For every move by the male to increase his success at the expense of the female, she would normally make a countermove. But when the females' countermoves were blocked, the males' interest prevailed. The findings complement long-standing evidence for behavioral warfare between the sexes, such as forced copulations in ducks and deceitful matings in some primates, notes John Alcock, a behavioral ecologist at Arizona State University in Tempe: "This study demonstrates the level of chemical warfare that exists as well."

The quick genetic response of the evolving males to the sitting-duck females also "shows the speed with which males can exploit females for their own reproductive advantage," says Alcock. And Howard suggests that the speedy evolutionary change means that "barriers to fertilization [between species] can also arise quickly. In fact, you can see such a barrier in its initial stages right there in Rice's experiment." Because the supermales fertilized most of the eggs, normal males were effectively shut out and no longer contributed their genes to the population. When genes don't move freely between groups, the populations are considered reproductively isolated—and thus separate species. Indeed, the reproductive tract right after insemination may be the setting where barriers to gene flow first arise, say Howard, Rice, and many other researchers in evolutionary biology and genetics. "Intersexual competition can thus be seen as a major engine of speciation," says Rice.

But although Rice's demonstration of the evolutionary duel between the sexes wins plaudits all around, not everyone agrees that this intersexual warfare is driving speciation. For example, Chung-I Wu, an evolutionary geneticist at the University of Chicago, puts his money on male-male competition as having a bigger role in speciation, because male reproductive traits evolve faster. "The genetic blueprint for males to make sperm changes at a much more dramatic rate than do equivalent genes in females. It's 10 times faster," he notes. Counters Rice, "Even if sexual coevolution only contributes 10% to the process, that's still significant."

To show that the female's response is also an important factor in driving evolutionary change, Rice may have to reverse his experiment, and hold the males in check and let the females evolve. Sexual conflict theory more or less predicts that the females will turn into the equivalent of fruit fly Ice Queens, mating with only one or two males to limit the amount of toxins they receive and to get "just enough sperm to get the job done," Rice speculates. Will the male partners of such superfemales also meet an early death? Stay tuned: The battle of the sexes is far from over.

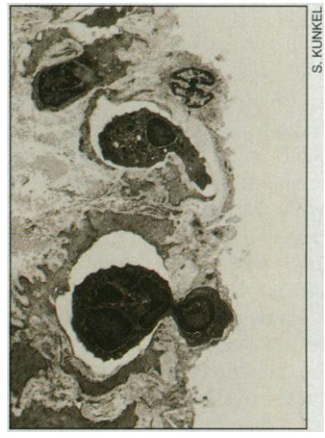
**–Virginia Morell**

IMMUNOLOGY

# Chemokines Take Center Stage in Inflammatory Ills

As any actor knows, becoming a star often requires more than just good looks and talent. Being "discovered" can also take a lucky break. That's true even for biomedicine's stars, such as proteins that may hold the key to new therapies. Take the chemokines, a family of proteins that act as magnets for white blood cells and thus play a key role in eliciting inflammatory responses.

Although immunologists discovered the first chemokine nearly a decade ago, the proteins did not take center stage until late last year when Robert Gallo's group at the National Cancer Institute (NCI) found that certain chemokines suppress production of the AIDS virus, raising the possibility that they might be used to treat the immunodeficiency disease (*Science*, 15 December 1995, p. 1811). "Before chemokines were associated with AIDS, the field was restricted to a small group of investigators doing a very good job of investigating [the proteins]," says Anthony Fauci, chief of the National Institute of Allergy and Infectious Diseases (NIAID). "Now, all of a sudden, everybody is talking about chemokines." They are finding a lot to talk about.




**Tight squeeze.** Neutrophils leave a blood vessel, presumably in response to a chemokine.

Work reported just last week, for example, suggests an explanation for how the chemokines inhibit AIDS virus reproduction: They may bind to and block a cell-surface protein that the virus must latch onto in order to get into cells (*Science*, 10 May, pp. 809 and 872). But the proteins usually play a different, more widespread role in the body.

