IMMUNE THERAPIES

IL-12 Holds Promise Against Cancer, Glimmer of AIDS Hope

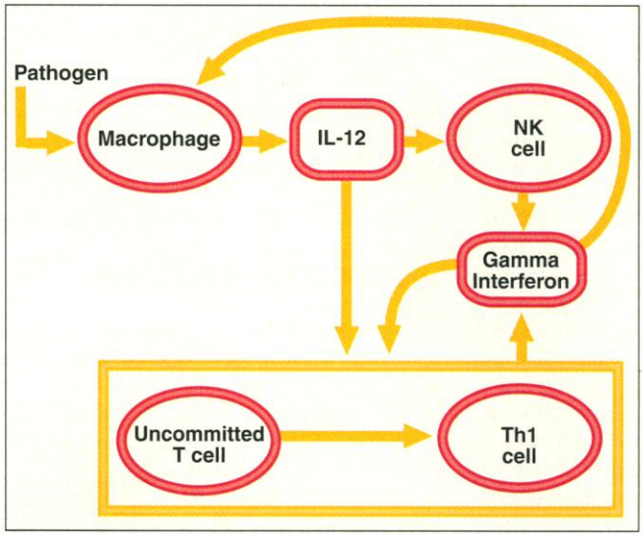
Could it be déjà vû all over again? Yogi Berra used the phrase about baseball, but last week the question was being asked at a conference on an entirely different subject: the cytokine interleukin-12. This crucial immune-system molecule, discovered in the late 1980s, is months—if not weeks—away from Phase I clinical trials as an anticancer and anti-AIDS agent. And an air of enthusiasm is spreading in the research community about the drug's potential to inhibit tumor growth.

But some cautious researchers are reminding colleagues that they've seen this kind of enthusiasm before: 10 years ago, when the cytokine IL-2 was greeted with hyperbole. Although initial tests seemed so promising that a 1985 *Fortune* magazine cover story hailed the substance as a "Cancer Breakthrough," expanded clinical trials showed that IL-2 offered limited efficacy—at a price of severe toxicity. Although the Food and Drug Administration (FDA) approved the drug as a treatment for renal cell carcinoma in 1992, its usefulness is now viewed as modest. Is the same fate in store for IL-12?

"Sometimes I have the sense that we're walking down a path we've walked down before," said Maurice K. Gately, who headed IL-2's preclinical studies at Hoffmann-La Roche Inc. a decade ago and now heads the company's IL-12 preclinical research team in Nutley, New Jersey. Despite his cautionary tone, Gately argued that this time the eagerness is justified. Animal studies of the drug's surprisingly potent antitumor effects and apparently manageable toxicity, said Gately, allow a "realistic optimism about the therapeutic potential of IL-12."

If the optimism is realistic, some of the most interested parties will be on Wall Street, site of the recent 2-day workshop, held in a conference room at Merrill Lynch & Co. The setting was coincidental (a board member of the Cancer Research Institute, the meeting's sponsor, had arranged for the facility), but the Wall Street background was appropriate. Both Hoffmann-La Roche and the biotech company Genetics Institute Inc. of Cambridge, Massachusetts—once collaborators, now David-and-Goliath competitors—are readying the molecule for tests in humans. A Roche spokeperson would say only that clinical trials against metastatic cancer are planned for "sometime this year"; a spokesperson for Genetics Institute says the company will file an Investigational New Drug application with the FDA "soon" and hopes to launch trials by the end of June.

The possibility of clinical trials is a reflection of the enormously rapid progress researchers have made in parsing the molecule's biology since IL-12 was discovered in the mid-1980s by several groups. The human gene was cloned in 1991 and, unlike the case with most cytokines, much of the basic biology was first worked out using the human molecule rather than in animal systems; that work includes the identification of the IL-12 receptor, reported at the meeting by Ueli Gubler of Hoffmann-La Roche.



Center stage. Macrophages, the first immune cells to see pathogens, release IL-12, which has a central role in immune responses. It causes natural killer (NK) and T cells to release gamma interferon and uncommitted T cells to shift toward the "TH1" phenotype.

