MICROBIOLOGY AND MOLECULAR BIOLOGY REVIEWS, Mar. 1998, p. 71–109 Vol. 62, No. 1
1092-2172/98/$04.00+0

Oral Microbial Ecology and the Role of Salivary Immunoglobulin A

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INTRODUCTION

The indigenous microbiota plays an important role in health and diseases of humans and animals. It contributes to the development of the immune system and provides resistance to colonization by allochthonous or pathogenic microorganisms (95, 299, 323, 420, 495). It also constitutes a reservoir of potentially pathogenic bacteria that may infect host tissues (44, 299, 495).

In the oral cavity, indigenous bacteria are often associated with the etiology of two major oral diseases, which are endemic in industrialized societies and are increasing in developing countries (514). Oral diseases seem to appear after an inbalance among the indigenous microbiota, leading to the emergence of potentially pathogenic bacteria. To define the process involved in caries and periodontal diseases, it is necessary to understand the ecology of the oral cavity and to identify the factors responsible for the transition of the oral microbiota from a commensal to a pathogenic relationship with the host (299, 322). The regulatory forces influencing the oral ecosystem can be divided into three major categories: host related, microbe related, and external factors (299).

Secretory immunoglobulin A (SIgA) constitutes the predominant immunoglobulin isotype in secretions, including saliva. It is considered to be the first line of defense of the host against pathogens which colonize or invade surfaces bathed by external secretions (320, 328). The main function of SIgA antibodies seems to be to limit microbial adherence as well as penetration of foreign antigens into the mucosa (59, 320, 323, 328). Naturally occurring SIgA antibodies reactive with a variety of indigenous bacteria have been detected in saliva (55, 59, 108, 174, 293, 296). Furthermore, indigenous bacteria of the oral cavity have been found to be coated with SIgA (55, 108). The role of these antibodies in the colonization and the regulation of the indigenous microbiota is still controversial. Despite the presence of SIgA antibodies, a resident microbiota persists in the oral cavity. Indigenous bacteria can survive in the oral cavity because they are less susceptible to or can avoid immune mechanisms (30, 44, 87, 141, 142). It is also possible that SIgA has an effect on indigenous bacteria but that it is only a minor force among the multiple factors that maintain the homeostasis of the indigenous microbiota (87).

Since caries and periodontal diseases are associated with indigenous bacteria, defining the role of SIgA in the control of the oral indigenous microbiota is a prerequisite for the elaboration of effective vaccines against these diseases. Until now, studies that evaluated the role of SIgA in the microbial ecology of the oral cavity gave contradictory results. In vitro experiments have shown that SIgA may inhibit (222, 383) or promote (222, 270) the adherence of oral bacteria to teeth. Experiments with animal models showed that salivary IgA induced against *Streptococcus mutans* leads to a reduction in the colonization of this bacterium and to the prevention of caries (328). More recent studies indicate that the immunity is not maintained (392). IgA-deficient humans were found to be more or less susceptible to caries and periodontal diseases (90, 393, 394).

The present review describes the oral ecosystems, the major factors that might control the oral microbiota, the basic aspects of the secretory immune system, and the biological functions of SIgA and finally focuses on experiments related to the role of IgA in the microbial ecology of the oral cavity. To present an overall picture of the current knowledge of oral microbial ecology, this review is not limited to human studies but includes in vitro systems such as the chemostat and the artificial mouth, as well as results obtained with animal models, such as rodents and primates, including our study on a murine model.

MICROBIAL ECOLOGY OF THE ORAL CAVITY

Ecology is the science that studies interrelationships between organisms and their living (biotic) and nonliving (abiotic) environment (20).

An ecosystem consists of the microbial community living in a defined habitat and the abiotic surroundings composed of physical and chemical elements. In its simplest expression, the oral ecosystem is thus composed of the oral microorganisms and their surroundings, the oral cavity (495).

Within an ecosystem, the development of a community usually involves a succession of populations. The process begins with the colonization of the habitat by pioneer microbial populations. In the oral cavity of newborns, streptococci (*S. mitis* biovar 1, *S. oralis*, and *S. salivarius*) are the pioneer organisms (73, 367a). Pioneer microorganisms fill the niche of this new environment and modify the habitat, and as a result, new populations may develop. As the process continues, the diversity and the complexity of the microbial community increase. Succession ends when no additional niche is available for new populations. At this stage, a relatively stable assemblage of bacterial populations is achieved. It is called a climax community.

The concept of a stable or climax community does not imply static conditions. The stability is based upon homeostasis, which implies compensating mechanisms that act to maintain steady-state conditions by a variety of controls aimed at counteracting perturbations that would upset the steady state. The concepts of homeostasis and bacterial succession are important in oral microbiology. Some factors, such as a high-sucrose diet, may cause an irreversible breakdown in the homeostasis of the oral ecosystem, resulting in the initiation of caries (299).

ORAL ECOSYSTEM OF MAMMALS

Habitats

The oral cavity is a moist environment which is kept at a relatively constant temperature (34 to 36°C) and a pH close to neutrality in most areas and thus supports the growth of a wide variety of microorganisms. However, the mouth must not be considered a uniform environment. There are several habitats in the oral cavity, each being characterized by different physicochemical factors and thus supporting the growth of a different microbial community. This is partly due to the great anatomical diversity of the oral cavity and the interrelationship between the different anatomic structures. The oral cavity possesses both hard (teeth) and soft (mucosa) tissues. The tooth can be described as a nonshedding hard surface that offers many different sites for colonization by bacteria below (subgingival) and above (supragingival) the gingival margin. In contrast, the oral mucosa is characterized by a continuous desquamation of its surface epithelial cells, which allows rapid elimination of adhering bacteria. The mucosa that covers the

cheek, tongue, gingiva, palate, and floor of the mouth varies according to the anatomical site. The epithelium may be keratinized (palate) or nonkeratinized (gingival crevice) (495). The tongue, with its papillary surface, provides sites of colonization that are protected from mechanical removal. The area between the junctional epithelium of the gingiva and teeth, referred to as the gingival crevice, also provides a unique colonization site that includes both hard and soft tissues.

The oral surfaces are also constantly bathed by two important physiological fluids, the saliva and the gingival crevicular fluid. These fluids are essential for the maintenance of the oral ecosystems by providing water, nutrients, adherence, and antimicrobial factors. The supragingival environment is bathed by saliva, while the subgingival environment (gingival crevice) is bathed mainly by the gingival crevicular fluid. Saliva is a complex mixture that enters the oral cavity via the ducts of three pairs of major salivary glands, the parotid, the submandibular, and the sublingual, and the minor salivary glands. Saliva contains 99% water but also contains glycoproteins, proteins, hormones, vitamins, urea, and several ions. The concentrations of these components will vary according to the salivary flow. Generally, a slight increase in the secretion rate leads to an increase in sodium, bicarbonate, and pH and a decrease in potassium, calcium, phosphate, chloride, urea, and proteins (103, 347). At higher secretion rates, the concentrations of sodium, calcium, chloride, bicarbonate, and proteins increase while the concentration of phosphate decreases. Saliva helps maintain tooth integrity by providing ions such as calcium, phosphate, magnesium, and fluoride for the remineralization of tooth enamel.

Gingival fluid is an exudate originating from plasma that passes through the gingiva (junctional epithelium) to reach the gingival crevice and flows along teeth. The diffusion of gingival fluid in healthy gingiva is slow but increases during inflammation. The composition of the gingival fluid is similar to that of plasma; it contains proteins, albumin, leukocytes, Igs, and complement.

