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Novel insights into TNF receptor, DR3 and progranulin pathways in arthritis and bone remodeling

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Introduction

About 30 members of tumor necrosis factor receptor superfamily (TNFRSF) have been identified. They are transmembrane proteins with cysteine-rich motifs in their extracellular domains that bind to their cognate ligands [1]. They are categorized into three groups; death domain-containing receptors, decoy receptors, and TNF receptor-associated factor-binding receptors. Only eight TNFRSF members contain a death domain (TNFR1, DR3, DR4, DR5, DR6, Fas, NGFR, EDAR) of which TNFR1 and DR3 constitute the principle focus of this article. Interactions between tumor necrosis factor superfamily (TNFSF) ligands and TNFRSF receptors help maintain tissue homeostasis by controlling survival, proliferation, differentiation, and effector function of immune cells. Here the authors limit their review to recent advances and novel insights into the role of TNFR1 and DR3 in bone and joint biology.

Bone cells (osteoblasts, osteoclasts and osteocytes), fibroblast-like synoviocytes, chondrocytes and immune cells that infiltrate the arthritic joint will at different times express a wide range of TNFRSF members and TNFSF ligands. An overview of the current status of our knowledge in this regard is provided in **Table 1**. The impact of TNFR1 activation on bone and inflammatory joint diseases has been researched in great depth [2, 3], but other more recently discovered TNFRSF members such as TROY, EDAR and XEDAR have little or no published data in the field. The unexpected interaction between Progranulin (PGRN) and both TNFR1 and TNFR2 is particularly interesting in the context of arthritis-associated bone pathology. PGRN levels are elevated in synovial fluid of patients with rheumatoid arthritis, osteoarthritis and other arthropathies [4-6], and it has been shown to inhibit TNFα-induced osteoclastogenesis and promotes osteoblast differentiation in mice [7]. However, PGRN has a higher binding affinity for TNFR2 (anti-inflammatory with osteoprotective function) than TNFR1 (predominantly pro-

inflammatory with degenerative function) suggesting conflicting actions. The potential overall impact of these divergent PGRN signaling pathways upon the architecture of the arthritic joint are evaluated [8].

Death receptor (DR3) and its TNFSF ligand TL1A contribute to the pathogenesis of autoimmune and rheumatic diseases [9], however, research in this area is very much in its infancy. Inhibition of DR3 reduces osteoclastogenesis and protects bones against the development of erosive pathology in experimental models of arthritis [10]. A soluble form of DR3, produced by osteoblasts, regulates osteoblast apoptosis under tightly controlled conditions [11, 12]. TL1A levels are elevated in serum from patients with rheumatoid arthritis versus healthy controls. This review provides further insight to DR3's role in bone remodeling and arthritis.

PGRN/TNFR interactions in arthritis and bone remodeling

PGRN, also known as granulin–epithelin precursor (GEP), proepithelin, acrogranin, and GP88/PC-cell derived growth factor (PCDGF), is a 593-amino-acid autocrine growth factor. PGRN contains seven-and-a-half repeats of a cysteine-rich motif (CX5–6CX5CCX8CCX6CCXDX2HCCPX4CX5–6C) and forms a unique "beads-on-a-string" structure [13]. PGRN was first found to bind to TNFR in a yeast two-hybrid screening for PGRN-binding proteins[14]. The interaction was subsequently validated in human cells. Surface plasmon resonance (SPR) analysis revealed that PGRN bound to both TNFR1 and TNFR2 and with greater affinity than TNFα to TNFR2 [8, 14]. Three fragments of PGRN and their adjacent linkers enable the ligand to bind to TNF receptors [15]. Notably, PGRN showed therapeutic effects in several TNF-mediated inflammatory arthritis models, including collagen-induced

arthritis, collagen antibody induced arthritis, and spontaneous arthritis in the TNF-transgenic mouse model [14, 16, 17]. Furthermore a novel PGRN-mimetic called Atsttrin (**Fig. 1**) had a more pronounced beneficial effects than PGRN on inflammatory arthritis [14]. Currently marketed anti-TNF therapies bind to the TNFα ligand, in contrast, Atsttrin binds to TNFR and not to TNFα itself. Atsttrin was more efficacious than current anti-TNFα therapies, including etanercept, in several preclinical inflammatory arthritis models tested [14].

