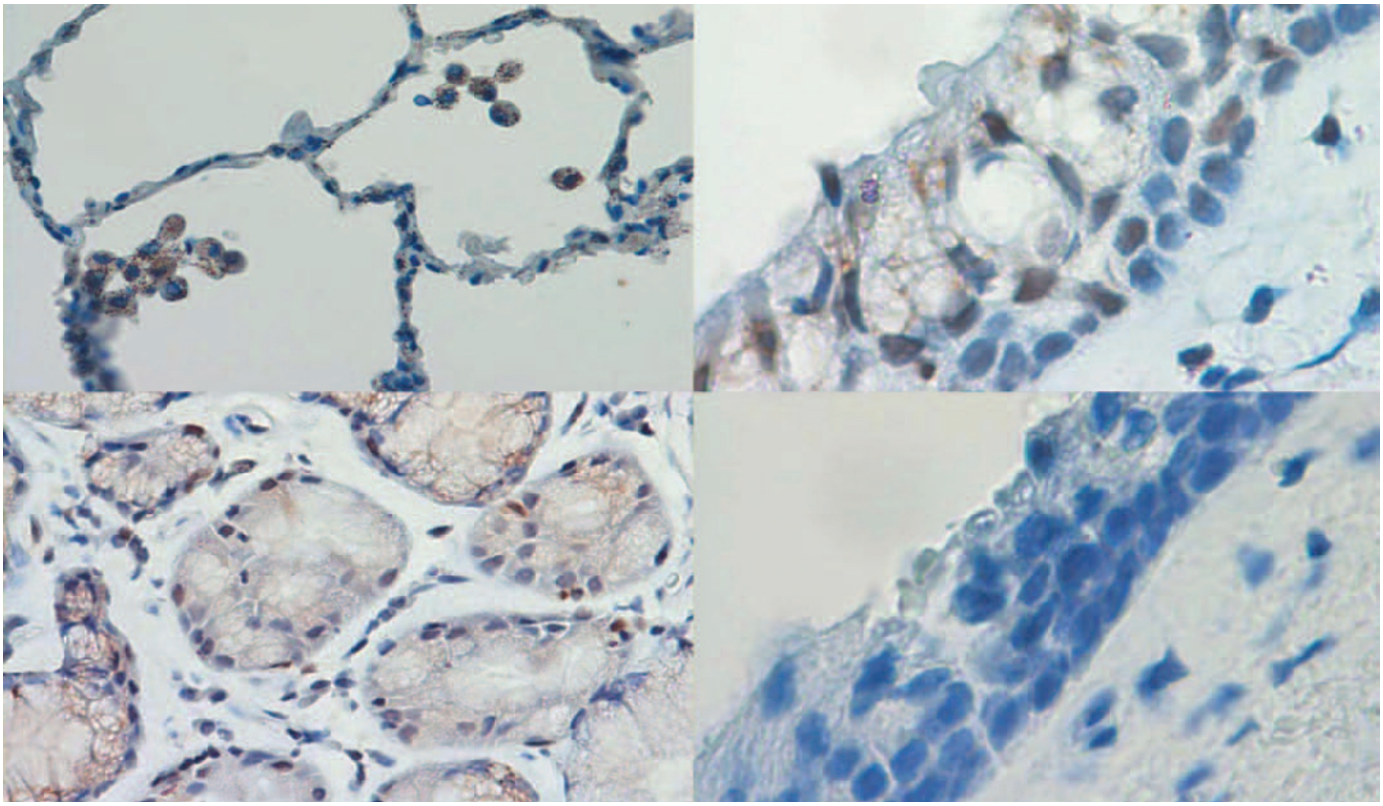


IMPERIAL COLLEGE LONDON

Immune cells taken from the lung seen expressing the protein Nrf2 (brown) which acts as a master switch for genes that encode protective antioxidants.

BIOCHEMISTRY

A radical treatment

Researchers are counting on drugs that activate a master switch for antioxidant genes to protect lung tissue of COPD patients from an onslaught of free radicals.