Oral Microbiota in Healthy Individuals

Humans. The oral microbiota of humans is highly complex and diverse. It is composed of more than 300 bacterial species, to which may be added protozoa, yeasts, and mycoplasmas. Some of the more frequently isolated microorganisms are listed in Table 1. Their distributions vary qualitatively and quantitatively according to the habitat. Mutans streptococci (*S. mutans*, *S. sobrinus*, *S. cricetus*, and *S. rattus*) and *S. sanguis* are found in larger numbers on teeth, while *S. salivarius* is isolated mainly from the tongue (446). *S. mutans* and *S. sanguis* appear in the oral cavity only after eruption of the teeth (446).

(i) Teeth. On teeth, microorganisms colonize in a dense mass forming dental plaque (299, 350, 495). Dental plaque consists of microbial communities organized in a complex matrix composed of microbial extracellular products and salivary compounds. The microbial composition of dental plaque varies according to the site and the sampling time. Dental plaque develops preferentially on surfaces protected from mechanical friction, such as the area between two teeth (approximal surface), the subgingival area (gingival crevice), and the pits and fissures of the biting surfaces. The predominant organisms isolated from supragingival dental plaque are gram-positive, facultatively anaerobic bacteria, particularly *Actinomyces* spp. and streptococci (299, 350, 495). Gram-negative bacteria of the group *Veillonella*, *Haemophilus*, and *Bacteroides* are regularly isolated but in lower proportions (45, 495).

In a healthy subgingival crevice, the total number of cultivable bacteria is relatively small (10^3 to 10^6 CFU/crevice). The subgingival plaque is also dominated by gram-positive organisms (*Actinomyces* and streptococci). It seems that the microbiota from the gingival crevice is an extension of supragingival plaque (44). Black-pigmented gram-negative rods including *Porphyromonas gingivalis*, *Porphyromonas endodontalis*, *Prevotella melaninogenica*, *Prevotella intermedia*, *Prevotella loescheii*, and *Prevotella denticola* are rarely isolated from a healthy gingival crevice (299, 437).

(ii) Mucosal surfaces. Little information is available on the microbiota of mucosal surfaces. The oral mucosa of the gingiva, palate, cheeks, and floor of the mouth are colonized with few microorganisms (0 to 25 CFU/epithelial cell) (495). Streptococci constitute the highest proportion of the microbiota in these sites, with a predominance of *S. oralis* and *S. sanguis*. The genera *Neisseria*, *Haemophilus*, and *Veillonella* have also been isolated (495). On the tongue, a higher bacterial density (100 CFU/epithelial cell) and diversity is found (44, 495). In all studies, *Streptococcus* spp. (*S. salivarius* and *S. mitis*) and *Veillonella* spp. were the predominant members of the microbiota (44, 495). Other major groups isolated include *Peptostreptococcus* spp., gram-positive rods (mainly *Actinomyces* spp.), *Bacteroides* spp., and other gram-negative rods. Black-pigmented obligate anaerobic rods and spirochetes, which are closely associated with periodontal diseases, have been recovered in small numbers (511). It has been suggested that the tongue could act as a reservoir for microorganisms that are implicated in periodontal diseases (511).

Organisms that are found in saliva are derived from the dislodgement of bacteria colonizing the various oral sites. The microbial composition of saliva is similar to that of the tongue (347, 500).

Animal models. Animal models have been widely used in dental research (347, 350, 475). In general, information on their resident oral microbiota has been only partial or has been obtained during experimental protocols (44, 347, 350, 533). However, some studies have characterized the oral resident microbiota of monkeys (26, 61, 438), rats (204), and mice (150, 396, 500) more extensively. These results are summarized in Table 1. It is interesting that two genera, *Lactobacillus* and *Streptococcus*, are common to all laboratory animals.

Nonhuman primates have a dentition and an oral microbiota similar to that of humans and, for this reason, represent the most suitable model for dental research (347, 350). Several primates, such as the macaque (306, 438), the marmoset (61), and the squirrel monkey (26, 84), have been used in dental research. Studies of their oral microbiota have been limited principally to their subgingival plaque, and the predominant groups isolated were streptococci, *Actinomyces* spp. and obligate anaerobic gram-negative rods (26, 306, 438). Among the gram-negative rods, black-pigmented species dominated while high proportions of *Fusobacterium* spp. and *Alcaligenes* spp. were also recovered (26, 306, 438).

Although the oral anatomic structures of humans and rodents differ in certain respects, rats and mice are often used in dental research due to their availability at low cost and the ability to use inbred animals. Isogai et al. (204) isolated more than 15 bacterial species from the oral cavity of rats (Wistar Kyoto strains). The predominant types of bacteria isolated from the saliva, tongue dorsum, buccal mucosa, and gingival crevice of rats were *Streptococcus* spp., *Lactobacillus* spp., *Veillonella* spp., and *Neisseria* spp. *S. salivarius* was found in higher proportions in saliva, on the tongue dorsum, and the buccal mucosa. *S. mitis* was found in high proportions on the tongue dorsum and the buccal mucosa. *S. sanguis* and *S. mutans* were found in low proportions and only in the gingival crevice.

TABLE 1. Comparative oral microbiota in human and animals

Group	Microbial genus	Present in:			
		Humans[a]	Monkeys[b]	Rats[c]	Mice[d]
Gram-positive cocci					
Aerobic or facultative	*Streptococcus*	++[e]	++	++	++
	Staphylococcus	−	+	+	++
	Enterococcus	+	+	+	++
	Micrococcus	+	−	−	−
Obligate anaerobes	*Peptostreptococcus*	+	+	−	−
	Peptococcus	+	+	−	−
Gram-positive rods					
Aerobic or facultative	*Lactobacillus*	+	+	++	++
	Corynebacterium	+	+	+	−
	Actinomyces	++	+	+	−
	Arachnia	+	−	−	−
	Rothia	+	−	−	−
	Alcaligenes	−	−	+	−
Obligate anaerobes	*Eubacterium*	+	+	−	−
	Propionibacterium	+	+	+	−
	Bifidobacterium	+	−	−	−
	Bacillus	±	±	++	±
	Clostridium	±	±	±	±
Gram-negative cocci					
Aerobic or facultative	*Neisseria/Branhamella*	+	+	++	−
Obligate anaerobes	*Veillonella*	++	+	++	−
Gram-negative rods					
Aerobic or facultative	*Enterobacteriaceae*	−	+	+	±
	Campylobacter	+	+	−	−
	Eikenella	+	−	−	−
	Actinobacillus	+	+	−	−
	Capnocytophaga	+	+	−	−
	Haemophilus	+	+	−	−
	Simonsiella	+	−	−	−
Obligate anaerobes	*Bacteroides*	++	+	+	−
	Fusobacterium	+	+	+	−
	Porphyromonas	+	++	−	−
	Prevotella	+	++	−	−
	Leptotrichia	+	+	−	−
	Wolinella/Selenomonas	+	+	−	−
Other microorganisms	*Mycoplasma*	+	NA	NA	−
	Candida	+	NA	NA	−
	Spirochetes	+	+	+	−
	Protozoa	+	+	+	−

[a] Data from references 299, 350, and 495.
[b] Combined data from the macaque (306, 438) marmoset (61), and squirrel monkey (26).
[c] Data from reference 204.
[d] Combined data from six mouse strains (BALB/c, CD-1, C3H/He, C57BL/6, DBA/2, and C57BL/10) (150, 396, 500).
[e] ++, isolated frequently and may constitute a high percentage of the total oral microbiota; +, isolated; ±, appears as transient; −, not isolated; NA, data not available.

Gram-negative bacteria, such as *Bacteroides* spp., were found in low proportions. Studies of the oral microbiota of other rat strains were less detailed, but similar results were obtained for the rice rat (347).

