Introduction

Approximately 30 members of the 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 3 groups: death domain–containing receptors, decoy receptors, and TNFR-associated factor–binding receptors. Only 8 TNFRSF members contain a death domain (TNFR type I [TNFRI], death receptor 3 [DR-3], DR-4, DR-5, DR-6, Fas, nerve growth factor receptor, and ectodysplasin A receptor [EDAR]), of which TNFRI and DR-3 constitute the principal focus of this article. Interactions between TNF superfamily (TNFSF) ligands and TNFRSF receptors help maintain tissue homeostasis by controlling survival, proliferation, differentiation, and effector function of immune cells. We limit our review to recent advances and novel insights into the roles of TNFRI and DR-3 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 TNFRI activation on bone and inflammatory joint diseases has been researched in great depth (2,3), but little or no data in the field have been reported on other more recently discovered TNFRSF members such as TROY (TNFRSF expressed on the mouse embryo; TNFRSF19), EDAR, and XEDAR (X-linked ectodysplasin receptor; TNFRSF27). The unexpected interaction between progranulin (PGRN) and both TNFRI and TNFRII is particularly interesting in the context of arthritis-associated bone pathology. PGRN levels are elevated in the synovial fluid of patients with rheumatoid arthritis (RA), osteoarthritis (OA), and other arthopathies (4–6), and PGRN has been shown to inhibit TNF-induced osteoclastogenesis and promote osteoblast differentiation in mice (7). However, PGRN has a higher binding affinity for TNFRII (anti-inflammatory with osteoprotective function) than for TNFRI (predominantly proinflammatory with degenerative function), which suggests conflicting actions. The potential overall impact of these divergent PGRN signaling pathways on the architecture of the arthritic joint has been evaluated (8).

DR-3 and its TNFSF ligand TNF-like molecule 1A (TL1A) contribute to the pathogenesis of autoimmune and rheumatic diseases (9); however, research in this area is very much in its infancy. Inhibition of DR-3 reduces osteoclastogenesis and protects bones against the development of erosive pathology in experimental models of arthritis (10). A soluble form of DR-3, produced by osteoblasts, regulates osteoblast apoptosis under tightly controlled conditions (11,12). TL1A levels are elevated in serum from patients with RA compared
| Receptor       | Ligand          | Association with arthritis | Osteoblasts | Osteoclasts | Osteocytes | Fibroblast-like synoviocytes | Chondrocytes | Leukocyte subsets | References† |
|---------------|-----------------|-----------------------------|-------------|-------------|------------|-----------------------------|--------------|------------------|-------------|
| TNFR1 (TNFRSF1A) | TNF (TNFSF2), LTα (TNFSF1), PGRN | RA, OA, SpA, arthropathies | Yes         | Yes         | Yes        | Yes                         | Yes          | Yes              | 2–6, 14, 77, 78 |
| Fas (TNFRSF6) | FasL (TNFSF6), NGF | RA, OA, arthropathies       | Yes         | Yes         | No         | No                          | No           | No               | 79–82       |
| NGFR (TNFRSF16) | EDA (TNFRSF27) | RA, arthropathies           | Yes         | No          | No         | No                          | No           | CD4+ T cells, Treg cells, CD8+ T cells, IgM+ B cells, macrophages (inducible), neutrophils | 2, 79, 83, 84 |
| DR-3 (TNFRSF25) | TL1A (TNFSF15), PGRN | RA, OA, SpA, arthropathies | Yes         | Yes         | No         | No                          | No           | Macrophage subsets | 10–12, 37, 50, 78 |
| DR-4 (TNFRSF10A) | TRAIL (TNFSF10) | RA, OA, SpA, arthropathies | Yes         | Yes         | No         | Yes                         | Yes          | Activated T cells | 78, 79, 85, 86 |
| DR-5 (TNFRSF10B) | TRAIL (TNFSF10) | RA, OA, SpA, arthropathies | Yes         | Yes         | No         | Yes                         | Yes          | T cells          | 78, 85, 86   |
| DR-6 (TNFRSF21) | APP (TNFRSF10) | None                        | Yes         | Yes         | No         | No                          | Yes          | T cells, B cells, dendritic cells | 87          |

* TNFRSF = tumor necrosis factor receptor superfamily; LTα = lymphotoxin α; PGRN = progranulin; RA = rheumatoid arthritis; OA = osteoarthritis; SpA = spondyloarthritis; NGFR = nerve growth factor receptor; EDAR = ectodysplasin A receptor; DR-3 = death receptor 3; TL1A = TNF-like molecule 1A; APP = amyloid precursor protein.

† The reference list presented in this table was limited by the requirements of the journal; as such, the citations for the expression of TNFRSF and TNFSF ligands by cells are not comprehensive.
with that from healthy controls. This review provides further insight into the role of DR-3 in bone remodeling and arthritis.

**PGRN–TNFR interactions in arthritis and bone remodeling**

PGRN, also known as granulin-epithelin precursor, proepithelin, acrogranin, and GP88/PC cell–derived growth factor, is a 593–amino acid autocrine growth factor. PGRN contains 7.5 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-2-hybrid screening for PGRN-binding proteins (14). The interaction was subsequently validated in human cells. Surface plasmon resonance analysis revealed that PGRN bound to both TNFRI and TNFRII and with greater affinity than TNF to TNFRII (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 models of TNF-mediated inflammatory arthritis, including collagen-induced arthritis (CIA), collagen antibody–induced arthritis, and spontaneous arthritis in the TNF-transgenic mouse model (14,16,17). Furthermore, a novel PGRN mimetic called Atsttrin (Figure 1) had a more pronounced beneficial effect than PGRN in inflammatory arthritis (14). Currently marketed anti-TNF therapies bind to the TNF ligand; in contrast, Atsttrin binds to TNFR and not to TNF itself. Atsttrin was more efficacious than current anti-TNF therapies, including etanercept, in several preclinical inflammatory arthritis models tested (14).

