Inflammation and Cancer: The Link Grows Stronger

Research into a long-suspected association between chronic inflammation and cancer reveals how the immune system may be abetting tumors

Hepatitis B virus infects hundreds of millions of people worldwide, causing jaundice, fatigue, liver damage, and joint pain. More ominously, investigators have indicted it in another role: as co-conspirator in a far-ranging case they've been building for years linking chronic inflammation and cancer. Researchers have long known that patients with persistent hepatitis B infections experience inflammation and scarring of liver tissue and an increased risk of liver cancer. Other sources of chronic inflammation, including the ulcer-causing bacterium *Helicobacter pylori* and an immune disorder known as ulcerative colitis, predispose patients to cancers of the stomach and colon.

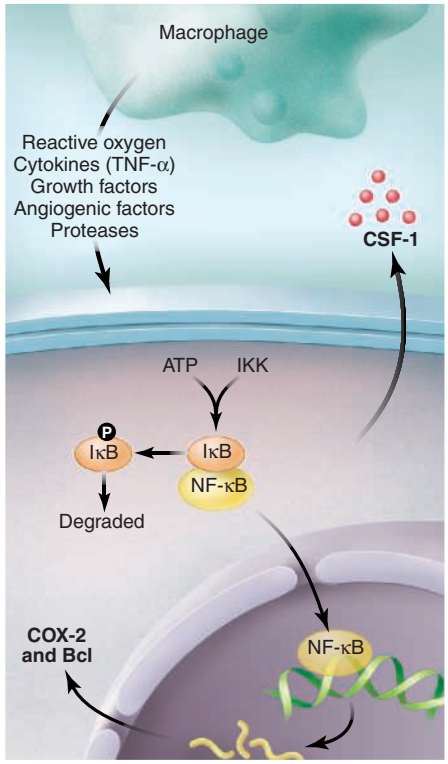
Based on their experience with these diseases, researchers estimate that inflammation contributes to the development of at least 15% of all cancers. Much less clear, however, is exactly how it does its dirty work. The inflammation-cancer connection is especially puzzling in light of other work suggesting that in some circumstances the immune system, which sustains inflammation, has the opposite effect: inhibiting tumor development. A flurry of results published over the past few months may now be resolving the mystery.

The new work has implicated an inflammation-induced protein called NF-κB as a key player. It is an intermediary in promoting the cellular changes leading to the uncontrolled growth of cancer cells and also to later changes that help metastatic cells escape from the original tumor and spread to new sites in the body.

Other studies have pointed to a source of trouble in the inflammatory cells that creep into a growing tumor, suggesting that they produce numerous substances that can contribute to tumor growth and survival, including some that might trigger increased NF-κB activity. Research into the inflammation-cancer link "is a very exciting area that is developing very rapidly," says Raymond DuBois of Vanderbilt University Medical Center in Nashville, Tennessee.

The excitement stems in part from the expectation that this emerging understanding could lead to improved cancer prevention and treatment. Epidemiological studies have already shown that people who regularly take NSAIDs—nonsteroidal anti-inflammatory drugs—have a lower risk of developing cancer than people who don't take the drugs. But the efficacy of NSAIDs is not ideal, and the first generation of these drugs, such as aspirin, can cause life-threatening stomach ulcers. Now, even the newer NSAIDs—the so-called COX-2 inhibitors, which were designed to avoid that side effect—may have problems: On 30 September, the pharmaceutical company Merck removed its blockbuster COX-2 inhibitor Vioxx from the market because it increased patients' risks of having heart attacks and strokes.

Researchers hope that if they learn how chronic inflammation leads to cancer, they will be able to design new drugs that counter its effects. The information may also aid the development of vaccines and other strategies to enhance immune attacks on tumors. But researchers will have to toe a fine line: The new work suggests that some approaches might enhance the growth of tumors rather than kill them. "It's a complicated field, but it's extremely important," says Albert Baldwin of the University of North Carolina School of Medicine in Chapel Hill (UNC).



Vicious cycle. Macrophages produce several substances that can enhance tumor growth, including TNF-α, which can turn up NF-κB activity in both target tissue cells and in macrophages themselves. Tumor cells produce substances such as CSF-1 and COX-2 that give a further boost to inflammatory processes, as well as proteins such as Bcl that inhibit apoptosis.

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Jekyll-and-Hyde macrophages

One line of evidence linking inflammation to cancer comes from the somewhat surprising finding that immune cells can foster tumor development. Lisa Coussens, Douglas Hanahan, and their colleagues at the University of California School of Medicine in San Francisco have found that this can happen in a model of skin cancer they devised in mice that ordinarily develop invasive skin carcinomas by age 1.