Chemokines belong to a large class of intracellular messengers called cytokines that carry regulatory signals from cell to cell. But unlike some better known cytokines, such as interleukin-1 and -2 (IL-1 and -2), which act early in immune responses and tend to activate many kinds of white blood cells, chemokines come into play later and appear to have a much more specialized role in attracting inflammatory cells to damaged or infected areas. "Chemokines are key to mobilizing [white blood cells] to sites of inflammation," says Joost Oppenheim, whose team at the NCI co-discovered the first two true chemokine molecules. "They are a fundamental part of the inflammatory host defenses."

That puts them in the frontlines of the body's defenses against a wide variety of invading pathogens in addition to HIV. These include numerous other viruses, bacteria, and parasites such as the malaria-causing *Plasmodium vivax*. Just how chemokines work against these other invaders varies, sometimes dramatically, from pathogen to pathogen, but in general, the chemical messengers pull white blood cells out of the bloodstream, trigger some of them to spew out a potent mix of digestive agents that kill or maim live pathogens, and encourage others to gobble up the remains of tissue damaged by infection or injury. These actions cause the characteristic redness, soreness, and other symptoms of inflammation, which is normally a protective response, causing only limited damage in the interests of clearing up an infection.

But these initially protective chemokine effects can turn into an overzealous attack on healthy tissue, contributing to the damage in a wide range of inflammatory diseases, including short-term conditions like septic shock and persistent disorders such as rheumatoid arthritis. "The response may backfire and cause a lot of symptoms of illness. These are illnesses caused by overenthusiastic inflammatory and immune responses," Oppenheim says.

This dual role of the chemokines, as both fighters and perpetrators of disease, has caught the eye of the pharmaceutical industry. Dozens of companies, ranging from small biotech firms such as LeukoSite Inc. and Repligen Corp., both located in Cambridge, Massachusetts, to industry giants such as San Francisco–based Genentech, have joined the chemokine hunt in the hopes of finding novel therapeutic drugs.

Although researchers hope that the chemoattractors themselves might be useful in AIDS therapy, most current drug development efforts have been spurred by animal studies indicating that chemokine inhibitors may be useful for blocking the destructive inflammation in pneumonia, asthma, and other lung conditions as well as in arthritis and various cardiovascular diseases. "There is an enormous effort by pharmaceutical companies on this," says Charles Mackay,

director of immunology at LeukoSite. "There are more than 20 programs by major pharmaceuticals on [the chemokines] MCP-1 and IL-8 alone."

**Discovery and disillusion.** One reason for the enthusiasm is that, at least at first, the molecules appeared to be very picky about the type of white blood cells they attract. In fact, the first indications that chemokine signaling might be so specialized came more than a century ago when researchers began linking different illnesses with characteristic immune cell types.

Immunology pioneers found, for example, that the damage done to the joints of rheumatoid arthritis patients is perpetrated mainly by neutrophils, whose normal job is to destroy invading bacteria. The lung-clogging inflammation of pneumonia is caused by another cell type, the monocytes, which ordinarily help rid tissues of intracellular pathogens, including the influenza virus and the tuberculosis-causing bacterium, as well as clearing out dead or dying cells. "These [white blood cell] patterns were defined pathologically years ago, but nobody knew the factors that caused the patterns," says immunologist and molecular biologist Phil Murphy, who studies chemokines and their receptors at NIAID.