Accumulating evidence indicates that TNF orchestrates OA pathology (18). Recent findings support the notion that PGRN could also modulate the etiopathogenesis of OA. PGRN is an important regulator of cartilage development (19,20) and was identified as an OA-associated growth factor in a genome-wide screen for differentially expressed genes in OA (21), and its
deficiency in aging mice led to a 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. Intraarticular transplantation of Atstrrin-transduced mesenchymal stem cells inhibited TNF-mediated catabolic response, ameliorating OA development (23). One chondroprotective mechanism has been proposed, namely, that PGRN increases the levels of anabolic biomarkers and suppresses the inflammatory action of TNF in cartilage and chondrocytes via activation of the ERK-1/2 signaling pathway (19).

The direct impact of PGRN on bone remodeling has yet to be determined, with current knowledge derived from a model of bone healing. In mice at least, PGRN deficiency delayed bone healing, while recombinant PGRN enhanced bone regeneration (24). Furthermore, PGRN-mediated bone formation was dependent upon TNFRII but not TNFRI. In that same study, Zhao et al showed that PGRN blocked osteoclastogenesis in TNF-transgenic mice. Taken together, these findings imply that PGRN exerts dual action on bone during inflammatory arthritis, first, by inhibiting TNF-induced bone erosion by osteoclasts, and second, by promoting osteoblast-dependent mineral apposition via TNFRII. Findings of a recent study using Atstrrin incorporated into 3-dimensional–printed alginate/hydroxyapatite scaffolds imply that PGRN stimulates bone regeneration by inhibiting TNF signaling (25).

The inflammatory and catabolic actions of TNF are largely mediated through its interaction with TNFRI. However, we continue to have limited understanding of the impact of TNFRII-mediated signaling. Recent studies indicate that TNFRII signaling is beneficial and protects against joint destruction (26,27). Studies also reveal differential roles of TNFRI and TNFRII in PGRN-mediated fracture healing and OA (22,24,28). Although PGRN and TNF exhibit comparable binding affinity to TNFRI, the binding affinity of PGRN for TNFRII is ~600-fold higher than that of TNF (14). Since PGRN and TNF compete for binding to the same extracellular cysteine-rich domains (CRDs) of TNFR, CRD2 and CRD3 (8), PGRN acts as a naturally occurring antagonist of TNF and disturbs the binding of TNF to TNF receptors. More importantly, PGRN also acts as a ligand of TNFRII and directly activates the PGRN/TNFRII protective and antiinflammatory pathway. TNFRII has been shown to be critical for PGRN-mediated protection in OA. Injection of sTNFRII inhibits both TNF and PGRN. Furthermore, PGRN may be more inhibited than TNF, as the binding affinity of PGRN to TNFRII is much higher than that of TNF.

Unlike etanercept, mouse monoclonal antibody to TNF (infliximab) and humanized monoclonal antibody to TNF (adalimumab) are specific for TNF and have been shown to be protective against OA in animal models (30). The opposite effects of TNF-specific (i.e., infliximab and adalimumab) and nonspecific (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 TNF, PGRN, and their receptors in the pathogenesis of arthritis and bone remodeling, which not only will improve our understanding of TNF signaling in the pathogenesis of these musculoskeletal disorders but also may lead to innovative therapies via selective targeting of distinct TNFR pathways.

**TL1A–DR-3 interactions in arthritis and bone remodeling**

DR-3 (TNFRSF25, Apo-3, lymphocyte-associated receptor of death, TNFR-like molecule 3, TNFR-related apoptosis-mediating protein, WSL-1) was discovered simultaneously by multiple groups in the middle-to-late 1990s, when a combination of BLAST homology searches to Fas and TNFRI (32,33) and a yeast-2-hybrid library screening using a TNFRI death domain as bait (34) identified a closely related protein. Subsequently, DR-3 emerged as the closest structural homolog to TNFRI, containing an equivalent 4 CRDs as well as an intracellular death domain. However, unlike TNFRI, whose cellular distribution is widespread and whose surface expression can be controlled by the generation of soluble forms through cleavage, DR-3 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 remains unclear.

The identification of ligand(s) for DR-3 has been complicated by the number of potential candidates and their altering nomenclature (36), but prior to the discovery of PGRN, one TNFSF member, TL1A (TNFSF15) (37), had withstood stringent biochemical and functional scrutiny for DR-3 specificity (10,38). TL1A is the product of a longer alternative messenger RNA transcript for a protein initially named vascular endothelial growth
inhibitor (TL1), so named for its capacity to inhibit angiogenesis and induce apoptosis of endothelial cells (39). As its name and nomenclature suggest, TL1A is closely related in structure to TNF, encoding a type II transmembrane protein with a metalloproteinase cleavage site allowing release of a soluble molecule, but it also has distinct expression patterns as it is found in ng/ml concentrations in serum from healthy individuals (40), which suggests that it has physiologically different levels of production and functional regulation. In this regard, there may also be significant differences between species, as decoy receptor 3, the decoy ligand for LIGHT (TNF ligand superfamily member 14), TL1A, and FasL, is found only in humans and not in mice. It is in this context that the function of DR-3 and its potential for therapy should be interpreted.

