Disrupting atherosclerosis

By Tim Fulmer, Senior Writer

A team of German and U.S. researchers has designed a peptide that decreases atherosclerotic lesions in mice by disrupting interactions between two platelet-derived chemokines.¹ The findings have led to the formation of **Carolus Therapeutics Inc.**, which is developing the peptide for cardiovascular and inflammatory disorders.

Atherosclerotic lesions and plaques occur as a result of a series of inflammatory processes on the walls of blood vessels.² In particular, adhesion of circulating leukocytes and platelets to inflamed arterial endothelial cells increases vessel permeability to plasma lipid components like plaque-forming low-density lipoprotein (LDL).

Over time, plaques accumulate, causing a narrowing of the vessel lumen. Occasionally, plaques rupture and release debris, triggering the formation of a thrombus, which can obstruct blood flow and result in myocardial infarction or stroke (see **Figure 1, “Targeting chemokines in cardiovascular disease”**).

Marketed atherosclerosis drugs, like statins, target plasma lipids and are thought to trigger anti-inflammatory effects via mechanisms that are not fully elucidated.

Recently, researchers have also begun to focus on the role that known inflammatory pathways play in atherosclerosis, and at least three biotech companies are running clinical trials of compounds that target proinflammatory chemokine receptors to treat the disease.

As described in *Nature Medicine*, a team led by Christian Weber, professor and director of the Institute for Cardiovascular Research at **Aachen University** and a researcher in the **Cardiovascular Research Institute Maastricht at Maastricht University**, took yet a different tack and opted to target the chemokines themselves.

The paper builds on previous work in which the same researchers showed that circulating platelets can deposit chemokine (C-C motif) ligand 5 (CCL5; RANTES) and platelet factor 4 (CXCL4; PF4) on arterial endothelial cells. There, the two chemokines form heterodimers that recruit leukocytes to the vessel wall and promote formation of atherosclerotic lesions.^{3,4}

In the *Nature Medicine* paper, the researchers set out to disrupt formation of CCL5-CXCL4 heterodimers in order to prevent recruitment of immune cells, slow or even arrest disease progression and hopefully avoid immunological side effects typical of targeting single chemokine receptors.

They initially used NMR spectroscopy and molecular dynamics simulations to generate a model of the heterodimer interaction that could guide

the design of a peptide antagonist. This led to the development of CKEY2, a peptide that competitively bound CCL5 with a K_d of 100 nM and prevented it from interacting with CXCL4 *in vitro*.

The researchers then designed a mouse peptide ortholog of CKEY2 called MKEY for *in vivo* testing. In apolipoprotein E-deficient mouse models of diet-induced atherosclerosis, intraperitoneal MKEY decreased atherosclerotic lesions in the aorta compared with the effect of scrambled control peptide.

To look for immunological side effects potentially associated with chemokine blockade, the researchers studied MKEY in nonatherosclerotic HSV-infected mice. They found that the peptide did not affect HSV-induced interferon- γ (IFN- γ) production or antigen-specific T cell proliferation. Nor did the compound impair clearance of the virus.

Thus, the team concluded that the findings “establish selective inhibition of CCL5-CXCL4 heteromerization as a feasible approach to counteracting atherosclerotic lesion progression.”

Weber told *SciBX* that “blocking chemokine ligand-ligand interactions potentially has a key therapeutic advantage over the more well-known approach of blocking chemokine receptor-ligand interactions. By targeting only the ligands, the normal functioning of the receptor remains intact during treatment, which could potentially avoid the side effects that often come with blocking a receptor that mediates multiple immunologically important processes.”

Weber’s strategy caught the eye of **Avalon Ventures**, which put up the seed capital last year to help Weber and colleagues found Carolus. The biotech holds an exclusive license to IP based on Weber’s research.

“Given the ubiquity of chemokine receptors, combined with the fact that each receptor typically binds an array of ligands, it’s probably not surprising that blocking such a receptor can trigger immunological side effects in addition to potentially reducing inflammation at the vessel wall,” said Joshua Schultz, VP of research at Carolus.

Easy does it

Biotech companies polled by *SciBX* wanted to see long-term safety and efficacy data for the ligand-targeting peptide in animal models of established cardiovascular disease.

“Weber and colleagues have identified what may be an example of a new layer of chemokine regulation in the CCL5-CXCL4 heterodimer interaction. Moreover, they show that targeting the interaction is possible and generates a therapeutic effect,” said Thomas Schall, president and CEO of chemokine therapeutic developer **ChemoCentryx Inc.** “A key next step will be demonstrating efficacy in animal models of established, well-progressed atherosclerosis.”