BY KEN GARBER

Smoking is the most common cause of chronic obstructive pulmonary disease (COPD), a progressive condition characterized by lung damage, a narrowing of the airways and difficulty breathing. No surprise given that each puff of cigarette smoke contains more than 10^{15} free radicals — atoms or molecules with unpaired electrons that can react violently with other molecules, setting off chain reactions that damage proteins, lipids, and DNA. "In excess, [free radicals] will cause injury, and will cause impairment in the repair process," says biochemist Irfan Rahman of the University of Rochester in New York. "That's what happens in lungs in response to tobacco smoke."

COPD continues to progress long after a person quits smoking, and free radicals are partly to blame. COPD involves chronic inflammation, and inflammatory cells release abundant free radicals. As a result, the lungs of COPD patients exist in a constant state of oxidative stress — an imbalance of free radicals and antioxidants. Antioxidant enzymes convert free radicals to less reactive molecules, and antioxidant free radical scavengers donate electrons to halt free radical chain reactions. In COPD, the shower of free radicals overwhelms these antioxidants, causing cell death and tissue damage.

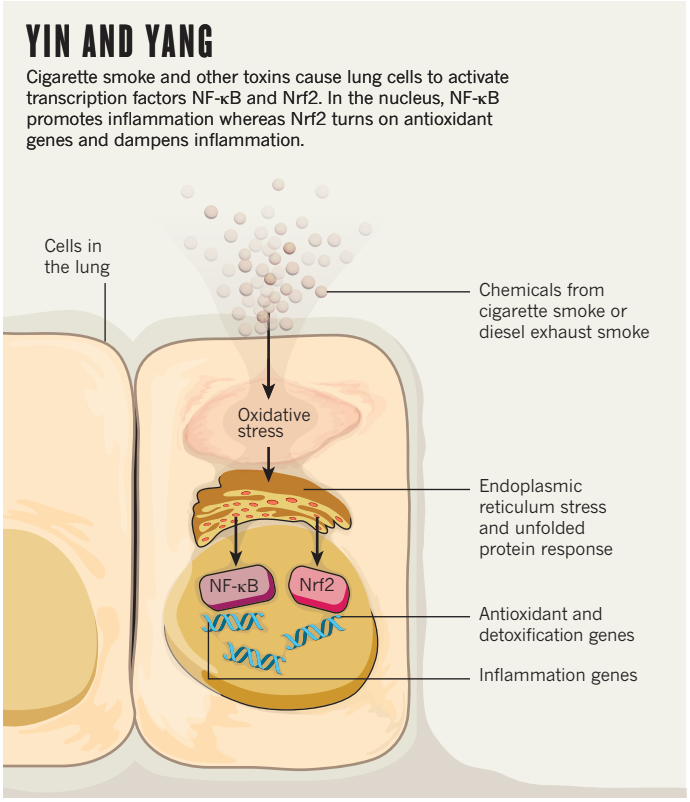
But clinical trials of antioxidants have mostly failed to prove they work as a COPD treatment. That may be because each chemical antioxidant molecule can extinguish only one target molecule; it is therefore impossible to quench more than a fraction of the excess free radicals even using high doses of antioxidants. Such antioxidants behave in a "sacrificial" manner, says Paul Kirkham, a biochemist at Imperial College London. "Once it's been used, it's gone." Another problem, says Kirkham, is that individual antioxidants may not reach the cellular compartment where they are needed most.

A new approach to COPD promises to solve these problems. Several drug companies are developing compounds that activate the DNA-binding protein Nrf2 (nuclear erythroid–related factor 2, also known as Nfe2l2), which acts as a master switch of genes that encode antioxidants. Nrf2 emerged from obscurity in 1997, when biochemist Masayuki Yamamoto at the University of Tsukuba in Japan showed that it activates an entire class of detoxifying enzymes. Among these enzymes are many that generate critical antioxidants.

Drugs that activate Nrf2, in theory, would solve the problems of dose and compartmentalization that have so far derailed other antioxidant therapies. To begin with, Nrf2-activated enzymes aren't spent each time they do their job. "They can effectively regenerate themselves — that's the beauty of an enzyme," says Kirkham. "So you need much less of it." Together, these enzymes neutralize a variety of free radical molecules, not just one, and in all important cell compartments. Finally, Nrf2 induces the expression of proteins that can repair some of the damage inflicted by free radicals.

