IL-12 at the Crossroads

In animal tests, this immune molecule shows dramatic powers against a wide range of infectious diseases prevalent in developing countries—but market forces may inhibit its development

Since its discovery in the late 1980s, the potent cytokine interleukin-12 (IL-12) has enjoyed all the trappings of a hot molecule. Its crucial role in shaping the earliest moments of the immune response has led to an explosion of articles in immunological journals. As a drug, it has already entered human clinical trials against cancer and AIDS. Researchers recently bestowed the sobriquet "magic bullet" on IL-12 because of its power in inhibiting the blood vessels that feed tumors in animal experiments, powers noted in the *Wall Street Journal* last April.

Recently it was the turn of infectious-disease experts at a National Institutes of Health (NIH)–sponsored meeting* to sing the praises of IL-12 as a potential therapeutic agent and vaccine adjuvant. But one sour note was discernible amid that chorus of praise. Representatives of pharmaceutical companies made clear at the meeting that although IL-12 has shown promise against infectious diseases prevalent in developing countries, economic pressures suggest it will probably be developed first for more lucrative markets closer to home.

Genetics Institute Inc. (G.I.) of Cambridge, Massachusetts, began Phase I testing against kidney cancer and AIDS a year ago, and Hoffmann-LaRoche Inc. of Nutley, New Jersey, began tests against kidney cancer last December in Europe. But researchers hoping to hear preliminary data from those clinical tests were disappointed; both companies chose not to reveal anything about initial safety and toxicity studies. Nonetheless, participants heard plenty about IL-12's broad powers against protozoa, worms, fungi, bacteria, and viruses.

Early switch

Since 1989, when the first report appeared in the literature, IL-12 has been shown to be produced by monocytes, macrophages, neutrophils, and dendritic cells (the first cells to encounter a foreign antigen during an infection), as well as by antibody-producing B cells. IL-12 in turn activates natural killer cells and T cells and seems particularly potent in its ability to induce production of interferon γ (IFN-γ), a cytokine that helps shape the immune response. The combination of IFN-γ and IL-12 sends a powerful signal to naive precursor cells of the T helper lineage, shifting an unfolding immune response toward cell-mediated immunity, one of the two major arms of acquired immunity (the other being a humoral, or antibody-associated, form).

Thus, early in infection IL-12 functions as a switch, its presence along with IFN-γ nudging uncommitted T helper cells toward a cellular, or T_H1, response. In contrast, another early cytokine, interleukin-4 (IL-4), pushes T helper cells toward an antibody-associated, or T_H2, response. That role is important, because a cell-mediated response is especially effective at identifying and eliminating cells infected with pathogens such as parasites, bacteria, and some viruses. One researcher refers to IL-12 as "the jump-starter of cell-mediated immunity."

And a remarkable jump-starter it is. "From the perspective of an experimentalist, it's the most potent cytokine I've ever worked with," said Alan Sher, whose lab is at the National Institute of Allergy and Infectious Diseases (NIAID). "This is like an atomic bomb. It modifies the course of one infection after another." In virtually every infectious disease presented at the meeting the message was the same: Resistance to pathogens increases when IL-12 is present to drive a T_H1 response.

Take leishmaniasis, a protozoal disease common in Central and South America, southern Europe, Africa, and India. IL-12 may offer an alternative to the existing drug, Pentostam, which is reasonably effective but can produce side effects such as heart arrhythmias and kidney dysfunction. In animal experiments with *Leishmania major*, Phillip Scott of the University of Pennsylvania has shown that BALB/c mice, normally susceptible to leishmania, control the infection with IL-12 treatment; this immunity is long-lasting. Scott's group has now shown that the combination of Pentostam and IL-12, delayed until well after infection, seems to shift the response back to a T_H1 type that allows the animals to recover. "It looks like a good switch from T_H2 to T_H1 in vivo," said Scott, "which is the first time that has been seen."

* "IL-12 in Infection: Prospects for Prophylactic and Therapeutic Intervention," 15–17 May 1995, Bethesda, Maryland

IL-12: REMARKABLY BROAD POWERS

Disease and Infectious Agent (type)	Est. Number of Infections Worldwide	Effects of Interleukin-12	Status of Clinical Trials
AIDS Human Immunodeficiency Virus (Viral)	14 million	Addition of IL-12 to peripheral blood mononuclear cells from HIV+ individuals results in increased T_H1-type cytokine production (IL-2) and interferon γ, plus increased cell proliferation	Phase I
Leishmaniasis *Leishmania* (protozoan)	12 million	Reduces or prevents growth of parasites if administered early in infection (mice); immunizes against infection when given with antigen (mice); synergizes with existing chemotherapy in curing animals of disease (mice).	None
Malaria Plasmodium family of parasites (protozoan)	300–500 million new infections annually	Prevents growth of parasites if administered early in infection; inhibits development of liver and blood stages of disease (mice)	None
Tuberculosis *Mycobacterium tuberculosis* (mycobacterium)	8 million new infections annually	Reduces bacterial load, reduces lung pathology, and prolongs survival (mice)	None
Schistosomiasis *Schistosoma mansoni* (helminth)	200–250 million	Blocks immune-associated pathology in liver, reducing granuloma formation and fibrosis (mice); enhances vaccine protection against challenge infection	None

