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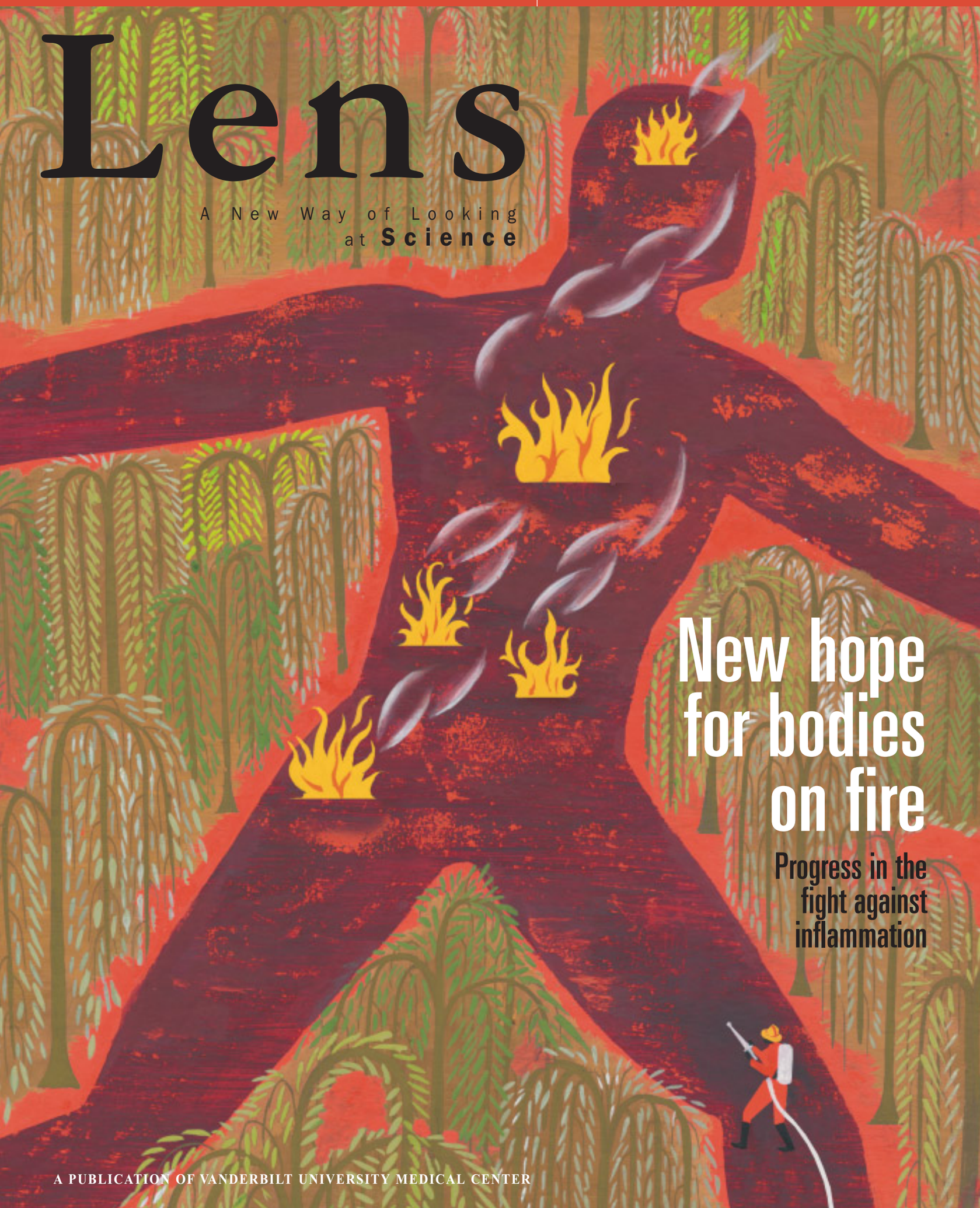
# Lens

A New Way of Looking at **Science**



## New hope for bodies on fire

### Progress in the fight against inflammation

A PUBLICATION OF VANDERBILT UNIVERSITY MEDICAL CENTER

Lens –
A New Way of Looking
at **Science**

**AUTUMN 2004**

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**About the cover: A body afflicted by "hot spots" of chronic inflammation is surrounded by willow trees, source of the ancient remedy salicin. Scientists are learning new ways to quench the "fires within."**

**Illustration by Philippe Lardy.**

One never notices what has been done; one can only see what remains to be done.

– MARIE CURIE

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**Our goal: to explore the frontiers of biomedical research, and the social and ethical dimensions of the revolution that is occurring in our understanding of health and disease. Through our *Lens*, we hope to provide for our readers – scientists and those who watch science alike – different perspectives on the course of discovery, and a greater appreciation of the technological, economic, political and social forces that guide it.**

Lens

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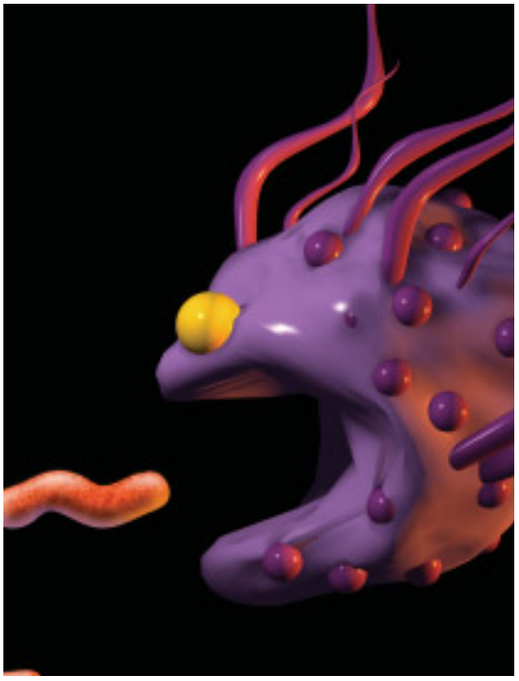

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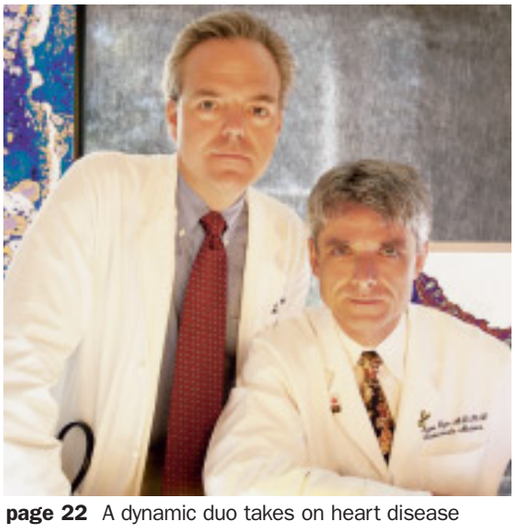

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DEAN DIXON

# Exploring inflammation

## A modern-day "Corps of Discovery"

**By Lawrence J. Marnett, Ph.D.**

Director
Vanderbilt Institute of Chemical Biology

Mary Geddes Stahlman Professor
of Cancer Research

Inflammation is a series of biochemical and cellular events that constitute our body's response to infection. Inflammatory cells surround invading pathogens and generate highly reactive and toxic chemicals including Clorox (sodium hypochlorite) and chlorine gas. They also synthesize antibodies to help clear bacteria, viruses, and other noxious stimuli, and they produce a range of signaling molecules such as prostaglandins and cytokines to amplify the inflammatory response.

This vigorous attack causes some collateral damage to surrounding tissue but normally it is local and transient. However, prolonged exposure to inflammatory stimuli or incorrect regulation of the inflammatory response leads to chronic and occasionally systemic tissue damage. As outlined elsewhere in this issue, this contributes to many important human diseases.

There is a rich history of research on the cause and treatment of inflammation, which illustrates the role that trained observation, serendipity, initiative, and hard work play in science and medicine. Some very interesting personalities have devoted their lives to inflammation research and their discoveries have had enormous impact on human health.

Drugs that treat inflammation are among the most prescribed therapeutic agents, and pharmaceutical companies spend billions of dollars trying to improve them. The complexity of the inflammatory response offers many potential strategies and targets for new drug development. So this is a very exciting and rewarding area for research.

The hallmarks of inflammation – pain, swelling, redness, and heat – and methods for its treatment were documented over 3,000 years ago. The Ebers Papyrus (1534 B.C.) describes the use of an infusion of dried myrtle for rheumatic and back pain. Hippocrates of Kos (400 B.C.) recommended a tea extract from the bark of the willow tree for pain and fever.

In 1763, an English clergyman, the Rev. Edward Stone, reported in a letter to the Royal Society, Britain's national academy of science, that powdered willow bark administered in water was effective in reducing fever in a clinical study of 50 of his parishioners. A major component of these plant extracts, called salicin, was isolated in 1828 by the German chemist, Johannes Buchner. Salicin is converted in the body to salicylic acid, which is the actual anti-inflammatory agent.

A small German dye company founded by Friedrich Bayer developed an industrial scale synthesis of salicylic acid in the late 1800s, but it was too harsh on the mouth and stomach to be very useful as a drug. A chemist at Bayer, Felix Hoffman, added an acetyl group to form acetylsalicylic acid (i.e., aspirin) and with a pharmacologist, Heinrich Dresser, found that it had promising anti-inflammatory activity.

Bayer began marketing aspirin as a drug in 1899. At first sold by prescription only, it became available over the counter in 1915. The synthesis and marketing of aspirin is viewed by many as the birth of the modern pharmaceutical industry.

In the mid-1960s, John Vane was a British pharmacologist studying the mechanism of action of non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin. He was particularly interested in the effect of NSAIDs on the production of prostaglandins.

Prostaglandins were first discovered in 1930 as muscle-contracting components of human semen by the American gynecologists, Ralph Kurzrok and Charles Lieb.

**Bayer, along with all German companies, lost its patent and trademark rights as part of the reparations for World War I. It was able to repurchase them in most countries, except for the United States, so Bayer aspirin was actually manufactured and sold here by Sterling-Winthrop for most of the 20th Century. Bayer eventually repurchased its trademark from Sterling in 1994.**

ANNE RAYNER

Nearly 30 years later, the structures of prostaglandins were elucidated by the Swedish biochemists, Sune Bergstrom and Bengt Samuelsson, and it was discovered that prostaglandins are made in many parts of the body including inflamed tissues.

Vane noticed that prostaglandins caused some of the symptoms of inflammation and that NSAIDs inhibited those symptoms. He hypothesized that anti-inflammatory drugs inhibit the production of prostaglandins, designed a set of experiments to test this, and in 1971 published results that demonstrated it was true. These straightforward biochemical experiments defined the basis for the treatment of inflammation by a class of drugs that had been known for millennia. For their discoveries, Vane, Bergstrom and Samuelsson were awarded the Nobel Prize in medicine in 1982.

Vane's discovery provided a rapid test-tube screen for new anti-inflammatory drugs and many began to emerge from pharmaceutical companies. Although they were more potent than aspirin, their safety wasn't much better. All NSAIDs cause stomach toxicity in a significant fraction of people who take them. This is due to their ability to reduce prostaglandin production by inhibiting the enzyme, cyclooxygenase, abbreviated COX.

Since the mechanism of their anti- inflammatory activity and their gastrointestinal toxicity is the same, there didn't appear to be much one could do to reduce NSAID side effects. But Philip Needleman and Michael Holtzman from Washington University provided evidence for the existence of a second COX enzyme, and in 1991, Dan Simmons at Harvard and Harvey Herschman at UCLA simultaneously identified the new gene – COX-2.

COX-2 was found to be expressed in stimulated inflammatory cells but not in the stomach, whereas COX-1 was found in stomach and many other tissues. This suggested that COX-2 might be the target for the anti-inflammatory action of NSAIDs, whereas COX-1 might be the target for their toxicity. So the race was on to develop a selective COX-2 inhibitor!

Eight years and $200 million later, Monsanto brought Celebrex to market. Celebrex was the biggest drug launch in history, selling approximately $1.5 billon in its first year. Merck marketed Vioxx six months later, and the sales of these two blockbusters grew to nearly $6 billion annually. These selective COX-2 inhibitors appear to have an improved gastrointestinal safety profile for individuals who cannot tolerate non-selective NSAIDs, but their utility for individuals who are not highly sensitive to NSAID toxicity is the subject of considerable debate.

In September, Merck pulled Vioxx off the market after finding that patients in a long-term study who took the drug had an increased risk of heart attack and stroke. Some experts believe all COX-2 inhibitors can cause cardiovascular problems in certain groups of patients. So it will be important to determine the cardiovascular risks of other COX-2 inhibitors and define the patient populations that should or should not be taking these drugs.

Studies of inflammation and treatments for it represent a classic example of the bi-directional translation of scientific discovery, from clinic to bench top and back again. Physicians, molecular biologists, pharmacologists, biochemists, and chemists focus on different aspects of how inflammation arises, what are the important molecular players, how their production can be minimized, and how one can optimize the structure of drugs that do this.

Scientists from all over the world bring their skills to this effort individually or collectively as part of multi-investigator teams. By focusing on the key events in inflammation, scientists can identify the most important studies to be conducted, and they can be assured that the results will have important clinical implications. This makes research in inflammation very exciting because one can see the impact of one's scientific discoveries on improved human health. Since inflammation contributes to many chronic diseases, this impact is further magnified.

The pace of scientific investigation is accelerating dramatically thanks in part to the development of new tools and technologies, which enable us to plan and conduct experiments that were unthinkable only a few years earlier. Information exchange is nearly immediate via the Internet, so the most exciting findings are communicated rapidly to investigators worldwide. But the basic currency of science remains – the formulation of good ideas and the design of experiments to test them, coupled with the hard work and diligence to complete them.

Good scientists are also good innovators. They not only think about what their experimental results mean but they also ask how they can use the new findings to do something that has never been done before. This means they are frequently traveling in uncharted territory, which is simultaneously terrifying and exhilarating. It is also essential to the translation of good science into better medicine. **LENS**

In a world brimming with disease-causing germs, inflammation is our body's best defense against dangerous microbes. However, when it goes awry, it can also be our worst enemy.

# COLLATERAL DAMAGE

## Saving the innocent bystander in the battle against infection

BY MELISSA MARINO



Humans have been battling inflammation since the dawn of time. Yesterday's willow tree bark is today's plethora of pills and tablets. According to IMS Health, a pharmaceutical information and consulting company, more than $17 billion are spent annually in the United States on prescription anti-inflammatory drugs.

The quest for better medicine continues today. Dozens of compounds are moving through the drug-development pipeline to address a growing list of inflammatory conditions.

Meanwhile, scientists are deciphering the interplay of cells, chemical messengers and genes that makes up the language of inflammation. Along with this understanding comes an increased capability to intervene – to treat and ultimately to prevent the disabling, potentially life-threatening and costly consequences of chronic inflammation.