Accumulating evidence indicates that TNFα orchestrates osteoarthritis (OA) pathology [18]. Recent finding support the notion that PGRN could also modulate the aetiopathogenesis of OA. PGRN is an important regulator of cartilage development [19, 20], was identified as an OA-associated growth factor in a genome-wide screen for differentially expressed genes in OA [21], and in aging mice PGRN deficiency led to spontaneous OA-like phenotype characterized by severe breakdown of cartilage structure[22]. The OA-like pathology was attenuated by the local delivery of a recombinant PGRN protein. Intra-articular transplantation of Atsttrin-transduced mesenchymal stem cells inhibited TNFα-mediated catabolic response, ameliorating OA development [23]. One chondro-protective mechanism has been proposed, namely that PGRN increased the levels of anabolic biomarkers and suppresses inflammatory action of TNFα in cartilage and chondrocytes via activation of the ERK1/2 signaling pathway[19].

The direct impact of PGRN upon bone remodeling is yet to be determined, with current knowledge derived from a bone-healing model. In mice at least, PGRN deficiency delayed bone healing, while recombinant PGRN enhanced bone regeneration [24]. Furthermore, PGRN-mediated bone formation was dependent upon TNFR2, but not TNFR1. In this same study, Zhao et al showed that PGRN blocked osteoclastogenesis in TNF-α transgenic mice. Taken together these findings imply that PGRN exerts dual action on bone during inflammatory arthritis namely;

inhibiting TNF-α induced bone erosion by osteoclasts and promoting osteoblast-dependent mineral apposition via a TNFR2. A recent report using Atsttrin, incorporated into 3D-printed alginate/hydroxyapatite scaffolds, implies that PGRN stimulates bone regeneration by inhibiting TNF signaling [25].

TNFα's inflammatory and catabolic actions are largely mediated through its interaction with TNFR1. However, understanding of the impact of TNFR2-mediated signaling remains largely unclear. Recent studies indicate that TNFR2 signaling has a beneficial and protective role in joint destruction [26, 27]. Studies also reveal differential roles of TNFR1 and TNFR2 in PGRN-mediated fracture healing and OA[22, 24, 28]. Although PGRN and TNFα exhibit comparable binding affinity to TNFR1, PGRN has an approximately 600-fold higher binding affinity for TNFR2 than TNFα[14]. Since PGRN and TNFα compete for binding to the same extracellular cysteine-rich domains (CRD) of TNFR, CRD2 and CRD3 [8], PGRN acts as a naturally-occurring antagonist of TNFα and disturbs the binding of TNFα to TNFRs. More importantly, PGRN also acts as a ligand of TNFR2 and directly activates the PGRN/TNFR2 protective and anti-inflammatory pathway. TNFR2 has been shown to be critical for PGRN-mediated protection in OA and bone fracture healing [22, 24, 28]. Recent paper showing that local injection of soluble TNFR2 (sTNFR2, etanercept) resulted in more severe joint destruction in a mouse model of OA [29] also suggest the importance of PGRN-mediated protection in OA. Injection of sTNFR2 inhibits both TNFα and PGRN. Further, PGRN may be more inhibited than TNFα, as PGRN has a much higher binding affinity to TNFR2 than TNFα. Unlike etanercept, mouse TNFα monoclonal antibody (infliximab) and humanized TNFα monoclonal antibody (adalimumab) are specific for TNFα, and have been shown to be protective against OA in animal models [30]. The opposite effects of TNFα specific (i.e. infliximab and adalimumab) and non-

specific (i.e. etanercept) inhibitors in OA indicate the critical protective role of other ligand(s) of TNFR, i.e. PGRN, in the pathogenesis of OA [31]. Thus, future studies are warranted to clarify the complex interplay between TNFα, PGRN and their receptors in the pathogenesis of arthritis and bone remodeling, which will not only better our understanding of TNFR signaling in the pathogenesis of these musculoskeletal disorders, but may lead to innovative therapies via selectively targeting distinct TNFR pathways.

TL1A/DR3 interactions in arthritis and bone remodeling

Death Receptor 3 (DR3, TNFRSF25, Apo3, LARD, TR3, TRAMP, WSL-1) was discovered simultaneously in the mid-to-late 1990s by multiple groups, when a combination of BLAST homology searches to Fas and TNFR1 [32, 33] and a yeast-two hybrid library screen using a TNFR1 death domain as bait [34], identified a closely related protein. Subsequently, DR3 emerged as the closest structural homolog to TNFR1, containing an equivalent 4 CRDs as well as an intracellular death domain. Unlike TNFR1, however, whose cellular distribution is widespread and surface expression of which is controlled by the generation of soluble forms through cleavage, DR3 has a more restricted tissue distribution and is regulated by the expression of multiple activation-induced splice variants, including soluble and death-domain containing transmembrane forms with excision of the membrane proximal CRD [33, 35]. The exact function of these splice variants remain unclear.

