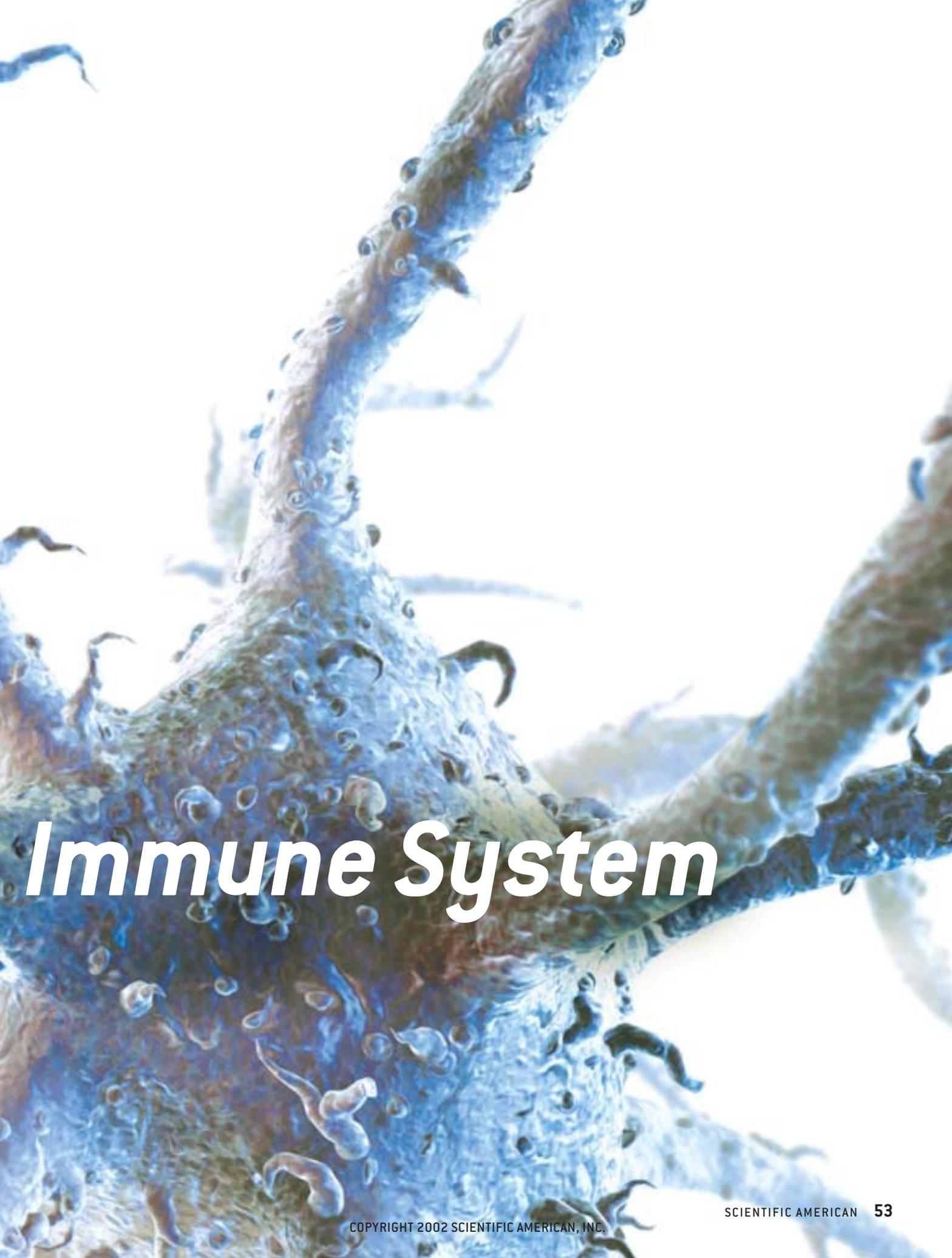
A microscopic image showing several long, branching dendritic cells. The cells are light blue and have a textured, bumpy surface. They are set against a bright, white background. The cells are arranged in a way that suggests they are reaching out or 'catching' something, which is the metaphorical 'long arm' mentioned in the title.

The **LONG** **ARM** *of the*

Dendritic cells catch invaders and tell the immune system when and how to respond. Vaccines depend on them, and scientists are even employing the cells to stir up immunity against cancer

By Jacques Banchereau



Immune System

They lie buried—their long, tentaclelike arms outstretched—in all the tissues of our bodies that interact with the environment.