### The language of inflammation

Inflammation begins when white blood cells – mast cells, neutrophils, macrophages – residing in tissue respond to the site of an injury or infection. They produce waves of chemicals – called inflammatory "mediators" – that can kill germs and sound the alarm for other populations of inflammatory cells.

Unfortunately, these mediators also "can put tissue on 'fire,' and cause 'collateral damage,'" says Jacek Hawiger M.D., Ph.D., who is leading the search for inflammation-related genes at Vanderbilt University Medical Center.

Inflammation is a complex, multiple signaling pathway phenomenon, but it can be broken down into three basic groups of mediators: reactive oxygen species (ROS) such as hydrogen peroxide and "free radicals" (atoms with unpaired electrons); eicosanoids including the prostaglandins and leukotrienes; and protein messengers called cytokines.

The production of free radicals by neutrophils and macrophages is called oxidative or oxidant stress, and it is a critical part of infection control.

**Pictured left:** A macrophage, part of the first line of defense against infection, prepares to engulf an *H. pylori* bacterium in the stomach lining. Unfortunately, the bacterium will survive this encounter, as well as other inflammatory assaults that end up damaging surrounding tissue. Eventually an ulcer may result.

Illustration by Pat Britten.

# A survival advantage

## The origin of inflammation

**Immunity and inflammation are almost as old as life itself.**

**Even amoebae, the single-celled organisms thought to be one of the first forms of life on Earth, are capable of distinguishing between members of their own species and other species they can eat. This capacity to distinguish "self" from "non-self" is what normally prevents our more complicated immune system from attacking our own tissues.**

**The cellular foundations of our immune system were laid at the time the first multi-cellular life forms, primordial worm-like creatures, wriggled their way along the ocean floor. These organisms developed primitive immune cells that held a constant vigil against invading germs. Prototypes of the more sophisticated germ-eating macrophages and dendritic cells of our advanced immune system, these cells provided a form of protection we now call "innate" immunity.**

**A major evolutionary milestone occurred in jawed fishes such as sharks and rays, which developed "adaptive" immunity, the ability of immune cells to generate diverse responses against pathogens. These responses include production of antibodies and stimulation of subpopulations of "T" lymphocytes that can seek out and destroy germ-harboring cells.**

**Adaptive immunity provides a survival advantage – the ability to "remember" a germ to which the human or animal host had previously been exposed, and to mount a bigger and stronger response the next time the pathogen invaded. But the cells that fight off attacking microbes also can damage normal tissue under circumstances that often involve chronic inflammation.**

**It seems evolution has handed us a loaded gun in our immune system and the inflammatory response. Without it, we would be defenseless. With it, we may just be shooting ourselves in the foot.**

– MELISSA MARINO

"Oxidative stress goes hand in hand with inflammation," says Vanderbilt researcher Jason D. Morrow, M.D., who has made major contributions to current understanding of inflammatory mediators. "The first response is the neutrophil or macrophage engulfing a bacterium. Then, as a consequence, is the oxidative burst that leads to the generation of hydrogen peroxide, superoxide and other free radicals that acutely damage the invading organism."

Free radicals are important in killing bacteria, but, in doing so, they can damage surrounding tissue. Oxidative damage has been linked to the development of Alzheimer's disease, certain forms of cancer, and other inflammatory diseases, which suggests that antioxidants might be useful in preventing or treating such disorders.

Free radicals don't last very long in tissues, making it difficult to measure their impact directly. However, their damage can be assessed indirectly. Morrow and Vanderbilt colleague L. Jackson Roberts, M.D., have identified and characterized a unique class of oxidatively-damaged fats, called isoprostanes. Circulating levels of isoprostanes provide an accurate and non-invasive index of oxidative stress in humans.

Recently, Morrow and colleagues at Oregon State University found that the extreme exercise involved in an ultra-marathon (a 32-mile run) caused increased production of isoprostanes and inflammatory cytokines. Runners who took high doses of antioxidant vitamins had significantly lower levels of isoprostanes in their blood compared to runners who took a placebo, but the vitamins had no effect on measures of inflammation.

"We de-linked the protein (cytokine) component of inflammation from the oxidative stress component," Morrow explains. "This says that inflammation is a multi-factor pathway and not all of those pathways are regulated in the same way – if we block one pathway (such as oxidative stress) we may still have another pathway that can be damaging."

One of the other potentially destructive arms of inflammation involves lipid-derived molecules called eicosanoids. These molecules, which include the prostaglandins and leukotrienes, are produced by nearly every cell in the body. They act as local hormones, regulating many biological functions including smooth muscle contraction.

Prostaglandins are also released by damaged cells and by macrophages, and they contribute to the cardinal signs of inflammation. They stimulate pain receptors in the damaged tissue, promote vasodilation (heat and redness), and increase capillary permeability, leading to the accumulation of fluid in tissue (swelling).

Prostaglandins are made from arachidonic acid by the cyclooxygenase (COX) enzymes, which come in two forms. In the early 1990s, researchers determined that prostaglandin production by one of the enzymes, called COX-1, protects the stomach lining, whereas activation of a related enzyme, COX-2, in other tissues can lead to inflammation, pain and tumor growth.

The finding led to the development and the marketing of drugs that specifically inhibit the COX-2 enzyme without affecting the activity of COX-1. Thus, they were designed to relieve pain and inflammation without causing stomach upset and gastrointestinal bleeding, a problem with aspirin and other non-steroidal, anti-inflammatory drugs (NSAIDs) that block both enzymes.

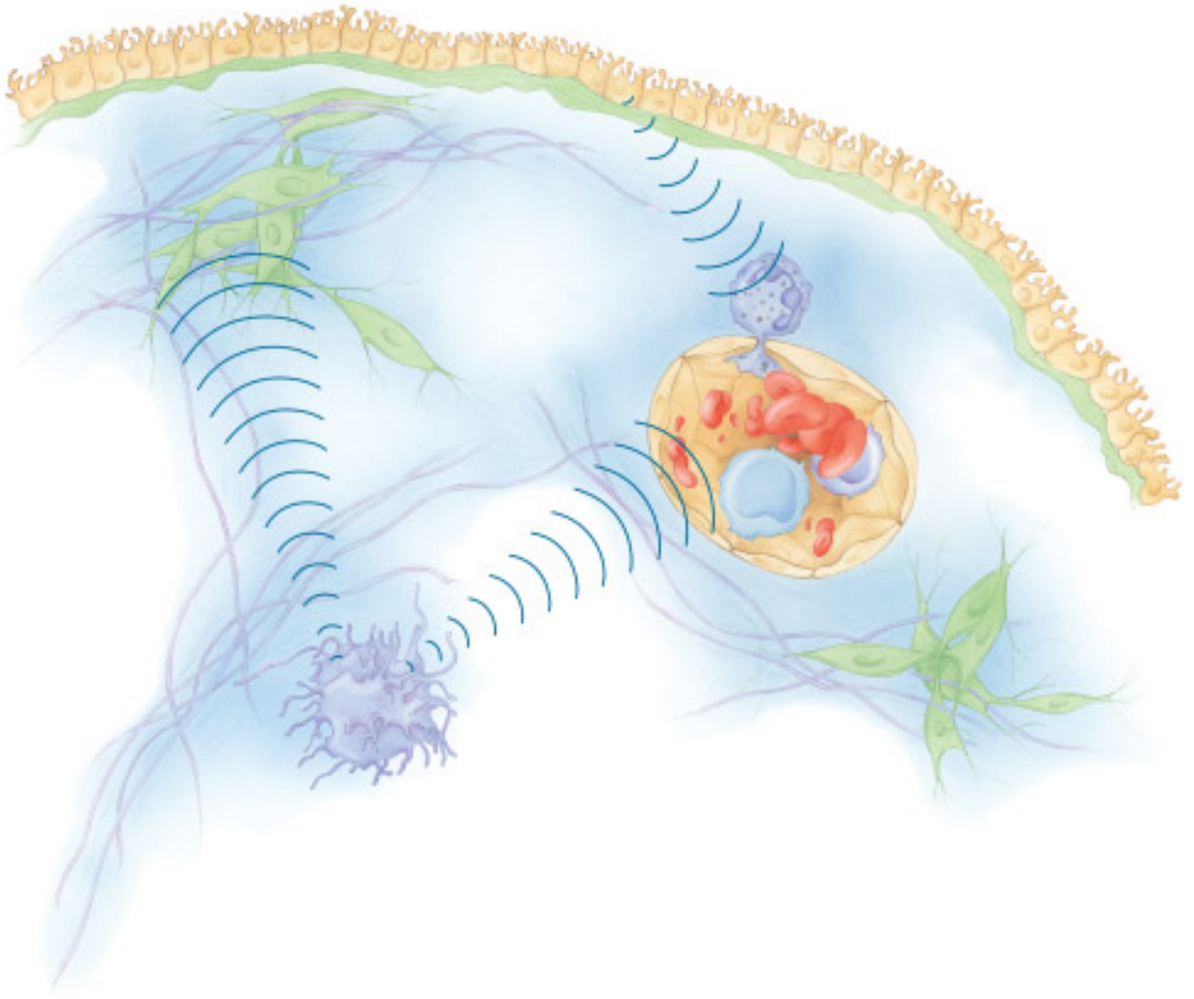
The third arm of inflammatory mediators includes the cytokines, a large family of messenger proteins that – among other things – are involved in reproduction, the development of the embryo, maturation of blood cells and the immune response.

Cytokine production also is an essential part of the inflammatory response to infection or injury. Produced by a variety of cells in response to injury or infection, they communicate messages that help coordinate the processes of healing and fighting pathogenic invaders.

Cytokines involved in the inflammatory response include the interferon and interleukin families and tumor necrosis factor-alpha (TNF-alpha). Interferons and interleukins play a variety of roles; some of them "fuel the fire" of inflammation, while others dampen it. Various treatments are being developed to either block pro-inflammatory members of the interleukin family or enhance the anti-inflammatory activities of others.

TNF-alpha, named for its ability to kill tumor cells, is thought to be at the center of inflammatory signaling. It can help recruit white blood cells to the site of inflammation, thereby boosting production of perhaps dozens of other cytokines.

**Hawiger's group has developed a peptide that can bring production of inflammatory messengers – commonly called the "cytokine storm" – to a screeching halt.**



**Pictured left:** The messages transmitted between cells by cytokines are an essential part of the inflammatory response. Represented here as signal beams, cytokines produced by the epithelium (top right) can "recruit" neutrophils (circulating white blood cells, right) that can "rev up" inflammation. Meanwhile, a macrophage (bottom left) signals the blood vessel and fibroblasts (structural cells, top left) to play their various roles in fighting infection and repairing damaged tissue.

Illustration by Marie Guibert for Jean-Marc Cavaillon, Ph.D., Institut Pasteur.



Within the last few years, several drugs that shut off TNF-alpha signaling have come to market for the treatment of rheumatoid arthritis and other chronic inflammatory disorders such as Crohn's disease. Since TNF-alpha signaling appears to be altered in a number of other inflammatory conditions, including cancer, multiple sclerosis and diabetes, the impact of these drugs may be far-reaching.

## Stopping the cytokine storm

Cytokines are not the only chemical messengers involved in inflammation. Chemokines and cell adhesion molecules help attract and direct the "first responders" to the source of injury or infection, and to bring in the reinforcements once the inflammatory response has begun.

These factors aren't always around, however. "They have to be produced on demand – just in time when you have inflammation," says Hawiger, the Oswald T. Avery Distinguished Professor of Microbiology and Immunology at Vanderbilt and chair of the department.

To provide inflammatory signals only "as needed," the genes that express them must be switched on and off. This realization has led Hawiger and his colleagues to the nucleus where, ultimately, inflammation begins.

The gene switch is operated by "stress-responsive transcription factors," proteins that – upon activation by pro-inflammatory stimuli such as oxidative stress – slip into the nucleus and turn on the genes for inflammatory cytokines, chemokines and the like.

Hawiger's group has developed a peptide or protein fragment called cSN50 that can keep the transcription factors out of the nucleus. This brings production of inflammatory messengers – what is commonly called the "cytokine storm" – to a screeching halt.

Recently they tested cSN50 in mice exposed to a bacterial toxin that can cause a life-threatening systemic inflammatory response called toxic shock. By shutting down cytokine and chemokine production, the peptide dramatically reduced liver damage and mortality, they reported in the *Journal of Biological Chemistry* in April.

The genomic approach may have broad implications, since excessive cytokine and chemokine production is a central theme in every inflammatory disease. About 250 genes that mediate inflammation have been identified so far. That's just the tip of the iceberg, Hawiger says.

In 2001, a multidisciplinary group of Vanderbilt researchers led by Hawiger was formed to find genes that mediate inflammation, with the ultimate goal of identifying new targets for anti-inflammatory drugs. The NIH-funded "Functional Genomics of Inflammation" program so far has developed a number of tools – including gene arrays, bioinformatics techniques and "knockout" mice – to aid in the search for the "inflamed gene."

"We are currently pursuing between eight and 12 genes," Hawiger says. The scientists plan to characterize the proteins expressed by these genes using proteomics techniques to determine their precise roles in inflammation.

"This … will help us to solidify a new paradigm of inflammation based on its 'control center' in the cell's nucleus," Hawiger says. "Thus, we will move closer to the development of a new generation of more effective and, hopefully, safe anti-inflammatory drugs tailored to tackle the key gene players in inflammatory cells." **LENS**

# THE INFECTION CONNECTION

## MICROBIAL TRIGGERS OF CHRONIC INFLAMMATION

What is the spark that lights the fires of chronic inflammation? Is it defective genes? Too many bacon-cheeseburgers? Toxic chemicals in our air, water and food?

BY MELISSA MARINO

Genetic and environmental factors certainly may contribute to inflammatory conditions, but there is another, albeit controversial explanation for them – persistent, and often silent, infections.

One of the most outspoken proponents of this theory, Paul Ewald, Ph.D., of the University of Louisville, argues that defective genes are an unlikely cause of chronic diseases because, over evolutionary time, they should have disappeared from the human population. Although there are exceptions in which a mutation may be beneficial (such as the mutation for sickle cell anemia which provides resistance to malaria), Ewald thinks that infectious agents are more likely culprits.

"Bad genes and bad environments have often been falsely accused, or, at least, they have taken more than their share of the blame. Viruses and bacteria are the primary offenders," he writes in his 2002 book *Plague Time: The New Germ Theory of Disease.*

Perhaps the most convincing evidence for his argument comes from the example of ulcer disease. For years, the medical community believed that stress and spicy food caused most ulcers. However, in the 1980s a team of researchers led by Barry Marshall, M.D., at the University of Western Australia made a radical proposal – that a curiously curvy bacterium called *Helicobacter pylori* was the primary cause of gastric ulcers.

Marshall proved that this bacterium was the cause of ulcers by guzzling an *H. pylori* cocktail. He subsequently developed gastritis, an inflammation of the stomach lining and precursor to ulcer disease, which was cured by a course of antibiotics.

Marshall's revolutionary idea didn't catch on immediately. "It probably took five or six years for the medical community to grasp the concept that ulcer disease was indeed an infectious disease," says Richard Peek, M.D., associate professor of Medicine and Cancer Biology at Vanderbilt University Medical Center.

