Gastrointestinal Blues

Research finds bugs that inflame the human gut

By KATHLEEN FACKELMANN

A thriving colony of microscopic organisms lives in the gut of the average person. These bugs don't cause any trouble for most people, but they send some 1 million U.S. residents running to the bathroom in pain from a chronic, debilitating inflammation of the intestines. Researchers think they have now identified two specific microorganisms that trigger such disease.

Inflammatory bowel disease is a general term for ulcerative colitis and Crohn's disease. Ulcerative colitis is usually confined to the inner surface of the colon, whereas Crohn's disease can involve all layers of the entire gastrointestinal tract. Both can cause recurring attacks of diarrhea, severe abdominal pain, and weight loss. Drugs can quell the illness temporarily, but in severe cases, doctors must remove the diseased bowel.

Though the new research will not provide immediate relief for people with ulcerative colitis or Crohn's disease, it may ultimately lead to a better way to treat the diseases.

Joel D. Taurog, a rheumatologist at the University of Texas Southwestern Medical Center in Dallas, and his colleagues weren't thinking about colitis when they set out to genetically engineer a population of rats with arthritis, another inflammatory disease. They inserted into the animals a human gene, *HLA-B27,* that has been linked to the disease. The rats, as expected, developed arthritis.

They also developed a progressive inflammation of the intestines.

Taurog's team had serendipitously created rats with ulcerative colitis, thus giving scientists an animal model in which to study the human disease. They described their work in the Nov. 30, 1990 CELL.

Taurog and his colleagues went on to show that when they raised HLA-B27 rats in a sterile environment, the animals failed to develop arthritis or colitis. In contrast, HLA-B27 rats living in standard laboratory cages—and hence exposed to germs—did get the disorders.

Those results, published in the December 1994 JOURNAL OF EXPERIMENTAL MEDICINE, suggested a microbial link to ulcerative colitis.

But which microbes? The gut is home sweet home to trillions of bugs. How could scientists find the ones that aided in the assault?

Enter R. Balfour Sartor, a gastroenterologist at the University of North Carolina at Chapel Hill. Sartor and his colleagues teamed up with Taurog's group to find the answers.

The researchers again turned to the HLA-B27 rats. They decided to expose the rats that lived in a sterile environment to specific mixtures of bacteria.

"We carefully picked the cocktails to represent the most common bacteria in the normal intestine," Sartor told SCIENCE NEWS. They also included bacteria associated with gastrointestinal inflammation.

The researchers dosed one set of rats with a cocktail that represents the normal rat's intestinal flora. They exposed another group to certain bacteria taken from humans who suffered from intestinal inflammation. The third group of rats got that cocktail with an added ingredient—*Bacteroides*. These bacteria, which are common residents of the human intestines, produce a colitislike disease in certain guinea pigs.

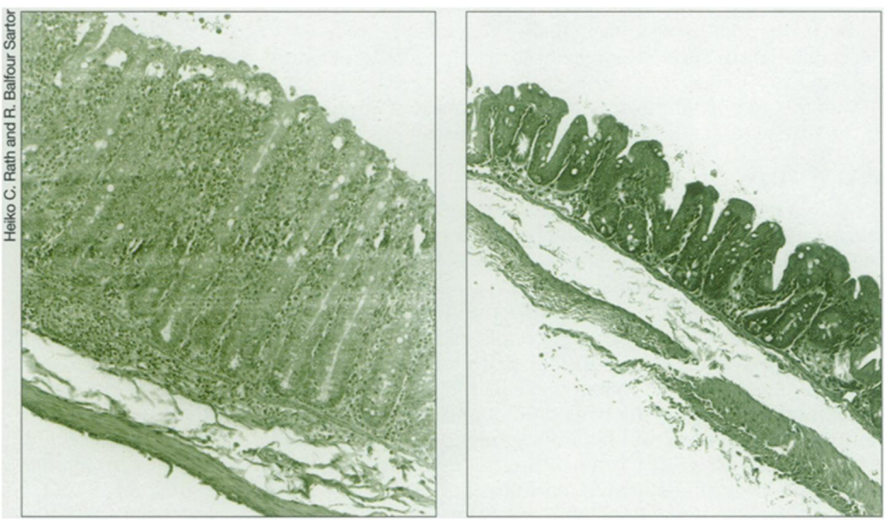
The rats given the cocktail containing normal intestinal flora got diarrhea. So did the rats given the human and guinea pig microbes. Both cocktails contained *Bacteroides.*

The one cocktail that did not have *Bacteroides* failed to produce significant inflammation in the rat gut. Indeed, rats that got this mixture had intestines that looked as healthy as the HLA-B27 rats kept in a germfree environment, Sartor notes.