In the lining of our nose and lungs, lest we inhale the influenza virus in a crowded subway car. In our gastrointestinal tract, to alert our immune system if we swallow a dose of salmonella bacteria. And most important, in our skin, where they lie in wait as stealthy sentinels should microbes breach the leathery fortress of our epidermis.

They are dendritic cells, a class of white blood cells that encompasses some of the least understood but most fascinating actors in the immune system. Over the past several years, researchers have begun to unravel the mysteries of how dendritic cells educate the immune system about what belongs in the body and what is foreign and potentially dangerous. Intriguingly, they have found that dendritic cells initiate and control the overall immune response. For instance, the cells are crucial for establishing immunological “memory,” which is the basis of all vaccines. Indeed, physicians, including those at a number of biotechnology companies, are taking advantage of the role that den-

dritic cells play in immunization by “vaccinating” cancer patients with dendritic cells loaded with bits of their own tumors to activate their immune system against their cancer. Dendritic cells are also responsible for the phenomenon of immune tolerance, the process through which the immune system learns not to attack other components of the body.

But dendritic cells can have a dark side. The human immunodeficiency virus (HIV) hitches a ride inside dendritic cells to travel to lymph nodes, where it infects and wipes out helper T cells, causing AIDS. And those cells that become active at the wrong time might give rise to autoimmune disorders such as lupus. In these cases, shutting down the activity of dendritic cells could lead to new therapies.

Rare and Precious

DENDRITIC CELLS are relatively scarce: they constitute only 0.2 percent of white blood cells in the blood and are present in even smaller proportions in tissues such as the skin. In part because of their

rarity, their true function eluded scientists for nearly a century after they were first identified in 1868 by German anatomist Paul Langerhans, who mistook them for nerve endings in the skin.

In 1973 Ralph M. Steinman of the Rockefeller University rediscovered the cells in mouse spleens and recognized that they are part of the immune system. The cells were unusually potent in stimulating immunity in experimental animals. He renamed the cells “dendritic” because of their spiky arms, or dendrites, although the subset of dendritic cells that occur in the epidermis layer of the skin are still commonly called Langerhans cells.

For almost 20 years after the cells’ re-discovery, researchers had to go through a painstakingly slow process to isolate them from fresh tissue for study. But in 1992, when I was at the Schering-Plough Laboratory for Immunology Research in Dardilly, France, my co-workers and I devised methods for growing large amounts of human dendritic cells from bone marrow stem cells in culture dishes in the laboratory. At roughly the same time, Steinman—in collaboration with Kayo Inaba of Kyoto University in Japan and her colleagues—reported that he had invented a technique for culturing dendritic cells from mice.

In 1994 researchers led by Antonio Lanzavecchia, now at the Institute for Research in Biomedicine in Bellinzona, Switzerland, and Gerold Schuler, now at the University of Erlangen-Nuremberg in Germany, found a way to grow the cells from white blood cells called monocytes.

Overview/*Dendritic Cells*

- Dendritic cells—named for their long arms, or dendrites—exist in many tissues, particularly the skin and mucous membranes. They reel in invaders, chop them into pieces called antigens and display the antigens on their surfaces.
- Antigen-bearing dendritic cells travel to lymph nodes or the spleen, where they interact with other cells of the immune system—including B cells, which make antibodies, and killer T cells, which attack microbes and infected cells.
- Cancer vaccines composed of dendritic cells bearing tumor antigens are now in clinical trials involving humans. Scientists are also hoping to turn off the activity of dendritic cells to combat autoimmune diseases such as lupus.

Scientists now know that monocytes can be prompted to become either dendritic cells, which turn the immune system on and off, or macrophages, cells that crawl through the body scavenging dead cells and microbes.

The ability to culture dendritic cells offered scientists the opportunity to investigate them in depth for the first time. Some of the initial discoveries expanded the tenuous understanding of how dendritic cells function.

There are several subsets of dendritic cells, which arise from precursors that circulate in the blood and then take up residence in immature form in the skin, mucous membranes, and organs such as the lungs and spleen. Immature dendritic cells are endowed with a wealth of mechanisms for capturing invading microbes: they reel in invaders using suction cup-like receptors on their surfaces, they take microscopic sips of the fluid surrounding them, and they suck in viruses or bacteria by engulfing them in sacks known as vacuoles. Yong-Jun Liu, a former colleague of mine from Schering-Plough who is now at DNAX Research Institute in Palo Alto, Calif., has found that some immature dendritic cells can also zap viruses immediately by secreting a substance called interferon-alpha.