We have extensively studied the composition of the oral microbiota of mice (34, 94, 150, 294, 295, 296, 396, 398, 500). We have isolated more than 20 species from six different mouse strains (BALB/c, CD-1, C3H/He, C57BL/6, DBA/2, and C57BL/10) originating from different suppliers. Generally, no more than four or five species predominated at any one time in the oral cavity of any one group of mice. The most predominant and frequently isolated bacteria from the whole oral cavity (cheeks, tongue, and teeth) of mice were *Lactobacillus murinus*, *Streptococcus* spp., *Enterococcus faecalis*, and staphy-

lococci (*Staphylococcus epidermidis*, *S. conhii*, and *S. sciuri*). *L. murinus* was found in higher proportions on the mucosa, and *E. faecalis* was found in higher proportions on teeth (500). In contrast other experimental models, no obligate anaerobic bacteria were isolated. Wolff et al. (533) isolated obligate anaerobic bacteria from the gingival plaque of two mice strains (STR/N and Swiss-Webster). In our studies, a less diversified microbiota was found, probably because specific-pathogen-free (SPF) mice were used. An SPF mouse colony is initiated from germfree mice inoculated with a defined bacterial cocktail from a supplier. The original cocktail contains nonpathogenic bacteria that have been previously isolated from conventional mice. We are currently using this mouse model to study the effect of different factors on the homeostasis of the oral microbiota. Although the oral microbiota is not representative of that of humans, the basic principles that govern the mechanisms involved in the maintenance of homeostasis in the oral cavity are probably similar in the mouse model and in humans. It could also be argued that because of their coprophageous habits, the microbiota of the rodents would be significantly modified. However, our results (295) show that this does not appear to be the case. It would be interesting, however, to study more systematically the influence of coprophagy on the oral microbial biota of rodents.

Oral Microbiota Associated with Oral Diseases

It is now well established that caries and periodontal diseases are infectious diseases associated with resident microorganisms of the dental plaque (299). In deciding upon therapy, such as vaccination, it is important to determine if one or several microorganisms cause the diseases. There are two major hypotheses about the role of plaque in oral diseases (301). The specific plaque hypothesis proposes that only a few microorganisms are involved in the oral disease process, while the other hypothesis (nonspecific) considers that diseases result from the interaction of the whole plaque with the host. Several experiments have attempted to describe the oral microbiota associated with caries and periodontal diseases and to identify the specific etiological agents. The current knowledge of the microorganisms involved in caries and periodontal diseases process has been obtained from the studies of humans and of laboratory animals, such as monkeys, hamsters, rats, and mice.

Caries. Dental caries is a bacterial disease of the dental hard tissues; it is characterized by a localized, progressive, molecular disintegration of the tooth structure. The development of caries is associated with dental plaque of smooth coronal surfaces, pits, and fissures. Caries may also appear on root surfaces that are exposed to the oral environment as a result of gingival recession. The demineralization of teeth (enamel, dentine, and cementum) is caused by organic acid produced from the bacterial fermentation of dietary carbohydrates. The frequent ingestion of carbohydrates may lead to the selection of bacteria that are acidogenic (capable of producing acid from carbohydrates) and aciduric (capable of tolerating acid) and concurrently to a low-pH environment. These conditions favor the solubilization of tooth minerals. The pH at which this demineralization begins is known as the critical pH and ranges between pH 5.0 and 5.5 (275).

Laboratory animals have been particularly valuable in elucidating the microbiological origin of dental caries. In a series of experiments, Keyes and his collaborators demonstrated that rodents developed caries when infected with cariogenic streptococci and fed a high sucrose diet (139, 221). The "caries-inducing streptococci" isolated by Keyes and colleagues were subsequently identified as a mutans streptococcus (*S. cricetus*). Many bacterial species have been similarly tested for their cariogenic potential in conventional animals (monkeys, rats, gerbils, hamsters, and mice) and in gnotobiotic rodents (germfree rodents monoassociated with a known bacterial species). Among 30 bacterial species tested, mutans streptococci including *S. mutans*, *S. sobrinus*, *S. cricetus*, and *S. rattus* were shown to be the most cariogenic. Other cariogenic bacterial species include *Lactobacillus acidophilus*, *Lactobacillus casei*, *Actinomyces naeslundii*, *A. naeslundii* genospecies 2 (formerly *Actinomyces viscosus*), *S. salivarius*, *S. sanguis*, and *E. faecalis* (275). It may be misleading to extrapolate the results obtained in animals to the situation prevailing in humans. In contrast to humans, the caries process is induced rapidly in animals by feeding them a high bacterial inoculum and a high-sucrose diet. Furthermore, the use of gnotobiotic animals does not account for the multiple microbial interactions that occur among the human oral resident microbiota. Bacteria that are less acidogenic, such as *Actinomyces*, may be cariogenic under such experimental conditions but not in humans.

The complexity of the bacterial community in dental plaque of humans has made it difficult to determine the single bacterial agent of caries. However, there is considerable evidence that mutans streptococci (particularly *S. mutans* and *S. sobrinus*) and *Lactobacillus* are involved in the initiation and progression of caries (275). These two bacterial groups are able to rapidly metabolize carbohydrates into acid, primarily lactic acid, and to tolerate a low-pH environment. Cross-sectional studies demonstrated that a large number and isolation frequency of mutans streptococci and *Lactobacillus* are associated with increasing prevalence of enamel lesions. Most of the longitudinal studies revealed that the appearance of enamel caries is preceded by an increased level of mutans streptococci (275). The increase in *Lactobacillus* is generally slower, and *Lactobacillus* reaches a high level only after the lesion can be detected clinically (69). These findings suggest a microbial succession in which mutans streptococci are implicated in caries initiation and *Lactobacillus* is implicated in caries progression (353). However, coronal caries also appears to develop in the absence of mutans streptococci and lactobacilli. Species such as *Actinomyces* spp., *S. mitis*, *Veillonella* spp., and *Candida* spp. have been associated with enamel caries (353). Other studies also suggest that the microflora of root surface caries is complex (43). In addition to mutans streptococci and lactobacilli, a broad range of microorganisms may be isolated from root lesions, and *Actinomyces* occasionally constitutes the predominant species (43, 353). Furthermore, a high level of *S. mutans* has been found in dental plaque without evidence of caries (353).

To reconcile these findings, Marsh proposed the "ecological plaque hypothesis" (299, 301, 303). A factor(s) will trigger a shift in the proportions of the resident microbiota and therefore predispose a site to disease. At neutral pH, mutans streptococci and lactobacilli are weakly competitive and constitute only a small percentage of the total plaque microbial community. The frequent consumption of fermentable carbohydrates may lead to frequent conditions of low pH in the plaque. Such conditions lead to decreased proportions of acid-sensitive bacteria such as *S. sanguis*, *S. oralis*, and *S. mitis* and to increased proportions of mutans streptococci and lactobacilli. Such a population shift predisposes a surface to dental caries. The increased numbers of *S. mutans* and *Lactobacillus* lead to the production of acid at a higher rate, enhancing the demineralization of the tooth. The sequence of events explains the lack of total specificity in the microbial etiology of caries and the bacterial succession observed in longitudinal studies. The apparent absence of caries observed in the presence of high levels

of *S. mutans* may be due to differences in flow rate, buffer capacity, or composition of saliva or to the presence of a high level of lactate-metabolizing and base-generating bacterial species in dental plaque (299). Some studies suggest that the presence of *Veillonella*, a lactate-metabolizing bacteria, is associated with a lower prevalence of caries (353).

