Coagulation as a Common Thread in Disease

Coagulation may be a general host response that contributes to the pathology of heart disease, cancer, and inflammation

Coagulation research is not just for hematologists anymore. Such was the message brought home by a recent meeting on "Coagulation, Cancer, and Inflammation."* "The coagulation pathways are not just the turf of people studying thrombosis," said meeting chairman Thomas Edgington of the Research Institute of Scripps Clinic, "but they are a central host response system."

Not only is coagulation a necessary defense to prevent blood loss following injury, but it now appears that activation of clotting systems is an integral part of the host's immune response. The contributions of abnormal coagulation to diseases of the blood vessels, including coronary heart disease, have long been appreciated. The new work indicates that the clotting pathways may also be involved in the pathology of allergy and inflammation. In addition, clot deposition around cancer cells may aid the progressive growth of malignant tumors.

The impetus for having the meeting, Edgington says, was the growing awareness during the past 3 or 4 years of the major contributions made by cells to the activation of the coagulation and fibrinolytic (clot-dissolving) systems. Cells, including macrophages and cancer cells, can participate in the activation of the serine proteases, plasma enzymes that are required for coagulation or fibrinolysis to occur. These discoveries have led to a greater appreciation of the importance of the systems to diseases in tissues outside the vascular system. For example, macrophages, which are conspicuous participants in inflammatory responses, can go anywhere in the body and initiate coagulation there.

These cells, as Zanvil Cohn of Rockefeller University pointed out in his opening presentation, are amazingly versatile in their contributions to inflammatory responses. They can destroy foreign matter and other cells directly by engulfing them. Or they can do so indirectly. Cohn listed some 50 biologically active substances found by his laboratory and others to be released by macrophages. In fact, macrophages are sometimes called "circulating hepatocytes" because they are as active in this regard as liver cells.

Some of the substances released attract other immune cells to inflammatory sites. Others, including activated oxygen products such as hydrogen peroxide and enzymes such as lysozyme, cause tissue damage by attacking cellular constituents. And the macrophage very actively produces clot-promoting substances, such as tissue factor.

Production of procoagulant activity by macrophages may provide an explanation for the deposition of fibrin, which forms the insoluble matrix of blood clots, in inflamed sites. Moreover, initiation of coagulation by macrophages may be a necessary component of at least some inflammatory responses. For example, Gary Levy, who originally worked with Edgington at Scripps and who is now at the University of Toronto School of Medicine, presented data indicating that the severity of a viral disease, mouse hepatitis, is correlated with the ability of macrophages to trigger coagulation in the liver. The virus reproduces in the liver cells of all the mouse strains studied, but only one strain, which is genetically very susceptible to the disease, suffers a high degree of liver damage. Macrophages from these mice respond to virus by producing proteases of the coagulation pathway. Mice that are extremely resistant to the hepatitis do not suffer liver damage nor do their macrophages initiate coagulation. And a third strain is intermediate for both.

Edgington says, "The only correlation that we have is between the coagulation response and the degree of injury. We don't know exactly how the generation of coagulation proteases causes the damage but we suspect there is vascular injury, which would add further insult to the virus-infected cells, leading to their death."

Activation of coagulation pathways might contribute in additional ways to the damage. According to Cohn and to Dolph Adams of Duke University serine proteases, such as those required for fibrin deposition, can enhance cell-killing by macrophages that release activated oxygen products. Moreover, the products released during the formation of fibrin from fibrinogen, its soluble protein precursor, can attract additional inflammatory cells. And Harold Dvorak and his colleagues at Beth Israel Hospital in Boston found that fibrin deposits promote angiogenesis, the growth of new blood vessels. Although this is part of normal healing, it could also serve to facilitate further the entry of inflammatory cells into the damaged area.

