in spontaneous inflammation featuring the formation of lymph node-like structures in the lung [89]. Similar lymphocytic aggregates called ectopic lymphoid-like structures (ELS; also called tertiary lymphoid structures, TLS) are a histopathological hallmark of tissue inflammation in a number of autoimmune diseases, cancers and infection [90]. New approaches such as ultrasound-directed small-needle synovial biopsy, combined with histopathological assessment of joint inflammation, has provided new insight into disease heterogeneity in rheumatoid arthritis [91]. Here, based on cellular and molecular signatures, synovitis can be classed into three pathotypes called '*follicular*', '*diffuse*' and '*pauci-immune*'. While *diffuse* pathology is characterized by a typical random infiltration of leukocytes primarily composed of macrophages and some T cells, the *follicular* pathotype features highly-organized and segregated aggregates of T and B cells accompanied by CD21+ follicular dendritic cell networks, active germinal centers and high endothelial venules (HEV). ELS are associated with the local priming of immune cells and autoantibody responses [92, 93].

Notably, our recent evaluation of *IL27* and *EBI3* expression (encoding IL-27p28 and EBI3 respectively) in the *diffuse* synovial pathotype mirrored previous studies that identified heightened levels of IL-27 in rheumatoid arthritis joint tissues as compared with control osteoarthritis joints [60]. However, compared to patients with *diffuse* pathology, the *follicular* form of disease was associated with reduced expression of *IL27*. Notably, *IL27RA* was highly expressed in the *follicular* form of rheumatoid arthritis, and cells expressing the IL-27R were localized at ELS. These observations suggest that distinct cytokine networks govern the development of synovial pathotypes, and that the absence of a regulatory IL-27 signal may contribute to the development of a *follicular* form of disease that is linked with severe local and peripheral inflammation and inferior responses to biological therapy (e.g., anti-TNF) [94-96].

Early investigations into the endogenous role of IL-27 in inflammatory arthritis revealed a pro-inflammatory role in proteoglycan-induced arthritis [97]. While this observation in IL-27R-deficient mice appears to contradict the therapeutic effect that has been observed following treatment with IL-27 in experimental arthritis [58, 59, 65, 79, 98], this may reflect the importance of a robust Th1 cell response for driving the proteoglycan-induced arthritis model. Our studies using IL-27R-deficient mice in the mBSA antigen-induced arthritis model revealed that these mice develop exacerbated joint inflammation, synovial hyperplasia, and cartilage and bone erosion that was accompanied by elevated peripheral Th17 cell and mBSA-specific antibody responses [60]. Reflecting the observation that *IL27* expression was reduced in synovial biopsies from rheumatoid arthritis patients with a follicular-rich

form of disease, IL-27R-deficient mice developed synovial ectopic lymphoid-like structures that were associated with the expression of homeostatic cytokines (e.g., *Lta*, *Ltb*) and chemokines (e.g., *Cxcl13*, *Ccl21*) [60]. Thus, while IL-6 can promote ELS development in inflamed tissues, IL-27 is a negative regulator of ELS. An inhibitory role for IL-27 at ELS is consistent with observations in other models of inflammation that have linked heightened Th17-type effector responses (e.g., elevated expression of IL-17, IL-17F, IL-22, IL-21) with ELS development [99-101]. ELS development in antigen-induced arthritis was associated with the local expression of IL-17 and IL-21 [60], and effector cytokines linked with the Th17 programme (e.g., IL-17F, IL-21, IL-23, IL-22) have also been implicated in synovial lymphoid neogenesis in clinical rheumatoid arthritis [102]. The inhibitory control of effector Th17-type responses by IL-27 may therefore offer opportunities to identify new therapeutic targets for the treatment of the follicular form of rheumatoid arthritis.

