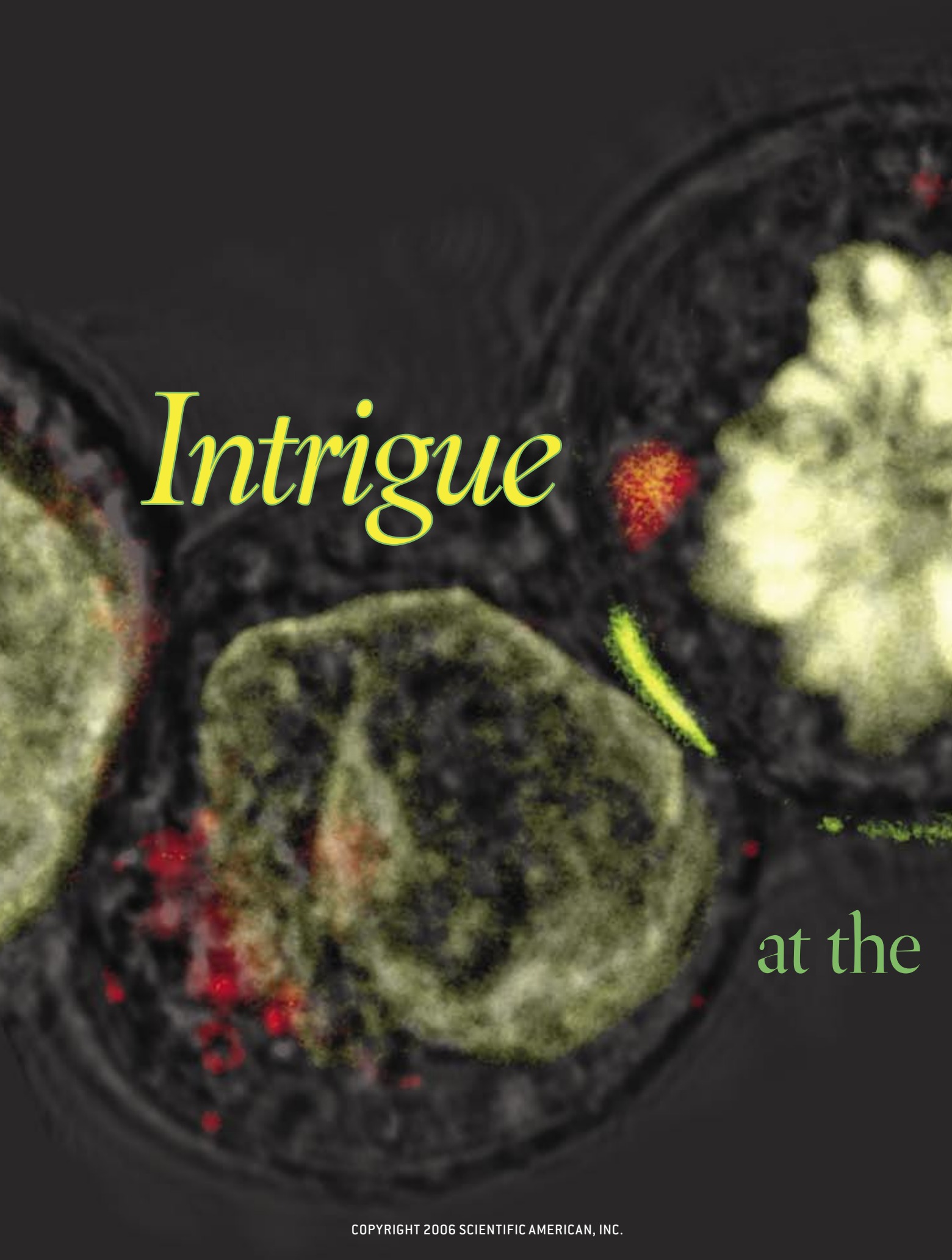
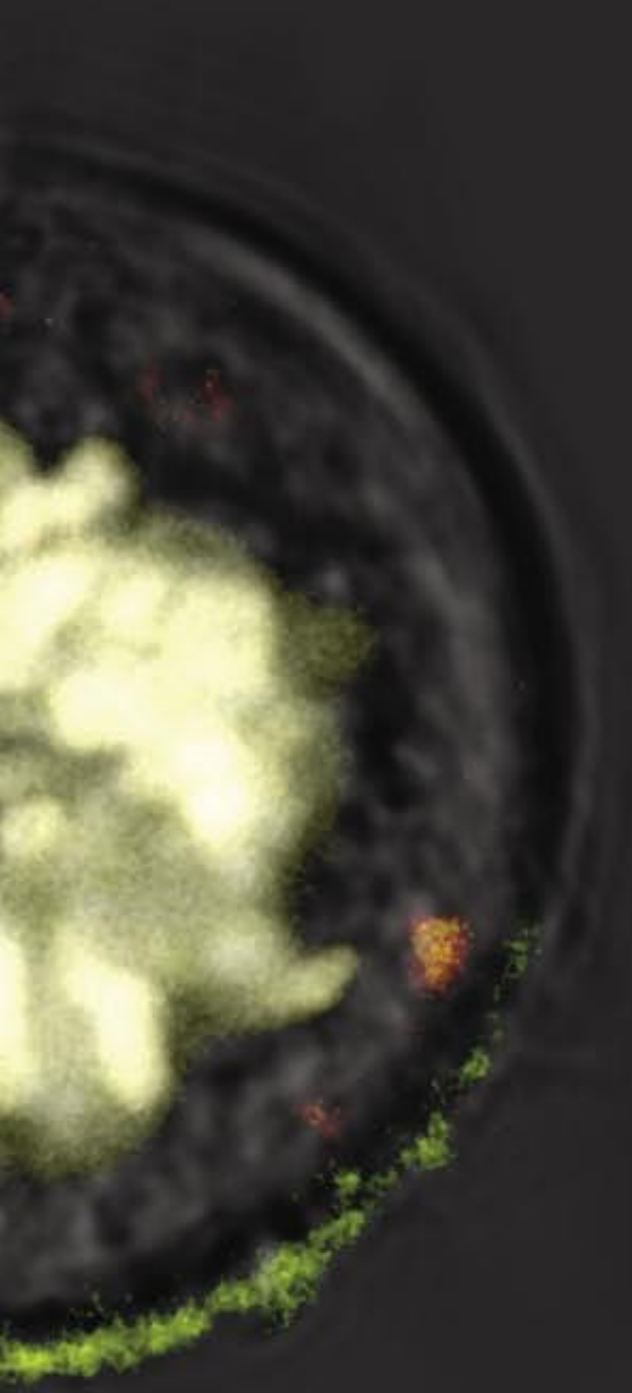




