New Clues to Asthma Therapies

Identification of major players in the inflammatory cascade that damages the lungs in asthma offers targets that may produce better treatments for the disease

A rising pollen count means itchy eyes, scratchy throats, and stopped-up sinuses for many allergy sufferers, but for those whose allergies trigger asthma, this means more than just discomfort. For them, exposure to usually harmless pollen or other allergens can set off a life-threatening attack in which the airways leading to the lungs close up—making the sufferers feel, they say, as if they are trying to breathe with a full-grown person standing on their chest. These frightening attacks are becoming more and more common. Since 1980, the prevalence of asthma has almost doubled in the United States. Today, it afflicts more than 14 million people in this country alone, and costs almost 5000 lives each year—with no signs of leveling off.

Researchers don't have a clear idea of what is causing this increase (see sidebar). Nor have they worked out what predisposes some people to asthma in the first place. But on one front, they are making real headway. They are beginning to pinpoint many of the key biological players that take part in asthma attacks. And that in turn is providing researchers with openings for new ways to treat asthma, some of which are just entering clinical use. "There is a story that's coming out," says Yale pulmonologist Jack Elias. "It's beginning to hang together."

The main theme of the story is inflammation. Doctors have known for years that asthma attacks are often triggered by allergens, such as cockroaches, dust-mite feces, pollen, or animal hair. Now, researchers are working out the exact cascade of events that these allergens—and other nonallergen triggers such as cold air, viral infections, and exercise—set in motion in the lungs. At the top of the cascade is a particular type of immune cell called the T lymphocyte, which responds to the noxious substances by sending out more than a dozen chemical signals: so-called cytokines, which attract inflammatory cells to the airways of the lungs.

These warriors, in particular those called the eosinophils, release chemical weapons of their own. This second wave of signals, including histamine and small, fatty molecules called leukotrienes, causes blood vessels to leak and lung tissues to swell, contracts the smooth muscles of the airways—cutting off the air supply like squeezing a hose—and encourages mucus production, further clogging already constricted airways. Each crisis causes an immediate difficulty in breathing, and repeated crises over time lead to permanent lung changes that may make the next attack even worse.

Current asthma treatments are aimed at the end result. Bronchodilators open the airways, and antihistamines and steroids reduce inflammation. But by dissecting the chain of command that leads to an attack, researchers have identified a whole new set of promising targets for asthma drugs. "The therapy is moving back closer and closer to the beginning of the inflammation cascade," says Harold Nelson, an allergist and immunologist at the National Jewish Medical and Research Center in Denver. The hope is that these therapies, because of their improved specificity, will be more effective and less liable to cause dangerous side effects than current treatments.

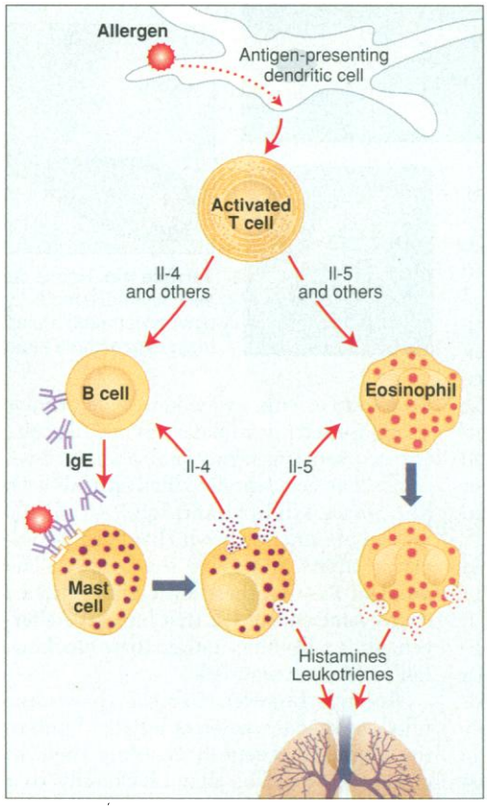


ILLUSTRATION: K. SUTLIFF

Overzealous warriors. The T lymphocyte helps command the immune cells—including mast cells and eosinophils—that react to pollen, cold, exercise, and other stimuli to trigger an asthma attack.

