ORAL INTERACTIONS

The molecular basis for bacterial binding

By JULIE ANN MILLER

Second of two articles

Adhesion of bacteria to different surfaces is key to many infectious diseases, but it is especially important in the mouth, where free-floating microbes are rapidly washed away. Which oral bacteria stick to various human tissues, and to bacteria of other genera, is a matter of specific structures on the surfaces, research over the last decade has revealed. Scientists now are applying the tools of molecular biology to identify these components and to find ways to intervene.

The oral bacteria may be considered as scraps of fabric covered with snaps of many different sizes. The scraps are scattered in a drafty room containing furniture also bearing snaps, but of only a few sizes. The fabric pieces that have snaps matching those on the furniture can be fastened to the furniture so they will not blow away. Some of the other scraps can next be attached to different-sized snaps on the fastened group, and others can then be affixed to this second layer. Further layers can be snapped on, preventing the loss of the attached fabric.

What is the actual nature of the biological "snaps"? Most commonly, half of the snap is a carbohydrate (sugar) structure called a receptor. The other half, sometimes called an adhesin, is a lectin, a protein with sites that bind specific sugars.

The importance of carbohydrate binding was indicated in 1979 by Floyd C. McIntire and his colleagues at the University of Colorado Health Sciences Center in Denver. They discovered that, in laboratory experiments, the binding between bacterial genera can often be inhibited by an abundance of a specific sugar in the solution.

Lectin-carbohydrate recognition systems are widespread in biology. They are important, for example, in the fertilization of plants and enable many flowering plants to prevent self-pollination.

The oral interaction most extensively explored is between two bacterial genera that are early colonizers of the tooth surface above the gum line. "This work is going to serve as a prototype for other studies investigating why different microorganisms are found in certain niches," says Stephan E. Mergenhagen of the National Institute of Dental Research (NIDR) in Bethesda, Md.

This well-studied recognition system comprises a carbohydrate on *Streptococcus sanguis* and a lectin on *Actinomyces viscosus*. The lectins of the *Actinomyces* bacteria are located on filaments called fimbriae, which are the equivalent of pili in other types of bacteria. When the fimbriae are removed, or when mutant bacteria have no fimbriae, the bacteria no longer bind to *Streptococci*.

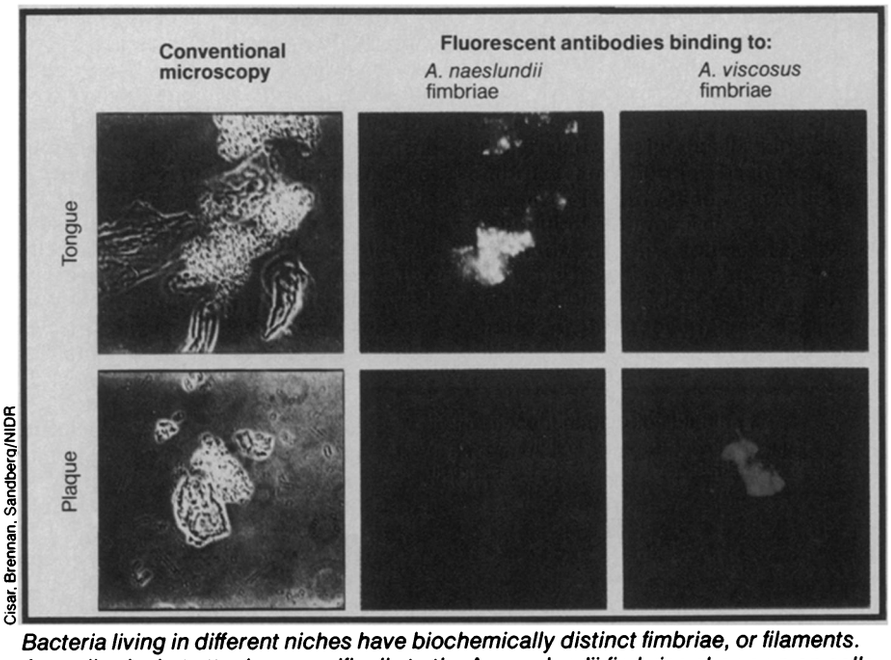
Individual bacteria of the species *Actinomyces viscosus* actually have two types of fimbriae, John O. Cisar of NIDR and his colleagues discovered when they made specific (monoclonal) antibodies to the bacterial surface. One of the fimbriae, called type 1, seems to provide the link between the bacterium and a saliva-coated tooth.

