Cytokines Are Two-Edged Swords in Disease

Without the molecules known as cytokines the body could not mount its defenses against infection and injury. Cytokines, as transmitters of regulatory signals among cells, participate in the activation of the various types of immune cells and also mediate other typical responses to infection, such as fever and inflammation.

However, the agents are now being implicated in the etiologies of several of the most common human ailments, including rheumatoid arthritis, diabetes, and atherosclerosis. A sampling of presentations from the recent "International Workshop on Monokines and Other Non-Lymphocytic Cytokines" illustrates some of the ways in which cytokines have been linked to disease development.*

Does Interleukin-1 Play a Role in Atherosclerosis?

Although interleukin-1 is not the only cytokine thought to play a role in causing disease, its name seems to crop up the most frequently in this regard. With another cytokine, tumor necrosis factor, it may even contribute to the development of atherosclerosis—and thus of heart disease and stroke—according to Helen Vlassara and Michael Brownlee of Rockefeller University.

The investigators have found that as the proteins of blood vessel walls age they are modified by the addition of the sugar glucose. In diabetic individuals, who have increased concentrations of blood glucose at least part of the time, the glucose addition is accelerated. When long-lived proteins, such as collagen, undergo the modification, they cross-link with other proteins and become difficult to degrade.

There is nonetheless a way of eliminating the modified proteins, which are called "AGE-proteins" (where AGE stands for advanced glycosylated end product). Vlassara and Brownlee have identified a specific high-affinity receptor on macrophages that recognizes and binds AGE-proteins. As a result, they are taken into the cells by means of endocytosis and destroyed. "Macrophages have a mechanism for recognizing senescent proteins," Vlassara says.

The question then raised was: do the macrophages that bind AGE-proteins also become activated to release cytokines? The answer, the Rockefeller workers now find, is yes. "When the glycosylated proteins bind to the macrophage receptor," Vlassara told the cytokine workshop participants, "they trigger the macrophages to secrete tumor necrosis factor and interleukin-1."

The formation of AGE-proteins on blood vessel walls and the consequent attraction and activation of macrophages may contribute to atherosclerosis development in several ways. For one, interleukin-1 may stimulate the proliferation of the cells of the vessel wall. Such proliferation is a characteristic feature of atherosclerotic plaques.

For another, AGE-proteins can cross-link with the low-density lipoproteins (LDLs), the cholesterol carriers that foster plaque formation. "The type of modification that we have discovered can trap LDLs in the wall so that they can't be metabolized," Vlassara says. Moreover, the trapped LDLs may themselves become glycosylated and attract still more macrophages, thereby setting up a vicious circle that may eventually culminate in vessel blockage by atherosclerotic plaques.

More work will be required to confirm the scheme proposed by the Vlassara-Brownlee group for plaque formation. Meanwhile, Stefan Endres of Charles Dinarello's group at Tufts University and the New England Medical Center reported at the workshop that there may be a way of reducing interleukin-1 production, should that prove desirable. Endres and his colleagues found that when healthy volunteers supplement their normal diets (the usual graduate student diets consisting of pizza and hamburgers, Dinarello notes) with a fish oil concentrate containing about 3 grams of eicosapentaenoic acid (EPA), the volunteers' white blood cells show a 70% reduction in interleukin-1 production.

EPA is one of the "omega-3" fatty acids, which are so called because they have a double bond between carbons 3 and 4. Epidemiological studies had previously linked the omega-3 fatty acids to a reduced risk of coronary and inflammatory disease in Eskimos, who have a low incidence of such conditions even though they eat high-fat diets. They also eat a great deal of fish that contain EPA and its relatives, a circumstance that has led to the proposal that omega-3 fatty acids may protect against heart disease and arthritis.

If interleukin-1 contributes to the development of those diseases, as proposed, then the Tufts results may help explain the protective effects of EPA. Whether dietary supplementation with EPA might reduce the risk of heart disease in non-Eskimo populations remains to be established. Also unknown is whether it might have untoward effects, reducing the ability to fight off infections, for example.