Researchers have been studying Nrf2 in animal models of COPD for more than a decade, and in human tissue. It now appears that Nrf2

activators may potentially benefit COPD patients beyond triggering antioxidant and detoxification enzymes. Following Yamamoto's discovery, researchers assumed that Nrf2 activators would need to be administered early in the disease — in particular, before the lung tissue destruction typical of emphysema, one of the two main manifestations of COPD. (The other is bronchitis, inflammation of the bronchi, the main airways that go into the lungs.) But work by toxicologist Shyam Biswal at the Johns Hopkins University School of Public Health in Baltimore, Maryland, and others suggests that Nrf2 activators might be effective not only in preventing COPD progression but in treating advanced cases. And, unlike bronchodilator drugs, says Biswal, Nrf2 activators might not just treat the disease's symptoms but also arrest its course.



In 2004, Biswal's group found that mice genetically engineered to lack Nrf2 developed early-onset emphysema with more severe inflammation than did wild-type mice, and that this was a result of oxidative stress[1]. Moreover, exposing the mice without Nrf2 to cigarette smoke over six months caused comparatively more lung damage. Four years later, three different research groups reported that Nrf2 activity declines as COPD progresses in humans — suggesting some protective role for Nrf2. Then Biswal's group found that lack of Nrf2 activity in human COPD lungs caused defective protein clearance, which led in turn to more oxidative stress and cell death. Adding an Nrf2 activator to cells prevented these effects. The overall picture emerging from these studies is that Nrf2 is a key stress-response factor whose absence worsens COPD.

Nrf2 may also help clear harmful bacteria from the lungs of COPD patients. Such bacteria can cause COPD exacerbations — acute and potentially fatal bouts of increased coughing, mucus production and shortness of breath. Biswal's group found that adding an Nrf2 activator to cultures of macrophages (crucial host defense cells) taken from the lungs of COPD patients restored the cells' ability to clear bacteria in culture and in mice[2]. Biswal's group went on to show that Nrf2 in macrophages activates a scavenger receptor that recognizes pathogenic bacteria and enables the macrophages to clear infection — thus, in theory, limiting COPD exacerbations.

Finally, Nrf2 plays a crucial role in overcoming treatment resistance to corticosteroids, a mainstay in asthma therapy, which doctors often prescribe to COPD patients. Unfortunately these steroid hormones provide little or no therapeutic benefit in COPD. In fact, says Biswal, "steroids cause more problems in COPD than they help," mainly because steroid treatment can lead to pneumonia. An explanation for this corticosteroid resistance emerged in 2005, when pulmonologist Peter Barnes at Imperial College London reported that oxidants in cigarette smoke inactivated the enzyme histone deacetylase 2 (HDAC2), which normally blocks the expression of inflammatory genes. Corticosteroids reduce inflammation by signalling through a receptor that recruits HDAC2 and represses inflammatory gene expression. But in smokers and COPD patients, oxidative stress inactivates HDAC2 resulting in continuous inflammation and tissue damage.

"You've got to do the clinical trial and then see what happens."

By reducing oxidative stress, Nrf2 activators can reverse corticosteroid resistance, at least in cell culture. Biswal and his Johns Hopkins colleague Robert Wise are planning a clinical trial of treating COPD patients with the steroid prednisone and the Nrf2 activator sulforaphane. "This would be a logical approach," says Barnes. Sulforaphane is already in a phase II clinical trial for COPD, sponsored by the US National Institutes of Health. Cells extracted from the lungs of COPD patients will be tested to see if the drug raises levels of Nrf2.

But sulforaphane is not an ideal Nrf2 activator. It does activate Nrf2, but not always very potently, and because it targets many other proteins it could potentially cause collateral damage. And although safe at low doses (it is derived from broccoli sprouts), sulforaphane can be toxic at high doses. Hence an intense search for more selective Nrf2 activators is underway.