The identification of ligand(s) for DR3 has been complicated by the number of potential candidates and their altering nomenclature [36], but prior to the discovery of PGRN, one TNFSF member, TNF-like protein 1A (TL1A, TNFSF15) [37], had withstood stringent biochemical and

functional scrutiny for DR3 specificity [38, 39]. TL1A is the product of a longer alternative mRNA transcript to a protein initially named vascular endothelial growth inhibitor or VEGI (TL1), so named for its capacity to inhibit angiogenesis and induce apoptosis of endothelial cells [40]. As its name and nomenclature suggests, TL1A is closely related in structure to TNFα, encoding a type II transmembrane protein with a metalloprotease cleavage site allowing release of a soluble molecule, but also has distinct expression patterns as it is found in ng/ml concentrations in serum from healthy individuals [41] that suggests physiologically different levels of production and functional regulation. In this regard, there may also be significant differences between species as DcR3, the decoy ligand for TL1A, FasL and LIGHT (discussed above), is only found in man and not mouse. It is in this context that interpretations of DR3 function and its potential for therapy should be taken.

The generation of transgenic mice genetically deficient for DR3, TL1A or overexpressing TL1A or dominant negative forms of DR3 have given rise to many *in vivo* studies describing the essential requirement for the DR3/TL1A pathway in models of multiple autoimmune and inflammatory diseases. These have supported an ever-growing list of *in vitro* human functional and genetic studies that have associated DR3 and TL1A with human diseases ranging from inflammatory bowel disease and primary billiary cirrhosis to leprosy (comprehensively reviewed in [42]). Of significance for this review were findings that suggested alternate respective ligands for DR3 and TL1A. This included the apparent greater protection against experimental autoimmune encephalomyelitis afforded by $DR3^{-/-}$ [38] compared to $TL1A^{-/-}$ mice [43] in otherwise similar models of disease and the DR3-independent triggering of TNFR2 expression by TL1A in kidney organ cultures [44]. The underlying conclusion was that there were still

unknown interactions for this complex of proteins, which will have to be discovered and dissected in detail before their full potential as therapeutic targets can be understood.

With specific regard to disorders of the bone, initial genetic studies suggested DR3 gene duplication [45] and a mutation predicted to destabilize DR3 [46] were linked to development of rheumatoid arthritis (RA), while synovial cells from RA patients exhibited a hypermethylated DR3 gene suggestive of activation [47], however, genome wide association studies (GWAS) have had less success with supporting this connection. Two early investigations associated genetic variation around the DR3 (TNFRSF25) locus with RA [48, 49], but more recent ones have not. In contrast, genetic variation at the TL1A (TNFSF15) locus has not been associated with RA, but has been linked to another bone disorder, ankylosing spondylitis [50]. Irrespective, increased levels of TL1A have been reported in the serum of patients suffering from both of these arthritides [41, 51, 52], as well as the synovial tissue and synovial exudates of rheumatoid factor positive RA patients [53, 54].

The functional consequences of raised TL1A levels in these disorders have generally been associated with a range of outcomes dependent on the type and differentiation state of the DR3-expressing cell to which TL1A is binding and signaling. Here, we will cover those cell types specifically associated with bone physiology irrespective of the inflammatory context, although it should be noted that there may also be secondary effects as TL1A can induce TNFα [55], thereby having the capacity to trigger a broad range of secondary effects associated with other pro-inflammatory cytokines. The DR3/TL1A axis was first described as a T cell co-stimulator [37], but its effects on Th17 cells, drivers of osteoclastogenesis and therefore inflammatory bone resorption [56], highlighted the complexity in the outcome of TL1A signaling. Initial reports in $TL1A^{-/-}$ mice suggested that TL1A regulated Th17 differentiation [43], but more extensive *in*

vitro studies in both DR3$^{-/-}$ mice [57] and healthy human subjects indicated that Th17 differentiation from naïve CD4^{+} T cells was impaired, while maintenance of the response once T cells were Th17 committed was enhanced, by TL1A [58]. Intriguingly, recent reports have shown that TL1A-driven Th17 differentiation from naïve CD4^{+} T cells occurs in samples from RA patients [52, 59]. Why these differences have been observed remain an area of debate, though the underlying theme is that TL1A promotes the Th17 response in RA.

