Waste management for lupus

By Lev Osherovich, Senior Writer

Systemic lupus erythematosus and other autoimmune diseases are usually viewed as disorders of acquired immunity marked by production of autoantibodies. Now, independent German and Swedish teams have found that components of the innate immune response—neutrophils and invariant NK T cells—contribute to SLE in mice and humans.

Turning the discoveries into therapies will require drilling down to specific targets that modulate the innate immune cells.

Both studies show that “everything starts with cell debris,” said Ann Rothstein, professor of rheumatology at the **University of Massachusetts Medical School**. Innate immune mechanisms ordinarily dispose of cell debris to prevent the acquired immune system from seeing self-antigens, but “failure to clear out the trash can lead to autoimmunity,” she said.

In the first study, researchers at the **Max Planck Institute for Infection Biology** found that debris-like protein-DNA complexes secreted by neutrophils to entrap bacterial pathogens also can trigger autoimmunity in certain SLE patients.¹

Separately, **Karolinska Institute** researchers uncovered a role for lipid-sensing invariant NK T (iNKT) cells in preventing autoantibody production.² The involvement of iNKT cells in B cell inhibition was a surprising finding and suggests that endogenous lipids found in cell debris may restrain autoimmunity.

Garbage strike

The Max Planck group, led by Institute for Infection Biology head Arturo Zychlinsky, tested how antimicrobial complexes called neutrophil extracellular traps (NETs) affected SLE. NETs consist of neutrophil DNA, chromatin proteins and antimicrobial peptides, and they are secreted by dying neutrophils as a last-ditch effort to ensnare and destroy pathogens. Excess NETs are usually degraded by nucleases.³

Because some SLE patients develop antibodies against NET components, Zychlinsky suspected these individuals might be unable to get rid of the surplus of NETs. To test this idea, the team developed an *in vitro* serum assay of NET degradation.

Whereas serum from most SLE patients underwent rapid NET degradation when treated with a nuclease, the serum from a subset of patients didn't respond at all.

Importantly, patients unable to degrade NETs had higher autoantibody titers and a greater incidence of renal complications due to SLE

than patients who could degrade NETs.

Results were reported in the *Proceedings of the National Academy of Sciences*.

Zychlinsky suspects that failing to degrade NETs creates a positive-feedback loop that causes more NET-derived autoantigens to pile up.

“We showed that 30% of patients we tested had problems with NET degradation, leading to a recurrent loop of autoantigen presentation,” Zychlinsky told *SciBX*.

Safety NET

The simplest idea for treating patients with extra NETs would be to increase nuclease levels. However, Zychlinsky's team has found that the serum of nondegrading patients contains a potent inhibitor of DNase I, the nuclease that ordinarily degrades NETs. The team hopes to biochemically isolate the inhibitor, which could be an autoantibody against the enzyme or against another protein altogether.

A second tactic could be to block the B cell-stimulating effect of NETs. For this approach, the missing puzzle piece is whether NETs act through toll-like receptors (TLRs), which help immune cells recognize pathogen-derived molecules.

Rothstein said that TLR7 and TLR9, which recognize extracellular DNA, are the “most likely to be engaged by NETs,” although this remains to be proven.

If NETs indeed stimulate autoimmunity through TLR7 and TLR9, antagonizing these receptors might be especially effective in patients with high NET levels.

Sudhir Agrawal, president, CEO and CSO of **Idera Pharmaceuticals Inc.**, suggested testing the effect of high NET levels on the production

of cytokines associated with TLR activation.

The DNA component of NETs “could activate TLRs and lead to engagement of B cells, which leads to activation of antibodies,” said Agrawal. “What's missing is the correlation between lack of NET degradation and cytokine induction.”

Idera's IMO-3100 TLR7 antagonist is in preclinical development to treat lupus.

Renee Martin, senior director of clinical and translational sciences at **Anthera Pharmaceuticals Inc.**, said that an alternative explanation for Zychlinsky's findings could be that sicker patients might have abnormally high neutrophil activity and thus more NETs.