Once they devour foreign objects, the immature cells chop them into fragments (antigens) that can be recognized by the rest of the immune system [see illustration on next two pages]. The cells use pitchfork-shaped molecules termed the major histocompatibility complex (MHC) to display the antigens on their surfaces. The antigens fit between the tines of the MHC, which comes in two types, class I and class II. The two types vary in shape and in how they acquire their antigen cargo while inside cells.

Dendritic cells are very efficient at capturing and presenting antigens: they can pick up antigens that occur in only minute concentrations. As they process antigens for presentation, they travel to the spleen through the blood or to lymph nodes through a clear fluid known as lymph. Once at their destinations, the cells complete their maturation and present their antigen-laden MHC molecules

to naive helper T cells, those that have never encountered antigens before. Dendritic cells are the only cells that can educate naive helper T cells to recognize an antigen as foreign or dangerous. This unique ability appears to derive from costimulatory molecules on their surfaces that can bind to corresponding receptors on the T cells.

Once educated, the helper T cells go on to prompt so-called B cells to produce antibodies that bind to and inactivate the antigen. The dendritic cells and helper cells also activate killer T cells, which can destroy cells infected by microbes. Some of the cells that have been educated by dendritic cells become “memory” cells that remain in the body for years—perhaps decades—to combat the invader in case it ever returns.

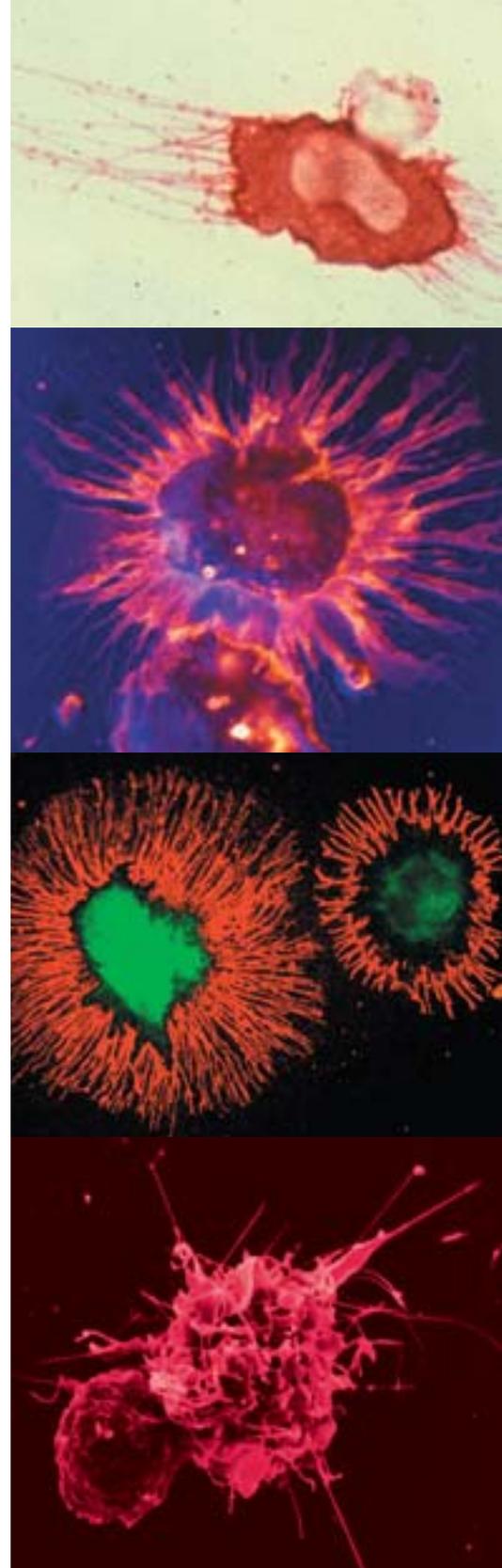
Whether the body responds with antibodies or killer cells seems to be determined in part by which subset of dendritic cell conveys the message and which of two types of immune-stimulating substances, called cytokines, they prompt the helper T cells to make. In the case of parasites or some bacterial invaders, type 2 cytokines are best because they arm the immune system with antibodies; type 1 cytokines are better at mustering killer cells to attack cells infected by other kinds of bacteria or by viruses.

If a dendritic cell prompts the wrong type of cytokine, the body can mount the wrong offense. Generating the appropriate kind of immune response can be a matter of life or death: when exposed to the bacterium that causes leprosy, people who mount a type 1 response develop a mild, tuberculoid form of the disease, whereas those who have a type 2 response can end up with the potentially fatal lepromatous form.

