Spring 1983

Stalking The Macrophage: [Dr. Zanvil A. Cohn]

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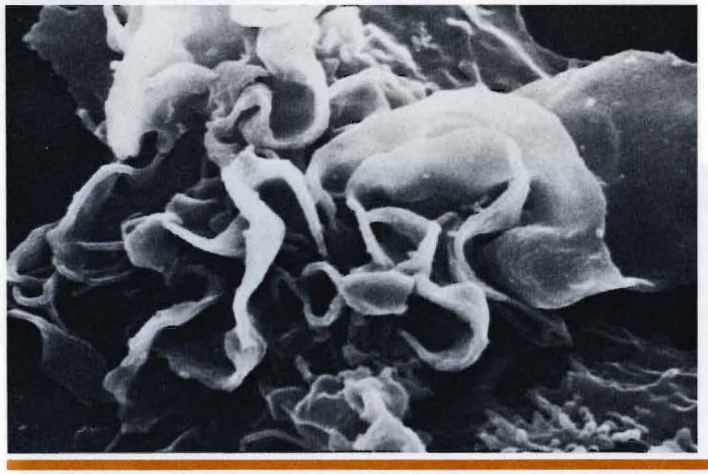


Macrophage

THE ROCKEFELLER UNIVERSITY

RESEARCH PROFILES

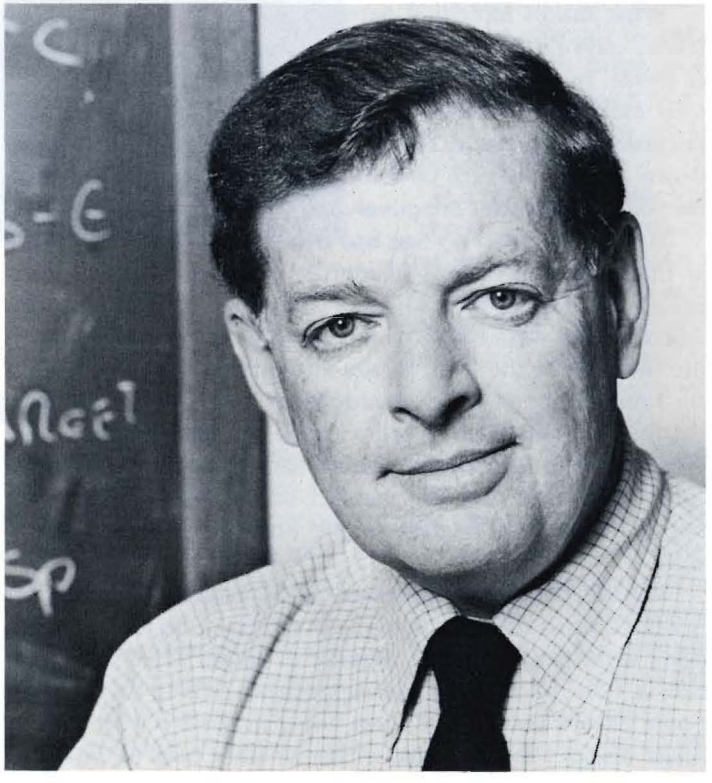
SPRING 1983

Stalking The Macrophage

This spring Professor Zanvil A. Cohn and members of his Rockefeller University laboratory flew to Brazil. They went to follow up clues, uncovered during an earlier trip, as to why certain cells of the immune system that should kill leprosy bacteria fail to do so. If the clues are correct, they may help strengthen some current ideas about the incompletely understood mechanisms of the body's immune defense. They have already provided a useful new tool for diagnosing leprosy.

Among Dr. Cohn's co-workers are scientists who, in addition to leprosy, have been studying Legionnaires' disease, Chagas' disease, leishmaniasis, and cancer. Central to their research is the macrophage, a white cell named for its large size and its most obvious activity—eating. The macrophage belongs to the cell-mediated immune system, a network that normally prevents hostile forces such as microbes and tumors from gaining a foothold in body tissue.

