tion of this pathway during cardiac organogenesis. Most aspects of cardiac development were normal, but there was a factor of 2.5 increase in heart size. The massive growth was attributable to hyperplasia (cell number) and not hypertrophy (cell size), was evident by mid-gestation, and affected both the left and right ventricle, compartments whose developmental origins involve separate spatial fields, signals, and transcriptional mechanisms. Fibroblasts and smooth muscle cells in the heart were unaffected, as were cardiac progenitor cells, and no effect on apoptosis was seen. Ablation of Salv was inferred to affect cardiac muscle cells but not their precursors, and to involve cell division, not cell survival. And conditional deletions of Mst1/2 pathway kinases—either Mst1/2 or just Lats2 (mouse ortholog of Wrts)—resulted in similar myocardial expansion.

Heallen *et al*. determined that in the absence of Salv, numerous genes controlled by the transcription factor β-catenin had increased expression, reflecting increased activity of a signaling pathway controlled by Wnt family growth factors. Recently, cross-talk between Mst1/2 and canonical Wnt cascades was described in which phosphorylated Taz (a mouse relative of Yki) suppressed canonical Wnt signals by interfering with activation of Dishevelled (Dvl1), a component of the Wnt pathway (*13*). In mice genetically engineered to have decreased expression of both β-catenin and Salv, Heallen *et al*. observed that even partial reduction of β-catenin in cardiomyocytes rescued muscle wall thickness, proliferation rates, and the expression of β-catenin–dependent genes. They also noted association between β-catenin and nonphosphorylated Yap (which localizes to the nucleus) in the mouse embryonic heart, and that the complex associates with genes that control cell proliferation.

Repression of the growth-promoting Wnt signaling pathway by the Mst1/2 pathway raises questions about what combination of positive and negative signals tunes the timing of heart growth. It is also of interest to identify genes that are responsible for Mst1/2 function in the heart. Extrapolating from *Drosophila*, these potentially include orthologs of genes that encode the cell surface adhesion proteins Dachsous and Fat, and the cytoplasmic proteins Expanded, Merlin, and Kibra, which convey signals to Hpo. It will also be important to evaluate the binding partners of Yap—the cytoskeletal protein α-catenin (*14*), and in the fly, the membrane protein Crumbs (*15*). The precise β-catenin and Yap effector genes that hold greatest importance for cardiac muscle cell number also remain to be determined. A further question is whether the operation of interconnected Mst1/2-Wnt pathways impinges just on cardiac muscle cell division, or also imparts the irreversibility of cardiac cell cycle exit. In the newborn mouse heart, whereas regeneration remains briefly possible, do injury signals repress components of the proliferation-repressive Mst1/2 pathway? Conceivably, the Mst1/2 pathway might offer therapeutic opportunities for adult cardiac myocyte renewal.

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MEDICINE

Progranulin Resolves Inflammation

A molecule binds to a cytokine receptor and limits the cytokine from eliciting inflammatory responses by immune cells.

Hao Wu[1] and Richard M. Siegel[2]

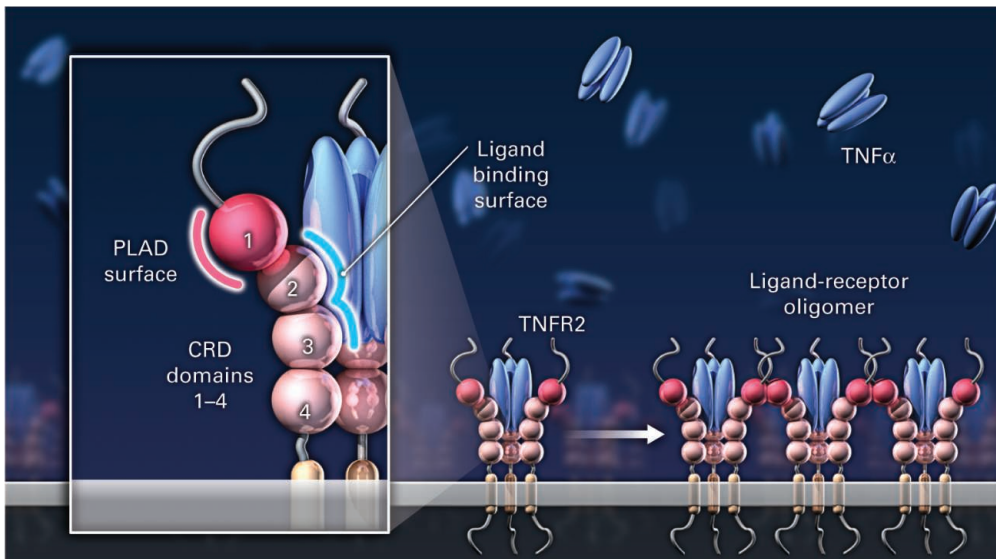
The cytokine tumor necrosis factor α (TNFα) is a major driver of rheumatoid arthritis and related inflammatory diseases. Therapeutic agents that block TNFα or mimic a soluble receptor for the cytokine are effective in these diseases, but whether other endogenous modulators of TNFα action exist that could be targets for therapeutic intervention is not clear. On page 478 of this issue, Tang *et al*. (*1*) show that progranulin, a secreted molecule with some cytokine-like properties, binds to TNF receptors and limits the action of TNFα in inflammatory arthritis.