The development of the main effectors of bone resorption, osteoclasts, are also regulated by the DR3/TL1A axis, at least in an inflammatory setting. While osteoclastogenesis driven by M-CSF and RANK-L was unaffected in DR3$^{-/-}$ mice, these animals exhibited resistance to cartilage destruction and bone erosions in a model of antigen-induced arthritis (AIA) [39, 60]. Furthermore, DR3$^{-/-}$ mice were resistant to exacerbation induced by exogenous addition of TL1A, while antagonism of the pathway with anti-TL1A mAb ameliorated disease in collagen-induced arthritis (CIA) [39]. Addition of exogenous TL1A also exacerbated CIA [54]. The direct nature of this signaling in myeloid cells has been demonstrated, with DR3 expression being induced during the process of macrophage differentiation and TL1A signaling resulting in the DR3-dependent production of the gelatinase MMP-9 [61]. The DR3/TL1A pathway may also control other aspects of macrophage differentiation that promote the arthritic process. Thus, DR3 regulates the expression of scavenger receptors on macrophages [62], which have been implicated in AIA-induced cartilage destruction [63].

Finally, DR3 also modulates osteoblast function. Human osteoblast cell lines were first reported to express DR3 in 2003 [64], which were then used to demonstrate differential regulation dependent on cell culture conditions. Crosslinking induced apoptosis at low density, but differentiation at high density [11]. The subsequent reported association between TL1A and

ankylosing spondylitis [50] and breeding of the DR3$^{-/-}$ genotype on a DBA/1 background, which spontaneous develops ankylosing enthesopathy [65], led to a recent study on the role of DR3 in osteoblast function *in vitro* and *in vivo*. Indeed, DBA/1 DR3$^{-/-}$ mice showed significantly lower thoracic spine-specific bone formation *in vivo*, while DR3$^{-/-}$ osteoblast cultures exhibited reduced levels of alkaline phosphatase, osteopontin and mineral apposition [12]. Thus, the DR3/TL1A axis is involved in the direct regulation of every major cell type involved in bone physiology, recent data suggesting it has an important homeostatic role in this tissue as well as its more established function in inflammatory disease.

PGRN/DR3 Interactions in arthritis and bone remodeling

Screening the associations of Atsttrin with all members of the TNFR subfamily led to the discovery that in addition to TNFR, PGRN/Atsttrin also directly binds to DR3 and inhibits TL1A activity [66]. Structural modeling of DR3 predicts a similar structure to TNFR1 in which primary contacts with TL1A are in the 2nd and 3rd CRD [46]. In addition, a mutation linked to rheumatoid arthritis at the end of CRD3 is in a region critical for structural integrity of ligand–receptor complexes [46]. The first three CRD domains of the extracellular potion of DR3, i.e. CRD1, CRD2 and CRD3, are all required for interacting with Atsttrin. PGRN was also found to directly bind to DR3 in an *in vitro* binding assay, as it did to TNFRs [66]. Atsttrin dose-dependently inhibited TL1A-stimulated expressions of TL1A-target genes C1qTNF3 and βigH3. In addition, Atsttrin effectively neutralized TL1A-promoted osteoclastogenesis *in vitro*[66].

The associations of PGRN with TNFR and DR3 also led to investigations on the immunological mechanisms underlying PGRN mediated anti-inflammatory and protective activities in

autoimmune diseases[67-69]. Since both animal and human studies have demonstrated that regulatory T cells (Tregs) play a critical role in the prevention of autoimmunity and other pathological immune responses, the effects of PGRN on Treg differentiation and function were first determined. PGRN protects Tregs from a negative regulation by TNFα and these protective effects are primarily mediated by TNFR2[68, 69]. In contrast, PGRN-antibodies, opposite to recombinant PGRN, led to an increase of TNFα-induced down-regulation of FOXP3 in $CD4^{+}CD25^{hi}$ Tregs [70]. In addition, PGRN was able to stimulate the conversion of CD4+CD25- T cells into induced Tregs (iTregs) in a dose-dependent manner *in vitro*. Further, PGRN showed synergistic effects with TGF-β1 on the induction of iTreg[69]. PGRN was required for the immunosuppressive function of Tregs, since PGRN-deficient Tregs have a significant decreased ability to suppress the proliferation of effector T cells. PGRN deficiency caused a marked reduction in Tregs number in the course of inflammatory arthritis [69]. In a bone marrow chimera and $CD4+CD45Rb^{hi}$ T cell transfer model, lack of PGRN signaling in CD4+ T cells also exacerbated experimental colitis. In addition, PGRN-mediated protective effect was compromised in the absence of IL-10 or TNFR2 signaling[68]. It is noted that PGRN mediated regulation of Tregs appears to be inflammation-dependent, because PGRN deficiency does not alter the numbers of CD4+CD25+Foxp3+ Treg cells *in vivo* under physiological conditions [69]. Progranulin inhibits expression and release of chemokines CXCL9 and CXCL10 in a TNFR1 dependent manner CD4+ T cells [67]. The DR3 pathway may also contribute to PGRN-mediated protective effect in inflammatory diseases, since a recent report showed that agonistic antibody to DR3 expanded CD4(+)FoxP3(+) Tregs *in vivo*, which in turn suppressed immune responses. In addition, a neuropathology develops with age in both DR3-/- [71] and PGRN deficient mice[72]. Intriguingly, transgenic overexpression of TL1A in both the myeloid and T cell

lineage results in *in vivo* expansion of Tregs, though these eventually become dysregulated and intestinal inflammation develops [10].

