A century after they were first described, macrophages and the questions they pose are being revealed in their complexity.
It was exactly a century ago that the Russian biologist Elie Metchnikoff identified phagocytes, scavenger cells in the bloodstream. His “big eaters” were long the objects of disdain. Even after he became a Nobel laureate, he was vilified for suggesting that these white corpuscles, including the large, potent microbe-eaters he called macrophages, were important to immunity.

In 1906, two years before Metchnikoff’s Nobel prize, George Bernard Shaw made him and his theory the butt of a satire, The Doctor’s Dilemma. “We must stimulate the phagocytes,” insists Shaw’s bumbling physician, Sir Ralph Bloomfield Bonington. When Bonington’s ministrations kill his patient, the doctor wonders, “Have we over-stimulated the phagocytes?” Nonetheless there have always been some scientists who have insisted on studying macrophages, and in recent years their persistence has been paying off. A new understanding of macrophages has grown in tandem with other advances in cellular and molecular biology, and it reveals macrophages to be far more potent and versatile than even Metchnikoff had imagined. Macrophages, says one scientist in the field, are the rediscovered cells of the 1980s.

The scavengers among white blood cells have been divided into two classes, the neutrophils and the mononuclear phagocytes. Neutrophils are kamikaze cells; they die in the process of killing. The mononuclear phagocytes, or monocytes, are much less numerous, being about 10 percent as abundant as neutrophils in the circulating blood. But monocytes mature as macrophages, and a macrophage can live for months, routinely filtering the blood for unwanted particles all the while.

**Complex roles**

Life for the macrophage begins in the bone marrow. There precursor cell matures and is released into the blood as a monocyte, which circulates briefly and then settles in tissue to become a resident macrophage. Macrophages are everywhere. They are in the liver, the lungs, the peritoneum lining the abdominal cavity, the reproductive tract, and even the retina.

Whenever there is inflammation, a rapid influx of new monocytes begins and local, resident macrophages multiply. It is still not clear which of these cells, visitors or residents, become legions of killers devouring pathogens or anything out of the ordinary in the region of inflammation. If the irritation persists, the macrophages may collect in lumps, known as granulomas, which are the disfiguring nodules of leprosy and other diseases.

In their usual routine, most monocytes and macrophages are sanitation engineers. In the course of a day, they clear away 300 billion aging red blood cells inside any healthy human, and they do away with untold bacteria that (probably for that very reason) are unimportant in human disease. Macrophages are also associated with many major diseases: arthritis, atherosclerosis, allergies, emphysema, cancer, and perhaps even such neuromuscular wasting diseases as multiple sclerosis. Do they cause damage in these diseases, or is their role to help to clear away wreckage and rebuild normal tissue? The answer is seldom clear because both roles, in different situations, belong to the mononuclear phagocytes.

More than just killers and eaters, macrophages are producers; they secrete more biologically important substances than any other type of body cell outside the liver. Besides being the likeliest source of pyrogens, which cause fever, macrophages now are known to be sources of more than 50 substances. Among these substances are the molecules that provoke asthma and those that contribute to the clotting and de-clotting of blood.

What stimulates these cells? What does stimulation mean, and what are its consequences? How does a macrophage receive and process the signals that evoke any one of its many capacities? These questions are still current a century after Metchnikoff posed them.

Metchnikoff tirelessly called the “mobile cells” into action by inserting rose thorns or bacterial injections into a starfish larvae, water fleas, and a host of other primitive organisms. “In all cases where the organism enjoys an immunity,” he wrote, “the introduction of infectious microbes is followed by an accumulation of mobile cells, white blood corpuscles in particular, which incorporate the microbes and destroy them.”

Metchnikoff’s contemporaries were certain that macrophages were involved in spreading infection, not attacking it. Most immunologists then took for granted that all immunity was what they called humoral. Derived from the ancient physiology of Galen, this term had come to mean soluble, or blood-borne. German bacteriologist Paul Ehrlich had demonstrated the humoral, or soluble, presence in the blood of molecules called antibodies. These antibodies recognized and locked selectively onto toxins or foreign substances called antigens. That, the immunologists declared, was how infection is fought. In contrast the image of a generalized purge directed by the immunological equivalent of maggots was viewed as not only odd but ugly. (The Nobel committee prudently sidestepped the dispute; Ehrlich and Metchnikoff shared the Nobel Prize in physiology and medicine in 1908.)