According to the U.S. Centers for Disease Control and Prevention, *H. pylori* is now considered to be responsible for 80 percent to 90 percent of ulcers and is associated with a two- to six-fold increased risk of gastric cancers. Approximately two-thirds of the world's population is infected with the bacterium.

Yet most people who are infected never develop ulcers or gastric cancers. Why not? Peek believes the body's inflammatory response to the infection may be the answer.

"It appears that disease results from the dynamic interactions between a particular virulent strain (of *H. pylori*) and a susceptible host," he says. "Most of these host genetic differences we are finding are within inflammatory genes."

One bacterial virulence factor Peek and colleagues have identified is a group of linked genes called the cag island. Bacteria that express cag genes are able to trigger the production of inflammatory cytokines by gastric epithelial cells.

"Persons who have certain polymorphisms in cytokine genes can produce increased amounts of these molecules in response to the bacterial infection," Peek says. This causes an enhanced inflammatory response, which is thought to be the direct cause of gastric ulcers.

*(continued on page 10)*

Illustration by Matt Gore
Based on the work of Andreas Vesalius

# HOW INFLAMMATION ATTACKS:

**ALZHEIMER'S DISEASE**
Amyloid plaques that form in brains of those with AD show significant amount of associated inflammation.

**ASTHMA**
Chronic inflammation of the airways, due to allergens or irritants, makes the airways super-sensitive. Later exposure can trigger swelling of the airways that obstructs airflow.

**DIABETES**
In type 1 diabetes, the body's immune system attacks the islet cells of the pancreas. Recently, inflammatory processes have also been linked to type 2 diabetes.

**HEART DISEASE**
Inflammation is likely involved in all aspects of heart disease – from early plaque formation within the arteries to thrombosis, the cause of a heart attack.

**INFLAMMATORY BOWEL DISEASES**
**IBD, Crohn's disease, ulcerative colitis**
Chronic inflammation of the intestines may damage the bowel wall, allowing bacteria to "leak" through into the circulation. This may cause problems in other body areas (including joints, skin, eyes).

**MULTIPLE SCLEROSIS**
The body's immune system attacks and destroys the insulating covering of nerve cells (myelin sheath). This causes extensive scarring which, in turn, slows or blocks nerve impulses.

**PRE-TERM LABOR**
Infections in the reproductive tract during pregnancy can trigger an inflammatory response that can initiate uterine contractions and premature labor.

**RHEUMATOID ARTHRITIS AND OSTEOARTHRITIS**
In both types of arthritis, enzymes that break down cartilage are activated by inflammatory cytokines.

This combination – of a highly virulent bacterium and a host that overreacts to the infection – might be the answer to this vexing problem.

While identifying the infectious agent responsible for ulcers and gastric cancer was relatively easy (because *H. pylori* is virtually the only bacterial species that can colonize the normal stomach), the picture gets much cloudier when dealing with other tissues. For example, Peek describes, "in the large bowel, there are approximately one trillion bacteria per gram of tissue, so trying to pinpoint one species that causes a chronic inflammatory disorder that affects this organ, such as Inflammatory Bowel Disease (IBD), is prohibitive."

Finding the cause of inflammatory neurodegenerative diseases like Alzheimer's disease, multiple sclerosis (MS) and the myasthenias (which cause muscle weakness) is hampered by the long time course – often 20 years – from the onset of disease to severe disability.

MS is a progressive demyelinating disease with periodic relapses and remissions in which the protective myelin around the nerves is destroyed or damaged, resulting in a range of neurological symptoms.

Although the cause is unknown, MS has been classified as an "autoimmune" disease in which the immune system mistakenly attacks body tissues that bear an auto-antigen it recognizes as foreign. For some diseases, like the myasthenias, an auto-antigen has been identified. But, so far, no convincing auto-antigen has been found for MS.

"The inference that an inflammatory disease is autoimmune is largely by default since we are unable to find an infectious agent," says Subramanian Sriram, M.D., professor of Neurology, and Microbiology & Immunology at Vanderbilt.

Just because scientists haven't found a causative organism(s) doesn't mean it doesn't exist, however. "We may have overlooked them," Sriram says. "To use the cliché, 'absence of evidence is not evidence of absence.'"

Both a virus (human herpesvirus 6) and a bacterium (*Chlamydia pneumoniae*) have been proposed as inciting silent infections that may underlie MS. However, no definitive link has yet been shown for either of these candidates.

Although at least five labs, including Sriram's, have found *C. pneumoniae* in the spinal fluid of MS patients, several others have not. While he thinks that the bacterium definitely plays a role in the disease, the nature of MS makes the link difficult to establish.

"We do not think it's the cause of MS," Sriram says. "We think it's a cofactor – a secondary mediator, or possibly one of the polymicrobial infectious agents in the disease process."

Sriram and his colleagues recently completed a pilot study, which suggested that a six-month course of antibiotics might stabilize the brain lesions and brain atrophy characteristic of MS. The researchers are planning a larger, longer-term study, which will be necessary to prove a pathogenic basis for MS.

"In my studies with MS, the problem in finding a link is that we have very little tissue of patients with MS in early stages. MS is not a fatal disease, and so we obtain postmortem brain tissue from people who've had the disease for 40 to 50 years," Sriram said.

"It is impossible to identify what factors caused these lesions to develop 30 to 40 years ago. What we see is the result of something having happened – 'the aftermath of a battle'."

To address this problem, the Mid South Chapter of the National MS Society is setting up a donor program to collect brain tissue from MS patients who die of causes unrelated to the disease. This tissue will allow scientists to examine the initial events that cause MS.

As in the *H. pylori* story, the disease process likely depends on an interaction between the germs and the host's response to them. In general, Sriram agrees with Ewald's theory of infectious agents causing chronic diseases but adds, "we can't blame it all on the pathogen. The host bears some responsibility in the ultimate outcome."

> "A unique circumstance of the fetus is that it must live in an infected environment. That problem is often solved by initiating labor."
>
> ROBERTO ROMERO, M.D., NIH

Premature labor, the leading cause of perinatal morbidity and mortality worldwide, also appears to have an inflammatory origin. According to Roberto Romero, M.D., chief of the Perinatology Research Branch at the National Institute of Child Health and Human Development, "one of every four premature babies are born to women with subclinical infections of the amniotic cavity."

Romero's studies have shown that chronic, often silent infections within the amniotic cavity incite inflammation of the membranes and the fetus. The most frequent offenders are the normal bacterial residents of the vagina – *Ureaplasma urealyticum, Mycoplasma hominis*, and *Fusobacterium* species.

"For unclear reasons, bacteria from the lower genital tract cross the cervix, get into the uterus, and cross intact fetal membranes to gain access to the amniotic cavity. Microorganisms within the amniotic cavity may infiltrate the fetus when it breathes in infected amniotic fluid, through the ear or even through the skin," Romero says. This prompts the fetus to mount an exaggerated inflammatory response, known as Fetal Inflammatory Response Syndrome.

Inflammatory cytokines produced by intrauterine tissues and the fetus are believed to mediate the key aspects of normal labor and delivery: uterine contractions, cervical dilation, and rupture of the membranes. However, if the fetus has a systemic inflammatory response due to intra-amniotic infection, this response may trigger premature birth.

Romero and colleagues have found increased levels of several cytokines in the amniotic fluid of women with infection. These cytokines stimulate the synthesis of prostaglandins, which can induce contractions, cervical dilation and rupture of membranes.

"A unique circumstance of the fetus is that it must live in an infected environment. That problem is often solved by initiating labor," he says. Premature delivery has its own attendant risks, however, including respiratory distress syndrome, heart problems and blindness.

Antibiotic therapy has not been effective in preventing preterm labor, possibly because the infection is not typically found until preterm labor has already begun. Instead, studies in animals suggest that anti-inflammatory therapies – including the use of anti-oxidants and COX-2 inhibitors – may be the best bet for stopping premature labor and improving neonatal outcome. **LENS**

# The best defense ...

## is a good inflammatory response

BY MELISSA MARINO

Perhaps the best evidence for the importance of inflammation is what can happen in its absence.

For 16-year-old Allen Grimes of Hopkinsville, Ky., a rare genetic disorder that results in an insufficient inflammatory response has left him nearly defenseless against infections.

When he was three weeks old, Allen was diagnosed with chronic granulomatous disease (CGD), a condition caused by a defective enzyme in his phagocytes, infection-fighting white blood cells. The enzyme helps phagocytes produce toxic chemicals like hydrogen peroxide and bleach that kill bacteria. Without it, CGD patients are plagued with recurrent, often life-threatening infections.

ANNE RAYNER

**Allen Grimes knows first-hand the value of a healthy inflammatory response.**

To help his body compensate for this deficiency, Allen takes an antibiotic, an anti-fungal drug and gamma-interferon to bolster his immune system. He also avoids situations that could expose him to harmful bacteria and fungi.

Because crops grown in his rural community are rife with molds and fungi, "I can't work on a farm, which is something I always wanted to do," Allen says. He also can't have pets, swim in area lakes or be out in the sun because of his illness and the medications he takes.

But that hasn't kept him from participating in Little League, band and now varsity football. His family and doctors have always encouraged his active lifestyle. "We think it's the best way to keep him healthy," says his mother, Deanna Grimes.

Before the 1970s, CGD was known as "fatal granulomatous disease of childhood," according to John I. Gallin, M.D., director of the Clinical Center at the National Institutes of Health. Two percent of CGD patients died each year.

Fortunately, by the time Allen was born, Gallin and other researchers had determined the underlying genetic defect and how to treat it. Studies of prophylactic antibiotic therapy in CGD patients in the early 1970s were the first major step. "Low doses of antibiotics reduced life-threatening infections from one (per patient) every year to about one every four years," Gallin says.

Studies of gamma-interferon yielded an even more dramatic effect – a 70-percent reduction in the number of infections.

In an apparent paradox, CGD patients often form areas of chronic inflammation called granulomas that can lead to life-threatening blockages in the esophagus, digestive system, urinary tract and lungs. Gallin, who has studied CGD and related disorders for more than 30 years, describes the disease as "one of the great examples of the good and the bad that can come of inflammation."

Bone marrow transplants can cure the disease, but are limited by matching donors. Replacing the defective gene through gene therapy showed some early potential, but the recovery was short-lived. Researchers led by Harry Malech, M.D., chief of the Laboratory of Host Defenses at the National Institute of Allergy and Infectious Diseases, are now looking for new viral vectors that will deliver the normal gene to patients' blood stem cells more effectively.

With an incidence of only about one or two cases per million people, why have researchers spent so much time studying CGD? "I believe that if you can understand how inflammation is dysregulated in CGD, you might be able to determine the mechanisms involved in other chronic inflammatory diseases like atherosclerosis, Crohn's disease, arthritis and certain types of cancer," Gallin says.

Allen and his family chalk up his current good health to a little luck, a strong faith in God and constant vigilance against infection. They also are strong supporters of research.

Allen has participated in some of the treatment studies, and he hopes for a day without needles, pills or IVs. A day when he can swim without worrying about getting sick. A day when he can stand in the sun.

"He'll do whatever it takes to bring awareness to the disease, and to help find a cure," his mother says. **LENS**

When John Condeelis, Ph.D., first watched tumor cells in living tissue under the microscope, he was amazed by what he saw. The cells were speeding along like little cars on fibrous "superhighways." Their destination: a newly formed blood vessel surrounded by macrophages.

# A New View of Cancer

## THE ROLE OF INFLAMMATION

By Bill Snyder

Illustration by Philippe Lardy.

Macrophages are a type of white blood cell normally involved in inflammation and fighting infection. In this case, they appeared to be attracting the tumor cells to the vessel. "It's almost as if the macrophages are sending a 'come hither' signal," says his colleague Jeffrey W. Pollard, Ph.D., deputy director of the Albert Einstein Cancer Center in New York.

The pictures, achieved through the convergence of multi-photon laser microscopy and the generation of transgenic mice with fluorescent tumors, are providing some of the first visual, real-time evidence linking cancer to inflammation in living animals, says Condeelis, a biophysicist who directs Einstein's Analytical Imaging Facility.

Many questions remain, but new technologies and a flood of research studies are redefining the old, relatively static model of cancer. Tumors are astonishingly dynamic and versatile. They depend in absolutely crucial ways on interactions with surrounding normal tissue. This new view of cancer is shattering old assumptions, while at the same time raising hopes for earlier diagnosis, better treatment and, perhaps most provocatively, prevention.

"Prevention is going to become the dominant approach to cancer within the next 20 to 30 years … just as it has for cardiovascular disease," predicts Ernest Hawk, M.D., MPH, who leads gastrointestinal research in the National Cancer Institute's Division of Cancer Prevention. "That will come about through an improved understanding of the various pathways … that influence inflammation … And that's where it really starts to get exciting."

"How do these inflammatory cells potentiate tumor development at the molecular level? What is the precise role of the microenvironment? We don't have answers to that." RAY DUBOIS, M.D., PH.D.

For nearly a century and a half, scientists have suspected that many cancers are caused by inflammation – the complicated process by which the body heals wounds and fights off invading pathogens.

Prime examples: colorectal cancer in patients with inflammatory bowel disease; lung cancer that follows chronic inflammation from inhaled asbestos particles; and tumors of the stomach and liver – among the most common cancers worldwide – that are linked to pathogen-induced inflammation. Inflammation also may contribute to the development of prostate cancer, which, next to lung cancer is the second leading cancer killer in American men.

Another link in this causal chain was forged about 10 years ago, when population-based studies detected a 40 percent to 50 percent drop in the relative risk of developing colorectal cancer among people who regularly used aspirin.

The finding came at a fortuitous moment: during the previous two decades, scientists had learned that aspirin worked by blocking production of prostaglandins, ubiquitous lipid-signaling molecules that are involved in a host of physiological processes. Two prostaglandin-generating enzymes, the cyclooxygenases, had been identified, and one of them – COX-2 – was known to play an important role in pain and inflammation.

In 1994, Ray DuBois, M.D., Ph.D., and his colleagues at Vanderbilt University Medical Center found high levels of COX-2 in cancerous colon tissue. Three years later, they had stopped human colon cancer cells from growing in the laboratory by blocking the COX-2 enzyme.

By this time, Celebrex – the first selective COX-2 inhibitor – was on its way to market for treatment of rheumatoid arthritis. Researchers began testing the drug in patients with familial adenomatous polyposis (FAP), an inherited condition characterized by the appearance of multiple polyps in the colon that nearly always become malignant. In June 2000, an international team of researchers, including DuBois and Hawk, reported that the use of Celebrex led to a significant reduction in the number of polyps in patients with FAP.

The landmark finding, published in *The New England Journal of Medicine*, opened the floodgates for studies aimed at preventing other cancers by blocking production of prostaglandins. Since then, DuBois and his colleagues have found that one of the members of the prostaglandin family, PGE2, seems to be specifically involved in spurring the proliferation and invasive potential of colorectal cancer cells, and it may do so – at least in part – through the receptor for epidermal growth factor (EGF), which stimulates cell growth.