Immunologists are learning that sometimes macrophages ingest these pathogens but do not kill them as they should; instead, they provide the invaders with a sheltering nest in which to thrive and multiply. This phenomenon, which was observed in Legionnaires' disease by Marcus Horwitz in Dr. Cohn's lab, also occurs in tuberculosis and leprosy, as well as with protozoan parasites. Dr. Horwitz found that macrophages from human lung tissue, infected with the bacteria of



Zanvil Cohn

Samuel Silverstein

Legionnaires' disease, could multiply as much as 10,000-fold, causing the macrophages to burst within only three days. Samuel C. Silverstein, a member of the laboratory for many years, likened macrophages in this state to "Trojan horses."

In cell-mediated immunity, the white cells that are supposed to recognize and apprehend pathogens are called T lymphocytes. (This is analogous to the humoral immune system that operates in body fluids and tissues where B lymphocytes secrete antibody molecules. These lock into antigen "keyholes." Antigens are the molecules on the surface membrane of invading organisms against which antibodies are produced.) Some T cells are killer cells, while some summon macrophages. Some T cells help macrophages, and some, immunologists now realize, actually suppress immune responses.

"What makes leprosy a particularly interesting model for study," says Dr. Cohn, "is that it appears in a whole spectrum of forms. In the lepromatous form—the most virulent—bacteria multiply uninhibitedly, with the unfortunate results we normally associate with the disease. Then there are gradations down to the mildest or tuberculoid form, in which the immune system does effectively limit bacterial spread.

"For some reason no one had ever really looked very hard at the local skin lesions of leprosy patients. Diagnosis has usually been based on analysis of cells in the blood stream. So when we first went to Brazil, we decided that we would examine the cells in the skin. In the virulent lepromatous lesions we found bacteria-filled macrophages together with a large number of suppressor T cells. As we studied patients in the intermediate stages of the disease and on to the tuberculoid form, we found that the number of so-called helper cells began to increase relative to the number of suppressor T cells.

"We think that the suppressor cells produce a factor which, in effect, turns off the macrophage. Or else, the macrophages don't work because of the absence of helper cells. The question is, Can you suppress the suppressors or help the helpers? Some experiments we've been doing in the lab with cells of other diseases indicate that either or both may be possible, but we need to know a lot more about the bacteria and the immune cells before we can manipulate them clinically. In Brazil, where leprosy is widespread, new cases are admitted to hospitals all the time, which makes it possible to get cells for study before they are modified by treatment.

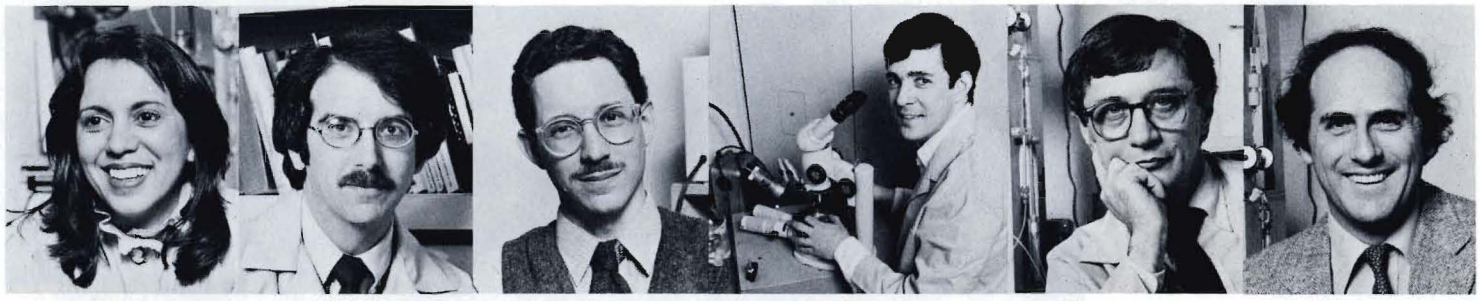
"What we can learn about these suppressor cells," Dr. Cohn explains, "is also of great interest because there's a fair amount of evidence in many animal models that suppressor cells modify the ability of a host to fight off tumors, and that if you can destroy the suppressor cells, you'll allow normal body defenses to come back and destroy the tumor."

