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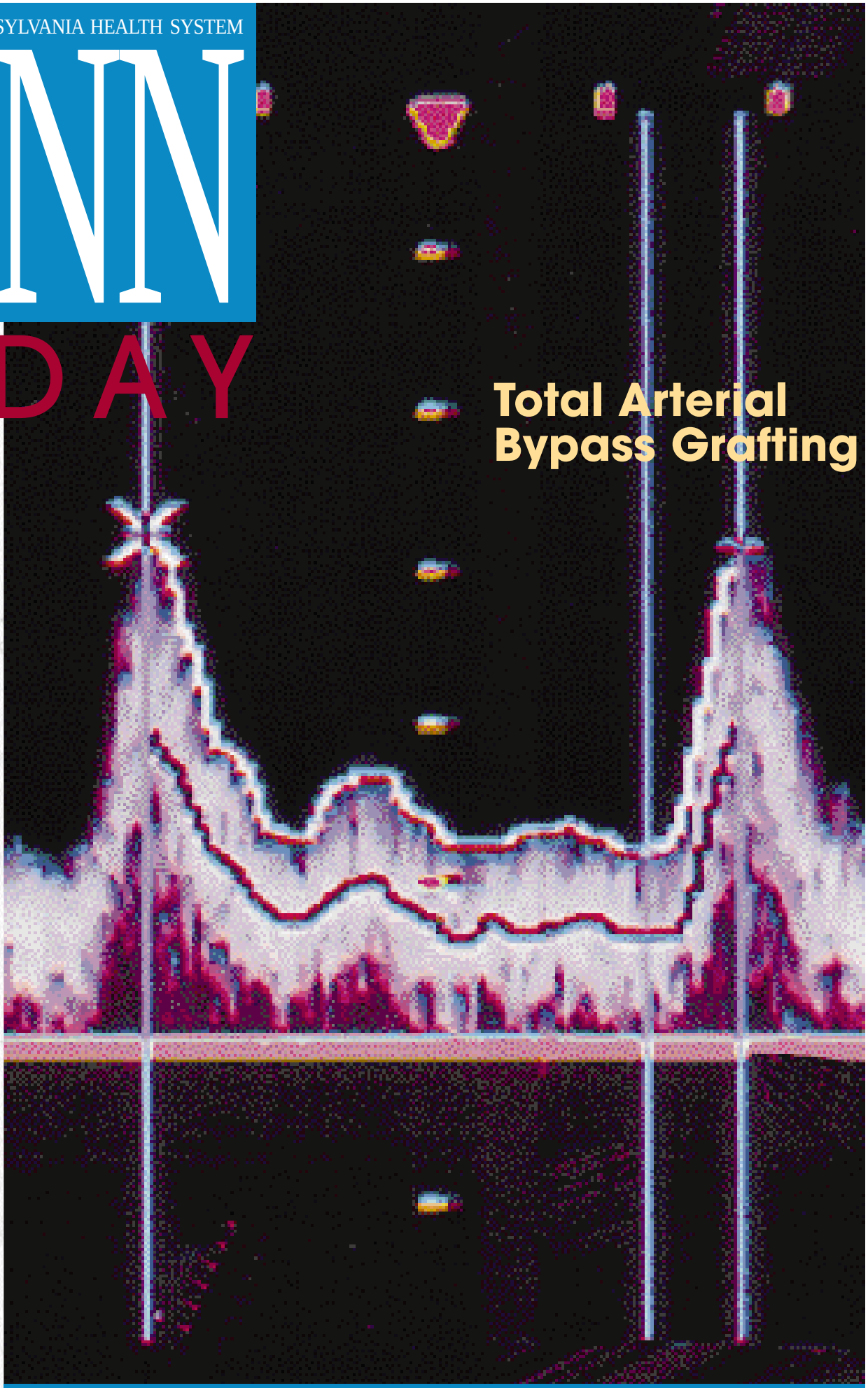