Although most of the treatments aimed at the inflammatory cascade are a few years away from patients' medicine cabinets, two have already made it to the shelves. Both drugs, which were approved late last year by the U.S. Food and Drug Administration (FDA), got ahead of the crowd for the simple reason that their targets, the leukotrienes, were implicated in the cascade nearly 50 years ago.

Released by activated eosinophils and other immune-system soldiers recruited to asthmatic lungs, the leukotrienes have several effects that contribute to the airway constriction and inflammation of asthma. They recruit other inflammatory cells, for example. But they are particularly effective in contracting the smooth muscle of the bronchi, the tubes carrying air from the trachea into the lungs. Molecule for molecule, says pulmonologist Jeffrey Drazen of Brigham and Women's Hospital in Boston, the leukotrienes are the most potent bronchoconstrictors ever described—a fact, he adds, "that was not lost on the drug companies," which set out to develop inhibitors.

The two approved last year are Zileuton, which blocks a vital enzyme needed for leukotriene synthesis and is marketed by Abbott Laboratories in Chicago, and Zafirleukast, which blocks the lipid's receptors on smooth muscle and other cells and is produced by Zeneca Pharmaceuticals, based in the United Kingdom. Two more receptor blockers, from Merck and from SmithKline Beecham, are awaiting FDA approval.

The drugs have worked miracles in some hard-to-treat patients, Drazen says. "In some patients it's like manna from heaven. We treated a veterinarian allergic to dogs and cats who was just miserable. On this treatment, he is a normal person again." But for reasons that are currently unclear, only about half of all patients respond to the drugs; in the other half, there is almost no change, says Sally Wenzel of National Jewish, who helped conduct several of the preapproval clinical trials.

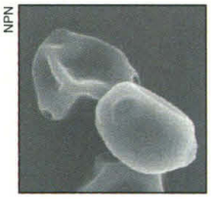
Stopping inflammation early

Patients who receive no relief from the leukotriene inhibitors still have reason to hope, however. While the leukotrienes act late in the inflammatory cascade, other ef-

forts are aimed at interrupting it before it gets established. One development propelling that research is the recognition that a particular subset of T lymphocytes seems to be a major culprit in asthma and other allergic diseases, responding with undue vigor to apparently harmless invaders.

In work done nearly a decade ago, researchers working with T cells from mice found they could divide the cells into two groups based on the cytokines they produce. Members of one set, which they called T_H1 cells, produce a set of signals that orchestrate attacks on unfamiliar cells, protecting the body against bacteria and tumor cells. Those in the other set—the T_H2 cells—produce inflammatory signals normally directed against parasitic invaders. They also encourage the antibody-producing B cells to secrete IgE antibodies, the hallmark of allergies, which help trigger the inflammatory responses.


EFA


NPN

As researchers learned more about these activity patterns, it became clear that T_H2 overactivity is a major factor in asthma. High levels of IgE, for example, are common in asthma patients. And one of the key T_H2 cytokines, called interleukin-5 (Il-5 for short), helps trigger the eosinophils that can wreak havoc in asthmatic lungs. Although the distinction between T_H1 and T_H2 cells is not as cut-and-dried as many might like—many human T cells seem to produce both T_H1 and T_H2 signals—Yale's Elias says the concept "has opened doors in thinking about asthma" and about potential new therapies.

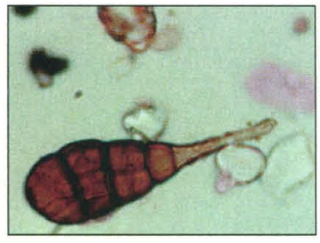
Some of those efforts are aimed directly at thwarting the effects of T_H2 cytokines. For example, Il-5 appears to be a good target. If the cytokine's action in mice is blocked, either by inactivating the gene that codes for the protein or by giving the animal antibodies that prevent Il-5 from binding to and activating eosinophils, the animal's airways do not react to allergens, says immunologist David Huston at Baylor College of Medicine in Houston. This suggests several possible approaches to asthma treatments.