The generation of transgenic mice genetically deficient for DR-3 or TL1A or overexpressing TL1A or dominant-negative forms of DR-3 has given rise to many in vivo studies describing the essential requirement for the DR-3/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 DR-3 and TL1A with human diseases ranging from inflammatory bowel disease (IBD) and primary biliary cirrhosis to leprosy (comprehensively reviewed in ref. 41). Of significance for this review were findings that suggested alternate respective ligands for DR-3 and TL1A. This included the apparent greater protection against experimental autoimmune encephalomyelitis afforded to DR-3−/− mice (38) compared to TL1A−/− mice (42) in otherwise similar models of disease and the DR-3–independent triggering of TNFRII expression by TL1A in kidney organ cultures (43). The underlying conclusion was that there were still unknown interactions for this complex of proteins, which would have to be discovered and dissected in detail before their full potential as therapeutic targets could be understood.

With specific regard to disorders of bone, initial genetic studies suggested that DR-3 gene duplication (44) and a mutation predicted to destabilize DR-3 (45) were linked to development of RA, while synovial cells from RA patients exhibited a hypermethylated DR-3 gene suggestive of activation (46); however, genome-wide association studies have had less success with supporting this connection. Two early investigations associated genetic variation around the DR-3 (TNFRSF25) locus with RA (47,48), 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 (49). Regardless, increased levels of TL1A have been reported in the serum of patients with both of these arthritides (40,50,51) as well as in the synovial tissue and synovial exudates of rheumatoid factor–positive RA patients (52,53).

The functional consequences of raised TL1A levels in these disorders have generally been associated with a range of outcomes that depend on the type and differentiation state of the DR-3–expressing cell to which TL1A is binding and signaling. In this review, we will cover those cell types specifically associated with bone physiology irrespective of the context of inflammation, although it should be noted that there may also be secondary effects as TL1A can induce TNF (54), thereby having the capacity to trigger a broad range of secondary effects associated with other proinflammatory cytokines. The DR-3/TL1A axis was first described as a T cell costimulator (37), but its effects on Th17 cells, which are drivers of osteoclastogenesis and therefore inflammatory bone resorption (55), highlighted the complexity in the outcome of TL1A signaling. Initial reports in TL1A−/− mice suggested that TL1A regulated Th17 cell differentiation (42), but more extensive in vitro studies in both DR-3−/− mice (56) and healthy human subjects indicated that Th17 cell differentiation from naive CD4+ T cells was impaired by TL1A, while maintenance of the response once T cells were committed to becoming Th17 cells was enhanced by TL1A (57). Intriguingly, recent studies have shown that TL1A-driven Th17 cell differentiation from naive CD4+ T cells occurs in samples from RA patients (51,58). Why these differences have been observed remains an area of debate, although the underlying theme is that TL1A promotes the Th17 cell response in RA.

The development of the main effectors of bone resorption, osteoclasts, is also regulated by the DR-3/TL1A axis, at least in a setting of inflammation. While osteoclastogenesis driven by macrophage colony-stimulating factor and RANKL was unaffected in DR-3−/− mice, these animals exhibited resistance to cartilage destruction and bone erosion in a model of antigen-induced arthritis (AIA) (10,59). Furthermore, DR-3−/− mice were resistant to exacerbation of disease induced by exogenous addition of TL1A to DR-3−/− animals, while antagonism of the pathway with anti-TL1A monoclonal antibody ameliorated disease in CIA (10). Addition of exogenous TL1A also exacerbated CIA (53). The direct nature of this signaling in myeloid cells has been demonstrated, with DR-3 expression being induced during the process of macrophage differentiation and TL1A signaling resulting in DR-3–dependent production of the gelatinase matrix metalloproteinase 9 (60). The DR-3/TL1A pathway may also control other aspects of
Both animal and human studies have demonstrated that PGRN-mediated anti-inflammatory and pro-tective activities in autoimmune diseases (66–68). Since PGRN deficiency caused a marked reduction in Treg cell numbers in the course of inflammatory arthritis (68). In a bone marrow chimera and CD4+CD45RB<sup>hi</sup> 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 interleukin-10 (IL-10) or TNFRII signaling (67). It is noted that PGRN-mediated regulation of Treg cells appears to be inflammation dependent, because PGRN deficiency does not alter the numbers of CD4+CD25+FoxP3+ Treg cells in vivo under physiologic conditions (68). PGRN inhibits expression and release of the chemokines CXCL9 and CXCL10 in a TNFRI-dependent manner in CD4+ T cells (66).

The DR-3 pathway may also contribute to PGRN-mediated protective effect in inflammatory diseases, since a recent study showed that agonistic antibody to DR-3 expanded CD4+FoxP3+ Treg cells in vivo, which in turn suppressed immune responses (70). In addition, a neuropathology develops with age in both DBA/2<sup>2</sup> and PGRN-deficient (72) mice. Intriguingly, transgenic overexpression of TL1A in both the myeloid and T cell lineage results in in vivo expansion of Treg cells, although these eventually become dys-regulated and intestinal inflammation develops (10).

In contrast to Treg cells, the frequency of Th17 cells was significantly decreased in spleens of mice treated with recombinant PGRN in a CIA model (67, 68). In addition, the serum IL-17 level was also significantly decreased in PGRN-treated mice. Further, both TNFRI and DR-3 pathways were found to be involved in PGRN inhibition of IL-17–producing cells. Taken together, PGRN and its Atstrin mimic appear to exert their anti-inflammatory activities through multiple pathways: 1) by activation of the PGRN/TNFRII protective pathway and...
Figure 2. Proposed model illustrating the multiple signaling pathways by which progranulin (PGRN) and its derivative Atsttrin exert their protective actions in autoimmunity. PGRN (or Atsttrin) binds to tumor necrosis factor receptor type II (TNFRII) and stimulates the formation and function of Treg cells, but may antagonize TNF-like molecule 1A (TL1A)/death receptor 3 (DR-3) signaling in these cells. PGRN (or Atsttrin) also antagonizes TNF/TNFRI and TL1A/DR-3 signaling and inhibits their inflammatory activities. RORγt = retinoic acid receptor-related orphan nuclear receptor γt.