Images of interacting immune cells reveal structured connections similar to the ones neurons use to communicate. Studying these synapses is providing new insights into how the cells form an information-sharing network to fight disease

By Daniel M. Davis

Immune Synapse

PROTEINS (*yellow*) cluster at the point where two immune cells meet to trade information. The proteins at this synapse tell a natural killer cell (*bottom left*) that the B cell (*right*) is healthy and should be spared. For other cells that fail this test, the natural killer cell has acidic organelles (*red*) ready to move toward the synapse and deliver a lethal injection.

Comic-book fans know well that the most sought after editions are those in which a superhero appears for the first time. A comic book published in 1962 featuring the first appearance of Spider-Man, for example, recently sold at auction for $122,000. Sadly, publications representing the first appearance of an important scientific fact generally do not command similar prices, but to scientists these firsts are equally treasured.

Just such a moment occurred in 1995, when Abraham "Avi" Kupfer of the National Jewish Medical and Research Center in Denver stood before an unsuspecting group of a few hundred immunologists gathered for one of the prestigious Keystone symposia, named for a U.S. ski resort. Kupfer's presentation included the first three-dimensional images of immune cells interacting with one another. As the crowd watched in stunned silence, Kupfer showed them image after image of proteins organized into bull's-eye patterns at the area of contact between the cells.

To the group, the pictures were instantly understandable and unequivocal: like the synapses that form the critical junctures between neurons in neural communication networks, the contacts between the immune cells involved organized aggregates of proteins. Both outer rings of molecules keeping the cells adhered to one another and inner clusters of interacting proteins particular to the discussion between the cells were clearly visible.

The idea that immune cells—which must exchange and store information in the course of searching for and responding to disease—might share mechanisms with those consummate communicators, the cells of the nervous system, had been put forth before. But here, at last, was proof of structures to go with the theory. When Kupfer was finished, the room erupted in prolonged applause, followed by a barrage of questions.

A decade later these structured synapses formed by immune cells are still generating questions: about how cellular machinery or other forces produce the synaptic architecture, how the architecture, in turn, might regulate cell-to-cell communication, how its malfunction could lead to disease, and even how pathogens might exploit the mechanism to their own advantage.

Discovery of the immune synapse and its ongoing exploration has been made possible by new high-resolution microscopy techniques and computer enhancement of older imaging methods. Now the realization that a thought, the sensation of a touch, or the detection of a virus in the bloodstream all require similar choreography of molecules is providing a compelling new framework for understanding immunity.

Seeking Direction

LONG BEFORE the immune synapse was seen, the possibility that immune cells might be able to target their communication was apparent. Scientists knew that immune cells secreted protein molecules called cytokines to talk with one another and with other types of cells. Yet at least some of these molecules did not seem to function like hormones, which diffuse throughout the body broadcasting their message widely. Rather cytokines could barely be detected in the blood and seemed to act only between cells that were touching.