“The whole era of humoral immunity took central stage for years, and any other kind of immunity was not recognized or appreciated,” says George Mackaness, a

---

**Macrophage activity.** Macrophage-antibody binding through the constant-region receptor (left), and an activated, spread macrophage. Trypanosoma cruzi, the organism of trypanosomiasis, is captured for killing.
pioneer of macrophage research, which he calls “a field without literature until 1948.”

Nor was there much being said about macrophages in 1958, when a young physician named Zanvil Cohn arrived at Rockefeller University, the seat of American cell biology at that time. Like many other macrophage researchers who began as doctors curious about the mechanisms of infection, Cohn wanted to understand more about phagocytes. Why did they eat some pathogens but not others? Why did they fail to respond in certain patients?

“Nobody had taken the time to separate the cells or characterize them,” Cohn recalls. “The life history of the monocyte was not known, nor its origin in the bone marrow.”

Today many scientists credit Zanvil Cohn with almost singlehandedly shaping the amorphous study of macrophages into a modern science. Cohn demurs: “I was in the environment of Rockefeller University. The atmosphere was stimulating, and you felt you had to do something interesting.” Whatever the reason, Rockefeller became the cradle of macrophage research, where anyone with a serious interest in the field had to work, if only on sabbatical.

Hooked on macrophages

A year after Cohn arrived at Rockefeller, a young Australian immunologist arrived to refresh his acquaintance with the macrophage. George Mackaness had become hooked on macrophages while at Oxford University several years earlier. He had been studying tuberculosis, an enigma that humoral immunity could not resolve. Scientists could not culture antibodies that would transfer resistance to the disease from one person to another, though macrophages did seem to be involved in resistance; when, in the skin test for tuberculosis, a tubercle bacillus was injected into the skin of someone already infected, the area became inflamed and tender, and macrophages were abundant.

Animal studies done by Mackaness and others, showed that these macrophages were not all the same. Macrophages taken from susceptible animals seemed to ignore the tubercle bacillus. Those from resistant animals, however, were unguided weapons; after an injection of the bacillus they would kill almost any bacterium, including the one that caused tuberculosis. This response was called delayed hypersensitivity. It was somehow related to immunity, and it was a mystery to the researchers.

“There was something screwy about this,” recalls Mackaness, who is now president of the Squibb Institute for Medical Research. “These cells were never involved in the immune response except as effectors. How could you link that to the immune response? There had to be something very specific going on—and yet clearly the response was not specific.”

Today some of his colleagues speak almost eulogistically of Mackaness, seeing him as the one person who had been willing to roll up his sleeves and tackle an untidy problem that was bothering everybody but attracting nobody. The problem was the way infection with a single kind of pathogen could lead to this angry, nonspecific barrage of scavenger cell. Attacking it required Mackaness to return to using whole animals, which seemed like drudgery to most cell biologists.

Mackaness re-read everything he could find about phagocytes, from Metchnikoff forward. To speed his experiments, he embarked on a long search for a bacterium that would grow faster than the tubercle bacillus. Ultimately he wound up working with an esoteric bug called Listeria, which causes the livestock diseases known as staggers and circling. Though relatively unimportant to humans, Listeria, like the tubercle bacillus, could live inside macrophages, was undaunted by antibodies, and provoked a strong delayed hypersensitivity.

Mackaness found first that, as is the case with tuberculosis, antibodies from Listeria-resistant rabbits could not transfer resistance. He went on to find, though, that if he extracted immune cells called lymphocytes from the spleens of resistant rabbits and injected these into rabbits that had never encountered Listeria, there was an immediate response from the macrophages. All the signs of delayed hypersensitivity appeared. The injected rabbits behaved as though Listeria were an old and familiar enemy.

