Evasin invasion

By Kai-Jye Lou, Staff Writer

Researchers at **Merck KGaA** have found that ticks, parasites better known as transmission vectors for diseases such as Lyme disease, may be a source of therapeutically useful molecules. They have identified several selective chemokine-binding proteins identified in tick saliva, dubbed evasins, that have demonstrated anti-inflammatory properties in mice.¹

Merck is hoping evasins' chemokine multiselectivity and potential for low immunogenicity will be key advantages over existing chemokine-targeting molecules. Although the company has preclinical programs evaluating the compounds, other inflammation researchers polled by *SciBX* feel that more data on pharmacokinetics and immunology are needed before proceeding into the clinic.

Chemokines are small secreted proteins that direct the movement of leukocytes to sites of injury or inflammation. Aberrant signaling to various chemokine receptors is associated with a range of inflammatory and autoimmune conditions, from acute antigen- or ischemia-induced inflammation to chronic diseases like atherosclerosis, multiple sclerosis (MS) and rheumatoid arthritis (RA).^{2,3} As a result, human chemokines and their receptors constitute an attractive class of therapeutic targets (see **Table 1, "All about evasins"**).

As published in the *Journal of Experimental Medicine*, a group at the

Merck Serono Geneva Research Center assessed the binding properties of three tick-derived chemokine-binding proteins: evasin-1, evasin-3 and evasin-4. The group was led by Amanda Proudfoot, principal scientist of immunology and head of chemokine biology for the center's drug development programs.

Protein-binding assays and surface plasmon resonance studies showed that evasin-1 inhibited chemokine (C-C motif) ligand 3 (CCL3), CCL4 and CCL18 activity, whereas evasin-3 inhibited chemokine (C-X-C motif) ligand 1 (CXCL1) and CXCL8. Evasin-4 was shown to bind to CCL5 and CCL11.

In *in vitro* assays and mouse studies, the evasins inhibited chemokine-mediated neutrophil recruitment. For example, evasin-1 lowered inflammation in mouse models of psoriasis and lung injury, and evasin-3 inhibited inflammation in a mouse model of arthritis.

"Our study was based on the assumption that ticks have designed proteins to be immunologically silent since they do not elicit an immune response during their feeding period," said Proudfoot. Indeed, ticks can feed for long periods of time without eliciting an immune response from the host.¹

Proudfoot said Merck has preclinical programs evaluating evasin-1, evasin-3 and evasin-4 to treat inflammatory diseases. The company also is now studying the anti-inflammatory properties of evasin-4, which were not characterized in the *JEM* paper.

Evasin advantage

Although the data in the *JEM* article demonstrate the efficacy of evasins across multiple mouse models of inflammation, other researchers wanted to see additional pharmacokinetic and safety data, plus more comprehensive immunogenicity data.

Table 1. All about evasins. Merck KGaA (Xtera:MRK) thinks its newly discovered evasins hit multiple chemokines at once. Below are selected compounds for autoimmune and inflammatory conditions that target chemokines and chemokine receptors affected by evasins, including chemokine (C-C motif) receptor 1 (CCR1), CCR3, CCR5, chemokine (C-X-C motif) receptor 2 (CXCR2), eotaxin-1 and IL-8.

Company	Product	Description	Indication	Status
Dompé Farmaceutici S.p.A.	Reparixin	IL-8 antagonist	Renal transplant rejection	Phase II
Pfizer Inc. (NYSE:PFE)	Maraviroc	CCR5 antagonist	Rheumatoid arthritis (RA)	Phase II
Pharmacopeia Inc. (NASDAQ:PCOP)/ Schering-Plough Corp. (NYSE:SGP)	PS291822 (SCH 527123)	CXCR2 antagonist	Asthma; chronic obstructive pulmonary disease (COPD)	Phase II
Topigen Pharmaceuticals Inc.	TPI ASM8	Dual modified phosphorothioate antisense oligonucleotides that downregulate the expression of CCR3 and the common β -chains of the receptors for IL-3, IL-5 and granulocyte/macrophage colony-stimulating factor (GM-CSF)	Asthma	Phase II
iCo Therapeutics Inc. (TSX-V:ICO)/ MedImmune Inc. unit of AstraZeneca plc (LSE:AZN; NYSE:AZN)	Bertilimumab (iCo-008; CAT-213)	Human mAb against eotaxin-1	Vernal keratoconjunctivitis	To start Phase II
Genmab A/S (CSE:GSN)	HuMax-IL8	Human antibody targeting IL-8	Palmoplantar pustulosis	Phase I/II
ChemoCentryx Inc./GlaxoSmithKline plc (LSE:GSK; NYSE:GSK)	CCX354	Small molecule inhibitor of CCR1	RA	Phase I

Amgen Inc., which has multiple marketed drugs for autoimmune and inflammatory indications, said it was too early to determine whether evasins have therapeutic value.

