in pellets obtained after the supernatant from low-speed centrifugation of the water overlay was centrifuged at 100,000*g* for 60 minutes.

Although the number of capsomeres could not be enumerated, many properties of these particles—the presence of the envelope, the core of DNA, the nature of the replicative cycle, the likely cubic symmetry, and the diameter of the capsid—strongly suggest that the particles are a herpes-type virus belonging to the family Herpesviridae (*7*).

In broad outline, the events that occur during the replicative cycle are not unlike those found in other herpes viruses (*8, 9*). Several unusual features of the replicative cycle were noted, but most of these have been reported to occur in various other herpes-type viruses. Cytoplasmic particles surrounded by two unit membranes have been reported by Shipkey *et al.* (*8*). The nuclear membrane is considered the site for permanent envelopment of most herpes-type viruses; but temporary envelopment by nuclear membranes, as well as the cytoplasmic acquisition of a coat, have been reported for the viruses associated with frog renal adenocarcinoma (*10*). Many herpes-type virions are described as essentially spherical, varying from 145 to 205 nm in diameter (*11*). The extracellular particles found in *Thraustochytrium* sp. cultures are elongate (280 nm long), with the capsids eccentrically located in the envelopes (Fig. 1, d to f).

Herpes-type viruses have not been described in organisms other than the vertebrates. The presence of these particles in a fungus considerably extends the host range for this viral group [similar particles may be present in another monocentric phycomycetous fungus, *Schizochytrium aggregatum* (*12*)]. The infection of a unicellular organism by a herpes-type virus may prove useful as a tool for studying host-virus interactions; this is especially significant because some herpes viruses have oncogenic activity (*13*). As far as we are aware, all viruses infecting fungi thus far studied have cores of double-stranded RNA, and not of DNA as is found in the virus infecting *Thraustochytrium* sp. Viruses infecting marine fungi have also not been previously described.

No method to assess the viral titer has been devised, but observations of thin sections, as well as our inability to obtain concentrated virus pellets despite numerous attempts, suggest that it is low. In spite of the apparent low titer and the low number of infected cells found in the electron microscopic preparations (approximately 16 percent of 500 cell profiles examined were found to contain particles), repeated attempts have failed to yield single zoospore cultures free of the viruses. No virus particles have yet been observed in mature fungal thalli or in suspensions of zoospores obtained less than 5 hours after the cultures were flooded. These results suggest that the fungal cultures are "virogenic," with some cells becoming productive under as yet unidentified permissive conditions during certain phases of the fungal life cycle.

FREDERICK Y. KAZAMA
KATHLEEN L. SCHORNSTEIN
Virginia Institute of Marine Science, Gloucester Point 23062

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Inhibition of Bacterial Adherence by Secretory Immunoglobulin A: A Mechanism of Antigen Disposal

Abstract. *Preparations of secretory immunoglobulin A (S-IgA) isolated from human parotid fluid specifically inhibited the adherence of* Streptococcus *strains to epithelial cells. Since bacterial adherence is a prerequisite for colonization of mucous surfaces, S-IgA–mediated inhibition of adherence would limit bacterial colonization. This mechanism can explain how secretory immunoglobulins function in the disposal of bacterial antigens.*

Secretory immunoglobulin A (S-IgA) is the predominant class of antibody in secretions bathing mucous membranes (*1, 2*). S-IgA has been demonstrated to serve a protective function against viral infections, but the mechanism by which S-IgA antibodies function in the disposal of bacterial antigens is not understood (*1, 2*). Although S-IgA has been reported to bind to and agglutinate bacteria to which it is specifically directed, S-IgA is not generally considered to be bactericidal, to mediate complement-dependent bacterial lysis, to bind to macrophages, or to enhance phagocytosis (*1, 2*).

It is possible that by merely binding to, and aggregating bacteria, S-IgA may exert an immune protective action. Bacteria colonizing an exposed mucous surface must attach to the surface to prevent being washed away by bathing secretions. Bacterial species exhibit surprising specificity in the degrees to which they can attach to surfaces, and this property directly influences their colonization (*3–5*). The recognized ability of S-IgA to specifically bind to bacterial cells and effect their aggregation could influence their adherence and, hence, their colonization of a mucosal surface. We now report that human parotid S-IgA does, in fact, interfere with bacterial adherence to epithelial surfaces in vitro and our evidence suggests that this effect also occurs in vivo. This function per se would limit the colonization of indigenous organisms as well as exogenous pathogens on mucosal surfaces and could be the major mechanism of immunity mediated by secretory immunoglobulins.