In cell culture, the rabbit macrophages quickly set about to kill Listeria as well as Salmonella, which causes food poisoning, and Brucella, which causes infectious abortions in livestock. Using a clearly specific immunological signal, Mackaness

Wingerson is a New York-based science writer and a correspondent for New Scientist.
had brought forth a population of voracious macrophages. "They weren't normal; normal ones would support Listeria and allow it to kill them," he says. "These were very, very active, and they chewed the heck out of things. I didn't know what to call them." The term he finally chose was "activated macrophages."

The discovery of this cellular immunity had implications in several fields, including transplantation and cancer research. It led to a rush of interest in macrophages in the late 1960s. Before long a number of teams had shown that a substance taken from the broth of cell cultures of lymphocytes that had been exposed to antigens worked just as well as whole bacteria in activating macrophages. This substance was named lymphokine.

The most unexpected and provocative offshoot of all this research was a 1970 report from John Hibbs and Jack Remington at Stanford University. They had found that activated macrophages would also kill tumor cells, spontaneously and specifically, while leaving normal tissue unharmed. No one had anticipated this effect (except Metchnikoff, who had predicted it without supporting evidence). The term "activated" took on a new significance.

Obviously macrophages can no more destroy all cancer cells than they can resolve all bacterial infections. People suffer and die from tuberculosis and cancer, despite the presence of protector cells. Just as some bacteria can escape the action of macrophages, tumor cells have defenses of their own.

For instance Ralph Snyderman at Duke University Medical Center discovered that both mouse and human tumors produce proteins that inhibit the substances that normally summon macrophages to a site of inflammation. Other research has shown that tumors can also prevent macrophages from producing lethal, oxygen-derived poisons.

Nonetheless the activated macrophage may be a logical candidate to send after metastases, the few traveling cancer cells that can evade surgery and other treatments and cause fatal secondary tumors. Joshua Fidler of the M. D. Anderson Tumor Institute in Houston has worked to turn mouse macrophages against metastases by feeding them liposomes, small envelopes of lipid, or fat, that contain chemical triggers of activation.

**Traveling factories**

Macrophages do not swallow tumor cells as they do bacteria. They simply kill them, which have a wide range of effects on cells in the blood, and interleukin 1, a molecular signal to lymphocytes and other cells in the liver, joints, and brain. The macrophages are also major producers of the leukotrienes important in asthma. This implies that besides causing late delayed hypersensitivity, macrophages probably participate in very early inflammation. (See "Leukotrienes and the Teams That Tamed Them," *Mosaic*, Volume 14, Number 1.)

Normally substances from macrophages get further credit for reconstruction of damaged tissue. Out of control, however, these molecules can be devastating. Gordon showed this when he found that macrophage proteinases can destroy the fatty myelin sheaths that surround and protect nerve cells. Such inflammatory demyelination is characteristic of such serious neuromuscular diseases as multiple sclerosis.

**Provoking substances**

To researchers studying the macrophage in cell culture, which is far removed from real animals and real delayed hypersensitivity, the nagging problem today is to define what provokes activation and its panoply of responses. There is no doubt that the macrophage is remodeled after activation. Normally the cell's membrane is ruffled, wavy, and in constant flux, turning itself inside out and back again every half hour. The activated macrophage, however, is smooth and flat. Some researchers familiar with the activated cell often say it looks angry. Its membrane recycling accelerates and its oxygen consumption increases. To Mackaness activated cells appeared "bigger, better, and faster," doing their usual thing with unusual vigor and seemingly determined to engulf and devour the coverslip before he could focus his microscope.

"Nobody agrees what all the things that should be measured are," says Paul Edelson, a pediatrician studying macrophages at New York Hospital-Cornell Medical Center. "Everyone has his own pet meaning for activation." As Page Morahan, a macrophage researcher at the Medical College of Pennsylvania, puts it, "One paper says the activated macrophage does such-and-such; another says it does ABC. You'd tear your hair out trying to figure out what cell it was talking about."