CHARTING THE TERRITORY

Zanvil Cohn has been stalking white cells for most of his professional career. He grew up on Long Island, where he still lives, and where he and his wife and family relax by deep-sea fishing. He came to Rockefeller in 1958 after earning a medical degree *summa cum laude* at Harvard and serving a stint in uniform as head of a unit at the Walter Reed Army Institute for Research. While he was still in medical school, his interest in research had been spurred by a series of technological advances that were dramatically expanding the scope of cell biology. His interest in Rockefeller stemmed from the fact that many of the advances, in electron microscopy, cell fractionation, and immunology, were happening there.

Today, with scientists manipulating molecules and recombining genes, it's easy to lose sight of the fact that, until recently, the inner world of living cells was essentially uncharted territory. Less than twenty years ago, James Hirsch, formerly co-leader with Dr. Cohn of the laboratory of cellular physiology and immunology, was quoted as saying: "For the first time now, in the past decade, techniques have been developed that allow us to look inside the cell with some degree of precision and reliability. A whole new field has opened up and we have only scratched the surface."

Dr. Cohn and Dr. Hirsch both began their Rockefeller careers under the late René Dubos, whose microbiological



Nadia Nogueira *Marcus Horwitz* *Carl Nathan* *Jay Unkeless* *William Scott* *Ralph Steinman*

studies were critical to the development of antibiotics. In those days, the stunning success of the "wonder drugs" in fighting bacterial infection was accompanied by a lack of any real knowledge of how they worked. Dr. Cohn's first project in Dr. Dubos' lab, conducted with the late Steven Morse, was to ascertain that polymorph leukocytes, white-cell cousins of macrophages (although that was not known then), are the cells that kill the bacteria of "staph" infections.

Polymorphs are front-line defenders in the immune system. Like macrophages, they are phagocytes, cells that ingest their prey. A couple of years after coming to Rockefeller, Dr. Cohn collaborated with Dr. Hirsch to find out, among other things, whether the tiny granules that could be seen within polymorphs were, as was suspected, the instruments of microbe killing. Before they could learn that, however, they had to get the granules out of the cells intact, something no one had been able to do. After months of effort, Dr. Cohn found the solution. With the granules free, their content of toxic chemicals and digestive enzymes could be identified, establishing the granules as a type of lysosome or cellular "stomach." After microbes are internalized, the cell delivers its granule contents into the phagocytic vacuole—the killing ground.

Macrophages, which Dr. Cohn began exploring about twenty years ago, are bigger and more complicated than polymorphs. They live longer and make more chemical products, some fifty of which have been identified to date, many in the Cohn laboratory. They begin life in the bone marrow, deriving from the same precursor cells as polymorphs. As young cells, they are monocytes, named for their single nuclear shape, in contrast to the many different nuclear shapes of polymorphs. Monocytes circulate in the blood for a time before maturing into macrophages of various types, which take up residence in different body tissues. The phagocytic family tree was plotted a decade ago by Dr. Cohn with Dr. Ralph van Furth, now working in The Netherlands.

THE "THIRTY-MINUTE FRENZY": RECEPTORS AND "ZIPPERS"