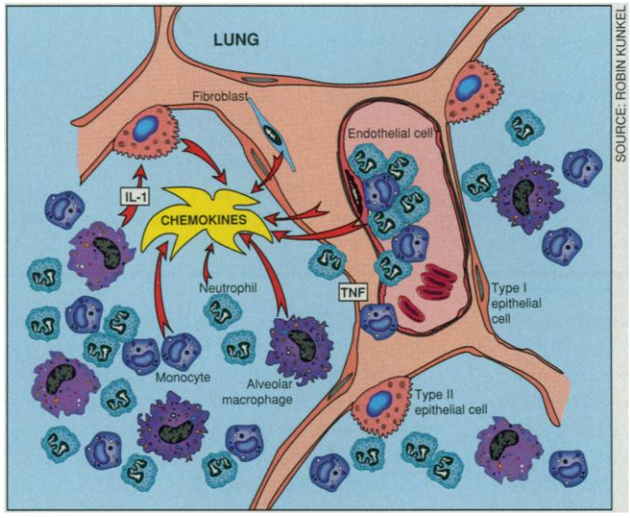
Early attempts to find the chemical signals that could attract one immune cell type without pulling in others were not successful. It wasn't until the late 1980s that two collaborating teams of researchers at the NCI stumbled onto the first one.

Chemokines can be made by "almost any nucleated cell" in response to infection or other tissue insult, Oppenheim says. This includes inflammatory cells themselves. As the first cells move into an area of tissue damage in response to a chemokine signal, they may make either other chemokine family members or more of the same chemokine that initially brought them in. This both boosts the inflammatory attack and customizes it to the particular foreign invader.

In 1987, the two NCI teams—one including Oppenheim and his colleague Kouji Matsushima, and the other led by Teizo Yoshimuro and Ed Leonard—were trying to pin down the identity of a chemoattractive protein released by monocytes that appeared selective for neutrophils. At the time, they thought it was almost certainly IL-1. But, as Oppenheim recalls, "we kept getting less [neutrophil-attracting] activity the more we purified IL-1. Then we realized the neutrophil chemotactic activity was separate from IL-1." Ultimately, the researchers purified the correct protein, which was eventually called interleukin-8 (IL-8).

After cloning and sequencing IL-8's gene, the researchers then returned to their same mixtures of monocyte products in hopes of finding an analogous chemoattractor for the monocytes themselves. But, because the two teams had split up—"you know how these things sometimes go," Oppenheim says—the hunt for the monocyte selective factor became a contest rather than a collaboration. It finished in a dead heat in 1989, when both teams simultaneously purified a protein that was later named monocyte chemoattractant protein-1 (MCP-1).

As a result of these discoveries, says NIAID's Murphy, "everyone began jumping up and down," touting the chemoattractants as a new breed of apparently selective immunoregulatory molecules. Their selectivity excited researchers because it seemed that a drug that blocked a single chemokine might inhibit just the cells causing a specific inflammatory response—say the neutrophils in rheumatoid arthritis—without upsetting the rest of the immune system. In addition, the discovery of IL-8 and MCP-1 touched off a massive hunt for additional chemokines, as researchers could now use segments of the first two genes as probes for fishing out the genes for related proteins.




**Potent signals.** The chemokines that draw inflammatory cells like neutrophils and monocytes into tissues can be made by many cells, including the inflammatory cells themselves.

The search has paid off—the total number of chemokines now stands just short of 30. "This is the biggest family of cytokine genes," Murphy says. And the quest may not be over, he adds: "We're still trying to figure out how big the [chemokine] family really is."

But as the chemokine list grew, researchers made a discovery that dampened their enthusiasm—at least temporarily—for the idea of trying to design chemokine inhibitors: Chemokine communication turned out to be much less selective than originally thought. Not only could the same white blood cell be attracted by several different chemokines, but, says Caroline Hébert, a research scientist at Genentech, "a particular chemokine may act on several types of cells." For example, MIP-1α (which stands for macrophage inflammatory protein-1α) recruits at least six other white blood cell subgroups and two noninflammatory cell types in addition to macrophages. Even IL-8 proved to be less than totally selective.