Even the definition of activation itself is in dispute, with a question of experimental technique at its heart. The technique, called sterile inflammation, is a way to harvest vigorous, spreading macrophages. It involves injecting a sterile culture
medium, thioglycollate, into a mouse's peritoneum and collecting the responding macrophages. For a time this nonbacterial inflammation was a popular shortcut to what appeared to be activation. It remained popular until it became clear that macrophages derived this way may be activated by some criteria, but they do not kill other cells.

Seeking signals

In what sense then are the cells activated? Purists still reserve the term for the effect noticed by Mackaness and Hibbs: broadscale killing. Many others would agree with what Zanvil Cohn said in the Journal of Immunology in 1978: "One can begin to consider as activated properties those . . . that exceed the baseline values exhibited by the resident unstimulated peritoneal macrophage." Cohn relates this particularly to secretory patterns.

The place to begin to understand activation from the macrophage's point of view is at the membrane, the envelope through which the cell receives signals and first encounters what it will engulf, attack, or ignore. Macrophage specialists hope to discover some change in this membrane, some marker that will tell conclusively whether macrophages in culture are activated in the same way as they are in nature.

The search has been greatly abetted by the creation of monoclonal antibodies, biological probes tailored to recognize an antigen of a scientist's choice. These antibodies are made in vast numbers by strange, single-purpose hybrids formed by fusing tumor cells and antibody-making cells taken from animals immunized to the antigen under study. For macrophage research, pieces of macrophage membrane are used as the antigen. (See "Putting Antibodies to Work," in Mosaic, Volume 12, Number 1.)

Siamon Gordon, who is now at the Sir William Dunn School of Pathology in Oxford, has created an especially broad collection of monoclonal antibodies that latch on to macrophages. They have helped him to discover, among others things, a membrane protein unique to these cells. He calls this protein F4/80, and he uses it as a sort of macrophage identification tag.

Protein F4/80, though ubiquitous, has no known importance to the activities of macrophages. Other antigens are being found, however, that give useful clues to the biological events of activation. For instance one class of molecule prominent on the surface of bacteria is glycoprotein, and the macrophage surface is dotted with receptor molecules specific for particular types of glycoproteins. The union of the two molecules stimulates the macrophage to engulf its receptor and thereby the bacterium itself. Activated macrophages have fewer of these receptors, although they do show a threefold increase in a cell-surface protein called la; the immune system uses la to distinguish self from nonself. The plasma membrane of the macrophage is altered profoundly but selectively.

The message Gordon derives from those revelations is that activated macrophages are less interested in eating; they seek instead to "influence their environment by secreting enzymes and poisons."

Activation also changes one of the most celebrated membrane components in biology, a molecule called 5' nucleotidase, which is present on nearly every known cell. The molecule is called an ectoenzyme, because its active portion sits outside the cell. There it lops phosphate groups off nucleotide molecules, which are subunits of DNA.

Paul Edelson became intrigued by the absence of this ectoenzyme on the surface of macrophages from mice injected with the sterile-culture medium thioglycollate. He realized that the absence of this common marker for membranes might itself be a way to identify activation. He found that resident macrophages from mouse spleens, livers, and lungs all bore the ectoenzyme, but that activated cells did not.

"What intrigued us was that the pattern of expression seemed to be inversely correlated with the capacity to enter the cell cycle," Edelson says. Activated macrophages can be triggered to divide, but resident cells cannot. The absence of the enzyme reflects this change.

Probably the best-understood feature of the macrophage membrane is its receptor for antibodies. Even George Bernard Shaw knew that macrophages prefer to eat bacteria "buttered," as he put it, with antibodies. The antibody receptor itself was not isolated until the late 1970s, but its workings are now understood in detail, mostly because of research by Jay Unkeless at Rockefeller.

Cells enraged

When a macrophage grabs an antibody, it takes it by the tail, which forms the base of the antibody's Y shape. This tail is invariant from one antibody to another of the same class or subclass and is therefore called the constant region. The two arms of the Y, though, occur in the tremendous variety of forms needed to recognize the limitless number of different antigens in nature; the arms are called the variable region. Through its constant-region receptor, a macrophage can capture any antibody of a subclass, regardless of what the antibody is carrying with its arms. (See "Betaγ: There at the Beginning," in this issue of Mosaic, and "Gene Segments on the Move," Mosaic, Volume 12, Number 1.)