This ability to trade chemical signals with just a particular neighbor is important for immune cells. Unlike neurons, which tend to form stable, long-term junctions with other cells, immune cells make fleeting contacts as they constantly roam the body seeking out signs of disease and exchanging information about present dangers. When an immune cell charged with identifying illness bumps into another cell, it may have only a couple of minutes to decide whether its target is healthy or not. If not, the immune cell, depending on its type, might kill the sick cell directly or raise an alarm, calling other immune soldiers to come do the job. Getting the communication wrong might lead to immune cells mistakenly killing healthy cells, as happens in autoimmune diseases such as multiple sclerosis, or allowing cancer cells to continue growing unchecked. Thus, immu-

Here, at last, were structures to go with the theory.

Overview/*The Structured Dialogue*

- High-resolution microscope images of immune cells contacting other cells have revealed temporary membrane structures similar to the "synapse" connections nerve cells make with one another for communication.
- Investigations of these immune cell synapses focus on mechanisms that might control their configuration and on how the structures modulate communication between cells.
- Observing the real-time interactions of individual immune cells is a new avenue for understanding how they share and process information to defend the body against disease.

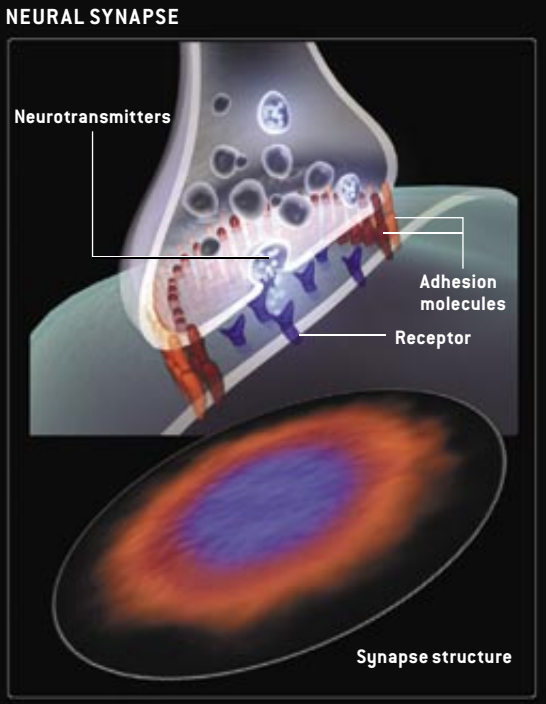
COURTESY OF BJÖRN ÖNFELT AND DANIEL M. DAVIS (*preceding pages*)

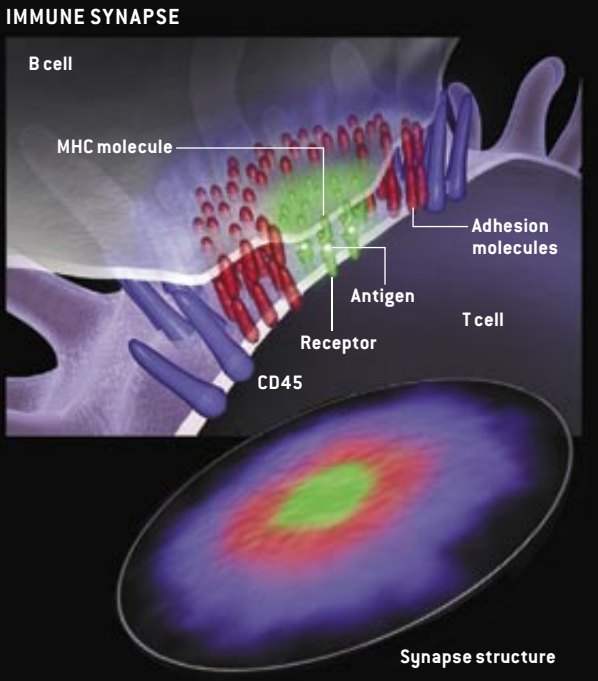
SYNAPSES UP CLOSE

Derived from two Greek words meaning "to join together" and "to fasten," a synapse is the point of contact where two cells exchange molecular signals and are often physically bound to one another by linked proteins. Between neurons, these connections are generally long-term, whereas immune cells make temporary bonds for quick dialogues. Immune synapse configurations can vary depending on cell type, and their formation proceeds in stages that may also regulate the cells' conversation.