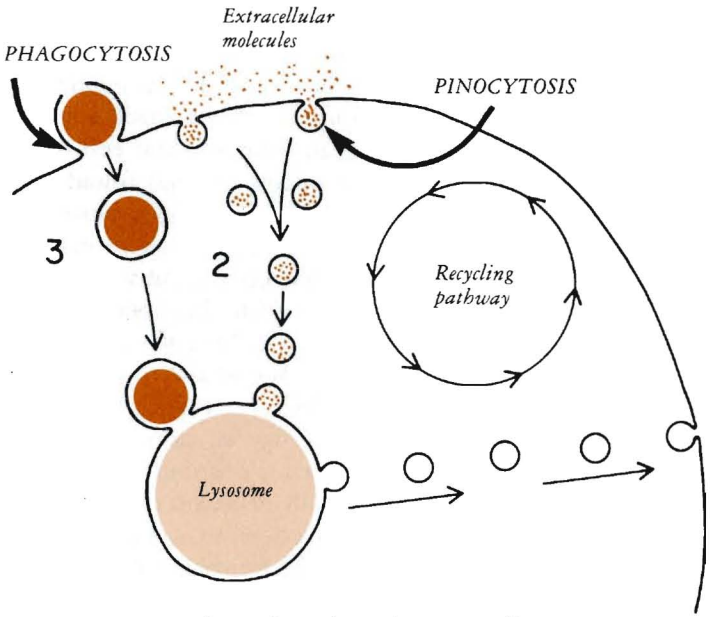
Macrophages are the most omnivorous of the phagocytes. In addition to eating pathogens, they eliminate aged body cells. In the human system this includes some 300 billion defunct red blood cells each day. Macrophages achieve this awesome intake by the process of endocytosis. When a portion of the cell's outer membrane comes in contact with a particle, the membrane surrounds the particle to form a sac, called a vacuole, that tucks into the cell. Small vacuoles are fluid filled and are part of cell drinking or pinocytosis, the ingesting of particles in solution. Large vacuoles enclose solid particles as big as whole cells. The vacuole then separates from the cell surface and fuses with the lysosome to initiate the process of intracellular digestion.

"During our studies of endocytosis," Dr. Cohn says, "we wanted to find out how much membrane flows into the cell, at what rate, and what happens to it. Ralph Steinman, who joined the lab in 1970, devised elegant methods for following the uptake of labeled enzymes by pinocytosis. As a result, we learned that the cell takes in its entire surface area, as small vesicles, every thirty-three minutes. Then we wondered whether the membrane is destroyed in the lysosome and new membrane synthesized, or whether it is recycled. We worked out a pretty neat trick for labeling only the inner membrane of the lysosome, based upon a procedure of a former student, Ann Hubbard, who's now on the faculty of Johns Hopkins. If the membrane was recycling, we knew the isotope would show up sooner or later on the surface of the cell. And within a period of five minutes that's exactly what happened."

The efficiency and speed of endocytosis helps to explain how the macrophage can envelop, in its "thirty-minute phagocytic frenzy" (as one researcher phrased it), a quantity of matter equal to half its own mass. But chaos would ensue if the macrophage had no means for discriminating what should or should not be devoured. Jay Unkeless, another member of the group, has been isolating from the cell membrane the receptor molecules, the "taste buds," through which a macrophage perceives that a particle is fit for consumption.

The receptor binds to microbes and cells that are coated with specific antibodies, which is the way the macrophage is able to recognize "foreignness." Receptors have been isolated from both mouse and human cells through the use of monoclonal antibodies, exquisitely precise reagents which zero in on specific molecules. It is hoped that through the use of recombinant DNA technology enough receptor will be produced to allow the determination of its structure.

Once the prey is attached to the macrophage's surface-membrane receptors, it is engulfed by a "zipper mechanism," a process discovered by Samuel Silverstein. Antigens on the prey continually combine with phagocyte receptors, resulting in a flow of membrane around the particle. When the prey is completely surrounded by host-cell membrane, fusion occurs and the phagocytic vacuole is formed.

