Interleukin-1 Genes Are Cloned

The cloning work indicates that there is a family of interleukin-1 proteins but raises more questions about how the lymphokine might work

Nowadays it is the gene cloners who often provide the first good look at important proteins. The recent cloning of genes coding for interleukin-1 molecules is a case in point. Interleukin-1 is a lymphokine, a member of a diverse group of proteins that transmit growth and differentiation signals among immune cells. Without the lymphokines, normal immune responses could not be mounted, but the agents are produced in such small amounts that the purification of quantities sufficient for biochemical characterization has proved difficult.

The problem seemed particularly acute in the case of interleukin-1 because so many different activities have been attributed to the protein. It participates in the activation of both major classes of immune effector cells, the antibody-producing B cells and the various T cells, including regulatory and killer cells.

Moreover, interleukin-1 apparently works in the brain to induce fever and may also mediate the headaches and body pains that commonly accompany infections. The lymphokine's effects on connective tissue cells suggest that it may be a two-edged sword, one that promotes wound-healing but may contribute to the tissue damage of inflammation if not properly controlled. "It's part of the host defenses," explains Charles Dinarello of the New England Medical Center of Tufts University School of Medicine. "But you pay a price for it."

Interleukin-1 has attracted a great deal of commercial interest because of all these activities. A drug that stimulates or mimics its effects might be useful as a promoter of wound-healing, for example. Perhaps even more important, it might be possible to design interleukin-1 antagonists that would provide new and more specific drugs for treating inflammatory diseases such as arthritis.

Before any of this can come to pass, however, a number of questions about interleukin-1 have to be answered. Immunologists especially want to know if the many activities ascribed to the agent reside in a single protein or if there is a family of interleukin-1's, each with somewhat different functions.

The cloning work is now resolving this issue. So far, three groups have identified interleukin-1 clones—and they apparently represent two different genes. Peter Lomedico of the Roche Research Center in Nutley, New Jersey, Steven Mizel of Pennsylvania State University, and their colleagues obtained a clone for a mouse interleukin-1 from macrophages, cells that help to initiate immune responses and are important producers of the lymphokine, and have used it to derive the amino acid sequence of the protein (*1*). Meanwhile, Dinarello, Philip Auron of the Massachusetts Institute of Technology, Andrew Webb of Wellesley College, and their colleagues determined the amino acid sequence of a human interleukin-1, also from a clone prepared from macrophages (*2*).

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Overall about 35 percent of the amino acids encoded by the two genes are identical, Auron says, with the identical amino acids tending to cluster in particular regions, especially in the carboxyl portions of the proteins. The regions of close similarity may be needed for the interleukin-1 activities.

According to Auron, the sequence comparisons do not permit a definitive conclusion about whether these two clones represent different genes or are mouse and human versions of the same gene. However, Mizel, Lomedico, and their co-workers have now obtained an additional human interleukin-1 clone. It is very similar to their original mouse clone but appears to be different from the human interleukin-1 clone of Auron, Dinarello, and Webb.

The conclusion that there are at least two human interleukin-1 genes is further buttressed by as yet unpublished work by a group of investigators from the Immunex Corporation in Seattle and the Syntex Corporation in Palo Alto who have obtained and sequenced two human interleukin-1 clones of their own. One of these is related to that studied by Auron, Dinarello, and Webb. "It's clear that one of the genes we cloned is similar, but not identical, to the one they've cloned," says Christopher Henney of Immunex. The other is similar to the mouse clone.

The proteins encoded by the Dinarello and Mizel group clones contain about 270 amino acids and thus have a molecular weight of about 31,000. The evidence suggests that these larger proteins are subsequently split to proteins with a molecular weight of about 18,000. According to Auron, amino acid 117 is the first amino acid of this smaller species of human interleukin-1.

In a related development, J. Van Damme, A. Billiau, and their colleagues at the University of Leuven and the State University of Ghent in Belgium have reported the sequence of the first 39 amino acids of a human protein that has many of the classic earmarks of an interleukin-1 (*3*). This sequence closely corresponds, but is not identical, to amino acids 117 to 155 of the human interleukin-1 of Auron, Dinarello, and Webb, and is thus another piece of evidence favoring the existence of multiple interleukin-1 genes. In addition, the sequence analysis of the Belgian workers suggests that their interleukin-1 preparation contained two or more related proteins, one of which might be the same as that of the Dinarello group.

One of the more puzzling features of the interleukin-1 proteins is their apparent lack of "signal sequences," which help to direct newly synthesized proteins to their ultimate destinations, whether in or out of the cell. The absence of signal sequences raises the question of how the interleukin-1's get out of macrophages and other cells.

They may not be actively secreted by living cells. "Macrophages are probably suicidal," Dinarello suggests. "They fight infection to their death and may release interleukin-1 when they die." This type of release may be particularly important in inflammation. Dead and dying cells, including macrophages, are found at inflammatory sites.