SYNAPTIC SIMILARITIES

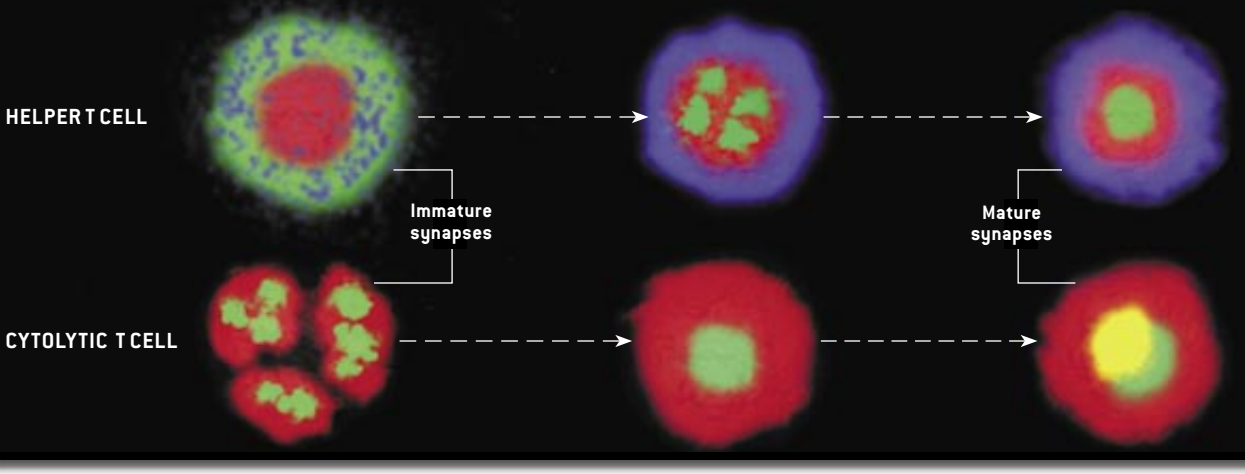
In the classic synapse between two neurons, adhesion proteins hold the membranes of two interacting cells close together. When the first neuron is stimulated, packets of signaling molecules called neurotransmitters move toward the membrane to release their contents, which travel to receptors on the second neuron. In an immune synapse, adhesion molecules also hold cell membranes close together while other proteins interact. In this example, major histocompatibility complex (MHC) molecules on a B cell present protein fragments called antigens to a T cell's receptors. Proteins called CD45 that normally suppress signaling are shunted to the synapse periphery. Viewed as if from inside one of the cells, the synapse structures resemble bull's-eye patterns.





DYNAMIC DEVELOPMENT

The helper T cell's synapse starts with adhesion molecules (*red*) clustered at its center and receptors (*green*) in an outside ring, an order that will reverse in the mature synapse, where CD45 proteins (*blue*) form the outermost ring. Another immune cell type, the cytolytic T cell, is charged with killing unhealthy cells. When it meets one, its receptors and adhesion proteins begin to cluster, then form a ring. Finally, granules containing toxic molecules (*yellow*) travel from inside the cell to the center of the synapse to release their lethal load. Investigators want to learn what role these stages of synapse formation may play in regulating immune cell communication.



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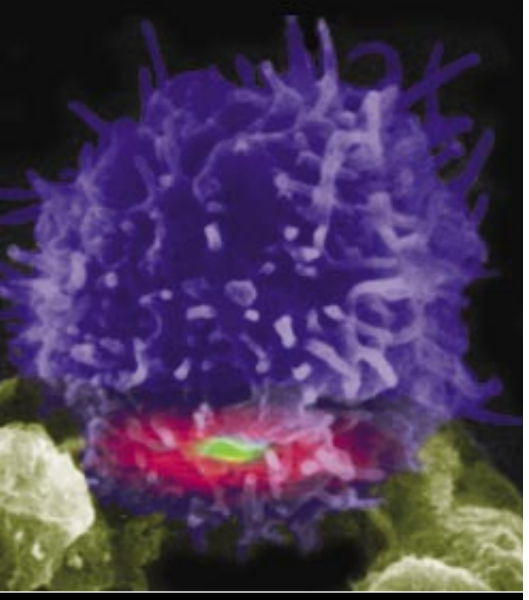


IMMUNE CELLS CONNECT for an exchange that will cause a T cell (*blue*) to become activated if it recognizes an antigen presented by the larger dendritic cell (*gold*). In this electron micrograph merged with a live-cell fluorescence image, T cell receptors interacting with antigen are clustered at the synapse center (*green*), and a ring of adhesion molecules (*pink*) holds the two cells together.

nologists have a keen interest in figuring out not only which molecules are involved in these dialogues but how they interact to enable such critical decisions.

In the early 1980s scientists in the Laboratory of Immunology at the National Institutes of Health began exploring the idea that a structured interface could allow immune cells to direct their secretion of cytokines to another cell. Because the cellular membranes, made largely from fat and protein molecules, are fluid, proteins could certainly move easily up to the point of contact between two cells and form an organized architecture there, as happens when neurons create a connection to another cell.

The NIH group's hypothesis grew from critical experiments showing that clustering specific proteins together at the surface of immune cells called T cells was sufficient to trigger activation of those cells. In a paper published in 1984, NIH investigator Michael A. Norcross first formally articulated the possibility that the nervous and immune systems have a common mechanism of communication through synapses. Unfortunately, it appeared in a journal that was not widely read, and some of his molecular details were off, so his early synaptic model of immune cell communication was soon forgotten. But curiosity about whether and how immune cells might target their messages remained.