In contrast to Tregs, Th17 cell frequency was decreased significantly in the spleens of mice treated with recombinant PGRN in a collagen-induced arthritis model[68, 69]. In addition, the serum level of IL-17 was also decreased significantly in PGRN-treated mice. Further, both TNFR1 and DR3 pathways were found to be involved in the PGRN inhibition of IL-17 cells. Taken together, PGRN and its derived Atsttrin appear to exert their anti-inflammatory activities through multiple pathways: **1**) by activation of PGRN/TNFR2 protective pathway, and **2**) by inhibition of TNF/TNFR1 and TL-1A/DR3 inflammatory signaling (**Fig.** 2).

Clinical perspective

Because TNFα is one of the key main inflammatory mediators, it is no surprise that alterations of its physiologic antagonist PGRN have a direct impact on the initiation and progression of arthritis. The effect of TNFα antagonism should be at least comparable to conventional TNFα-blockers [14]. The additional specific inhibition of the TL1A/DR3 interaction and activation of TNFR2 anti-inflammatory pathway by PGRN or its derivate [66] is a unique characteristic and might represent a significant advantage over conventional TNFα inhibitors particularly for patients with refractory or relapsing disease under conventional TNFα-blockers. Blocking the TL1A/DR3 interaction probably offers additional positive effects by the reduction of proinflammatory cytokines, reduction of autoantibody formation and by the reduction of osteoclastogenesis [10, 54].

A potential disadvantage of PGRN or Atsttrin compared to anti-TNFα antibodies might be, that anti-TNFα antibodies can trigger apoptosis of proinflammatory T-lymphocytes by binding to membranous TNFα. This effect, which is also missing for TNFR/Fc fusion proteins, appears to play a particular role in inflammatory bowel diseases (IBD) and less in arthritis [73]. The question is whether the administration of PGRN or a derivative thereof has a higher risk of iatrogenic induced neoplasms than conventional TNFα blockers. Usage of conventional TNFα-blockers results in an elevated risk for reactivation of latent infections such as mycobacteria, viral hepatitis, or for the development of opportunistic infections [74]. The effects of administered recombinant PGRN or its derivative on the risk for opportunistic infections remain speculative and are not further discussed in this review.

Another question arises through the discovery of progranulin-autoantibodies: Can recombinant PGRN or Atsttrin be administered to patients with preexisting PGRN-antibodies? Frequently occurring PGRN antibodies have been identified in a wide spectrum of autoimmune diseases including rheumatoid arthritis and surprisingly psoriatic arthritis, which had been regarded as a seronegative disease [5, 75]. PGRN-antibodies occur in relevant titres, belong predominantly to IgG1 subclass (in IBD also IgA), have a neutralizing effect on PGRN plasma levels and thus are likely to act in a proinflammatory fashion.

Epitope mapping identified a binding region within the N-terminal 112 amino acids of PGRN as a target of progranulin antibodies in all patients. This means that PGRN-autoantibodies target the anti-inflammatory progranulin and possibly co-target only mature granulin G, the most N-terminal granulin motif. Despite the structural similarity of granulin G with the other six granulins, no binding against granulin motifs other than granulin G was detected [75]. With regard to Atsttrin, no antibodies were detected so far directed against those parts of progranulin

which are constitutive of Atsttrin, i.e. granulin F, granulin A, granulin C and the appropriate linker regions [14]. Nevertheless, epitope spreading and immunogenicity should be monitored closely in preclinical and clinical trials addressing the therapeutic effects of Atsttrin administration. A potential binding of patient derived, preexisting PGRN-antibodies against Atsttrin itself has not yet been tested to our knowledge and should be excluded.