Figure 3. A, Balance of tumor necrosis factor (TNF) and TNF-like molecule 1A (TL1A) and their antagonist progranulin (PGRN) in a healthy control. B, Dysbalance of proinflammatory TNF and TL1A and antiinflammatory PGRN due to overexpression of proinflammatory TNF and TL1A and diminished antagonistic effects of PGRN due to hyperphosphorylation of PGRN at Ser81 and induction of neutralizing antibodies to PGRN. TNF-R1/2 = TNF receptors type I and type II; DR-3 = death receptor 3.
2) by inhibition of TNF/TNFRI and TL1A/DR-3 inflammatory signaling (Figure 2).

**Clinical perspective**

Because TNF is one of the key main mediators of inflammation, 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 TNF antagonism by PGRN should be at least comparable to that of conventional TNF blockers (14). The additional specific inhibition of the TL1A–DR-3 interaction and the activation of the TNFRII antiinflammatory pathway by PGRN or its derivate (65) are unique characteristics and might represent a significant advantage over conventional TNF inhibitors, particularly for patients with refractory or relapsing disease who are taking conventional TNF blockers. Blocking the TL1A–DR-3 interaction probably offers....

| Key points | References |
|------------|------------|
| PGRN       | 14, 16, 17 |
| Also known as granulin-epithelin precursor, proepithelin, acrogranin, and GP88/PC cell-derived growth factor | |
| Autocrine growth factor with 593 amino acids | |
| Contains 7.5 repeats of a cysteine-rich motif | |
| Involved in embryogenesis, wound healing, countering inflammation, host defense, acting as neurotrophic factor | |
| High levels associated with several human cancers | |
| PGRN as ligand of TNFRI, TNFRII, and DR-3 | 14, 65, 68 |
| PGRN acts as ligand of TNFRI, TNFRII, and DR-3 and as physiologic antagonist of TNF, LTα, and TL1A | |
| Inhibits TNFRI and DR-3 pathways, but activates TNFRII pathway | |
| Binding affinity of PGRN for TNFRII is ~600-fold higher than that of TNF | |
| PGRN affinity for TNFRI, TNFRII, and DR-3 originates from granulins F, A, and C with linker regions | |
| Atsttrin is smallest recombinant derivate of PGRN and is synthesized from granulins F, A, and C and linker regions P3, P4, and P5 of PGRN with preserved antiinflammatory effect | |
| PGRN attenuates TNF-induced down-modulation of CD4+CD25highFoxP3+ Treg cells | |
| PGRN stimulates conversion of CD4+CD25− T cells into induced Treg cells | |
| PGRN, TNFRI, and TNFRII in OA | 30, 31 |
| Low PGRN levels yield spontaneous OA | |
| High PGRN levels yield anabolic function | |
| Catabolic effect of TNF is mainly mediated via TNFRI | |
| TNFRII pathway is both antiinflammatory and osteoprotective | |
| Administration of sTNFRII-Fc fusion protein neutralizes TNF and PGRN and leads to exacerbation of OA | |
| Administration of anti-TNF monoclonal antibodies neutralizes TNF specifically and ameliorates OA | |
| PGRN accounts for the opposite effects of sTNFRII-Fc fusion protein and anti-TNF monoclonal antibodies | |
| TL1A/DR-3 | 10, 51, 58, 59 |
| High levels of TL1A induce Th17 cell response in RA | |
| DR-3−/− mice are resistant to cartilage destruction in AIA | |
| CIA is exacerbated by TL1A and ameliorated by anti-TL1A monoclonal antibody | |
| TL1A/DR-3 activation induces MMP-9 and CCL3 | |
| Decoy receptor 3 decoy ligand for TL1A, FasL, and LIGHT is found only in humans and not in mice, making results from mouse models difficult to translate | |
| PGRN isoform hyperphosphorylated at Ser81 and anti-PGRN antibodies | 5, 75, 76 |
| Neutralizing antibodies directed against a binding region within the N-terminal 112 amino acids of PGRN | |
| occur frequently in various autoimmune diseases | |
| Anti-PGRN antibodies are induced by a second, transiently occurring PGRN isoform hyperphosphorylated at Ser81 | |
| PGRN isoform hyperphosphorylated at Ser81 lacks affinity for TNFRI, TNFRII, and DR-3 and thus antagonizes TNF and TL1A | |
| These phenomena result in dysbalance of proinflammatory TNF and TL1A and antiinflammatory functional PGRN in various inflammatory diseases | |

**References**

5, 75, 76

* PGRN = progranulin; TNFR = tumor necrosis factor receptor; DR-3 = death receptor 3; RA = rheumatoid arthritis; OA = osteoarthritis; SpA = spondyloarthritis; LTα = lymphotoxin α; TL1A = TNF-like molecule 1A; sTNFRII = soluble TNFRII; AIA = antigen-induced arthritis; CIA = collagen-induced arthritis; MMP-9 = matrix metalloproteinase 9; LIGHT = TNF ligand superfamily member 14; TNFRSF = TNFR superfamily.

*PGRN: progranulin; TNFR: tumor necrosis factor receptor; DR-3: death receptor 3; RA: rheumatoid arthritis; OA: osteoarthritis; SpA: spondyloarthritis; LTα: lymphotoxin α; TL1A: TNF-like molecule 1A; sTNFRII: soluble TNFRII; AIA: antigen-induced arthritis; CIA: collagen-induced arthritis; MMP-9: matrix metalloproteinase 9; LIGHT: TNF ligand superfamily member 14; TNFRSF: TNFR superfamily.
additional positive effects through reduction of proinflammatory cytokines, reduction of autoantibody formation, and reduction of osteoclastogenesis (10,53).