Cancer Killers

ACTIVATING NAIVE helper T cells is the basis of vaccines for everything from pneumonia to tetanus to influenza. Scientists are now turning the new knowledge of the role that dendritic cells play in immunity against microbes and their toxins into a strategy to fight cancer.

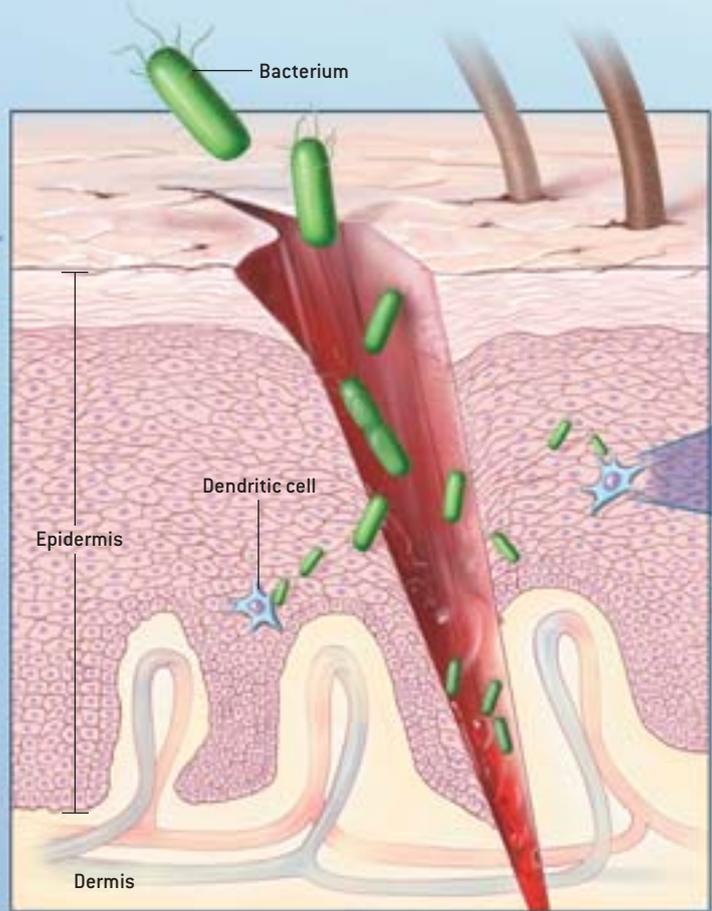
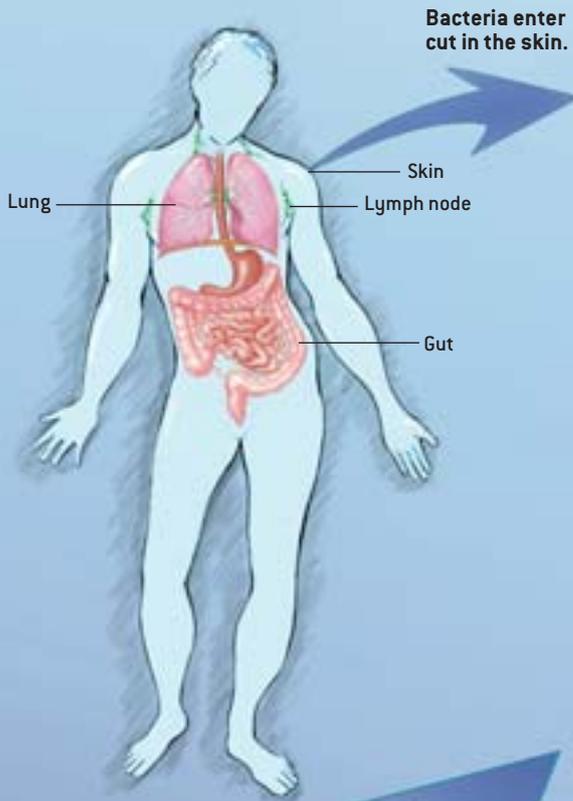
Cancer cells are abnormal and as such are thought to generate molecules that