The tumors also show infiltration by immune cells, an inflammatory response the researchers initially viewed in a positive light as the body's attempt to destroy the tumor cells. But when they turned down the immune response by crossing their mice with another strain lacking a particular type of T immune cell, the resulting animals showed reduced tumor formation rather than the expected increase. "If we abate inflammation, we abate cancer development," Coussens says.

Similarly, Jeffrey Pollard and his colleagues at Albert Einstein College of Medicine in New York City have evidence that infiltration with inflammatory cells called macrophages promotes both the development of breast cancers and their eventual spread to other sites in the body. The team showed this by knocking out the gene for colony-stimulating factor 1 (CSF-1), a so-called cytokine that attracts macrophages, in a mouse strain genetically engineered to develop mammary cancers.

Compared to controls, Pollard says, "the incidence and rate of early [tumor] growth in the resulting animals was not changed, … but progression to malignancy was slowed, and there was almost no metastasis." Consistent with this, Pollard's team and others have also shown that CSF-1 levels are elevated in human breast, ovarian, and uterine cancers and that this elevation correlates with a poor prognosis.

Why the immune system can promote cancer development at times and in other cases help keep it in check remains unclear. But activating macrophages appears to be especially dangerous; many of the weapons these immune system scavengers release can promote cancer. The noxious substances include highly reactive forms of oxygen that can cause carcinogenic mutations. Cells are especially vulnerable to these assaults when they are dividing rapidly, as may be the case when

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tissues try to repair the damage caused by viral or bacterial infections. Macrophages also produce growth factors, enzymes that can help cancer cells escape from tumors and migrate through the body, and still other proteins that stimulate the formation of blood vessels needed for tumor growth.

Inflammation hallmark implicated

Although the overall picture is far from complete, recent evidence has now firmly tied one protein, NF-κB, into the cancer-promoting action of inflammatory cells. Suspicion fell on the protein several years ago. It is highly active in both inflammatory cells, such as macrophages, and in other cells of inflamed tissues. Indeed, NF-κB activity is "almost a hallmark of inflammation," says a researcher involved in this work, Yinon Ben-Neriah of Hebrew University-Hadassah Medical School in Jerusalem, Israel.

Researchers have also found that the protein is abnormally active in some cancers and that that portends a bad prognosis. This may be due to the fact that NF-κB activity leads to inhibition of the programmed cell death (apoptosis) that can eliminate defective cells, thus contributing both to cancer development and resistance to drug and radiation therapies.

But the case against NF-κB was largely circumstantial—until this summer, when Michael Karin and his colleagues at the University of California, San Diego, helped uncover a smoking gun. They found that the protein contributes to cancer development in two distinct ways. (The results appeared in the 6 August issue of *Cell*.)

For these experiments, the researchers turned to a mouse model of colitis-associated cancer developed about 8 years ago by a team led by Isao Okayasu of Kitasato University School of Medicine in Japan. This involves giving young mice a single injection of a cancer-triggering chemical and then dosing them repeatedly with a salt that irritates the intestinal lining, causing chronic inflammation of the colon. This treatment produces numerous colon tumors.

To test the involvement of NF-κB, the Karin team used two different strains of genetically altered mice; in one, the gene for an essential NF-κB activator enzyme called IKKβ had been knocked out only in the cells that give rise to macrophages, and in the second strain the same gene was knocked out in the epithelial cells lining their intestines. In the absence of the *IKKβ* gene, neither cell type could activate its NF-κB.

Loss of NF-κB function in macrophages reduced tumor incidence in the animals by about 50%, and the tumors that did form were smaller than those in controls. Further work traced this reduced tumor growth to loss of growth factors produced by inflammatory cells in response to NF-κB activation.

The picture was different in mice whose intestinal epithelial cells couldn't activate NF-κB. These animals "had an 80% reduction in tumor incidence," Karin says. That difference was not due to reduced intestinal inflammation because it was more severe in the knockouts than in the controls. Instead, with NF-κB eliminated, apoptosis was no longer inhibited in intestinal cells, which presumably helped the knockout animals eliminate cells with cancer-promoting mutations. "Apoptosis is sort of like a safety mechanism," Karin explains. "It makes sure tumor formation is low."

Something similar appears to be going on in inflammation-associated liver cancer, as described by Ben-Neriah and his colleagues in *Nature* (published online on 25 August). These researchers started with a genetically altered strain of mice that develop severe liver inflammation—and cancer. Then they made a second genetic alteration, adding a gene for a natural NF-κB inhibitor called IκB that carried a regulatory sequence allowing the gene to be turned off at will by giving the animals an antibiotic.