A potential disadvantage of PGRN or Atstrin compared to anti-TNF antibodies might be that anti-TNF antibodies can trigger apoptosis of proinflammatory T lymphocytes by binding to membranous TNF. This effect, which is also missing for TNFR-Fc fusion proteins, appears to play a particular role in inflammatory bowel diseases and less of a role in arthritis (73). The question is whether administration of PGRN or a derivative confers a higher risk of iatrogenic induced neoplasms than administration of conventional TNF blockers. Use of conventional TNF blockers results in an elevated risk of reactivating latent infections such as *Mycobacterium tuberculosis* or viral hepatitis, or of developing opportunistic infections (74). The effects of administered recombinant PGRN or its derivative on the risk of opportunistic infections remain a subject of speculation and are not discussed further in this review.

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

Epitope mapping identified a binding region within the N-terminal 112 amino acids of PGRN as a target of antibodies to PGRN in all patients. This means that autoantibodies to PGRN target the antiinflammatory PGRN and possibly cotarget only mature granulin G, the most N-terminal granulin motif. Despite the structural similarity of granulin G and the other 6 granulins, no binding was detected against granulin motifs other than granulin G (75). With regard to Atstrin, no antibodies have been detected so far that are directed against those parts of PGRN that are constitutive of Atstrin (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 Atstrin administration. To our knowledge, a potential binding of patient-derived, preexisting antibodies to PGRN against Atstrin itself has not yet been tested, and this possibility should be tested for and excluded.

As a reason for the breakdown of self-tolerance against PGRN, a second immunogenic PGRN isoform, hyperphosphorylated at Ser81, was identified exclusively in an anti-PGRN antibody–positive patient (76). This hyperphosphorylated PGRN is caused by inactivated protein phosphatase 1. Interestingly, phosphorylation of PGRN at Ser81 prevents interaction with TNFRI, TNFRII, and DR-3, so hyperphosphorylated PGRN has lost its antiinflammatory function. Considering these facts, it seems that a reasonable therapeutic strategy would be to compensate for the imbalance of pro- and antiinflammatory molecules due to lack of functional PGRN (caused by anti-PGRN antibodies or hyperphosphorylation of PGRN at Ser81) and/or excessive secretion of TNF and TL1A by administering a recombinant PGRN derivate that cannot be neutralized by preexisting autoantibodies to PGRN (Figure 3).

In conclusion, PGRN and its interaction with TNF/TNFRI/TNFRII and TL1A/DR-3 represent attractive new therapeutic targets (Table 2). When we consider the underlying theory and the known preclinical data, Atstrin could be a therapeutic alternative in cases of refractory or recurrent arthritis.

**AUTHOR CONTRIBUTIONS**

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published.