Drug companies are already heavily involved. In December 2011, in one of the largest preclinical deals ever, Abbott Laboratories, headquartered in Abbott Park, Illinois, agreed to pay US$400 million to Reata Pharmaceuticals, based in Irving, Texas, to license Reata's second-generation Nrf2 activators. Abbott executive vice president Thomas Freyman told investors in January 2012 that he expected the first of those compounds to enter clinical trials later in the year. Meanwhile, Cureveda, a Baltimore, Maryland, biotech firm cofounded in 2010 by Shyam Biswal, is screening compound libraries from the UK pharmaceutical company GlaxoSmithKline for pro-Nrf2 activity. Other companies are interested in targeting Nrf2, including Pfizer and Novartis, which are part of a consortium studying new therapeutic strategies for treating COPD, according to Kirkham.

Despite their great potential in COPD, Nrf2-activating drugs present possible safety issues. Nrf2 is usually inactive in cells, but is activated by spikes in oxidative stress. Using drugs to keep Nrf2 turned on could disrupt beneficial oxidative processes in cells. "The cell is actually maintained in a very fine redox [oxidation-reduction] balance, because it actually needs oxidative stress to do some of its signaling," says Kirkham. Biswal concedes that continuous activation of Nrf2 could cause side effects. "You've got to do the clinical trial and then see what happens," he says.

Then there is the evidence that Nrf2 might increase the risk of cancer. In 2008, a group at Japan's National Cancer Research Institute in Tokyo reported apparent Nrf2-activating mutations in human lung tumours and in head and neck tumours[3]. In their report, they contend that ongoing activation of Nrf2 might give cancer cells "undue protection from their inherently stressed microenvironment." In 2011, two groups reported that in mouse models Nrf2 is indirectly activated by mutations that cause hereditary kidney cancer in humans. And a group from the Cambridge Research Institute, part of Cancer Research UK, found that three common cancer-causing genes activate Nrf2, which may promote tumorigenesis by reducing oxidative stress in

pre-cancerous cells[4].

But, paradoxically, activated Nrf2 can also have anticancer effects. Several studies have found that Nrf2-activating compounds can prevent or suppress cancer in mouse models. And a recent study found that sulforaphane did not promote lung cancer in mice. Pharmacologists Mike Sporn and Karen Liby at Dartmouth College in Hanover, New Hampshire, explain that Nrf2 can either promote or suppress cancer depending on the cellular context[5].

Safety concerns about Nrf2 activators in COPD have been lessened by trials of two Nrf2-activating drugs in other diseases. Weston, Massachusetts-based Biogen Idec recently completed phase III clinical trials of dimethyl fumarate (DMF) for multiple sclerosis. Although DMF wasn't designed as an Nrf2 activator, the company claims it works at least partly that way. And Abbott and Reata are jointly developing bardoxolone methyl, another unintentional Nrf2 activator that's now in phase III trials for chronic kidney disease in type 2 diabetes. Side effects of bardoxolone methyl have been mild. Side effects reported for DMF include abdominal pain, diarrhea, flushing and headaches. On the other hand, it is too early to assess the risk of cancer — any cancers would likely take many years to appear.

Clinical trials should soon determine if Nrf2 can help resolve the many problems in COPD, from oxidative stress to inflammation. "COPD is a multifactorial disease — it's one of the worst," says Biswal. Harnessing the power of Nrf2, that prolifically protective protein, may eventually prove to be the solution. ■

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1. Rangasamy, T. *et al. J. Clin. Invest.* **114**, 1248–1259 (2004).
2. Harvey, H. *et al. Sci. Transl. Med.* **3**, 78ra32 (2011).
3. Shibata, T. *et al. Proc. Natl. Acad. Sci.* **105**, 13568–13573 (2008).
4. DeNicola, G. M. *et al. Nature* **475**,106–109 (2011).
5. Sporn, M. B. & Liby, K. T. *Nat. Rev. Cancer* **8**, 564–571 (2012).
6. Lee, S.H. *et al. Nature Med.* **13**, 567–569 (2007).
7. Shan, M. *et al. Sci. Transl. Med.* **1**(4):4ra10 (2009).
8. Chen, K. *et al. PLoS One* **6**, e2033 (2011).
9. Shan, M. *et al. Sci. Transl. Med.* **4**, 117ra9 (2012).