"This is what I'm really excited about," says DuBois, the Hortense B. Ingram Professor of Molecular Oncology at Vanderbilt. "It just continues to support our whole concept from 1994, that (COX-2) is really playing a role" in colon cancer.

COX-2 is not the only performer in this drama. During the past half-century, scientists have chronicled a burgeoning list of growth factors, cytokines, genetic switches and protein-chomping enzymes that play interacting – and overlapping – roles in inflammation and cancer.

These chemicals are generated in response to emergencies such as injuries and infection by a variety of cell types and through a variety of signaling pathways, including activation of the COX-2 enzyme and the resulting production of prostaglandins. They include:

- Growth factors that trigger cell proliferation, and which block signals that otherwise would lead to cell death, a process known as apoptosis;
- Enzymes that repair and "remodel" tissue after an injury, and which spur the growth of new blood vessels to feed it; and
- Immune regulators, including cytokines, that call in the reinforcements of immune and inflammatory cells to fight infection and help heal wounds, and others that "call off the troops" to limit collateral damage to surrounding healthy tissue.

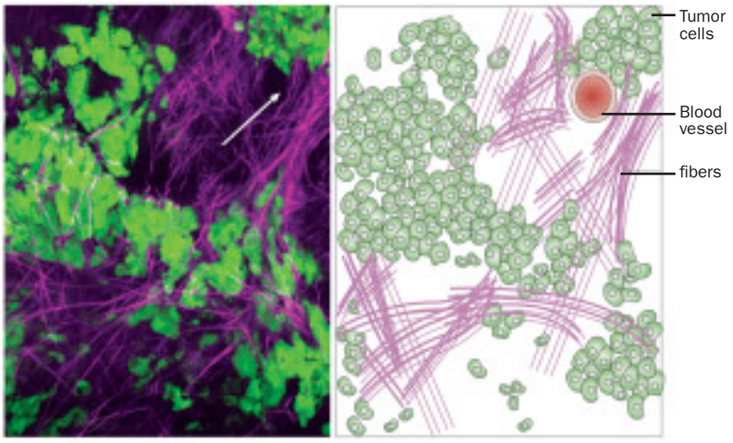
These pathways exist in exquisite balance. When the balance is tipped, or when certain genetic switches are turned on, some growth factors and cytokines may be "over-produced" while others are suppressed. The result can be chronic inflammation, damage to tissues and abnormal growth. A growing number of scientists believe, therefore, that understanding the unique molecular and cellular "micro-environment" in which the tumor thrives is key to improving cancer therapy and prevention.

This is not an easy puzzle to solve. The "imbalance" of growth factors and cytokines that promotes cancer may differ depending on where in the body the tumor began and its stage of growth. The role of inflammation also may differ depending upon the circumstance.

In some cases, chronic inflammation may trigger tumor growth. Inflammatory cells, including mast cells, neutrophils and macrophages, can generate highly reactive molecules of oxygen and nitrogen that can literally punch holes in bacteria. Too much of this firepower, however, can damage the DNA of nearby cells, leading to out-of-control growth. Limiting inflammation in these cases could be an effective way to prevent the development of cancer.

**Pictured right:** Using a multi-photon microscopy technique they developed, John Condeelis, Ph.D., and his colleagues at the Albert Einstein College of Medicine showed that tumor cells (green) move on fibers in the extracellular matrix (purple), some of which converge on a blood vessel (arrow). The in-vivo imaging technique has enabled the study of invasive tumor cells in real time.

From *Nature Reviews Cancer* (2003)
Courtesy John Condeelis, Ph.D., and *Nature*




Mutations in other cases may result from exposure to irradiation, carcinogenic chemicals or viral proteins. In this case, inflammation may be a "promoting" rather than initiating event. There is increasing evidence that the tumor can attract inflammatory cells, and use their growth-promoting factors to help it grow and spread. In this sense, the tumor "hijacks" the normal functions of inflammation for its own ends.

There is some evidence that tumor cells send out chemical signals – called chemokines – that attract inflammatory cells. According to one model, macrophages literally roll along the lining of blood vessels, following a trail of increasingly concentrated chemokines, like bloodhounds tracking a scent.

When the macrophages reach the site of the tumor, they squeeze through the blood vessel lining into the underlying tissue. Normally, they would sound the alarm, calling other elements of the immune system to attack the tumor. Instead, they are "re-educated" by the tumor to produce a variety of factors that nourish the tumor and enable it to break through the connective tissue that restrains it.

These "tumor-associated macrophages," as they are called, also can release immunosuppressive factors – various members of the interleukin family of cytokines – that blunt the ability of the body's immune surveillance system to detect and attack the tumor. So can other white blood cells (lymphocytes) that infiltrate tumors.

One possible way to stop this process and prevent tumor progression is to block expression of chemokines that chronically recruit inflammatory cells to the site where the tumor is developing, suggests Ann Richmond, Ph.D., professor of Cancer Biology at Vanderbilt. Richmond is co-discoverer of one of the first chemokines that has been shown to affect melanoma tumor growth.

Three years ago, Pollard and his colleagues at the Albert Einstein Cancer Center reported that colony stimulating factor-1 (CSF-1), a major macrophage growth factor and chemokine, seemed to be essential in a mouse model of breast cancer for "metastasis," spread of a tumor from its site of origin to other parts of the body.

Mice that lacked the gene for CSF-1 still developed mammary tumors, but the spread of the metastatic tumors to the lungs was significantly delayed. In addition, these tumors did not have the normal infiltration of macrophages seen in mice with the normal CSF-1 gene. When the gene was reintroduced into the mice, metastasis – in conjunction with macrophage infiltration – was restored. "At least in this model," Pollard explains, "macrophages are bad news."

Macrophages are not the only cell type that can fan the inflammatory flames of cancer. Zena Werb, Ph.D., Lisa Coussens, Ph.D., and their colleagues at the University of California, San Francisco have found that cells in the connective tissue surrounding the tumor also can send out signals that help it grow and spread. The cells "talk" to each other, just as they do during development of mammary glands or hair follicles. "The environmental side and the tumor side co-evolve," explains Werb, professor and vice chair of Anatomy at UCSF.

Most cancers arise from the epithelium, the layer of cells that separate underlying tissues from the outside world. The epidermis of the skin, the linings of the lungs and digestive tract – all are examples of epithelial tissues. Underneath is the stroma, which provides the connective tissue, blood vessels, nerves and other vital physiological functions. Between cells is the extracellular matrix, which plays a role in tissue development and healing. It is here that the cells travel on their fibrous "superhighways" from one location to another.

An example of this cellular "cross-talk" is the tissue remodeling that occurs during development or wound healing with the help of the matrix metalloproteinases (MMPs), a family of enzymes involved in a wide range of physiological processes. "Not only do they degrade the matrix, they release biologically active factors that are involved in calling in inflammatory cells and in angiogenesis," says Lynn Matrisian, Ph.D., chair of Cancer Biology at Vanderbilt who, as a post-doctoral fellow, cloned the first full-length MMP in the mid-1980s.

MMPs also are believed to contribute to metastasis, the major cause of death from cancer, by helping to increase the tumor's blood supply and means of escape to other parts of the body. The first synthetic MMP inhibitor was tested in humans in 1992, but by 2002, several clinical trials had failed to show any survival benefit.

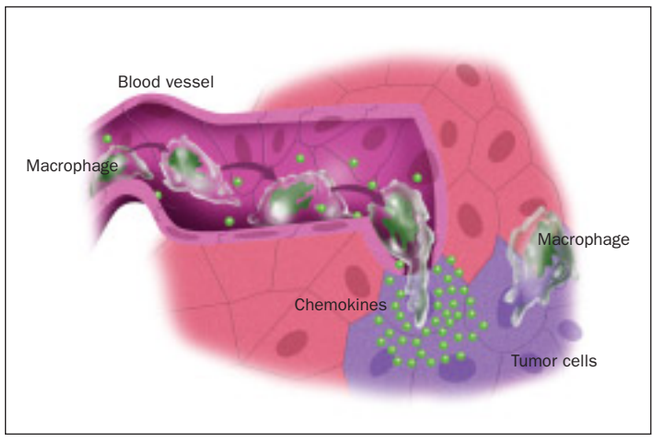
That's not surprising, says Matrisian, current president of the American Association of Cancer Research. MMP inhibitors "don't stop cancer in its tracks," she says. "What we learned is they change the rate of progression (of the disease)."

Two years ago in *Science* magazine, Matrisian, Coussens and their Vanderbilt colleague Barbara Fingleton, Ph.D., predicted that MMP inhibition would help prolong survival to the point that patients died of "old age" before they died of cancer. "But you can't give it at the ninth hour," Matrisian says. "You have to give it earlier, or you have to give it in combination (with other drugs) … or you have to think about prevention."

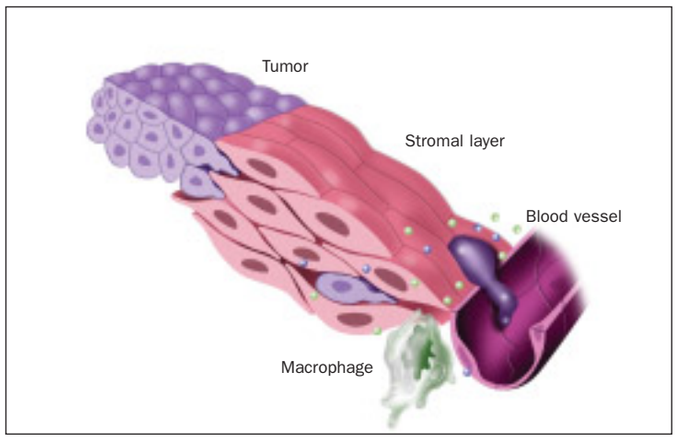
Research is continuing. Matrisian and colleagues around the world are developing biomarkers and optical imaging techniques to determine how effectively

## Rolling along – a nefarious journey

ILLUSTRATIONS BY DOMINIC DOYLE




According to one model, macrophages roll along the lining of blood vessels, "tracking the scent" of chemical messengers called chemokines released by tumor cells. Once at the tumor site, they are "re-educated" by the tumor to produce factors that help it grow and spread.




Guided by factors in their "micro-environment," some tumor cells find their way to a blood vessel. Once in the bloodstream, a chemokine trail – or possibly a blood cell "chaperone" – leads them to another tissue. This process is known as metastasis.

the inhibitors block the enzymes, and new animal models that more closely resemble the human condition.

Other researchers are tackling metastasis from the point of view of the errant tumor cell that makes it into the bloodstream. In patients with cancer, "there are (an estimated) million or so tumor cells circulating at any one time, and yet only one or two of those end up as metastases," Pollard says. "Does that mean that (only) one in a million cells … is able to metastasize, or that there are a million cells able to metastasize but only a one-in-a-million chance of them lodging in the right place?"

One theory is that once out in the bloodstream or lymphatic system, the tumor cell tracks a "trail" of chemokines to a specific tissue that is producing the signal – essentially reversing the course that inflammatory cells took to get to the primary tumor. "Another possibility which is a bit … more outlandish," he says, "is that tumor cells are 'chaperoned' by (blood) cells." In either case, the spread of cancer may be guided by specific chemokines released by various tissues.

Pollard hopes the new imaging technology the Einstein group has developed will help solve the mysteries of metastasis. "We should be able to follow cells as they move around the body in ways that we've not been able to do before," he says.

Ultimately, the control of cancer may depend on a better understanding of the complex chemical signals that guide these cells. "These are the big questions," says DuBois. "How do these inflammatory cells potentiate tumor development at the molecular level? What is the precise role of the microenvironment, and what regulates the inflammatory cell recruitment into the neoplastic (tumor) microenvironment? We don't have answers to that."

With the help of techniques such as DNA microarray, scientists are identifying genes that play a role in inflammation and cancer. They're studying what happens when the genes are "knocked out" in mouse models. One goal is to learn how to "manipulate" macrophages, for example, by altering the balance of cytokines in a way that would turn them from the tumor's friend to a foe.

On the clinical side, researchers are trying to identify patterns of proteins in blood samples from patients that can be correlated with types and stages of cancer, and their response to treatment. Another approach seeks to identify groups of patients with genetic differences, called polymorphisms, who would be most likely to benefit from targeted cancer therapies.

With these approaches, "you will have determined on some level what genetic pathways or patterns of protein expression are altered in the patient's tissue," Richmond explains. "Then you might be able to predict which regime to use for that patient by targeting the defect in that specific patient's tumor."

For example, if over-production of pro-inflammatory cytokines and chemokines can be detected, and if drugs that specifically target these signaling molecules or their receptors can be given to block this step in tumor progression and metastasis, "we will likely increase our success with both prevention and therapeutic intervention in cancer," she says.

What's needed, says DuBois, is a unifying theme. "There are different components of the inflammatory response that play a role in cancer," he says. "We've been looking at them individually. Hopefully people can go back and look at this, and try to see if they can make sense of some of the research that's been done." **LENS**

# the war on CANCER

## A STATUS REPORT

FORTUNE magazine created a stir in the research community last March with a cover story entitled "Why we're losing the war on cancer (and how to win it)."

The story's author, Clifton Leaf, one of magazine's executive editors and a cancer survivor, described "a dysfunctional 'cancer culture' ... that pushes ... physicians and scientists toward the goal of tiniest improvements in treatment rather than genuine breakthroughs."

Leaf criticized current research efforts for "isolated (and redundant) problem-solving instead of cooperation," and for focusing on shrinking tumors instead of the more difficult problem of metastasis, which is "the thing that kills people."

Scientists interviewed for this issue of *Lens* disputed the "dysfunctional" label, but they said they could make faster progress if there were greater incentives for collaboration among researchers, clinicians and drug companies.

"Patients are going to benefit the most from combinational approaches," yet current patent and regulatory constraints make it difficult to test new drugs in combination, notes Lisa Coussens, Ph.D., a cancer researcher at the University of California, San Francisco. "You have to test them as single agents and if they don't demonstrate efficacy, they're not going to go any further."

Junior faculty also are discouraged from collaborating with each other because they have to demonstrate independence in order to be promoted and win research grants, Coussens says. Yet that's exactly what's needed to make progress, maintains Ernest Hawk, M.D., MPH, of the National Cancer Institute's Division of Cancer Prevention.

"I'm not talking revolution here, but it's something the whole culture needs to take a look at," Hawk says. "Getting researchers working together rather than quite so independently will reduce redundancy and perhaps maximize the effort we're putting forward."

Toward that end, the NCI is serving as a "catalyst" – bringing scientists from diverse fields together to develop new research strategies, and pursuing partnerships with pharmaceutical companies to hasten drug discovery and development.

The discovery that Celebrex can inhibit pre-cancerous polyps in high-risk patients emerged from just that kind of partnership. "That was just a six month trial, very fast, very small, very efficient," says Hawk, who participated in the research, "and yet it had a profound impact ... both for immediate clinical care of a high-risk group and then more broadly for the potential of many others."