TGF-β_2 May Cause Immune Suppression in Glioblastoma Patients

Patients who have a glioblastoma, a highly malignant type of brain tumor, also show signs of immune suppression, especially of the cell-mediated branch of the immune system. According to Adriano Fontana of University Hospital in Zurich and his colleagues, the immune suppression may be the result of the tumor cells secreting the cytokine known as transforming growth factor–β_2 (TGF-β_2).

The Zurich workers have shown that human glioblastoma cells, when grown in culture, produce a protein factor that inhibits the proliferation of the T cells of the immune system in response to activating agents such as interleukin-2. The effect of the inhibition would be to depress immune responses that depend on the T cell activities.

Determination of a partial amino acid sequence of the suppressive factor indicated that it is related to TGF-β. The cloning of the factor gene, and the determination of its complete sequence, have now confirmed that possibility and pinpointed the protein as the TGF-β_2 variant.

In addition to providing an example of how the immune and nervous systems can interact, the result has a number of implications for the development of glioblastomas. Although TGF-β was originally identified as a stimulator of cell division, it was subsequently found to inhibit the division of most types of cells. "In many systems, TGF-β seems to act as a kind of brake," says Michael Sporn of the National Cancer Institute. Researchers have postulated that a loss of responsiveness to TGF-β may contribute to cancer development by allowing cells to grow out of control.

Whether or not that is the case, the immunosuppressive effects of the cytokine might contribute to cancer development. "TGF-β is an important immune suppres-

*The workshop was held on 6 to 10 December 1987, on Hilton Head Island, South Carolina.

sor, both in vivo and in vitro," Sporn explains. "It is at least 1000 times more potent than cyclosporine." (Cyclosporine is a drug that is used to suppress the immune systems of organ transplant recipients and of patients with autoimmune diseases.)

In addition to inhibiting T cell activation, TGF-β depresses the activities of the "natural killer" cells of the immune system. Natural killer and cytotoxic T cells may help to destroy cancer cells before they have a chance to multiply to form a life-threatening tumor. If the TGF-β_2 that is produced by glioblastoma cells suppresses the activities of these immune cells in human beings, the result might be to allow a glioblastoma to grow out of control.

Interleukin-1 May Contribute to Diabetes Development

Over the past few years, researchers have come to a growing acceptance of the hypothesis that insulin-dependent diabetes is caused by an autoimmune attack that destroys the insulin-producing beta cells of the pancreas. However, there is still a great deal of uncertainty about what initiates the attack and which components of the immune system carry it out. At the cytokine meeting, Thomas Mandrup-Poulsen of Steno Memorial Hospital in Gentofte, Denmark, described his group's evidence implicating interleukin-1 in the early stages of beta cell destruction.

Although diabetes patients often have antibodies and cytotoxic T cells that are directed against beta cells, Mandrup-Poulsen says that there are difficulties with the idea that these immune effectors have a primary role in wiping out the insulin-producing cells. Mandrup-Poulsen and his colleagues are focusing instead on macrophages as possible mediators of beta cell destruction. Diabetic islets contain large numbers of these cells, which are prominent producers of interleukin-1.

In early work, the Gentofte group showed that the culture fluids in which macrophages have been grown contain a substance that is toxic to beta cells. That substance apparently is interleukin-1. The pure recombinant material is also toxic to cultured beta cells. Other cytokines, such as lymphotoxin, γ interferon, and tumor necrosis factor, all of which kill some cell types, have little effect on beta cells by themselves, although tumor necrosis factor potentiates the effects of interleukin-1.

Mandrup-Poulsen proposes that damage to beta cells causes a release of some antigen that would not normally be exposed to the immune system. This in turn leads to infiltration of the islets by immune cells, including macrophages, that release interleukin-1. The cytokine is not only directly toxic to beta cells but can also attract still more macrophages into the islets. Ultimately all the beta cells will be killed and diabetes will result.

What causes the initial beta cell damage is still a matter for speculation. Viral infections and toxic chemicals have both been proposed as initiating agents. And interleukin-1 itself might even play that role, according to Mandrup-Poulsen.