"Our conclusion was that *Bacteroides* was important," Sartor says. "The *Bacteroides* had by far the most impressive ability to induce colitis." The team detailed its work in the August JOURNAL OF CLINICAL INVESTIGATION.

Even more important, when the researchers killed the infected rats and looked at intestinal tissue under a microscope, they saw inflammatory cells. Such cells are part of the immune system's response to infection.

This finding fit with the theory that colitis is an autoimmune disorder. Scientists know that people who inherit the *HLA-B27* gene run the risk of developing inflammatory diseases. Sartor speculates that in such people, white cells recognize *Bacteroides* in the gut. Their immune response leads to the production of natural chemicals that inflame the



Heiko C. Rath and R. Balfour Sartor

Left: A magnified section of intestinal tissue taken from an HLA-B27 rat living in a standard laboratory cage. The tissue is thickened and shows the invasion of immune cells. Right: In contrast, intestinal tissue taken from an HLA-B27 rat raised in a germfree environment shows no such thickening.

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intestines, he says.

Sartor stresses that *HLA-B27* is a normal gene. "This is not a mutation," he says, noting that about 4 percent of people carry the gene. *HLA* genes are known to regulate the immune system, but no one knows exactly how *HLA-B27* may create a vulnerability to colitis and arthritis, Taurog adds.

The HLA-B27 rats infected with *Bacteroides* in the most recent study did not develop arthritis. However, Sartor believes that joint swelling would have developed as the animals aged. Previous studies with these genetically engineered rats showed that they developed a gut inflammation first and then showed signs of arthritis.

"It's very exciting work," comments microbiologist Andrew D. Onderdonk of Harvard University.

Onderdonk and his colleagues had demonstrated in 1981 that a specific type of *Bacteroides*, the one used in Sartor's third cocktail, helped trigger a colitislike disease in some guinea pigs. Sartor's work is "putting the pieces of the puzzle together in a way that may allow for some novel therapeutic interventions that will be of value to patients," Onderdonk says.

Sartor and his colleagues have already started down the therapeutic pathway. In unpublished research, the team describes administering a *Bacteroides*-killing antibiotic to rats with inflamed intestines. They gave the rats daily doses of metronidazole for as long as 4 months. The treatment significantly calms the inflammation of the colon, Sartor told SCIENCE NEWS.

Whether an antibiotic treatment would work for humans with colitis remains unclear. A key question is whether *Bacteroides* would develop resistance to an antibiotic and thus foil any long-term attempt to quiet the disease.

Ultimately, Sartor believes, a two-pronged approach might work for colitis patients. He suggests giving a short-term dose of a *Bacteroides*-killing antibiotic. Then, after the *Bacteroides* has died, he would add to the intestine a harmless bacterium that occupies the same niche. If *Bacteroides* tried to muscle in again, the competing microbe would crowd it out. His team is now using *Lactobacillus*, a beneficial bacterium found in active yogurt cultures, to compete with *Bacteroides*.

This isn't the first time researchers have linked an inflammatory bowel disease to a microbe.

Previously, researchers had linked Crohn's disease to an organism called *Mycobacterium paratuberculosis*. This microbe (not to be confused with *M. tuberculosis*, which underlies infectious tuberculosis in humans) causes a disorder in cattle and sheep called Johne's disease. Infected livestock develop an inflammation of the intestines that looks remarkably like Crohn's disease.

In the late 1980s, doctors treated Crohn's disease patients with drugs aimed at mycobacterial infection, but the patients showed no improvement. After that, most doctors ruled out *M. paratuberculosis* as the cause of Crohn's disease.