Tests of other potential drugs have been disappointing, in part because of the way they are tested both in animals and humans. "When we give a mouse cancer, we start treating immediately," says Lynn Matrisian, Ph.D., chair of Cancer Biology at Vanderbilt, whereas experimental drugs traditionally are tested first in patients with advanced disease.

What's needed is the development of smaller clinical trials looking at earlier stages of disease, says Harold L. Moses, M.D., founding director of the Vanderbilt-Ingram Cancer Center. The studies should measure biological markers or "correlates" of drug activity, and be flexible enough to change course quickly if it becomes apparent that the drug is most effective in a subgroup of research subjects.

"The idea is to do it better, more quickly and with less expense," says Moses, current president of the Association of American Cancer Institutes.

This fall, the Association of American Cancer Institutes, in partnership with the American Association of Cancer Research and the American Society of Clinical Oncology, hosted a workshop on "designing a smart clinical trials system for the 21st Century." Attendees included representatives of the pharmaceutical industry, patient advocacy groups and the research community.

In the past, "there's been a lack of communication between the clinicians and the basic scientists," Matrisian explains. "We're looking for new and better ways to make this happen."

– BILL SNYDER

**Above:** Lynn Matrisian, Ph.D., (right) watches Barbara Fingleton, Ph.D., perform surgery on a mouse used in cancer research.

Photo by Dana Johnson

Sometimes great discoveries emerge slowly, after decades of trials and errors. At other times, ingenious ideas seem to strike from out of the blue. Such was the case with the Nobel Prize-winning findings by the late Sir John Vane, who – over the course of a weekend – cracked a 70-year-old mystery about how aspirin relieves pain and inflammation.

By Lisa A. DuBois

# IMPROBABLE BEGINNINGS

## Sir John Vane and the value of blue-sky thinking

**Pictured left, clockwise from top right:**

John Vane in his office at the Wellcome Research Laboratories in Beckenham, southeast of London, and (center photo) in his Wellcome lab – early 1980s.

Vane with longtime colleague Professor Gustav Born at The Royal Society in London. Like Vane, Born is a Fellow of The Royal Society, the United Kingdom's national academy of science.

Colleagues gather outside Vane's office in Beckenham, mid-1970s. Back row, second through fourth: Philip Needleman, Ph.D., credited with the development of Celebrex; John C. McGiff, M.D., chair of Pharmacology, New York Medical College; and Miles Weatherall, Ph.D., Wellcome Research Laboratories. Front row, left, Sergio Ferreira, Ph.D., whose research helped lead to the development of ACE inhibitor drugs to treat high blood pressure; and – third from left, next to Vane – Professor Harold Burn, Vane's mentor from Oxford.

Photos courtesy of Professor Rod Flower FRS and The William Harvey Research Institute

Vane's discovery was the "tipping point," the culmination of knowledge and technique that led to an immediate explosion in the field of pharmacology, as well as to some of the most exciting medical research going on today. It also proved the value of serendipity and "blue sky" thinking in biomedical research.

"He had an uncanny nose for going after the right kind of scientific problem," says Philip Needleman, Ph.D., who helped pioneer the current generation of pain relievers. Needleman was one of the first Americans to study under Vane, who died last month from complications following a fall.

Vane's entry into science began humbly enough. Born in 1927, the son of a businessman who ran a small company making portable buildings, Vane grew up on the outskirts of Birmingham, England. Even in his early childhood he was intrigued by experimentation, and at the age of 12 his parents gave him a junior chemistry set for Christmas.

The gift was not without its costs in terms of a learning curve. One of Vane's early experiments (involving a makeshift Bunsen burner attached to his mother's gas stove) exploded, splattering hydrogen sulfide all over the kitchen walls. Shortly thereafter, his father built a shed in the back yard – a suitable distance from the house and complete with gas and water – for young John to use as a laboratory.

Vane eventually went on to the University of Birmingham where he developed an extreme distaste for chemistry. By the time he graduated he realized that it was scientific discovery through experimentation that thrilled him, not academic exercises in theory. As a result he jumped when an opportunity arose for him to study pharmacology at Oxford – even though he had absolutely no biological training at the time. He was hungry to return to the laboratory bench.

As a doctoral student at Oxford in the 1950s, Vane learned to use bioassays, which detect and measure the natural sensitivity of pieces of tissue to hormones and other biologically active compounds. At that time, the instruments for bioassay were highly complicated and answers to research questions came only after weeks of laborious tests.

Vane, who in those early days was studying the biological activity of snake venom, quickly grew impatient with the cumbersome biomedical technology necessary for his research. He was determined to find a way to reach answers more quickly, in particular, to find an easier method for examining unstable compounds.

In 1956, after moving into a junior faculty position in Experimental Pharmacology at the University of London's Royal College of Surgeons, he developed a groundbreaking technique, the "cascade superfusion bioassay," which allowed him to investigate the release of hormones and other substances in the bloodstream in "real time."

Says Needleman, currently associate dean for special research projects at Washington University Medical School in St. Louis: "Vane's methodology was a perfection of existing biological bioassay methods and was so spectacular that it allowed us to ask biological questions with some specificity and get instant gratification. It required the interplay of using different responding tissues that could recognize different body chemicals, and play them off against known bioactive compounds."

"At the time, this was a revolutionary technique of enormous sensitivity and versatility," explains Rod Flower, Ph.D., a former protégé and longtime colleague of Vane's, and professor of biochemical pharmacology at the William Harvey Research Institute in London. "John had used this idea to measure the release and disappearance of hormones in the circulation and also to measure the release of substances from other perfused organs, such as the perfused lung."

From the moment of its invention, Vane thoroughly enjoyed the quick reward his bioassay provided. In fact, many times his lab members could find out the results of their endeavors in a single day. Flower recalls that at one point Vane installed a closed circuit television camera in the lab with the lens aimed at the chart recorder, and he would watch the monitor from his office. As the pen moved over the chart and the tissue began to contract, Vane would phone from his command center and reel off instructions to the people working in the lab – such as the next best dose to try on that tissue sample.

Flower recalls, "As young technicians and graduate students we used to ape around behind the camera, suddenly switching to a more serious demeanor as we moved into its perceived field of view."

About this time, investigators in London and elsewhere were making significant strides into understanding prostaglandin biology.

Prostaglandins are lipid molecules found in virtually all tissues and organs, which have powerful physiological effects. Often produced in response to trauma, stress or disease, these so-called mediators can affect smooth muscle activity, for example, in blood vessel walls and the uterus, and they play a role in a host of other metabolic processes.

Scientists at the time knew that prostaglandins were useful in obstetrics for both inducing labor and terminating pregnancies, and that the compounds could inhibit ulcer formation in the stomach and cause fever and inflammation in animals. Some of the most interesting prostaglandins were notoriously difficult to study, however, because they are potent for only a few seconds before they are rendered inactive and excreted. Even after four decades of research, the specifics of prostaglandin activity had yet to be worked out.

By the mid-1960s, some of those questions were being answered. Sune Bergstrom of the Karolinska Institute in Stockholm had purified the first prostaglandins and determined their structure, and his student Bengt Samuelsson was examining the various components within this newly discovered biological system.

One weekend in 1971, Vane had a remarkable idea. He knew that aspirin, one of the world's most widely used drugs, reduced pain and relieved fever and inflammation – although no one understood exactly how. Vane hypothesized that aspirin might work by inhibiting the generation of prostaglandins – and he realized he could easily test his theory by using his cascade superfusion assay.

"It was a brilliant experiment. It immediately gave us a conceptual framework by which to evaluate the role of prostaglandins in inflammation," says John Oates, M.D., an internationally known prostaglandin researcher at Vanderbilt University Medical Center. "We could use his assay as a tool for identifying prostaglandin activity in any number of processes," Oates says. "It linked prostaglandins to fever and analgesia."

Also, Vane's work laid the cornerstone for three decades' worth of new directions in research, including the current focus on the role of cyclooxygenase (COX) enzymes. Today researchers are looking at evidence that such nonsteroidal anti-inflammatory drugs (NSAIDs) as aspirin, Advil and Motrin that block COX activity might reduce the risk for some kinds of cancers and for Alzheimer's and other diseases. "None of this would have made sense without Vane's early experiments on prostaglandins," Oates says.

A year after his findings were published, Vane left academia for industry, accepting a position at the British pharmaceutical company, the Wellcome Foundation, and bringing a number of outstanding colleagues with him. Needless to say, Vane caught tremendous flak from his academic colleagues for his decision, but he remained undeterred.

During his 13 years at Wellcome, Vane was in on the development of many new products, including prostacyclin, a prostaglandin derivative important in cardiovascular medicine because of its action in dilating constricted blood vessels and inhibiting platelet aggregation, or blood

**Pictured here:** (Far left) In his office in Beckenham in the mid-1970s, John Vane visits with long-time friend and colleague Professor Ryszard J. Gryglewski of Jagiellionian University Medical College in Cracow, Poland. (Left) Vane in his lab at the Royal College of Surgeons in the 1960s.

clots. Vane engineered a collaboration between Wellcome and its competitor Upjohn to introduce prostacyclin into the medical marketplace.

Needleman says, "He was driven. And it was quite natural for anyone working in prostaglandins, whose research was strong enough, to be in direct competition with John Vane. A lot of people melted."

To many of his colleagues and students, however, Vane's competitive spirit and verve was invigorating. "John had a lively, productive lab," Oates says. "He drew around him a remarkably talented and energetic group of fellows."

When dealing with young investigators, Vane gladly shared his scientific credo: "Always do the simple experiment first!" Flower says, "He was a master of the clever, low-tech, high-thought experiment that involved nothing more complicated than a small strip of artery or similar tissue moving a lever or transducer."

Needleman adds, "John Vane was like a symphony conductor. He was a great scientific strategist. The week I spent with him in 1972, I learned the bioassay methods very quickly, then we spent day and night talking about strategy."

While Vane was fiercely loyal to those who worked for and with him, he was never one to suffer fools gladly. With a big booming voice and all the confidence of a British aristocrat (although he was raised in a decidedly middle class family), Vane could command attention long before he became renowned for his research.

Needleman recalls one international pharmacology meeting in Switzerland in 1969. In keeping with the free-spirit attitudes of that era, the meeting organizers planned to allow a free-flow of ideas in the large amphitheater – an open, unstructured discussion among the hundreds of attendees.

Unfortunately, this was a disastrous idea. "It was bedlam," Needleman recalls. "Everyone was talking at once, nobody had the floor. Suddenly John Vane stood up and in this wonderful English baritone announced, 'I have a question!' Everyone stopped talking to hear his question. Vane asked, 'WOULD YOU PLEASE PAUSE LONG ENOUGH SO THAT I CAN LEAVE THIS MEETING?'"

At that point, Vane turned on his heels and headed for the exit. The others in the audience applauded and followed him out the door.

In 1982, Vane, Bengt and Samuelsson shared the Nobel Prize for medicine for their discoveries in prostaglandin synthesis. Oates finagled his schedule and attended the ceremony in Stockholm, Sweden, with a group of his international associates, cheering on their Nobel Prize-winning prostaglandin cronies. It was, he says, one heck of a party.

In 1986, Vane retired from the business world and devoted his energies towards preserving and advancing scientific research. He recruited some of his old lab buddies to form The William Harvey Research Institute, an organization designed to bridge the gap between academics and industry.

In 1990, Flower, who had spent several years as chairman of Pharmacology at the University of Bath, joined the Institute, this time on equal footing with his beloved mentor as a member of the board of directors. The purpose of the institute, an affiliate of the United Kingdom's Association of Medical Research Charities, has been to encourage creative approaches to basic research, present new data and foster collegiality among medical scientists.

For all of his accomplishments, Vane's greatest legacy may be the people he trained.

Flower worked on understanding the biology of such autoimmune inflammatory diseases as rheumatoid arthritis and asthma. Sergio Ferreira, Ph.D., professor of Medical Biochemistry at the Federal University of Rio de Janeiro, is internationally renowned for his contributions to the collection of ACE inhibitor drugs for lowering blood pressure. John Hughes, Ph.D., shared the prestigious Lasker Award in 1978 for the discovery of endogenous opioid peptides involved in the body's regulation of pain.

Salvador Moncada, Ph.D., currently director of the Wolfson Institute for Biomedical Research of the University College London, pioneered research into nitric oxide, now considered a "super-molecule" because of the role it plays in the immune and nervous systems, in inflammation and in programmed cell death (apoptosis).

Needleman went on to hold executive positions in the pharmaceutical giants, Pharmacia, Monsanto and Searle, and was involved in the development of such drugs as the COX-2 inhibitors Celebrex, Bextra and Dynastat, and Inspra, a blood pressure drug that blocks the actions of the hormone aldosterone.

Needleman says, "The years I scientifically jousted with John Vane more than prepared me for a career in industry where I would be dealing with CEOs, boards of directors and industry analysts. To survive a scientific interaction with John Vane you had to be at the top of your game. He was a great influence."

Throughout his career, Vane was, first and foremost, an activist for scientific freedom.

Recalling his days in training, Flower says, "John's attitude to drug discovery was that if you gave bright scientists (creative freedom) then they would come up with the goods sooner or later. We had few formal departmental meetings or departmental seminars, and yet somehow we seemed to know more about what we were individually doing, and what our colleagues out there were doing, than at any other time.

"Despite these factors, which no doubt would horrify a head of department today, the department was undoubtedly the friendliest, the fairest, and the safest I have ever worked in."

Vane echoed this point of view in his speech at the 1982 Nobel banquet – expressing ideas that still resonate 22 years later: "The medicines of today," he said, "are based upon thousands of years of knowledge accumulated from folklore, serendipity and scientific discovery. The new medicines of tomorrow will be based on the discoveries that are being made now, arising from basic research in laboratories around the world ...

"In many countries now, research in universities is under severe financial restraint. This is a shortsighted policy. Ways have to be found to maintain university research untrammeled by requirements of forecasting application or usefulness. Those who wish to study the sex-life of butterflies, or the activities associated with snake venom or seminal fluid should be encouraged to do so. It is such improbable beginnings that lead by convoluted pathways to new concepts and then, perhaps some 20 years later, to new types of drugs." **LENS**


Sergio Fazio, M.D., Ph.D.
Cardiovascular Medicine

# THE HEAT THAT HURTS

## Inflammation and the development of heart disease

BY HAROLD OLIVEY

When it comes to heart disease, fat in the bloodstream is one of the major culprits. Yet as many as 50 percent of people with atherosclerosis – artery blockage that can lead to a heart attack – do not display traditional risk factors such as high cholesterol.