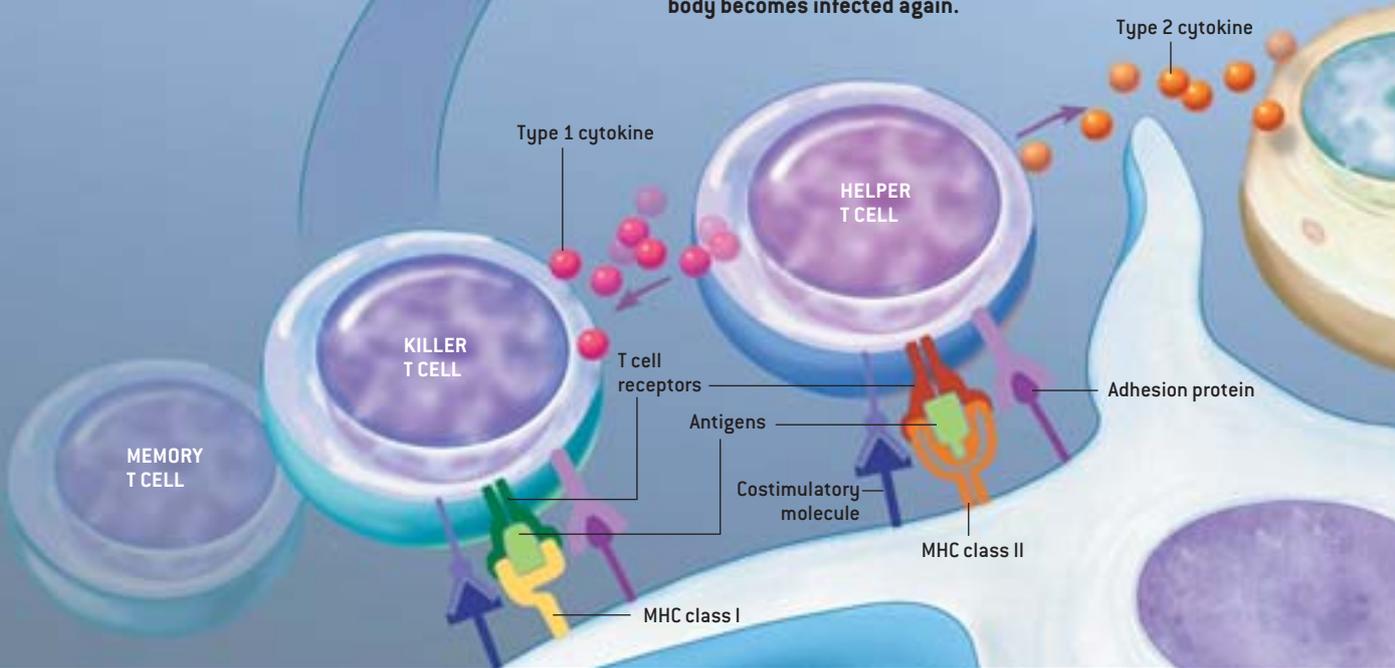
SPIKY ARMS are common to mature dendritic cells from humans (top and top middle), mice (bottom middle) and rats (bottom). The rat dendritic cell is interacting with what is probably a helper T cell. Through such interactions, dendritic cells teach the immune system what it should attack. Cells matured in the laboratory, such as the one at the top middle, are being used in cancer vaccines.

