Mighty Macrophages • Plasmas • Solidifying Molten Metals • Tree Lignin

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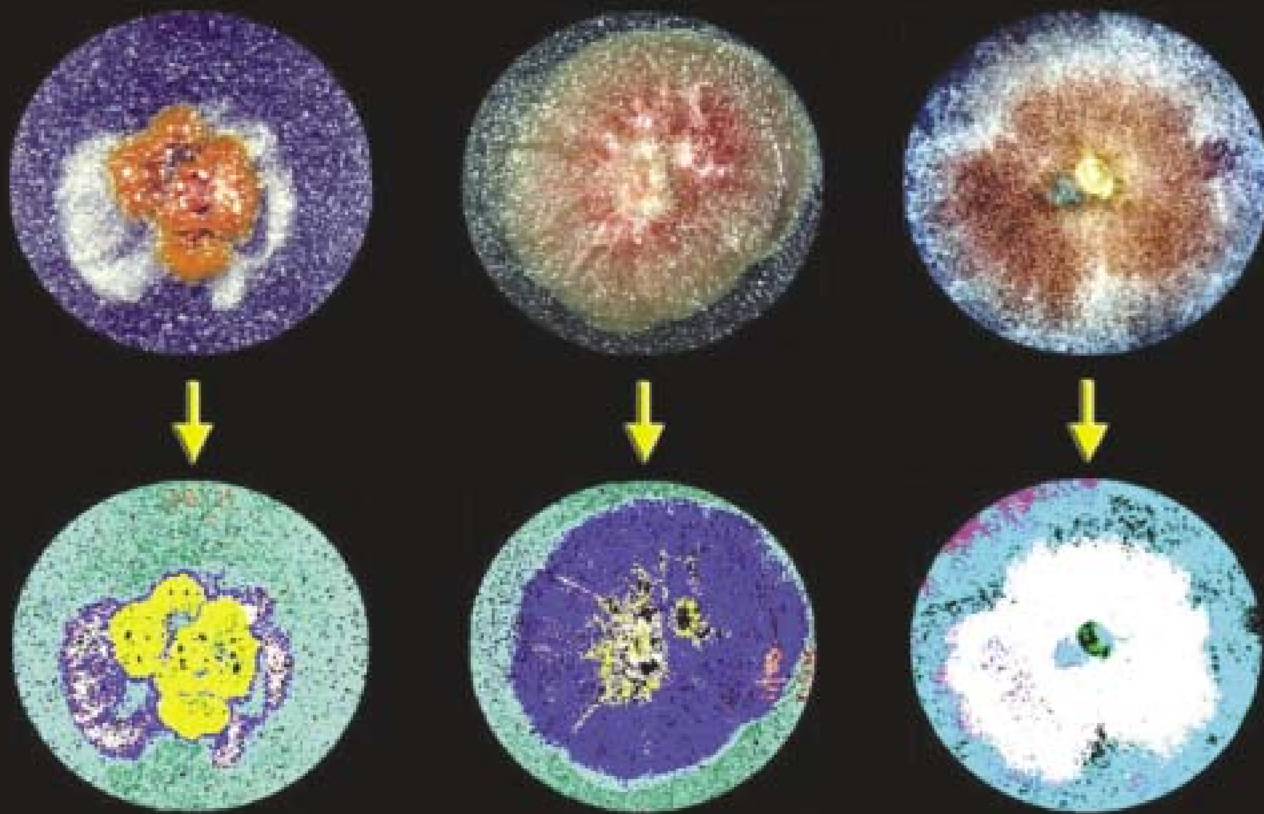
Securing the Home Planet



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Probing the Inner Universe of the Mighty Macrophage

The macrophage is a white blood cell critical for our defense against infection and skeletal well-being. Understanding the behavior of macrophages in microgravity could lead to treatments for immune and bone disorders, and help astronauts remain healthier on long spaceflights.

For cell biologists, the inner space within the boundaries of cellular membranes holds as many unanswered questions and places for exploration as the distant reaches of outer space. One type of white blood cell, known as the macrophage, has particularly captivated NASA investigator Stephen Keith Chapes, leading him beyond Earth's atmosphere to probe the cell's inner secrets.

