EBI2: the race is on

By Chris Cain, Staff Writer

A trio of pharma companies are converging on an autoimmune target called EBI2 and are starting to flesh out the orphan GPCR's role in disease. **GlaxoSmithKline plc** kicked off the action two months ago with the identification of a molecule that blocked receptor activity.¹ Now, separate teams from **Johnson & Johnson** and **Novartis AG** have identified an oxysterol as the natural ligand for EBI2.^{2,3}

The pharma companies have not disclosed their plans for G protein-coupled receptor 183 (GPR183; EBI2) and its ligand. However, the new data will allow them to test the therapeutic potential of modulating the receptor in disease models.

EBI2 was identified more than 15 years ago when researchers at **Harvard University** found that it was highly upregulated in Epstein-Barr virus (EBV)-infected lymphoma cells.⁴

In the last few years, additional lines of evidence have linked polymorphisms in or near the *EBI2* gene with type 1 diabetes and acute myelogenous leukemia (AML).^{5,6} Moreover, reports in 2009 showed EBI2 was required to localize B cells within lymphoid organs and produce a normal antibody response.^{7,8}

However, the precise link between EBI2 and disease has remained unclear.

To help determine EBI2's mechanistic connection to inflammation and disease, independent teams from J&J and Novartis sought to identify a ligand that could regulate EBI2's function *in vivo*.

Both turned to brute-force screening, using pig and sheep spleen extracts and biochemical fractionation to identify a compound that could bind and activate EBI2. They both identified an oxysterol derivative of cholesterol called $7\alpha,25$ -dihydroxycholesterol that activates the receptor at subnanomolar concentrations.

In cell culture, this oxysterol acted as a chemoattractant and stimulated the migration of B cells, T cells and dendritic cells. It had no effect in mouse cells lacking *Ebi2*.

To verify the role of the oxysterol in mice, the research teams each deleted a distinct enzyme required for its production and observed a phenotype similar to the one seen with *Ebi2* deletion.

Results were published in *Nature*.^{2,3}

Robert Brink, associate professor of immunology at the **Garvan Institute**, said that if this oxysterol is blocked, immune cells will not move toward the chemoattractant signal, thus potentially limiting inflammation. He added that, counterintuitively, an agonist of the receptor might induce a similar result because if the chemoattractant

signal appears to be everywhere, immune cells will not know where to go.

Brink led one of the two teams that first demonstrated EBI2's role in B cell migration.

GSK and collaborators at the **University of Copenhagen** are already pursuing molecules that dampen receptor activity. In June, the team reported the synthesis and *in vitro* characterization of an EBI2 blocker in *The Journal of Biological Chemistry*.¹

Moving toward models

Researchers now need to explore the therapeutic potential of targeting the GPCR in models of autoimmune disease.

"The key to answering whether EBI2 is functionally connected to disease is to inhibit the receptor or its ligand in rodent disease models,"

said Andreas Sailer, senior investigator at Novartis and senior author of one of the ligand identification studies. "We also want to look at patient samples to correlate ligand levels or expression of the receptor with disease states or disease progression, for example in patients with arthritis. For us it is very important to get the human disease connection."

Sailer said the role of EBI2 needs to be studied in many autoimmune disorders, including multiple sclerosis (MS), lupus and

rheumatoid arthritis (RA), but he did not say where Novartis will focus its efforts.

Brink's lab is continuing to study the role of EBI2 in mouse models of disease and also is looking to see whether EBI2 has functions in addition to directing cell migration.

Mette Rosenkilde, professor of neuroscience and pharmacology at the University of Copenhagen and senior author of the study characterizing GSK's EBI2 antagonist, is focusing on the role of EBI2 in the context in which it was originally discovered—EBV infection. "We don't really know why it is upregulated by the virus; it could be benefitting the virus, or the cell could be trying to protect itself from the virus," she said.

Peter Holst, associate professor of immunology, microbiology and international health at the University of Copenhagen and Rosenkilde's collaborator, added, "Whether EBI2 plays a role in EBV-associated pathology is completely unknown, but it is tempting to speculate so."

Rosenkilde will continue to follow up on cell culture data she published suggesting that EBI2 could be driving B cell proliferation, which could be part of the role it is playing during EBV infection. She is also working with Holst on using GSK's EBI2 blocker in mouse models of disease.

J&J and GSK declined to disclose their next steps or the patent status of their work. Novartis has filed a patent application covering its findings.

Cain, C. *SciBX* 4(32); doi:10.1038/scibx.2011.895
Published online Aug. 18, 2011

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