Carbohydrates in Cell Recognition

Telltale surface sugars enable cells to identify and interact with one another. New drugs aimed at those carbohydrates could stop infection and inflammation

by Nathan Sharon and Halina Lis

In 1952 Aaron Moscona of the University of Chicago separated the cells of a chick embryo by incubating them in an enzyme solution and swirling them gently. The cells did not remain apart; they coalesced into a new aggregate. Moreover, Moscona saw that when retinal cells and liver cells were allowed to coalesce in this way, the retinal cells always migrated to the inner part of the cellular mass. Three years later Philip L. Townes and Johannes Holtfreter of the University of Rochester performed a similar experiment with cells from amphibian embryos, which re-sorted themselves into tissue layers like those from which they had come.

Those experiments and countless other observations testify to the keen ability of cells to recognize one another and to respond accordingly. Sperm, for example, can distinguish eggs of their own species from those of others, and they will bind only with the former. Some bacteria settle preferentially in the intestinal or urinary tract; others fancy different organs.

It is not surprising, then, that decoding the language of cellular interactions holds profound interest for researchers in many areas of biology and medicine. Although we still do not understand the chemical basis of most cell-recognition phenomena, clear explanations for some have emerged during the past decade. Proteins, which mediate most of the chemical reactions inside living organisms, appear on the cell surface as well, and they certainly play a part.

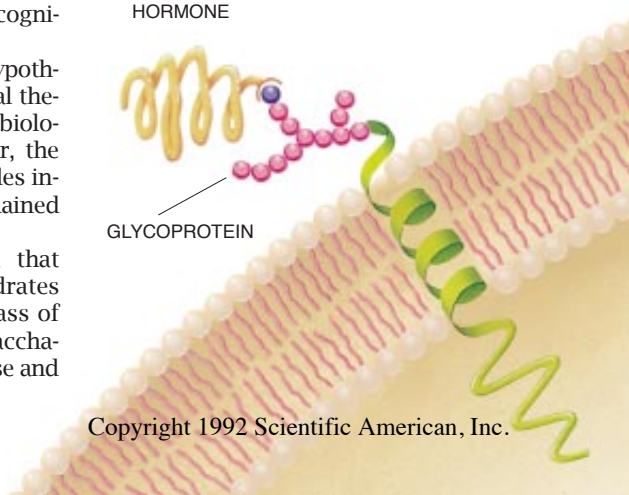
Yet the accumulating evidence also suggests that in many cases carbohydrates (frequently referred to as sugars) are the primary markers for cell recognition. Discoveries about the involvement of specific sugars in recognition will have practical applications to the prevention and treatment of a variety of ailments, including cancer.

Biologists generally accept that cells recognize one another through pairs of complementary structures on their surfaces: a structure on one cell carries encoded biological information that the structure on the other cell can decipher. That idea represents an extension of the lock-and-key hypothesis formulated in 1897 by Emil Fischer, the noted German chemist. He used it to explain the specificity of interactions between enzymes and their substrates. Pioneering immunologist Paul Ehrlich extended it in 1900 to account for the highly specific reactions of the immune system, and in 1914 Frank Rattray Lillie of the University of Chicago invoked it to describe recognition between sperm and eggs.

By the 1920s the lock-and-key hypothesis had become one of the central theoretical assumptions of cellular biology. Yet for many years thereafter, the nature and identity of the molecules involved in cellular recognition remained a complete mystery.

To most biologists, the idea that the molecules might be carbohydrates seemed farfetched. That large class of compounds consists of monosaccharides (simple sugars such as glucose and fructose) and of oligosaccharides and polysaccharides, which are composed of linked monosaccharides. Until the late 1960s, carbohydrates were thought to serve only as energy sources (in the forms of monosaccharides and storage molecules such as the polysaccharide starch) and as structural materials (the polysaccharides cellulose in plants and chitin in the exoskeletons of insects). The two other major classes of biological materials—nucleic acids, which carry genetic information, and proteins—were obviously far more versatile. By comparison, carbohydrates looked like dull, second-class citizens.

Interest in carbohydrates was further discouraged by the extraordinary complexity of their structures. In contrast to the nucleotides in nucleic acids and the amino acids in proteins, which can interconnect in only one way, the monosaccharide units in oligosaccharides and polysaccharides can attach to one another at multiple points. Two identical monosaccharides can bond to form 11 different disaccharides, whereas two amino acids can make only one dipeptide. Even a small number of monosaccharides can create a staggering diversity of compounds, including many with branching structures. Four different nucleotides can make only 24 distinct tet-



NATHAN SHARON and HALINA LIS have been members of the biophysics department of the Weizmann Institute of Science in Rehovot, Israel, for more than 30 years. During most of that time, they have collaborated closely on the study of complex carbohydrates and lectins, proteins that bind selectively to carbohydrates. In addition to their many joint scientific papers, they have written several widely cited review articles on those topics, as well as the recent book *Lectins* (Chapman & Hall, London). This is Sharon's fifth article for *Scientific American*.

ranucleotides, but four different monosaccharides can make 35,560 unique tetrasaccharides.