“There's a huge spread in NET levels in the population” in Zychlinsky's study, said Martin. High NET levels “could be a signal of whether you've had a recent infection” and thus might not have much to do with the underlying disease.

Anthera's A-623, an antagonist of the B cell surface receptor BLYS (BAFF), is expected to start Phase II trials to treat SLE this year. The product is partnered with **Amgen Inc.**

The most advanced BLYS antagonist for SLE is Benlysta belimumab, an antibody from **Human Genome Sciences Inc.** and **GlaxoSmithKline plc**. The partners hope to submit a BLA and an MAA this quarter.

Although therapeutics that block NETs are far off, Zychlinsky thinks

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**—Ann Rothstein,
University of Massachusetts
Medical School**

monitoring NET degradation could help identify patients at risk of especially severe SLE.

"If we were able to diagnose a patient who is unable to degrade NETs, we could put this patient on a more aggressive immunotherapy" before renal complications appear, he said.

"It would be useful to follow patients over time to see if being unable to degrade NETs accelerates or increases risk" of renal disease, added Rothstein. "This would really validate NETs as a biomarker."

Zychlinsky has filed a patent on the *in vitro* NET degradation assay. The IP is available for licensing. His team is also testing whether NETs play a role in mouse models of SLE and whether getting rid of them can help prevent disease.

Oil spill

Meanwhile, a team led by Mikael Karlsson, associate professor of medicine at Karolinska, found that iNKT cells can suppress autoimmunity. iNKT cells are a type of helper T cell best known as innate immune sensors of lipid antigens. Karlsson's findings, reported in *The Journal of Experimental Medicine*, imply that endogenous lipids may tone down autoimmunity.

Starting from the prior observation that SLE patients have abnormally low levels of iNKT cells, Karlsson's team tested whether such cells were needed to prevent autoimmunity in a mouse model of SLE.

The group found that injecting debris from apoptotic cells into mice with defective iNKT cells raised autoantibody levels and caused renal damage compared with injecting debris into wild-type mice.

A series of adoptive transfer experiments showed that iNKT cells are likely to interact directly with B cells that express cluster of differentiation 1D (CD1D), which is a lipid-binding receptor. Indeed, mice with B cells that lacked Cd1d developed autoimmunity when injected with cell debris compared with mice that had Cd1d-expressing B cells. In contrast, Cd1d knockout mice implanted with B cells with intact Cd1d were resistant to autoimmunity compared with mock-treated controls.

"The novel finding here is that iNKT cells regulate autoantibody production by interacting with B cells directly," said Karlsson. "This is a new point of regulation of autoimmunity."

The finding suggests at least one potential route to treating autoimmunity: turning on iNKT cells to turn off autoantibody production

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by B cells.

"The idea that iNKT cells keep overenthusiastic autoreactive B cells in check makes this a very interesting therapeutic candidate," said Elizabeth Leadbetter, assistant member of the **Trudeau Institute**, an immunology research center. "If you can enhance their activity, you could bring back some of this inhibition. The question is how."

Mariolina Salio, clinical research fellow at the **University of Oxford**, suggested that "increasing iNKT cell numbers *ex vivo* followed by adoptive transfer could be a therapeutic approach worth exploring."

Leadbetter said that upping iNKT cell activity may also require raising levels of the endogenous lipid presented by CD1D. The catch is that the identity of that lipid is unknown.

"The exact mechanism involved in iNKT cell action is not completely clear," said Salio. "Which lipids do iNKT cells recognize following apoptotic cell injection?"

Karlsson said his team is now searching for the mystery lipid, which he thinks will be key to testing the therapeutic potential of iNKT cells. He has not filed for patents on the discoveries reported in the study.

Osherovich, L. *SciBX* 3(20); doi:10.1038/scibx.2010.606
Published online May 20, 2010

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COMPANIES AND INSTITUTIONS MENTIONED

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Anthera Pharmaceuticals Inc. (NASDAQ:ANTH), Hayward, Calif.
GlaxoSmithKline plc (LSE:GSK; NYSE:GSK), London, U.K.
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