"We find evasins to be an interesting family of molecules, scientifically intriguing, but still very poorly understood in terms of how they actually work," Tomas Mustelin, VP of research, inflammation, told *SciBX*.

Philip Murphy, chief of the Laboratory of Molecular Immunology at the NIH's **National Institute of Allergy and Infectious Diseases**, suggested that "the main attraction to these compounds is that they seem to already play a role in preventing inflammation from ticks." The problem, he said, is that "we don't really know that these are the factors that prevent inflammation when ticks attach to the host."

Murphy pointed out that there may be other compounds in tick saliva that allow the parasites to feed undetected by the host immune system.

However, he noted that compounds that have multiple targets, such as evasins, hold more therapeutic promise than single-target compounds. "What's needed are compounds that are multispecific, and in that light, that's exactly what these evasins provide."

Proudfoot agreed, adding that "the main advantage of the evasins over strategies such as neutralizing antibodies is that they target not only one ligand, but two or three, whilst not interfering with chemokines that recruit other cell types" like viral chemokine-binding proteins.^{4,5}

Proudfoot also noted that the small size of evasins should confer a solubility advantage over antibodies and larger therapeutic proteins.

A potential drawback of multitargeted compounds is that activity against individual targets occurs in a fixed ratio. This can complicate dose adjustment in cases where activity against a single target needs to be changed.⁶

Evaluating immunogenicity

Given the broad range of potential applications for evasins, a clear question is which indications would be good starting points.

"The immune system is very good at recognizing foreign proteins, and evasins are tick proteins, not human proteins," Murphy told *SciBX*. Thus, he said, long-term evasin therapy could elicit neutralizing antibodies.

"One can still imagine acute settings where evasins would be useful—where the specific immune response would not come into play,"

Murphy added. For example, he said the molecules could be used to prevent inflammation that occurs during and shortly following cardiac revascularization procedures.

Proudfoot said it remains to be seen whether the body will mount a specific immune response against evasins after prolonged exposure. In any case, she argued that host neutralization of evasins "would not be life-threatening, since at worst the inhibitory properties would be lost."

Going forward, Murphy thinks Proudfoot's group "needs to do the basic homework of finding out if evasins can be delivered in animal models in a manner that is efficacious." Regarding safety, he wanted to see whether using evasins would lead to undesirable immunosuppression. "From there, I think it would be on to the clinic," he said.

Proudfoot said the ideal delivery route and dosing frequency of evasins still need to be determined. Other steps, she said, could include half-life extension and further protein optimization.

"Lastly we will focus on the question of immunogenicity, using *in silico*, *in vitro* and *in vivo* approaches," she said. "The only definitive answer will be obtained in the clinic."

Merck KGaA's **Merck Serono S.A.** unit has multiple patents pending for the use of evasins to treat inflammatory diseases. The compounds are unavailable for licensing.

REFERENCES

1. Deruaz, M. *et al. J. Exp. Med.*; published online Aug. 4, 2008; doi:10.1084/jem.20072689
Contact: Amanda E.I. Proudfoot, Merck Serono Geneva Research Centre, Geneva, Switzerland
e-mail: amanda.proudfoot@merckserono.net
Contact: Christine A. Power, same affiliation as above
e-mail: christine.power@merckserono.net
2. Charo, I.F. & Ransohoff, R.M. *N. Engl. J. Med.* **354**, 610–621 (2006)
3. Luster, A.D. *et al. Nat. Immunol.* **6**, 1182–1190 (2005)
4. Lucas, A. & McFadden, G. *J. Immunol.* **173**, 4765–4774 (2004)
5. Alcamí, A. *Nat. Rev. Immunol.* **3**, 36–50 (2003)
6. Lou, K.-J. *SciBX* **1**(22), 9–10; June 26, 2008

COMPANIES AND INSTITUTIONS MENTIONED

Amgen Inc. (NASDAQ:AMGN), Thousand Oaks, Calif.
Merck KGaA (Xetra:MRK), Darmstadt, Germany
Merck Serono S.A., Geneva, Switzerland
National Institute of Allergy and Infectious Diseases, Bethesda, Md.
National Institutes of Health, Bethesda, Md.