**REFERENCES**

1. Bossen C, Ingold K, Tardivel A, Bodmer JL, Gaide O, Hertig S, et al. Interactions of tumor necrosis factor (TNF) and TNF receptor family members in the mouse and human. J Biol Chem 2006;281:13964–71.
2. Espirito Santo AI, Ersek A, Freidin A, Feldmann M, Stoop AA, Horwood NJ. Selective inhibition of TNFR1 reduces osteoclast numbers as is differentiated from anti-TNF in a LPS-driven model of inflammatory bone loss. Biochem Biophys Res Commun 2015;464:1145–50.
3. Schling P, Rudolph C, Heimerl S, Fruth S, Schmitz G. Expression of tumor necrosis factor alpha and its receptors during cellular differentiation. Cytokine 2013;33:239–45.
4. Cerezo LA, Kuklova M, Hulejova H, Vernerova Z, Kasprikova N, Veigl D, et al. Progranulin is associated with disease activity in patients with rheumatoid arthritis. Mediators Inflamm 2015;2015:740357.
5. Thurner L, Zaks M, Preuss KD, Fadle N, Regitz E, Ong MF, et al. Progranulin antibodies entertain a proinflammatory environment in a subgroup of patients with psoriatic arthritis. Arthritis Res Ther 2013;15:R211.
6. Yamamoto Y, Takemura M, Serrero G, Hayashi J, Yue B, Tsuji A, et al. Increased serum GP88 (Progranulin) concentrations in rheumatoid arthritis. Inflammation 2014;37:1806–13.
7. Noguchi T, Ebina K, Hiroi M, Kawase R, Ohama T, Yamashita S, et al. Progranulin plays crucial roles in preserving bone mass by inhibiting TNF-α-induced osteoclastogenesis and promoting
osteoblastic differentiation in mice. Biochem Biophys Res Commun 2015;456:638–43.
8. Jian J, Zhao S, Tian Q, Gonzalez-Gugel E, Mundra JJ, Uddin SM, et al. Progranulin directly binds to the CRD2 and CRD3 of TNFR extracellular domains. FEBS Lett 2013;587:3428–36.
9. Siakavellas SI, Siikakos PP, Bamias G. The TL1A/DR3/DcR3 pathway in autoimmune rheumatic diseases. Semin Arthritis Rheum 2015;45:1–8.
10. Bull MJ, Williams AS, Mecklenburgh Z, Calder CJ, Twohig JP, Elford C, et al. The death receptor 3-TNF-like protein 1A pathway drives adverse bone pathology in inflammatory arthritis. J Exp Med 2008;205:2457–64.
11. Borsyenko CW, Garcia-Palacios V, Girisold RD, LI Y, Iyer AK, Yaroslavskiy BB, et al. Death receptor-3 mediates apoptosis in human osteoblasts under narrowly regulated conditions. J Cell Physiol 2006;209:1021–8.
12. Collins FL, Williams JO, Bloom AC, Stone MD, Choy E, Wang EC, et al. Death receptor 3 (TNFRSF25) increases mineral apposition by osteoblasts and region specific new bone formation in the axial skeleton of male DBA/1 mice. J Immunol Res 2015;2015:901679.
13. Harbail R, Chen Z, James S, Bennett HP, NI F. The hairpin stack fold, a novel protein architecture for a new family of protein growth factors. Nat Struct Biol 1996;3:747–52.
14. Tang W, Lu Y, Tian QY, Zhang Y, Guo FJ, Liu GY, et al. The growth factor progranulin binds to TNF receptors and is therapeutic against inflammatory arthritis in mice. Science 2011;332:478–84.
15. Tian Q, Zhao Y, Mundra JJ, Gonzalez-Gugel E, JIa J, Uddin SM, et al. Three TNFR-binding domains of PGRN act independently in inhibition of TNF-alpha binding and activity. Front Biosci (Landmark Ed) 2014;19:1176–85.
16. Liu CJ. Progranulin: a promising therapeutic target for rheumatoid arthritis. FEBS Lett 2011;585:3675–80.
17. Liu CJ, Bosch X. Progranulin: a growth factor, a novel TNFR family member. Cytokine Growth Factor Rev 2012;23:138–48.
18. Liu-Bryan R, Terkeltaub R. Emerging regulators of the inflammatory response in osteoarthritis. Curr Opin Rheumatol 2014;66:2657–60.
19. McCann FE, Porocheau DP, Ruspi G, Blazek K, Davies ML, Feldmann M, et al. Selective tumor necrosis factor receptor I blockade is antiinflammatory and reveals immunoregulatory role of tumor necrosis factor receptor II in collagen-induced arthritis. Arthritis Rheumatol 2014;66:2728–38.
20. Zhao YP, Tian QY, Liu B, Cuellar J, Richborough B, Jia TH, et al. Progranulin knockout accelerates intervertebral disc degeneration in aging mice. Sci Rep 2015;5:9102.
21. Olson SA, Furman BD, Kraus VB, Huebner JL, Gukil F. Therapeutic opportunities to prevent post-traumatic arthritis: lessons from the natural history of arthritis after articular fracture. J Orthop Res 2015;33:1266–77.
22. Zhang Q, Lv H, Chen A, Liu F, Wu X. Efficacy of infliximab in a rabbit model of osteoarthritis. Connect Tissue Res 2012;53:355–8.
23. Wei JL, Buza J III, Liu CJ. Does progranulin account for the opposite effects of etanercept and infliximab/adalimumab in osteoarthritis? J Orthop Res 2016;34:12–4.
24. Marsters SA, Sheridan JP, Donahue CJ, Piti RM, Gray CL, Goddard AD, et al. Apo-3, a new member of the tumor necrosis factor receptor family, contains a death domain and activates apoptosis and NF-kB. Curr Biol 1996;6:1699–706.
25. Screaton GR, Xu XN, Olsen AL, Cowper AE, Tan R, McMichael AJ, et al. LAF-1, a new lymphokine-inducible death domain containing receptor regulated by alternative pre-mRNA splicing. Proc Natl Acad Sci U S A 1997;94:4615–9.
26. Kitson J, Raven T, Jiang YP, Goeddel DV, Giles KM, Pun KT, et al. A death-domain-containing receptor that mediates apoptosis. Nature 1996;384:372–5.
27. Wang EC, Kitson J, Thern A, Williamson J, Farrow SN, Owen MJ. Genomic structure, expression, and chromosome mapping of the mouse homologue for the WSL-1 (DR3, Apo3, TRAMP, LARD, TR3, TNFRSF12) gene. Immunogenetics 2001;53:59–63.
28. Wang EC. On death receptor 3 and its ligands. Immunology 2012;137:114–6.
29. Migone TS, Zhang J, Luo X, Zhuang L, Chen C, Hu B, et al. TL1A is a TNF-like ligand for DR3 and TR6/Dr3 and functions as a T cell costimulator. Immunity 2002;16:479–92.
30. Meylan F, Davidson TS, Kahle E, Kinder M, Achariya K, Jankovic D, et al. The TNF-family receptor DR3 is essential for diverse T cell-mediated inflammatory diseases. Immunity 2008;29:79–89.
31. Yue TL, Ni J, Romanic AM, Gu JL, Keller P, Wang C, et al. TL1A, a novel tumor necrosis factor-like cytokine, induces apoptosis in endothelial cells: involvement of activation of stress protein kinases (stress-activated protein kinase and p38 mitogen-activated protein kinase) and caspase-3-like protease. J Biol Chem 1999;274:1479–86.
32. Bamias G, Siakavellas SI, Stamatopoulos KS, Chrysochouou E, Papamichael C, Siikakos PP. Circulating levels of TNF-like cytokine 1A (TL1A) and its decoy receptor 3 (DR3) in rheumatoid arthritis. Clin Immunol 2008;129:249–55.
33. Richard AC, Ferdinand JR, Meylan F, Hayes ET, Gabay O, Siegel RM. The TNF-family cytokine TL1A: from lymphocyte costimulator to disease con-sporator. J Leukoc Biol 2015;98:333–45.
34. Pappu BP, Borodovsky A, Zheng TS, Yang X, Wu P, Dong X, et al. TL1A-DR3 interaction regulates Th1 cell function and Th1-mediated autoimmune disease. J Exp Med 2008;205:1049–62.
35. Al-Lamki RS, Wang J, Tolkovsky AM, Bradley JA, Griffin JL, Thang S, et al. TL1A promotes and protects from renal inflammation and injury. J Am Soc Nephrol 2018;19:953–60.
36. Osaka K, Takami N, Shiozawa K, Hashiramoto A, Shiozawa S. Death receptor 3 (DR3) gene duplication in a chromosome region 1p36.3; gene duplication is more prevalent in rheumatoid arthritis. Genes Immun 2004;5:439–45.
37. Borsyenko CW, Furey WF, Blazek HC. Comparative modeling of TNFRSF25 (DR3) predicts receptor destabilization by a mutation linked to rheumatoid arthritis. Biochem Biophys Res Commun 2005;328:794–9.
46. Takami N, Osaka K, Miura Y, Komai K, Taniguchi M, Shiraiishi M, et al. Hypermethylated promoter region of DR3, the death receptor 3 gene, in rheumatoid arthritis synovial cells. Arthritis Rheum 2006;54:779–87.