Two pharmaceutical companies, Schering-Plough and SmithKline Beecham, have been working on the development of human versions of mouse anti–Il-5 antibodies, and have been getting promising results in trials with animals—including primates. In the animal trials, the anti–Il-5 antibodies have prevented both eosinophil inflammation and airway constriction. Human trials "are imminent," Huston says.

In addition, Huston and his colleagues, as well as a number of industry groups, are working to engineer an inactive version of Il-5 itself that would bind to the cytokine's receptor on eosinophils without triggering the cells, while also preventing the native molecule from binding.

Perhaps closer to pharmacy shelves is an antibody that blocks IgE itself. After T_H2 signals trigger B-cell production of an IgE with a particular specificity, the antibody attaches to mast cells, and when it encounters a protein it recognizes as threatening, it triggers the mast cells to unleash their weapons, including leukotrienes and histamine. If there were some way to block the IgE trigger, researchers reasoned, the whole battle could be avoided.

But disarming IgE has proved to be a tricky business. When researchers tried to inactivate it with antibodies, some of their efforts turned out to have just the opposite effect, even triggering fatal allergic reactions. "I've accidentally killed animals with [the wrong kind of] anti-IgE," says Paula Jardieu of Genentech, who has led her company's efforts to develop the therapy. The problem was that these antibodies attached to the same part of IgE that binds the allergen, thus triggering, rather than blocking, IgE's effects on mast cells.


STEVE KAGAN

Terrible trio. House dust mite *(top left)*, *Alternaria* mold *(center)*, and birch pollen *(left)* are all common triggers of asthma attacks.

Recently, however, researchers have identified the specific region of IgE that binds to the mast cell receptor, enabling them to produce antibodies that block only that site. Buoyed by promising results in mice, they went on to build a human version of the mouse antibody. Through DNA manipulation, they were able to transplant the IgE-binding region of the mouse molecule onto the base of a human antibody. They tested the resulting "humanized" antibody by giving it to monkeys allergic to ragweed and found that it prevented the typical skin sensitivity to the pollen.

Initial trials, designed to test the safety of this antibody in humans, have been very positive, says Jardieu. The 40 patients who received doses of the antibody suffered only mild reactions when the research team blew allergens into their lungs, with "absolutely no indication of any side effects," Jardieu says. Results from a second round of trials, which tested the antibody's ability to protect 400 asthma patients from natural exposures to allergens, should be published later this summer, says Jardieu, and she expects phase III trials—testing anti-IgE against the best available treatment—to begin this fall.

Tipping the balance against asthma

Another therapeutic strategy currently being investigated aims to short-circuit misplaced T_H2 attacks. T_H1 and T_H2 activities are mutually suppressive: Signals from one cell type inhibit the activity of the other. So several researchers are attempting to take advantage of certain bacteria that induce vigorous T_H1 responses, causing the immune system to pump out messengers, such as interleukin-12 and interferon-γ, that inhibit T_H2 cell activity.

Some, including Steven Holgate of Southampton University in the United Kingdom, Julian Hopkin of Oxford University, and Graham Rook of University College, London, are working with whole bacteria. They have just begun a series of studies in which they will attempt to protect allergic volunteers from the perils of allergy season by injecting them with a harmless bacterium of the *Mycobacterium* genus, which—like many bacteria—is a strong T_H1 inducer. The hope, says Holgate, is that "if we give this to asthmatic subjects, maybe it can switch off the allergies."

Immunologists found a few years ago that it is particular sequences in the bacterial DNA that induce such a strong T_H1 response. Those sequences play a key role in a therapy under development by immunologists Eyal Raz and Dennis Carson and allergist David Broide of the University of California, San Diego. The team is attempting to devise a more effective means of desensitizing people to their allergies, which currently involves repeatedly injecting them with small amounts of the allergen, often for years. To bolster this effect, the researchers have designed a small circular piece of DNA, called a plasmid, that includes both the DNA encoding any of several common allergen proteins and fragments of bacterial DNA.

