Shockproofing against sepsis

By Kai-Jye Lou, Staff Writer

Activation of toll-like receptors by pathogens can trigger excessive release of proinflammatory cytokines that can progress to sepsis and septic shock. Researchers at the **Technical University Munich** now report they have stopped the cytokine storm before it begins in mice, using the simultaneous blockade of toll-like receptor 2 and toll-like receptor 4 to protect the mice against Gram-negative sepsis and septic shock.¹

Companies in the space now want to see additional *in vivo* data in models relevant to human sepsis and called for experiments to determine whether the strategy would work against other types of sepsis.

The toll-like receptor (TLR) system recognizes a broad range of pathogens and alerts the host immune system about impending infections. But some studies have linked excessive activation of certain TLRs to sepsis, and a handful of companies are developing compounds targeting either the TLRs or downstream proinflammatory cytokines in the TLR signaling pathway such as tumor necrosis factor- α (TNF- α).

As the German researchers reported in the *Journal of Experimental Medicine*, simultaneous treatment with mAbs against TLR2 and TLR4 protected against fatal shock in wild-type mice infected with *Salmonella enterica* or *Escherichia coli*, two common strains of Gram-negative bacteria.

In contrast, infected mice receiving either TLR2 or TLR4 antibodies separately, or no treatment altogether, died within 72 hours ($p < 0.004$).

Cell-culture studies showed that the dual blockade also lowered TNF- α release in murine macrophages and human peripheral blood mononuclear cells (hPBMCs) compared with that seen in untreated controls.¹

Monotherapy with a TLR4 antibody prior to *E. coli* challenge did block TNF- α release in hPBMCs. Although a TLR4 blockade thus protected against sepsis when given prior to infection, only the dual blockade offered protection after infection, as long as it was provided before the onset of full-blown sepsis.

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Kirschning, principal investigator on the paper and a private docent in the Institute of Medical Microbiology, Immunology, and Hygiene at Technical University Munich (TUM).

Inhibiting early signaling events "should therefore have broad action in dampening inflammation as well as consequential apoptosis and immunoparalysis seen at later stages of sepsis," said Greg Elson, a coauthor on the paper and section head of manufacturing and hybridoma technologies and project head of the TLR4 program at **NovImmune S.A.**

NovImmune's NI-0101, a human mAb targeting TLR4, is in preclinical development for an undisclosed indication.

As a next step, Elson suggested testing in rabbits.

"While mouse *in vivo* and human *in vitro* data have provided a strong rationale for evaluating TLR2- and TLR4-neutralizing mAbs in the clinic, the use of larger, more relevant laboratory species for *in vivo* sepsis studies would be beneficial," he said. Those studies would "clearly define a treatment window and hence target patient populations more likely to respond to such treatment."

Elson noted that rabbits have lipopolysaccharide (LPS) responses similar to humans and can be used to monitor key physiological parameters during inflammation.

NovImmune has used the rabbit model to demonstrate the efficacy of an undisclosed TLR4-neutralizing mAb in LPS-dependent ventilator-induced lung injury.

Yann Echelard, VP of research at **GTC Biotherapeutics Inc.**, also cautioned against using the mouse data to speculate on the potential effects of sepsis therapeutics in humans

because the results generally do not translate.

"Use baboon models or the human endotoxemia model," he said. "For sepsis, you really need a good set of immunological data."

GTC's ATryn, a recombinant antithrombin, is in Phase III testing for disseminated intravascular coagulation, which is a complication of severe sepsis. The product is partnered with **Leo Pharma A/S**.

Kirschning's research group is trying to create a line of transgenic mice that express human TLR2 antibodies. He agreed that existing mouse models of sepsis clearly have shortcomings and that there also is a need for a transgenic mouse line expressing both human TLR2 and TLR4.

Window of opportunity

In addition to the desire for studies with larger animals, other key questions from sepsis companies include how early in the cascade the dual TLR antagonist approach would need to be used and which subsets of sepsis to pursue.

Jim Glover, director of scientific affairs at **Protherics plc**, noted that "a product targeting TLRs must be used promptly before the sepsis cascade has progressed too far. Thus there may be only a small treatment window in the early stages of sepsis."