As a reason for the breakdown of self-tolerance against PGRN, a second immunogenic PGRN isoform, hyperphosphorylated at Ser81 was exclusively identified in a PGRN-antibody-positive patients [76]. This hyperphosphorylated PGRN is caused by inactivated PP1. Interestingly, the phosphorylation of Ser81 PGRN prevents interaction with TNFR1 & 2 and DR3, so hyperphosphorylated PGRN has lost its anti-inflammatory function. Considering these facts, it seems a reasonable therapeutic strategy would be to compensate the imbalance of pro- and anti-inflammatory molecules due to either lack of functional PGRN, caused by neutralizing PGRN-antibodies, Ser81 hyperphosphorylation of PGRN, and/or excessive secretion of TNFα and TL1A, by the administration of a PGRN derivate, which cannot be neutralized by pre-existing PGRN-autoantibodies (**Fig. 3**).

In conclusion it can be stated that PGRN and its interaction with TNFα/TNFR1&2 and TL1A/DR3 represent an attractive new therapeutic target (**Table 2**). Looking at the underlying theory and the known preclinical data, Atsttrin could be a therapeutic alternative in cases of refractory or recurrent arthritis.

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Table 1 Cellular expression of death domain containing TNFRSF members and their association with arthritis

Receptor	Ligand	Association with arthropathies	Osteoblast	Osteoclast	Osteocyte	Fibroblasts-like synoviocytes	Chondrocytes	Leukocyte subsets	¶References
TNFR1 (TNFRSF1A)	TNFα (TNFSF2) LTα (TNFSF1) PGRN	RA, OA, SA, Arthropathies	✓	✓	✓	✓	✓	All	[2-6, 14, 77, 78]
Fas (TNFRSF6)	FASL (TNFSF6)	RA OA Arthropathies	✓	✓	✓	✓	✓	All	[79-82]
NGFR (TNFRSF16)	NGF	RA OA SA Arthropathies	✓	✓	X	X	X	T cells	[2, 79, 83]
EDAR (TNFRSF27)	EDA	RA Arthropathies	X	X	X	X	X	Macrophage subsets	[84]
DR3 (TNFRSF25)	TL1A (TNFSF15) PGRN	RA OA SA Arthropathies	✓	✓	X	X	X	CD4+ T, Treg, CD8+ T cells, B cell subset, macrophages (inducible),	[10-12, 37, 51, 78]

								neutrophils	
DR4 (TNFRSF 10A)	TRAIL (TNFSF 10)	RA OA SA Arthropathies	✓	✓	X	✓	✓	Activated T cells	[78, 79, 85, 86]
DR5 (TNFRSF 10B)	TRAIL (TNFSF 10)	RA OA SA Arthropathies	✓	✓	X	✓	✓	All	[78, 85, 86]
DR6 (TNFRSF 21)	APP	X	APP	APP	X	X	✓	T cells, B cells, dendritic cells	[87]

Table 2 Summary of key points about Progranulin and TNFR and DR3 pathways in rheumatoid arthritis, osteoarthitis, spondyloarthritis and other arthropathies

#	Key points	References
1.	**<u>Progranulin (PGRN)</u>**	
	• also known as granulin–epithelin precursor (GEP), proepithelin, acrogranin, and GP88/PC-cell derived growth factor (PCDGF) • 593-amino-acid autocrine growth factor • seven-and-a-half repeats of a cysteine-rich motif • Involved in: embryogenesis, wound healing antiinflammatory, host defense, neurotrophic factor • High PGRN levels associated with several human cancers	[14, 16, 17]
2.	**<u>PGRN ligand of TNFR1, TNFR2 and DR3</u>**	
	• PGRN ligand of TNFR1, TNFR2 and DR3 and physiologic antagonist of TNF-α, LTα and TL1a • Inhibition of TNFR1 and DR3 pathways, but activation of TNFR2 pathway by PGRN • 600 times higher affinity towards TNFR2 by PGRN compared to TNF-α • PGRNs affinity to TNFR1, TNFR2 and DR3 originates from granulins F, A and C with linker regions • Atsttrin: smallest recombinant derivate of PGRN synthesized of granulins F, A, C and linker regions P3, P4 and P5 of PGRN with preserved antiinflammatory effect • PGRN attenuates TNF-α induced downmodulation of $CD4^+CD25^{hi}$ $FOXP3^+$ Tregs • PGRN stimulates conversion of $CD4^+CD25^-$ T cells into induced Tregs (iTregs)	[14, 66, 69]
3.	**<u>PGRN, TNFR1 and TNFR2 in Osteoarthritis</u>**	[30, 31]
	• low PGRN levels -> spontaneous OA • high PGRN levels -> anabolic function • catabolic effect of TNF-α mainly mediated via TNFR1 • TNFR2 pathway anti-inflammatory & osteoprotective • administration of sTNFR2/Fc fusion protein neutralizes TNF-α and PGRN and leads to exageration of OA • administration of Anti-TNF-α Mabs neutralizes TNF-α specifically and ameliorates OA • PGRN account for the opposite effects of sTNFR2/Fc fusion protein and Anti-TNF-α Mabs	
4.	**<u>TL1a/DR3</u>**	
	• High levels of TL1A induce TH17 response in RA • $DR3^{-/-}$ mice resistant of cartilage destruction in AIA • CIA exaggeration by TL1a, amelioration by Anti-TL1a Mab • TL1a/DR3 activation induces MMP9 and CCL3	[52, 59] [60] [39]

