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# CF: new roles for neutrophils

*By Michael J. Haas, Senior Writer*

Although cystic fibrosis is caused by a mutation in the cystic fibrosis transmembrane conductance regulator channel, disease progression is thought to stem primarily from pulmonary damage caused by elastase. The prevailing theory is that elastase is released from necrotic neutrophils that are recruited to the lungs and fail in the fight against bacterial infection. But new work published in the *Proceedings of the National Academy of Sciences* by researchers at **Stanford University School of Medicine** instead suggests living, but dysfunctional, neutrophils are the primary source of lung-damaging elastase in cystic fibrosis.[1]

Under the necrotic neutrophil theory, elastase is passively released from dying neutrophils, a process so far downstream toward pulmonary damage that it presents no obvious drug targets. Typical strategies to prevent damage rely either on antibiotics to target infection, which minimize neutrophil recruitment to the lungs in the first place, or on compounds that target elastase after its release. But bacteria in the lungs often develop resistance to antibiotics, and inhibiting extracellular elastase may be too far downstream of where the damage starts, resulting in a case of too little, too late.

Anti-inflammatory compounds such as *N*-acetylcysteine—an endogenous precursor of the antioxidant glutathione—have shown some promise in treating cystic fibrosis (CF), but have yet to overcome safety and/or formulation obstacles.[2]

The new findings imply it may be possible to target dysfunctional neutrophils directly and thus prevent the release of elastase into the lungs.

"The main—and contentious—issue has been to decide whether elastase was released by dying or active cells," said Rabindra Tirouvanziam, an instructor at the medical school and leader of the research team. "Nobody has shown that elastase is released by necrotic neutrophils. A key point we've shown in our research is that elastase release is occurring in viable neutrophils."

He said neutrophil elastase ordinarily does not encounter host tissues but instead remains inside the neutrophil, where it plays a role in phagocytosis. Tirouvanziam also cited a 2005 study in the *Annual Review of Immunology* by A.W. Segal at **University College London**, which shows that necrotic neutrophils are unlikely to release their elastase.[3]

Using flow cytometry, the Stanford team performed *ex vivo* analyses of sputum samples from induced expectoration from the lungs of CF patients. Tirouvanziam said the fraction of viable neutrophils in the samples varied from 10% to 70% of the total neutrophil count and noted that the number of active neutrophils "correlates significantly" with the amount of elastase present.