47. Shiozawa S, Hayashi S, Tsukamoto Y, Goko H, Kawai H, Wada T, et al. Identification of the gene loci that predispose to rheumatoid arthritis. Int Immunol 1998;10:1891–5.

48. Cornelis F, Faure S, Martinez M, Prud'homme JF, Fritz P, Dib C, et al. New susceptibility locus for rheumatoid arthritis suggested by a genome-wide linkage study. Proc Natl Acad Sci U S A 1998; 95:10746–50.

49. Zinovieva E, Bourgain C, Kadi A, Letourneur F, Izac B, Said-Nahal R, et al. Comprehensive linkage and association analyses identify haplootypes, near to the TNFSF15 gene, significantly associated with spondyloarthritides. PLoS Genet 2009;5:e1000528.

50. Konsta M, Bamias G, Tektonidou MG, Christopoulos P, Shiozawa S, Hayashi S, Tsukamoto Y, Goko H, Kawasaki H, et al. New susceptibility locus for rheumatoid arthritis (RMA1) on chromosome 20q11. J Rheumatol 2014;41:581–9.

51. Xiu Z, Shen H, Tian Y, Xia L, Lu J. Serum and synovial fluid levels of tumor necrosis factor-like ligand 1A and decoy receptor 3 are increased in ankylosing spondylitis and rheumatoid arthritis. J Immunol 2007;178:7325–33.

52. Cassatella MA, Pereira-da-Silva G, Tinazzi I, Facchetti F, Scapini P, Calzetti F, et al. Soluble TNF-like cytokine (TL1A) production by immune complexes stimulated monocytes in rheumatoid arthritis. J Immunol 2007;181:5325–33.

53. Zhang J, Wang X, Fahmi H, Wojcik S, Fikes J, Yu Y, et al. Role of TL1A in the pathogenesis of rheumatoid arthritis. J Immunol 2009;183:5535–70.

54. Reichwald K, Jorgensen TZ, Tougaard P, Skov S. TL1A induces TCR independent IL-6 and TNF-α production and growth of PLZF− leukocytes. PloS One 2014;9:e85793.

55. Sato K, Suematsu A, Okamoto K, Yamaguchi A, Morishita Y, Kadono Y, et al. Th17 functions as an osteoclastogenic helper T cell subset that links T cell activation and bone destruction. J Exp Med 2006;203:2673–82.

56. Wang EC, Thern A, Denzel A, Kitson J, Farrow SN, Owen MJ. TNFR, DR-3, AND PGRN IN ARTHRITIS AND BONE REMODELING 2855

57. Wang EC, Thern A, Denzel A, Kitson J, Farrow SN, Owen MJ. Soluble TNF-like cytokine 1A in ankylosing spondylitis. Rheumatology (Oxford) 2013;52:448–51.

58. Wang EC, Thern A, Denzel A, Kitson J, Farrow SN, Owen MJ. Soluble TNF-like cytokine 1A in ankylosing spondylitis. Rheumatology (Oxford) 2013;52:448–51.

59. Zhang J, Wang X, Fahmi H, Wojcik S, Fikes J, Yu Y, et al. Role of TL1A in the pathogenesis of rheumatoid arthritis. J Immunol 2009;183:5535–70.

60. McLaren JE, Calder CJ, McSharry BP, Sexton K, Salter RC, et al. New susceptibility locus for rheumatoid arthritis suggested by a genome-wide linkage study. Proc Natl Acad Sci U S A 1998; 95:10746–50.

61. Van Lent PL, Hofkens W, Blom AB, Grevers L, Sloetjes A, McLaren JE, Calder CJ, McSharry BP, Sexton K, Salter RC, et al. New susceptibility locus for rheumatoid arthritis suggested by a genome-wide linkage study. Proc Natl Acad Sci U S A 1998; 95:10746–50.

62. Van Lent PL, Hofkens W, Blom AB, Grevers L, Sloetjes A, McLaren JE, Calder CJ, McSharry BP, Sexton K, Salter RC, et al. New susceptibility locus for rheumatoid arthritis suggested by a genome-wide linkage study. Proc Natl Acad Sci U S A 1998; 95:10746–50.

63. Bu R, Borysenko CW, Li Y, Cao L, Sabokbar A, Blair HC. Expression and function of TNF-family proteins and receptors in human osteoblasts. Bone 2003;33:760–70.

64. Lories RJ, Matthys P, de Vlam K, Derese I, Luyten FP. Ankylosing enthesitis, dactylitis, and onychoparathyrosis in male DBA/1 mice: a model of psoriatic arthritis. Ann Rheum Dis 2004;63:595–8.