Concluding remarks—

Cytokines that signal via the Jak-STAT pathway are increasingly viewed as therapeutic targets for the treatment of autoimmune diseases, infection and cancer. These include drugs that block IL-6 (e.g., olokizumab, clazakizumab, tocilizumab), IL-12p40 (e.g., ustekinumab), IL-21, IL-23p19 (e.g., risankizumab, guselkumab, tildrakizumab, mirikizumab) or GM-CSF (e.g., mavrilimumab) signaling, or members of the Jak protein family (e.g., tofacitinib, baracitinib, ruxolitinib). When considering the immuno-modulatory or anti-inflammatory properties of IL-27 it is tempting to consider how IL-27 intervention would supplement these therapies. Here, the capacity of IL-27 to inhibit Th17 development, and to promote the expression of checkpoint regulators and Treg activity, mirrors the therapeutic responses linked with tocilizumab treatment [3, 5]. However, further investigations are required to assess the context-dependent inflammatory activities of IL-27. These may require clinical trials in humans. While primary clinical endpoints will undoubtedly fixate on improvements in local tissue inflammation and damage, the wider implications of systemic inflammation are becoming equally important. For example, a metabolic shift associated with the systemic activation of T cells in PD-1-deficient mice was recently shown to impact the generation of brain monoamines and changes in emotional behavior [103]. In this respect, the bioactivity of IL-27 is interesting since IL-27 promotes the expression of PD-1 ligand PD-L1 [104]. Thus, an IL-27 intervention may offer opportunities to explore whether IL-27 can bring about improvement in disease activity and patient wellbeing. Such strategies would be relevant to clinical indications where IL-17 or Th17 driven outcomes promote disease progression (e.g., psoriasis). The question is whether supplementation with recombinant IL-27 can be used as a standalone intervention or an adjunct therapy in conditions where biological drugs that target IL-6, IL-12, IL-17 or IL-23 are effective. Several of the benefits associated with IL-6 blockade relate to the

impact of therapy on altered metabolic processes (e.g., anaemia through altered iron metabolism), fatigue and patient wellbeing. It is unclear whether recombinant IL-27 would elicit similar outcomes.

In summary, IL-6 and IL-27 appear to work in coordinated fashion, with IL-27 often suppressing the action of IL-6. These differences in biological activities reflect changes in the control of transcription factors STAT1 and STAT3 and may also relate to differences in the cytokine receptor subunits. While further work is required to fully appreciate the associations between IL-6 and IL-27, the current data offers interesting perspectives on how an IL-27 intervention may supplement existing biological drug therapies against IL-6, or members of the IL-12 cytokine family.

Acknowledgements –

GWJ and SAJ are supported by an Arthritis Research UK Career Development Fellowship (reference 20305) and programme grant (reference 20770) respectively. DGH is supported by a Medical Research Council PhD studentship and Life Science Research Network Wales, a research initiative funded through the Welsh Government's Sêr Cymru program.

Competing interests –

SAJ has received funding support from Hoffman la Roche, GSK, Ferring Pharmaceuticals and NovImmune SA, and during the last 5 years he has acted as an advisory consultant for Roche, Chugai Pharmaceuticals, NovImmune SA, Genentech, Sanofi Regeneron, Johnson & Johnson, Janssen Pharmaceuticals, Eleven Biotherapeutics and UCB. GWJ has received funded for GSK and undertakes collaborative research with MedImmune. DGH and ACF declare no competing interests.