In 1988 the late Charles A. Janeway, Jr., and his colleagues at Yale University performed a beautiful experiment to confirm that immune cells could indeed secrete proteins in a specific direction. They fitted T cells tightly into the pores of a membrane dividing a chamber containing solution. By adding a stimulant to the solution on only one side of the membrane, they activated the T cells, which subsequently started secreting proteins toward the source of the stimulant but not into the stimulant-free solution on the other side of the membrane.

Encouraged by this key observation, in 1994 NIH researchers William E. Paul and Robert A. Seder resurrected the idea that the immune synapse is the communicating junction between immune cells and other cells. They described the synapse as two cell surfaces in close proximity with a structured arrangement of receptor proteins on one cell surface, opposite their binding partners on the contacting cell. Acknowledging that immune cells move about far more than neurons, Paul spoke of the immune synapse as a "make and break" union in contrast with longer-term neuronal connections.

Thus, by the mid-1990s the immune synapse was established as a provocative concept for which a structure still needed to be seen experimentally. Then Avi Kupfer presented his slide show at the Keystone symposium. His images showed interactions between immune cells called antigen-presenting cells (APCs) that specialize in breaking up proteins belonging to an invader, such as a virus, and displaying the protein fragments to T cells, which become activated when they recognize one of the antigens. Hence, Kupfer dubbed the bull's-eye patterns of protein molecules formed at the interface of the two cells supramolecular activation clusters, or SMACs.

Independently, Michael L. Dustin, Paul M. Allen and Andrey S. Shaw of the Washington University School of Medicine in St. Louis, with Mark M. Davis of Stanford University, had also been imaging T cell activation, but with an interesting twist. Instead of observing two cells interacting together, they replaced the APC with a surrogate membrane composed of lipid molecules from a real cell laid out flat on a glass slide. To this glass-supported lipid membrane, they added the key proteins normally found at the surface of APCs, each tagged with a different colored fluorescent dye. They then watched the organization of these labeled proteins as T cells landed on the membrane [*see illustration below*].

Dustin's group also saw bull's-eye patterns of proteins emerge as the T cells surveyed the proteins within the supported membrane. Clearly, a structured synapse did not require the effort of two

IN ONE OF THE EARLIEST imaging experiments to reveal immune synapse structure, Michael L. Dustin and his colleagues employed fluorescently labeled proteins within an artificial cell membrane. These assume a synapse formation when an unseen T cell on the membrane's opposite side begins interacting with them.

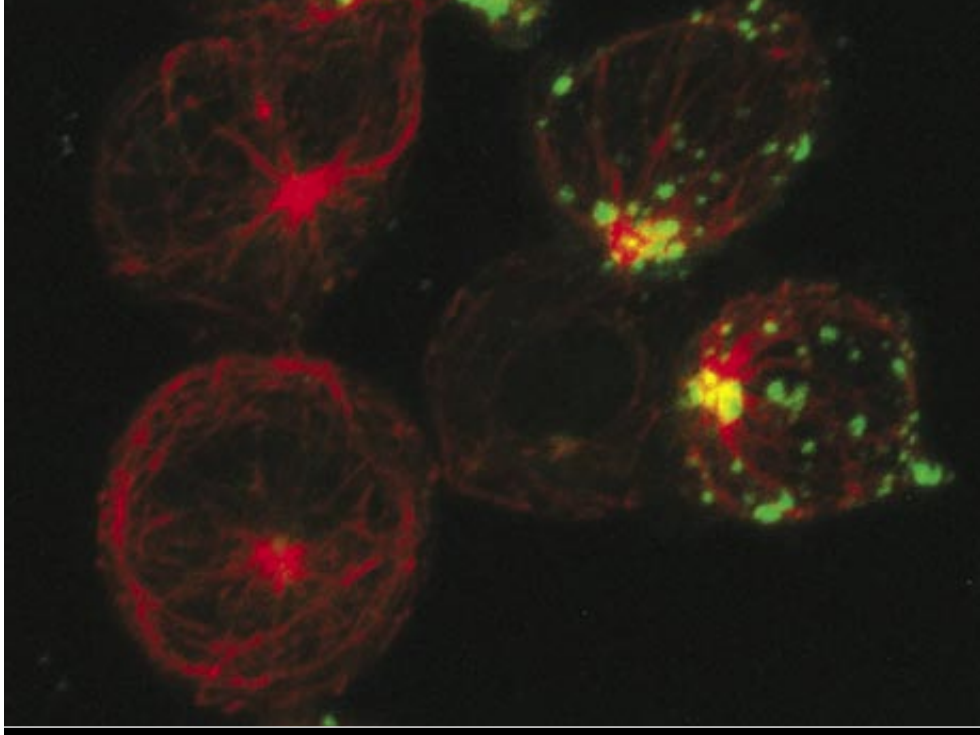
Proteins bearing an antigenlike molecule (*green*) first form an outer ring, with adhesion molecules (*red*) clustered at the center. Over a 60-minute interval, their arrangement reverses, mimicking stages of interaction at a synapse between T cells and real antigen-presenting cells.