DENDRITIC CELLS AND INFECTION

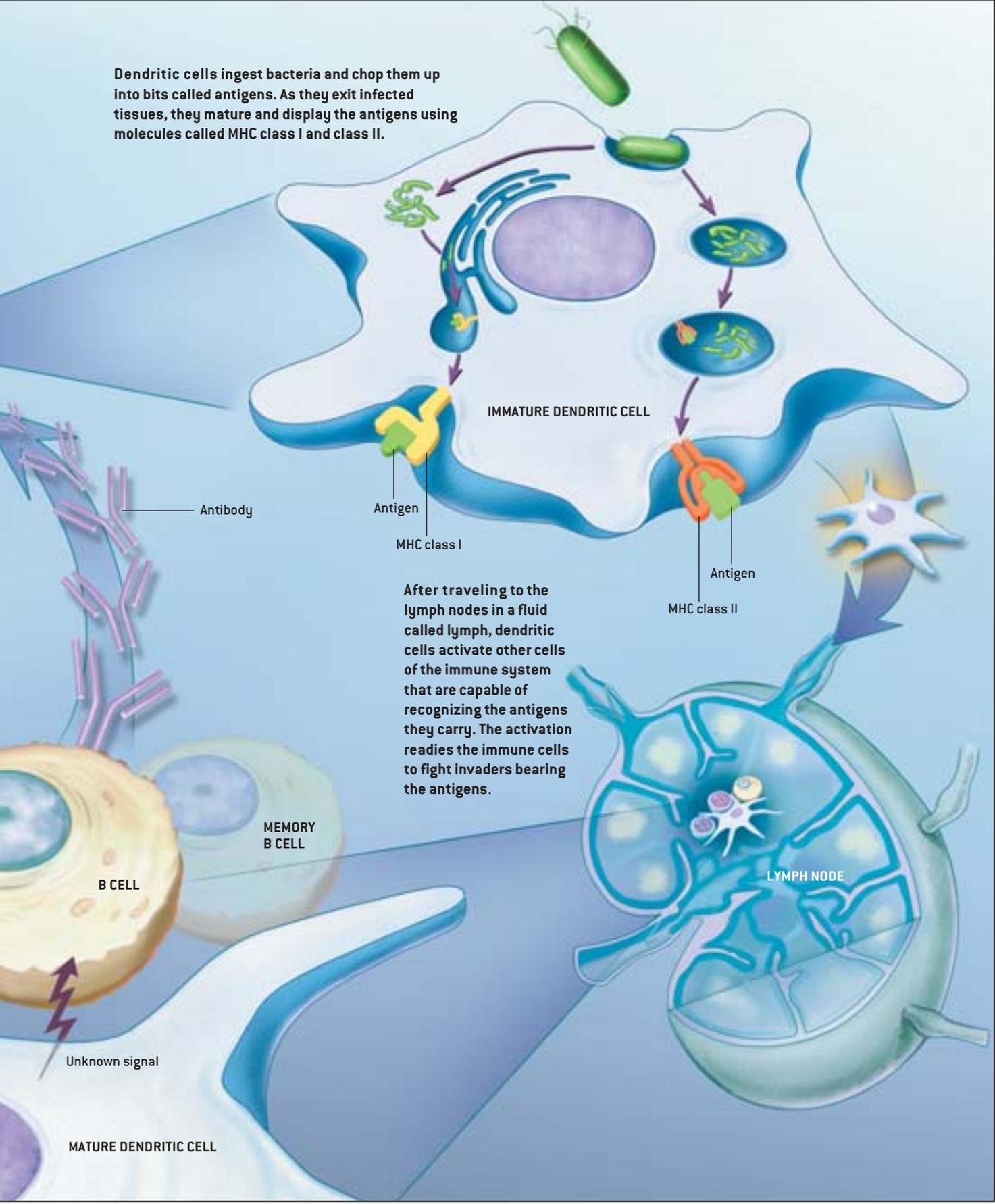
PRESENT IN THE LUNGS, skin, gut and lymph nodes, dendritic cells orchestrate the immune response against invaders (here, bacteria entering a cut in the skin).



Dendritic cells bind to helper T cells, killer T cells and—perhaps—B cells. The binding prompts the helper T cells to make substances called cytokines that stimulate killer T cells and cause B cells to begin making antibodies. The antibodies and killer T cells migrate to the cut to fight the infection. Memory cells persist in case the body becomes infected again.



Dendritic cells ingest bacteria and chop them up into bits called antigens. As they exit infected tissues, they mature and display the antigens using molecules called MHC class I and class II.



After traveling to the lymph nodes in a fluid called lymph, dendritic cells activate other cells of the immune system that are capable of recognizing the antigens they carry. The activation readies the immune cells to fight invaders bearing the antigens.

Dendritic Cell Cancer Vaccines under Development

COMPANY NAME	HEADQUARTERS	STOCK SYMBOL	CANCER TYPE	STATUS*
ML Laboratories	Warrington, England	LSE: MLB	Melanoma	Entering phase I tests
Dendreon	Seattle	Nasdaq: DNDN	Prostate, breast, ovary, colon, multiple myeloma	Phase III (prostate), phase II (prostate, multiple myeloma), phase I (breast, ovary, colon)
Genzyme	Framingham, Mass.	Nasdaq: GZMO	Kidney, melanoma	Phase I (kidney), phase I/II (melanoma)
Immuno-Designed Molecules	Paris	Privately held	Prostate, melanoma	Phase II tests
Merix Bioscience	Durham, N.C.	Privately held	Melanoma	Entering phase I
Oxford BioMedica	Oxford, England	LSE: OXB	Colorectal	Phase I/II
Zycos	Lexington, Mass.	Privately held	DNA-based vaccine against various cancers	Phases I and II

*Phase I tests evaluate safety in a small number of patients; phases II and III assess ability to stimulate the immune system and effectiveness in larger numbers of patients.

healthy cells don't. If researchers could devise drugs or vaccines that exclusively targeted those aberrant molecules, they could combat cancer more effectively while leaving normal cells and tissues alone—thereby eliminating some of the pernicious side effects of chemotherapy and radiation, such as hair loss, nausea and weakening of the immune system caused by destruction of the bone marrow.