The binding of an antibody to a macrophage's constant-region receptor quite literally pulls a trigger. It causes ingestion and also an angry respiratory burst, which is one of the most remarkable events of activation.

Carl Nathan, also at Rockefeller, has spent years scrutinizing the metabolic explosion that follows the pulling of the trigger. During the explosion the macrophage's oxygen uptake swells, feeding a side reaction of glucose oxidation. In muscle
cells, the object of glucose oxidation is energy. Here it is invoked to create potent cellular poisons, such as the oxygen by-products hydrogen peroxide, superoxide, and hydroxyl radical. These volatile molecules can disrupt cells literally by ripping electrons out of their membranes. Lacking a good assay, Nathan could not establish until 1976 that oxygen intermediates indeed participate in killing by macrophages. It is now clear, though, that these molecules do destroy bacteria and tumor cells.

Clearly, however, macrophages can act independently of antibodies, as Metchnikoff knew. They have been found to have a backup system that works by way of the receptor for one component of a set of substances, collectively called the complement, which are important to humoral immunity. The complement receptor binds particles, but it does not trigger ingestion.

This characteristic made the receptor a useful tool for Frank Griffin of the University of Alabama and Celso Bianco of the New York Blood Center in their study of phagocytosis, the process of ingestion. (The work was done in collaboration with Sam Silverstein of Rockefeller.) They discovered that macrophages derived with thioglycollate could phagocytize under certain circumstances. The cells did this not through the usual pathways, but through their receptors for the complement component called C3.

"This was not very useful to us," Griffin recalls. "It said that under some conditions, macrophages would phagocytize through their C3 receptors, but it left zillions of questions unanswered. Was it through a different differentiation pathway? What were we doing with the thioglycollate? We hoped to identify techniques by which we could alter C3-receptor function—turn things off and on in vitro so that we could dissect the mechanisms responsible for this activation of a receptor function."

Griffin later discovered a lymphokine that could stimulate macrophages to ingest something that linked up with a C3 receptor. There were conditions, however. The macrophage already had to be in direct contact with lymphocytes as well as with an antibody by way of a constant-region receptor somewhere in the macrophage plasma membrane. These conditions, Griffin realized, are very similar to those in sites of inflammation, where the antibody receptors that normally trigger ingestion may all become occupied. "All the constant-region receptors may quickly be used up," Griffin says. "The lymphokine may then be a mechanism for ingestion through the..."
C3 receptor—and ingestion of debris may trigger this pathway."

But what does the lymphokine do? The answer relates to another peculiarity of the C3 receptor: It is one of the few membrane proteins known to be fixed, and not free to migrate around the cell surface. Freedom of movement might be essential to ingestion through a receptor, Griffin reasoned, and this might be what the lymphokine conferred on the C3 receptor.

To learn whether he was right, Griffin borrowed a technique from Sam Silverstein and Josef Michl of Rockefeller: He coated a glass coverslip with ligands to the C3 receptor. These ligands are substances that would bind to the receptors and thereby trap the macrophages to the coverslip. Griffin found that even in the presence of the lymphokine, the macrophages bound to the coverslips lost their ability to bind and ingest red blood cells through their C3 receptors.

This finding implied that treatment with lymphokine had made the C3 receptors mobile within the plasma membrane and free to travel toward the coverslip, where they were trapped and useless for phagocytosis. Added to evidence from other laboratories, Griffin's result suggests that mobility within the plasma membrane must be vital to a receptor's phagocytic function. Without mobility a receptor may be unable to transmit a signal to the inside of the cell that alerts its internal phagocytic "machinery."

Griffin's macrophages had been activated, as it were, by thioglycollate. Researchers differ about whether change in the C3 receptor also occurs after classical activation. If it does not, Griffin thinks, his finding is further evidence that there are subsets of activated cells. Furthermore it is one of the increasing indications that whatever disputes macrophage and antibody immunity have produced in science, they do cooperate in nature.

**Steps to activation**

The same is true of tumor killing. Carl Nathan has found that activated macrophages can kill tumor cells with oxygen intermediates provoked by antibodies. It also seems that the activated macrophages can do the same, though more slowly.