References–

1. Heinrich, P.C., et al., *Principles of interleukin (IL)-6-type cytokine signalling and its regulation*. Biochem J, 2003. **374**(Pt 1): p. 1-20.
2. Pflanz, S., et al., *IL-27, a heterodimeric cytokine composed of EBI3 and p28 protein, induces proliferation of naive CD4+ T cells*. Immunity, 2002. **16**(6): p. 779-90.
3. Yoshida, H. and C.A. Hunter, *The immunobiology of interleukin-27*. Annu Rev Immunol, 2015. **33**: p. 417-43.
4. Pflanz, S., et al., *WSX-1 and glycoprotein 130 constitute a signal-transducing receptor for IL-27*. J Immunol, 2004. **172**(4): p. 2225-31.
5. Hunter, C.A. and S.A. Jones, *IL-6 as a keystone cytokine in health and disease*. Nat Immunol, 2015. **16**(5): p. 448-57.
6. Jones, L.L. and D.A.A. Vignali, *Molecular interactions within the IL-6/IL-12 cytokine/receptor superfamily*. Immunologic research, 2011. **51**(1): p. 5-14.
7. Gearing, D.P. and D. Cosman, *Homology of the p40 subunit of natural killer cell stimulatory factor (NKSF) with the extracellular domain of the interleukin-6 receptor*. Cell, 1991. **66**(1): p. 9-10.
8. Lucas, S., et al., *IL-27 regulates IL-12 responsiveness of naive CD4+ T cells through Stat1-dependent and -independent mechanisms*. Proc Natl Acad Sci U S A, 2003. **100**(25): p. 15047-52.
9. Artis, D., et al., *The IL-27 receptor (WSX-1) is an inhibitor of innate and adaptive elements of type 2 immunity*. J Immunol, 2004. **173**(9): p. 5626-34.
10. Chen, Q., et al., *Development of Th1-type immune responses requires the type I cytokine receptor TCCR*. Nature, 2000. **407**(6806): p. 916-20.
11. Takeda, A., et al., *Cutting edge: role of IL-27/WSX-1 signaling for induction of T-bet through activation of STAT1 during initial Th1 commitment*. J Immunol, 2003. **170**(10): p. 4886-90.
12. Yoshida, H., et al., *WSX-1 is required for the initiation of Th1 responses and resistance to L. major infection*. Immunity, 2001. **15**(4): p. 569-78.
13. Villarino, A.V., et al., *IL-27 limits IL-2 production during Th1 differentiation*. J Immunol, 2006. **176**(1): p. 237-47.
14. Hall, A.O., et al., *The cytokines interleukin 27 and interferon-gamma promote distinct Treg cell populations required to limit infection-induced pathology*. Immunity, 2012. **37**(3): p. 511-23.
15. Stumhofer, J.S., et al., *Interleukins 27 and 6 induce STAT3-mediated T cell production of interleukin 10*. Nat Immunol, 2007. **8**(12): p. 1363-71.
16. Villarino, A., et al., *The IL-27R (WSX-1) is required to suppress T cell hyperactivity during infection*. Immunity, 2003. **19**(5): p. 645-55.
17. Young, A., et al., *Cutting edge: suppression of GM-CSF expression in murine and human T cells by IL-27*. J Immunol, 2012. **189**(5): p. 2079-83.
18. Stumhofer, J.S., et al., *Interleukin 27 negatively regulates the development of interleukin 17-producing T helper cells during chronic inflammation of the central nervous system*. Nat Immunol, 2006. **7**(9): p. 937-945.
19. Iyer, S.S., A.A. Ghaffari, and G. Cheng, *Lipopolysaccharide-mediated IL-10 transcriptional regulation requires sequential induction of type I IFNs and IL-27 in macrophages*. J Immunol, 2010. **185**(11): p. 6599-607.
20. Patin, E.C., et al., *IL-27 Induced by Select Candida spp. via TLR7/NOD2 Signaling and IFN- β Production Inhibits Fungal Clearance*. J Immunol, 2016. **197**(1): p. 208-21.
21. Clement, M., et al., *Cytomegalovirus-Specific IL-10-Producing CD4+ T Cells Are Governed by Type-I IFN-Induced IL-27 and Promote Virus Persistence*. PLoS Pathog, 2016. **12**(12): p. e1006050.
22. Hamano, S., et al., *WSX-1 is required for resistance to Trypanosoma cruzi infection by regulation of proinflammatory cytokine production*. Immunity, 2003. **19**(5): p. 657-67.
23. Batten, M., et al., *Cutting edge: IL-27 is a potent inducer of IL-10 but not FoxP3 in murine T cells*. J Immunol, 2008. **180**(5): p. 2752-6.