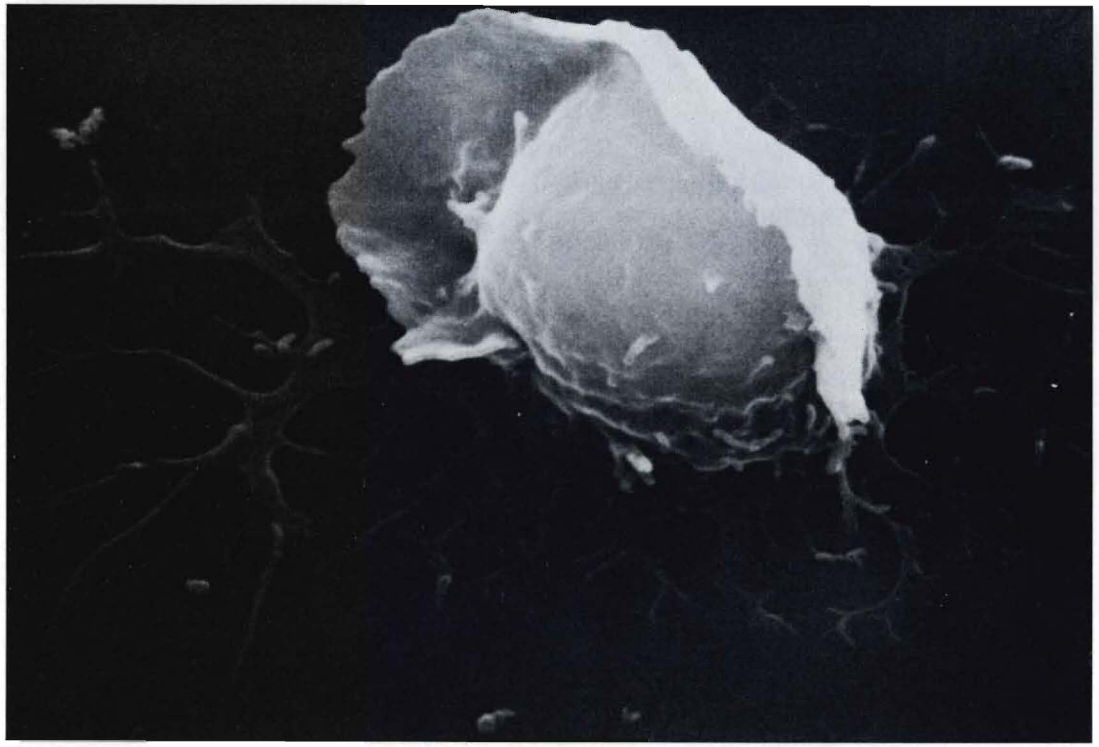


Endocytosis and membrane recycling

NATURE'S ADJUVANTS

While examining the spleen macrophages of mice, Dr. Steinman and Dr. Cohn discovered an entirely new class of immune cells. "These represented a tiny population and because of their branched appearance we named them dendritic cells," Dr. Cohn explains. "After a number of years of work, Ralph and his group have been able to isolate and characterize these cells in both animals and man. Of great interest is their unique ability to stimulate the cell division of lymphocytes. In other words they are accessory cells— 'adjuvants,' Ralph calls them—needed to initiate an immune response. Since they are the most potent stimulators of transplantation reactions, we think it likely that they play an important role in graft rejection."

Zanvil Cohn and Ralph Steinman



Dendritic cell

SHARPSHOOTERS: TO PROTECT OR DESTROY?

A major immunological finding, which has come primarily from work in Dr. Cohn's laboratory, is that macrophages are not just eaters. In addition to the chemicals they make to kill and degrade the cells they ingest, they also secrete many molecules into their surrounding environment, affecting the activity of other cells.

"This is an important part of the inflammatory process, which may lead either to wound healing and tissue repair or to *destruction* of tissues," says Dr. Cohn. "Among the secreted chemicals are those that stimulate the formation of blood cells and blood vessels; pyrogens, the fever-producing substances; and enzymes involved in lipoprotein metabolism. A delicate balance exists in the amount and nature of the secretory products. When present in excessive amounts disease states such as rheumatoid arthritis, glomerulonephritis, and even atherosclerosis may be potentiated.

"We've also learned that macrophage membrane is one of the richest sources in the body of arachidonic acid and its metabolites. William Scott and Nicholas Pawlowski have been studying arachidonic acid metabolites, the two main classes of which are the leukotrienes and the prostaglandins. Both have profound effects on the inflammatory process. Some of these macrophage products may directly influence the smooth muscles of lung and blood vessels. Some stimulate the production of prostaglandins by other cells leading to a 'metabolite cascade.' One of the leukotrienes they've isolated acts strongly to attract circulating polymorphs and monocytes to the lungs in response to infections and tumors. Obviously, knowing how to control the production of these potent mediators of inflammation will have clinical applications.