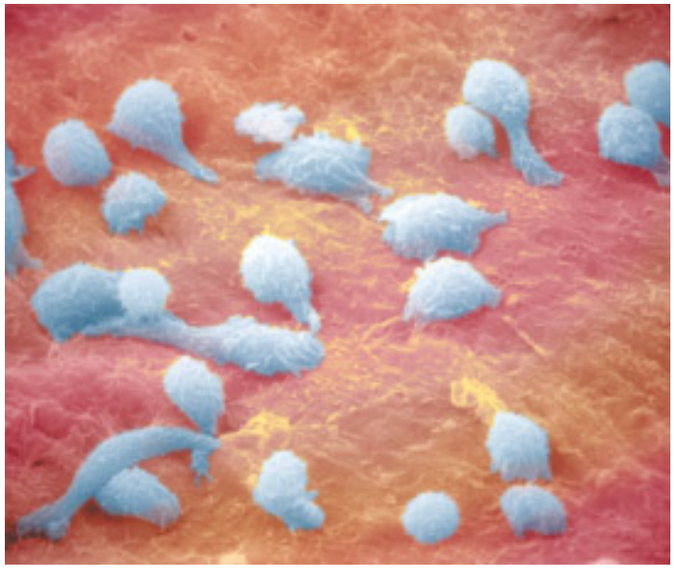
Thanks to recent technological advances, scientists are now able to take a closer look at what stubbornly remains the nation's leading disease killer. What they are finding may surprise you.

Inflammation, incited by a plethora of infection-fighting and wound-healing blood cells and molecules, seems to play a major role in atherosclerosis. For example, high levels of C-reactive protein (CRP), a circulating marker of inflammation, are associated with an increased risk for heart attack and stroke.

That doesn't mean a once-a-day anti-inflammatory pill to prevent heart disease is right around the corner. Researchers are hopeful, however, that their pursuit of inflammation may lead to better ways of treating and preventing heart disease and other ailments of the Western lifestyle – including type 2 diabetes.

**Pictured left:** MacRae Linton, M.D., and Sergio Fazio, M.D., Ph.D., surrounded by images of cholesterol-engorged "foam cells," have found tantalizing molecular and cellular clues supporting a link between inflammation, heart disease and metabolic syndrome.

Photo illustration by Dean Dixon



Colorized microscopic image shows monocytes "diving" into an atherosclerotic lesion in the lining of a blood vessel. The monocytes have been "activated" by inflammatory signals on the blood vessel lining. Arm-like pseudopods drag the monocytes into the gaps between endothelial cells and down into the atherosclerotic plaque beneath the endothelium. Here they become macrophages, vacuum up excess lipid and add to the size of the plaque.

Image courtesy of Jay Jerome, Ph.D., director of the Research Electron Microscopy Resource at Vanderbilt; colorization by Deborah Doyle

## The Vanderbilt connection

Vanderbilt's contributions to the field of inflammation and heart disease began more than a decade ago, when, as a resident physician, MacRae Linton, M.D., became interested in atherosclerosis. "I would see all these people having bypass surgery, and nobody was thinking about their risk factors," recalls Linton, now professor of Medicine and Pharmacology at Vanderbilt.

Linton's interest led him to pursue an endocrinology fellowship at the renowned Gladstone Institute of Cardiovascular Disease at the University of California, San Francisco. There he met Sergio Fazio, M.D., Ph.D., another research fellow who was studying how the body handles cholesterol.

"The real excitement came from understanding the complexity of lipid metabolism," recalls Fazio, an Italian native whose doctorate is in Molecular Biology. "But when you look at it from the point of view of clinical relevance, what's important is the damage that lipid metabolism can do to the vessel wall. It was clear that we needed to become vascular biologists."

Linton and Fazio decided early in their careers to take a team approach to their research. Since joining the faculty at Vanderbilt in 1993 (Fazio is a professor of Medicine and Pathology), they have published several seminal papers in the field of atherosclerosis, and they co-direct the medical center's Atherosclerosis Research Unit.

In one of their highest profile papers, published in the journal *Science* in 1995, Fazio, Linton and James Atkinson, M.D., Ph.D., professor of Pathology, reported that apolipoprotein E (apoE), a protein important in lipoprotein metabolism, seemed to protect mice from developing atherosclerosis.

The largest supply of apoE comes from the liver, Linton says. But the protein is also made by macrophages, and thus may participate in the inflammatory response.

To determine what, if any, role apoE expressed in macrophages played in the development of atherosclerosis, Linton and Fazio studied a strain of mice that lacked both copies of the apoE gene. These mice develop significant atherosclerosis, unlike their genetically normal – or wild type – counterparts. The researchers irradiated the apoE deficient mice to kill their bone marrow, the source of macrophages, then gave them transplants of bone marrow cells from wild type mice.

Mice deficient in apoE that received the transplants did not develop atherosclerotic plaques. "The small amount of apoE that came from the bone marrow was enough to cure the mice," says Linton.

After the study published in *Science*, "we became more interested in genes related to cholesterol homeostasis – enzymes, proteins, receptors," he continues. "Recently we've expanded that into an interest in inflammation and how macrophages and other cells may play a role in the inflammatory process of atherosclerosis."

## "Living wounds" that will not heal

Atherosclerotic plaques form when blood vessels are injured by chemicals (such as those found in cigarette smoke), high blood pressure or high levels of plasma lipids (fats, like cholesterol).

These plaques are living wounds that can trigger clot formation inside the blood vessel. When a clot forms in an artery that supplies the heart with blood, a heart attack ensues, leading to death of heart muscle. Understanding the cellular and molecular events that lead to atherosclerosis will be critical to making progress against the disease.

Atherosclerotic plaques contain a variety of cell types. These include endothelial cells that line the blood vessels and make up the endothelium, and vascular smooth muscle cells that give form and resilience to the blood vessels. Other cells found in plaques, such as pro-inflammatory macrophages and lymphocytes, do not normally reside in the vessel wall. Instead, they remain in the bloodstream and stand ready to mediate inflammatory responses at sites of injury and infection.

As part of the innate immune response system, macrophages are among the first line of defense at sites of injury. Derived from circulating monocytes, these specialized cells engulf and destroy pathogenic organisms and damaged cells. When circulating monocytes encounter injured endothelium, they migrate underneath the endothelium. This invasion of monocytes starts the formation of the atherosclerotic plaque.

Once inside the vessel wall, monocytes differentiate into macrophages that become "activated" and recruit other monocytes and T helper lymphocytes to enter the plaque. They also ingest cholesterol. As macrophages become engorged with cholesterol, they take on a characteristically foamy appearance, and thus are referred to as "foam cells."

As an atherosclerotic lesion becomes more advanced, an increasing number of foam cells are found in the plaque due to the continual recruitment of macrophages into the lesion. A thin fibrous cap of smooth muscle cells and collagen forms over the plaque, and smooth muscle cells underlying the damaged endothelium

*(continued on page 26)*

# C Reactive Protein:

## The Next Big Thing?

by Harold Olivey

A 70-year-old diagnostic test has become the latest tool for predicting a person's future risk of developing cardiovascular disease. It measures levels of C-reactive protein (CRP), which is made by the liver during periods of inflammatory activity in the body.

Atherosclerosis is thought to raise the plasma levels of CRP as any other inflammatory process would. Until recently, however, the relatively small increase in CRP resulting from vessel disease was below the detection range of the standard test method.

A method was developed in the late 1990s to increase the sensitivity of the CRP assay. This new diagnostic tool, called the high-sensitivity CRP test (hsCRP), has become a recommended standard for the care of individuals at risk for developing coronary artery disease.

Cardiologist Paul Ridker, M.D., Eugene Braunwald Professor of Medicine at Harvard University, helped develop the hsCRP test and has been its main champion.

Ridker and his colleagues measured CRP levels in blood samples from a subset of participants in the Physicians' Health Study, which assessed the benefits of aspirin and beta-carotene in reducing the risk of adverse cardiovascular events in several thousand male physicians. They found that participants with elevated CRP, as detected by the hsCRP assay, were at increased risk for heart attack and stroke.

Intriguingly, the elevated risk was independent of other known risk factors like high blood levels of low-density lipoprotein (LDL) cholesterol and smoking. Subsequent studies have largely supported this finding.

Last year the American Heart Association and U.S. Centers for Disease Control and Prevention (CDC) published a scientific statement suggesting that physicians could reasonably test for CRP in patients considered to be at moderate risk for developing cardiovascular disease.

More recently, however, researchers led by John Danesh, M.B., Ch.B., of Cambridge University performed a meta-analysis of prospective studies of coronary artery disease, and found that CRP concentration "added only marginally" to the predictive value of established risk factors like smoking and serum LDL concentration.

"Recent recommendations regarding the use of measurements of C-reactive protein in the prediction of coronary heart disease may need to be reviewed," the researchers reported in April in *The New England Journal of Medicine*.

Sergio Fazio, M.D., Ph.D., professor of Medicine and Pathology at Vanderbilt University Medical Center, does not believe that current data conclusively support a causal link between CRP and atherosclerosis. In comparison, LDL cholesterol is both a circulating marker and causative agent of atherosclerosis. CRP is, however, the best of several diagnostic tools available to detect inflammation, a recognized component of atherosclerosis, he says.

CRP also may be useful in the diagnosis of metabolic syndrome, a clustering of risk factors related to insulin resistance that is recognized as a predictor of future risk for developing cardiovascular disease and diabetes.

People diagnosed with metabolic syndrome possess at least three of the following conditions: elevated serum triglycerides (TG), low levels of high-density lipoprotein (HDL, often called the "good" cholesterol), visceral obesity, elevated blood pressure, and insulin resistance or glucose intolerance (see table).

Obesity may help tie inflammation to heart disease and diabetes.

Adipose (fat) tissue releases pro-inflammatory cytokines such as interleukin-6 (IL-6), which may contribute to the development of atherosclerosis and heart disease. IL-6 also may interfere with the body's ability to respond to insulin, a hormone that regulates glucose metabolism. Resistance to insulin signaling is a hallmark of metabolic syndrome, and can eventually lead to type 2 diabetes.

"As a relatively specific marker of inflammation, (CRP) does help identify people" at elevated risk of cardiovascular disease, says Doug Vaughan, M.D., chief of the Division of Cardiovascular Medicine at Vanderbilt.

"It helps you change your perceptions about a given patient and perhaps move them from a moderate risk category to a higher risk category. And that in turn precipitates a more aggressive intervention to reduce risk. That's the value of it." **LENS**

### Hallmarks of Metabolic Syndrome

| RISK FACTOR | VALUE IN MEN | VALUE IN WOMEN |
|---|---|---|
| Fasting blood triglycerides | 150 mg/dl | 150 mg/dl |
| Low blood HDL cholesterol | <40 mg/dl | < 50 mg/dl |
| Central obesity (waist circumference) | >40 inches | >35 inches |
| High blood pressure | >130/85 mmHg | >130/85 mmHg |
| Fasting blood glucose | >110 mg/dl | >110 mg/dl |

Source: American Heart Association –www.americanheart.org.

"I wouldn't be surprised if we develop a panel of 20 to 30 genes that we routinely look at … to define and predict risk." DOUG VAUGHAN, M.D.

*(continued from page 24)*

begin to proliferate, expanding the volume of the plaque.

Stable atherosclerotic plaques are less likely to cause an acute cardiovascular event such as a heart attack or stroke. Although they restrict blood flow through the lumen of the blood vessel, they rarely cause total occlusion. Instead, plaques provide a site within the vessels where clots can form. Platelets, blood cells involved in clotting, do not attach to the wall of healthy blood vessels. However, they will attach to atherosclerotic lesions.

As foam cells within a lesion die, the center of the plaque becomes necrotic, weakening the overlying fibrous cap and increasing the risk of rupture. The interior of an atherosclerotic plaque contains molecules that attract platelets and provide ample sites for attachment.

Thus, when an atherosclerotic plaque ruptures, a clot can quickly form and completely occlude the blood vessel. Acute cardiovascular events are most often precipitated by the rupture of the thin fibrous cap that covers the atherosclerotic plaque.

## Clot blocker

Aspirin, the classic anti-inflammatory drug, can prevent clot formation over atherosclerotic plaques by inhibiting the enzyme cycoloxygenase-1 (COX-1) in platelets. That, in turn, reduces formation of a powerful, pro-coagulant prostaglandin called thromboxane A2.

Aspirin also inhibits the related COX-2 enzyme, which produces other pro-inflammatory prostaglandins at sites of inflammation. Long recognized for its role in chronic inflammatory processes like arthritis, COX-2 is also expressed by cells within atherosclerotic plaques, but not elsewhere in the circulatory system.

Linton and Fazio recently have reported that COX-2 contributes to the pathology of atherosclerosis in mouse models of the disease. Inhibiting the enzyme in mice with high cholesterol levels, either pharmacologically or genetically, retards early atherosclerotic plaque formation. These data suggest that blocking inflammation could suppress the progression of atherosclerosis.

But Linton cautions that the tale is not so cut-and-dry. "It's tough to say (whether COX-2) is just good or bad. It probably depends on which cell is expressing it and at what time." For example, "COX-2 is expressed by basically all the players in the artery wall – smooth muscle cells, endothelial cells, macrophages," he says.

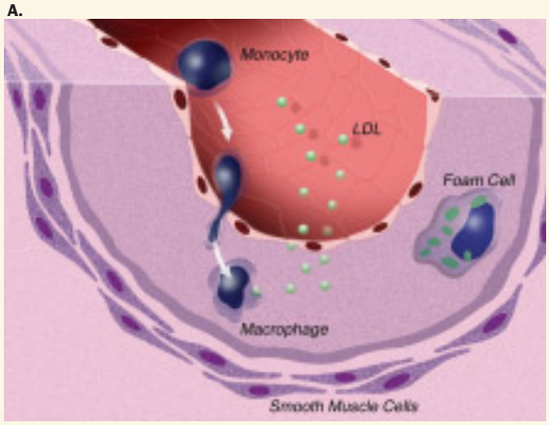
In addition, macrophages down-regulate their pro-inflammatory activities and lose COX-2 expression when they become foam cells. Other studies have suggested that blocking COX-2 activity does little to ameliorate the symptoms of more advanced atherosclerotic lesions.

This change in macrophage gene expression may come out of necessity: "When it's overloaded with cholesterol, the macrophage has to focus on getting

## Tale of a thrombus

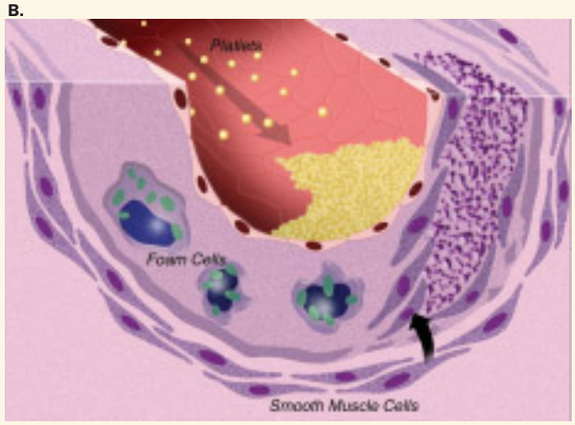
ILLUSTRATIONS BY DOMINIC DOYLE

A.




