Number 12 Steps up to Bat

Will this immune system messenger hit a grand slam?

By ELIZABETH PENNISI

In Africa, a teenager first notices he is sick when he begins spitting up blood. Like 200 million other people worldwide, he suffers from schistosomiasis, a chronic infection caused by a parasitic worm. Eventually, the young man's liver fails and he dies, one of 800,000 such fatalities each year.

Thousands of miles away, mourners gather to pay their last respects to a grandmother who died of cancer. In the United States alone, 500,000 people a year suffer a similar fate.

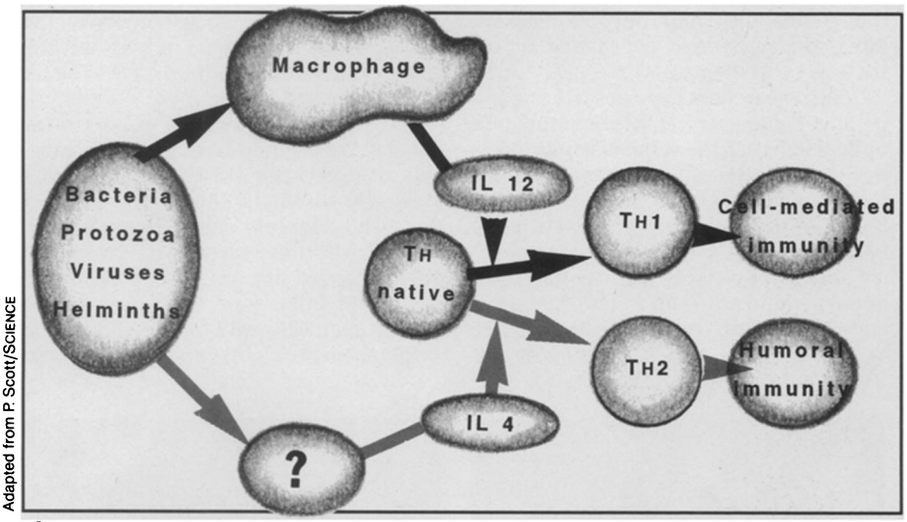
In Massachusetts, a brother stitches a quilt square for his sister, dead of AIDS. He will add this needlework to the NAMES Project quilt commemorating those lost to this modern epidemic. Worldwide, an estimated 16 million people carry HIV, the AIDS virus; 939,500 have been diagnosed with AIDS.

These three very different diseases may one day face a common adversary.

In addition, some researchers hope to harness this molecule's apparent power to make many kinds of vaccines more effective and to fight allergies. On the flip side, blocking this interleukin's activity may prevent the devastating symptoms of septic shock. "It seems to have possibilities for use in quite a wide spectrum of disorders," says Maurice K. Gately of Hoffman-La Roche in Nutley, N.J.

IL12 isn't the first messenger molecule to raise the hope of a wonder drug. Early in the biotechnology revolution, interferon made headlines as a possible panacea. More recently, interleukin 2 (IL2) engendered enthusiastic support. Neither has lived up to researchers' initial expectations.

Even if the new interleukin, too, falls short of its therapeutic potential, it nevertheless "is creating a huge stir in immunology," says Thomas A. Wynn, a molecular immunologist at the National Institute of Allergy and Infectious Diseases (NIAID) in Bethesda, Md.



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***A**lien organisms either stimulate macrophages to make IL12, which causes T helper (TH1) cells to activate a cell-mediated response, or cause IL4 to rev up TH2 cells for increased antibody production, part of humoral immunity.*

Laboratory tests with a recently discovered immune system messenger called interleukin 12 (IL12) indicate that this one molecule may effectively combat AIDS, cancer, and many parasitic infections. IL12 can reduce the inflammation caused by the schistosome worm, activate intrinsic anticancer defenses, and restore immune system function in cells infected with the AIDS virus.

Researchers have named the 15 known interleukins in the order in which they were discovered. To immunologists, each new one represents another piece of the incredibly complex puzzle called the immune system.

These molecules signal some white cells, or lymphocytes, to proliferate; yet they may inhibit the replication of others. They sometimes do this by altering the production or efficiency of other messenger molecules, also called cytokines.

IL12 was first discovered in the 1980s by two immunologists, neither of whom recognized it immediately for what it was. Gately was scrambling to find a substance that would rev up the immune system much as IL2 does, but without the toxic effects that had stalled development of IL2 as a potential anticancer tool. He called his group's discovery "cytotoxic lymphocyte maturation factor" because of the effects they saw.

Meanwhile, the other discoverer, Giorgio Trinchieri of the University of Pennsylvania School of Medicine in Philadelphia, had dubbed it "natural killer cell stimulatory factor."

