TLR4 signaling in allergic asthma

By Lauren Martz, Staff Writer

Baylor College of Medicine researchers have uncovered a pathway in allergic asthma that opens up the potential for developing therapeutics that target the causes of the disease rather than just its symptoms.[1] The team now has to show that the pathway, which involves degradation of the blood clotting protein fibrinogen by fungal allergens to cleavage products that activate toll-like receptor 4, also applies to other allergens.

Allergic asthma involves a magnified immune response to airway allergens that results in airway hyperresponsiveness, inflammation and constriction. Standard of care includes corticosteroids for general immune suppression and a combination of short- and long-acting bronchodilators to relax the airway smooth muscles and decrease constriction.

All standard-of-care drugs treat symptoms resulting from the inflammatory reaction rather than the source of the disease. Moreover, corticosteroids can cause increased susceptibility to infection, and bronchodilators can induce increased heart rates and hyperactivity.

Targeting the underlying causes of asthma has been hampered by an incomplete understanding of the mechanisms linking allergen exposure to the inflammatory reaction in the lungs.

Previous work by David Corry and Farrah Kheradmand at Baylor and by groups elsewhere has shown that proteinases from common allergens induce an adaptive immune response involving proinflammatory T helper type 2 (Th2) cell activation and production of proinflammatory cytokines.[2]

Corry is director of the Biology of Inflammation Center, chair in immunology, chief of immunology, allergy and rheumatology and a professor of medicine and of pathology and immunology at Baylor. Kheradmand is professor of medicine and immunology at Baylor.

One strategy to prevent Th2 cell activation is to agonize certain toll-like receptors (TLRs) including TLR7 and TLR9. Activating these receptors on dendritic cells polarizes naïve helper T cells to the Th1 subtype, thus decreasing the Th2 subtype that contributes to asthma.

There are at least six TLR7 or TLR9 agonists in clinical and preclinical development to treat asthma (*see* **Table 1, "TLR9 and TLR7 agonists in development for asthma"**).

Previous studies have found that another TLR, TLR4, may also play a role in asthma,[3] although the mechanism underlying TLR4 activation in the disease has remained elusive.

Now, Corry, Kheradmand and colleagues have established a mechanistic link for the allergen-triggered activation of TLR4 and identified new therapeutic targets within the pathway to treat allergic asthma.

In mice, knockout of *Tlr4* prevented allergic airway disease induced either by a fungal proteinase from *Aspergillus oryzae* or the proteinase-free allergen ovalbumin.

In mouse bone marrow–derived macrophages, knockout of *Tlr4* or its adaptor proteins *myeloid differentiation primary response gene 88* (*Myd88*) and *toll-like receptor adaptor molecule 1* (*Ticam1*; *Trif*) prevented the antifungal response following stimulation with the *A. oryzae* proteinase, whereas wild-type expression or knockout of only one of the adaptor molecules did not.

Interestingly, in the cell experiments the researchers noticed that serum was required for the antifungal response, which led them to hypothesize that some serum factor was required for the TLR4-dependent antifungal response.

Previous work had shown that one such serum factor, fibrinogen, activates TLR4 *in vitro*.[4] Indeed, the team determined that culture of the macrophages with fibrinogen and the allergic proteinases alone was sufficient to produce the antifungal response.

More precisely, fibrinogen cleavage products produced by the proteinases from fungal allergens or the airway itself triggered the antifungal response (*see* **Figure 1, "Proteinase and nonproteinase allergen–induced inflammation"**).

Wild-type mice treated with fibrinogen cleavage products formed by proteinases but not the allergic proteinases themselves exhibited an innate immune cell response that included TLR4 activation, but they did not show asthma symptoms. Asthma-associated Th2 cell activation did not occur, suggesting that proteinases independently activate the fibrinogen-TLR4 pathway and a Th2 cell response and that activation of both is required for asthma development.

In mice, a proteinase inhibitor blocked development of asthma symptoms caused by the *A. oryzae* proteinase or proteinase-free ovalbumin, suggesting that proteinase-free allergens must activate endogenous airway proteinases to induce allergic inflammation.

Results were published in *Science*. The team included researchers from the **Michael E. DeBakey VA Medical Center** and **The University of Texas Health Science Center at Houston**.