Periodontal diseases. Periodontal diseases is a general term describing the inflammatory pathologic state of the supporting tissues of teeth. Periodontal diseases can be grouped into two major categories, gingivitis and periodontitis. Each can be further divided according to the age of the patient (prepubertal, juvenile, adult), disease activity and severity (rapid, acute, chronic), and distribution of lesions (localized or generalized) (299, 439). Gingivitis is defined as an inflammation of gingival tissues which does not affect the attachment of teeth. Periodontitis involves the destruction of the connective tissue attachment and the adjacent alveolar bone (439). In periodontitis, the gingival crevice is deepened to form a periodontal pocket due to the apical migration of the junctional epithelium along the root surface (495). The induction and progression of periodontal tissue destruction is a complex process involving plaque accumulation, release of bacterial substances, and host inflammatory response (156, 157, 299). Although bacteria rarely invade tissues, they may release substances that penetrate the gingivae and cause tissue destruction directly, by the action of enzymes and endotoxins, or indirectly, by induction of inflammation. The host inflammatory response to bacterial antigens is both protective and destructive in periodontal diseases. Tissue damage may be caused by the release of lysosomal enzymes from phagocytes and by the production of cytokines that stimulate connective tissue cells to release metalloproteinases (including collagenases) or cytokines that activate bone resorption. Among the bacteria regularly isolated from periodontal pockets, those producing such virulence factors are generally gram-negative rods and include *Porphyromonas*, *Prevotella*, *Fusobacterium*, *Actinobacillus actinomycetemcomitans*, *Capnocytophaga*, and *Wolinella.*

Experimental models have been widely used to understand the etiology of periodontal diseases. Rodents do not represent an attractive model for human periodontal diseases, in part because hair, food, and litter accumulated in their gingival crevices may induce inflammation and destruction of periodontal tissues. However, it has been demonstrated that oral inoculation of germfree rodents with several suspected periodontal pathogens including *A. actinomycetemcomitans*, *Porphyromonas gingivalis*, *Capnocytophaga sputigena*, *Eikenella corrodens*, and *Fusobacterium nucleatum* increases alveolar bone destruction. Antibiotic therapy was effective in preventing or arresting periodontal destruction, thus confirming the role of microorganisms in periodontal diseases (232). Other species, usually not recognized as periodontopathogens (such as *Streptococcus* and *Actinomyces*), also cause bone loss in gnotobiotic rodents, suggesting that the spectrum of periodontal pathogens in humans may be wider than generally accepted. Periodontal diseases associated with accumulations of indigenous plaque may also develop in rodents, dogs, and monkeys. Primates appear to be more suitable models for studies of periodontal diseases because of the similarity of the inflammatory response to that of humans. These animals develop periodontal diseases naturally, but the disease process may be accelerated by the subgingival placement of a silk ligature around the teeth. The destruction of periodontal tissues in primates is associated with an increase in gram-negative anaerobic rods including *Fusobacterium*, *Capnocytophaga*, *A. actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Prevotella intermedia* (26).

In humans, gingivitis is also associated with plaque accumulation around the gingival margin. When oral hygiene is restored, the gingival tissue quickly returns to a state of health, demonstrating that dental plaque is responsible for gingival inflammation and is not a result of the disease. In a healthy gingival crevice, the total number of microorganisms is small and the microbiota is dominated by facultative gram-positive bacteria. The number of bacterial cells in plaque associated with gingivitis is 10- to 20-fold larger than in healthy sites. The facultative gram-positive bacteria still dominate, but there is an increase in the proportion obligately anaerobic gram-negative bacteria (439). The microbiota increases in diversity, but no specific group seems associated with the diseases (336, 339). The predominant gram-positive bacteria are *Actinomyces naeslundii* genospecies 2 (formerly *A. viscosus*), *A. naeslundii*, *S. sanguis*, *S. mitis*, and *Peptostreptococcus micros*. Gram-negative rods include *F. nucleatum*, *P. intermedia*, *Veillonella*, *Wolinella*, *Capnocytophaga*, and *Haemophilus*. Although it is not clear whether gingivitis is essential for the development of more advanced forms of periodontitis, some species that predominate in periodontitis have been found in small numbers in gingivitis. Chronic adult periodontitis is the most common form of advanced periodontal disease. The microbiota is extremely diverse and may be composed of more than 150 different species, among which are large numbers of obligately anaerobic gram-negative rods and spirochetes (337). The microbiota differs in composition between pockets within a patient and between patients (337, 338). The predominant species include *P. gingivalis*, *P. intermedia*, *Bacteroides forsythus*, *A. actinomycetemcomitans*, *W. recta*, *E. corrodens*, *Treponema denticola*, and *P. micros*. Chronic periodontitis probably results from the microbial activity of a mixture of microorganisms, particularly the obligately anaerobic gram-negative rods. Other more acute and rapid forms of periodontal diseases may also arise due to different predisposing conditions such as hormonal changes or depressed immune systems. These diseases seem more associated with particular microbial groups, such as in localized juvenile periodontitis which is closely associated with high numbers of *A. actinomycetemcomitans* (440).

The plaque ecologic hypothesis may also be applied to explain the role of microorganisms in periodontal diseases (299, 301, 303). In the healthy gingival crevice, suspected periodontopathogens such as *P. intermedia*, *A. actinomycetemcomitans*, *P. gingivalis*, and spirochetes are undetectable or found in very small numbers. In the absence of oral hygiene, the accumulation of plaque can lead to inflammation and an increase in the flow of gingival crevicular fluid. This fluid may provide nutrients for bacteria and favor the growth of fastidious obligately anaerobic gram-negative bacteria implicated in periodontal destruction. It has been demonstrated that cultures of subgingival plaque in serum allowed the enrichment of suspected periodontopatogens that were previously undetected in the primary inoculum (493). This finding might explain the observed succession of microorganisms from healthy gingiva and gingivitis to periodontitis and the difficulty in identifying specific etiologic agents in periodontal diseases.

FACTORS INFLUENCING THE ORAL ECOSYSTEM

The growth of oral microorganisms is influenced by a variety of factors such as temperature, pH, oxidation-reduction potential, the availability of nutrients and water, the anatomy of the oral structures, salivary flow, and antimicrobial substances. Each factor in a given oral habitat influences the selection of oral microorganisms and helps maintain the equilibrium

among bacterial populations. The result of these selective pressures has already been observed in the differences in the oral microbiota among the different sites of the oral cavity.

The formation of dental plaque on smooth surfaces has been widely studied in vitro and in vivo and represents a good example of the force involved to maintain the homeostasis of the oral ecosystems. The development of dental plaque follows a general bacterial succession pattern under the control of several factors (reviewed in reference 353). After tooth brushing, dental plaque is initiated by the deposition of an acellular proteinaceous film, termed the acquired pellicle (353). The major constituents of the pellicle are components of saliva and gingival crevicular fluid such as proteins (albumin, lysozyme, proline-rich proteins), glycoproteins (lactoferrin, IgA, IgG, amylase), phosphoproteins, and lipids. Bacterial components such as glucosyltransferase are also present. The bacteria colonize the pellicle within the first 2 to 4 hours after cleaning. The pioneer microorganisms consist mainly of streptococci (*S. sanguis*, *S. oralis*, and *S. mitis*) and, in smaller numbers, *Neisseria* and *Actinomyces*. The bacteria with low affinity for the pellicle are eliminated by salivary flow. After initial colonization, the pioneer microorganisms grow rapidly, forming microcolonies that are embedded in an extracellular matrix composed of bacterial and host molecules. During this process, the alteration of the environment by pioneers allows the colonization by other bacterial groups such as *Veillonella* and *Haemophilus* (48 h after tooth brushing). Several bacterial interrelationships including coaggregation, production of antibacterial substances, and food chains contribute to increase the diversity of the bacterial community. Also, the consumption of oxygen by aerobic species favors the colonization of obligately anaerobic microorganisms such as *Fusobacterium*, *Bacteroides*, and spirochetes (1 to 2 weeks). If the plaque is left to accumulate, the complexity of the microflora increases until a climax community has been established (2 to 3 weeks). As described above, an inbalance in the plaque ecosystem may lead to the development of oral diseases. For example, the uncontrolled development of supragingival plaque in the absence of oral hygiene may cause gingivitis and subsequent colonization of the subgingival crevice by periodontopathogens. A high sucrose intake may lead to a higher colonization of plaque by *S. mutans* and *Lactobacillus* and to the development of caries.

