Chemical Signals in the Immune System

Activation of immune cells depends on a complex interplay of proteins that regulate the growth and differentiation of the cells

The past few years have seen a surge of interest in the lymphokines, proteins that transmit growth and differentiation signals between immune cells. Until recently the agents were poorly characterized "factors"; but with improved techniques for preparing the proteins and studying their biochemical characteristics and biological activities, it has become clear that the agents are essential for immune responses. Cloning of the genes for the lymphokines interleukin-2 and γ-interferon has added further respectability. "Five or six years ago, people were reluctant to say that they worked on these factors," says William Paul of the National Institute of Allergy and Infectious Diseases (NIAID), "but now it is obvious that they play a central role in the immune system."

Still to be determined is whether insufficient production of lymphokines contributes to the development of immunodeficiency or whether these peptides have any therapeutic effects on immunodeficiency diseases. There have been some very preliminary reports that interleukin-2 may improve the functioning of immune cells from individuals with acquired immune deficiency syndrome (AIDS), although it is still too early to tell whether the lymphokine will improve the clinical symptoms or the survival of individuals who suffer from this devastating disease.

Researchers from NIAID and the Food and Drug Administration (FDA), using a test tube assay, have recently found that interleukin-2 improved the function of T cells from six AIDS patients. "Interleukin-2 produced a remarkable reconstitution of the cytotoxic effects of T cells from AIDS patients," says NIAID's Anthony Fauci. One of the major defects in these patients is a lack of T cells, primarily of the helper class, which are needed for the activities of other immune cells, including both antibody production in response to some antigens and the cytotoxicity of other T cells. Fauci suggests that the added interleukin-2 may have worked by replacing a lymphokine that would otherwise have been produced by the patients' missing helper cells.

The FDA and NIAID investigators are beginning a phase I clinical trial with interleukin-2 in AIDS patients. Such trials are designed to determine the length of time a potential therapeutic agent will remain in the patient's system, its toxicity, if any, and the size of the doses tolerated. Therapeutic efficacy may then be determined in later trials.

Meanwhile, investigators at Sloan-Kettering Memorial Institute treated 13 patients, six of them with AIDS, with interleukin-2. "Our overall attitude is to be cautiously optimistic. It is nontoxic and appears to have biological activity," concludes Roland Mertlesman. For example, the ratio of helper T cells to suppressor T cells, which is very low in AIDS patients, increased after the treatment. With only a limited amount of interleukin-2 available for their study, the Sloan-Kettering physicians could not determine whether the material would produce any lasting clinical improvement in the patients.

Both the FDA-NIAID studies and those at Sloan-Kettering have used relatively impure preparations of interleukin-2. However, large quantities of pure material may become available for further studies as a result of the gene engineering work that is under way in many academic and commercial laboratories.

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Most investigators credit work done in the mid-1970's by Robert Gallo and his colleagues at the National Cancer Institute (NCI) for paving the way to the current progress in basic research on interleukin-2. They discovered that human lymphocytes, which were stimulated to divide, release into the culture medium in which they are growing a material that allows T cells to grow in culture for indefinite periods of time. This discovery was important because it led to the identification of T cell growth factor, which was subsequently renamed interleukin-2, and to the development of methods for cloning T cells. Large, pure populations of a single type of T cell could then be produced for studying lymphokine effects and T cell function generally. Before the availability of cloning techniques only heterogeneous T cell populations were available for lymphokine research, a situation which led to a great deal of confusion about the identities of various lymphokines, their sources and targets, and the manner in which they worked. "The ability to have a homogeneous cell line as target meant we were able to purify and characterize T cell growth factor," explains Kendall Smith of Dartmouth Medical School.

The better techniques for assaying interleukin-2 activity soon make it clear that several factors, which had been given different names on the basis of their apparently diverse actions on the less well characterized, heterogeneous T cell populations, were in fact the same, that is, they were all interleukin-2.

Biochemical characterization of human interleukin-2 indicated that it has a molecular weight of roughly 15,500, a figure which has now been confirmed by the cloning of the gene for the lymphokine. Tadatsugu Taniguchi and his colleagues at the Japanese Foundation for Cancer Research in Tokyo and the Central Research Laboratories of Ajinomoto Co., Inc., cloned a DNA copy of messenger RNA for human interleukin-2 and determined its nucleotide sequence. The cloned gene codes for a protein containing 153 amino acids, including a probable signal sequence of 20 amino acids that would be clipped from the final product. Removal of the putative signal sequence from the interleukin-2 molecule would leave a protein with a calculated molecular weight of 15,420.