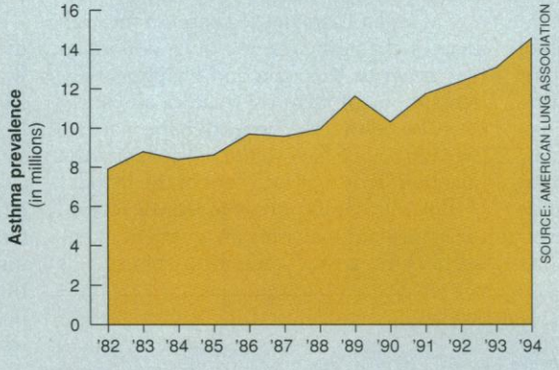
In early tests, the team injected the plasmids into mice, whose skin cells took up the DNA. There the plasmid started producing the antigen protein. "It's like immunotherapy," says Raz, "but instead of having to give it repeatedly, you give it only twice or three times and it is there permanently." At the same time, the researchers hoped, the bacterial DNA in the plasmids would crank up the suppressive effects of the treatment by

Why the Rise in Asthma Cases?

Asthma is a disease of the industrialized 20th century. First described in the mid-1800s, it may have existed before that time, but was very rare. It is still rare in developing countries. But in the developed world in the last 2 decades, asthma rates have skyrocketed—doubling in the United States since 1980. "Asthma and allergies have become representative of the westernization of our society," says William Busse, an allergist at the University of Wisconsin, Madison.

Researchers do not yet know why. They have come a long way in dissecting the sequence of events that leads to individual asthma attacks: the activation by an allergen or other trigger of certain immune cells, which in turn marshal other cells that mount inflammatory attacks on the lungs (see main text). But why some people are predisposed to such attacks—and why their numbers are now increasing—remain mysteries, although researchers have some clues.

Increased exposure to environmental allergens and immune system changes due to fewer childhood infections may play a role, say some. And geneticists are closing in on a host of genes that have been linked to increased asthma susceptibility. The search for a cause is urgent, says Busse, because it might point to ways of preventing children from developing the disease in the first place. For the moment, he says, "we are treating the consequences of the disease, not preventing it from occurring."



Asthma ascending. Researchers are struggling to explain asthma's dramatic increase.

One of the most popular theories holds that asthma has increased partly because of greater exposure to allergens such as house dust mites or cockroaches. Allergist Thomas Platts-Mills of the University of Virginia notes that nowadays children spend more time indoors in front of the television in close contact with carpets and upholstered furniture crawling with dust mites. Still, Platts-Mills says, this "Annette Funicello" effect, as he calls it, "can't explain the rise by itself." The asthma increase is just too great and has occurred even in dry regions where the dust mite is uncommon.

Another feature of modern life might also be contributing: the fall in childhood infections. Early infections, say proponents of this idea, may stimulate a kind of immune response that suppresses later allergic reactions. Earlier this year, Oxford pulmonologist Julian Hopkin and colleagues at the Wakayama Medical Center in Wakayama, Japan, found that children who responded strongly to a skin test indicating that they had been exposed to tuberculosis are less likely to suffer from asthma or other allergic diseases (*Science*, 3 January, p. 77). Similarly, in a study of 1600 Italian soldiers, Paolo Matricardi of the Laboratory of Immunology and Allergy in Rome and his colleagues found that soldiers who tested positive for antibodies to hepatitis A virus—a sign of more childhood infections in general, say the authors—had significantly fewer allergies. (The results appeared in the April *British Medical Journal*.)

Researchers have also turned up other hints that early immunological experience can affect a child's chances of developing asthma—very early experience, if Jill Warner at the University at Southampton in the United Kingdom is right. When she and her colleagues studied immune cells from premature and terminated fetuses, they found that cells from fetuses as young as 22 weeks could multiply when exposed to house dust mites and birch pollen—suggesting that they recognized the allergens from a previous exposure. Warner is currently studying whether limiting a mother's exposure to common allergens can protect her unborn child from later developing allergies and asthma.

Still, environmental influences can't be the full answer, because asthma susceptibility is well known to run in families. A number of all-out hunts are now under way for asthma-susceptibility genes, which might be interacting with environmental factors to drive the rising incidence. So far, only one team—at Sequana Therapeutics Inc. in San Diego—says it has pinpointed a gene, and team members are keeping details of their find under wraps (*Science*, 30 May 1997, p. 1327). But several more public searches are closing in on genes.