In the following sections, each factor that may influence the physicochemical environment and the colonization of the oral cavity will be described in detail. All these factors are interrelated and depend on host and microbial activities as well as external factors such as diet or oral hygiene. For better clarity, these factors have been divided into four categories: physicochemical, host related, microbial, and external.

Physicochemical Factors

The physicochemical factors result from the combined action of host, microbial, and external factors. In all in vivo and in vitro systems, the growth of microorganisms is influenced by five important variables: temperature, pH, availability of water, availability of nutrients, and oxidation-reduction potential (20). As the mouth is constantly bathed by saliva and crevicular fluid, water is not considered to be a limiting factor.

Temperature. The temperature in the oral cavity is relatively constant (34 to 36°C), which allows a wide range of microorganisms to grow. The temperature may be more variable on the mucosal and tooth supragingival surface. During food intake, microorganisms colonizing these sites are exposed to hot and cold meals and probably must adapt to these extreme variations of temperature. However, to our knowledge, no data are available on the effect of this short period of temperature variation on the metabolism of oral bacteria.

pH. The pH or hydrogen ion concentration of an environment affects microorganisms and microbial enzymes directly and also influences the dissolution of many molecules that indirectly influence microorganisms. Microorganisms generally cannot tolerate extreme pH values. In the oral cavity, the pH is maintained near neutrality (6.7 to 7.3) by saliva. The saliva contributes to maintenance of the pH by two mechanisms. First, the flow of saliva eliminates carbohydrates that could be metabolized by bacteria and removes acids produced by bacteria. Second, acidity from drinks and foods, as well as from bacterial activity, is neutralized by the buffering activity of saliva. Bicarbonate is the major salivary buffering system of saliva, but peptides, proteins, and phosphates are also involved. Increases in pH also result from bacteria that metabolize sialine and urea into ammonia. Acids that are produced by the microbial metabolism of carbohydrates may accumulate in dental plaque because of the slow diffusion of saliva through dental plaque. Following sugar intake, the pH of dental plaque may decrease to below 5.0.

The pH is an important parameter in oral microbial ecology (42, 47, 186). Frequent sugar intake favours the growth of aciduric bacteria such as *Lactobacillus* and *S. mutans* and predisposes to caries formation (299). An increased colonization by *S. mutans* was demonstrated by simply rinsing the mouth with low-pH buffers (462). In vitro studies have also shown that gradual decreases in pH in glucose-pulsed cultures favored *S. mutans* and *Lactobacillus* while populations of *S. sanguis*, *S. mitior*, *P. intermedia*, and *F. nucleatum* were reduced (42, 317). When the pH of the chemostat was controlled at 7.0, the glucose pulse had little effect on the microbial populations, suggesting that the low pH generated from carbohydrate metabolism, rather than carbohydrate availability per se, is responsible for the shift in composition of the oral microbiota in vivo (47, 322).

The subgingival area is bathed by gingival fluid and is not controlled by the buffering salivary activity. The pH in the gingival crevice may vary between 7.5 and 8.5, while the crevicular fluid ranges from pH 7.5 to 7.9. An alkaline pH in gingival crevices and periodontal pockets may exert a selective force towards the colonization of periodontopathogens (186, 316).

Oxidation-reduction potential and anaerobiosis. Many enzymatic reactions are oxidation-reduction reactions in which one compound is oxidized and another compound is reduced. The proportion of oxidized to reduced components constitutes the oxidation-reduction potential or redox potential (E_h). The E_h is greatly influenced by the presence or absence of molecular oxygen, which is the most common electron acceptor. Anaerobic bacteria need a reducing environment (negative E_h) for growth, while aerobic bacteria need an oxidizing environment (positive E_h). The mouth is characterized by a wide range of oxidation-reduction potentials, allowing the growth of aerobic, facultative anaerobic, and anaerobic bacteria (495). In general, the dorsum of the tongue and the buccal and palatal mucosa are aerobic environments with positive E_h, thus better supporting the growth of facultative anaerobic bacteria. The gingival crevice and the approximal surfaces of the teeth (surfaces between teeth) possess the lowest E_h and the highest concentration of obligately anaerobic bacteria. The E_h values vary between +158 to +542 mV in saliva but may reach −300 mV in gingival crevices (495). The E_h also varies during plaque formation, changing from positive values (+294 mV) on clean tooth surfaces to negative values (−141 mV) after 7 days (218). The fall in E_h during plaque formation is the result of oxygen

consumption by facultative anaerobic bacteria as well as a reduction in the ability of oxygen to diffuse through the plaque. This explains in part the increased in number of obligately anaerobic bacteria during plaque formation.

Nutrients. Chemostat studies (42, 145, 186, 316) and a study with mice (34) suggest that the levels of most bacterial populations are strongly controlled by substrate availability. Each bacterial species must be more efficient than the rest in utilizing one or a few particular substrates under certain conditions. According to Liebig's law of the minimum, the growth of each organism is limited by the required substrate that is present in the lowest concentration (20). In the oral cavity, microorganisms living in the supragingival environment have access to nutrients from both endogenous (saliva) and exogenous (diet) origin. Saliva is an important source of nutrients and can sustain normal growth of microorganisms in the absence of exogenous nutrients (105, 454). Saliva contains water, carbohydrates, glycoproteins, proteins, amino acids, gases, and several ions including sodium, potassium, calcium, chloride, bicarbonate, and phosphate. Among exogenous dietary components, carbohydrates and proteins have the greatest influence on the composition of the oral microbiota (34, 335, 457).

The gingival crevice is not exposed to dietary components and saliva, and its principal source of nutrients is the gingival crevicular fluid. The crevicular fluid originates from plasma and is an excellent source of nutrients for fastidious microorganisms. It contains growth factors such as hemin and vitamin K required by *P. gingivalis*, a gram-negative rod associated with adult periodontitis.

Many nutritional interrelationships also occur between microorganisms. Some microorganisms cooperate for the degradation of nutrients. Some bacteria also use nutrients and other substances produced by other microorganisms.

Host Factors

Host defense mechanisms. The supragingival environment of the oral cavity is controlled primarily by saliva. The continuous flow of saliva increased by the muscular activity of the lips and tongue removes a large number of bacteria from teeth and mucosal surfaces. Saliva also contains several specific and nonspecific defense factors. SIgA is the principal specific defense factor of saliva, and its role is discussed more extensively below. The nonspecific defense factors include mucins, nonimmune salivary glycoproteins, lactoferrin, lysozyme, peroxidase, histatins, and cystatins.