Neurokinin Linked to Interleukin-1 and Arthritis

Probably the strongest link between a cytokine and a disease is that between interleukin-1 and the chronic inflammatory disease, rheumatoid arthritis. The interleukin can not only contribute to inflammation in several ways, but it is also found in increased concentrations in the joints of rheumatoid arthritis patients.

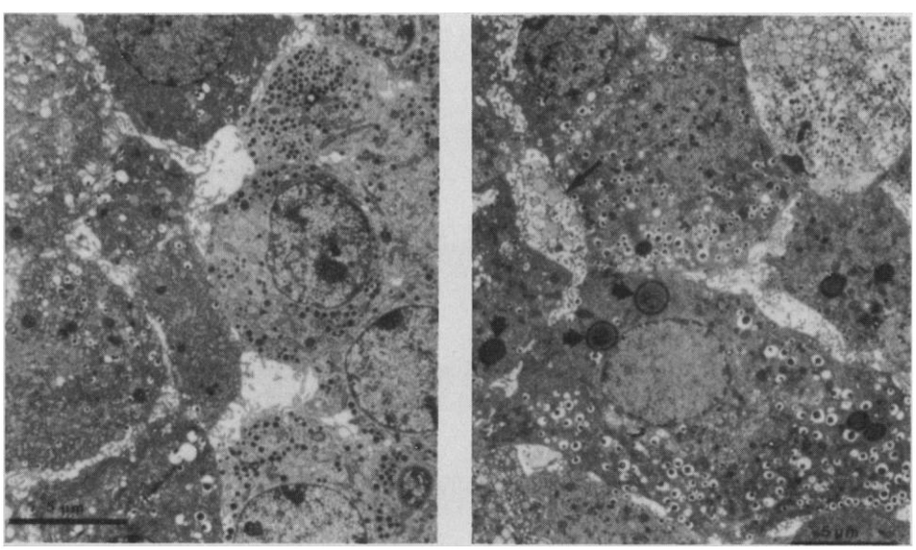
Interleukin-1 does not act alone, however. It usually works in conjunction with other cytokines. In addition, according to Edward Kimball and his colleagues at McNeil Pharmaceutical in Spring House, Pennsylvania, interleukin-1 interacts with neuroactive agents such as substance P. The research illustrates once again that there is often considerable overlap between the agents that mediate nerve cell activities and those that mediate immune responses.

Substance P is best known as a transmitter of pain impulses in the nervous system, but, Kimball says, it has a variety of inflammatory effects "that are highly reminiscent of what one would expect of interleukin-1." Substance P activates macrophages, for example, to secrete agents such as prostaglandins and oxygen-free radicals that are involved in inflammation.

Macrophages are also a major source of interleukin-1. Kimball and his colleagues have now shown that substance P stimulates macrophage secretion of the cytokine. Neurokinins A and B have a similar effect, although neurokinin B is somewhat less active than the other agents. The joints are innervated by substance P–releasing neurons, and the neurotransmitter may thus be released at the target tissue for the inflammation of rheumatoid arthritis.

In addition, the McNeil workers find that substance P potentiates the stimulation by interleukin-1 of fibroblast proliferation. Although such proliferation is a necessary part of wound-healing, this is another cytokine activity that may contribute to inflammation if it is not properly limited.

"There are two ways that neurotransmitters such as substance P may affect inflammation," Kimball concludes, "by increasing secretion of cytokines and by potentiating their effects." The work suggests that blocking substance P's activities may be a way of treating arthritis, although that possibility must still be tested. ■ JEAN L. MARX



Effects of interleukin-1 on beta cells. *The left micrograph shows normal islet tissue. The insulin-producing beta cells are the darker cells on the left. The right micrograph is of beta cells that were incubated with interleukin-1 for 24 hours. Most of them contain abnormal, dark-staining bodies (heavy arrowheads) and some beta cells (slender arrows) have disintegrated.* [*Reprinted with permission from T. Mandrup-Poulsen* et al., Acta Pathol. Microbiol. Immunol. Scand. Sect. C **95**, *55 (1987)*]