What is interesting about macrophages? Well, they do have busy workdays. Macrophages are part of an individual's early defense mechanism, and act as cellular garbage disposals by gobbling up spent red blood cells. They also respond to the daily torrent of pathogens by wolfing down bacteria and by carrying foreign proteins to cells that make antibodies. In fact, macrophages link the fate of the immune system and the fate of the skeleton by producing cytokines, immune proteins that perform double-duty by fighting infection and promoting skeletal health.


credit: Joseph Chapes

Stephen Keith Chapes stands in front of the Space Hardware Optimization Technology (SHOT) CellCult Bioreactor and Advanced Separation (ADSEP) Processing Facility hardware, a fully automated cell-culture system to be used during spaceflight to study how human immune cells called macrophages develop in microgravity. The ADSEP hardware grows bone marrow stem cells in a controlled environment for 7.5 days, to preserve how stem cells in space morph into macrophages compared to ones on Earth.

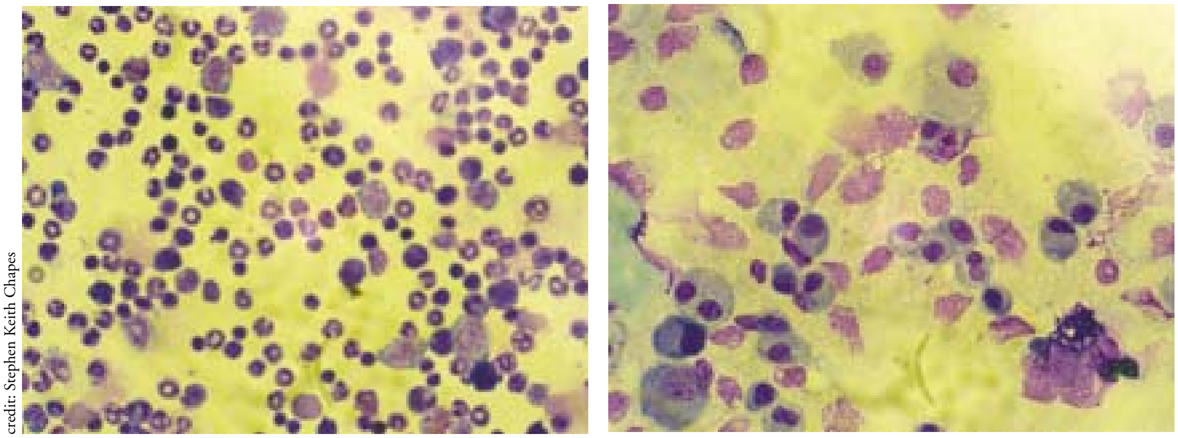
While scientists understand the macrophage duty roster, these cells still hold abundant secrets. Only in the late 1990s, for instance, did researchers discover that macrophages could, with the addition of a certain protein, continue down the differentiation pathway to become osteoclasts, one of two types of bone cells critical for maintaining skeletal strength. That research spawned the discovery of new receptors on the surfaces of macrophage cells and new pathways inside the cells — work that continues today. Chapes, a biology professor at Kansas State University in Manhattan, is contributing to new discoveries in macrophage biology by using microgravity as a research tool.

From Stem Cells to Macrophages

It is well-known that red blood cells do not develop as well in microgravity as they do on Earth. But scientists know less about the fate of other cells such as macrophages in the near absence of gravity. That is why Chapes and his team are studying how macrophages grow from stem cells, formative cells found in several tissues such as the spleen and bone. Stem cells, depending on the chemical signal, morph into mature white blood cells, such as lymphocytes and macrophages. In microgravity, if stem cells cannot differentiate and become mature macrophages, the immune system and the skeleton will be compromised.

Like other blood cells, macrophages are made in the bone marrow during what is called hematopoiesis, a process during which stem cells progress through a series of changes in biochemistry, structure, and metabolism that transform them from immature cells indistinguishable from one another to an array of highly-specialized cells. Macrophages are formed by dividing and differentiating through a complicated pathway that creates both immature cells called monocytes (which leave the marrow and patrol the blood stream) and macrophages (which take up residence in tissue as mature cells). Scientists already know that prolonged spaceflight or long bed rest weakens the skeleton, and any changes to the skeleton could weaken the marrow as well. When hematopoiesis is disrupted, every tissue in the body is at risk.