At first, this compound didn't seem that special compared to other immune messengers, in part because it took a while for researchers to get enough of the material to study it thoroughly. "It was very nontrivial to get it purified," says Stanley F. Wolf of Genetics Institute in Cambridge, Mass. He worked with Trinchieri to determine the compound's amino acid sequence, and with that information they were able to figure out its genetic code. To their surprise, two genes hold the code for what would later be named IL12.

All other known cytokines are the product of just one gene, even if they consist of two amino acid chains twisted or knotted together. But IL12's two chains — called p35 and p40 for the number of amino acids in each — arise independently and "must then find each other in the cell," Wolf explains.

Also, the p35 chain exists in many kinds of cells, whereas only certain cells make p40 chains — and then only under certain conditions, says Wolf. Some white cells — specifically, B cells and macrophages — produce lots of p40 in response to bacterial infection, then join some to p35 to make IL12, Wolf and his colleagues reported in March at a meeting on IL12 held in New York City.

The lighter chain resembles other cytokines, whereas p40 looks like a section of a cell's molecular docking sites for cytokines. Wolf thinks IL12 evolved when this section escaped from cells and

merged with a primitive cytokine. Somehow, the pair got drafted as an immune messenger.

Even before Gately, Trinchieri, Wolf, and others picked up the IL12 scent, researchers were hot on the trail of T cells, discovering just how diverse these white cells could be. They had known about T helper cells, so called because they prod other lymphocytes to act, and cytotoxic T cells, which attack and destroy undesirable cells. They knew, too, that different T cells wear identifying molecules, called receptors, on their surfaces and that many respond to just a single foreign substance, or antigen, in the body. The lymphocytes made famous by AIDS, for example, are CD4 T helpers, which carry CD4 molecules.

Then in 1986, scientists at the DNAX Research Institute in Palo Alto, Calif., noticed that two types of T helper cells exist in mice, each associated with a distinctive bouquet of chemical messengers. Stimulation of the first type, T helper 1 (TH1), led to an increase in interleukin 2 and a messenger called interferon gamma. Activation of the other, T helper 2 (TH2), resulted in the production of interleukins 4, 5, and 10.

Five years later, Sergio Romagnani, a clinical immunologist at the University of Florence in Italy, found the equivalent types in people. He realized that certain infectious agents, such as parasites, stimulated increases in the number and activity of TH2 cells, while some bacteria made populations of TH1 cells thrive. In retrospect, having two kinds of T helper cells makes a lot of sense.

When faced with a foreign invader, the immune system mounts either of two defenses. One, humoral immunity, involves primarily B cells. These white cells recognize a particular antigen, then make antibodies that bind to that molecule. The other depends heavily on T cells – not helper, but cytolytic CD8 T cells – that can destroy tumors or cells infected with viruses or bacteria. These assassins, including natural killer cells, become part of the cell-mediated immune response.

T helper cells are the sergeants that roust T or B cells into action. As helpers form in the thymus, each becomes sensitive to just one antigen trigger. They drift through the bloodstream or hang out in lymph nodes in a "naive" state until they meet the antigen they were primed to recognize. At that moment, a helper cell's fate is sealed as either a TH1 or a TH2, or so some researchers think. If it becomes a TH1, the cell then readies cytolytic T cells to do battle, generating the TH1 response. As a TH2 cell, it initiates humoral immunity, the TH2 response.

"Essentially, it seems like in the Western world, the condition of health is linked to a strong TH1 profile," explains Mario Clerici, an immunologist now at the University of Milan in Italy. These beneficial assassins can destroy a cell that has been tricked into harboring pathogens where antibodies and TH2 components can't get at them.

In contrast, when chronic diseases develop, they often signal a loss of TH1 defenses and an augmentation of TH2 power. Antibodies produced by B cells and their cellular collaborators cause sniffles, rashes, and other symptoms, leading allergy sufferers to wonder: Why have TH2 capability at all?

The answer is unknown. Some researchers suggest that TH2 responses may have helped primitive humans fight off parasites and infections now relatively rare in developed countries. Others theorize that TH2 may serve to keep TH1 in check.

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— M. Lotze

Immunologists don't know exactly what determines a particular helper's destiny. They suspect that these cells take on different roles depending on the chemical mentors they encounter either early in their development or when they are exposed to antigen. They do know, for example, that interleukin 4 stimulates a TH2 response and the subsequent production of antibodies.

Researchers also realized a few years ago that IL2 and interferon gamma help elaborate the TH1 reaction. But they did not know what caused the production of interferon gamma to increase. That's why IL12 proved so exciting. "It is the critical cytokine that's involved in inducing a TH1 response," says Wynn.