Protherics' CytoFab, a TNF- α -neutralizing sheep polyclonal Fab antibody fragment, is in Phase II testing to treat severe sepsis. The compound is partnered with **AstraZeneca plc**.

Kirschning agreed with Glover and thinks it will be important to administer the dual blockade either prior to or during treatment with antibiotics.

"It should not be used after antibiotics are applied," he said, because the dual blockade becomes less effective if the inflammatory cascade is already triggered.¹

Kirschning noted that antibiotics themselves can trigger a proinflammatory reaction, as some bacteria respond to antibiotics with a rapid release of so-called pathogen-associated molecular patterns (PAMPs), bacterial factors that in turn activate TLRs and accelerate the progression to sepsis and shock. This effect is known as the Jarisch-Herxheimer reaction.²

At the same time, he added, the strategy "should not be used without antibiotics." Kirschning cited his own data showing that the dual blockade significantly accelerated disease progression compared with what was seen in controls without antibiotic treatment ($p < 0.004$).¹

In the clinical setting, both Glover and Kirschning think the combination of TLR2 and TLR4 inhibitors would primarily be used with antibiotics and would be focused on preventing sepsis and shock in at-risk individuals, such as patients recovering from surgery.

Elson was more cautious about predicting treatment protocols for humans based on the mouse data. His reservations are based on a recently published paper in *Science* showing that Myeloid differentiation primary response gene 88 (MYD88)-deficient children were not severely immunocompromised and normally were resistant to most common bacteria, viruses, fungi and parasites. By contrast, MyD88-deficient mice were vulnerable to almost all tested pathogens.³

The paper cited other studies showing MyD88-deficiency results in abolished TLR4 and a greatly lowered TLR2 response.

Another open question, said Glover, is whether "a product targeting a particular TLR, like TLR4, is only likely to be effective against certain types of infections, like Gram-negative infections. Many infections that cause sepsis are fungal in origin."

Indeed, various pathogens that activate different TLRs can set off the proinflammatory cascade that leads to sepsis.⁴⁻⁶ TLR2 is typically activated by Gram-positive bacteria whereas TLR4 is activated by Gram-negative bacteria.

For TLR2 and TLR4 antagonists, Echelard said that the "narrow mechanism of action may mean that these types of agents may apply to small subsets of patients."

Kirschning disagreed. "Gram-negative bacteria still contribute to a large proportion of septic shock cases, with *E. coli* being the primary culprit," he said.

Building more blocks

Although Kirschning and Elson acknowledged the protective effects

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of the dual TLR blockade in the *JEM* paper centered on Gram-negative bacteria, they think the general methodology of blocking TLRs and other pattern recognition receptors (PRRs) should be applicable to additional types of sepsis.

"It seems reasonable that other PRRs are working along with TLR2 in the recognition of bacteria," Elson told *SciBX*.

Kirschning said his group is searching for PRRs relevant to protecting against Gram-positive sepsis and studying the effects of TLR2 and TLR4 blockade in the later phases of the

cascade.

Elson noted that "future clinical strategies may include combining anti-TLR therapies with targets of late mediators of sepsis such as MIF [macrophage migration inhibitory factor] and HGMB1 [high-mobility group box 1]."

"I don't think there is any question that there will be a synergy between MIF inhibition and the TLR blockade," said Vidal de la Cruz, VP of business development at **Cytokine PharmaSciences Inc.**

The company has a small molecule MIF inhibitor in preclinical development. The company has yet to finalize an indication.

Cruz noted that MIF is known to upregulate TLR4 expression and cited research showing how blocking MIF activity protects against a broad range of conditions, including sepsis.⁷ He also noted that numerous human case studies have associated high levels of MIF with higher and accelerated mortality in sepsis patients.^{8,9}

The work reported in *JEM* used the TLR2 blocking mAb T2.5. **Amgen Inc.** and TUM share ownership of patents to the mAb and its derivatives, according to the company. The compounds currently are available for licensing through the Service Centre for Research Support & Technology Transfer at TUM.

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COMPANIES AND INSTITUTIONS MENTIONED

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AstraZeneca plc (LSE:AZN; NYSE:AZN), London, U.K.
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GTC Biotherapeutics Inc. (NASDAQ:GTCB), Framingham, Mass.
Leo Pharma A/S, Ballerup, Denmark
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