Interleukin-2 is produced by T cells and stimulates the division of T cells. To show this, investigators first had to distinguish its effects from those of the lymphokine interleukin-1 and from those of antigens, which are the natural initiating triggers for immune responses.

Exposure to antigen alone or to lectins, plant proteins sometimes used as laboratory stand-ins for antigens, is not sufficient to stimulate T cell division, investigators found. Macrophages, another type of immune cell, are also needed to present the antigen to the T cells. In addition to presenting antigen, the macrophages supply a chemical signal in the form of interleukin-1, a protein with molecular weights in the range of 12,000 to 16,000. "Interleukin-1 is an augmenting rather than a primary signal," says Joost Oppenheim of the Frederick (Maryland) Cancer Research Center of NCI. "It acts only when you stimulate T cells with antigen or some other stimu-

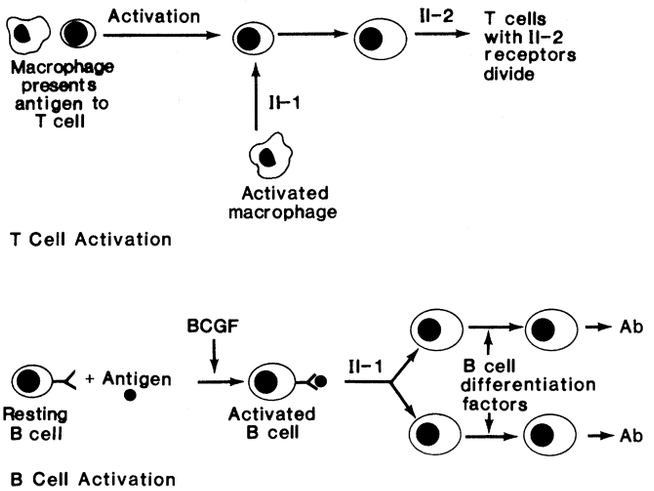
lus." Interleukin-1 does not stimulate T cell division directly, however. It induces the production of interleukin-2 by the activated T cells, and it is this lymphokine that stimulates the T cells to proliferate.

In addition to producing an increase in the size of T cell populations, interleukin-2 stimulates the production by the cells of immune interferon (also called γ-interferon), according to Oppenheim and William Farrar, who is also at Frederick. Immune interferon is itself a lymphokine and has a number of effects in the immune system, including enhancing the cytotoxic activities of T cells, macrophages and natural killer cells.

According to Oppenheim and Patricia Steeg, who is now at NCI, the interferon also increases expression on the surfaces of macrophages of a type of class II histocompatibility molecule that is needed for presentation of foreign antigen. In that way, immune interferon can further amplify the response to a particular antigen by enhancing its presentation. In effect there is a cascading loop of lymphokine actions, each of which serves to amplify the response to the triggering antigen. This is important because the interaction between a triggering antigen and an immune cell is very specific and only a very few members of the total population will be able to recognize it. But once the cells are activated they can respond to the nonspecific lymphokines, and a large expansion of the antigen-specific cell population can occur.

The manner in which the lymphokines stimulate cell division is unclear, although more is known about the action of interleukin-2 than of interleukin-1. "Interleukin-2 is acting exactly like a polypeptide hormone," Smith says. He and his colleagues have shown that T cells have receptors for the agent. "The intriguing thing is," he explains, "in contrast to all other major polypeptide receptor system's, whose receptors are always expressed, the resting lymphocyte does not express receptor for interleukin-2. The only cells that will respond to interleukin-2 are cells that are antigenically stimulated." What happens after the lymphokine binds to its receptor is still a mystery, however. The assumption is that interleukin-1 will also act through a specific receptor, although this has not yet been demonstrated.

A major way in which interleukin-1 and interleukin-2 differ is in the much broader range of sources and targets of the former. Interleukin-2 is released by T cells and its effects are apparently limited to T cells, but interleukin-1, Oppenheim says, "looks like a factor that has multiple activities and is made by many cell types." For example, his group finds that interleukin-1, or a protein that resembles it very closely, is made by epithelial cells, including the keratin producing cells of the skin, corneal cells, and the cells lining the mouth cavity.



