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Immune dampening at the source

By Joanne Kotz, Senior Editor

Two U.S. academic groups have separately identified small molecule RAR-related orphan receptor inhibitors that block production of proinflammatory T helper type 17 cells and decrease disease severity in a mouse model of multiple sclerosis.[1,2] The researchers believe targeting T helper type 17 cells may provide increased efficacy over blocking only one cytokine produced by the cells, such as IL-17. Independently, **Merck & Co. Inc.** and **Lycera Corp.** announced a partnership last month to co-develop inhibitors for one of these receptors in autoimmune disease.

T helper type 17 (Th17) cells, which are part of the immune response to pathogens, have been previously linked with the development of autoimmune diseases,[3] and targeting IL-17 (IL-17A), the major cytokine produced by Th17 cells, has been identified as a strategy for treating autoimmunity. Indeed, there are at least three disclosed antibody and small molecule inhibitors of IL-17 in Phase II testing.

However, Th17 cells produce additional proinflammatory cytokines such as IL-22. Th17 cells also have cytokine-independent roles in promoting inflammation. Thus, targeting Th17 cells directly, rather than blocking individual cytokines, could potentially shut down inflammation more effectively.

The identification in 2006 and 2008 of two nuclear hormone receptors as key regulators of Th17 cell differentiation provided possible targets to do just that. One is RAR-related orphan receptor C (RORC; RORγ; RORγT), which is required for Th17 cell differentiation,[4] and the other, RAR-related orphan receptor A (RORA), cooperates with RORγT to mediate differentiation.[5]

A major difference between antagonizing RORs and blocking IL-17 is that the former approach depletes all of the cytokines produced by the proinflammatory Th17 cells, said Vijay Kuchroo, professor of neurology at **Harvard Medical School** and associate immunologist at **Brigham and Women's Hospital**.

The strategy is akin to the "difference between hitting one molecule made by a factory versus hitting the factory itself," said Kuchroo, who is also a cofounder of **Tempero Pharmaceuticals Inc.**, a company targeting Th17 cells for autoimmune disease. Tempero has not disclosed the specific targets it is attacking.

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> ***—Spiros Jamas, Tempero Pharmaceuticals Inc.***

RORing into the lead

Based on genetic evidence that blocking RORγT, either alone or together with RORA, could have therapeutic benefit in autoimmune diseases,[4,5] two academic teams set out to identify ROR antagonists.

One team, led by Dan Littman, conducted a cell-based screen of about 5,000 compounds and found that digoxin, a cardiac glycoside, antagonized RORγT activity.[1] Digoxin did not block RORA activity in the same assay.

Littman is a professor of pathology, molecular immunology and molecular pathogenesis at the **New York University School of Medicine** and an investigator with the **Howard Hughes Medical Institute**.

Another team, led by Thomas Burris, developed a compound with dual selectivity for RORγT and RORA.[2] The compound, SR1001, is an inverse agonist of the two targets.

Burris is a professor in the Department of Molecular Therapeutics at **Scripps Florida**.

In mouse $Cd4^+$ T cells cultured under conditions that promoted differentiation to Th17 cells, either digoxin or SR1001 decreased both Th17 cell differentiation and Il-17 expression compared with vehicle controls.

The reduction in Th17 cell differentiation *in vitro* translated to a therapeutic effect *in vivo*. In an experimental autoimmune encephalomyelitis (EAE) mouse model of MS, digoxin or SR1001 delayed disease onset and decreased disease severity compared with vehicle controls.

In both the Th17 cells and MS mouse model, SR1001 lowered expression of additional cytokines produced by Th17 cells, including IL-22, compared with vehicle.

Both studies were published in *Nature*.

The two groups are not alone in targeting RORs. Merck and partner Lycera plan to discover, develop and commercialize small molecules targeting RORγT for autoimmune diseases. The companies will focus on rheumatoid arthritis (RA), psoriasis, inflammatory bowel disease (IBD) and MS.

Both companies declined to comment for this article.

Balancing act

An open question is whether the increased immune suppression caused by ROR antagonists could pose safety risks. Fine-tuning selectivity for RORγT and RORA may be an important part of the equation.

"Approximately 85% of IL-17 production is under the control of RORγT, and only 15% is under the control of RORA. Therefore, targeting RORγT is likely to inhibit most of the IL-17 production," said Spiros Jamas, president and CEO of Tempero. "Since IL-17 controls important host defenses against bacteria and fungi, inhibiting all IL-17 production could be associated with an increased risk of infection. Targeting RORγT selectively may provide the right balance of inhibiting the proinflammatory effects of IL-17 while leaving beneficial host defenses intact," he added.

"In our studies, RORA does not appear to have a significant role in induction of IL-17. Moreover, RORA has important roles in the development of the nervous system. Therefore we think that an RORγT-

selective inhibitor will have full efficacy and increased safety compared with a dual RORγT/RORA inhibitor," added Littman.

"Since both RORA and RORγT are required for optimal Th17 cell differentiation, targeting both of these may offer some therapeutic advantage," Burris noted. "However, there may also be disadvantages such as a less attractive side-effect profile," he agreed.

According to Krzysztof Masternak, head of biology at **NovImmune S.A.**, the balance of efficacy and safety may vary between autoimmune diseases.

In terms of effector cytokines, antagonizing RORγT would result in a decrease of not only IL-17 levels but also IL-22 levels. In diseases like psoriasis or MS in which both IL-17 and IL-22 may contribute to pathology, RORγT antagonists could become a therapeutic alternative to IL-17 mAbs, said Masternak.

However, in the case of IBD, "IL-22 is one of the principal factors responsible for barrier integrity and innate immunity in the gut mucosa, and the cytokine plays a protective role in IBD. Here IL-17 mAbs could be a better therapeutic option," Masternak noted.

NovImmune's anti-IL-17 mAb, NI-1401, is exclusively licensed to the **Genentech Inc.** unit of **Roche** and is in preclinical development for autoimmune diseases.

Next steps

Both groups plan to move forward with optimization and preclinical testing of ROR-modulating molecules.

Digoxin is difficult to synthesize. Thus, in addition to digoxin, Littman's team has identified two other chemical classes of RORγT-selective antagonists. For the most promising class, the lead molecule "completely inhibits differentiation of human $CD4^+$ T cells from cord blood at submicromolar concentrations and is a good template for medicinal chemistry optimization," said Littman.

His group plans to test the efficacy of the compound in animal models of colitis, asthma and graft-versus-host disease (GvHD).

Littman also told *SciBX* that his group is close to reporting the as yet unidentified natural ligand for RORγT, which "could open up biosynthetic targets."

The NYU School of Medicine has filed patents covering RORγT modulators, and the compounds are available for licensing.

In addition to dual RORγT and RORA inverse agonists, the Scripps Florida team has identified RORγT-selective inverse agonists. "All of our scaffolds are chemically tractable, and most examples have good pharmacokinetic properties," said Burris.

Scripps Florida has filed for patents covering dual-selective and RORγT-selective compounds. The molecules are available for licensing, said Burris. "We are evaluating partnership and licensing opportunities as well as options for a new biotechnology company," he added.

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