A team led by Carol Ober, a geneticist at the University of Chicago, reported at the recent American Thoracic Society meeting that its work with the South Dakota Hutterites, a religious group of 5000 descended from 64 18th-century ancestors, has linked asthma or asthmalike conditions to specific regions on chromosomes 2, 13, and 21. And in a wider study of the general population, the multicenter Collaborative Study on the Genetics of Asthma reported in the April issue of *Nature Genetics* that its researchers have linked asthma in various ethnic groups to a half-dozen different chromosome regions. Other studies have found linkages to regions on chromosomes 11 and 12 containing genes known to code for important players in the inflammation that is part of asthma pathology.

The linkages, like all the other clues, are a long way from solving the asthma riddle, but they are a start. "Everyone knew [the gene search] was a black hole," says Susan Banks-Schlagel, manager of asthma research at the National Heart, Lung, and Blood Institute. "They said, 'Oh, you'll never find anything.' But some interesting things are starting to happen." –G.V.

eliciting production of interferon-γ and other T_H2 suppressors.

Again, initial results are promising. Mice receiving the novel immunotherapy have less IgE in their blood, fewer eosinophils in their lungs, and less evidence of T_H2-type cytokines when they are exposed to the substance to which they were allergic. Raz and his colleagues have formed a company, called Dynavax, and plan to begin human trials in collaboration with researchers at Johns Hopkins University as soon as they receive FDA approval—expected "within the year," says Raz.

But the T_H2 model that has inspired these new treatments may not be a complete answer to the asthma puzzle. Viral infections, for example, have been blamed for 80% of severe asthma attacks, says Daniel Rotrosen of the National Institute of Allergy and Infectious Diseases. But viruses have usually

been considered a trigger of a T_H1-type response. Some researchers believe that viruses are not the immediate trigger, but contribute to asthma susceptibility by attacking the lining of the lungs, leaving the inner layers more exposed to environmental allergens or other traditional asthma triggers—which would then activate T_H2 cells and the other responses they orchestrate. Others believe the viruses may have an inside role, activating certain genes in the nucleus that exacerbate or trigger the inflammatory cascade.

Those unanswered questions might explain why new treatments such as the leukotriene inhibitors will not work for everyone. But the fact that the drugs don't help some patients may be as important as the help they do give some people: "That is where it gets really interesting," Drazen says. "Up until now, we have graded asthma as mild, moderate or severe," which is only of limited help to physicians trying to determine the best course of treatment.

Patients' different responses to the various drugs may help doctors sort out what many suspect is the case: Asthma is not a single disease. Like pneumonia or anemia, Brigham and Women's Drazen says, asthma is a set of symptoms that has varied causes. The new treatments, by getting closer to those causes, may help doctors divide patients into subgroups based on how they respond to treatments, he adds. That, in turn, will help researchers determine how to treat each patient most effectively—a development, certainly, that will help millions breathe easier.

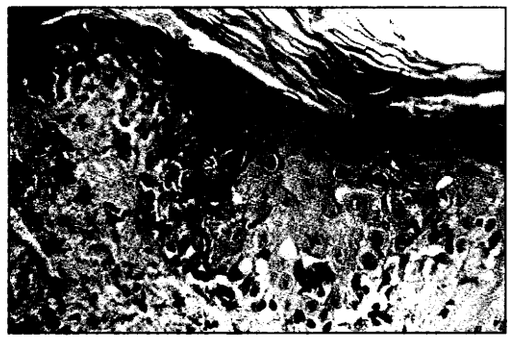
–Gretchen Vogel

IMMUNOLOGY

New Lead to Safer Marrow Transplants

Bone-marrow transplants have become a mainstay of medicine's battle against blood-cell cancers, such as leukemias and lymphomas, as well as against certain noncancerous blood diseases. But in at least half of all patients, the donor immune cells turn against the recipient's own tissues, triggering a deadly ailment called graft-versus-host disease (GVHD). Now a team of doctors led by hematologist Claudio Bordignon at the San Raffaele Scientific Institute in Milan, Italy, may have found a solution to this problem.