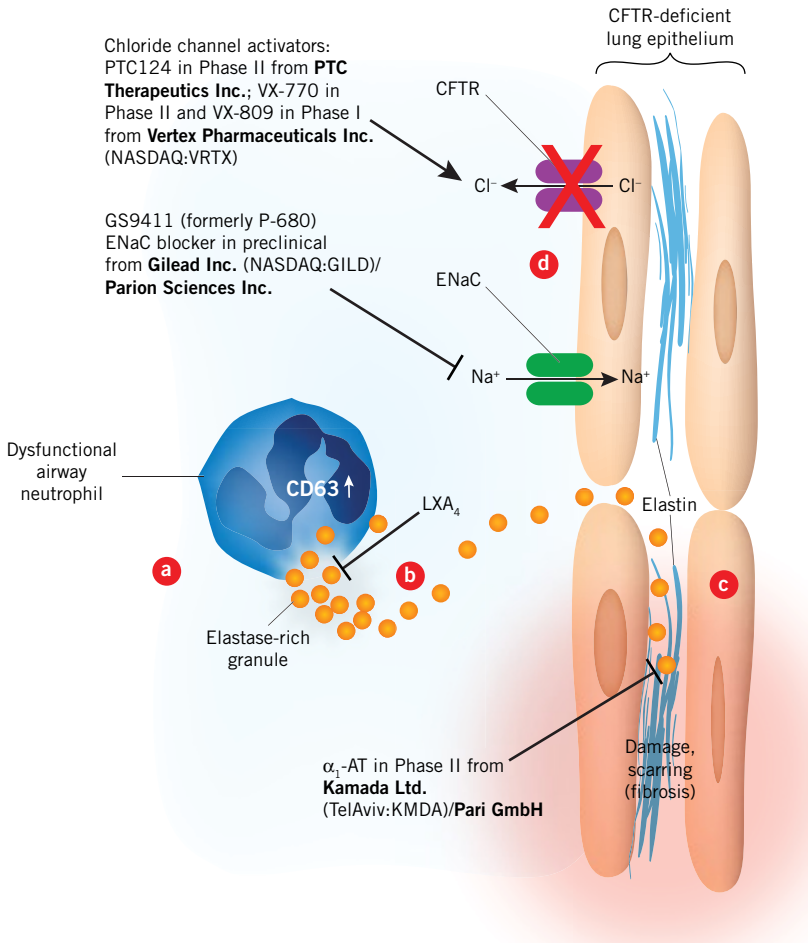


**Figure 1. CD63-induced neutrophil dysfunction in the CF airway.** Airway neutrophils undergo functional and signaling changes in the vicinity of the cystic fibrosis transmembrane conductance regulator (CTFR)-deficient membrane in a CF patient's airway [**a**]. CD63 is upregulated, which promotes excretion of neutrophil elastase (NE)-rich granules [**b**]. NE degrades elastin in the pulmonary structures, leading to damage and scarring (fibrosis) [**c**]. A recent study[1] proposes the use of lipoxin $A_4$ ($LXA_4$) and similar compounds to inhibit mobilization of these NE-rich granules. Current CF treatment strategies include the use of $\alpha_1$-antitrypsin ($\alpha_1$AT), a specific inhibitor of elastase, to prevent proteolysis and fibrosis of pulmonary structures. Chloride channel activators or epithelial sodium channel (ENaC) blockers [**d**] also are used to promote proper movement of fluids across CFTR-deficient membranes.

The team also found several alterations in the surface receptors expressed by CF airway neutrophils compared with blood neutrophils or neutrophils from the lungs of healthy volunteers. That result suggests the neutrophils in CF patients were dysfunctionally reprogrammed upon moving from the blood to the airways.

A significant change was increased expression of CD63, which promoted the extracellular release of elastase and subsequent tissue damage. CD63 is a signal transducer of cell development, activation, growth and motility.

Thus, the researchers suggest that compounds blocking the release of elastase from neutrophils—such as lipoxin $A_4$ ($LXA_4$)—could prevent CF-associated pulmonary damage before it starts. $LXA_4$ is an endogenous anti-inflammatory molecule derived from arachidonic acid (*see* **Figure 1, "CD63-induced neutrophil dysfunction in the CF airway"**).

Moreover, Tirouvanziam told *SciBX* the findings suggest "an early, primary role of neutrophils in making the bed for opportunistic infection. The environment of CF airways conditions the neutrophils to dysfunction" in response to any inflammatory stimulus in the lungs, he said.

Elastase released by these dysfunctional neutrophils degrades the pulmonary structure and creates a breeding ground for opportunistic bacteria such as *Haemophilus influenzae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* that eventually chronically infect CF patients.

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> ***—Trevor Hansel, Imperial College London***

"We think of neutrophils as defenders against infection," said Trevor Hansel, a group leader at the National Heart and Lung Institute, a division of **Imperial College London**. "To find that they are perpetuators of a disease is a new insight."

Hansel is focused on the role of eosinophils in allergies and asthma. In 1991 he and his colleagues at the **Swiss Institute of Allergy and Asthma Research** published a study in *Clinical and Experimental Immunology* that shows blood and airway eosinophils in asthmatics have different cell-surface receptors from one another.[4]

Hansel told *SciBX* the findings reported in the *PNAS* paper "are exactly analogous to the role of eosinophils, which have been shown to be activated in sputum from asthmatics." Eosinophils are longer lived than neutrophils, but they can migrate to a site of allergic response, produce cytokines, take part in tissue remodeling and interact with fibroblasts and other cells, he said.

Nevertheless, Hansel was cautious about targeting neutrophils in CF. "CF patients are vulnerable to recurring or chronic infections. You have to be careful about targeting neutrophils" so that a correct immune response is not compromised, he said.

"If you could selectively target the dysfunctional neutrophils, that would be OK," Hansel added. "But the molecules proposed in the paper don't seem very selective. More work would be needed to figure out what's different about the dysfunctional neutrophils and how to target them selectively."