The evidence for IL12's role in the immune system comes from several laboratories. Some researchers use mice bred to lack certain immune cells or to be susceptible to particular diseases. Others depend on tests done on immune cells grown in laboratory dishes.

At the Washington University School of Medicine in St. Louis, Kenneth Murphy used a transgenic mouse strain to study the different ways the immune system reacts to an alien cell or substance. He and his colleagues verified that bacterial products stimulate the TH1 response and that this response involves roving scavenger cells called macrophages. They also demonstrated that something the macrophages secrete – and not the cells themselves – causes this response. That something is IL12, they reported last year.

In a test tube, IL12 influences immature T helper cells, causing them to make much more interferon gamma than they would otherwise, says Roberto Manetti of the University of Florence in Italy. After a week of exposure to IL12, cells become "committed" to making this interferon, even without further prodding by IL12, he and his colleagues reported in the April JOURNAL OF EXPERIMENTAL MEDICINE.

By predisposing young cells to become TH1, IL12 can influence the whole tenor of the immune system: When interleukin 12 revs up TH1, TH2 lags. "It's the regulator of TH1 and TH2 balance," Gately explains.

Studies involving the exposure of two strains of mice to the protozoan parasite leishmania drove this point home for Gately and others. Mice of one strain fend off infection by these organisms, mounting a TH1 response. But the other mice augment their TH2 defenses, getting sicker and sicker until they die. Treating that vulnerable strain with IL12 shifts the balance, dampening TH2 while activating TH1. The mice can then fight off the parasite.

IL12 also impresses scientists because it recruits natural killer cells into the fight against disease. Even in mice bred to lack T cells, IL12 could stimulate a protective immune response to another protozoan parasite, *Toxoplasma gondii*, Alan Sher and his colleagues at NIAID discovered. This messenger caused natural killer cells to arm themselves for battle and to increase the production of interferon gamma. That interferon, in turn, stimulated more production of IL12 and suppressed the proliferation of TH2 cells.

Because IL12 stimulates a TH1 response even without T cells around and dampens TH2 reactions, many researchers have set out to determine IL12's role in a variety of diseases.

At the Pittsburgh Cancer Center, Michael T. Lotze and his colleagues knew that the immune system could fight cancer if stimulated properly. They built an artificial gene that carried DNA for both chains of IL12 and tested the IL12 protein in mice with different cancers. "The mice would cure themselves of tumor and reject [new tumors]," says Wolf. "That's

Continued on p.124

Continued from p.121

pretty dramatic." Other research over the past 6 months supports those results.

In May, Lotze began testing IL12's safety in patients with incurable tumors. In June, the National Institutes of Health Recombinant DNA Advisory Committee gave Lotze permission to pursue IL12 gene therapy.

"Of all the cytokines that I have had a chance to evaluate, IL12 appears to have the greatest promise," Lotze says. "I suspect there are going to be a large number of IL12 protocols."

Those protocols already include efforts to stem AIDS. In May, physicians at the Universities of California in Los Angeles and San Francisco began testing the safety of IL12 in 80 people with AIDS. Laboratory data (see sidebar) suggest that IL12 will stimulate a TH1 response and make HIV infection less devastating to the immune system. IL12 does not kill the HIV virus, but it may make the body less susceptible to the cancers and infections that characterize AIDS, the researchers hope.

Even as observations linked AIDS to a disruption of IL12 and T helper balance, other scientists were demonstrating IL12's potential for fighting much larger pathogens.

The eggs from schistosomes can become lodged in blood vessels in key organs, such as the liver. Primarily because of the TH2 immune response, the body walls off these eggs with an aggregation of cells called a granuloma. If the immune system gets carried away, the granuloma can block blood flow and cause problems more serious than the parasitic infection itself, says Wynn.

To try to prevent that overreaction, he and his colleagues injected mice first with eggs and IL12 and then, 4 to 12 weeks later, with just eggs. Much smaller granulomas formed in vaccinated mice than in untreated mice, and in vaccinated mice the aggregates disappeared within 2 weeks, the team reports in the May JOURNAL OF EXPERIMENTAL MEDICINE. They suspect that IL12's stimulation of interferon gamma biases the immune system away from building big granulomas.

Others envision IL12 enhancing the power of vaccines. Most vaccines stimulate the development of antibodies, products of a TH2 response. Those antibodies work best when free-floating toxins or other substances besiege the body. A vaccine that sets off a TH1, cell-mediated response would instead target cells making these toxic materials, says Wolf, thus making the vaccine more protective.