"This *Science* paper unravels a clear link between the proteinase activity of certain allergens and activation of TLR4 through proteolytic cleavage of fibrinogen. These components were known to be linked with allergic inflammation, but until now we didn't know how they fit together," said Philippe Saudan, VP of research at **Cytos Biotechnology AG**.

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Table 1. TLR9 and TLR7 agonists in development for asthma.

Company	Product	Description	Status
Cytos Biotechnology AG (SIX:CYTN)	CYT003	Virus-like particle containing an oligonucleotide agonist of toll-like receptor 9 (TLR9)	Phase II
GlaxoSmithKline plc (LSE:GSK; NYSE:GSK)	GSK2245035	TLR7 agonist	Phase II
AstraZeneca plc (LSE:AZN; NYSE:AZN)/**Dainippon Sumitomo Pharma Co. Ltd.** (Tokyo:4506; Osaka:4506)	AZD8848	Inhaled TLR7 agonist	Phase I
Idera Pharmaceuticals Inc. (NASDAQ:IDRA)	IMO-2134	TLR9 agonist	Phase I
Dynavax Technologies Corp. (NASDAQ:DVAX)/AstraZeneca	AZD1419	TLR9 agonist	Preclinical
InDex Pharmaceuticals AB	DIMS 9054	DNA-based immunomodulatory sequence (DIMS) binding to TLR9	Preclinical

Hirohito Kita, a professor of pulmonary medicine, medicine and immunology at the **Mayo Clinic**, added, "This work addresses a very fundamental question that people are trying to answer. Researchers want to know how allergens cause the immune system to pathologically activate. This is the first evidence of this pathway involvement, and I expect that similar studies are likely to come up now."

Common allergens

The Baylor group now needs to show that the mechanism applies to more common asthma-causing allergens than those from fungi.

"Fungal infections generally cause complications in asthma patients but are not the primary cause, so it is important to prove that this mechanism more generally applies to other aero allergens. These results are convincing, but we have to keep in mind that this is just a model system," noted Saudan.

Cytos' CYT003, a TLR9 agonist that blocks Th2 cells, is in Phase IIb testing to treat moderate to severe allergic asthma not sufficiently controlled by standard therapy.

Kita added that "many other allergens are active in causing allergic asthma such as dust mites, pollen, cats and cockroaches. They will need to determine whether these other allergens are using the same or similar TLR4-dependent mechanisms or if they induce an allergic response by signaling through different receptors, in which case treatments interfering with the fibrinogen-TLR4 pathway would have limited effect for most asthma patients."

Corry expects the results will indeed be broadly applicable. He said the team "tested three distinct allergens: the fungus *A. niger*, a proteinase derived from *A. oryzae* and chicken egg ovalbumin. All induced allergic disease through the same fibrinogen-TLR4 pathway despite being different biochemically. We therefore believe most, if not all, allergic reactions depend on this mechanism."

He added, "Dust mite and pollen allergens are just more proteinases, so although not formally tested, it is virtually certain that these allergens work through the fibrinogen-TLR4 pathway."

Another question is whether the pathway itself exists in humans.

According to Saudan, TLR4 signaling in humans may be different than that in mice. For instance, humans show an increased TLR4 response to lipopolysaccharide (LPS), whereas mice do not.

"To determine whether it might apply to humans, one could attempt to see whether there is a correlation between fibrinogen cleavage products in the lung and the level of allergic inflammation by comparing healthy individuals and asthma patients. Such a correlation would be a good hint that a similar phenomenon occurs in humans," he said.