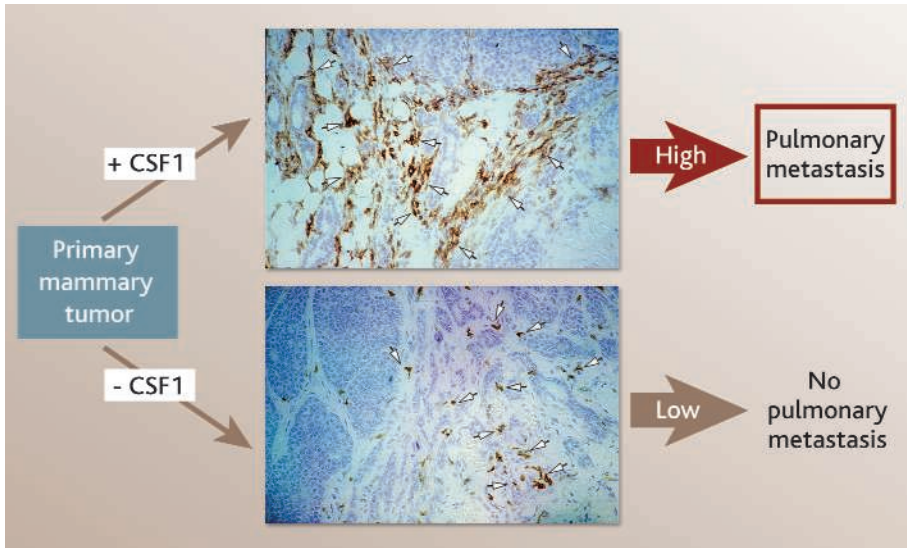
When the IκB gene was on—and NF-κB was inactive—the livers of the mice showed early precancerous changes such as increased cell division and the formation of small growths called adenomas for 7 months. But compared to controls, very few of those adenomas—about 10%—progressed to full-fledged cancers.

When the researchers then turned the IκB gene off, Ben-Neriah says, "we started to see tumors as if there had been no [earlier] NF-κB switch-off." This suggests that the protein is not required for the early changes that put cells on the path to cancerous transformation but is required for later progression to malignancy. Again, it apparently promotes liver cancer development by inhibiting apoptosis.

What's more, NF-κB may be needed for one of the most dangerous features of cancer cells: their ability to spread throughout the body and seed new tumors. Some of this work, described in the August *Journal of Clinical Investigation*, comes from a team led by Thomas Wirth of the University of Ulm, Germany. Working with cultured mammary epithelial cells that had been transformed with the cancer-causing Ras oncogene, the researchers showed that the NF-κB protein is needed for something called the epithelial-mesenchymal transition, in which normally sedentary epithelial cells undergo changes that allow them to migrate.

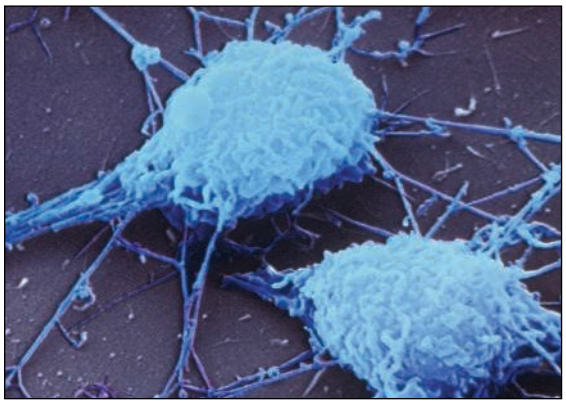
Consistent with those findings, the German team also showed that cells in which NF-κB had been inhibited by addition of an active IκB gene form far fewer lung metastases when injected into mice. "NF-κB may be involved both early and late" in cancer development, Wirth says. If so, it would be a good target for potential therapeutic drugs.

Karin's team provides further confirmation that inflammation-induced NF-κB activity is needed for growth of tumor metastases. To mimic metastatic growth, they injected colon cancer cells into mice and observed them forming metastases in the animals' lungs. When the researchers also injected a bacterial lipopolysaccharide (LPS) to stimulate an inflammatory response in the mice, the metastases "pretty much doubled in size," Karin says. That response also required a protein called tumor



Metastasis link. Breast cancer cells producing CSF-1 (top) contain copious numbers of macrophages (brown stain) and produce numerous lung metastases. Cells that don't make the protein (bottom) contain few macrophages and show little tendency to metastasize.