24. Findlay, E.G., et al., *Essential role for IL-27 receptor signaling in prevention of Th1-mediated immunopathology during malaria infection*. J Immunol, 2010. **185**(4): p. 2482-92.
25. Sun, J., et al., *CD4+ T cell help and innate-derived IL-27 induce Blimp-1-dependent IL-10 production by antiviral CTLs*. Nat Immunol, 2011. **12**(4): p. 327-34.
26. Liu, F.D., et al., *Timed action of IL-27 protects from immunopathology while preserving defense in influenza*. PLoS Pathog, 2014. **10**(5): p. e1004110.
27. Batten, M., et al., *Interleukin 27 limits autoimmune encephalomyelitis by suppressing the development of interleukin 17-producing T cells*. Nat Immunol, 2006. **7**(9): p. 929-36.
28. Stumhofer, J.S., et al., *Interleukin 27 negatively regulates the development of interleukin 17-producing T helper cells during chronic inflammation of the central nervous system*. Nat Immunol, 2006. **7**(9): p. 937-45.
29. Diveu, C., et al., *IL-27 blocks RORc expression to inhibit lineage commitment of Th17 cells*. J Immunol, 2009. **182**(9): p. 5748-56.
30. Fonseca, M.M., et al., *(153) The interleukin 27 (IL-27) protects mice from neuropathic pain development through up-regulation of anti-inflammatory cytokine IL-10*. The Journal of Pain. **18**(4): p. S14-S15.
31. Mascanfroni, I.D., et al., *IL-27 acts on DCs to suppress the T cell response and autoimmunity by inducing expression of the immunoregulatory molecule CD39*. Nat Immunol, 2013. **14**(10): p. 1054-63.
32. Morandi, F., et al., *IL-27 in human secondary lymphoid organs attracts myeloid dendritic cells and impairs HLA class I-restricted antigen presentation*. J Immunol, 2014. **192**(6): p. 2634-42.
33. Wang, S., et al., *Augmentation of antigen-presenting and Th1-promoting functions of dendritic cells by WSX-1(IL-27R) deficiency*. J Immunol, 2007. **179**(10): p. 6421-8.
34. Kalliolias, G.D., R.A. Gordon, and L.B. Ivashkiv, *Suppression of TNF-alpha and IL-1 signaling identifies a mechanism of homeostatic regulation of macrophages by IL-27*. J Immunol, 2010. **185**(11): p. 7047-56.
35. Kalliolias, G.D. and L.B. Ivashkiv, *IL-27 activates human monocytes via STAT1 and suppresses IL-10 production but the inflammatory functions of IL-27 are abrogated by TLRs and p38*. J Immunol, 2008. **180**(9): p. 6325-33.
36. Kalliolias, G.D., et al., *Interleukin-27 inhibits human osteoclastogenesis by abrogating RANKL-mediated induction of nuclear factor of activated T cells c1 and suppressing proximal RANK signaling*. Arthritis Rheum, 2010. **62**(2): p. 402-13.
37. Robinson, C.M. and G.J. Nau, *Interleukin-12 and interleukin-27 regulate macrophage control of Mycobacterium tuberculosis*. J Infect Dis, 2008. **198**(3): p. 359-66.
38. Dibra, D., et al., *Expression of WSX1 in tumors sensitizes IL-27 signaling-independent natural killer cell surveillance*. Cancer Res, 2009. **69**(13): p. 5505-13.
39. Seita, J., et al., *Interleukin-27 directly induces differentiation in hematopoietic stem cells*. Blood, 2008. **111**(4): p. 1903-12.
40. Yoshimoto, T., et al., *Antiproliferative activity of IL-27 on melanoma*. J Immunol, 2008. **180**(10): p. 6527-35.
41. Jones, S.A., J. Scheller, and S. Rose-John, *Therapeutic strategies for the clinical blockade of IL-6/gp130 signaling*. J Clin Invest, 2011. **121**(9): p. 3375-83.
42. Heink, S., et al., *Trans-presentation of IL-6 by dendritic cells is required for the priming of pathogenic TH17 cells*. Nat Immunol, 2017. **18**(1): p. 74-85.
43. Stumhofer, J.S., et al., *A role for IL-27p28 as an antagonist of gp130-mediated signaling*. Nat Immunol, 2010. **11**: p. 1119-26.
44. Garbers, C., et al., *An interleukin-6 receptor-dependent molecular switch mediates signal transduction of the IL-27 cytokine subunit p28 (IL-30) via a gp130 protein receptor homodimer*. J Biol Chem, 2013. **288**(6): p. 4346-54.
45. Oyanedel, C.N., et al., *Peripheral and central blockade of interleukin-6 trans-signaling differentially affects sleep architecture*. Brain, Behavior, and Immunity, 2015. **50**(Supplement C): p. 178-185.