Antigens that occur only on cancerous cells have been hard to find, but researchers have succeeded in isolating several of them, most notably from the skin cancer melanoma. In the early 1990s Thierry Boon of the Ludwig Cancer Institute in Brussels, Steven A. Rosenberg of the National Cancer Institute and their colleagues independently identified melanoma-specific antigens that are currently being targeted in a variety of clinical trials involving humans.

Such trials generally employ vaccines made of dendritic cell precursors that have been isolated from cancer patients and grown in the laboratory together

with tumor antigens. During this process, the dendritic cells pick up the antigens, chop them up and present them on their surfaces. When injected back into the patients, the antigen-loaded dendritic cells are expected to ramp up patients' immune response against their own tumors.

Various researchers—including Frank O. Nestle of the University of Zurich and Ronald Levy and Edgar G. Engleman of Stanford University, as well as scientists at several biotechnology companies [see box above]—are testing this approach against cancers as diverse as melanoma, B cell lymphoma, and tumors of the prostate and colon. There have been glimmers of success. In September 2001, for instance, my co-workers and I, in collaboration with Steinman's group, reported that 16 of 18 patients with advanced melanoma to whom we gave injections of dendritic cells loaded with melanoma antigens showed signs in laboratory tests of an enhanced immune response to their cancer. What is more, tumor growth was slowed in the nine pa-

tients who mounted responses against more than two of the antigens.

Scientists are now working to refine the approach and test it on larger numbers of patients. So far cancer vaccines based on dendritic cells have been tested only in patients with advanced cancer. Although researchers believe that patients with earlier-stage cancers may respond better to the therapy—their immune systems have not yet tried and failed to eradicate their tumor—several potential problems must first be considered.

Some researchers fear that such vaccines might induce patients' immune systems to attack healthy tissue by mistake. For instance, vitiligo—white patches on the skin caused by the destruction of normal pigment-producing melanocytes—has been observed in melanoma patients who have received the earliest anti-melanoma vaccines. Conversely, the tumors might mutate to “escape” the immune onslaught engendered by a dendritic cell vaccine. Tumor cells could accomplish this evasion by no longer making the antigens the vaccine was designed to stimulate the immune system against. This problem is not unique to dendritic cells, though: the same phenomenon can occur with traditional cancer therapies.

In addition, tailoring a dendritic cell vaccine to fight a particular patient's tumors might not be economically feasible.

THE AUTHOR

JACQUES BANCHEREAU has directed the Baylor Institute for Immunology Research in Dallas since 1996. The institute aims to manipulate the human immune system to treat cancer as well as infectious and autoimmune diseases. Before 1996 Banchereau led the Schering-Plough Laboratory for Immunology Research in Dardilly, France. He obtained his Ph.D. in biochemistry from the University of Paris. Banchereau holds many patents on immunological techniques and is a member of the scientific advisory board of Merix Bioscience, a biotechnology company based in Durham, N.C.

But many scientists are working to circumvent the costly and time-consuming steps of isolating cells from patients and manipulating them in the laboratory for reinjection.

One approach involves prompting dendritic cell precursors already present in a person's body to divide and start orchestrating an immune response against their tumors. David H. Lynch of Immunex in Seattle (recently acquired by Amgen in Thousand Oaks, Calif.) and his co-workers have discovered a cytokine that causes mice to make more dendritic cells, which eventually induce the animals to reject grafted tumors. Other scientists, including Drew M. Pardoll of Johns Hopkins University, have observed that tumor cells that have been genetically engineered to secrete large amounts of cytokines that activate dendritic cells have the most potential as cancer vaccines.

Shutting Immunity Down

IN THE MEANTIME, other scientists are looking at ways to turn off the activity of dendritic cells in instances where they exacerbate disease instead of fighting it. Usually, in a phenomenon known as central tolerance, an organ in the chest called the thymus gets rid of young T cells that happen to recognize the body's own components as foreign before they have a chance to circulate. Some inevitably slip through, however, so the body has a backup mechanism for restraining their activity.