Mutant bacteria lacking type 1 fimbriae could not bind to an artificial tooth surface, whereas mutants lacking type 2 fimbriae attach in the same way normal bacteria. In addition, normal bacteria in the presence of antibody to type 1 fimbriae do not bind to the model tooth.

Type 2 fimbriae of *A. viscosus* consist of the lectin involved in interbacterial coaggregation. Mutants lacking type 2 fimbriae, and normal bacteria in the presence of antibody to type 2 fimbriae, do not bind *Streptococci*.

McIntire and his colleagues have recently purified the receptor on *S. sanguis* for the *A. viscosus* lectin. It is a polysaccharide with a repeating six-sugar unit.

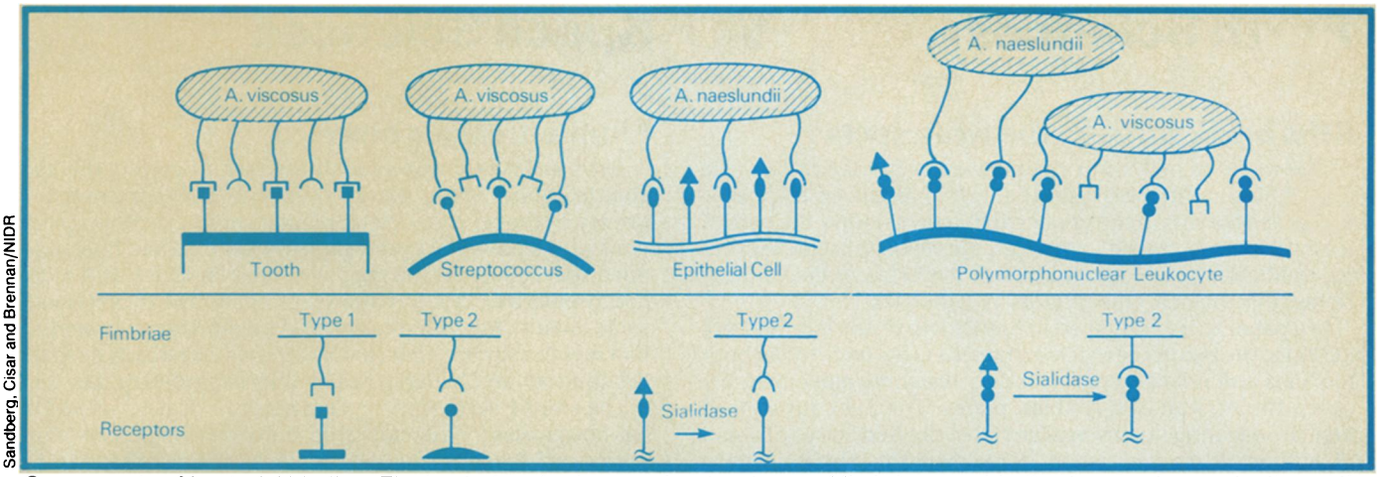
In contrast to *A. viscosus*, which inhab-



Cisar, Brennan, Sandberg/NIDR

Bacteria living in different niches have biochemically distinct fimbriae, or filaments. An antibody that attaches specifically to the A. naeslundii *fimbriae shows up on cells taken from the tongue, and not on plaque from a tooth. In contrast, an antibody to* A. viscosus *fimbriae binds to plaque and not to tongue cells.*

Science Service, Inc. is collaborating with JSTOR to digitize, preserve, and extend access to *Science News*. www.jstor.org



Sandberg, Cisar and Brennan/NIDR

Components of bacterial binding: The bacterium A. viscosus *has two types of fimbriae, one binding to receptors on teeth and the other binding to receptors both on streptococcal bacteria and on white blood cells (polymorphoneuclear leukocytes) after they are treated with the enzyme sialidase. The bacterium* A. naeslundii *has only one type of fimbriae, which binds to tongue (epithelial) cells and white blood cells. It resembles the* A. viscosus *type 2 fimbriae.*