To activate T cells, macrophages release interleukin-1 (Il-1), which stimulates production of interleukin-2 (Il-2) by T cells that have been triggered by antigen. The Il-2 stimulates division of receptor-bearing T cells. B cell activation requires both B cell growth factor (BCGF) and Il-1 to evoke division of triggered cells. Specific differentiation factors then elicit antibody (Ab) production.

In addition, the lymphokine has a wide range of effects on nonlymphocytic cells, all of which could contribute to inflammation. Steven Mizel of Pennsylvania State University says, "What excited us is that this mediator seems to play a major role in the inflammatory response." For example, interleukin-1 stimulates proliferation of fibroblasts and might contribute to fibrosis, the abnormal deposition of fibrous connective tissue that is often seen at inflamed sites.

In addition, Lawrence Lachmann and his colleagues at M. D. Anderson Hospital and Tumor Institute observed that interleukin-1 stimulates production by fibroblasts of such protein-splitting enzymes as collagenase and plasminogen activator. In this way, the agent might promote the normal cleaning up of debris that is part of wound healing. But if the effects of the agent were prolonged they might lead to chronic inflammation.

In related findings, the Mizel group reported that interleukin-1 stimulates release by synovial cells of collagenase and prostaglandins, potent chemicals that have also been implicated as mediators of inflammation. And the Oppenheim group has shown that fluids washed from inflamed gums have higher concentrations of the lymphokine than fluids from normal gums.

Fever may be another effect of interleukin-1 production, which would mean that the agent acts on brain cells in the temperature control region of the hypothalamus. According to Charles Dinarello of Tufts University, endogenous pyrogen, a macrophage factor that elevates body temperatures has many of the same properties as the lymphokine. And Patrick Murphy of Johns Hopkins University School of Medicine finds that interleukin-1 causes fever in rabbits. It now appears that endogenous pyrogen and interleukin-1 are identical.

There have been reports that increased temperatures in the range typical of fever greatly increase T cell proliferation in the test tube. By fever induction then, interleukin-1 may further facilitate the cellular proliferation it sets in motion by eliciting interleukin-2 release.

It is not yet known whether all the effects now being attributed to interleukin-1 are produced by exactly the same molecule or whether there is a family of interleukin-1 molecules with related, but different, structures. Cloning of the gene, which can then be used to detect related DNA's in the genome, may help to resolve this issue. In the case of interleukin-2, the Taniguchi group has preliminary evidence that there is only one gene.

Regulation of B cell proliferation and differentiation resembles that of T cells in some respects, according to studies by several investigators, including Fauci, Paul, and Maureen Howard of NIAID; Abby Maizel of M. D. Anderson; and Tadamitsu Kishimoto of Osaka University. The B cells must first be activated by antigen. Then the activated cells can respond to B cell growth factor, which must act in conjunction with interleukin-1, and proliferate.

The B cell growth factor is produced by T cells and for some time there was confusion about whether it was a discrete factor or was actually interleukin-2. But more recent research from the various laboratories has eliminated the latter possibility. "The T and B cell growth factors are distinct entities," Maizel notes. The activities of the two lymphokines could be separated. "If you

take activated T cells to absorb out T cell growth factor, B cell growth factor remains in the medium. You could also absorb B cell growth factor with activated B cells," he continues. Results such as these also suggest that there are specific receptors for B cell growth factor just as there are for interleukin-2, the T cell growth factor. According to Paul, interleukin-2 elicits production by T cells of B cell growth factor and this may have been the source of the original confusion about whether interleukin-2 has B cell growth factor activity.

Although B cell growth factor and interleukin-1 stimulate proliferation of B cells, they are not sufficient to cause the cells to differentiate to the state in which they can produce antibody. Additional lymphokines, which are called B cell differentiation factors and secreted by T cells, are required for that.

There are indications, from Kishimoto's laboratory and that of Ellen Vitetta at the University of Texas Health Science Center in Dallas, among others, that the differentiation factors may be specific for the production of different antibody classes. For example, Vitetta's group has identified a factor that induces activated B cells to secrete antibodies of the immunoglobulin M (IgM) class and another factor that induces the secretion of an immunoglobulin G (IgG) molecule. These factors, Vitetta says, appear to work by increasing production of the messenger RNA's for the antibodies.