46. Peters, M., A.M. Muller, and S. Rose-John, *Interleukin-6 and soluble interleukin-6 receptor: direct stimulation of gp130 and hematopoiesis*. *Blood*, 1998. **92**(10): p. 3495-504.
47. Collaboration, I.R.G.C.E.R.F., et al., *Interleukin-6 receptor pathways in coronary heart disease: a collaborative meta-analysis of 82 studies*. *Lancet*, 2012. **379**(9822): p. 1205-13.
48. Consortium, C.A.D., et al., *Large-scale association analysis identifies new risk loci for coronary artery disease*. *Nat Genet*, 2013. **45**(1): p. 25-33.
49. Fishman, D., et al., *The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis*. *J Clin Invest*, 1998. **102**(7): p. 1369-76.
50. Stahl, E.A., et al., *Genome-wide association study meta-analysis identifies seven new rheumatoid arthritis risk loci*. *Nat Genet*, 2010. **42**(6): p. 508-14.
51. Posadas-Sánchez, R., et al., *Interleukin-27 polymorphisms are associated with premature coronary artery disease and metabolic parameters in the Mexican population: the genetics of atherosclerotic disease (GEA) Mexican study*. *Oncotarget*, 2017. **8**(38): p. 64459-64470.
52. Zhang, M., et al., *Association of 3 Common Polymorphisms of IL-27 Gene with Susceptibility to Cancer in Chinese: Evidence From an Updated Meta-Analysis of 27 Studies*. *Medical Science Monitor : International Medical Journal of Experimental and Clinical Research*, 2015. **21**: p. 2505-2513.
53. Zicca, E., et al., *Interleukin 27 polymorphisms in HCV RNA positive patients: is there an impact on response to interferon therapy?* *BMC Infect Dis*, 2014. **14 Suppl 5**: p. S5.
54. Paradowska-Gorycka, A., et al., *Association of single nucleotide polymorphisms in the IL27 gene with rheumatoid arthritis*. *Scand J Immunol*, 2014. **80**(4): p. 298-305.
55. Garbers, C., et al., *The interleukin-6 receptor Asp358Ala single nucleotide polymorphism rs2228145 confers increased proteolytic conversion rates by ADAM proteases*. *Biochim Biophys Acta*, 2014. **1842**(9): p. 1485-94.
56. Esteve, E., et al., *Polymorphisms in the interleukin-6 receptor gene are associated with body mass index and with characteristics of the metabolic syndrome*. *Clin Endocrinol (Oxf)*, 2006. **65**(1): p. 88-91.
57. Song, Y., et al., *The interaction between the interleukin 6 receptor gene genotype and dietary energy intake on abdominal obesity in Japanese men*. *Metabolism*, 2007. **56**(7): p. 925-30.
58. Niedbala, W., et al., *Interleukin 27 attenuates collagen-induced arthritis*. *Annals of the rheumatic diseases*, 2008. **67**(10): p. 1474-9.
59. Pickens, S.R., et al., *Local expression of interleukin-27 ameliorates collagen-induced arthritis*. *Arthritis Rheum*, 2011. **63**(8): p. 2289-98.
60. Jones, G.W., et al., *Interleukin-27 inhibits ectopic lymphoid-like structure development in early inflammatory arthritis*. *J Exp Med*, 2015. **212**(11): p. 1793-802.
61. Moreland, L., et al., *Results of a phase-I/II randomized, masked, placebo-controlled trial of recombinant human interleukin-11 (rhIL-11) in the treatment of subjects with active rheumatoid arthritis*. *Arthritis Res*, 2001. **3**: p. 247-252.
62. Tanida, S., et al., *IL-27-producing CD14(+) cells infiltrate inflamed joints of rheumatoid arthritis and regulate inflammation and chemotactic migration*. *Cytokine*, 2011. **55**(2): p. 237-44.
63. Wong, C.K., et al., *Effects of inflammatory cytokine IL-27 on the activation of fibroblast-like synoviocytes in rheumatoid arthritis*. *Arthritis Res Ther*, 2010. **12**(4): p. R129.
64. Shen, H., et al., *Increased levels of interleukin-27 in patients with rheumatoid arthritis*. *Arthritis Rheum*, 2011. **63**(3): p. 860-1.
65. Moon, S.J., et al., *In vivo action of IL-27: reciprocal regulation of Th17 and Treg cells in collagen-induced arthritis*. *Exp Mol Med*, 2013. **45**: p. e46.
66. Vasconcellos, R., et al., *IL-12p35 subunit contributes to autoimmunity by limiting IL-27-driven regulatory responses*. *J Immunol*, 2011. **187**(6): p. 3402-12.
67. Kallen, K.-J., *The role of transsignalling via the agonistic soluble IL-6 receptor*. *Biochim Biophys Acta*, 2002. **1592**: p. 323-343.