Stimulated parotid saliva from a single donor was clarified, dialyzed, and lyophilized. Parotid S-IgA was separated from other salivary immunoglobulins and isolated by sequential chromatography on a column of Bio-Gel A-15

and Sephadex G-200 successively, each equilibrated with 0.01*M* phosphate buffered saline (PBS), *p*H 6.0. Fractions were monitored by reaction with monospecific antiserum to IgA (Hoechst Corp.). The S-IgA preparation obtained was found to be free of immunoglobulins G and M (IgG and IgM) when tested by capillary precipitation and agar double diffusion assays with the use of monospecific antiserums to IgG and IgM (Hoechst). Approximately 0.5 mg of a preparation of S-IgA protein was obtained per 100 ml of stimulated parotid fluid, as determined spectrophotometrically, assuming an extinction coefficient (1 percent solution at 280 nm) of 13.9 (*1*). Examination of the S-IgA preparation by standard polyacrylamide disc electrophoresis (*6*) revealed three slowly migrating bands near the cathode, an indication that some contaminating materials were present.

Strains of *Streptococcus* species indigenous to human oral mucous surfaces were screened for agglutination with the S-IgA preparation by means of a macroscopic tube technique (*7*). Strains of each species which were or were not agglutinated by the S-IgA preparation were selected for study. The ability of the S-IgA preparation to inhibit the attachment of these strains to human buccal epithelial cells was determined as follows. Washed bacterial cells were prepared from 24-hour cultures in Trypticase soy broth (Baltimore Biological Laboratory), and suspended in 0.067*M* PBS to a final cell density of 10^8 organisms per milliliter, as determined by direct microscopic count. The S-IgA was added to suspensions of each organism at a final concentration of approximately 65 μg of protein per milliliter. Control bacterial suspensions were treated with 0.067*M* PBS. The suspensions were incubated for 1 hour at 35°C and then for 1 hour at 4°C. The bacteria were then washed and restandardized in PBS. Their ability to attach to human buccal epithelial cells was determined by means of an in vitro assay system (*3*).

Control cell suspensions of all strains which were first incubated in buffer adhered to the epithelial cells in significant numbers, averaging between 60 to 150 bacteria per epithelial cell. The S-IgA preparation had no effect on the adherence of streptococcal strains which were not aggregated (Table 1). However, the S-IgA preparation inhibited the adherence of all strains which it agglutinated (Table 1). Thus the ability of the S-IgA preparation to inhibit bacterial attachment was strain-specific and correlated with agglutinating activity. The inhibiting effect of the S-IgA preparation on the adherence of *S. salivarius* strain 9GS2 was studied further. Addition of as little as 2 μg of the S-IgA preparation to suspensions of this organism inhibited its attachment to epithelial cells (Table 1). The adherence-inhibiting effect of the S-IgA preparation was eliminated when the IgA was specifically inactivated by prior incubation with an equal volume of monospecific antiserum (*8*) directed against either κ or λ light chains, or the heavy chains of IgA (Table 1). These data indicate that the S-IgA in the preparation was specifically and solely responsible for inhibition of bacterial adherence. Although S-IgA predominates in secretions, it is likely that other immunoglobulins, if present, would function in a similar manner, for we have observed that specific typing antiserum to the M antigen of streptococcus inhibited the adherence of *S. pyogenes* to epithelial cells (*7*), and hyperimmune rabbit serum directed against *S. salivarius* strain 9GS2 specifically impaired attachment.

Because surface epithelial cells are continually shed, colonization of a mucous surface in vivo requires continuous bacterial reattachment. Hence, an organism whose adherence was reduced by S-IgA or other immunoglobulins in secretions would be expected to be eliminated relative to a serotypically different strain not affected. If their innate capacity to attach is assumed to be equal, a small degree of inhibition in adherence of one bacterium relative to another would lead to the predominance of the unaffected serotype in a manner somewhat analogous to the selective pressures exerted by small differences in bacterial growth

Table 1. Strain-specific inhibition of bacterial adherence to human buccal epithelial cells by S-IgA and parotid fluid. The relative bacterial adherence is equal to 100 × (average number of bacteria per cell)/(average number of control bacteria per cell).

Organism	Strain	Reactivity with S-IgA or parotid fluid*	Prior bacterial treatment	Relative bacterial adherence
S. sanguis	RSGA	+	Buffer control	100
	RSGA	+	65 μg S-IgA	32
	34	−	Buffer control	100
	34	−	65 μg S-IgA	110
S. mitis	B	+	Buffer control	100
	B	+	65 μg S-IgA	37
	26	−	Buffer control	100
	26	−	65 μg S-IgA	111
S. salivarius	RSSA	+	Buffer control	100
	RSSA	+	65 μg S-IgA	32
S. salivarius	9GS2	+	Buffer control	100
	9GS2	+	65 μg S-IgA	24
	9GS2	+	2 μg S-IgA	49
	9GS2	+	S-IgA + antiserum to IgA	98
	9GS2	+	S-IgA + antiserum to κ chain	104
	9GS2	+	S-IgA + antiserum to λ chain	54
	9GS2	+	S-IgA + antiserum to κ chain + antiserum to λ chain	90
	9GS2	+	Antiserum to IgA (control)	114
	9GS2	+	Antiserum to κ chain (control)	109
	9GS2	+	Antiserum to λ chain (control)	93
	9GS2	+	Normal rabbit serum (control)	90
S. salivarius	9GS2	+	Parotid fluid†	56
	9GS2	+	Parotid fluid + antiserum to IgA	89
	SSD3	−	Buffer control	100
	SSD3	−	Parotid fluid	87

* Reactivity was determined by macroscopic agglutination. † Fourfold concentration.

rates. This mechanism of bacterial elimination would therefore be highly efficient, requiring only small amounts of S-IgA. This may account for the low titers of S-IgA and other immunoglobulins that are usually detected in secretions.