65. Liu C, Li XX, Gao W, Liu W, Liu DS. Progranulin-derived Atstrin directly binds to TNFRSF25 (DR3) and inhibits TNF-like ligand 1A (TL1A) activity. PloS One 2014;9:e92743.

66. Munder H, Jian J, Bhagat P, Liu C. Progranulin inhibits expression and release of chemokines CXCL9 and CXCL10 in a TNFR1 dependent manner. Sci Rep 2016;6:21115.

67. Wei F, Zhang Y, Jian Y, Munda JJ, Tian Q, Lin J, et al. PGRN protects against colitis progression in mice in an IL-10 and TNFR2 dependent manner. Sci Rep 2014;4:7023.

68. Wei F, Zhang Y, Zhao W, Yu X, Liu C. Progranulin facilitates conversion and function of regulatory T cells under inflammatory conditions. PloS One 2014;9:e112110.

69. Thurner L, Stoger E, Fadle N, Klemm P, Regitz E, Kemele M, et al. Proinflammatory progranulin antibodies in inflammatory bowel diseases. Dig Dis Sci 2014;59:1733–42.

70. Wolf D, Schreiber TH, Tryphonopoulos P, Li S, Tzakis AG, Ruiz P, et al. Tregs expanded in vivo by TNFRSF25 agonists promote cardiac allograft survival. Transplantation 2012;94:569–74.

71. Twohig JP, Roberts MI, Gavalda N, Rees-Taylor EL, Giralt A, Adams D, et al. Age-dependent maintenance of motor control and corticospinal innervation by death receptor 3. J Neurosci 2010;30:3782–92.

72. Yin F, Dumont M, Banerjee R, Ma Y, Li H, Lin MT, et al. Mitochondrial deficits and progressive neuropathology in progranulin-deficient mice: a mouse model of frontotemporal dementia. FASEB J 2010;24:4639–47.

73. Van den Brakel JH, Braut H, van den Brink GR, Versteeg HH, Bauer CA, Hoedemaeker I, et al. Infliximab but not etanercept induces apoptosis in lamina propria T-lymphocytes from patients with Crohn’s disease. Gastroenterology 2003;124:1774–85.

74. Keane J, Gershon S, Wise RF, Mirabile-Levens E, Kassnica J, Schiermeier WD, et al. Tuberculosis associated with infliximab, a tumor necrosis factor α-neutralizing agent. N Engl J Med 2001;345:1098–104.

75. Thurner L, Preuss KD, Fadle N, Regitz E, Klemm P, Zaks M, et al. Progranulin antibodies in autoimmune diseases. J Autoimmun 2013;42:29–38.

76. Thurner L, Fadle N, Regitz E, Kemele M, Klemm P, Zaks M, et al. The molecular basis for development of proinflammatory autoantibodies to progranulin. J Autoimmun 2015;61:17–28.

77. Kitaura H, Kimura K, Ishida M, Kohara H, Yoshimatsu M, Takano-Yamamoto T. Immunological reaction in TNF-α-mediated osteoclast formation and bone resorption in vitro and in vivo. Clin Dev Immunol 2013;2013;181849.

78. Robinson LJ, Borysenko CW, Blair HC. Tumor necrosis factor family receptors regulating bone turnover: new observations in osteoblastic and osteoclastic cell lines. Ann N Y Acad Sci 2007; 1116:432–43.

79. Croft M, Benedict CA, Ware CF. Clinical targeting of the TNF and TNFR superfamilies. Nat Rev Drug Discov 2013;12:147–68.

80. Hashimoto H, Tanaka M, Suda T, Tomita T, Hayashida K, Takeuchi E, et al. Soluble fas ligand in the joints of patients with rheumatoid arthritis and osteoarthritis. Arthritis Rheum 1998;41:657–62.

81. Martinez-Lorenzo MJ, Ancl A, Saenz-Gutierrez B, Royo-Canas M, Bousque A, Alava MA, et al. Rheumatoid synovial fluid T cells are sensitive to APO2L/TRA1L. Clin Immunol 2007;122:28–40.

82. Wang L, Liu S, Zhao Y, Liu D, Liu Y, Chen C, et al. Osteoblast-induced osteoclast apoptosis by fas ligand/FAS pathway is required for maintenance of bone mass. Cell Death Differ 2015;22:1654–64.

83. Seidel MF, Herguijuela M, Forkert R, Otten U. Nerve growth and remodeling by death receptor 3. Arthritis Rheumatol 2014;66:2762–72.

84. Kitaura H, Kimura K, Ishida M, Kohara H, Yoshimatsu M, Takano-Yamamoto T. Immunological reaction in TNF-α-mediated osteoclast formation and bone resorption in vitro and in vivo. Clin Dev Immunol 2013;2013;181849.

85. Robinson LJ, Borysenko CW, Blair HC. Tumor necrosis factor family receptors regulating bone turnover: new observations in osteoblastic and osteoclastic cell lines. Ann N Y Acad Sci 2007; 1116:432–43.
triggered responses in the synovial fibroblasts of patients with rheumatoid arthritis. Arthritis Rheum 2011;63:904–13.

86. Colucci S, Brunetti G, Cantatore FP, Oranger A, Mori G, Piganti P, et al. The death receptor DR5 is involved in TRAIL-mediated human osteoclast apoptosis. Apoptosis 2007; 12:1623–32.

87. Xia WF, Jung JU, Shun C, Xiong S, Xiong L, Shi XM, et al. Swedish mutant APP suppresses osteoblast differentiation and causes osteoporotic deficit, which are ameliorated by N-acetyl-L-cysteine. J Bone Miner Res 2013;28:2122–35.

88. Wu H, Siegel RM. Progranulin resolves inflammation. Science 2011;332:427–8.