On page 1719, the group reports the first successful human test of a gene therapy designed to halt the attack of the donated cells on the recipient's tissues. The researchers genetically engineered the transplanted cells with a self-destruct button that enables doctors to kill them selectively with a drug if they turn mutinous. This allowed the team to wipe out GVHD in two of the three patients who developed it, and partially eliminate it in the third—without using immunosuppressive drugs.



Under attack. Multiple lymphocytes are invading the epidermis of human skin with graft-versus-host disease.

GEORGE SALE/FRED HUTCHINSON CANCER RESEARCH CENTER

That success is a boost for the struggling field of genetic therapy, says immunologist Drew Pardoll of the Johns Hopkins University School of Medicine in Baltimore, who calls the work "one of a very small cohort of examples in which gene therapy has been shown to have clinical utility." Indeed, if further studies bear out the early promise of the technique, it could make bone-marrow transplants much safer and more effective. Doctors might even start using such transplants more broadly, in patients with less advanced disease. The technique is "very exciting," says immunologist Philip Greenberg of the Fred Hutchinson Cancer Research Center in Seattle. "It has the potential to improve substantially the outcome of [bone-marrow] transplantation."

The strategy's seeds were planted in 1990, when Bordignon first heard about the problems with GVHD that were cropping up in the top bone-marrow transplant centers, particularly in patients who relapsed and required infusions of donor lymphocytes. Marrow transplants are needed because the high doses of chemotherapeutic drugs and radiation given to leukemia and lymphoma patients in an effort to rid them of all cancer cells also destroy the patients' bone marrow, the vital source of both the red cells and the infection-fighting white cells of the blood.

But unless the donor is an identical twin, the transplant may turn on a patient, causing GVHD, as the foreign white blood cells attack essential organs such as the liver, gut, and skin. Clinicians have sought to avoid this attack by sifting out all of the mature T lymphocytes from the foreign marrow before infusing it. Those are the cells that trigger GVHD, but their removal leaves the patient more vulnerable to infections or cancer relapse. If infection or cancer does develop, the patient can be infused with the donor T cells—again running the risk of GVHD.

Bordignon, a doctor trained in gene therapy, recalls that he asked himself, "How might one take advantage of gene-transfer technology to control this problem?" He set out in early 1992 to test whether he could introduce a "suicide gene" into these cells, then use the gene to kill the cells if they triggered GVHD. Results with cultured cells looked promising: Lymphocytes engineered with the gene for the enzyme thymidine kinase died when he doused them with the antiviral drug ganciclovir, which the enzyme converts to a deadly poison.

After showing that ganciclovir also kills the suicide gene–bearing lymphocytes in mice, Bordignon and colleagues began their pilot study in humans. In 1993, they infused donor lymphocytes bearing the thymidine kinase or suicide gene into 12 patients who, after receiving bone-marrow transplants, had suffered complications such as cancer relapse or virus-induced lymphomas. The lymphocytes survived in the patients for up to a year, battling the tumors to achieve complete or partial remissions in five of the eight patients for whom results are available.

Of the three patients who developed GVHD, ganciclovir totally shut down the immune attack in two; in the third, the disease was attenuated. The success may have been limited in the third patient, Greenberg speculates, because some of the infused lymphocytes may not have borne the suicide gene.

Still, if the new gene-therapy procedure helps two out of every three patients, it will be an improvement. Researchers caution, however, that tests in many more patients will be needed to determine just how effective the therapy is. Toward this end, Bordignon is organizing a multicenter European trial that he hopes will start by the end of 1997. But even that may not settle the question, says Pardoll, because transporting the Italian group's technique to other centers may be difficult: "I can count on one hand, with a couple of fingers missing, the number of groups that could do this [gene-transfer procedure] with high efficiency." He adds, however, that developing simple, reproducible protocols for the procedure could boost that number.

One thing is certain. The therapy has already shown sufficient promise, says Richard O'Reilly, a marrow-transplant pioneer at New York City's Memorial Sloan-Kettering Cancer Center, to ensure that it "will be looked at by many people."

–Ingrid Wickelgren