Mucins are high-molecular-weight glycoproteins produced by submandibular, sublingual, and numerous minor salivary glands. They are the principal organic constituent of mucus, the slimy viscoelastic material that envelopes all mucosal surfaces of the body. Saliva contains two forms of mucins, MG1 and MG2. The MG1 mucin, which has a molecular mass greater then 1,000 kDa, is involved mainly in tissue coating; MG2, which has a molecular mass of 125 kDa, affects the aggregation and adherence of streptococci. In the oral cavity, mucins provide a protective coating for both soft and hard tissues. The mucins form a viscous slime layer on oral mucosa that traps microorganisms and antigens, limiting their penetration into the tissues (466, 467). Potentially harmful microorganisms are thus eliminated by the continuous renewal of the mucous layer combined with the washing action of salivary flow. Mucins are also constituents of the acquired pellicle and may protect teeth from acid demineralization.

The role of mucins and other nonimmune salivary glycoproteins in bacterial adherence is complex. When salivary glycoproteins are adsorbed on solid surfaces, they may bind to bacteria and promote bacterial adherence. Conversely, some of these glycoproteins, when free in saliva, may prevent bacterial colonization by binding to their adhesins or by agglutinating bacteria in saliva (50, 344, 422). This type of aggregation may facilitate the removal of oral bacteria by swallowing. Saliva from subjects with low levels of mutans streptococci aggregate these bacteria more efficiently, suggesting a protective role for salivary agglutinins (128). In vitro, pretreatment of *S. sanguis* and *S. mutans* with salivary glycoproteins prevented their attachment to hydroxyapatite (267). The phenomena of adherence and aggregation may be mediated by two different binding mechanisms. Saliva agglutinin (300 kDa), which acts as a receptor for antigen I/II of *S. mutans*, mediates both aggregation and adherence. However, the interaction involves different regions of the antigen I/II and different binding specificities (50, 344).

Saliva also possesses defense factors with direct antimicrobial activity in vitro. A group of salivary proteins, lysozyme, lactoferrin, and peroxidase, act in conjunction with other components of saliva to limit the growth of bacteria or kill them directly.

Lysozyme is a small cationic protein that is present in all major body fluids; it is secreted by intercalated duct cells (290, 377). Lysozyme can lyse some bacterial species by hydrolyzing glycosidic linkages in the cell wall peptidoglycan. It may also cause lysis of bacterial cells by interacting with monovalent anions, such as thiocyanate, perchlorate, iodide, bromide, bicarbonate, nitrate, and fluoride, and with proteases found in saliva. The combination leads to destabilization of the cell membrane probably by activation and deregulation of endogenous bacterial autolysins (290, 377). In vitro, the bacteriolytic activity of the lysozyme-protease-monovalent anion system has been demonstrated against *S. mutans*, *L. casei*, and *F. nucleatum* (376, 377). In addition, lysozyme can aggregate oral bacterial cells and inhibit their colonization on mucosal surfaces and teeth (378). In vivo, an inverse correlation has been found between the concentration of lysozyme and the accumulation of dental plaque (208).

Lactoferrin is an iron-binding glycoprotein produced by intercalated duct cells. It inhibits microbial growth, probably by sequestering iron in the environment. In addition, iron-free lactoferrin (apolactoferrin) possesses a direct, iron-independent bactericidal effect against various oral bacterial strains including *S. mutans* (17, 18). Apolactoferrin was shown to agglutinate *S. mutans* but not other species of streptococci, *P. gingivalis*, or *A. actinomycetemcomitans* (456). Recent results with bovine lactoferrin indicate a bactericidal activity of the N-terminal portion of the lactoferrin molecule (111a, 195a).

Salivary peroxidase is an enzyme secreted by salivary gland acinar cells. It is part of an antimicrobial system that involves the oxidation of salivary thiocyanate to hypothiocyanite and hypothiocyanous acid by hydrogen peroxide, generated by oral bacteria. These oxidizing agents react with sulfhydryl groups of the enzymes involved in glycolysis and sugar transport (489). Salivary peroxidase removes toxic hydrogen peroxide produced by oral microorganisms and can reduce acid production in dental plaque (113). Salivary peroxidase has been shown to retain activity when adsorbed on hydroxyapatite and so should be effective at the enamel-plaque interface (487). The activation of peroxidase systems in vivo by addition of appropriate amounts of exogenous hydrogen peroxide reduces plaque accumulation, gingivitis and caries (198, 199).

Histatins (histidine-rich peptides) are a family of small basic peptides (3 to 5 kDa), with a high content of histidine, that are produced by acinar cells (359). They inhibit the development of *Candida albicans* from the noninfective to the infective form

(359). They can also inhibit coaggregation between *P. gingivalis* and *S. mitis* (343), aggregate oral streptococci, and inhibit the growth of *S. mutans* (367).

Cystatins are a family of cysteine-containing phosphoproteins that are secreted by acinar cells (190, 430). These proteins are also present in plasma and may reach the oral cavity via the gingival crevicular fluid (190). Cystatins act mainly as thiol protease inhibitors and can inhibit proteases produced by suspected periodontopathogens.

Saliva does not gain access to the gingival crevice, and this area of the oral cavity is almost essentially controlled by the antimicrobial factors of plasma. Cellular and humoral components of blood can reach the gingival crevice of the oral cavity by the flow of gingival fluid through the junctional epithelium. Even in the healthy state, there is a continuous flow of small quantities of fluid and leukocytes from the gingival capillaries through the crevicular epithelium into the gingival crevice. This flow increases greatly with inflammation induced by plaque accumulation (258). The continuous flow of gingival fluid from the crevice to the oral cavity removes nonadherent bacterial cells. Gingival fluid also contains antimicrobial substances including IgM, IgG, IgA, complement, and leukocytes. These factors are primarily protective against microbial invasion, but, as seen above, the inflammation may become destructive, resulting in loss of periodontal attachment.

The leukocytes in gingival crevicular fluid are composed of 90% polymorphonuclear leukocytes (PMNs) and 10% mononuclear cells. Among the mononuclear cells, 60% are B lymphocytes, 20 to 30% are T lymphocytes, and 10 to 15% are macrophages (478). About 80% of PMNs are viable and functional within the crevice. The cells are capable of phagocytosis and of killing microorganisms, although the efficiency of phagocytosis is reduced compared with that of blood neutrophils (258). The PMNs perhaps remain functional at a short distance from the gingival margin by the flow of gingival fluid along the tooth surface, but once neutrophils are in saliva, they degenerate, due to osmotic lysis (274). Lysozyme and peroxidase that are released from the lysosome of PMNs during phagocytosis might also control microbial growth in the gingival crevice.

Components of the complement cascade are present in the gingival crevicular fluid. In subjects with healthy gingivae, C3 and C4 components of complement can be detected. During gingival inflammation, C3a, C3b, and C5a appear, suggesting that complement activation may have occurred in vivo (258, 441). Complement factors may initiate bacterial cell lysis or enhance phagocytosis of microorganisms.

The IgG, IgM, and IgA antibodies directed against a variety of oral microorganisms have been detected in plasma and crevicular fluid even in healthy individuals (120, 219, 278, 342, 447). These antibodies may influence the oral microbiota by interfering with adherence or by inhibiting bacterial metabolism (258, 447). Furthermore, the IgG antibodies may enhance phagocytosis and killing of oral microorganisms through activation of complement or opsonization (12, 258, 313, 428). It has been demonstrated that systemic immunization of animals with periodontopathogens may reduce the colonization of these bacteria in the gingival crevice and reduce periodontal destruction (83, 131, 314, 371). However, since periodontal diseases are of multifactorial origin, systemic immunization with periodontopathogens may also enhance the destruction of alveolar bone (67, 122). The immune response itself may contribute significantly to the periodontal destruction, sometimes even more than the pathogens!

Some indications suggest that serum antibodies may also regulate the bacterial colonization of supragingival surfaces of teeth. Although conflicting evidence exists, some studies have reported an inverse correlation between the level of serum IgG antibodies against *S. mutans* and the level of this bacterium in dental plaque or the frequency of caries (2, 447). Also, systemic immunization of nonhuman primates with *S. mutans* antigens led to reduced levels of *S. mutans* and less caries (261, 262).