CREDIT: E. Y. LIN, V. GOUON-EVANS, A. V. NGUYEN, J. W. POLLARD, *JOURNAL OF MAMMARY GLAND BIOLOGY AND NEOPLASIA* 7, 147 (2002)



Partners in crime. Inflammatory cells such as the macrophages shown here are turning out to boost tumor growth and spread.

necrosis factor–α (TNF-α) that's made by macrophages. But when the researchers performed the same experiment with tumor cells bearing an NF-κB inhibitor, the tumors actually shrank following LPS injection due to increased apoptosis.

Although much evidence supports the idea that NF-κB promotes cancer by inhibiting apoptosis, it may contribute in numerous other ways as well. Some may be direct outgrowths of the protein's role in inflammation. The genes activated by NF-κB include the one that makes COX-2, an enzyme needed for the synthesis of a pro-inflammatory compound called PGE-2. This can bring in more immune cells to maintain the inflammation and further prod tumor growth. COX-2 also promotes blood vessel growth. Actions such as these may explain why COX-2 inhibitors have cancer-fighting effects.

Some NF-κB actions may be independent of inflammation, however. Although NF-κB activity is not necessary for cancerous transformation by Ras, Baldwin says, it does foster cancer growth, similar to what Karin's team found in their experiments. But when Ras activates NF-κB, the consequences may be different than when TNF-α or other inflammatory factors do. In work published in the 15 October issue of *Cancer Cell*, Baldwin and his colleagues analyzed the genes turned on by NF-κB following its activation by Ras. The genes affected included several that make growth-promoting proteins, but for reasons not yet understood, none of the inflammation-promoting genes typically activated in response to TNF-α. Although this fosters cancer growth, NF-κB activity in response to Ras apparently activates a different set of genes than when TNF-α is the activator.

However NF-κB works, it's looking more and more like a good target for anticancer drugs. "There are definitely ways to take advantage of this," Karin predicts. The pharmaceutical industry is currently working to develop NF-κB inhibitors, and even some low-tech compounds such as the active ingredients in green tea and red wine, which are thought to have anticancer properties, are turning out to act on the protein. "Almost every cancer preventive is an NF-κB inhibitor," Baldwin says. **–JEAN MARX**

Invasive Species

Expanding Trade With China Creates Ecological Backlash

Scientists in the United States and China are scrambling to cope with an unintended consequence of increasing economic ties—a two-way flow of unwelcome plants and animals

To the unknowing eye, the reeds growing along the Yangtze River near Shanghai—a burst of green in the summer, with tips that turn golden brown in the fall—belong on picture postcards. But ecologists know them as a biological experiment run amok. The salt marsh grass *Spartina alterniflora*, a native of eastern North America introduced in 1979 to check erosion, has now spread across southeastern China, choking estuaries, crowding out native grasses, and reducing feed and habitat for fish and migratory birds.

Across the Pacific, the tiny holes in the bark of maples, willows, and elms in New York, New Jersey, and Illinois come from the Asian longhorned beetle. *Anoplophora glabripennis* is an unwelcome hitchhiker from China that most likely arrived in the United States a decade ago aboard wooden shipping crates. Unless checked, the beetle threatens to bore its way through billions of dollars' worth of valuable timber, shade, and maple syrup trees.

Different continents, different species, different routes of introduction—but a common problem. Since China opened its doors to the West in the early 1980s, its burgeoning trade with the United States (see graph) has meant more marine organisms in ballast water and insects in packing crates accidentally transported across the Pacific in both directions. The influx has been supplemented by intentional introductions by commercial U.S. nurseries and horticultural collectors looking for exotic Chinese specimens. Many of these species flourish thanks to similar habitats and climates in the two countries. The result, says Li Bo, a plant ecologist at Shanghai's Fudan University, is that "American species can easily get established in China, and Chinese species can easily get established in America."

Many introduced species never expand beyond a beachhead, and most horticultural species are well behaved. But for reasons that are still being debated, some introductions lead to ecological disaster. This summer Chinese and U.S. scientists held two meetings* in hopes of sharing information and developing strategies that might help avert dire consequences from this two-way traffic. "There is a chance we could stop this wave of invasive species exchange between China and the United States" through stricter inspections and stiffer regulations, believes Peter Alpert, a plant ecologist at the University of Massachusetts, Amherst. But he says "there

Invaders. Arkansas rivers are filling with carp from China originally imported for aquafarming.

CREDITS (TOP TO BOTTOM): MANFRED KAGE/PETER ARNOLD INC.; USGS

*Beijing International Symposium on Biological Invasions, 8–15 June 2004. Biological Invasions: Species Exchanges between Eastern Asia and North America, Portland, Oregon, 2 August 2004, held in conjunction with the Ecological Society of America meeting.

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