Total Arterial Bypass Grafting

Volume 10, Number 1

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STOPPING THE CASCADE

Inappropriate Complement Activation

Xenotransplantation, cardiopulmonary bypass surgery, plasmapheresis. Some of the most innovative technologies under development have been plagued by one of the most evolutionarily ancient natural defense mechanisms - the complement cascade. A complex blood-based system of at least 30 proteins, complement is an important line of defense against pathogenic organisms and other foreign substances. Because activation of complement may also damage healthy tissue, it has also been linked to a wide range of diseases. Now, a compound discovered by Arvind Sahu, PhD, in the laboratory of John D. Lambris, PhD, offers hope of inhibiting complement activation and has shown promise in two models of extracorporeal circulation as well as in an ex-vivo xenotransplantation model.

The list of diseases associated with inappropriate complement activation is long. It includes experimental allergic neuritis, type II collagen-induced arthritis, myasthenia gravis, hemolytic anemia, glomerulonephritis, immune complex-induced vasculitis, and multiple sclerosis. Complement is also thought to contribute to the cellular destruction that occurs in adult respiratory distress syndrome, stroke, heart attack, and burn injuries. And, activation of complement may also occur when blood comes into contact with bioincompatible surfaces such as the tubing of a cardiopulmonary bypass oxygenator, initiating "whole body" inflammation; or when the body encounters incompatible transplanted tissue, initiating hyperacute rejection.

Inhibiting complement activation has thus been a long-sought "holy grail" for immunologists, yet one that has until recently evaded discovery. According to Lambris, small molecule complement inhibitors have been identified in vitro but have shown limited effectiveness and unacceptable toxicity in vivo.

Complement, found in all vertebrates and even some invertebrates, plays a role both in innate (non-specific) and adaptive (specific, antibody-based) immune systems and is the human body's first line of defense against foreign invasion. Since it was first discovered more than a century ago, scientists have attempted to define precisely the chemical reactions involved. They have described three enzymatic cascades in which one protein binds and activates another, which binds and activates another, and so on. The so-called "classical pathway" is triggered by the binding of IgM and IgG antibodies with antigen. The end result of the proteolytic cascade is lysis of the target cells. Other pathways of complement activation, the so-called "alternative pathway" and the "lectin pathway," do not involve antibodies but are triggered by the presence of proteins and carbohydrates on the surfaces of foreign invaders such as bacteria and viruses. In addition to triggering cell lysis, activation of the complement pathway also results in the production of other bioactive substances that both augment the body's defenses and further contribute to pathology by activating mast cells, neutrophils, and platelets.

Finding an Appropriate Target

In searching for an inhibitor of complement, Lambris and colleagues focused on one particular protein of the cascade called C3, which is cleaved into two fragments, called C3a and C3b. C3 seemed a particularly good target for inhibition because it is involved in all pathways of complement activation. The strategy Lambris used required screening billions of compounds derived from combinatorial libraries of

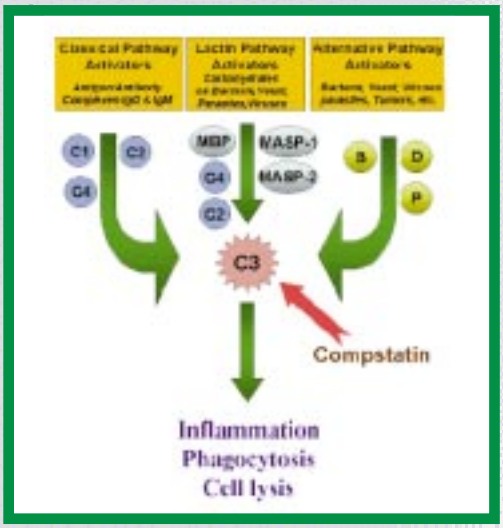

Figure 1: Schematic representation of the three pathways of complement activation. All the pathways converge at C3 activation step. Compstatin blocks the activation of C3, which results in inhibition of the activation process and thereby complement-mediated tissue injury.