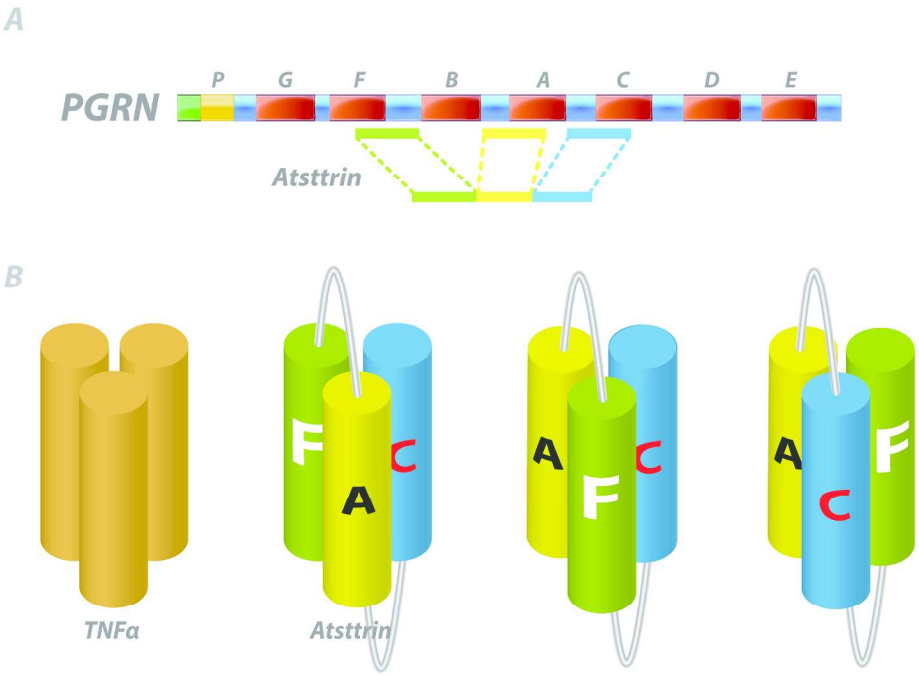
	• DcR3 decoy ligand for TL1A, FasL and LIGHT not in mice -> results from mouse models difficult to translate	
5.	**pSer81 PGRN and PGRN antibodies**	
	• Neutralizing PGRN antibodies directed against the N-terminal 112AA occur frequently in various autoimmune diseases • PGRN antibodies are induced by a second, transiently occuring hyperphosphorylated PGRN isoform (pSer81 PGRN) • pSer81 PGRN lacks affinity for TNFR1, TNFR2 and DR3 and thus antagonism of TNF-α and TL1a • -> dysbalance of proinflammatory TNF-α & TL1A and antiinflammatory functional PGRN in various inflammatory disesases	[5] [75, 76]
6.	**Clinical Perspective**	
	• Targeting of TNFR/TNF superfamily common therapeutic strategy • Possible advantages of rec. PGRN/Atsttrin compared to conventional TNF-blockers due to additional inhibition of DR3 and activation of TNFR2 • PGRN-autoantibodies regularly target the N-terminal 112 AA and thus not the parts constitutive for Atstrrin; however affinity has not been excluded • Risk of side effects concerning susceptibility to infectious diseases, emergence of new autoimmune phenomena or cancer remain unclear	[79]

Figure Legend

Fig. 1. A) Domain Structure and Organization of PGRN and Atsttrin. PGRN consists of 7½ repeats of a cysteine-rich granulin motif in the order of P-G-F-B-A-C-D-E, where A to G are full repeats, and P is the half motif. Atsttrin, derived from PGRN, consists of three half units of granulins A, C and F, and their accompanying linker regions. B) A proposed models for explaining the independent action of three TNFR-binding domains of PGRN. TNFα trimmers binds to receptors in a heterohexameric 3:3 complex[88]. The three fragments of Atsttrin act independently for interacting with TNFR, and changing the order of these fragments does not affect the ability to binding to TNFR[15]. It is proposed that each TNFR-binding domain may function as a single TNFα molecule, and the intact Atsttrin might resemble a TNF trimer through internal folding at their linker regions.