The interleukins are key molecules that communicate between immune cells and orchestrate both the activation and damping of the immune system in response to infection or injury. Although IL-12 came late in the chronology of discovery, it is one of the earliest acting and most critical cytokines. During the first hours of the immune reponse (the so-called natural immunity), macrophages—the first cells to see an infectious agent—begin to churn out IL-12. This burst stimulates proliferation of natural killer (NK) and T cells, enhances those cells' killing capacity, and triggers a surge of gamma interferon from both cell types.

It is its role as an "initiation cytokine" that has stimulated the most interest among IL-12 researchers, says Giorgio Trinchieri of the Wistar Institute, one of the meeting's cochairs. IL-12, he said, represents a "missing link" between the early, nonspecific immune reponse and the specific later response that confers immune memory. IL-12 pushes uncommitted T helper cells toward commitment as Type 1 helper cells; these "TH1" cells, which generate a characteristic constellation of cytokines, are associated with cell-mediated immunity. (Another important initiation cytokine, IL-4, pushes uncommitted T helper cells toward becoming Type 2 helper cells; the TH2 fate is associated with humoral, or antibody-driven, immunity.)

IL-12's role as a "TH switch" is what intrigues AIDS researchers. One group of those researchers thinks the progressive loss of TH1-like function, even very early on in asymptomatic individuals, may predict later clinical history—including onset of AIDS symptoms and death. What causes the loss of function remains unclear, but IL-12 may be part of the equation. At the meeting, Trinchieri reported that mononuclear cells from people infected with HIV produce only one-tenth as much IL-12 as cells from uninfected donors when they are stimulated in the test tube by bacteria. Gene Shearer of the National Cancer Institute summarized work by his group suggesting that in HIV-infected people who do not yet show AIDS symptoms, a shift toward a "TH2-like cytokine profile" seems to foreshadow progression to AIDS. Last December, the group also published evidence that IL-12 can restore HIV-specific cell-mediated immunity in the test tube to cells from people infected with HIV.

Shearer added that unpublished results from his lab now indicate that IL-12 can prevent programmed cell death, or apoptosis, in T cells from both infected and uninfected people following stimulation with agents that cause cells to divide. A combination of IL-4 and IL-10, on the other hand, can cause significant increases in programmed cell death, which some researchers think plays a role in the destruction of the immune system seen in AIDS. If these data hold up, says Trinchieri, they "could be very significant."

The most important question raised by this work, of course, is whether administration of IL-12 to people infected with HIV could shift them toward a protective, cell-mediated immune response and prevent development of symptoms. Although the evidence is still circumstantial, the possibility of such a shift is the rationale behind Genetics Institute's plans to launch an HIV trial using

IL-12, which will probably take place "at two sites," according to a company spokesman.

If the results in HIV research are intriguing, those involving IL-12 and cancer are dramatic. Researchers continue to be surprised at how broad a range of antitumor activity IL-12 shows in preclinical studies. Michael Brunda of Hoffmann-La Roche, whose group reported the first such results last October, summarized the data by saying that IL-12 "appears to have activity against a widespread group of tumors," including renal cell carcinoma, melanoma, colon adenocarcinoma, and 14 other malignancies.

In a mouse model of the B16F16 melanoma tumor, for example, Brunda has shown that researchers can wait 14 days after injecting malignant cells—by which time the subcutaneous tumors have grown larger than a centimeter in diameter—and still inhibit tumor growth with daily injections of IL-12. In a renal cell carcinoma model, direct injections of IL-12 into the tumor led to regression. "Initially we get stasis," says Brunda, "but after prolonged treatment, the tumors go away—and the animals are cured."

Michael Lotze's group at the Pittsburgh Cancer Institute has confirmed many of these results, including a systemic immune response from direct injection of IL-12 into a tumor. In a sarcoma model, where two tumors are generated on either side of a mouse, injection of IL-12 into one tumor causes both to regress. "We were astonished," says Lotze, "to see an effect on subcutaneous tumors. There are very few immunologic therapies capable of causing regression in subcutaneous tumors."