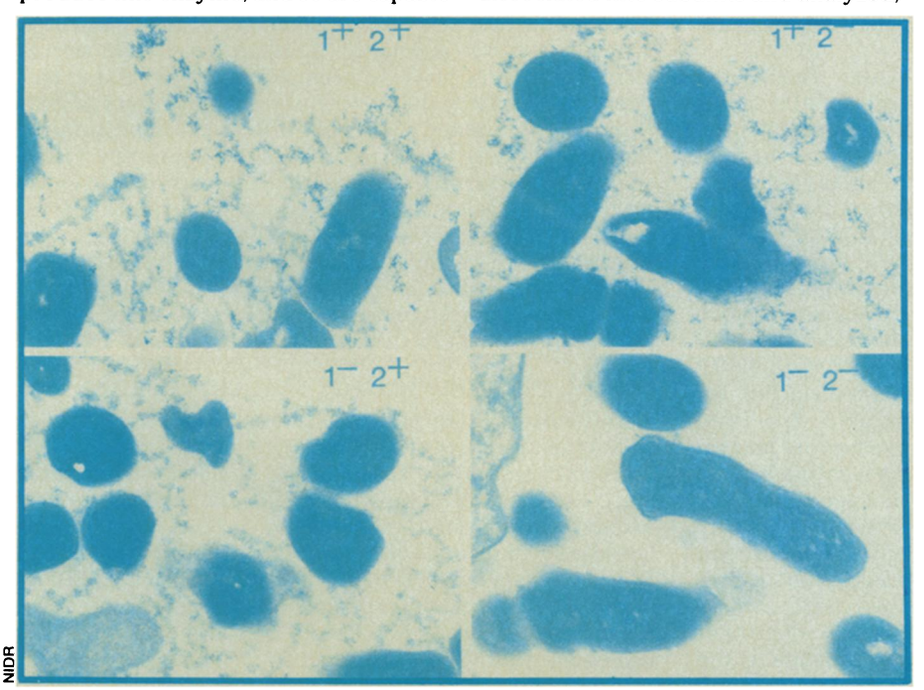
its the surface of teeth, another species of *Actinomyces,* called *A. naeslundii,* is found primarily on the tongue. Cisar, Mergenhagen and Ann L. Sandberg of NIDR have found that this bacterium possesses only type 2 fimbriae, which it employs in binding to tongue surface (epithelial) cells. These type 2 fimbriae are distinct from those of the bacteria that bind to other bacteria.

Effective binding to epithelial cells involves an additional step. The receptors on epithelial cells appear to be partially masked by a chemical called sialic acid. In laboratory studies Cisar and his colleagues discovered that binding by *A. naeslundii* is enhanced by epithelial cell treatment with an enzyme that removes this chemical. The bacteria themselves produce this enzyme, and so are capable of unmasking the carbohydrate receptors for their type 2 fimbriae.

The binding ability so crucial to the colonization of oral surfaces may sometimes be detrimental to bacterial survival. The type 2 fimbriae also attach the bacteria to a carbohydrate receptor on white blood cells, which are found in pockets between teeth and the surrounding gum. Like binding to epithelial cells, this binding also is enhanced by removing sialic acid to reveal the receptors. Once bound, the white blood cells engulf and destroy bacteria. This reaction may also result in destruction of oral tissues by the inflammatory reaction it induces.

"The structure of *A. viscosus* fimbriae has been difficult to study because neither type of fimbriae can be completely dissociated into subunits and analyzed," says Mergenhagen. But recently research teams at NIDR have isolated and transferred into the standard laboratory bacteria *Escherichia coli* the DNA segments that encode the two types of fimbriae. The genetically engineered bacteria now can produce sufficient amounts of fimbrial subunit for analysis.

"Utilizing these subunits, it should be possible to begin to examine the mechanisms of fimbrial secretion and assembly and ultimately the structural basis for bacterial adherence mediated by each fimbrial [type]," Mergenhagen says.



NIDR

The two types of A. viscosus *fimbriae show up between the bacterial cells in upper left micrograph. They are labeled with different antibodies. The other micrographs show mutant bacteria lacking one or both types of fimbria.*

Some scientists are already exploring the possibility of a vaccine against these oral bacteria. William B. Clark of the University of Florida in Gainesville and his colleagues immunized mice with a mixture of the two types of *A. viscosus* fimbriae. The mice produced antibodies that inhibited binding of the bacteria to model teeth, and were partially protected against subsequent infection by *A. viscosus.*

"These studies suggest that a fimbrial vaccine may modulate the colonization patterns of bacteria in the oral cavity," Mergenhagen says. Whether such a vaccine would prevent periodontal disease remains unclear.

Mergenhagen says it is plaque *below* the gum line that is the culprit in periodontal disease. But the plaque below the gum seems to grow down from *Actinomyces* adhering above the gum line. "If we could prevent supragingival [above-the-gum] bacteria from sticking, we might avoid all these diseases," he says.

Finding such a vaccine is not the main thrust of current dental research, Mergenhagen insists. "It's a possibility down the line," he says. "There are real payoffs in understanding oral ecology and trying to do something to change it. But we plan to stick with fundamental work for a while." □