"I like to think that if you impact hematopoiesis you are going to impact the host's ability to resist insults," explains Chapes. Among the many problems with space, continues Chapes, "Spaceflight is chock-full of insults. You are exposed to changes in pressure. You are exposed to an environment where aerosols and particles will [remain suspended and do not fall out] because there is no gravity so you inhale them. You are exposed to stress from being in a new environment." Also, he says, the body's circulation isn't quite normal because in microgravity blood redistributes towards the upper body instead of pooling toward our feet as it does on Earth.



credit: Stephen Keith Chapes

Bone marrow cells have been stained purple with a special dye and photographed at 40 times magnification under a microscope. Stem cells (left) from bone marrow before they have been grown in the culture hardware are uniformly small and circular. Cells after they have specialized and become macrophages (right) are larger and elongated. Since macrophages respond to microgravity in about 8 seconds by changing shape, Chapes and his group are also investigating how spaceflight might induce molecular changes inside the cells.

Many factors in the space environment can compromise the health of astronauts. Hematopoietic stem cells would be more susceptible to radiation damage if they were actively dividing, a situation that could be worsened if space increased cell division, says Chapes. Also, poor circulation and dust-filled air could add up to health problems if fewer mature macrophages patrol the lungs and nibble away at foreign particles. "Nobody has ever gotten so immunologically compromised [in space] that they're sick, but that doesn't mean there isn't an impact on the immune response," remarks Chapes.

Chapes knows from his ground-based research that genetically engineered mice lacking functioning macrophages are susceptible to microorganisms normal mice can fend off. Chapes also notes that several types of mice lacking macrophages also lack osteoclasts. Without osteoclasts around to hold up their end of skeletal remodeling these mice have osteopetrosis, a condition characterized by an overabundance of bone that is very brittle. If these observations of mice also hold true for people in microgravity, it might be difficult to keep astronauts healthy during prolonged exposure to the microgravity environment.

Macrophages in Microgravity

In 1987, Chapes and his group began to focus on how spaceflight affects the immune system. Experiments using macrophages in parabolic flight (see sidebar) revealed that macrophages respond to microgravity in less than 10 seconds after exposure to microgravity.

Investigations on several space shuttle flights revealed even more. For some experiments, he grew macrophages in "space test tubes" placed in special hardware and after the flight examined them in his laboratory. For other experiments, he flew rats to study changes in their bone marrow stem cells back on Earth. The results were significant: although marrow precursor cells divided better in microgravity than they did on Earth, they would not differentiate. "This led me to speculate there might be a direct effect of spaceflight on macrophage progenitors," explains Chapes.

Today, Chapes is preparing an experiment that will pinpoint at the molecular level where this defect in macrophage development occurs. This experiment was originally scheduled in May on the *Endeavour*, before all space shuttles were grounded following the destruction of the *Columbia*. On a future space shuttle flight, he will grow undifferentiated bone marrow precursors from mice and set up parallel experiments in his laboratory on Earth. In the first phase of the experiment, cells will be loaded into Space Hardware Optimization Technology (SHOT) CellCult Bioreactor and Advanced Separation (ADSEP) Processing Facility hardware, a fully automated cell-culture system. The cells will be kept at a low temperature, which will hold them in "neutral" until the experiment is ready to begin in microgravity. Once in orbit, an astronaut will flip a switch and the cells will be heated to 37 °C, or 98.1°F, snug warmth that mimics body temperature and encourages growth. The hardware automatically adds macrophage (CSF-1), a cytokine that pushes bone marrow stem cells along the differentiation pathway until they become mature macrophages. At the end of 7.5 days, plenty of time for marrow cells to mature, a fixative will be added to preserve cells for study on the ground.

Molecular Questions

After the space shuttle returns, both Earth-grown and space-grown cells will be analyzed for changes both inside and outside the cell membrane.

Chapes will first look for changes on the outer surface of the cell membranes at receptors, molecular "docking places" on inner and outer cell membranes where cytokines such as CSF-1 can bind and bring about some change in the cell. Once CSF-1 is bound to the receptor, a signal travels through an intracellular pathway telling the differentiation machinery to work. If c-*fms*, the receptor for CSF-1, is not working — or if fewer receptors are

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Cells on a Wire

In one set of experiments, Chapes asked whether microgravity affected macrophages and if so, how quickly. He and his group set up an experiment on NASA's KC-135 aircraft, which creates about 25 seconds of microgravity during the free-fall portion of parabolic flight. They placed macrophages on a silver electrode and passed a current through the wire. If the cells changed shape, that change could be measured as a change in resistance.

Just 7–10 seconds after the KC-135 entered its first freefall, the cells stayed attached to the wire and flattened out. When the aircraft returned to 1 *g*, the cells rounded back up.

Chapes wants to investigate the significance of this quick reaction to microgravity. For now, he knows that macrophages, stretchy cells to begin with, react quickly to the change in gravity.