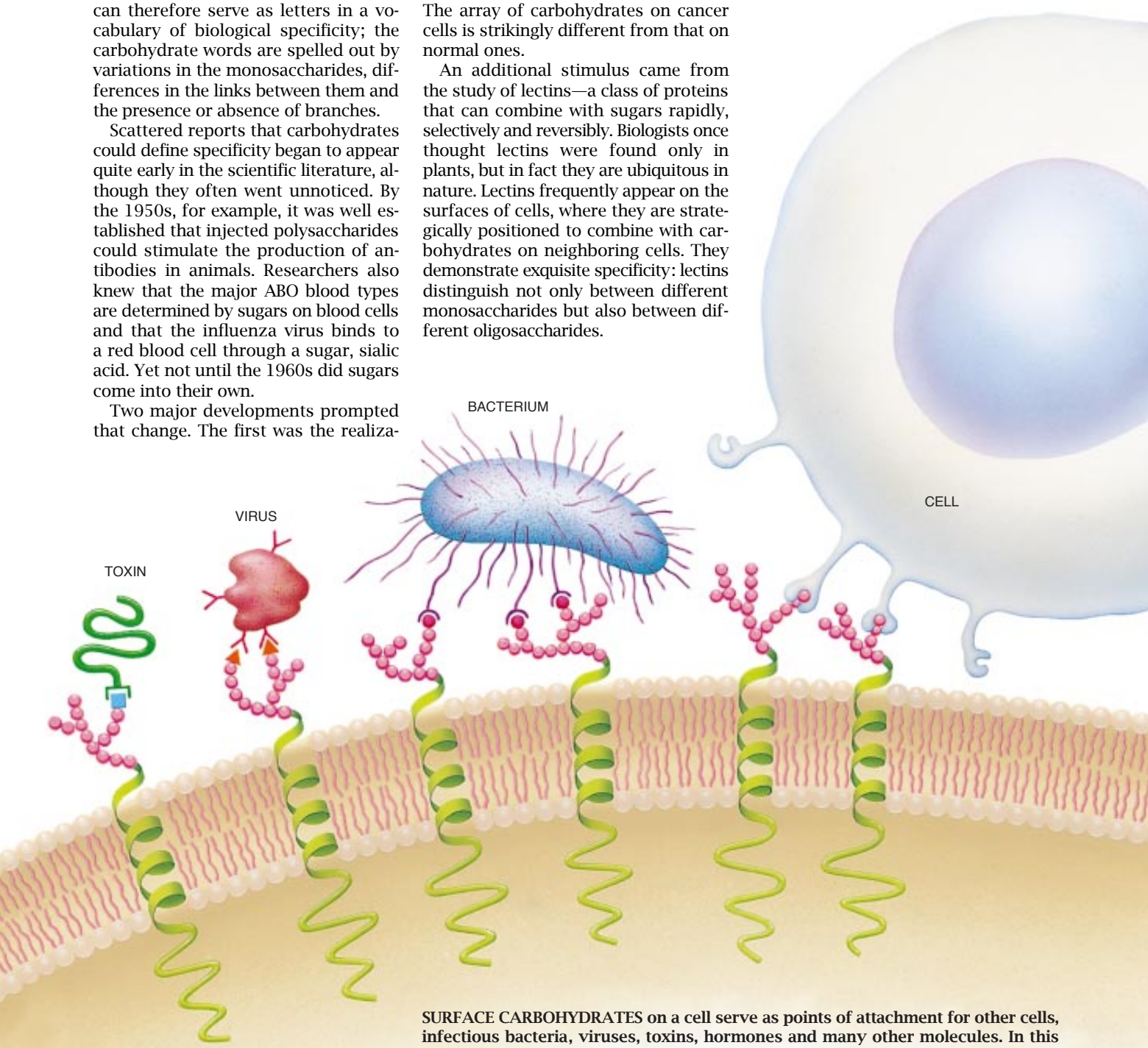
This potential for structural diversity is the bane of the carbohydrate chemist, but it is a boon to cells: it makes sugar polymers superbly effective carriers of information. Carbohydrates can carry much more information per unit weight than do either nucleic acids or proteins. Monosaccharides can therefore serve as letters in a vocabulary of biological specificity; the carbohydrate words are spelled out by variations in the monosaccharides, differences in the links between them and the presence or absence of branches.

Scattered reports that carbohydrates could define specificity began to appear quite early in the scientific literature, although they often went unnoticed. By the 1950s, for example, it was well established that injected polysaccharides could stimulate the production of antibodies in animals. Researchers also knew that the major ABO blood types are determined by sugars on blood cells and that the influenza virus binds to a red blood cell through a sugar, sialic acid. Yet not until the 1960s did sugars come into their own.

Two major developments prompted that change. The first was the realization that all cells carry a sugar coat. This coat consists for the most part of glycoproteins and glycolipids, two types of complex carbohydrates in which sugars are linked to proteins and lipids (fats), respectively. Several thousands of glycoprotein and glycolipid structures have been identified, and their number grows almost daily. This diversity is surely significant: the repertoire of surface structures on a cell changes characteristically as it develops, differentiates or sickens. The array of carbohydrates on cancer cells is strikingly different from that on normal ones.

An additional stimulus came from the study of lectins—a class of proteins that can combine with sugars rapidly, selectively and reversibly. Biologists once thought lectins were found only in plants, but in fact they are ubiquitous in nature. Lectins frequently appear on the surfaces of cells, where they are strategically positioned to combine with carbohydrates on neighboring cells. They demonstrate exquisite specificity: lectins distinguish not only between different monosaccharides but also between different oligosaccharides.

A landmark discovery about the role of lectin-carbohydrate interactions in cell recognition came from the work of G. Gilbert Ashwell of the National Institutes of Health and Anatol Morell of the Albert Einstein College of Medicine. In 1968 they enzymatically removed a few sialic acid molecules from certain



SURFACE CARBOHYDRATES on a cell serve as points of attachment for other cells, infectious bacteria, viruses, toxins, hormones and many other molecules. In this way, carbohydrates mediate the migration of cells during embryo development, the process of infection and other phenomena. Compounds consisting of carbohydrates that are chemically linked to proteins are called glycoproteins; those in which the carbohydrates are linked to fats are glycolipids.

blood plasma glycoproteins, then injected the glycoproteins into rabbits. Ordinarily, such molecules would persist in the animals' circulation for some time, but the sialic acid–deficient molecules quickly disappeared.

Ashwell and Morell found that the glycoproteins ended up in the liver. The removal of the sialic acids had unmasked galactose in the glycoproteins, and the exposed galactoses had attached to a lectin on the liver cells. Subsequently, the researchers learned that if they removed both the sialic acids and the uncovered galactoses from the glycoproteins, the rate at which the molecules were eliminated from the blood returned to normal. From those results, Ashwell and Morell concluded that carbohydrate side chains on proteins may serve as markers for identifying which ones should be removed from the circulation and eventually degraded.

Like the surface carbohydrates, the surface lectins go through changes that coincide with a cell's physiological and pathological states. For instance, in 1981 Reuben Lotan and Abraham Raz of the Weizmann Institute of Science showed that tumor cells from mice and humans carry a surface lectin not found on normal cells. They and other researchers later proved that this lectin is involved in the development of metastases.