There are data which imply that S-IgA functions in this manner in vivo. Bacteria present in human saliva are known to be coated with S-IgA (*9*), and thus they would be expected to be in a state of suppressed adherence. This appears so, for epithelial cells scraped directly from human cheeks contain only 10 to 15 bacteria per epithelial cell even though they are continuously exposed to concentrations of *S. salivarius, S. sanguis, S. mitis*, and other indigenous species approaching 10^8 cells per milliliter of saliva (*10*). However, as observed in this and a previous study (*3*), if epithelial cells are washed and incubated with species of these streptococci, grown in vitro, hundreds of bacterial cells attach to an epithelial cell within minutes. However, the high adherence in vitro of *S. salivarius* strain 9GS2 was inhibited by fourfold concentrated unfractionated parotid saliva (Table 1). The observed inhibition of adherence of this strain by parotid saliva appears to be mainly due to S-IgA, for addition of monospecific antiserum to IgA (*11*) reduced its inhibitory action (Table 1). Unfractionated saliva did not affect the adherence of serotypically different *S. salivarius* strain SSD3, which it did not agglutinate (Table 1). Consequently, it appears that parotid saliva constituents other than S-IgA have limited or no effect on the adherence of *S. salivarius*.

The studies of Freter also substantiate the concept of S-IgA–mediated inhibition of bacterial adherence (*12*). He observed that induced coproantibodies (primarily S-IgA) were protective against infection by *Vibrio cholerae*. When immune animals were challenged with *V. cholerae*, the organisms tended to remain free in the lumen, whereas in nonimmune animals they were adsorbed to the mucosa. Freter also observed that immune serum inhibited the attachment of *V. cholerae* to intestinal mucosa in vitro (*13*), but he did not consider the observed inhibition of bacterial adherence to be related to the protective action of intestinal S-IgA.

The mechanism proposed for the protective action of S-IgA can explain how this immunoglobulin functions in the disposal of bacterial antigens without requiring the bactericidal or opsonizing properties of other immunoglobulins. Its potential to influence the ecology of indigenous bacteria as well as of pathogens can explain the emergence and disappearance, which occur on naso-oral mucous surfaces and in the intestinal canal, of specific serotypes or phage types of bacteria (*14*). Moreover, it offers a rational basis for understanding how relatively small numbers of exogenous pathogens introduced as droplets on a mucous membrane can gain numerical predominance over the existing indigenous microflora. If one considers the indigenous flora to be in a state of partially suppressed adherence mediated by S-IgA, exogenously introduced serotypically distinct pathogens would be expected to enjoy a temporary immunologic selective advantage enabling them to adhere and colonize unimpeded by S-IgA. Once they attained a mass sufficient to stimulate production of specific S-IgA, their colonization would be inhibited, and an immune state created. However, should their colonization progress slowly so as to provide only a weak antigenic challenge, the small amounts of S-IgA produced would affect the pathogen comparably to indigenous bacteria, and a transient balanced or "carrier" state would result.

The secretions bathing mucous surfaces have been recognized to have a cleansing function. The concepts advanced in our report imply that immunoglobulins in these secretions facilitate the clearance of bacteria, and, as such, provide the basis of a primary defense mechanism. Should organisms elude this defense, and subsequently invade the host tissues, systemic defense mechanisms involving serum antibodies, complement, phagocytes, and other recognized systemic factors of immunity would assume importance.

R. C. WILLIAMS
R. J. GIBBONS
Forsyth Dental Center,
Harvard School of Dental Medicine,
Boston, Massachusetts 02115

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Nitrogen Excretion in Cockroaches: Uric Acid Is Not a Major Product

Abstract. *Thin-layer chromatographic and enzymatic spectrophotometric analyses have failed to detect uric acid in fecal extracts from American cockroaches on specified diets. Uric acid appears in the excreta when it is a dietary constituent, although most of it is apparently absorbed and stored internally. Surprisingly, ammonia is a major excretory product.*

Uric acid is generally thought to be the major nitrogenous waste product excreted by cockroaches (*1*), and for years students have been taught this (*2*). In contrast, our analyses with thin-layer chromatography (*3*) and enzymatic spectrophotometry (*4*), have shown that little or no uric acid is present in the nitrogenous materials excreted by the American cockroach, *Periplaneta americana* (L.). We have confirmed the presence of small quantities of materials, activated by ultraviolet light, in cockroach excreta (*5*) and have shown that they are not uric acid, nor are they allantoin, allantoic acid, urea, or any of the common purines, pteridines, or pyrimidines.