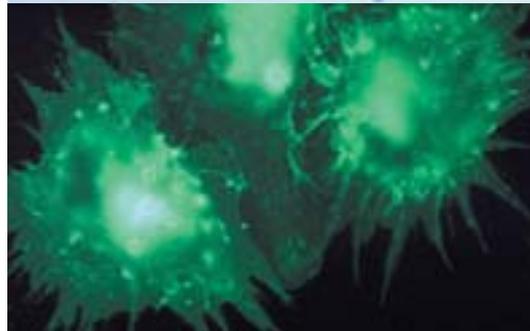
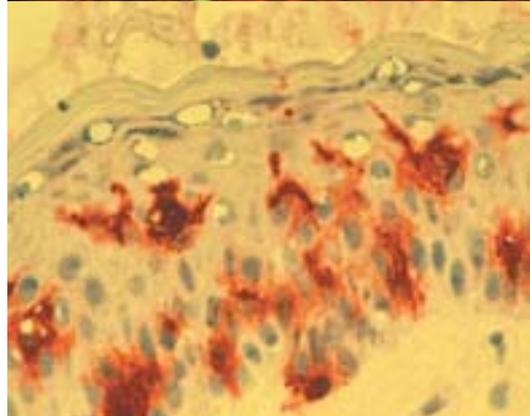
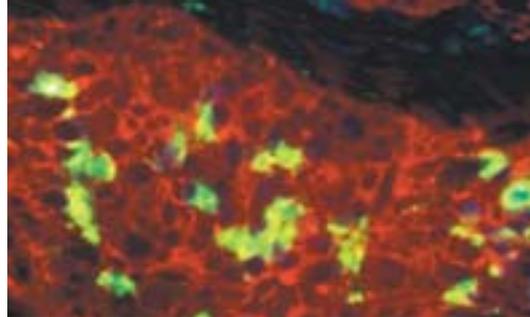
But this mechanism, termed peripheral tolerance, appears to be broken in patients with autoimmune disorders such as rheumatoid arthritis, type 1 diabetes and systemic lupus erythematosus. Last year my colleagues and I reported that dendritic cells from the blood of people with lupus are unnaturally active. Cells from these patients release high amounts of interferon-alpha, an immune-stimulating protein that causes precursors to grow into mature dendritic cells while still in the bloodstream. The mature cells then ingest DNA, which is present in unusual amounts in the blood of people with lupus, and that in turn causes the individual's immune system to generate antibodies against his or her own DNA.

These antibodies result in the life-threatening complications of lupus when they lodge in the kidneys or the walls of blood vessels. Accordingly, we propose that blocking interferon-alpha might lead to a therapy for lupus by preventing dendritic cell activation. A similar strategy might prevent organ transplant recipients from rejecting their new tissues.

A new treatment for AIDS might also rest on a better understanding of dendritic cells. In 2000 Carl G. Figdor and Yvette van Kooyk of the University Medical Center St. Radboud in Nijmegen, the Netherlands, identified a subset of dendritic cells that makes DC-SIGN, a molecule that can bind to the outer coat of HIV. These cells pick up HIV as they regularly prowl the mucous membranes and deep tissues. When they travel to the lymph nodes, they unwittingly deliver the virus to a large concentration of T cells. Drugs that block the interaction between DC-SIGN and HIV might slow the progression of AIDS.

Other infectious diseases—including malaria, measles and cytomegalovirus—also manipulate dendritic cells for their own ends. Red blood cells that have been infected by malaria parasites, for instance, bind to dendritic cells and prevent them from maturing and alerting the immune system to the presence of the invaders. Several groups of researchers are now devising approaches to prevent such microbes from hijacking dendritic cells; some are even seeking to use supercharged dendritic cells to fight the infections.

As we learn more about the molecules that control dendritic cells, we will find ways to harness their therapeutic potential. The increasing number of scientists and corporations working on dendritic cells portends that we will soon be able to maximize the biological power of these cells to treat and prevent the diseases that plague humankind. 



IMMATURE DENDRITIC CELLS can be stained to show up green in breast cancer tissue (top) or red in normal skin (top middle). As the cells mature, they make proteins that allow them to stick to one another (bottom middle). They also produce forklike receptors (green dots, bottom), which they use to show bits of invaders to other immune cells.

MORE TO EXPLORE

Dendritic Cells and the Control of Immunity. Jacques Banchereau and Ralph M. Steinman in *Nature*, Vol. 392, pages 245–252; March 19, 1998.

Dendritic Cells as Vectors for Therapy. Jacques Banchereau, Beatrice Schuler-Thurner, A. Karolina Palucka and Gerold Schuler in *Cell*, Vol. 106, No. 3, pages 271–274; August 10, 2001.

Background information on the immune system and on experimental cancer therapies such as those using dendritic cells can be found on the American Cancer Society's Web site: www.cancer.org