Many kinds of stimuli, including antigens such as bacterial lipopolysaccharides (LPS), complexes of antigen with antibodies, and certain chemicals, can activate procoagulant activity production by macrophages. Frederick Rickles of the University of Connecticut Health Center in Farmington says, "Macrophages are sensitive to a variety of perturbations. Tissue factor production is one of the earliest manifestations of their response."

According to the Scripps group, activation by most, if not all of these stimuli, requires the help of another type of immune cell, the T lymphocyte. The collaboration is restricted, meaning that the helper T cell and the macrophage must share a common histocompatibility antigen to interact. Edgington says, "In every case, only where they are compatible in the I-A [histocompatibility] locus, there is a response." In this regard, procoagulant release by macrophages resembles other immune responses, such as activation of cell-killing by T cells and of antibody production by B cells, both of which may require restricted collaboration with helper T cells.

Not everyone finds a requirement for T cell help for the procoagulant response to all stimuli, however. The collaboration may be required for activation by some stimuli, Rickles says, but not for all. "In our hands, lipopolysaccharide can act on the macrophage directly in the absence of any demonstrable T cells." Edgington does find a requirement for T cell cooperation in LPS activation and the reason for the discrepancy is currently unclear.

Nevertheless, procoagulant production, turned on as it is by a variety of antigenic stimuli, may be a general component of the host's immune responses. If so, it may help to explain why some types of infection are accompanied by clotting abnormalities, including a condition called disseminated intravascular coagulation (DIC), which is characterized, as its name suggests, by a generalized activation of clotting throughout the circulatory system. Often DIC results in hemorrhaging because the blood compo-

*Held in Airlie, Virginia, on 8 to 10 September under the aegis of the National Heart, Lung, and Blood Institute and the National Cancer Institute.

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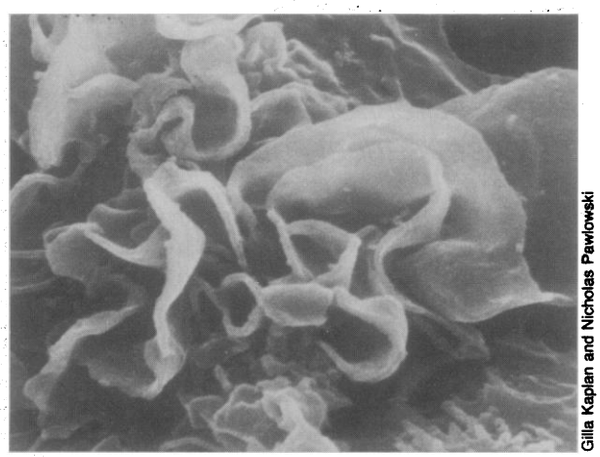

Gilla Kaplan and Nicholas Pawlowski

Human monocyte

The ruffles are characteristic of the circulating cell.

nents that should be available for clot formation have already been depleted. Alternatively, the clotting abnormalities may result in blockage of blood vessels.

Cancer patients frequently suffer from these problems, too. According to Julian Ambrus of Roswell Park Memorial Institute, clotting abnormalities were the major cause of death of some 18 percent of the 508 cancer patients who succumbed at his institution during a period of 1 year. The abnormalities contributed to the deaths of another 43 percent of the patients. Only infections, which were the major cause of death for 36 percent and contributory causes for another 68 percent, killed more patients than clotting abnormalities.

Production of tissue factor by activated monocytes (the immature form of macrophages that circulate in the blood stream) may contribute to the DIC of cancer patients, too. The Rickles group has found that the cells taken from patients with advanced cancer had increased levels of tissue factor when they were assayed in vitro. These levels correlated with the concentrations in the patients' blood of one of the peptides released during the conversion of fibrinogen to fibrin. What might activate the blood monocytes of the cancer patients is not known, but it may be a host response to the tumor cells.