DNA fragments and searching for a peptide fragment that would bind to C3.

"It's always compared with trying to find a needle in a haystack," he said. But in this case, the needle was found and

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not only did it bind to C3, it blocked its activity. "With a huge molecule like C3, we expected to find a lot of peptides that bind to different areas, and that would tell us about the functional domains of the C3 molecule," said Lambris. Surprisingly, only one peptide was isolated, a small molecular weight compound consisting of a ring of 11 amino acids. Even more surprising was the fact that it inhibited the activity of C3.

Further study of the structure of the peptide, which Lambris calls "Compstatin," has revealed more information about the structural basis of C3 activation and inhibition. For example, while most peptides are flexible in solution, Compstatin is rigidly held into its shape by virtue of its amino acid sequence. The solution structure of Compstatin has already been determined in collaboration with Dimitrios Morikis, PhD.

The next important step will be to determine the structure of C3-bound Compstatin. According to Lambris, this will help elucidate the C3-Compstatin recognition process, which will be important as investigators attempt to design other smaller molecules that will mimic the inhibitory activity of this peptide.

Figure 2. Solution structure of Compstatin.

In-vivo Studies

Compstatin has several properties that make it a promising candidate for clinical use. Because it is such a small molecule, it may be developed for oral delivery. Perhaps more important is that it resists degradation in serum, a drawback that has limited the effectiveness of some other peptide agents. Two recent studies from Lambris and collaborators in Sweden and Norway have demonstrated that Compstatin does prevent complement activation in models of extracorporeal circulation and xenotransplantation.

Complement is known to be activated during extracorporeal circulation, such as that used in dialysis and cardiopulmonary bypass surgery, resulting in a systemic inflammation reaction mediated by stimulation of neutrophils and platelets. Lambris and colleagues set up two different extracorporeal circulation models, in which blood was rotated through tubing similar to that used in heart bypass machines or dialyzers. When Compstatin was added to the system, complement activation was inhibited, as indicated by the reduction in cleavage products of C3 and inhibition of neutrophil activation. Further study of these models has also revealed more information about the process of complement activation when blood comes into contact with bioincompatible surfaces. For example, both the classical and alternative pathways appear to be activated, indicating that complement inhibitors that work only through one pathway would most likely be ineffective in these systems.

Similarly, both pathways of complement activation may be activated during transplantation, contributing to the inflammation and organ injury associated with hyperacute rejection. Inhibiting complement activation is thus a major hurdle that will need to be overcome in order for xenotransplantation to become a clinically useful technique. Lambris and colleagues tested Compstatin in a xenotransplantation model in which pig kidneys were perfused with human blood. The results were "unexpected," according to Lambris. The Compstatin-perfused kidneys survived to the maximum experimental time, nearly seven hours, in comparison with the control kidneys which survived only about an hour and a half. And, they showed fewer signs of complement-mediated damage.

The success of Compstatin in both the extracorporeal circulation and xenotransplantation systems has piqued the interest of both the scientific community and several biotechnology companies, said Lambris. He predicted that it will require another few years of preclinical work before clinical trials will be possible. And it may turn out that the compound currently being tested will be modified in order to make it even better.

"What we're going to do is modify the compound, see what is important in its interactions, and find out how it regulates C3," said Lambris. "We're trying to understand its molecular interactions and develop it into a useful therapeutic."

For more information, use PENNLine to call:
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To Read More...

"Solution structure of Compstatin; a potent complement inhibitor," Morikis D, Assa-Munt N, Sahu A, and Lambris JD. *Protein Science*. 7:1-9, 1998.

"Compstatin inhibits complement and cellular activation in whole blood in two models of extracorporeal circulation," Nillson B, Larson R, Hong J, Elgue G, Nilsson-Ekdahl K, Sahu A, and Lambris, JD. *Blood*. 92:1661-1667, 1998.