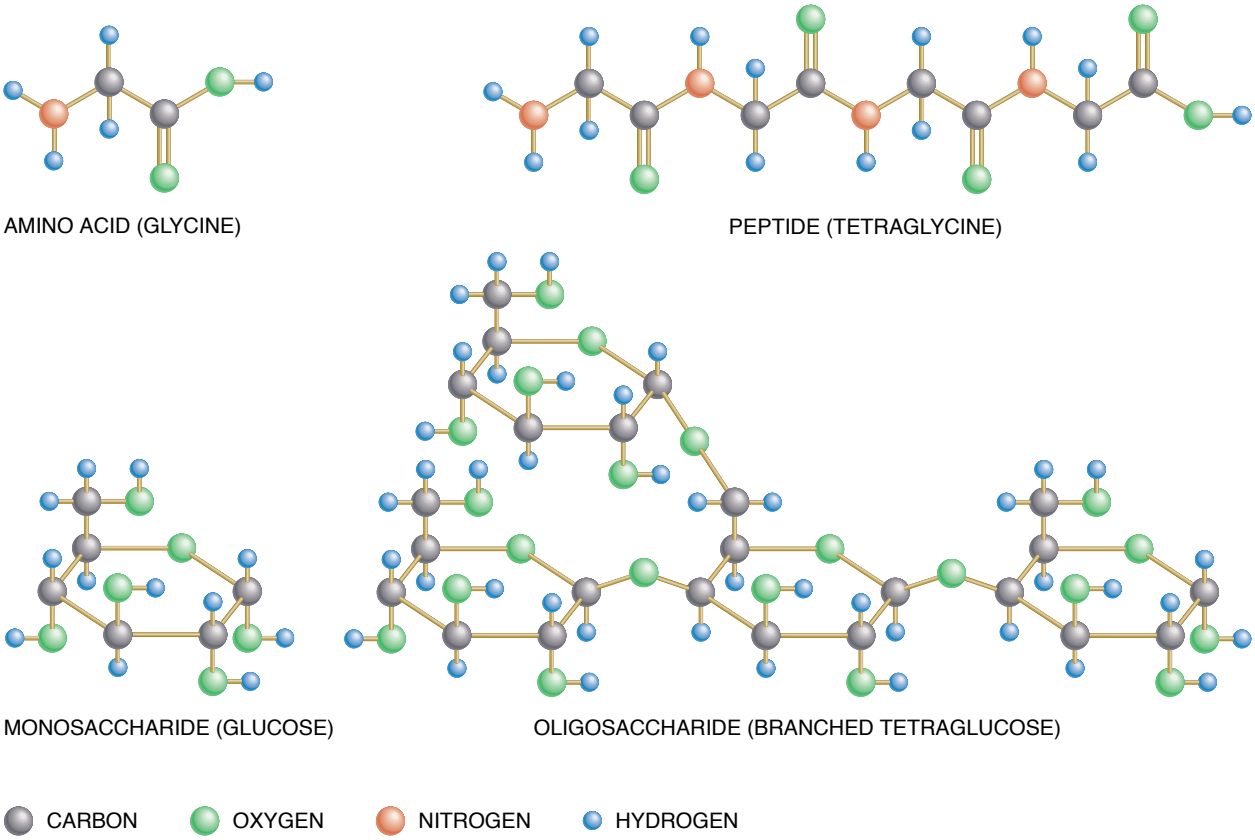
A striking recent illustration of the role of surface sugars and the molecules that bind to them comes from studies of embryo formation by Senitiroh Hakomori of the Fred Hutchinson Cancer Research Center in Seattle and by Ten Feizi of the Clinical Research Center in Harrow, England. Working with mouse embryos, they have shown that as a fertilized egg divides, the carbohydrate structures on the resulting embryonic cells change in characteristic ways. One of the carbohydrates is a trisaccharide known both as stage-specific embryonic antigen 1 (SSEA-1) and as Lewis^x (Le^x). It appears at the eight- to 16-cell stage, just as the embryo compacts from a group of loose cells into a smooth ball.

Hakomori's group has shown that a soluble compound carrying multiple units of the same trisaccharide inhibits the compaction process and disrupts embryogenesis. Closely related but structurally different carbohydrates have no effect. Thus, the Le^x trisaccharide appears to play a part in compaction.

Adhesive carbohydrates are therefore essential to embryonic development. As research continues, their role in that process will become more detailed. Today the two best-understood phenomena of that type are microbial adhesion to host cells and the adhesion

The Complexity of Carbohydrate Structures

Carbohydrates, nucleic acids and proteins all carry biological information in their structures. Yet carbohydrates offer the highest capacity for carrying information because they have the greatest potential for structural variety. Their component molecules, monosaccharides, can interconnect at several points to form a wide variety of branched or linear structures; in the example below, the branching carbohydrate is only one of many possible structures that can be made from four identical glucose molecules. The amino acids in proteins as well as the nucleotides in nucleic acids can form only linear assemblies, which restricts their diversity. The peptide (protein fragment) shown here is the only one possible made from four molecules of the amino acid glycine.



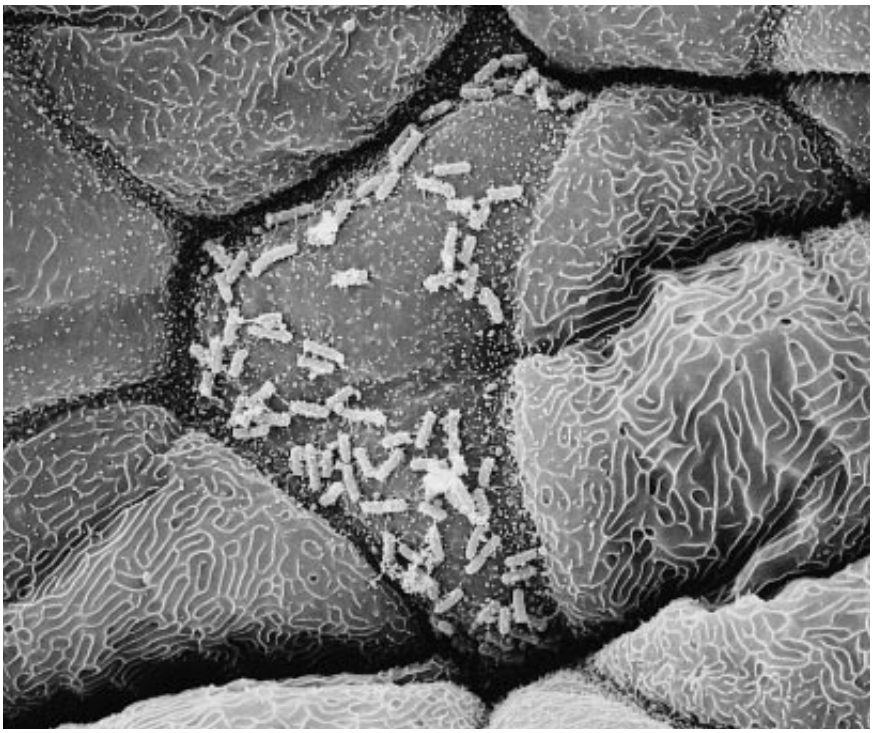
of white blood cells to blood vessels. The more thoroughly characterized of these interactions is microbial adhesion, which has been studied for nearly two decades and serves as a model for other forms of carbohydrate-mediated cell recognition.