Endothelium that is injured, in this case by high levels of low-density lipoprotein (LDL) cholesterol, releases factors that attract blood cells called monocytes. Once inside the tissue, the monocytes differentiate into macrophages. Part of their job is to sweep up excess cholesterol. As they become engorged with cholesterol, the macrophages take on a foamy appearance, and now are called foam cells.

B.




Interactions between foam cells and white blood cells trigger a chronic inflammatory process. Smooth muscle cells migrate to the arterial wall, proliferate and secrete proteins that form a fibrous plaque. As the foam cells die, the plaque weakens and can rupture. Platelets are attracted to the site of the rupture, and can quickly form a clot that blocks the vessel completely.

rid of cholesterol," Linton explains. "Before that, it may be more important to be an inflammatory cell involved in the recruitment of other cells and propagation of the inflammatory pathway."

Experiments on atherosclerotic mice have provided significant insight into the mechanisms behind cardiovascular disease, including the recent findings on the role of inflammation. Even so, Fazio is quick to point out that the mouse models of atherosclerosis offer only a pale reflection of the disease state in human beings.

"There is an issue in quality and in the extent and topography (of lesions in mice)," Fazio cautions. The majority of human cases of atherosclerosis, according to Fazio, are due to a combination of risk factors. This is in sharp contrast to atherosclerosis in mice induced experimentally by the targeted disruption of one or two genes.

### Talking back to fat

During the past decade, two new classes of drugs were developed to relieve pain and inflammation in patients with rheumatoid arthritis – specific inhibitors of the COX-2 enzyme, and blockers of the pro-inflammatory tumor necrosis factor (TNF). It remains to be seen, however, whether they also will be useful in preventing heart disease.

Although COX-2 is expressed in atherosclerotic lesions, chronic use of high doses of one of the COX-2 inhibitors has been linked to an increase in blood pressure, edema and serious heart problems in some patients. As for TNF-inhibitors, animal studies and at least one report in a patient suggest that TNF blockade may actually destabilize atherosclerotic plaques and precipitate heart attacks.

Statins, the blockbuster cholesterol-lowering drugs, also have anti-oxidant and anti-inflammatory properties that may protect against cardiovascular disease. To test this hypothesis, investigators in the multi-center JUPITER study are administering the statin drug Crestor to participants who have elevated circulating inflammatory markers including CRP, but normal LDL and triglyceride levels. The study is expected to be completed in about three years.

The newest targets for pharmacological treatment of atherosclerosis may come from studies of how adipose tissue (fat) regulates cholesterol metabolism and inflammation. Fazio and Linton point to recent studies that suggest adipose tissue, composed of fat cells, and macrophages in atherosclerotic plaques lead the inflammatory response in atherosclerosis and cardiovascular disease.

Some genes originally reported to be expressed primarily or exclusively in adipose tissue are also expressed in activated macrophages. Some of these genes, including the fatty acid binding protein aP2, are believed to be involved in insulin resistance, an early hallmark of type II (non-insulin dependent) diabetes. These recent reports suggest inflammation as a critical link between diabetes and atherosclerosis.

Individuals showing symptoms of insulin resistance are more likely to develop cardiovascular disease as well as diabetes. Patients with diagnosed diabetes are at elevated risk for adverse cardiovascular events such as heart attack and stroke. By targeting proteins expressed in fat cells and macrophages that seem to play a dual role in reducing insulin sensitivity and increasing inflammation, new therapies may reduce the incidence of both atherosclerosis and diabetes in at-risk populations.

"I wouldn't be surprised if we develop a panel of 20 to 30 genes that we routinely look at in individuals to define and predict risk," says Doug Vaughan, M.D., chief of Cardiovascular Medicine at Vanderbilt University Medical Center. "It's going to be a multi-factorial approach that includes … biochemical, physiological and genetic parameters." **LENS**

## An Ounce of Prevention: Emerging Tools in the Fight Against Cardiovascular Disease

Cardiovascular disease, like cancer, is a health problem best treated with prevention and early detection. Physicians successfully identify many at-risk patients by measuring the blood levels of markers like LDL cholesterol. However, for many patients, their first indication of cardiovascular disease is suffering a heart attack or stroke.

"The cornerstone of risk determination is found in the parameters that have been measured and validated through prospective epidemiological studies," says Doug Vaughan, M.D., the C. Sidney Burwell Professor of Medicine and chief of Cardiovascular Medicine at Vanderbilt. "Those (studies) have really defined the power of factors such as high HDL, low LDL, hypertension and smoking as authentic determinants of risk over time."

Yet an increasing number of patients develop cardiovascular disease in the absence of these traditional risk factors. "That has motivated and catalyzed a search for other important markers or determinants of risk," he says.

One emerging risk factor, insulin resistance, increases cardiovascular disease risk without significantly impacting lipid levels. "Generally, people with insulin resistance don't have high LDL; they've got a low HDL, they've got high TG," Vaughan says. Finding the link between insulin resistance and heart disease, therefore, is critically important.

One possibility: a serum protein involved in clot formation called plasminogen activator inhibitor-1 (PAI-1). "The neat thing about PAI-1 is that it is driven by so many different factors that contribute to cardiovascular disease in the 21st Century," Vaughan says, including inflammation and insulin resistance. PAI-1 levels track with CRP, making PAI-1 an "integrative marker of multi-factorial inputs that might influence your (heart disease) risk," he says.

Vaughan's laboratory has published several papers describing how the PAI-1 gene is regulated. When the PAI-1 gene becomes "switched on," the result is higher plasma levels of PAI-1 protein. Recent reports from Vaughan's laboratory have identified ways in which the PAI-1 gene might be switched on by inflammation. Others have reported that drugs designed to combat insulin resistance decrease circulating levels of PAI-1. "If you improve the lipid profile and if you reduce insulin resistance in patients," he says, "you would predict that their PAI-1 levels are going to come down."

Meanwhile, new technologies such as cardiac magnetic resonance imaging (MRI) and multi-slice computed tomography (CT), offer the promise of non-invasive, real-time diagnosis of coronary artery disease – at an earlier stage than ever before. Coupled with analysis of circulating markers of heart disease, perhaps even more patients can be spared the pain, expense, and morbidity of a heart attack or stroke.

– HAROLD OLIVEY

Q & A

# TEAMWORK

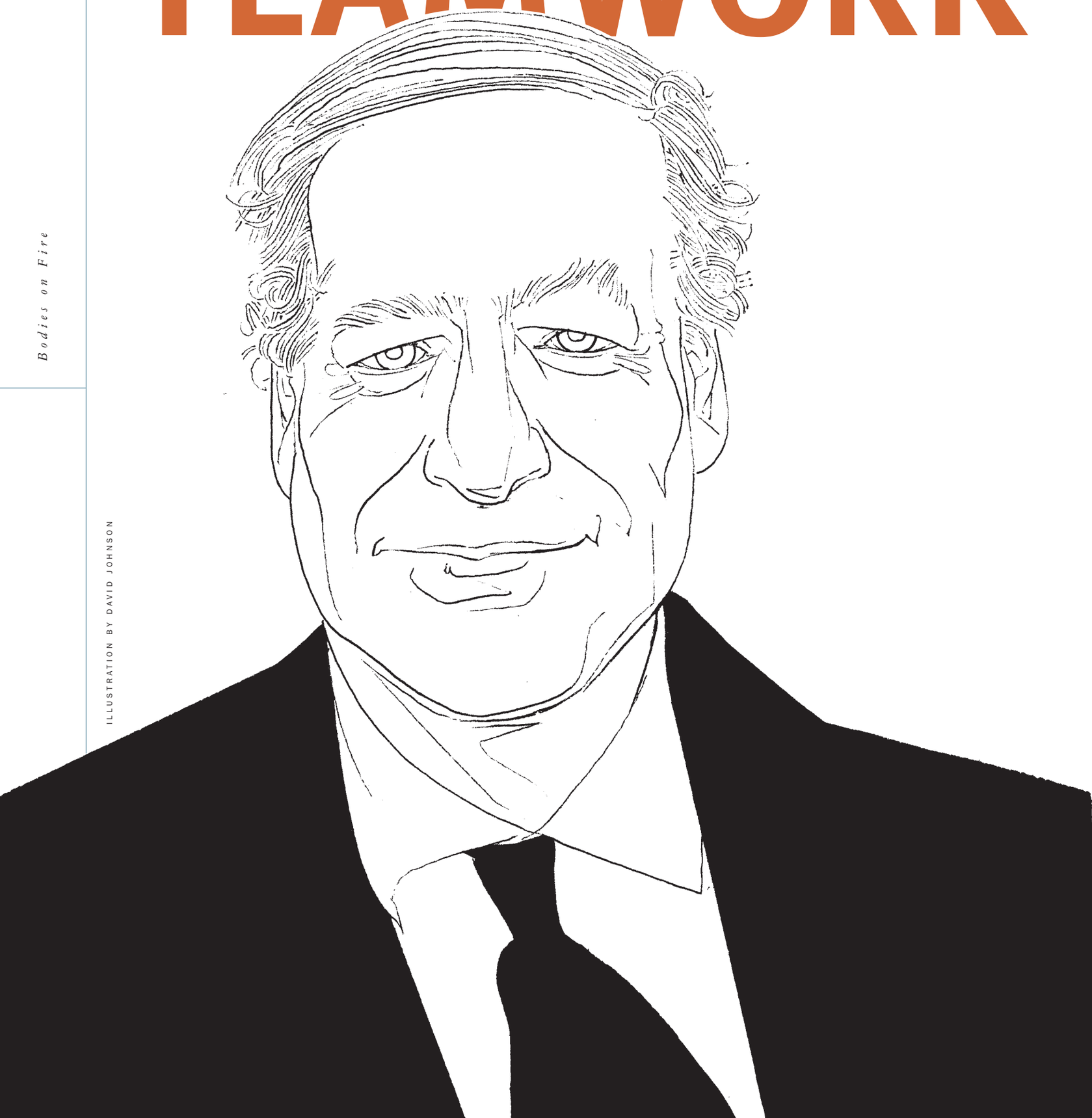


ILLUSTRATION BY DAVID JOHNSON

A conversation with Sir Ravinder Maini

# + TRUST

## THE DEVELOPMENT OF ANTI-TNF THERAPY

Emeritus Professor Sir Ravinder Maini, M.D., and Professor Marc Feldmann, Ph.D., of Imperial College London's Kennedy Institute of Rheumatology, received the 2003 Albert Lasker Award for Clinical Medical Research for their "discovery of anti-TNF (tumor necrosis factor) therapy as an effective treatment for rheumatoid arthritis and other autoimmune diseases."

Maini, who was born in India and has lived in the United Kingdom for 50 years, was knighted last year by Queen Elizabeth II for his groundbreaking work. Recently, he shared his thoughts with *Lens* editor Bill Snyder about the challenges of developing new treatments for inflammatory disorders, and the importance of collaboration.

**Is the current method of conducting clinical trials adequate for determining the impact of candidate drugs on a disease process like rheumatoid arthritis?**

A trial is by definition a very artificial entity in that you have exclusion and inclusion criteria, which define populations very rigidly. Some people would argue that defining patients before you enter them into a trial often means that you're loading that trial in favor of the patients that are most likely to respond, or patients that are least likely to show side effects ...

Already the regulatory authorities are mandating so-called phase four studies. Once the drug is licensed, companies are still required by regulatory authorities to keep information about adverse events, for example. For expensive drugs, I think more and more payers are insisting on some kind of evidence of effectiveness in the real-life situation …

In a world where resources are limited and health budgets are under strain, … some kind of objective evidence that they are doing good and not harm is part and parcel of what I call post-marketing surveillance. That's a different question from whether you can have better trials. And the answer to that is yes.

Traditionally in most diseases you start off with patients that are the sickest, as was the case with anti-TNF drugs. It isn't the best population to see the best result in, but these people are in a terrible mess usually by the time they get into the trial because they've failed everything else, they're often debilitated and sick as a result. Their resistance to infection is low because they've become immobile or they've put on weight – all the other factors that encourage what we call co-morbidity.

Obviously they are often not the best candidates to include in a trial. But if you have an agent of unknown safety, usually ethical issues demand that you start to gain full consent from a patient population where it's unlikely that you're going to do them harm. That's a difficult question.

**Can anti-TNF therapy reduce the risk of heart disease?**

We have reason to believe that coronary artery disease is inflammatory in nature, and that the inflammation in blood vessels affected by atherosclerosis is a process that is very similar to TNF-driven inflammation in other diseases. The prediction is that anti-TNF treatment may be beneficial in patients with coronary artery disease who are not in heart failure.

Such trials haven't yet been done, but it's likely that we'll get an answer from the registries that have been created to follow up patients on anti-TNF treatment ...

By the way, in rheumatoid arthritis, death from coronary artery disease is increased significantly. So what we would expect to see is that the population that is receiving anti-TNF will normalize and begin to resemble more the population that doesn't have an increased coronary artery disease.

**What about cancer?**

It turns out that patients with rheumatoid arthritis have an increased incidence of cancer of the lymphatic glands – lymphomas ...

In clinical trial evidence, there was (an) increased incidence of lymphatic cancer compared to the normal population. The FDA actually looked at this last year and concluded that the evidence at this stage was insufficient to tell us whether the rate was as expected in this disease because of the underlying disease, or whether there was an effect of anti-TNF therapy on the incidence of lymphatic cancer. Once again, we can only hope that the registry will tell us the answer to that ...

As far as any other type of cancer is concerned, epidemiological studies have not shown any increase in rheumatoid arthritis patients and so far no increase in any clinical trials or registry of any other kind of cancer. There is however some very interesting data in relation to COX inhibitors, which suggest that bowel cancer is reduced in patients that are taking regular NSAIDs …

The majority of patients with rheumatoid arthritis or most inflammatory diseases are on anti-inflammatory drugs anyway, and therefore we would expect a reduction in

> "PROGRESS ... REQUIRES MORE THAN THE INDIVIDUAL CAN EVER CONTRIBUTE. IT REQUIRES AN ORDERED SOCIETY THAT IS AWARE OF ITS RESPONSIBILITIES."

bowel cancer incidence in such people. So there is yet another confounding factor out there – whether anti-TNF, which is known to block COX-2 just as well as aspirin or Naprosyn or any of these kinds of agents, might have the same beneficial effect.

**Is there a concern that some candidate drugs may be abandoned because they do not show significance when evaluated independently, even though they may be useful in combination with other drugs?**

That's certainly true also for anti-rheumatic treatment. Even anti-TNF has been shown to work best when used with methotrexate, rather than as monotherapy. That's now proven for all three anti-TNF drugs. If we hadn't done such trials, we wouldn't know that. And it's possible there are other drug combinations with anti-TNF, which are going to be better than anti-TNF alone ...

**How can we encourage more testing of products in combination?**

Sadly, synergy between companies has not yet been a feature of drug development. Usually it means the academic community will do the clinical trial.

I must say I can't see the logic of it because you would imagine that if two drug companies thought there was a rationale for a combination, that it would present a win-win situation for them to get together and do such a trial.