Fig. 2. A proposed model illustrating the multiple signaling pathways by which PGRN (and its derivative Atsttrin) exerts its protective actions in autoimmunity. PGRN (Atsttrin) binds to TNF receptor 2 (TNFR2) and stimulates the formation and function of Tregs, but may antagonize TL1A/DR3 signaling in these cells. PGRN (Atsttrin) also antagonizes TNF/TNFR1 and TL1A/DR3 signaling and inhibits their inflammatory activities.

Fig. 3. A) Balance of TNF-α & TL1A and their antagonist progranulin in a healthy control. **B**) Dysbalance of proinflammatory TNF-α & TL1A and antiinflammatory PGRN due to overexpression of proinflammatory TNF-α & TL1A and diminished antagonistic effects of PGRN due to hyperphosphorylation of Ser81 of PGRN and induction of neutralizing PGRN-antibodies.



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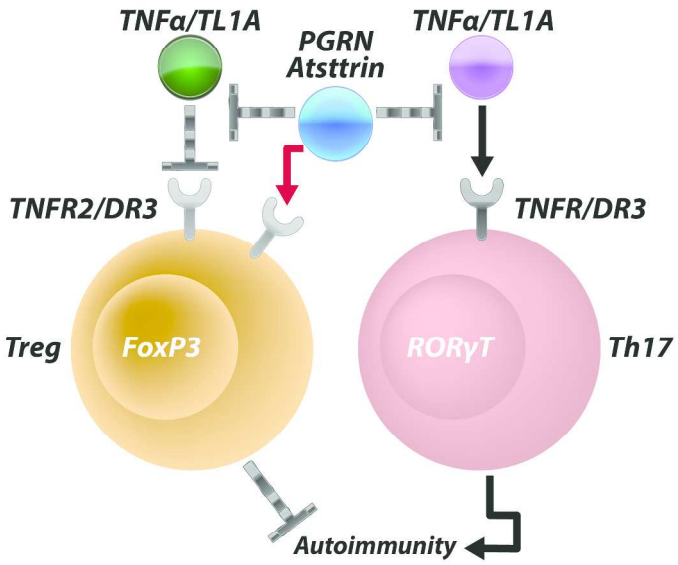

Fig. 2

279x215mm (300 x 300 DPI)

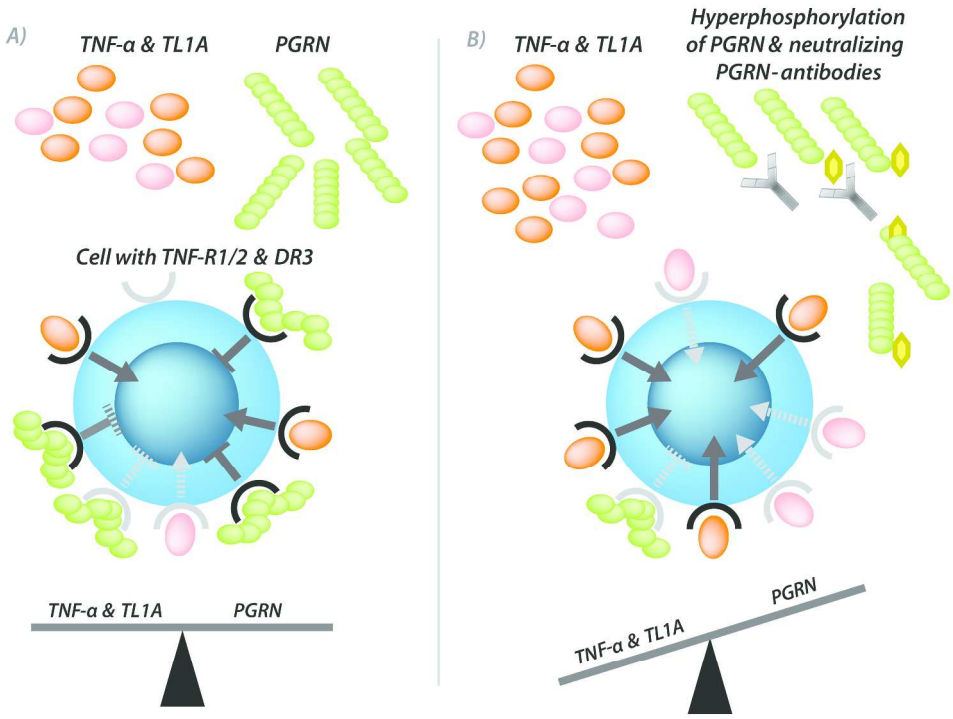

Fig. 3

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