To cause disease, viruses, bacteria or protozoa must be able to stick to at least one tissue surface in a susceptible host. Infectious agents lacking that ability are swept away from potential sites of infection by the body's normal cleansing mechanisms. Microorganisms in the upper respiratory tract, for example, may be swallowed and eventually destroyed by stomach acid; those in the urinary tract may be flushed out in the urine.

The first clues about the mechanism of bacterial adhesion sprang from a series of pioneering studies by J. P. Duguid of Ninewells Hospital Medical School in Dundee that began in the 1950s. Duguid demonstrated that many strains of *Escherichia coli* (a bacterial denizen of the intestines that can also colonize other tissues) and related bacteria adhered to cells from the epithelial lining of tissues and to erythrocytes, or red blood cells. In the presence of sticky bacteria, the erythrocytes would clump together—a phenomenon called hemagglutination. (Researchers still routinely use hemagglutination as a simple test for the adhesion of bacteria to animal cells.) To learn how the bacteria bound to the cells, Duguid exposed them to a wide range of compounds. He found that only the monosaccharide mannose and very similar sugars could inhibit hemagglutination.

Duguid also made the important observation that the bacterial strains responsible for mannose-sensitive hemagglutination had submicroscopic, hairlike appendages on their surfaces. These structures were five to 10 nanometers in diameter and several hundreds of nanometers long. He called them fimbriae, from the Latin word for fringe. Almost simultaneously, Charles C. Brinton, Jr., of the University of Pittsburgh described the same structures and named them pili, from the Latin word for hairs. Both terms are still in use.

Later, starting around 1970, Ronald J. Gibbons of the Forsyth Dental Center in Boston and his colleagues began reporting on the selective adhesion of bacteria to niches within the oral cavity. Gibbons observed that *Actinomyces naeslundii* colonizes both the epithelial surfaces of infants without teeth and the teeth of children and adults. Conversely, the related bacterium *A. viscosus* does not appear in the mouth until the teeth erupt from the gums; it exhibits a preference for teeth rather than oral epithelial surfaces.



BACTERIA ADHERE to tissues selectively. Hairlike protrusions called fimbriae on the bacteria bind exclusively to certain surface carbohydrates. These interactions determine which tissues are susceptible to bacterial invasion. Rod-shaped *Escherichia coli* bacteria are shown here on tissue from the urinary tract.

Today it is clear that the tissue specificity of bacterial adhesion is a general phenomenon. For example, *E. coli,* the most common cause of urinary tract infections, is abundant in tissues surrounding the ducts that connect the kidneys and the bladder, yet it is seldom found in the upper respiratory tract. In contrast, group A streptococci, which colonize only the upper respiratory tract and skin, rarely cause urinary tract infections.

Bacterial adhesion varies not only between tissues but also between species and sometimes between individuals of the same species, depending on their age, genetic makeup and health. In the early 1970s R. Sellwood and Richard A. Gibbons and their colleagues at the Institute for Research on Animal Diseases in Compton, England, studied the infectivity of the K88 strain of *E. coli*. Because those bacteria cause diarrhea in piglets, they are a costly nuisance for farmers. Gibbons's group found that the K88 bacteria adhered to the intestinal cells of susceptible piglets but not to those of adult pigs or of humans, which the bacteria cannot infect. Bacterial mutants that had lost the ability to bind to intestinal cells proved unable to infect the animals.

Moreover, as Gibbons's work showed, some piglets had a genetic resistance to K88 bacteria: even potentially virulent bacteria could not bind to cells from their intestines. By selecting genetically immune piglets for breeding, farmers were able to obtain K88-resistant progeny.

The gonorrhea organism, *Neisseria gonorrhoeae,* serves as another example of species and tissue specificity. It adheres to human cells of the genital and oral epithelia but not to cells from other organs or other animal species. That fact explains why humans are the exclusive host for *N. gonorrhoeae* and why other animals do not contract gonorrhea.

A strong impetus to the study of bacterial adhesion was a proposal made in 1977 by Itzhak Ofek of Tel Aviv University, David Mirelman of our department at the Weizmann Institute and one of us (Sharon). We suggested that bacterial adhesion is mediated by surface lectins on bacteria that bind to complementary sugars on host cells. That idea has proved to be generally valid. Work in many laboratories has shown that bacteria produce lectins specific for certain carbohydrates and that the bacteria depend on those lectins for adhering to a host's tissue as the first step in the process of infection.

Bacterial lectins have already been the focus of much study, although far

more remains to be done. The best-characterized lectins are the type 1 fimbriae of *E. coli*, which bind preferentially to surface glycoproteins containing mannose. Other research on *E. coli* during the past decade, primarily by Catharina Svanborg-Edén and her colleagues while working at the University of Göteborg, has described in detail the P fimbriae. Those fimbriae interact specifically with the P blood-group substance, an extremely common glycolipid containing the disaccharide galabiose. Research groups led by Karl-Anders Karlsson of the University of Göteborg and Victor Ginsburg of the NIH have mapped the specificities of lectins from a wide range of other bacterial species and strains.

These studies have shown that bacteria do not bind solely to the ends of surface carbohydrates—they can also sometimes bind to sugars located within the structure. Furthermore, different bacteria may bind to different parts of the same carbohydrate. Occasionally, only one face of an oligosaccharide may be exposed on a particular cell, and as a result the cell will bind bacteria of one kind and not other ones. The ability of cell-surface sugars to serve as attachment sites therefore depends not only on the presence of these sugars but also on their accessibility and their mode of presentation.

Considerable experimental evidence now greatly strengthens the conclusion that the binding of bacteria to host cell-surface sugars initiates infection. For example, uroepithelial cells from those rare individuals who lack the P blood-group substance do not bind to P-fimbriated *E. coli*. Such individuals are much less susceptible to infections from those bacteria than the rest of the population is. Experiments have shown, however, that the bacteria will bind if the epithelial cells are first coated with a synthetic glycolipid containing galabiose.

Similarly, intestinal cells from piglets that are resistant to the diarrhea-causing K88 *E. coli* lack the large carbohydrate to which the bacteria bind. Although the exact structure of this carbohydrate has not yet been elucidated, it is known to be present in susceptible piglets but absent in adult pigs. This explains why the bacteria were unable to attach to and colonize the intestines of the adult pigs, while they caused infection in the young piglets.