AUTOIMMUNITY

The T-cell connection

What exactly causes the destruction of lung tissue in emphysema? Free radicals definitely contribute, but for half a century, the prevailing hypothesis has been that emphysema, one of the two main aspects of COPD, develops when the activity of proteases — enzymes that digests proteins — falls out of balance with antiprotease activity. Proteases normally help maintain lung health by clearing roadblocks from the path of migrating immune cells hunting pathogenic viruses and bacteria. But cigarette smoke stimulates immune cells such as macrophages and neutrophils to release too many proteases, and wanton destruction of proteins ensues. Mysteriously and inexorably, this process continues in COPD patients even after they stop smoking, as emphysema slowly suffocates them.

That mystery is now beginning to be solved, as clues implicate autoimmunity. Th17 cells — a specialized class of $CD4^+$ T cells, the cell type that orchestrates the adaptive immune response — appear to be central to the pathology. Therefore, therapies targeting these cells might be a promising strategy.

Early evidence for autoimmunity in COPD came in 2007, when Farrah Kheradmand, a pulmonologist and immunologist at Baylor College of Medicine in Houston, Texas, isolated $CD4^+$ T cells from the blood of ex-smokers with COPD[6]. Elastin is an important structural protein in the lungs' connective tissue, and when Kheradmand added elastin fragments to these T-cell cultures, the T cells responded by releasing inflammatory cytokines as if the cells had encountered a microbial pathogen. "A large number of patients who have emphysema [who] stop smoking and continue to have the disease will have these autoreactive T cells," says Kheradmand.

Why the body attacks its own lung tissue remains unknown. But by isolating the cells driving this autoimmunity, scientists can identify potential drug targets. Th17 cells, which secrete the inflammatory cytokine IL-17, are the main drivers of autoimmunity in rodent models of rheumatoid arthritis, psoriasis and multiple sclerosis. Th17 cells are also present in COPD. In 2009, Kheradmand's group found that culturing certain cells of emphysema patients with their own T cells drove those T cells to release IL-17, which in turn led to the production of destructive proteases[7].

Evidence for IL-17 driving autoimmunity in COPD continues to be found. In 2011, University of Pittsburgh, Pennsylvania, pulmonologist Jay Kolls reported that mice genetically lacking the IL-17 receptor did not develop emphysema despite six months of exposure to cigarette smoke[9]. More recently, Kheradmand and collaborators at the MD Anderson Cancer Center in Houston, Texas, genetically engineered mice to overexpress one form of IL-17 — IL-17A — and then exposed these mice to cigarette smoke for four months[8]. The mice developed an especially severe form of emphysema along with producing more destructive proteases. Both studies fingered IL-17 as a culprit in COPD autoimmunity.

The picture emerging from these studies is that inhalation of cigarette smoke promotes the generation of T cells that express IL-17, which, in turn, causes the production of proteases that destroy lung tissue including elastin. Then, elastin fragments trigger an autoimmune response involving more IL-17-producing T cells, perpetuating a loop and more tissue destruction.

To disrupt this vicious cycle, pharmaceutical and biotech companies could test drugs they already have in hand that target these cells and the cytokines they produce. Antibodies specific for IL-17 and its receptor have been very effective in clinical trials for psoriasis. As for COPD, "I don't know if it is on the radar screen of these companies yet, but I think [IL-17] would be definitely an intriguing target," says Kolls. He is collaborating with an undisclosed company, testing whether anti-IL-17 receptor antibodies can treat COPD in mice.

Others are more ambivalent. "It would certainly be worth studying IL-17 or IL-17 receptor blockers," says Peter Barnes, a pulmonologist and COPD researcher at Imperial College London. He adds that such trials are planned. But he cautions that Th17 cells may not play the same role in human COPD as in the mouse version of the disease; indeed, he says, the evidence for increased Th17 cells in human COPD lungs is "not as striking" as it is in mice. "The evidence for Th17 cells playing an important role in COPD is not fully established," he adds.

Kheradmand disagrees. She says that IL-17 is "very much present" in lung tissue of human COPD patients. And Kheradmand points out that each case of emphysema is different, with not all manifesting autoimmunity. Her lab at Baylor College is developing an assay to identify COPD patients with an autoimmune form of the disease driven by Th17 cells, and hopes companies will eventually test their drugs in such patients. "Wouldn't it be wonderful," she asks, "if we can just identify this particular individual… who does have this autoimmune component, to then offer him these biologics?" — **K.G.**