Work by Emil Unanue and his colleagues at Washington University School of Medicine points toward another possibility (*4*). These investigators have identified active interleukin-1 on the outer surfaces of macrophages,

where it apppears to be an integral part of the membranes. They also find a secreted soluble form, which may be different from the membrane protein.

In any event, macrophages enter into direct and specific cell-to-cell contacts with their partners during the initiation of immune responses. The appearance of interleukin-1 on the macrophage membrane may thus help to focus this essential activating signal on just those immune cells that respond to a particular foreign antigen. This finding might explain how interleukin-1 could work without actually being secreted but does not explain how the protein arrives at the membrane in the first place.

Mizel, although he supplied an antibody needed for the Unanue group's work and thus appears as a coauthor on their paper, is not convinced that interleukin-1 works the way Unanue proposes. Mizel, suggests that the membrane-bound form may be on its way out of the cell and not an integral membrane component.

The cloning of the interleukin-1 genes has apparently settled one issue concerning this important lymphokine. There does indeed appear to be a family of the proteins. But many other questions have been raised. For example, the number of proteins that will ultimately be found to belong to that family is currently unknown, as is the manner of their release from the cell and the significance of the membrane-bound and soluble forms.

Also unknown is whether the various family members will differ in their activities. "The assumption that many activities can be ascribed to the one molecule will have to be reinvestigated," notes Steven Gillis of Immunex. Whereas the Immunex workers indicate that there may be differences in the range of activities of their two human interleukin-1's, Mizel notes that the mouse protein appears capable of all the known interleukin-1 functions.

The studies needed to resolve these issues will be greatly facilitated by the new ability to use the cloned genes to produce milligrams of the pure proteins in bacteria, yeast, or mammalian systems. "I have been involved in interleukin-1 research for 10 years," Mizel says. "The things we once dreamed about, we can now do."—**JEAN L. MARX**

References

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Fractal Fingers in Viscous Fluids

Highly unstable viscous fingers break up into many-branched structures that are suggestive of fractal geometry

An initially flat interface between two immiscible fluids does not move uniformly when pressure is applied to the less viscous of the fluids. In the most general case, the interface can become wavy and then break up into an array of fingers in which each fluid interpenetrates the other, although the average interface position moves in the direction of the pressure gradient.

During the last year, researchers have wondered under what conditions, if any, viscous fingers can exhibit fractal behavior. Fractal objects have a property called self-similarity—that is, they have similar features at all length scales and therefore look the same at all magnifications—and are characterized by an effective fractional dimension, rather than the 1, 2, and 3 of curves, surfaces, and volumes.

The first and so far strongest experimental example of fractal viscous fingers was reported in March by Johann Nittmann and Gérard Daccord of Dowell Schlumberger in St. Etienne, France, and H. Eugene Stanley of Boston University (*1*). Since then a number of results suggesting incipient fractal behavior under somewhat different experimental conditions have or are about to come out. An unanswered question is whether fractal viscous fingers are a general phenomenon or are peculiar to the particular types of fluids used by Nittmann, Daccord, and Stanley.

The three investigators also argued that the numerical model that researchers use to simulate the growth of a class of fractal objects known as diffusion limited aggregates likewise describes the fractal viscous fingers in their experiments. In an interesting turnabout, some theorists are finding that it is possible to extend the diffusion limited aggregation (DLA) model to simulate nonfractal viscous fingering, as well.

Apart from their curious properties, why should fractals be of interest? The answer lies in their self-similarity, which is a kind of symmetry—in this case invariance under a change in length scale. This sort of symmetry has an immense attraction to physicists at the moment, partly because of the crucial role it played in the development of the theory of second-order phase transitions (critical phenomena) in the late 1960's and early 1970's. Understanding how to accurately calculate the properties of physical systems near their critical points, where their behavior is strongly nonlinear, was one of the major triumphs of condensed matter science.

In critical phenomena, self-similarity is closely connected with another property, universality. Since a self-similar physical system looks the same under all magnifications, it has structures that are very large in comparison with the range of basic forces within it. The properties of these large-scale structures depend only on certain long-range symmetries and not on the local details of the forces. This implies that, near their critical points, quite different physical systems that show the same symmetries behave in the same way; that is, universally.

Following the connection of self-similarity with universality by Leo Kadanoff (now at the University of Chicago) and others, Kenneth Wilson of Cornell University was able to rigorously develop it in the renormalization group theory of critical pheonomena that later won him the 1982 Nobel Prize in physics. Now anything that smacks of self-similarity with its attendant promise of unifying diverse phenomena has considerable appeal.

Recently, for example, scientists have been fascinated by the behavior known as chaos that occurs in a wide range of dynamical systems (those that evolve in time), including fluids, that are described by nonlinear equations (*Science*, 5 November 1982, p. 554, and 8 July 1983, p. 140). The behavior is called chaotic because it seems to be wildly unpredictable or noisy, although in fact it is entirely