The Oppenheim team found that IL-8 recruits T cells, although in lower numbers than the neutrophils, and Leonard showed that it could also recruit basophils, white blood cells important in allergies. "Selecting the most promising therapeutic target can be difficult," says Hébert, who is currently studying the role of IL-8 in lung injuries. Similarly, researchers found that in animals, multiple chemokines are at work in many, if not all, inflammatory conditions.

Steve Kunkel, a biochemist at the University of Michigan, Ann Arbor, and co-discoverer of a chemokine known as ENA-78, has found in both laboratory culture and animal studies, for example, that during the development of persistent inflammatory diseases such as rheumatoid arthritis, as many as half a dozen chemokines may come into play, recruiting several types of white blood cells in characteristic patterns. "There is a road map or cascade for the response," Kunkel says. "It ultimately leads to the progression and maintenance of chronic inflammation."

The discovery of all this redundancy and complexity contributed to what Murphy calls "midterm pessimism" in the chemokine field. It raised worries that a specific chemokine inhibitor might either have no effect, because other chemokines could take over for the one whose action was blocked, or, alternatively, that the inhibitor would do too much, by blocking the response of classes of cells other than the intended target, perhaps leaving the patient open to infections.

**Renewed optimism.** Despite these concerns, however, optimism is slowly reinfusing the field. One reason is the discovery of a chemokine called eotaxin that appears to buck the nonspecificity trend. Eotaxin was originally detected about 3 years ago by Timothy Williams, of the National Heart and Lung Institute in London, as a chemoattractor for the white blood cells called eosinophils. And it may be clinically important as a cause of lung damage in asthma patients.

Mackay and his colleagues at LeukoSite have preliminary evidence that in asthma, a signaling mechanism involving eotaxin summons eosinophils into the lung airways.

There they damage healthy tissue by releasing toxins stored inside tiny intracellular granules. And the eotaxin signal appears to be specific, Mackay's group reported earlier this year.

They could find the receptor through which eotaxin exerts its effects only on eosinophils, unlike the receptors of nonspecific chemokines such as IL-8 and MCP-1, which are found on multiple cell types. Those findings imply, says Mackay, that eotaxin-blocking drugs might benefit asthma patients while having relatively few side effects on their immune systems. Indeed, Genentech's Hébert calls eotaxin "the hottest new target for drug development."

Another reason for renewed excitement about chemokines is evidence from animal studies that blocking chemokines can still have significant anti-inflammatory effects in spite of the redundancy. Normal mice infected with coxsackievirus develop a serious—sometimes fatal—inflammation of the heart, for example, while those infected with influenza virus develop pneumonia.

But when a team led by Oliver Smithies at the University of North Carolina genetically engineered mice in which the gene for MIP-1α was inactivated, or "knocked out," and then infected the animals with either of the two viruses, the animals came down with little or no heart or lung inflammation. They were protected even though the infected tissues' primary inflammatory cells release many other chemokines besides MIP-1α. "When you go in vivo and take out a single chemokine gene, you might predict that nothing happens due to the redundancy," says Murphy, "but that is not, in fact, what happens." Murphy and others say they are not sure why knocking out just one chemokine has such a drastic effect. "One possibility is that we just don't know enough about the system," he says.

The Smithies team's knockout experiment was encouraging in another way as well, for the animals lacking MIP-1α were still able to purge the virus from their tissues and did not seem to suffer serious problems as a result. "The viral [numbers] came down a little slower in the mutants," says Smithies. "But the animals still recovered. Thus, if one could block MIP-1α with a suitable drug, one might be able to protect the host from the overall [inflammatory] effect."

Blocking a single chemokine can apparently help in another condition as well: adult respiratory distress syndrome (ARDS), a currently untreatable condition that kills roughly half of the 200,000 patients who develop it every year in the United States. In ARDS, the lungs fill with fluid as a complication of any of a variety of insults such as sepsis, severe trauma, pneumonia, or aspiration of the stomach contents into the lungs, which is a particular problem for patients on respirators.