COURTESY OF MICHAEL L. DUSTIN, REPRINTED WITH PERMISSION FROM *SCIENCE*, VOL. 285; ©1999 A.A.A.S. (*top*); COURTESY OF MICHAEL L. DUSTIN, REPRINTED WITH PERMISSION FROM "THE IMMUNOLOGICAL SYNAPSE: A MOLECULAR MACHINE CONTROLLING T CELL ACTIVATION," BY GRAKOUI ET AL., IN *SCIENCE*, VOL. 285; ©1999 A.A.A.S. (*bottom*)

cells; instead it could form as one immune cell contacted and responded to an artificial array of proteins.

This work also revealed that the synapse itself is dynamic: arrangements of proteins change as the cell communication continues. For example, T cell receptors interacting with the antigen were first seen to accumulate in a ring surrounding a central cluster of adhesive proteins, creating an immature T cell synapse. Later, that structure inverted so that in the mature synapse the adhesive molecules formed an outer ring of the bull's-eye, surrounding a central cluster of interacting T cell receptors.

Since Kupfer and Dustin published their initial T cell synapse images, a variety of synapse structure patterns have also been seen between other types of immune cells. Indeed, my own contribution, while working with Jack Strominger of Harvard University in 1999, was to observe a structured synapse formed by a different kind of white blood cell—known as a natural killer (NK) cell—which helped to confirm the generality of their observations. Exploring how such changing arrangements of molecules occur and how they control immune cell communication is the new science opened up by the immune synapse concept.



TWO KILLER T CELLS (*right*) are captured as they prepare to destroy a diseased cell (*center*). Poisonous lytic proteins (*green*) cluster at the synapses between the T cells and their target, carried there by cytoskeletal proteins called microtubules (*red*). The lytic proteins will be injected into the target cell through the center of the synapse structures, which may also protect the T cells from poisoning themselves.

The patterns may transmit, or at least reflect, information.

Deciphering the Dance

OBSERVATIONS of the structure of immune synapses immediately spurred researchers to investigate what makes the cellular proteins move to the contact point between the cells and organize themselves into specific patterns. One driver of protein movements in all cells is a remarkable network of filaments known as the cytoskeleton, which is made up of long chains of proteins that can extend or shrink in length. Tethered to the cell surface by adapter proteins, the cytoskeleton can push or pull the cell membrane, enabling muscles to contract or sperm to swim.

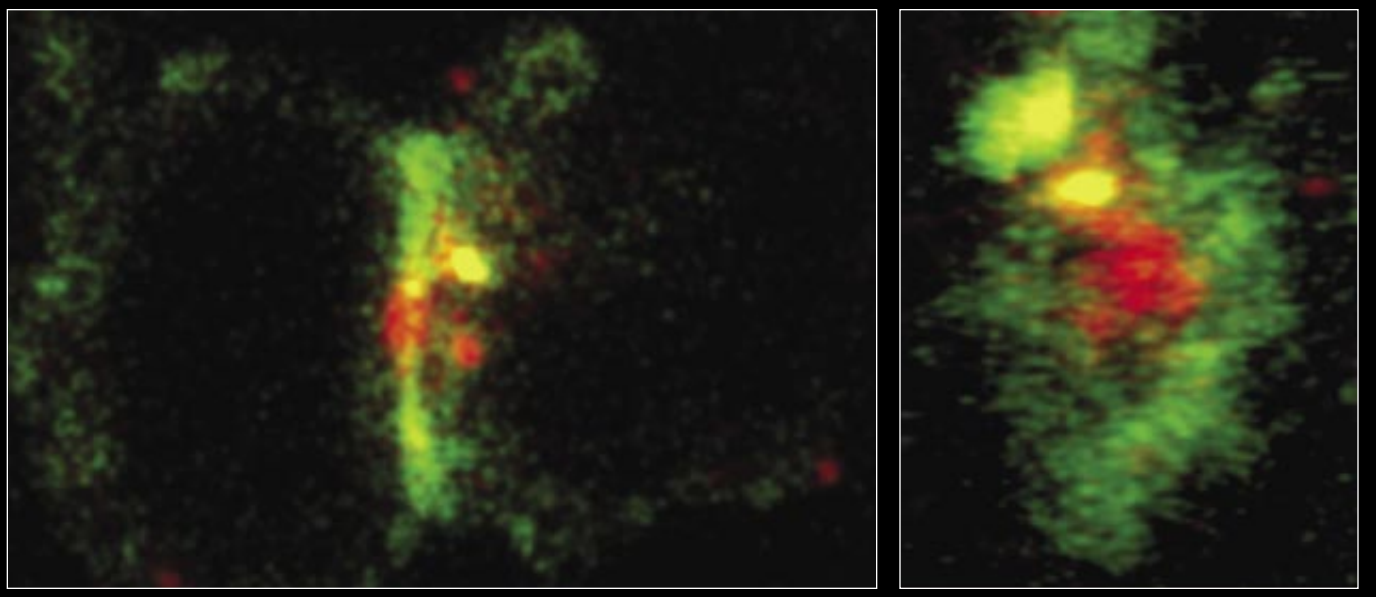
Experiments showed that when a cell's cytoskeleton was incapacitated by toxins, some proteins were no longer able to move toward the immune synapse, suggesting that movements of cytoskeletal filaments allow cells to control when and where the proteins accumulate at the synapse.