Malaria offered a promising scenario for both therapy and immunoprophylaxis—especially welcome news given the emergence of drug-resistant strains of the plasmodium parasite. A strain of mice known as A/J, for example, is highly susceptible to malaria: By the time the parasite's life cycle reaches the blood stage, malaria is normally 100% lethal in these animals by about the twelfth day after infection. But Mary Stevenson of McGill University in Montreal reported that 75% of otherwise susceptible mice survived when treated with a 5-day regimen of 0.1 micrograms of IL-12 beginning on the day of the infection.

In other animal experiments, Stephen L. Hoffman of the Naval Medical Research Institute tested a different strain of mice with the sporozoites of *Plasmodium yoelli*, which infect liver cells before disseminating through the bloodstream. As little as 10 to 30 nanograms of IL-12 daily for 5 days conferred 100% protection against blood-stage infection if treatment was initiated 1 to 2 days prior to the test. In July Hoffman expects to begin testing a preventive regimen against simian malaria in rhesus monkeys. The ultimate goal would be short-term prophylactic treatment that could be used by the estimated 27 million North Americans and Europeans each year who visit regions where malaria is endemic.

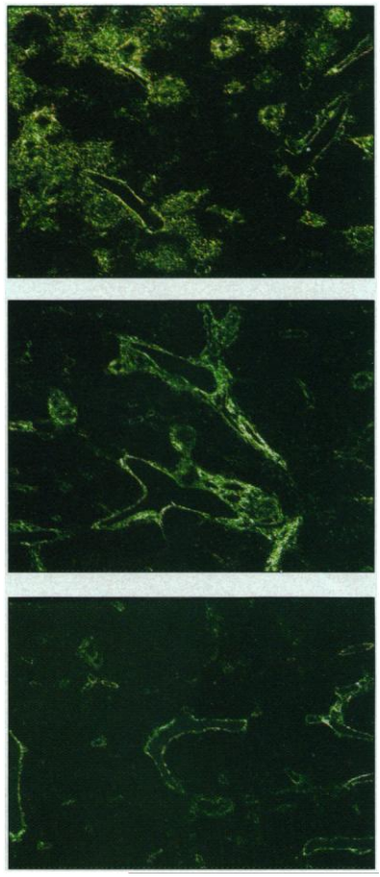
The drug has also shown promise against tuberculosis in mouse models used by Barry Bloom of Albert Einstein College of Medicine in New York and Ian Orme of Colorado State University. At the meeting, Orme reported "rather good protection" against tuberculosis in the lungs of mice with a 400-nanogram regimen of IL-12 every 2 days. Orme is now working on a TB vaccine based on a combination of TB proteins and several cytokines, including IL-12. In preliminary tests, he said, the combination seemed to "point T cells in the right direction" and achieved a statistically significant degree of protection.

In contrast to infection with parasites, the potential benefit of IL-12 in viral disease has been a question mark. But Christine Biron of Brown University in Providence, Rhode Island, has now shown that in one viral model, murine cytomegalovirus (MCMV), low doses of IL-12 are highly effective in controlling infection by inducing natural killer cells to clear the virus and reduce viral proliferation.

The jury is still out, however, on IL-12's potential for treating another viral disease: AIDS. Because the absence of IL-12 seems to play a role in AIDS pathogenesis, some researchers have argued that administering the cytokine to AIDS patients might promote strong cell-mediated immunity and a T_H1 response that could delay the progression of HIV-positive individuals to AIDS.

This complemented the working hypothesis of Gene Shearer at NIH that AIDS patients show an increasingly dominant T_H2 profile. That pattern, consistent with reduced production of IL-12, is ultimately associated with a dysfunction of the CD4+ class of T helper cells: When these cells encounter antigen, the result is apoptosis, or programmed cell death. Shearer has blocked programmed T cell death in cultures from an HIV-positive patient by adding IL-12 and blocking two T_H2-type cytokines, IL-4 and IL-10.

The clinical trials launched last year by G.I. should ultimately determine whether IL-12 lives up to its theoretical promise against AIDS. So far, however, the company is divulging no information about the preliminary findings.



ALLEN CHEEVER/NIAID

Bad, better, best. Collagen deposits *(bright areas)* damage liver in mice infected with the worm *Schistosoma mansoni (top)*. Damage decreases when mice are vaccinated before infection with the worm's eggs *(middle)* and decreases still further when IL-12 is used as a vaccine adjuvant *(bottom)*.