"Of the macrophage's cell-killing chemicals," he continues, "the group called toxic oxygen intermediates attack a whole variety of targets both inside and outside the cell. Carl Nathan, who was trained as an oncologist, has found that macrophages secrete large amounts of hydrogen peroxide, which kills tumors. But tumors, like microbes, vary in their susceptibility. Some, as we've learned, contain substances that block the effect of the hydrogen peroxide. In experiments in the test tube and with mice, we've been able to turn off the blocker synthesis in the tumor cells. We've also tried increasing the macrophage's hydrogen peroxide production. This is an area where interferon may one day be clinically useful, specifically gamma interferon, which is a strong macrophage stimulator." (Interferons are small proteins produced by virus-infected and immune cells as a defense mechanism. Scientists are now able to synthesize interferons using recombinant DNA technology.)

"The problem when dealing with human subjects is one of

Members of the Cohn lab on the first trip to Brazil. From left: Bradley Arrick, Gilla Kaplan, Wesley Van Voorhis, and Marcus Horwitz.

designing means to deliver chemicals safely and to specific target tissues within the body. This is one of the areas that Carl and others in the lab are working on."

"IT JUST SORT OF HAPPENED..."

For Nadia Nogueira, the trip to Brazil was a trip home. Like a number of others in Dr. Cohn's group, she came to the University as a Ph.D. student. Also like many of the group, she is a physician. She came because of her deepening frustration with trying to be a doctor in a part of the world where most of the diseases people suffer from cannot be cured, prevented, or, often, even treated. In Latin America, Chagas' disease afflicts 50 million people and can cause fatal heart damage. It is a parasitic disease, as is leishmaniasis, which is also rampant in Latin America and, in slightly different forms, in Africa, Asia, and the Middle East.

Dr. Nogueira can pinpoint the moment she was finally "pushed into research," as she says. "In the hospital in Rio where I was doing my internship, there were hundreds of patients with leishmaniasis. In its invasive form, it's a horrible, deforming disease, and there was nothing we could do for these patients. There had been a drug that worked reasonably well to contain the progression of the disease, but it had been taken off the market. It wasn't profitable. Later, it was again made available through governmental production, but by then I had already determined that we must have vaccines."

When she first came to the United States, she found that tropical medicine interested few American scientists. "When I told people that I wanted to work on Chagas' disease, they would say to me 'work on cancer.' Well, most people in my country rarely live long enough to die of cancer. There were suggestions in the clinical literature that macrophages were involved in Chagas' disease, so I went to Zan and asked if I could do my thesis in his lab. He asked me how I planned to go about it and how I would handle the problem of the high infectivity of the parasites. I told him, and he said, 'Okay, why don't you try it.' Then he gave me the ideal combination of total freedom to work and total availability when I needed help. His insight is unfailing. How did the leprosy project start? It just sort of happened naturally, I guess, after about a million conversations about macrophages and how they act."

For ten years, Dr. Nogueira has been exploring the interaction of macrophage and parasites, first in Chagas' disease and more recently in leishmaniasis. Lately, with the help of Dr. Unkeless, an expert in monoclonal antibody technology, and cell biologist Paul Lizardi, a member of another Rockefeller laboratory, she has identified the major surface antigen of these parasites and started cloning the genes coding for them. Vaccines may not be very far away. Dr. Cohn's support of young scientists is reflected in the large number of them in his laboratory and in his stewardship, for many years, of the University's joint MD.-Ph.D. program with Cornell University Medical College, which also reflects his commitment to clinical medicine.

One of the students currently in his group and in the MD.-Ph.D. program is Wesley Van Voorhis, who made the major contribution to the findings about leprosy gathered on the first trip to Brazil. Mr. Van Voorhis didn't go on the second trip. He stayed home to finish his thesis, on human dendritic cells.

RESEARCH PROFILES is published four times a year by The Rockefeller University. It is written and edited by Fulvio Bardossi and Judith N. Schwartz. This is issue Number 12, Spring 1983. Inquiries should be addressed to the University's Public Information Office, 1230 York Avenue, New York 10021, or phone (212) 570-8967. Photographs, Ingbert Grüttner pages 1 (bottom right), 2, 3, and 4; Gilla Kaplan pages 1 (top left) and 5. Designed by Angelica Design Group, Ltd.

Printed in the United States of America.