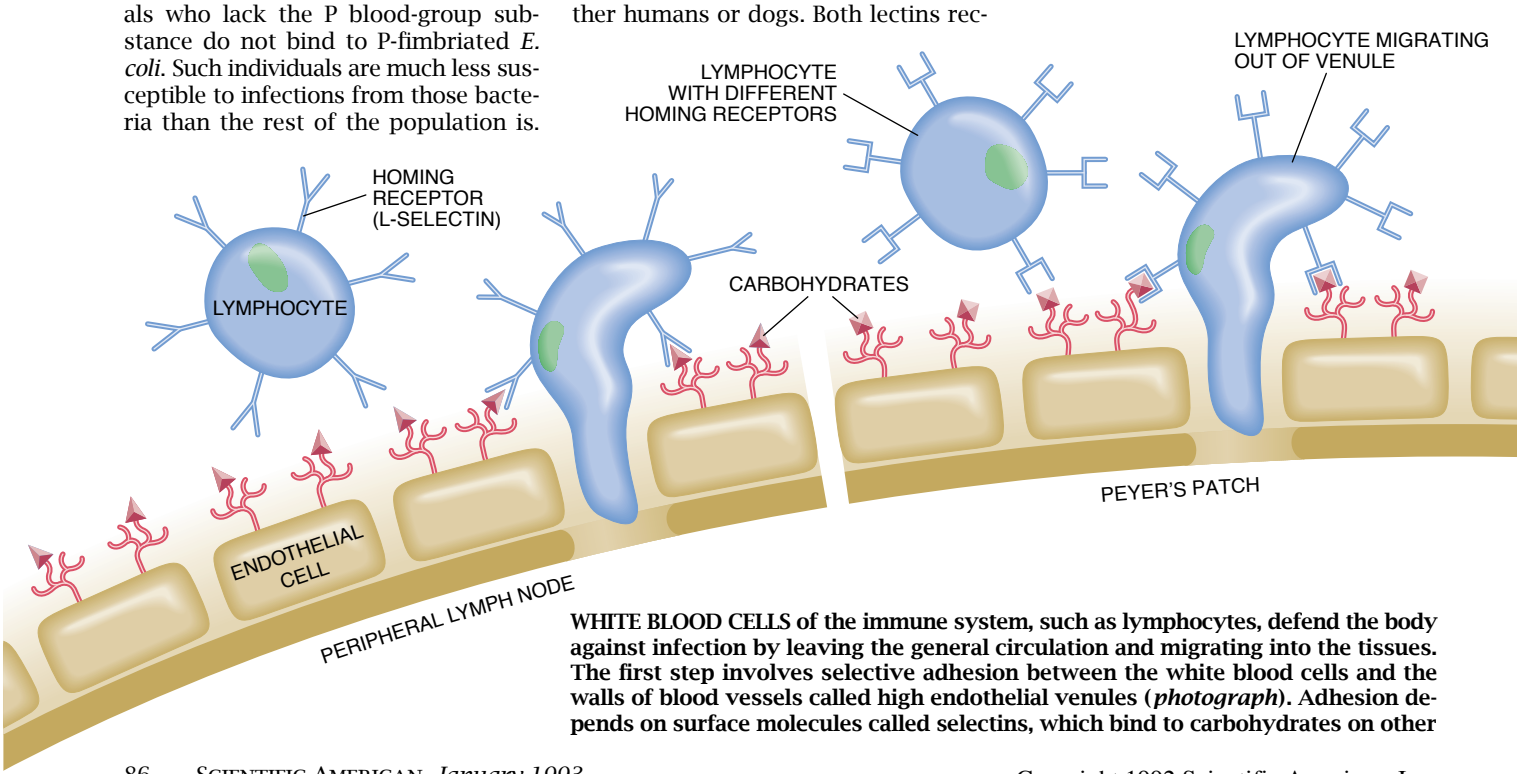
Another interesting case is that of the K99 strain of *E. coli*. Like the K88 strain, K99 causes diarrhea in farm animals but not in humans. It is less specific than K88, however, because it infects young calves and lambs as well as piglets. The K99 bacteria bind specifically to an unusual glycolipid that contains *N*-glycoloylneuraminic acid (a special type of sialic acid) linked to lactosylceramide. This glycolipid, which is present in piglets, calves and lambs, is absent from the cells of adult pigs and humans, which instead contain *N*-acetylneuraminic acid, a nonbinding analogue of sialic acid. Here a small difference between two highly similar sugars—the replacement of an acetyl group by a glycoloyl group—is readily detected by the bacteria and explains the host range of infection by the organism.

Further confirmation of the above conclusions was recently obtained in experiments on two fimbrial lectins from *E. coli* that infect the urinary tracts of either humans or dogs. Both lectins recognize galabiose, yet one binds only to the human uroepithelial cells and the other only to canine cells; the galabiose-bearing glycolipids on the surface of the cells are presented in subtly different ways. Those lectin-binding patterns accord with the host specificities of the *E. coli* strains.

Because bacterial adhesion is so critical to infection, medical researchers are seriously considering the use of sugars for prevention and treatment. Sugars that selectively inhibited adhesion could act as molecular decoys, intercepting pathogenic bacteria before they reached their tissue targets. Urinary tract infections have been the focus of particular attention because they are second only to respiratory infections in frequency.

In collaboration with Ofek, Moshe Aronson of Tel Aviv University and Mirelman, we performed the first study along those lines in 1979. We injected a mannose-specific strain of *E. coli* into the urinary bladder of mice. In some animals, we also injected methyl alpha-mannoside, a sugar that in the test tube inhibited bacterial adhesion to epithelial cells. The presence of the sugar reduced the colonization of the urinary tract by bacteria.

Svanborg-Edén has performed analogous experiments with P-fimbriated *E. coli* that infect the kidneys of mice. She incubated the bacteria in solutions of globotetraose, a sugar found in the glycolipid of kidney cells. When she subsequently injected those bacteria into



WHITE BLOOD CELLS of the immune system, such as lymphocytes, defend the body against infection by leaving the general circulation and migrating into the tissues. The first step involves selective adhesion between the white blood cells and the walls of blood vessels called high endothelial venules (*photograph*). Adhesion depends on surface molecules called selectins, which bind to carbohydrates on other

mice, they persisted in the kidneys for less time than untreated bacteria did. James A. Roberts of Tulane University obtained similar results in experiments on monkeys: incubation of P-fimbriated *E. coli* with a galabioselike sugar significantly delayed the onset of urinary tract infections.