All specific and nonspecific antimicrobial factors in the oral cavity do not act in isolation. Synergistic and antagonistic interactions among antimicrobial factors may influence their actions. Mucins serve to concentrate other antimicrobial substances, including lysozyme, IgA, and cystatins, at the mucosal surface (466). SIgA enhances the antimicrobial activity of lactoferrin, salivary peroxidase, agglutinins, and mucins. Similarly, the polycationic antimembrane effect of lysozyme may be enhanced by salivary peroxidase (377) and histatins (282). In contrast, salivary peroxidase may block the bactericidal effect of lactoferrin (255).

Age. The composition of the oral microbiota varies with the age of the host. Age-related changes in the oral microflora include those due to teeth eruption, changes in dietary habits, hormones, salivary flow, the immune system, or other factors.

The human oral cavity is usually sterile at birth. However, within 6 to 10 h after birth, microorganisms from the mother and to a lesser extent microorganisms from those present in the environment become established in the oral cavity. The pioneer species are usually streptococci, especially *S. mitis* biovar 1, *S. oralis*, and *S. salivarius* (73, 367a, 455). During the first year of life, the oral microbiota contains *Streptococcus*, *Neisseria*, *Veillonella*, *Staphylococcus*, and, to a lesser degree, *Actinomyces*, *Lactobacillus*, *Rothia*, *Fusobacterium*, and *Prevotella* (238, 455, 524). Some species, such as *S. sanguis*, *S. mutans*, and *A. naeslundii* genospecies 2 (formerly *A. viscosus*), colonize the oral cavity only after tooth eruption (446). Following tooth eruption, the number and isolation frequency of obligately anaerobic bacteria increase. The number of black-pigmented anaerobes and spirochetes in the gingival crevice increases more extensively during adolescence, and this could be due to hormonal changes (180, 455). Studies indicate that the next most important changes occur in the elderly and include an increased prevalence of staphylococci, lactobacilli, and *A. naeslundii* genospecies 2 (formerly *A. viscosus*) after the age of 70 years and an increase in the proportion of *Candida albicans* after 80 years (305, 369). The change in the oral microbiota of elderly individuals were not related to denture wearing, medication, or disease but may be caused by a decrease in salivary flow, an impaired immune system, or nutritional deficiencies (369, 370, 488).

Our research has focused on the development of the oral microbiota of BALB/c mice from birth to the age of 2 months (397). Staphylococci have been identified as the first colonizers, which are immediately followed by lactobacilli. The appearance of *Enterococcus faecalis* and members of the *Enterobacteriaceae* appeared to correspond to tooth eruption and the beginning of coprophagy. The proportion of *Lactobacillus murinus* sharply increased at weaning (20 days), probably due to changes in the composition and texture of the diet, from maternal milk to solid food (397). After the weaning period, no other significant changes were observed and the oral microbiota appeared to be completely stabilized when the mice reached the age of 6 to 8 weeks.

Hormonal changes. It is well known that in humans, puberty and pregnancy are characterized by increased levels of steroid hormones in plasma and subsequently in the crevicular fluid and saliva (130, 252). It is also well documented that pregnancy and puberty are associated with an increase in gingival inflammation which is accompanied by an increase in gingival exu-

date (539). It has been proposed that the exacerbations in gingival inflammation may be due to hormone-induced alterations in the microbiota of the gingival crevice (211, 241, 539). Microorganisms in the subgingival area that use hormones as growth factors may be favored during the period of hormone increase associated with puberty and pregnancy (242). Several investigators have described a transient increase in the number of black-pigmented gram-negative anaerobic bacteria in the subgingival microbiota during puberty (107, 180, 337, 529) or pregnancy (211, 242). Kornman and Loeshe (242) reported an increased proportion of *Prevotella intermedia* in the subgingival microbiota of pregnant woman, corresponding to an increased levels of estrogens and progesterone in plasma. They also demonstrated in vitro that progesterone or estradiol can substitute for vitamin K as an essential growth factor for *P. intermedia* (242). In contrast, other studies were unable to find any changes in the subgingival microbiota during puberty (536) and pregnancy (212).

In mice, we did not find any modifications in the oral microbiota during puberty (397) or pregnancy (94). However, in contrast to humans, mice do not usually harbor obligately anaerobic gram-negative rods. Furthermore, since the periods of puberty and pregnancy in mice are short, the bacteria might not be exposed to the hormones long enough to induce modifications in the oral microbiota.

Stress. Host stress may be associated with changes in hormones, salivary flow, dietary habits, and immune response (23, 51, 104, 254, 503). Few studies on the effect of stress on the indigenous microbiota have been performed, and most data have been obtained from studies of the intestinal microbiota of rodents. Stress in rodents is considered to be related to stimulation of the hypothalamic-pituitary-adrenocortical axis, which can lead to an elevation in corticosterone concentration. Crowding, fighting, and husbandry changes are some of the factors that are known to induce an increase in cortisol levels in plasma in mice and might play an important role in animal stress (51, 197, 254, 368, 503). These factors affect the behavior of mice in terms of feeding, sexual habits, grooming, rearing, and biting (503). The composition of the intestinal microbiota of rodents may be altered by a variety of unrelated disturbances, such as changes in environmental temperature, crowding in cages, and fighting among animals (239, 424). These changes in the intestinal microbiota include a reduction in numbers of lactobacilli (424, 474) as well as fusiform and segmented filamentous bacteria (230, 231, 239). In our studies, some evidence suggested that the oral microbiota of mice may also be influenced by stress. Various factors such as shipping to our animal facility, husbandry modifications, and low temperature (in nude mice) led to decreases in the proportions of *Lactobacillus murinus* among the total cultivable oral microbiota (150, 294, 295).

Genetic factors. The genetic background appears to influence the susceptibility to caries (249, 379) and periodontal diseases (161). This could in part be because the host genetic factors select for a microbiota with varying potential for causing oral diseases. The selection of a certain microbiota by the host is dependent on inherited immune factors, physiology, metabolism, mucus composition, or receptor-ligand interactions (337). Malamud et al. (289) produced evidence for the inheritability of agglutinin activity and parotid flow rate. Genetic factors also seem to influence the intestinal (230, 231, 308, 506) and oral (337, 425, 476) microbiota. Moore et al. (337) reported that the composition of subgingival microbiotas of monozygotic twins (11 to 14 years of age) was more similar than that of dizygotic twins. In contrast, we found that the genetic background does not seem to be an important factor in the composition of the oral microbiota of mice and that its influence is probably masked by environmental and husbandry factors (150). On the other hand, the oral indigenous microbiota of SPF mice is simple and the effect of the genetic background is perhaps observed only when the host is exposed to a more complex microbiota.