"I think that neutrophil elastase is an exciting target, so if there was a way to block elastase before release, that would be a good thing," said Clive Wood, EVP of discovery research and CSO at **Dyax Corp.** "The identification of dysfunctional neutrophils as a source of elastase might provide ways to do that."

Dyax has elastase inhibitors in early research to treat undisclosed respiratory conditions that do not include CF.

Fred Van Goor, research fellow at **Vertex Pharmaceuticals Inc.**, agreed that the dysfunctional nature of the neutrophils might be what makes them good drug targets.

"The usual role of neutrophils is to get rid of bacteria, so the fact that there are a lot of them in the lungs of CF patients—to fight infections—would seem to be a good thing," he said. "But if the neutrophils have defective biology in the CF lung, then preventing their activation might have some therapeutic or symptomatic benefit."

Vertex is developing two compounds to treat CF, both aimed at restoring the defective functions of cystic fibrosis transmembrane conductance regulator (CFTR). VX-770, a small molecule that potentiates the gating function of CFTRs on the cell surface, began a Phase Ia trial earlier this year. VX-809, a small molecule that increases the number of mutant DF508-CFTR channels on the cell surface, began a Phase IIa trial in 2007. The company has not disclosed the molecular targets of the compounds or completion dates for the trials.

As a cautionary note, Dyax's Wood told *SciBX* the sample collection used by the Stanford team has potential drawbacks.

"I'm always suspicious of induced sputum, because it can be contaminated by sources outside the airway, such as saliva," he said. "There are ongoing questions about how useful this method of sample collection is."

Wood said he would like to see data from another method, including histological analysis of lung cross-sections or bronchial lavage, that confirm airway neutrophils have the cell-surface receptors found in the sputum samples.

Hansel also expressed minor concerns about the sample collection method.

"Normally if we want to count sputum cells, we would liquefy the sputum with dithiothreitol (DTT)," which loosens up mucus to release neutrophils and other leukocytes, he said. But DTT also breaks down disulfide bonds—and thus the secondary structure of proteins—which "means that many surface markers become unraveled and nondetectable," he said.

Hansel noted the Stanford team avoided DTT and instead induced expectoration with saline, which "may select a population of sputum neutrophils that are activated. But it is pretty standard to induce sputum by inhaling hypertonic saline, so I am not particularly worried by it."

Tirouvanziam said his team is working to establish more accurate measures of the fraction of viable neutrophils in induced sputum samples. "But we need to do more studies to determine the modulation of neutrophil populations in different CF disease states" because the fraction of active neutrophils also appears to depend on the lung function of the individual, he said.

Van Goor of Vertex also was interested in the Stanford team's methods—but not because he harbored any concerns about them.

"It is worth looking at their methods of sample collection, neu-

trophil markers, and so on, because their approach might offer new biomarkers for therapies aimed at other drug targets along the CF disease pathway," he said. "To that end it would be important to do more studies on the relationship between the fraction of viable neutrophils in a sample and the patient's disease state," as the Stanford team is doing, he said.

The Stanford team is studying another set of signaling changes found on the dysfunctional neutrophils: increased expression of CD80, prostaglandin D2 receptor (CD294; CRTH2; GPR44) and major histocompatibility complex type II, which collectively suggests that the neutrophils are switching to a T cell–helping function.

Tirouvanziam noted that the expression of CD294 indicated that the neutrophils were polarized to a Th2 cell–helping function—usually considered an allergic response—instead of the Th1 cell–helping function associated with response to infection.

"There is possibly another layer of downstream immune dysfunction in CF giving improper cues to T cells," but further work is needed to ascertain whether this switch to Th2 cell–helping function does indeed have an exacerbatory influence on the disease, he said.

The team is also investigating the implications of their findings in other diseases.

"The release of elastase doesn't happen only in CF," Tirouvanziam noted. "It is observed in other diseases as well," such as rheumatoid arthritis and chronic obstructive pulmonary disease. "We're looking to see whether a similar neutrophil dysfunction occurs in RA."

Tirouvanziam said Stanford is considering whether to patent the findings reported in *PNAS*.

## REFERENCES

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## COMPANIES AND INSTITUTIONS MENTIONED

**Dyax Corp.** (NASDAQ:DYAX), Cambridge, Mass.
**Imperial College London**, London, U.K.
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