The TNF family of cytokines in humans comprises 19 ligands, for which there are 35 receptors. TNFα is expressed mainly by endothelial and immune cells as a membrane protein that can be cleaved off the membrane by metalloproteinases. The cytokine acts through two receptors, TNFR1 and TNFR2. TNFR1 is broadly expressed and promotes an inflammatory response. TNFR2 is expressed predominantly by lymphocytes and stimulates lymphocyte activation. It also inhibits the development and function of "inducible" regulatory T cells (which suppress immune system activation) (*2*). Mouse models of inflammatory arthritis support a primary role for TNFR1, with some contribution by TNFR2, to the pathogenic role of TNFα (*3*, *4*).

Progranulin (PGRN), also known as proepithelin or acrogranin, can be proteolyzed into small homologous subunits called granulins (GRNs) or epithelins. PGRN was originally identified as an autocrine growth factor for cancer cells and fibroblasts. Mutations in the human gene encoding PGRN are associated with some cases of frontotemporal dementia, a neurodegenerative disease (*5*). Other clues suggested that PGRN plays a role in inflammation. PGRN inhibits, whereas GRNs stimulate, the production of neutrophil-attracting chemokines. Cleavage of PGRN is promoted by enzymes including elastase, which is secreted by neutrophils. These findings suggested a role for GRN in amplifying acute inflammation, and PGRN in the resolution of inflammation and wound repair (*6*). Mice lacking PGRN have no overt immunological phenotype, but PGRN-deficient macrophages challenged with microbial lipopolysaccharide (and other agonists of Toll-like receptors expressed by macrophages) had increased proinflammatory cytokine production (*7*). But the receptors through which PGRN mediated these anti-inflammatory effects were unknown.

Tang *et al*. isolated TNFR2 in a screen for PGRN binding partners. Recombinant

[1]Department of Biochemistry, Weill Cornell Medical College, New York, NY 10065, USA. [2]National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, MD 20814, USA. E-mail: siegelr@mail.nih.gov



Ligands	TNF family receptors	Interaction domain	Effect on TNF receptor signaling
TNF cytokines	All	CRD2, 3 (ligand binding surface)	Positive
Preligand association domain (PLAD)	TNFR1, TNFR2, and other	CRD1 (PLAD)	Positive with full-length receptors; negative with decoy or soluble receptors
PGRN	Primarily TNFR2; less so for TNFR1	?	Negative

TNF signaling. TNFα binds to TNFR2, which may lead to oligomerization of ligand-receptor complexes, helping to propagate signaling. Some regulators of TNF family receptor signaling are listed.

PGRN bound to TNFR1 and TNFR2 with nanomolar affinity and blocked interaction with TNFα. PGRN also inhibited inflammation in disease models that depend on TNFα. PGRN-deficient mice are hypersensitive to arthritis (induced by collagen), and treatment with PGRN reduced clinical and histological features of this disorder. In a model of systemic inflammation and arthritis (driven by human TNFα), PGRN-deficient mice developed more severe disease, and treatment with PGRN slowed arthritis progression.

The effects of PGRN may be complicated by its cleavage into GRNs. No individual GRN domain of PGRN bound to TNFR2, suggesting that the proinflammatory effects of GRNs may be mediated through other receptors. However, strong binding to TNFR2 was observed with a fusion of three partial GRN subunits. This fusion protein, called antagonist of TNF-TNFR signaling via targeting to TNF receptors (Atsttrin), exhibited more potent anti-inflammatory activity than PGRN, perhaps because it does not contain any complete GRN domains that have proinflammatory function.

Some aspects of how PGRN exerts its anti-inflammatory effects remain unclear. Tang *et al*. determined that the effects of Atsttrin in collagen-induced arthritis depend on TNFR2. However, PGRN deficiency also enhanced disease in mice that overexpress human TNFα, which is thought to interact solely with mouse TNFR1. Studies with mice lacking PGRN and one or both TNF receptors may resolve these discrepancies.

The extracellular regions of TNF receptors are elongated structures with multiple cysteine-rich repeat domains (CRDs). TNF family ligands bind to receptors in a heterohexameric 3:3 complex in which each receptor subunit contacts two adjacent ligand subunits (see the figure) typically via CRD2 and CRD3, the "stalk" of the receptor. By contrast, the opposite side of the amino-terminal CRD1 contains the preligand association domain (PLAD), which mediates homotypic interactions among receptor chains and may also stabilize and propagate ligand-receptor contacts, thus producing higher-order oligomers critical for receptor signaling (*8*–*11*). Although interactions between receptors through the PLAD positively influence signaling, binding of decoy receptors or engineered soluble receptor homologs to the PLAD disrupts signaling by the targeted receptor (*12*, *13*).

Because PGRN and Atsttrin competitively bind TNF receptors, but apparently not TNFα itself, and potently block TNF-TNFR interactions in vitro, these molecules likely interact with the ligand-binding interface at CDR2 and CDR3. PGRN and Atsttrin are composed of multiple disulfide-stabilized GRN domains with some structural similarity to CRDs. Given their potential elongated shape, PGRN and Atsttrin could also interact with a surface on the opposite side of the ligand binding site of the receptor, including the PLAD, to induce allosteric changes that hinder TNFα binding. In this case, the mechanisms of action could inhibit both ligand binding and receptor oligomerization. The surfaces of GRN domains of PGRN and the CRDs of TNF receptors contain numerous positive and negative charges, which may facilitate either of these interactions. Also, the mechanisms of inhibition by PGRN versus Atsttrin may differ, the latter harboring multiple exposed, free cysteines. Structural studies of PGRN and Atsttrin bound to TNF receptors should resolve these issues. The work by Tang *et al*. suggests that there may be other endogenous regulators that dampen the dangerous consequences of excessive signaling by TNF receptors.

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