At least two other mechanisms could play a role in organizing proteins at the synapse, but the extent of their influence on immune cell communication is controversial. One set of proposals theorizes that small platforms made up of a few protein molecules each may be clustered in cell membranes and capable of moving around the cell surface together, most likely with help from the cytoskeleton. When these molecular "rafts" are brought together in the synapse with the key receptor proteins that detect disease in an opposing cell, their interaction could be what activates the immune cell. These preexisting platforms are contentious, however, because they are too small to see directly with an optical microscope, so evidence of their existence is somewhat indirect.

Another interesting possibility, with both indirect and direct support, is that the physical size of each type of protein forming the synapse can play an important role in determining where it goes when the cells come into contact. As proteins on one cell bind their counterparts on the opposing cell, the two cell membranes will be drawn together and the remaining gap between them will correspond to the size of the bound proteins. Thus, a central cluster of small proteins could bring the membranes close enough together to squeeze out larger proteins and hence segregate different types of protein to different regions of the synapse.

Arup K. Chakraborty and his colleagues at the University of California, Berkeley, used a mathematical model to test this idea by assessing the consequence of different-size proteins interacting across two opposing cell membranes. Although Chakraborty is not an

JANE C. STINCHCOMBE AND GILLIAN M. GRIFFITHS *University of Oxford*



AS A HUMAN T CELL leukemia virus (*red*) moves to an uninfected T cell from an infected one (*heading from right to left above*), the adhesion molecule talin (*green*) is seen concentrating where the cell membranes meet. In a view from inside the infected T cell (*above right*), the structure's similarity to an immune synapse suggests that HTLV and other viruses that prey on immune cells, such as HIV, may take advantage of cellular communication mechanisms to spread from cell to cell.

immunologist, a colleague had shown him images from Dustin's work, and the mathematician says that he became fascinated by the intriguing spatial patterns his immune cells might be forming whenever he had the flu. His group's analysis suggested that in fact the difference in size between proteins could be enough to cause bigger and smaller proteins to cluster in separate regions of the immune synapse.

Of course, immunologists also want to know what, if anything, these protein movements "mean" in the context of immune cell communication. The answer could be "nothing": the earliest conception of the immune synapse being a kind of gasket enabling immune cells to direct their secretion of cytokines to a target cell may be the sole purpose of the structure. Increasingly, however, evidence is suggesting that the synapse may also have other functions that, depending on the cells involved, could include initiating communication, or terminating it, or serving to modulate the volume, so to speak, of signals between two cells.

In 2002 Kupfer (now at the Johns Hopkins School of Medicine) observed, for example, that signaling between T cells and antigen-presenting cells before the SMAC begins to take shape fostered adhesion between the two cells but that a SMAC was necessary for the cells' interaction to produce T cell responses.

Yet Shaw and Allen, along with Dustin, now at New York University, and their co-workers have shown that productive signaling between T cells and APCs starts before the T cell receptors have clustered in their final position at the center of the synapse. In fact, some of the communication is done before the mature structure forms, implying that the mature synapse pattern might signal an end to the conversation.

These investigators and others have also been exploring what role synapse architecture might play in regulating the volume of dialogues between T cells and APCs. By pulling receptors away from their surface membrane during signaling, T cells can prevent themselves from being lethally overstimulated by too much antigen. Experiments have shown that T cells can reduce the number of receptors present in the synapse architecture to dampen signaling, or when only a small amount of antigen is available, T cells may cluster their receptors more closely within the synapse to amplify the signal.

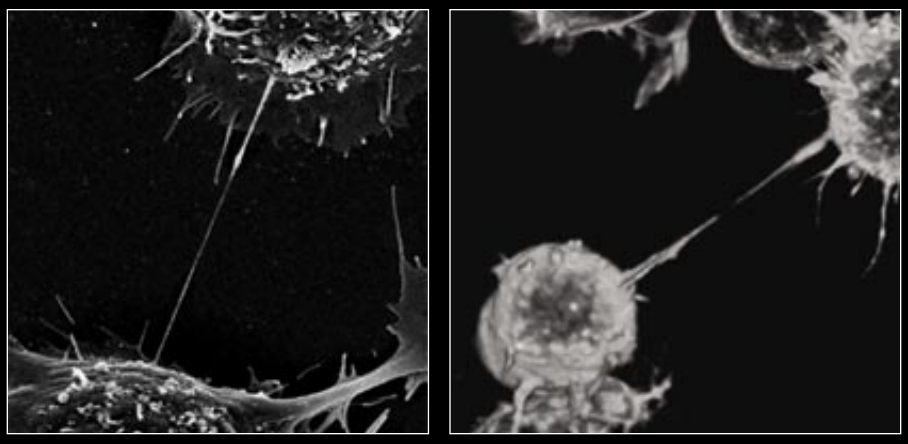
My own research group has been studying similar phenomena in natural killer cells, a type of immune cell that seeks and destroys cells damaged, for example, by a cancerous mutation or infected by a pathogen. These sick cells can lose the expression of some proteins on their surfaces, and NK cells recognize the loss as a sign of disease. We are finding that the amount of these proteins present on the target cell influences the pattern of the immune synapse formed by the NK cell. Different patterns correlate with whether or not the NK cell ultimately decides to kill the target cell, so the patterns may transmit, or at least reflect, information the NK cell uses to determine the extent of the target cell's illness.