Naturally, with results like these, researchers are eager to see what will happen when IL-12 is used in human trials. Yet hovering over the question of human trials is the issue of toxicity—the issue that caused IL-2 to lose its luster. Several months ago, rumors swept the IL-12 community that primates undergoing toxicology testing at Hoffmann-La Roche had died. In fact, as Roche's Gately explained at the workshop, one of several squirrel monkeys given a maximum daily dose of 50 micrograms per kilogram of body weight experienced lethargy and pulmonary edema and had to be sacrificed; the rest seemed to tolerate that and lower doses well. The main side effect of IL-2—pulmonary edema—has not been seen in any of the mouse toxicology studies, says Gately, who adds that "we think IL-12 is not simply another IL-2."

One side effect of IL-12, however, was underplayed at the meeting: the breakup of the collaboration between Genetics Institute and Hoffmann-LaRoche. The companies cross-licensed their patents in May 1992, when it became clear they were both working on the same molecule—IL-12—under different names: Genetics Institute called it "natural killer stimulatory factor," Hoffmann-LaRoche called it "cytotoxic lymphocyte maturation factor." After the cross-licensing there was initially a remarkable openness about sharing reagents. Last year, however, this happy marriage of scientists ended in a divorce brokered by lawyers. Although the companies are circumspect about the reasons, several researchers familiar with the breakup say Hoffmann favored a conservative approach to clinical trials, while Genetics Institute was in favor of a more aggressive posture.

While most researchers agree the split has not retarded science, there were a few grumbles that the New York meeting was not as open as past conferences and that crucial antibodies are not shared as generously as when feelings were warmer. "I'm not getting those reagents," said one researcher, "which a lot of other people in this room are getting." And, as clinical trials approach, researchers are being told they must choose which company's IL-12 they plan to use. "It's like a child in a divorce," says Lotze. "You have to choose one parent, and you like them both."

Unlike participants in divorces, however, the participants here aren't wasting time on recrimination; they're too busy concentrating on the future—specifically whether the IL-12 story will turn out differently from the tale of IL-2. The answer should come soon. "The real answer to the toxicity question will come in the Phase I trial," says Trinchieri, "and that will be answered this summer."

–Stephen S. Hall

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Additional Readings

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M. Kobayashi *et al.*, "Identification and Purification of Natural Killer Cell Stimulatory Factor (NKSF), a Cytokine with Multiple Biologic Effects on Human Lymphocytes," *Journal of Experimental Medicine* **170**, 827 (1989).

ANTHROPOLOGY

Mummy Settles TB Antiquity Debate

Christopher Columbus, transformed in our politically correct era from courageous hero to imperialist brute, can now be absolved of at least one sin: introducing tuberculosis (TB) to the New World. A group of scientists examining a Peruvian mummy who died 500 years before Columbus set foot on Hispaniola have found DNA specific to the TB bacteria. Their report, marking the first time DNA has been recovered from an ancient sample of a disease-causing organism, was published in the 15 March issue of the *Proceedings of the National Academy of Sciences*. And the research, utilizing the DNA-amplifying polymerase chain reaction (PCR) technique, opens a new avenue of inquiry for scientists trying to reconstruct epidemics of the past.

A. AUFDERHEIDE

New World microbes. This 900-year-old mummy had TB DNA.

Previously, paleopathologists had to rely on indirect evidence of disease, such as scars on old bones. "In the past we could only formulate hypotheses about the origin and spread of many diseases," says paleopathologist and physician Bruce Rothschild, director of the Arthritis Center in Youngstown, Ohio. But the advent of PCR, which makes it possible to probe ancient tissues for specific pathogens, changes all that. "Now," says Rothschild, "we can actually go out and test" those hypotheses.

In the case of TB, the direct test solves a longstanding puzzle. Ethnohistorians had suggested Europeans brought TB with them to the New World, because American Indians began suffering devastating TB epidemics in the early 1600s, soon after major European contact began. Paleopathologists, however, had found intriguing—but indefinite—clues indicating that TB was present in the Americas prior to 1492. These clues came from pathological examinations of bone and lung lesions found in some pre-Columbian skeletons and mummies. But those hints weren't conclusive, because many infectious fungi, parasites, and other diseases can leave behind similar marks.

Pathologist Arthur C. Aufderheide of the University of Minnesota's School of Medicine saw a chance for a more definitive answer when he gained access to a 40- to 45-year-old naturally mummified woman who died about 900 years ago. She was one of the Chiribaya people, who inhabited the south coast of Peru, and her fragmented remains had been recovered from a tomb there in 1990.