Glycopeptides can also interfere with the binding of bacteria to host tissues. In 1990 Michelle Mouricout of the University of Limoges in France and her co-workers showed that injections of glycopeptides taken from the blood plasma of cows can protect newborn calves from lethal doses of *E. coli*. The glycopeptides, which contain sugars for which the bacteria have affinities, decrease the adhesion of the bacteria to the intestines of treated animals.

Indeed, to interfere with bacterial adhesion, one need not even use a carbohydrate—any agent that competitively binds to either the bacterial lectin or the host cell's surface carbohydrate will do. For example, Edwin H. Beachey and his colleagues at the Veterans Administration Medical Center and the University of Tennessee at Memphis have used antibodies against mannose to prevent certain mannose-specific *E. coli* from infecting mice. The antibodies bind to mannose on the cells, thereby blocking the sites of bacterial attachment.

Those successful experiments make a clear case for antiadhesive therapies against microbial diseases. The application of this approach in humans is now the subject of intense research. Further studies of the sugars on host cells and of bacterial lectins should lead to the design of better adhesion inhibitors. One point about the approach is certain: because different infectious agents—even different bacteria within the same strain—can have a wide variety of carbohydrate specificities, a cocktail of inhibitors will undoubtedly be necessary to prevent or treat the diseases.

Carbohydrate-directed interactions between cells are not restricted to pathological phenomena; they are also crucially important to the healthy operation of the immune system. The immune system has many parts, but its most important soldiers are the cells called leukocytes. This group includes an array of diverse white blood cells—lymphocytes, monocytes and neutrophils—that act jointly to eliminate bacteria and other intruders and to mediate the inflammation response in injured tissues. All these cells circulate in the blood, but they accomplish their major functions in the extravascular spaces.

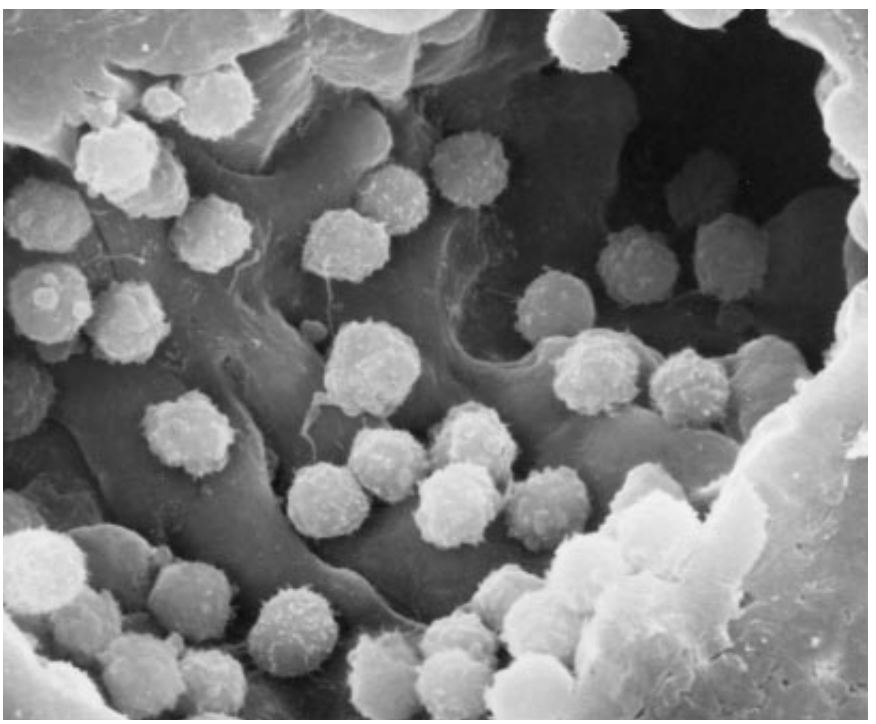
The picture emerging from research is that the inner lining of blood vessels, called the endothelium, actively snares white blood cells and guides them to where they are needed. This process requires an exquisitely regulated recognition between the circulating leukocytes and the endothelial cells.

Such recognition seems to be mediated by a family of structurally related lectins. Because this field of research is so new and because different laboratories often identify the same adhesion molecules simultaneously, the nomenclature is still in a somewhat chaotic state. Most researchers refer to these molecules as selectins because they mediate the selective contact between cells. Another name in vogue is LEC-CAMs, an acronym for leukocyte-cell (or lectin) adhesion molecules.

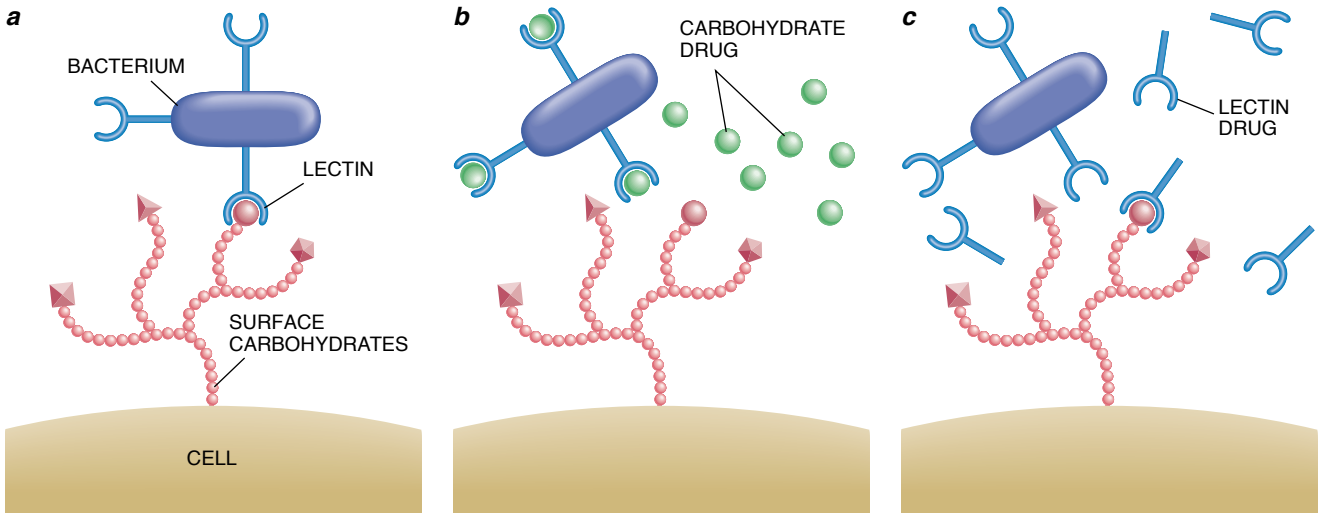
The selectins are highly asymmetric composite proteins, with an unusual mosaic architecture. They consist of three types of functional domains: one domain anchors the selectin in the cell membrane, and a second makes up most of the body of the molecule. The third domain, located at the extracellular tip of the molecule, structurally resembles animal lectins that work only in the presence of calcium ions. The binding of carbohydrate ligands to that domain is central to the function of selectins in interactions between cells.