68. Wong, P.K., et al., *Interleukin-6 modulates production of T lymphocyte-derived cytokines in antigen-induced arthritis and drives inflammation-induced osteoclastogenesis*. *Arthritis Rheum*, 2006. **54**: p. 158-168.
69. Hams, E., et al., *Oncostatin M receptor-beta signaling limits monocytic cell recruitment in acute inflammation*. *J Immunol*, 2008. **181**(3): p. 2174-2180.
70. Esashi, E., et al., *Oncostatin M deficiency leads to thymic hypoplasia, accumulation of apoptotic thymocytes and glomerulonephritis*. *Eur J Immunol*, 2009. **39**: p. 1664-1670.
71. West, N.R., et al., *Oncostatin M drives intestinal inflammation and predicts response to tumor necrosis factor-neutralizing therapy in patients with inflammatory bowel disease*. *Nat Med*, 2017. **23**(5): p. 579-589.
72. Schafer, S., et al., *IL11 is a crucial determinant of cardiovascular fibrosis*. *Nature*, 2017.
73. Hermann, J.A., et al., *Important immunoregulatory role of interleukin-11 in the inflammatory process in rheumatoid arthritis*. *Arthritis Rheum*, 1998. **41**: p. 1388-1397.
74. Walmsley, M., et al., *An anti-inflammatory role for interleukin-11 in established murine collagen-induced arthritis*. *Immunology*, 1998. **95**: p. 31-37.
75. Kondo, Y., et al., *Pre-treatment interleukin-6 levels strongly affect bone erosion progression and repair detected by magnetic resonance imaging in rheumatoid arthritis patients*. *Rheumatology (Oxford)*, 2017. **56**(7): p. 1089-1094.
76. Finzel, S., et al., *Interleukin-6 receptor blockade induces limited repair of bone erosions in rheumatoid arthritis: a micro CT study*. *Ann Rheum Dis*, 2013. **72**(3): p. 396-400.
77. Shukla, P., et al., *Interleukin 27 (IL-27) Alleviates Bone Loss in Estrogen-deficient Conditions by Induction of Early Growth Response-2 Gene*. *J Biol Chem*, 2017. **292**(11): p. 4686-4699.
78. Larousserie, F., et al., *Frontline Science: Human bone cells as a source of IL-27 under inflammatory conditions: role of TLRs and cytokines*. *J Leukoc Biol*, 2017. **101**(6): p. 1289-1300.
79. Park, J.S., et al., *Interleukin-27 suppresses osteoclastogenesis via induction of interferon-gamma*. *Immunology*, 2012. **137**(4): p. 326-35.
80. Kamiya, S., et al., *IL-27 suppresses RANKL expression in CD4+ T cells in part through STAT3*. *Immunol Lett*, 2011. **138**(1): p. 47-53.
81. Sims, N.A. and J.M. Quinn, *Osteoimmunology: oncostatin M as a pleiotropic regulator of bone formation and resorption in health and disease*. *Bonekey Rep*, 2014. **3**: p. 527.
82. Nicola, N.A. and J.J. Babon, *Leukemia inhibitory factor (LIF)*. *Cytokine Growth Factor Rev*, 2015. **26**(5): p. 533-44.
83. Pasquin, S., M. Sharma, and J.F. Gauchat, *Ciliary neurotrophic factor (CNTF): New facets of an old molecule for treating neurodegenerative and metabolic syndrome pathologies*. *Cytokine Growth Factor Rev*, 2015. **26**(5): p. 507-15.
84. Sims, N.A., et al., *Interleukin-11 receptor signaling is required for normal bone remodeling*. *J Bone Miner Res*, 2005. **20**(7): p. 1093-102.
85. Deng, C., et al., *Resistance to experimental autoimmune myasthenia gravis in IL-6-deficient mice is associated with reduced germinal center formation and C3 production*. *J Immunol*, 2002. **169**(2): p. 1077-83.
86. Kopf, M., et al., *Interleukin 6 influences germinal center development and antibody production via a contribution of C3 complement component*. *J Exp Med*, 1998. **188**(10): p. 1895-906.
87. Arkatkar, T., et al., *B cell-derived IL-6 initiates spontaneous germinal center formation during systemic autoimmunity*. *J Exp Med*, 2017. **214**(11): p. 3207-3217.
88. Batten, M., et al., *IL-27 supports germinal center function by enhancing IL-21 production and the function of T follicular helper cells*. *J Exp Med*, 2010. **207**(13): p. 2895-906.
89. Goya, S., et al., *Sustained interleukin-6 signalling leads to the development of lymphoid organ-like structures in the lung*. *J Pathol*, 2003. **200**(1): p. 82-7.
90. Jones, G.W., D.G. Hill, and S.A. Jones, *Understanding Immune Cells in Tertiary Lymphoid Organ Development: It Is All Starting to Come Together*. *Front Immunol*, 2016. **7**: p. 401.
91. Orr, C., et al., *Synovial tissue research: a state-of-the-art review*. *Nat Rev Rheumatol*, 2017. **13**(8): p. 463-475.