Another role: Vaccines

In addition to the consideration of IL-12 as a therapeutic agent, there was also considerable interest in using it to boost the protective effects of vaccines. Stanley Wolf of G.I. described experiments in which mice were treated with one microgram of IL-12 at the time of vaccination with a common soluble antigen (trinitrophenyl-modified keyhole limpet hemocyanin, or TNP-KLH); the mice produced a predictable T_H1-type response, with increases in IFN-γ and suppressed levels of the T_H2 cytokine IL-4. When they were retested with the same antigen a month later, the mice exhibited a T_H1-type recall response, but surprisingly, they also produced an antigen-specific antibody typical of a T_H2 form of immune memory: two forms of immune memory for the price of one. G.I. is not currently pursuing IL-12 as a vaccine adjuvant, but Wolf said, "We might actually move ahead very quickly in that regard."

Several other vaccine models also appear promising. In leishmania, University of Pennsylvania researcher Scott has shown that soluble leishmanial antigen (SLA) administered with IL-12 as an adjuvant completely protects otherwise susceptible BALB/c mice. On the basis of this and other work, Scott and co-workers began last November to test a leishmania vaccine with an IL-12 adjuvant in nonhuman primates in Kenya. Scott said dosage levels are still being worked out, and no data are yet available.

A vaccine approach even holds promise for diseases caused by worms, which are known to induce the same sort of T_H2 response associated with allergies. Thomas A. Wynn, from Alan Sher's laboratory at NIAID, described how an "anti-pathology vaccine" with IL-12 as an adjuvant reduced the tissue damage associated with schistosomiasis, a water-borne helminth infection. This damage is caused primarily by the immune system's reaction to the eggs of the parasite, which are deposited in the liver and induce a vigorous T_H2 response associated with formation of masses of inflamed tissue, or granulomas, and thickening of the connective tissue known as fibrosis.

Wynn reported that in mouse studies, immunization with egg antigens plus IL-12 sensitized the animals to such an extent that granuloma formation was almost completely suppressed when they were retested with eggs. In a follow-up study where three rounds of sensitization were followed by challenge with the live parasite, Wynn found that there was no effect on the number of worms or eggs in the tissue, but T_H2-type cytokines were almost completely suppressed. This resulted in a 30% decrease in granulomatous inflammation and an even more marked diminution of fibrosislike tissue scarring, which is the primary damage to the liver in active disease.

Clouds on the horizon

All these findings have kindled considerable enthusiasm for IL-12 as a therapeutic agent. But the enthusiasm is tempered by the realization that, like all cytokines, the molecule can exert potentially negative effects. One message from the meeting was that too much IL-12 in the wrong place, at the wrong time, may do more harm than good. Several researchers, including Biron and Stevenson, have reported that high doses of IL-12 in mice produce a systemic reaction that can resemble toxic shock syndrome. Luigina Romani of the University of Perugia in Italy,

who studies the fungal infection candidiasis, observed that IL-12 treatment in mice that normally have self-limited candida infections unexpectedly provoked disseminated disease. Robert Modlin of the University of California, Los Angeles, has argued that IL-12 can, in certain circumstances, set off an inflammatory pathway that promotes the formation of atherosclerotic plaques. "Does IL-12 have a role in atherosclerosis?" asked Joseph A. Kovacs of the NIH's Clinical Center. "That would concern me."

Side effects aside, there are clearly many promising avenues for clinical research, and the round-table discussion at the end of the meeting was pitched to identify two or three diseases meriting clinical trials. The discussion featured representatives of G.I. and Hoffman-LaRoche, the two companies that have filed for IL-12 patents and cross-licensed its development to one another. As one organizer, Lee Hall of NIAID, framed the final session's question, "You've heard 20 different models. How do you choose one?"

The answers could not have pleased the basic research community: Executives from the two companies chose none—for reasons ranging from possible side effects to the economic downside of trying to treat Third World diseases at this stage of IL-12's development.

John Ryan, who recently left Merck to become director of clinical development at G.I., warned that the success of IL-12 against, say, leishmaniasis at this point would be a "disaster" for the company: G.I. would be committed to scale up manufacturing costing millions of dollars and would probably wind up distributing the drug through the World Health Organization, which would in essence give it away, leaving the company with huge costs and little or no revenues. This prompted one scientist to ask: "So what the scientists should be finding are economically viable infectious diseases for you?"

At the end of the day, infectious-disease experts were left with an immensely powerful molecule and perhaps nowhere to go clinically until IL-12 proves its mettle against cancer or AIDS. What recourse do basic researchers have? "What's valuable to a company," said Stan Wolf, who cloned the gene at G.I., "is dose, schedule, and how to use a drug. ... How could people in academia help? Think from a pragmatic point of view, and supply that information back to the companies." That may sound like asking basic investigators to do product-oriented research, but in today's high-stakes drug development, the alternative may be no IL-12 to test.