About 10 years ago Eugene C. Butcher and Irving L. Weissman of Stanford University laid the foundation for our current understanding of how selectins (which were then unknown) direct lymphocyte traffic. Lymphocytes are unique among leukocytes in that they continuously patrol the body in search of foreign antigens (immunologically significant molecules) from bacteria, viruses and the like. For that purpose, lymphocytes leave the blood vessels and migrate through the lymph nodes, the tonsils, the adenoids, the Peyer's patches in the intestines or other secondary lymphoid organs. Different lymphocytes migrate selectively, or home, toward particular organs. To exit from the bloodstream, lymphocytes must first bind to specialized submicroscopic blood vessels less than 30 microns in diameter, known as high endothelial venules.

Using an assay technique developed by Hugh B. Stamper, Jr., and Judith J. Woodruff of the State University of New York in Brooklyn, Butcher and Weissman observed that the homing specificity of mouse lymphocytes is dictated by their selective interaction with the high endothelial venules in their targeted organs. Butcher and Weissman then developed a monoclonal antibody, MEL-14, that bound only to mouse lymphocytes that went to the peripheral lymph nodes. On slices of tissue, the antibody blocked the attachment of the lymphocytes to high endothelial venules



cells. The L-selectins, or homing receptors, on lymphocytes determine the endothelial cells to which a lymphocyte will stick: for example, some adhere only in peripheral lymph nodes or to the Peyer's patches in the intestines. After a lymphocyte has attached to the endothelium, it can migrate out of the blood vessel.



BLOCKING BACTERIAL ATTACHMENT is one strategy for combating infections. As a prelude to infection, bacterial surface proteins called lectins attach to surface carbohydrates on susceptible host cells (*a*). Drugs containing similar carbohydrates could prevent the attachment by binding to the lectins (*b*). Alternatively, drugs consisting of lectinlike molecules could have the same effect by innocuously occupying the binding sites on the carbohydrates (*c*).

from those tissues but not from other lymph organs. When injected into mice, MEL-14 inhibited the migration of lymphocytes into the peripheral lymph nodes.

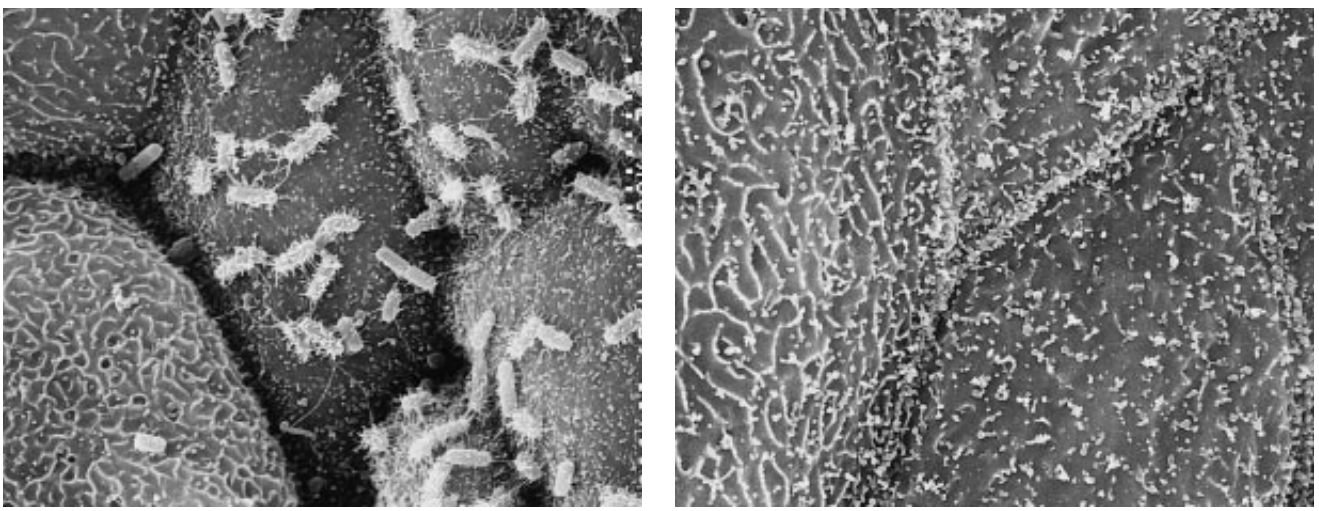
Butcher and Weissman went on to show that their antibody bound on the lymphocyte membrane to a single glycoprotein, now known as L-selectin. Because that glycoprotein is responsible for the specific binding of the lymphocytes to the high endothelial venules, it is also known as the homing receptor.

If high endothelial venules from the lymph nodes are exposed to solutions of L-selectin, lymphocytes cannot bind to them: the L-selectin molecules occupy all the potential attachment sites on the endothelial cells. Conversely, as Steven D. Rosen of the University of California at San Francisco has shown, certain small sugars and larger polysaccharides can also block interactions between lymphocytes and endothelial venules. In those cases, the sugars are binding to the L-selectin.

In 1989 separate experiments by Weissman and by Laurence A. Lasky of Genentech in South San Francisco in collaboration with Rosen proved conclusively that the homing receptor mediates the adhesion of lymphocytes to endothelial cells. The structure of the endothelial carbohydrate to which it binds is still unknown.

In contrast to the homing receptor, the two other known selectins are found mainly on endothelial cells, and then only when they are actively attracting leukocytes. One of these, E-selectin (ELAM-1), was discovered in 1987 by Michael P. Bevilacqua of Harvard Medical School. The third member of the group, P-selectin (previously known as GMP-140 and PADGEM), was independently discovered a couple of years later by Rodger P. McEver of the Oklahoma Medical Research Foundation and by Bruce and Barbara Furie of the Tufts University School of Medicine.