92. Takemura, S., *et al.*, *T cell activation in rheumatoid synovium is B cell dependent*. J Immunol, 2001. **167**(8): p. 4710-8.
93. Humby, F., *et al.*, *Ectopic lymphoid structures support ongoing production of class-switched autoantibodies in rheumatoid synovium*. PLoS Med, 2009. **6**(1): p. e1.
94. Thurlings, R.M., *et al.*, *Synovial lymphoid neogenesis does not define a specific clinical rheumatoid arthritis phenotype*. Arthritis Rheum, 2008. **58**(6): p. 1582-9.
95. Canete, J.D., *et al.*, *Clinical significance of synovial lymphoid neogenesis and its reversal after anti-tumour necrosis factor alpha therapy in rheumatoid arthritis*. Ann Rheum Dis, 2009. **68**(5): p. 751-6.
96. Dennis, G., Jr., *et al.*, *Synovial phenotypes in rheumatoid arthritis correlate with response to biologic therapeutics*. Arthritis Res Ther, 2014. **16**(2): p. R90.
97. Cao, Y., *et al.*, *IL-27 induces a Th1 immune response and susceptibility to experimental arthritis*. J Immunol, 2008. **180**(2): p. 922-30.
98. Rajaiyah, R., *et al.*, *Interleukin-27 and interferon-gamma are involved in regulation of autoimmune arthritis*. J Biol Chem, 2011. **286**(4): p. 2817-25.
99. Barone, F., *et al.*, *IL-22 regulates lymphoid chemokine production and assembly of tertiary lymphoid organs*. Proc Natl Acad Sci U S A, 2015.
100. Rangel-Moreno, J., *et al.*, *The development of inducible bronchus-associated lymphoid tissue depends on IL-17*. Nat Immunol, 2011. **12**(7): p. 639-46.
101. Bombardieri, M., *et al.*, *Inducible tertiary lymphoid structures, autoimmunity, and exocrine dysfunction in a novel model of salivary gland inflammation in C57BL/6 mice*. J Immunol, 2012. **189**(7): p. 3767-76.
102. Canete, J.D., *et al.*, *Ectopic lymphoid neogenesis is strongly associated with activation of the IL-23 pathway in rheumatoid synovitis*. Arthritis Res Ther, 2015. **17**: p. 173.
103. Miyajima, M., *et al.*, *Metabolic shift induced by systemic activation of T cells in PD-1-deficient mice perturbs brain monoamines and emotional behavior*. Nat Immunol, 2017. **18**(12): p. 1342-1352.
104. Hirahara, K., *et al.*, *Interleukin-27 priming of T cells controls IL-17 production in trans via induction of the ligand PD-L1*. Immunity, 2012. **36**(6): p. 1017-30.