Such model studies are important, said Schall, because “treatment of atherosclerosis often begins when there is already significant vessel pathology. An ideal atherosclerosis therapeutic will thus not only halt disease progression but also help re-establish normal homeostatic mechanisms that ensure proper vessel function.”

Schall said it will also be important to look at the safety of chronic administration of the peptide antagonist. “Potential immunological side effects associated with long-term treatment should be monitored beyond

Figure 1. Targeting chemokines in cardiovascular disease. Researchers suggest that targeting chemokine-chemokine interactions could be an effective strategy for decreasing or preventing inflammation in cardiovascular disease. Atherosclerosis can develop when excess plasma lipids accumulate in arterial walls, causing the formation of lipid-rich plaques that hinder blood flow and increase the risk of heart attack and stroke. Proinflammatory adhesion of immune cells to the arterial wall mediates the development of plaques by the following mechanism:

[a] Circulating platelets become activated by multiple ligands in the plasma.

[b] Once activated, platelets deposit chemokines on the endothelial cells that comprise the surface of the arterial wall.

[c] Two of these chemokines—chemokine (C-C motif) ligand 5 (CCL5; RANTES) and platelet factor 4 (CXCL4; PF4)—form heterodimers that bind receptors on circulating monocytes, causing adhesion of the monocytes to the vessel wall.

[d] Once fixed to the vessel surface, the monocytes can migrate into the wall, where they transform into macrophages.

[e] Macrophages then ingest circulating low-density lipoprotein (LDL) that enters the damaged wall, causing lipid-rich foam cells to form.

[f] Over time, the foam cells mature into atherosclerotic plaques that contain a mixture of lipids, lipoprotein particles, macrophages and vascular smooth muscle cells (VSMCs).

In the *Nature Medicine* paper, the authors designed a peptide that blocks formation of chemokine heterodimers at step [c], potentially preventing the subsequent cascade in [d–f] from occurring.

the 12 weeks in the paper,” he told *SciBX*. “Cardiovascular disorders often require chronic treatment regimens that stretch out over many months if not longer. That can pose potential challenges for therapies targeting the immune system.”

“Any time you target chemokine pathways you have to consider the potential for malignancies and opportunistic infections that come with the immunosuppressive effects of this drug class,” said Sandra Palreja, EVP and CMO at **Tobira Therapeutics Inc.** “Moreover, the likelihood of infections occurring increases in aged populations, who would typically be receiving an atherosclerosis treatment.”

ChemoCentryx focuses on shorter treatment duration by modulating chemokine pathways to treat vascular restenosis rather than atherosclerosis. “The restenosis cascade starts quite early and is potentially treatable with [a] short-term, 28-day chemokine blockade, which reduces the potential for serious immune deficiencies,” said Schall.

The company’s CCX140, a chemokine (C-C motif) receptor 2 (CCR2) antagonist, is in Phase I testing to treat vascular restenosis, with plans for a Phase II trial to begin in 2H09, Schall told *SciBX*.

Tobira’s TBR-652, a chemokine (C-C motif) receptor 5 (CCR5) antagonist in-licensed from **Takeda Pharmaceutical Co. Ltd.**, is in Phase IIa testing to treat HIV. CCR5 mediates entry of the HIV virus into CD4⁺ T cells.

Palreja told *SciBX* that Tobira is interested in eventually studying the effects of TBR-652 on inflammatory and atherosclerosis endpoints in HIV-infected patients, who are often at risk of chronic cardiovascular inflammation. “In the meantime, the company plans to study the compound in preclinical models of inflammatory disorders, first in HIV-infected animals and then in a non-HIV setting,” she said.

Next steps

With the CCL5-CXCL4 interaction established, Weber is now interested

in looking for other ligand-ligand interactions that could play a role in inflammatory disorders. “The *Nature Medicine* paper may provide a general paradigm for targeting other chemokine-chemokine interactions in disease using rationally designed antagonists,” he said.

“As for the CCL5-CXCL4 peptide antagonist,” Weber added, “we’ve shown that it selectively blocks a specific interaction in the nanomolar range, and additional optimization of that compound is likely unnecessary.”

Indeed, Carolus is now looking at the peptide antagonist—designated CAT-2008—in a variety of cardiovascular and noncardiovascular inflammation models. “In the longer term, we hope to find a pharma partner to help move the peptide into a potential cardiovascular trial,” said Jay Lichter, cofounder, president and CEO of Carolus and managing director at Avalon.

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COMPANIES AND INSTITUTIONS MENTIONED

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