**What's holding them back?**

I think it's usually competition fears, the fear that the market share of their individual drug will suffer. I think that … is being reflected in the big takeovers rather than company collaboration …

When Pfizer takes over another company, or Wyeth merges with Amgen, basically what's happening is that the pipeline of the two companies can then be combined ... But I don't see why (collaboration) can't happen between two independent companies.

**According to some, the traditional career path in academic science presents another challenge to collaboration because researchers must demonstrate their independence to win research grants and publish their results. Do you agree?**

Certainly there is some truth in it. The competitive grant system does encourage individuals to build their own little enterprise. And in that environment, sharing of knowledge is often regarded as a threat rather than as an opportunity. I think that it's further encouraged by the fact that discoveries now have commercial value, because you can patent your discoveries and exploit them.

But I do think that in practically every case that I can think of where major discoveries are made, either at the basic science level or at the clinical translational level, collaboration is more or less essential. Whether it was the Human Genome Project, or at a minor level, taking anti-TNF from the bench to the clinic, collaboration was essential.

What is usually needed is trust and enlightenment between the groups that will work together, and good management of the process so that the reward system is fair ... I think that in good environments, that is beginning to happen ...

I think teamwork is beginning to be appreciated more as a necessity in scientific achievement. In a properly managed environment, the career progress of individuals has to be taken care of. The real contributors have to be singled out ... I think that journals, for example, are becoming much more tough about accreditation of authorship. And similarly, where intellectual property is created, people are much more aware of and smarter about how that should be taken care of.

In one way, it's making the whole thing a little more commercially driven, but I take the view – rightly or wrongly – that in order to progress, resources of commercial backing are needed to take things from rudimentary bench to the clinic anyway. That's the reality. You can't do it without some involvement of commerce in it.

The whole business of technology transfer from academia to industry should be seen in my view as a duty of academic enterprise because, after all, the wealth of a nation depends in the end on how the brains of the country use that information for making progress. It is important that academics don't think we should be ashamed of enterprise. On the contrary, we should feel pleased to see this happen, and help to make it happen.

**How does this square with the traditional reluctance to mix academic and commercial activities?**

The traditional academic view, which has tended to regard commercialism as an undesirable mammon, I don't think actually represents a well-oiled, advanced nation's way of working.

There is no doubt at all that Britain in the Victorian Age demonstrated how exploitation of inventions was a key part of the Industrial Revolution. That remains true for the molecular revolution today.

What is more important is the transparency and freedom of access to information – that's of course absolutely vital, that an academic should be able to share and have access to information, reagents, and so on, so the process of invention and new discovery is not hindered, but rewarded.

It is a two-way street, and if managed properly, a win-win situation. Unfortunately, real life sometimes intrudes on that. Selfishness and greed can sour these things, can't they? But that shouldn't by itself be a hindrance to progress.

We're probably at a unique time in the history of biomedicine. The opportunities are absolutely fantastic, but the threats are there. Progress … requires more than the individual can ever contribute. It requires an ordered society that is aware of its responsibilities. **LENS**

# Breaking the COX code

## Using the team approach

Teamwork among scientists at Vanderbilt University Medical Center during the past 35 years has contributed much to current understanding of the role of the cyclooxygenase (COX) enzymes and their products – the prostaglandins – in human disease.

Prostaglandins, which were first isolated from the prostate gland in 1936, are very rapidly metabolized, or broken down, making measurement in the blood difficult. Researchers at Vanderbilt led by John Oates, M.D., developed methods for measuring levels of prostaglandin metabolites (breakdown products) in the urine using mass spectrometry.

Ray DuBois, M.D., Ph.D.
Photo by Dean Dixon

Using this technique, the research team – which by the late 1970s included L. Jackson Roberts, M.D. – identified prostaglandin D2 as a product of the human mast cell and demonstrated its release during allergic asthma.

With colleagues including Garret A. FitzGerald, M.D., now chair of Pharmacology at the University of Pennsylvania, Oates and Roberts showed that low doses of aspirin blocked the production of thromboxane, a prostaglandin made by platelets that causes blood clotting and constriction of blood vessels. Their findings supported the use of low dose aspirin to prevent heart attacks.

"All of this could not have been possible without the early and ongoing commitment on the part of Vanderbilt to mass spectrometry," says Jason Morrow, M.D., F. Tremaine Billings Professor of Medicine and Pharmacology at Vanderbilt. "John realized that in 1969 when (as director of the Division of Clinical Pharmacology) he brought the first mass spectrometer to Vanderbilt," Morrow says.

In the early 1990s, Vanderbilt researchers led by Ray DuBois, M.D., Ph.D., discovered a link between the COX-2 enzyme and colon cancer. That work helped lead to current tests of COX-2 inhibitors as a potential way to prevent cancer. Recently another group led by Morrow and David H. Johnson, M.D., director of the Hematology-Oncology division at Vanderbilt, reported that urine levels of a prostaglandin metabolite called PGE-M could predict the effectiveness of a COX-2 inhibitor in patients with non-small cell lung cancer. This suggests, says Morrow, "that the measurement of these inflammatory 'mediators' and their suppression may be useful in the treatment of lung cancer."

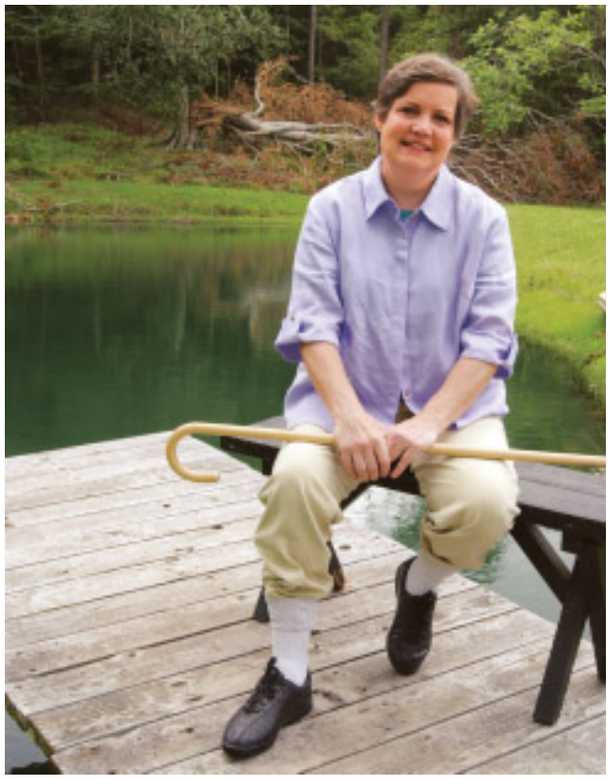
COX enzymes also may play a role in Alzheimer's disease. In addition to prostaglandins, the COX pathway can lead to the production of highly reactive molecular compounds called levuglandins, which, in turn, can form "adducts," or irreversible attachments to proteins that may be toxic to nerve cells.

In July, Oates and his colleagues at Vanderbilt and Johns Hopkins University reported that they found a 12-fold increase in the level of adducts in the brains of patients who had Alzheimer's disease compared to age-matched control brains.

"These are the first clear data showing that COX products are elevated in the brains of patients with Alzheimer's disease," says Oates, Thomas F. Frist Professor of Medicine and professor of Pharmacology.

Vanderbilt currently is participating in a national trial to see if long-term use of COX inhibitors will reduce the incidence of the disease.

– BILL SNYDER



**Pictured here:** Toni Locke on the family farm in Fayetteville, Tenn. Beyond the pond – one of the large trees toppled by a recent tornado.

Photography by Anne Rayner

# A tornado in the body

## Living in the wake of rheumatoid arthritis

By Toni Locke

My body's immune system has devastated the linings of my joints. It's like the tornado that cut a swath through our farm property this spring, toppling two pecan trees, a catalpa that had been in full bloom, and an American Beech that was 100-plus years old.

Like the tornado, rheumatoid arthritis caught me by surprise. But unlike damage from winds that can occur in seconds, this insidious disease worked on me for a while before I – or my doctors – realized what was happening.

In March of 2000 I was struck by an incredible fatigue. Outdoor activities I'd previously savored after my workdays in a windowless school library became impossible. Malaise and depression followed. I felt so miserable I did not even want to tell my doctor, fearing age to be the culprit. Eventually I let him know and I was treated for depression.

By August, my right hand had become so painful that even simple tasks like assigning texts became unbearably painful. I thought that I had overcompensated for my left hand, which had fractured.

But the pain and fatigue continued even after my left hand healed. I took a leave of absence and later resigned.

By the summer of 2002, my hands were waking me during the night with numbness and tingling sensations. An orthopedic surgeon diagnosed carpal tunnel syndrome in my right hand. It took two operations to relieve the pain. My physical therapist noted that both hands had stiffness, but because there was no rheumatoid arthritis in my family, I ignored his advice to see a rheumatologist.

In the summer of 2003, I began to limp from pain and swelling in my ankles. I had trouble rising from a seated position and getting in and out of a car. I would take shelter under a tornado warning, but until my inflamed ankles literally brought me to my knees with pain I was in denial.

Finally, I listened to my therapist and asked my doctor to refer me to a rheumatologist. In January 2004, Dr. Victor Byrd at Vanderbilt pulled my classic symptoms together into a diagnosis and treatment program for rheumatoid arthritis.

The medications are harsh on one's system and take a while to provide relief, but I am managing better. My pain has almost disappeared, and I am currently participating six days a week in a circuit workout to keep my joints flexible and to strengthen my bones. I have not had any energy, though, and still have to nap each afternoon to keep going at all.

The most aggravating damage is to my ankles. Losing weight will hopefully take some of the stress off these joints, so that is a goal I'm working on right now. I am very grateful that I finally have been diagnosed, and that I was able to spend 54 years without this pain and stiffness.

Be informed. Arthritis is the leading cause of disability in people over the age of 15. Read your body's warnings before rheumatoid arthritis or some other disease does irreparable damage. **LENS**

*Toni Locke is a former school librarian who lives in Fayetteville, Tenn.*

# Through our Lens

## LETTERS TO THE EDITOR

### Vanderbilt connections

I congratulate you on the recent issue of *Lens* on viral infections (Spring 2004), which is as fine a publication as I have seen from Vanderbilt in a long time. I found all the articles stimulating and informative, but I was particularly impressed by your article on Dr. Anthony Fauci and his laudatory efforts to deal with the ravages of HIV/AIDS.

Dr. Fauci paid tribute to Dr. Sheldon Wolff in several places in the article, including his statement that Dr. Wolff launched him on his career ...

It should be noted that Sheldon has an important relationship with Vanderbilt. He took his M.D. at Vanderbilt in 1957 and was later a medical intern and resident in medicine at Vanderbilt University Hospital. As your article states, Dr. Wolff had a distinguished medical career prior to his untimely death in 1994, including his being the director of the Laboratory of Clinical Investigation at the National Institute of Allergy and Infectious Diseases at NIH and finally the chief of medicine at Tufts University.

I am pleased to remember him as my dear friend and fellow house officer at Vanderbilt.

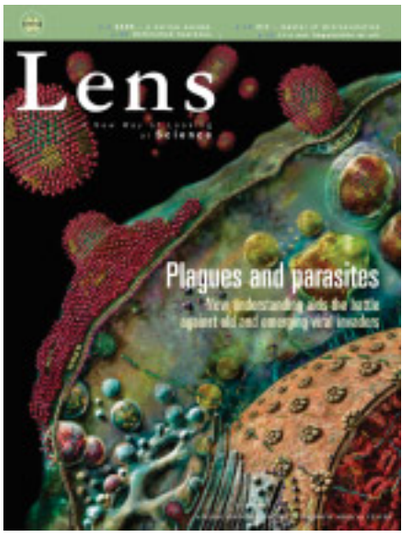
BOYD L. BURRIS, M.D.
*Clinical Professor of Psychiatry*
*George Washington University Medical Center*
*Georgetown University Medical Center*
*Resident in Psychiatry at Vanderbilt (1956-1959)*

### Eradicating polio

I am very impressed with the publication received this month from my beloved alma mater. However the article on polio by Lisa DuBois (page 25, Spring 2004 issue) leaves out the impact of Rotary International, whose members have given $600 million and much manpower to eradicate polio from the Earth.

I am aware that many of my Vanderbilt classmates are also Rotarians; many like me are past presidents of their respective clubs, and are very proud to be part of the elimination process.

PAUL HUCHTON, M.D.
*El Paso, Texas*
*Vanderbilt University School of Medicine, Class of '58*




### We goofed!

We misidentified the scientific illustrator whose colorful depictions of the influenza virus appeared on the cover and on page 23 of the last issue of *Lens*.

Proper credit should be given to Russell Kightley of Canberra, Australia (www.rkm.com.au).

We sincerely regret the error.

Letters may be mailed to:
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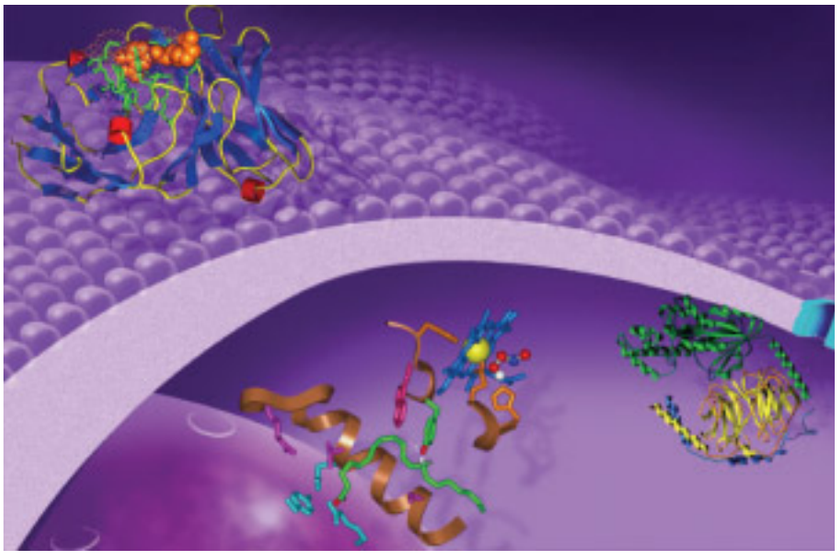
Or e-mailed to: william.snyder@vanderbilt.edu

**Targets for drug discovery: an antibody binding site on the cell surface (top left), the COX-2 enzyme inside the cell (bottom left), and a heterotrimeric G-protein in the cell membrane (bottom right). G-proteins are "molecular switches" that convert signals used to communicate between cells into signals that act inside the cell.**

**Illustration by Dominic Doyle. Courtesy of Lawrence J. Marnett, Ph.D., and the Vanderbilt Institute of Chemical Biology.**



## IN THE NEXT ISSUE:

**Linking bench to bedside**
University scientists are increasingly collaborating with their peers in industry to improve the search for new drugs.

**Dimming the switch**
How basic research led to the development of allosteric modulators and a redefinition of the term "medicate."

**Completing the circle**
The study of drugs on the market reveals insights into previously unrecognized biology – and to new pharmaceuticals.

Lens

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