Alongside these fascinating new insights into the possible functions of the immune synapse, disturbing news has emerged, too: another very recent observation is that the molecular dance that helps our immune cells communicate

THE AUTHOR

DANIEL M. DAVIS is a professor of molecular immunology at Imperial College London who specializes in high-resolution microscopy studies of immune cell interactions. Having started his scientific career as a physicist, he turned to immunology as an Irvington Institute postdoctoral research fellow in the Harvard University department of molecular and cellular biology. There, in 1999, he made the first images of the immune synapse structure in natural killer cells, which also provided the first sighting of synapse formation between two living cells. He has since written or co-authored more than 50 scientific papers in photophysics and immunology.

can be exploited by some viruses, including HIV. Charles R. M. Bangham of Imperial College London and his collaborators first showed that at the contact point between cells where viral particles are crossing over, proteins aggregate into a structure that resembles the immune synapse [*see illustration on opposite page*]. Several researchers have since observed similar "viral synapse" phenomena, and so it seems that viruses, which are known for hijacking cellular machinery to copy their genetic material, may also be able to co-opt cellular mechanisms for communication to propel themselves from one cell to another.



NANOTUBES made of cell membrane link two neural cells (*left*) and two immune cells (*right*). These recently discovered structures are still poorly understood but may constitute a novel mechanism for cells to communicate over long distances. Both immune and neural cells have been observed transferring proteins or calcium to one another through these nanotunnels, and viruses have been seen to travel from cell to cell within the tubes as well.

Healthy Voyeurism

THE DISCOVERY of the immune synapse has triggered a wave of research based on imaging immune cell interactions whose results have yet to be fully understood. But this fertile field is already producing new hypotheses and generating further research to test those. And the very idea of the synapse is already reshaping conceptions of the immune system, revealing it to be a sophisticated information-sharing network more like the nervous system than was previously realized.

Just using the synapse terminology to describe immune cell interactions has also encouraged neuroscientists and immunologists to compare notes, and they are finding that the two types of synapses use many common protein molecules. Agrin, for example, is an important protein involved in clustering other proteins at the synapse between neurons and muscle. Imaging experiments have shown that the same molecule also accumulates at immune synapses and can enhance at least some types of immune responses. Similarly, a receptor called neuropilin-1, known to participate in signaling between neurons, has been discovered at immune synapses. Experiments suggest that neuropilin-1 aids immune cells in their search for disease by helping to establish an immune synapse with other cells, but more research is needed to tease out the receptor's exact role in immunity.

My own team identified yet another intriguing similarity between neurons and immune cells when we observed that long tubes made of cell membrane readily form between immune cells and a variety of other cell types. Our investigation that led to this discovery was prompted by a report from German and Norwegian researchers of a similar phenomenon observed between neurons [*see illustration above*]. Neither we nor the neuroscientists know the function of these nanotubular highways, but finding out is a new goal for immunology and neuroscience alike.

These membrane nanotubes might, for example, constitute a previously unknown mechanism for immune cell communication by allowing directed secretion of cytokines between cells far apart. Simon C. Watkins and Russell D. Salter of the University of Pittsburgh School of Medicine have found that a population of immune cells could use such nanotubular highways to transmit calcium signals across vast (for cells) distances of hundreds of microns within seconds.

In the future, more studies of interactions among larger groups of immune cells could reveal additional aspects of immune cell communication networks. Imaging immune cell interactions as they traffic inside living organisms, rather than on a slide, is another important frontier for this line of research.

In a recent memoir, Nobel laureate John Sulston described using cutting-edge microscopy in the 1970s to understand worm development: "Now to my amazement, I could watch the cells divide. Those Nomarski images of the worm are the most beautiful things imaginable.... In one weekend I unraveled most of the postembryonic development of the ventral cord, just by watching."

High-resolution microscopy of immune cell interactions is still a very young field, and more surprises are surely in store. Virtually all the surface proteins involved in immune cells' recognition of disease have been identified and named. But the ability of scientists to now observe as these molecules play out their roles in space and time has revealed the immune synapse mechanism and reconfirmed the value of "just watching" as a scientific method. SA

MORE TO EXPLORE

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