Research has clarified how tissues use selectins to steer white blood cells where they are needed. When a tissue is infected, it defensively secretes proteins called cytokines, such as interleukin-1 and tumor necrosis factor. The cytokines stimulate endothelial cells in the venules to express P- and E-selectins on their surfaces. Passing white blood cells adhere to these protruding mole-



SELECTIVE EFFECTS of carbohydrates on bacteria are illustrated in these photographs. These *E. coli* have a lectin for the P glycolipid. Bacteria incubated in the sugar mannose can still cling to epithelial tissue (*left*). A constituent of the P glycolipid binds to the bacteria's lectin and prevents adhesion (*right*).

cules because their carbohydrate coat contains complementary structures. Once attached to the wall of a venule, a leukocyte can leave the bloodstream by squeezing between adjacent endothelial cells.

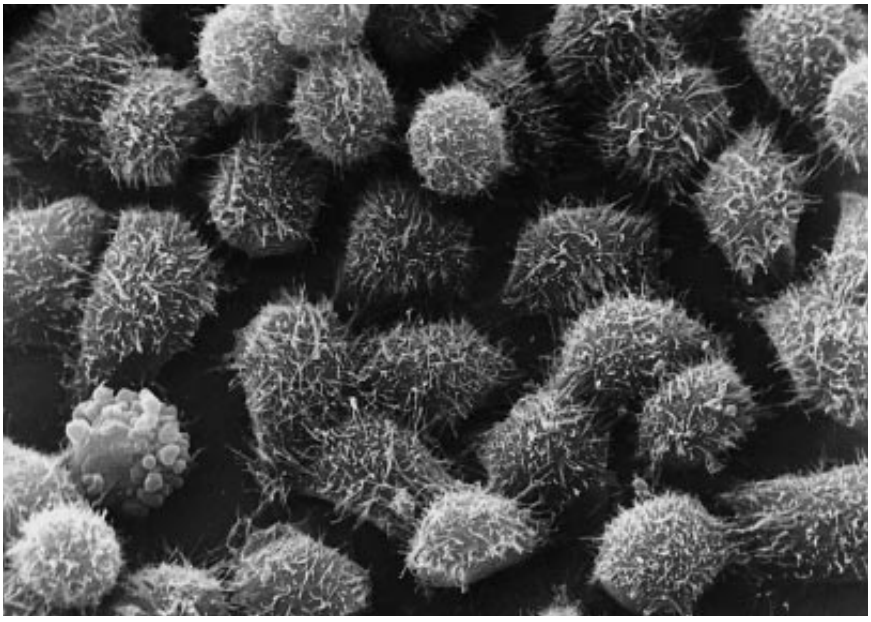
These two selectins appear on endothelial cells at different times and recruit different types of white blood cells. Endothelial cells have an internal stockpile of P-selectin that they can mobilize to their surface within minutes after an infection begins. P-selectin can therefore draw leukocytes that act during the earliest phases of the immunologic defense. In contrast, endothelial cells synthesize E-selectin only when it is required, so it takes longer to appear. That selectin seems to be most important about four hours after the start of an infection, after which it gradually fades away.

The mechanism that helps leukocytes to breech the endothelial barrier is indispensable to the fulfillment of their infection-fighting duties. Yet acting inappropriately, that same mechanism also allows leukocytes to accumulate in tissues where they do not belong, thereby causing tissue damage, swelling and pain.

The inflammation of rheumatoid arthritis, for instance, occurs when white blood cells enter the joints and release protein-chopping enzymes, oxygen radicals and other toxic factors. Another example is reperfusion injury, a disorder that occurs after the flow of blood is temporarily cut off from a tissue, such as during a heart attack. When the blood flow resumes, the white blood cells destroy tissues damaged by lack of oxygen.

The development of pharmaceutical reagents that would inhibit adverse inflammatory reactions holds major interest for the academic, clinical and industrial sectors. In theory, any drug that interferes with the adhesion of white blood cells to the endothelium, and consequently with their exit from the blood vessel, should be anti-inflammatory. The key to developing such drugs is the shape of the binding regions of the selectin molecules and the shape of the carbohydrates that fit into them. Work on determining those shapes is proceeding at a ferocious pace. In parallel research, intensive attempts are being made to synthesize carbohydrate inhibitors of the P- and E-selectins.

For an antiadhesive therapy to be successful, the drugs must simultaneously accomplish two seemingly incompatible ends. On the one hand, they must stop white blood cells from leaving the bloodstream inappropriately; on the other hand, they must still allow the cells to go where they are needed. Those goals may be achievable because the specificities of adhesion molecules vary in different tissues. One can envision, for example, a drug that keeps white cells from entering the joints but not other parts of the body.



CANCER CELLS have unusual carbohydrates on their surface, which may account for many of their invasive properties. Drugs that interfere with the adhesiveness of abnormal cells may someday be used in cancer therapies.

Aside from their involvement in inflammation, cell-adhesion molecules may play a role in other diseases, such as the spread of cancer cells from the main tumor throughout the body. For example, the carbohydrate recognized by E-selectin is expressed on cells from diverse tumors, including some cancers. Bevilacqua has recently reported that at least one type of human cancer cell binds specifically to E-selectin expressed on activated endothelium. Perhaps to promote their own metastasis, some malignant cells recruit the adhesion molecules that are part of the body's defenses.

If so, antiadhesive drugs may also turn out to be antimetastatic. One hopeful sign in that direction recently came from Hakomori's group, which was studying highly metastatic mouse melanoma cells that carry a lectin for lactose, the sugar in milk. The researchers found that by exposing the melanoma cells to compounds containing lactose before injecting them into mice, they could reduce the metastatic spread of the cells almost by half.

Although the importance of carbohydrates in cell recognition is immense, other modes of recognition that rely on a peptide language do exist. Some forms of attachment, for instance, involve surface proteins called integrins and complementary peptides. The existence of more than one system for binding activities lends greater flexibility to a cell's repertoire of interactions.

Biomedical researchers are still striving for a better understanding of the sugar structures on cell surfaces and of the specificities that lectins have for those structures. As they learn more, they will be in a better position to design highly selective, extremely powerful inhibitors of cell interactions. The day may not be far off when antiadhesive drugs, possibly in the form of pills that are both sugar-coated and sugar-loaded, will be used to prevent and treat infections, inflammations, the consequences of heart attacks and perhaps even cancer.

FURTHER READING

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