Biasing the body toward stronger TH1 responses may also help control allergies. IL12 treatments could cause the body to mount either no response or just a small TH2 one to pollen, mold, cat proteins, dust, and other allergens.

Dosing the body with IL12 could have a downside. After all, toxicity is what kept messenger IL2 out of the cancer clinic. Some researchers worry that exaggerated cell-mediated immunity could develop into autoimmune disease, in which the immune system attacks the body's own tissues. Or that the lack of TH2 capabilities could have unexpected negative consequences.

"Some of the direct, immediate effects of IL12 may not be the effects you [ultimately] want to get," Trinchieri warns. "Once you get it the wrong way, it may be difficult to reverse the effect."

"That's a concern I have about the hype that's starting to build up about IL12," Gately adds. "Clinical development for any drug is always a long and difficult road. I'd hate to see our hopes build too high."

"At the moment, though, it looks very promising," Trinchieri says. □

■ Boosting TH1: Key to thwarting HIV? ■

The scramble to treat and prevent AIDS has yielded much new knowledge about the immune system but no solution to this worldwide epidemic.

Now, however, some researchers hope that their latest findings about the chemical messenger interleukin 12 (IL12) and two kinds of T cells (TH1 and TH2) involved in immune responses will provide insights that will make a solution possible.

Last year, Gene Shearer and Mario Clerici at the National Cancer Institute (NCI) in Bethesda, Md., found that people infected with HIV showed decreased production of TH1 messenger compounds.

These and other results prompted the two to suggest that AIDS results when HIV shifts the balance between TH1 and TH2 cells to dramatically favor TH2. This shift would favor continued spread of HIV throughout the body.

Since then, Clerici and his colleagues have compared T cells in healthy people infected 6 or 7 years ago with the AIDS virus to cells from people infected at the same time who now have AIDS. These healthy people carry HIV yet maintain a strong TH1 defense, much like that seen in uninfected people, Clerici told SCIENCE NEWS. Those with AIDS lack functioning T cells and exhibit diminished TH1.

Without a strong TH1 response, TH2 reactions might proceed unchecked. This would result in increased production of TH2 substances that may trigger programmed cell death in T cells, resulting in the massive loss of T cells seen in AIDS patients, he adds.

Based on his own experiments, Sergio Romagnani, a clinical immunologist at the University of Florence in Italy, interprets HIV-driven changes in cytokines and T cells differently.

He finds that HIV preferentially invades TH2 cells. Therefore, he envisions a more complex scenario, one in which HIV does not directly cause a shift from TH1 to TH2 cells. Rather, far fewer than the usual number of precursor T cells mature into TH1 forms, his group proposes in the July 8 SCIENCE. The result is the same: stronger TH2 and more T cell death.

Another report, also in the July 8 SCIENCE, runs counter to both the Italian group and the Shearer-Clerici team. Cecilia Graziosi at the National Institute of Allergy and Infectious Diseases in Bethesda, Md., and her colleagues monitored cytokine concentrations in lymph nodes, where much HIV infection and immune response occur. They found no shift in the kinds of chemical messengers produced.

But Graziosi's finding has not slowed the pursuit of data supporting HIV's role in shifting the TH1-TH2 balance. When Romagnani analyzes the T cell makeup of people so sick that they have no CD4 helpers left, he finds that another T cell, called CD8, exhibits an unusual tendency to stimulate the TH2 response. Normally, these CD8 cells kill other cells and make interferon gamma, a TH1 messenger. But in AIDS victims, the CD8 cells make the TH2 messengers that stimulate the production of antibodies and eosinophils, cells associated with allergic reactions.

Romagnani and others also cite new data indicating that people with allergies and high amounts of circulating antibodies become sicker faster with AIDS. Existing TH1-TH2 imbalances may account for why AIDS became so rampant so quickly in Africa, says Clerici. Many people there already lack a strong TH1 response and so may develop AIDS more quickly after infection.

HIV also affects IL12. White cells from healthy people produce much more IL12 than white cells from individuals infected with HIV, report Jihed Chehimi of the Children's Hospital of Philadelphia and his colleagues in the April JOURNAL OF EXPERIMENTAL MEDICINE. This lack of IL12 may make the body more susceptible to opportunistic infections and perhaps even to the progression of AIDS itself, they suggest.

Indeed, adding IL12 to cells growing in the laboratory seems to help. It can prevent cell death initiated by antigens and amplified by TH2 chemicals, says Clerici. Also, IL12 revives unresponsive white cells taken from people infected with HIV. These cells regain their ability to react to flu viruses and HIV, the group finds. — *E. Pennisi*