B cells normally produce an IgM first. As they differentiate to their final forms they may either continue producing an IgM or switch to any of the other four immunoglobulin classes. The Texas workers find the IgG does not appear until after the cells have been exposed to the appropriate differentiation factor. Before that they make an IgM and Vitetta hypothesizes that the factors may be involved in the immunoglobulin class switch.

In addition to the lymphokines that act on T and B cells, there are a variety of agents that act on macrophages, to attract them to regions where they are needed, to help hold them there, and also to cause their activation. Studies of these agents are not yet as advanced as those of the interleukins and the B cell growth and differentiation factors.

Immune recognition of foreign antigen is highly specific, but amplifying the response of a few cells to the point at which they can mount an effective immune attack clearly requires a complex interplay of nonspecific chemical signals for growth and differentiation.

—JEAN L. MARX

Promising Animal Model for MS

While the causative agent of multiple sclerosis remains illusive, the search continues for a useful model system that might yield some important clues to the human disease. One promising candidate, which involves visna virus, is being scrutinized by workers at the Johns Hopkins University School of Medicine, led by Janice Clements and Opendra Narayan.

The symptoms of the sheep disease include progressive muscular weakness, which is punctuated by periods of remission: paralysis and death often occur. The lesions in the brain are scattered, discrete loci of inflammation followed by demyelination. These patterns echo the human disease.

What especially intrigues Clements and her colleagues is the pulselike progression of visna infection, which presumably accounts for the alternating remission and relapse in the animal. Infected animals eventually mount an antibody response against the virus, but the subsequent emergence of a new antigenic variant produces a new round of infection. The animal and the virus are locked in a constant battle in which the pathogen repeatedly runs ahead of the host by the generation of new variants.

Although certain pathogenic parasites, such as schistosomes, deploy a large repertoire of antigenic variants in prolonged infections of their hosts, they do so by shuffling genes around their relatively large genomes. Visna, which is a 10-kilobase long, nononcogenic retrovirus, is not so generously equipped and must generate variants by more modest means. A number of pathogenic viruses show shifts in antigenic status, but few display the progressive antigenic variation in a single host seen in visna virus infection.

It turns out the emergence of new variants in visna is the outcome of an accumulation of point mutations in the 3′ region of the virus genome, a region that almost certainly codes for the coat glycoprotein. Until sequencing is complete in the parental strain and the subsequent variants it will not be possible to determine how many point mutations produce each variant nor precisely where the base changes occur. Visna is a relatively unknown retrovirus and so the Hopkins team faces an enormous amount of background work in these analyses.

One especially interesting aspect of the antigenic variation in visna is that the repertoire of variants produced in different hosts is very similar, though the order in which they appear may differ. Clements and her colleagues interpret this as a reflection of a limited number of regions on the glycoprotein molecule that are of biological significance. Presumably, other mutants either produce no change in antigenic characteristics of the molecule or induce a shift that is lethal in some way. The window for selection of variants is very narrow, says Clements.

Each new virus variant carries the accumulated mutations of its predecessors. New mutations, it seems, alter the antigenic profile of the virus coat just sufficiently for the organism to escape neutralization by existing antibodies. One result of this is that the antibody spectrum directed against the virus becomes progressively broader as the disease continues.

The mode of visna infection in sheep is unusual and underlies the pathological picture produced. The viruses infect monocytes, which are cells of the immune system that mature into macrophages. The brain lesions are caused, suggests Clements, when the immune cells invade the nerve tissue following some other primary infection. An inflammatory response arises as a result of the primary and possibly the secondary infection, and then subsides, leaving a small demyelinated area.

Between 60 and 70 percent of sheep in this country have antibodies to visna virus, but only a small proportion show any pathological signs of disease. The progressive weakness might be seen as a rare complication of a widespread infection, says Clements.

Whether multiple sclerosis fits this etiological profile is, at present, mere speculation. The Hopkins workers are, however, attracted by the notion of a neurotropic retrovirus as the causative agent in the human disease. At some point they will begin rigorously to test cross reactions between multiple sclerosis sera and visna and related viruses and to look for nucleic acid hybridization.—ROGER LEWIN