Neutrophils have been implicated in the development of the condition. "In ARDS there is such massive damage to lung tissue by neutrophils that plasma leaks into the alveolar space," says Hébert. As a result, patients are not able to absorb oxygen from the blood.

Although neutrophils can be attracted by any of a half dozen chemokines, researchers, including Hébert and her colleagues at the University of California, San Francisco, have shown in animal studies that it's possible to reduce the neutrophil influx with antibodies to IL-8. In a rabbit model of ARDS, for example, the antibodies suppressed neutrophil-induced lung inflammation in up to 75% of the animals and eliminated their mortality. "Just because other chemokines are found at the scene of the crime does not mean that they are guilty," Hébert says. "If we block IL-8, that is enough to reduce inflammation."

**SOME INFLAMMATORY CELLS AND THEIR CHEMOKINES**

| Cell | Normal Function | Disease Involvement | Important Chemokines |
|---|---|---|---|
| Basophils | Release histamine, serotonin | Allergies | IL-8, MIP-1α MCP-1, -3, RANTES |
| Eosinophils | Destroy parasites | Asthma | IL-8, MIP-1α, MCP-3, RANTES, eotaxin |
| Neutrophils | Destroy bacteria | Rheumatoid arthritis, adult respiratory distress syndrome, reperfusion injury | IL-8, Groα, Groβ, -γ, ENA-78, others |
| Monocytes | Digest invading microorganisms and old, dying cells | Pneumonia, atherosclerosis | MIP-1α, MIP-1β, MCP-1, -2, -3, others |

**Disease role broadens.** Even as researchers find glimmerings that inhibiting chemokine action may aid in treating inflammatory conditions, the list of other diseases to which the molecules might contribute continues to grow. Among them are Alzheimer's disease, cancer, and atherosclerosis.

Some of the evidence linking chemokines to Alzheimer's comes from Bruce Gitter's group at Eli Lilly and Co. in Indianapolis. They've found that β-amyloid, a small protein that's a major suspect as a cause of the nerve cell degeneration of the disease, can, in conjunction with IL-1, stimulate IL-8 production by astrocytes, which are the brain's equivalent of monocytes. It's not yet clear what role, if any, the IL-8 might be playing in Alzheimer's.

One possibility is that IL-8 draws in inflammatory cells, because there is some evidence that inflammation can contribute to Alzheimer's pathology (*Science*, 18 June 1993, p. 1719). Or IL-8 may act directly on brain neurons themselves in some not-yet-understood way. Evidence that neurons might be IL-8 targets comes from the teams of Richard Horuk at Berlex, a Richmond, California–based biotech company, and Steven Pieper at the University of Louisville in Kentucky, who have detected IL-8 receptors in cells that may be degenerating neurons in Alzheimer's brains.

The cancer connection comes partly from Robert Strieter at the University of Michigan, Ann Arbor, who has evidence linking some chemokines to angiogenesis, the formation of new blood vessels needed for tumor growth. He and his colleagues showed that certain chemokines, including IL-8, stimulate blood-vessel sprouting in the corneas of rabbits and in laboratory cultures, presumably by acting on the endothelial cells needed to construct blood vessels. Strieter also showed that tumors such as human non–small cell lung cancer make the chemokine. If the presence of IL-8 does in fact help tumors grow, then blocking the angiogenic function of chemokines might then be a way of thwarting tumor growth by starving the malignant tissue of nutrient-supplying blood vessels.

"The simple message is that chemokines are going to be important far beyond inflammation and response to viral infection," says virologist Grant McFadden of the University of Alberta in Edmonton. "In the next few years, I think we will realize how much we have underappreciated the importance of these molecules." For the chemokines' sake, it is a shame that scientists don't award Oscars.

**–Trisha Gura**

*Trisha Gura is a reporter on leave from the* Chicago Tribune.

Additional Reading

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