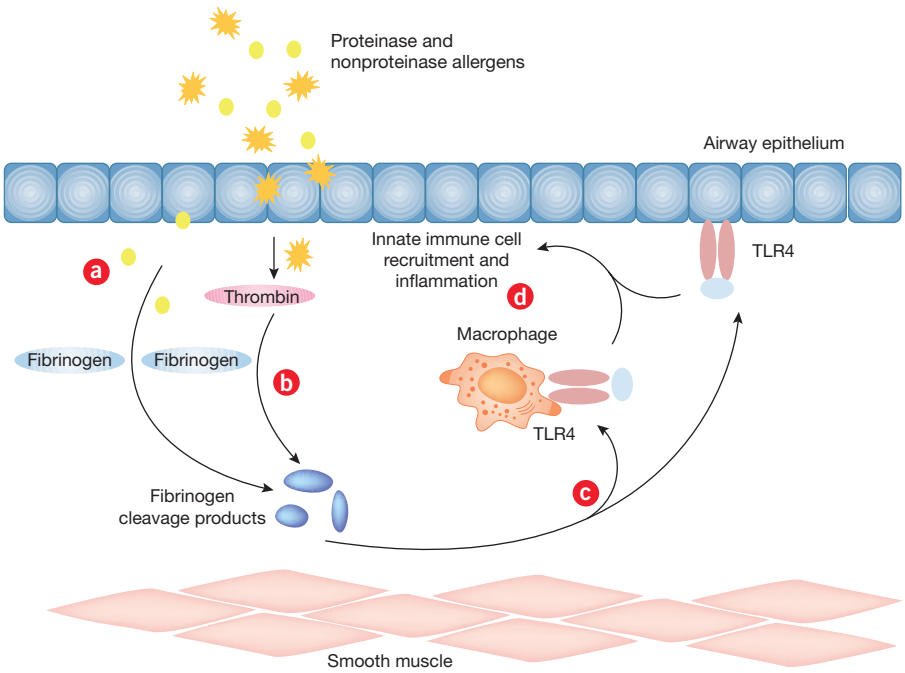

Figure 1. Proteinase and nonproteinase allergen–induced inflammation. Proteinase allergens cleave fibrinogen into fibrinogen cleavage products [**a**]. Nonproteinase allergens activate endogenous proteinases, such as thrombin [**b**], to cleave fibrinogen into cleavage products. Fibrinogen cleavage products act as ligands for toll-like receptor 4 (TLR4) expressed on cells including macrophages and airway epithelial cells [**c**]. Activated TLR4 stimulates recruitment of innate immune cells and airway inflammation [**d**].

Pathway intervention

The Baylor team's next task will be to identify a way to safely interfere with the pathway.

Corry said that two potential therapeutic interventions are anticoagulants delivered directly to the airway to block fibrinogen or

small molecules and antibodies that disrupt the interaction between fibrinogen cleavage products and TLR4.

The group thus plans to "identify the specific fragments of fibrinogen that signal through TLR4. This information is required for the development of a blocking agent that might ultimately be useful in asthma."

Both approaches could pose safety issues.

Jesper Wiklund, CEO of **InDex Pharmaceuticals AB**, noted that "pattern recognition receptors such as TLR4 are important for the immune host defense. As with all immunosuppressive therapies, an antagonistic therapy to TLR4 could impair the local immune defense, and caution should of course be taken."

InDex's DIMS 9054, a DNA-based immunomodulatory sequence that binds TLR9, is in preclinical testing to treat steroid-resistant pulmonary inflammation.

Despite these risks, Kita said, "our immune system has so much redundancy that blocking TLR4 may be safe—but it could also have a very detrimental effect."

Corry acknowledged that blocking the interaction between TLR4 and fibrinogen cleavage products could eliminate a patient's protective immunity against fungal infection.

"Interfering with a potential protective allergic immune response in these patients, while beneficial in the short term, could lead to fungal overgrowth in the long term and eventual loss of disease control," he said. "It is okay to inhibit the allergic inflammation as long as you give a second agent that kills off the fungus such as one of the existing antifungal agents. We used terbinafine or voriconazole with great success."

Separately, blocking fibrinogen cleavage could cause issues with normal blood clotting and lead to dangerous bleeding in the lungs. However, therapeutic strategies inhibiting the interaction between TLR4 and fibrinogen cleavage products involve blocking a molecule created after fibrinogen cleavage and remove the need for anticoagulation, making them more specific and safer approaches in terms of bleeding risks than strategies blocking fibrinogen cleavage, according to Corry.

Saudan added that the safety risks are compounded by the fact that the therapeutics would likely require chronic administration.

"It may be possible to treat allergic rhinitis sufferers only during their allergen season to prevent the chronic inactivation of these pathways, but chronic treatment would probably be required for most patients," he said. "This approach could be used prophylactically to actually prevent the allergic triggers of asthma attacks. This is beneficial, but it means that therapeutic developers would have to go into healthy patients and either interfere with TLR4 signaling or fibrinogen cleavage, which are two pathways important for clearance of infection and blood coagulation. Hence, it would be a quite challenging clinical development program."

Corry said that the findings are unpatented and are not available for licensing.

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COMPANIES AND INSTITUTIONS MENTIONED

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