Cancer cells themselves produce procoagulant activity, which might contribute to the DIC of the patients. It may also help tumor cells to escape destruction by the immune system, according to Dvorak, who with his colleagues, has recently confirmed and extended earlier reports of fibrin deposits in tumors. Dvorak told the meeting participants, "We have looked at a variety of solid tumors of the guinea pig and mouse and have found some fibrin in every one. But the acid test of findings of this sort is whether they apply to man."

And they do. The Dvorak group has found fibrin deposits in human breast cancers and in Hodgkin's lymphoma tumors. In the breast tumors, the fibrin deposits surrounded small clumps of tumor cells. Cells at the edges of the tumor had more extensive deposits but they were also seen around cells in the center. The arrangement implies that the fibrin deposits provide a physical barrier to protect the tumor cells from attack by immune cells. As Dvorak noted, "The ratio of lymphocytes to tumor cells was about five to one; yet the tumor cells are doing fine."

For fibrin to be deposited in a tumor, two requirements must be met. There must be an increase in the permeability of the small blood vessels to allow for passage into the tissue of plasma with its clotting factors and there must be the production of procoagulant activity. The Dvorak group finds that tumor cells produce an agent that increases permeability plus one with procoagulant activity. The latter was associated with membrane vesicles shed by the tumor cells. Other investigators have found clot-promoting agents of different types.

In addition to protecting tumor cells, fibrin deposits may promote tumor growth by stimulating the ingrowth of new blood vessels. Tumors, as is known from the work of Judah Folkman of Harvard Medical School and others, need to expand their blood supply as they grow and produce angiogenesis factors of their own. Fibrin deposition may help out with angiogenesis.

In the last presentation of the meeting, Daniel Rifkin of the New York University Medical School noted that angiogenesis and the invasion of growing tumors into surrounding tissue may be analogous. Both require, for example, that cells move through connective tissue with its collagen cement. The Rifkin group has shown that capillary endothelial cells, in response to stimulation with certain chemicals, produce a latent collagenase (an enzyme that dissolves collagen) and plasminogen activator, which converts inactive plasminogen to plasmin. In addition to dissolving clots by breaking down fibrin, plasmin can activate the latent collagenase, which could then help to break down connective tissue and facilitate the outgrowth of the capillary cells. Angiogenesis factors, including one supplied by Folkman, had the same effect on the endothelial cells. Many types of tumor cells also make plasminogen activator and latent collagenase, Rifkin notes, which could aid their invasion of normal tissue.

Because of suggestions that coagulation facilitates tumor development, a number of clinical trials, usually involving small numbers of patients, have been undertaken to determine whether anticoagulant treatment has therapeutic benefit for cancer patients. Most of these trials have produced negative results, but one study, described at the meeting by Leo Zacharski of the Veterans Administration Medical Center in White River Junction, Vermont, and the Dartmouth-Hitchcock Medical Center in Hanover, New Hampshire, was more promising.

Treatment with the anticoagulant Warfarin (Coumadin) doubled the survival time of patients with small cell carcinoma of the lung. Patients in the control group lived about 25 weeks, whereas those who received Warfarin lived about 50 weeks. The anticoagulant did not induce remissions of existing tumors, as chemotherapeutic drugs normally do, but instead appeared to slow the spread of the disease.

This is in accord with theories suggesting that the procoagulant activity produced by tumor cells may help an escaped cell to adhere to a blood vessel wall and seed a new tumor. Moreover, according to Dennis Cunningham of the University of California at Irvine, thrombin, the enzyme that converts fibrinogen to fibrin, stimulates the division of cells, an effect that could enhance the growth of both primary and secondary tumors. Other ways by which anticoagulants might act against tumors remain possible, however.

Not all cancer patients benefited from Warfarin therapy. Among the cancers of those that did were lung cancers other than small cell carcinoma, and cancers of the colon, prostate, head, and neck.

Anticoagulants may or may not prove to be of general value for cancer therapy. Nevertheless, coagulation and its reverse side fibrinolysis now appear to link such hitherto disparate—and common—conditions as inflammation, heart disease, and cancer.—JEAN L. MARX