---

_{Some players. Clockwise, from top: Nadia Nogueira, Carl Nathan and William Scott at Rockefeller; Page Morahan at the Medical College of Pennsylvania; Zanvill Cohn (left) and Ralph Steinman at Rockefeller; Frank Griffin at Alabama._}
Dolph Adams and his team at Duke University have been analyzing the kind of tumor killing that Hibbs and Remington noticed among macrophages. Watching time-lapse microscopic films, Adams has seen the languid dance of macrophages as they cluster and spread over tumor cells, apparently probing the surface and creating, he believes, pockets in which poisons can be trapped. After six hours, the tumor cells stop moving and dividing; then they swell, blister, and explode.

The Duke researchers have found that when antibodies that might trip the constant-region receptor are absent, activated macrophages do two things: First they capture the tumor cells by actively binding these targets to their surface. Second they release an enzyme that destroys membranes of tumor cells but not those of normal cells. Activation of macrophages for this process appears to happen in two steps: Lymphokines seem to stimulate ability for binding; to release the enzymes, though, the lymphokine-treated macrophage must also touch a tumor cell (or a lipopolysaccharide, a component of bacterial membranes).

This and other evidence makes Adams certain that activation is a complex series of events. Held in culture for more than two days or irritated by thioglycollate, macrophages are responsive to later stimulation, he says. They appear to be interested but not yet committed. It would take a big shove, such as large amounts of a bacterial stimulant called endotoxin, Adams declares, to get these cells fighting. Give them a lymphokine, though, or show them tuberculosis vaccine inside a live mouse, and they are primed for action. After that only one more poke, such as a little lipopolysaccharide or more lymphokine, and the primed macrophage becomes a killer.

Full circle

Not everyone subscribes to Adams' particular curriculum for the militarization of macrophages, but many researchers do endorse his general view of the matter. His view is that activation is an intricate process that has appeared nonspecific mostly because the tools used to study it were, until now, so primitive. "I think in most of our experimental systems, we deal with very crude signals," he says. "We set up a very abnormal situation. My model is that there are many things macrophages can do, depending on what signal they get. I would argue that when we define these things, every complex function will be specified by a finite list of capacities." Genetic engineering has been speeding progress toward that goal in a way no one dreamt of a decade ago. The term "lymphokine" then referred generally to the supernatant taken off antigen-stimulated lymphocytes. Purifying a lymphokine threatened to take decades more. Ten years ago the molecular weight and rough nature of a lymphokine were known but, says Nathan, "the assays were so difficult and the quantities so tiny that no one was able to say exactly what it was."

Today scientists have had the chance to test a battery of pure lymphocyte products, manufactured in bacteria working on orders from inserted human genes, for their activity as lymphokines. Such studies have persuaded the Rockefeller team that gamma interferon, a form of the antiviral agent interferon made only by lymphocytes, is the molecule that activates macrophages. "We've developed enough tools now to begin studying these cells in man," says Cohn. "That's what we've been working for." In his laboratory, then, macrophage science has come full circle, to the point of being applied to those human diseases in which macrophages are invaded and killed, rather than themselves engulfing and killing.

Leprosy is such a disease. In its most severe form, according to studies by Cohn and colleague Nadia Nogueira, people with the disease have lymphoid cells totally unable to produce gamma interferon. Not signaled that they are under attack, the macrophages are unable to act, and the leprosy parasite can colonize them.

Nogueira is determined to apply this knowledge to the disease, which is still prevalent in her native Brazil. There are also about 300 leprosy patients in New York City, some under treatment at Rockefeller University Hospital, and Cohn and his colleagues expect to be able to inject some of them with genetically engineered gamma interferon. They hope thereby to arouse the patients' uninformed macrophages to the challenge.

Not even the Rockefeller team, however, pretends that the gamma interferon finding will be the last word in macrophage activation. "The tidal wave hitting the literature is that this is the macrophage-activating factor," says Nathan, "but the undertow, as yet unpublished, says there's another one too." This condition is likely to mark much of macrophage research for some time to come.