–Stephen S. Hall

Stephen S. Hall is a science writer in New York.

LOW-TEMPERATURE PHYSICS

Helium-3 Crystals Captured on Video

AMSTERDAM—How can you get a good look at processes that only take place at temperatures just above absolute zero, when the very act of observing them creates heat? Raymond Wagner and Giorgio Frossati at the Kamerlingh Onnes Laboratory at the University of Leiden in the Netherlands have just solved the problem with some ingenious technology, obtaining the first video pictures of the growth of helium-3 crystals at temperatures below 0.001 kelvin. "It is obviously a technical tour de force to observe things at those temperatures," says low-temperature physicist David O. Edwards of Ohio State University.

This feat is more than just a record-setting achievement. At these temperatures, helium has some unusual properties: It becomes a superfluid—a substance with a viscosity of zero that can spontaneously form vortices and flow up and over the rim of a glass. Low-temperature physicists have long wondered how these properties affect the formation of crystals, because atoms in a superfluid can move easily to the place where the crystal is forming and crystallization is speeded up because the heat shed by atoms as they slot into place in the crystal is carried away instantaneously.

A couple of decades ago, they got their first look at crystals in superfluid helium-4, which has two protons and two neutrons in its nucleus and becomes superfluid at a relatively balmy 1.8 K. But helium-3, which has one less neutron, has been more elusive because it only becomes superfluid at 0.002 K. Researchers have been particularly keen to study the formation of helium-3 crystals because the atoms have different magnetic properties from those of helium-4.

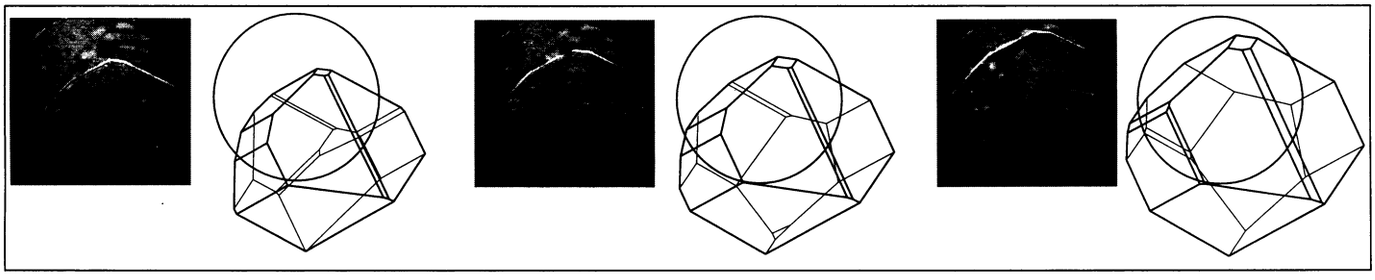
Jukke Pekola, Alexei Babkin, and Pertti Hakonen at Helsinki University of Technology in Finland managed to get the first single images of superfluid helium-3 in 1992. Now the Leiden team has gone one step further by obtaining video images of the growth of helium-3 crystals. "It's very difficult work," says Hakonen. "Before their work and ours, the minimum temperature at which pictures have been obtained was a factor of 40 higher."

To get pictures, the Leiden researchers had to prevent any radiation leaking in from the outside world. Pointing a camera in through a window was out of the question, so the researchers decided to mount a charge-coupled device (CCD) camera inside the cryostat. CCDs only work above 60 K, so the camera had to be cocooned inside a small, shielded, copper box inside the cryostat and heated up slightly. "The big difficulty was to combine the helium-3 at a temperature of 1 millikelvin and a camera that operated at a much higher temperature," says Wagner. Illuminating the crystal enough to take a picture also posed a problem, because of the heat from the light. The researchers used a tiny glass fiber to direct light pulses from an LED, specially shaped to reduce the light-emitting region, onto the crystal.

The sharp point of a needle inside the cryostat served as the nucleation site, and the group was able to observe different stages of the formation of the crystal. They observed three different facets of the crystal, two of them for the first time. (One first facet was observed by Sébastien Balibar at the Ecole Normale Supérieure in Paris at 0.07 K, a temperature at which helium-3 is not superfluid.) For Balibar, the observation of the other two facets confirms theory and is a key to understanding the crystal: "Helium crystals are real prototype crystals that allow us to study the general properties of all crystals."

–Alexander Hellemans

Alexander Hellemans is a writer in Amsterdam.



RAYMOND WAGNER

Cool pictures. Images of the growth sequence of helium-3 crystals at 0.002 K and associated 3D reconstructions.