So did Robert J. Greenstein, a researcher at the Veterans Affairs Medical Center in New York. When Dina Mishina, a postdoctoral student, approached Greenstein looking for a quick project in molecular biology, he suggested that she use a molecular technique to confirm that *M. paratuberculosis* does not cause Crohn's disease.

Mishina first obtained intestinal tissue from people with Crohn's disease. She used a technique called polymerase chain reaction (PCR) to copy a target piece of DNA and then searched for *M. paratuberculosis*.

It should have been a quick test, but it dragged on for months. Greenstein, thinking Mishina was having trouble, decided to shut the project down. "I was going to say, look, this experiment hasn't worked."

When Greenstein studied the results of Mishina's experiments, "it just blew my socks off."

What the data showed, contrary to the researchers' expectations, was a link between *M. paratuberculosis* and Crohn's disease. Mishina had tested tissue samples from a dozen patients—eight with Crohn's disease, two with colitis, and two with colon cancer.

She found *M. paratuberculosis* in all of the Crohn's and colitis samples. The tissues from the colon cancer patients showed no sign of this microbe.

The authors report the findings in the Sept. 3 PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES and offer a controversial explanation of why earlier studies did not show a link between *M. paratuberculosis* and inflammation.

Greenstein suggests that Crohn's disease comes in two forms.

One is the less severe, or indolent, form. The patient's main complaint is obstruction of the bowel. When people with this form of the disease eat anything but mashed foods, the chunks block the intestine.

Greenstein believes the indolent form of Crohn's disease results when the patient's immune system successfully fights an *M. paratuberculosis* infection. The infection starts with an inflammation but ends with a scarring of the intestinal tube that results in obstruction.

The other form of Crohn's is aggressive, leading to abnormal pockets in the intestine or an outright hole in the intestinal wall. This variety occurs when the immune system can't deter an *M. paratuberculosis* infection, Greenstein speculates.

Greenstein and his colleagues laid out the evidence for two forms of Crohn's disease in the Dec. 20, 1994 PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES. They showed that people with indolent Crohn's disease mount a stronger inflammatory response than people with the aggressive form. Although researchers believe that the immune system's response plays a key role in the development of Crohn's disease, they have not implicated *HLA-B27*.

If the aggressive form of Crohn's is due to a raging infection with *M. paratuberculosis*, antimicrobial drugs might be effective, Greenstein suggests. He points out that in previous studies, which did not distinguish between the forms of Crohn's, drugs showed no effect. While they may have benefited people with severe Crohn's, they would not have affected patients with the indolent form, whose infection had already been cleared, he says. Those patients might benefit from earlier therapy with anti-inflammatory drugs, a strategy that would prevent scar tissue from forming, he adds.

Reaction to this latest report linking *M. paratuberculosis* to Crohn's disease has been deeply skeptical.

"The evidence to date that *Mycobacteria* have anything to do with Crohn's disease is slim to nil," comments Lloyd Mayer, a microbiologist at Mount Sinai Medical Center in New York. To turn the skeptics into believers, Greenstein's theory needs more data, such as a large-scale study that examines the two forms of Crohn's disease separately.

Greenstein points out that researchers had been dubious about the link between the bacterium *Helicobactor pylori* and peptic ulcer disease. Now that link has been well established. Other groups have also published evidence of a microbial link to Crohn's disease. For example, investigators have shown that exposure to the measles virus may boost the risk of Crohn's.

Mayer has no doubt that a whole cast of microscopic organisms acts in this drama. It's very common, he says, to hear that both a husband and wife experienced diarrhea on a trip to Mexico but that only one went on to develop the recurrent symptoms of Crohn's disease.

"There may be multiple agents or organisms that start off the inflammatory process," Mayer says. "And then it's the genetics and the abnormal immune response that allow it to persist."

It may take years before such scientific investigations yield a practical solution to inflammatory bowel disease. Is it worth the wait?

Researchers like Sartor say it is. He points out that ulcerative colitis and Crohn's disease often strike people in the prime of life. Current drug treatment can only help temporarily. Sartor is looking for a lasting fix. □