Figure Legends-

Figure-1 – *The biological relationship between IL-6 and IL-27*

The illustration shows the composition of the IL-6 and IL-27 receptor complexes, and identifies the Signal Transducer and Activator of Transcription (STAT) factors triggered by both cytokines. Note the inclusion of gp130 in both receptors, and preferential induction of STAT1 and STAT3 activity (Bold text). For the IL-6 receptor cassette the reader should note the various IL-6 and IL-6R blocking therapies **currently** in clinical development or clinical utility. Proteins displayed in the orange box indicate biological entities that have been reported to engage with the IL-6 receptor, albeit at low affinity. Cytokines listed in the blue box showcase the protein composition of IL-27 related heterodimeric cytokines. Common subunits are colour coded. The IL-6:sIL-6R (and that of p28:sIL-6R) complex is not however stable and the cytokine-receptor undergoes association and re-association (indicated by the + symbol).

Figure-2 – *The functional properties of IL-6 and IL-27*

The biological properties of IL-6 and IL-27 have been broadly categorized under the terms 'Inflammation', 'Homeostasis', and 'Wellbeing'. Defined activities have been listed for each category and the heatmap identifies the relative contribution of IL-6 and IL-27 to each of these processes. The definition of the colour coding is listed. It should be noted that IL-6 and IL-27 may regulate similar or distinct outcomes in each process and the reader is referred to the manuscript text and review articles relevant to IL-6 or IL-27 (references [3,5]).

Figure-3 – *Immuno-modulatory action of IL-27 and the interface with IL-6*

IL-27 and IL-6 together coordinate adaptive immune responses, often with opposing biological outcomes. In an inflammatory microenvironment, and supported by accessory cytokines, IL-6 can promote the differentiation of Th1, Th2, Th22 and Th17 cells. In contrast, IL-27 counteracts the IL-6-driven expansion of Th17 cells and inhibits the development of Th2 and Th22 cells. However, IL-6 and IL-27 can both promote the secretion of IL-10 in a number of effector T cell subsets, and can drive the production of IL-21 in T helper cells. IL-27 drives immunosuppressive effector characteristics in T cells including the expression of the immune checkpoints PD-L1, PD1 and CTLA4. In contrast to the inhibitory action of IL-6 on Treg cells, IL-27 promotes the development of IL-10-producing T-bet⁺CXCR3⁺ Treg cells and Tr1 cells. IL-27 also has immunosuppressive roles at the DC:T cell synapse, for example through promoting expression of PD-L1 on DCs and inhibiting MHC-I expression. **Boxed areas highlight opposing roles of IL-27 and IL-6.** Figure adapted from Yoshida *et al.* (reference [3]).