Bacterial Factors

Adherence. To get established in the oral cavity, microorganisms must first adhere to teeth or to mucosal surfaces. Adherence is essential for providing resistance to the flow of saliva. Adherence is mediated by adhesins on the surface of bacteria and by receptors on the oral surface. Microbial adhesins consist of polysaccharides, lipoteichoic acids, glucosyltransferases, and carbohydrate-binding proteins (lectins). These adhesins are found as cell wall components or are associated with cell structures, such as fimbriae, fibrils or capsules. The receptors may be salivary components (mucins, glycoproteins, amylase, lysozyme, IgA, IgG, proline-rich proteins, and statherins) or bacterial components (glucosyltransferases and glucans) that are bound to oral surfaces (160, 405, 422). The adherence may result from nonspecific physicochemical interactions between the bacteria and the oral surfaces. For example, lipoteicoic acids on microbial surfaces may interact with negatively charged host components through calcium ions or through hydrogen or hydrophobic bonding. However, these interactions cannot alone explain the selective attachment of bacteria to various oral surfaces. It is believed that another mechanism accounts for this selective colonization, perhaps involving specific or stereochemical interactions between bacterial adhesins and host receptors. In this case, the same physicochemical forces intervene but now act between extremely small, highly localized, spatially well organized opposing molecular groups (70). It is probable that the bacteria first adhere by nonspecific interactions which are followed by stronger stereochemical interactions. A number of specific interactions have been identified in adhesion to human tooth surfaces (reviewed in reference 422). The stereochemical interactions involved in bacterial adhesion in the oral cavity are analogous to the interactions between antigen and antibody or between an enzyme and its substrate. For example, type 1 fimbriae of *Actinomyces naeslundii* genospecies 2 and surface antigen I/II (protein P1) of *S. mutans* can bind to proline-rich proteins (407), *Streptococcus gordonii* can bind to α-amylase (423), and *A. naeslundii* genospecies 2 and *Fusobacterium nucleatum* can interact with statherin (159, 160, 535). Another adherence strategy involves a lectin-like bacterial protein with the complementary carbohydrate receptor located on glycoproteins (344). The interaction may be inhibited in vitro by adding the specific carbohydrate. *S. sanguis* can bind to sialic acid-containing oligosaccharides of the low-molecular-weight salivary mucin (MG2) (345). Type 2 fimbriae of *Actinomyces* binds to the beta-linked galactose glycoprotein on epithelial cell surfaces (60).

Bacteria may also colonize host surfaces by adhering to other bacteria, and several examples of coaggregation between human oral bacterial species have been demonstrated in vitro (235–237). *Streptococcus* spp. aggregates with *Actinomyces* spp., *F. nucleatum*, *Veillonella*, and *Haemophilus parainfluenzae. F. nucleatum* binds with *A. actinomytemcomitans*, *Porphyromonas gingivalis*, *H. parainfluenzae*, and *Treponema* spp. Most coaggregates that have been studied in detail involve two strains from different genera; this is referred to as intergeneric coaggregation. Intrageneric coaggregation is seen almost exclusively within oral viridans streptococci (235, 237). Many of

these interactions appear to be mediated by a lectin from one cell type that interacts with a complementary carbohydrate receptor from the other cell type. Coaggregation may be important in the development of dental plaque because it allows the colonization of bacteria that are not able to adhere directly to the acquired pellicle. However, almost all the data on coaggregation derive from in vitro experiments. Indications that the phenomenon exist in vivo are the microscopic observations of dental plaque revealing the presence of two types of structures: gram-positive filaments covered by gram-positive cocci, referred to as corn cobs; and large filaments surrounded by gram-negative rods or short filaments, referred to as bristle brushes (353).

Another example of coaggregation is the synthesis of extracellular polysaccharides from sucrose by mutans streptococci. The glucosyltransferases that are bound to the surface of mutans streptococci synthesize glucans in the presence of sucrose. Thus, the polymers are cell associated and can bind to the tooth surface or to other bacteria via other glucosyltransferases or via independent glucan-binding components. These polysaccharides consolidate bacterial attachment to teeth and contribute to an increased stability of the plaque matrix. The synthesis of polymers by mutans streptococci in the presence of sucrose is probably one of the factors implicated in caries formation (299).

Bacterial interactions. A variety of beneficial and antagonistic interactions may help in maintaining the homeostasis of the oral microbiota. Most of these bacterial interrelationships have been characterized in vitro or in animal models, and it is assumed that they operate in the same way in the human oral cavity.

Coaggregation is one exemple of commensalism and synergism that occurs between microbial species. Coaggregation allows the indirect adherence of some bacteria on oral surfaces. In addition, it was demonstrated that coaggregated cells were more resistant to phagocytosis and killing by neutrophils in vitro and in vivo (354). Several other examples of positive interactions are likely to occur in the oral cavity. The utilization of oxygen by facultative anaerobic bacteria reduces the oxygen concentration and the E_h to levels that allow the colonization of anaerobic bacteria (495). Different bacterial species may also cooperate in the utilization of substrates that they could not metabolize alone. In laboratory studies, *P. gingivalis* and *F. nucleatum* were shown to hydrolyze casein synergistically (158). Chemostat studies indicated that glycoprotein degradation may involve the synergistic action of several species possessing complementary patterns of glycosidase and protease activities (509). The development of complex food chains also contributes to the diversity and stability of oral ecosystems (299, 495). For example, the metabolism of carbohydrates by *Streptococcus* and *Actinomyces* generates lactate, which may be used by *Veillonella*. The utilization of lactic acid by *Veillonella* produces vitamin K, required by black-pigmented gram-negative rods, and H_2, used by *Wolinella.*

Competition and antagonism mechanisms among oral resident bacteria may help to maintain the ecological balance by preventing the overgrowth of some resident bacterial species or the establishment of allochthonous bacteria (300, 495). It is more difficult to implant a bacterium in conventional animals than in axenic or antibiotic-treated animals (508). The barrier effect of the autochthonous microbiota against allochthonous and pathogenic species is known as colonization resistance (512). The competition for adhesion receptors, nutritional competition (322, 510), and the production of inhibitory substances (antagonism) are among the mechanisms involved in reducing bacterial colonization and preventing bacterial overgrowth. Recently, an inverse correlation was found between the proportion of salivary bacteria inhibiting *Streptococcus mutans* and the percentage of untreated carious teeth, suggesting a possible role of inhibitory substances in the maintenance of oral health (168). Inhibitory substances include organic fatty acids (112), hydrogen peroxide (296), lactic acid (365), antibiotics (417), enzymes (21), and bacteriocins (206, 366, 468, 469). The production of lactic acid by *S. mutans* and *Lactobacillus* generates low pH and inhibits the growth of *S. sanguis* and *S. oralis* as well as gram-negative bacteria. It has been suggested that the production of hydrogen peroxide by oral streptococci may reduce the growth of periodontopathogens. Bacteriocins are bactericidal proteinaceous substances, and those that are produced by streptococci may have a wide spectrum of activity against other gram-positive bacterial species (206, 366, 468). It has been argued that bacteriocins play a limited role in the ecology of the oral cavity because they are probably rapidly inactivated by the numerous proteases found in saliva (468). However, it has been found that some bacteriocins are not affected by human saliva (366). Many in vivo experiments with humans and animals indicate that the production of bacteriocins confers an ecological advantage. It is easier to implant strains of *S. mutans* that produce bacteriocins in the oral cavity than non-bacteriocin-producing strains (194, 228, 508).

External Factors

Diet. It is well documented that frequent consumption of a high-sucrose diet enhances the development of *S. mutans* and *Lactobacillus* (335, 347, 457). The fermentation of sucrose into lactate generates a low pH, favoring acidogenic and acidophilic bacteria. The substitution of sucrose with weakly fermentable sugar alcohols such as xylitol results in a reduction of *S. mutans* numbers and caries (205, 287). Xylitol appears to selectively inhibit carbohydrate metabolism in *S. mutans*, which reduces acid production and thereby stabilizes the composition of the oral microbiota (49). Apart from the effect of sucrose, very few studies have addressed the effect of other dietary components on the oral microbiota. During our research on the effects of various dietary components on the oral microbiota of mice (34), we found that a high-starch diet favored an increase in the proportion of *Enterococcus faecalis* while a high-protein diet favored an increase in *Lactobacillus murinus*. Variations in the vitamin, lipid and mineral content of the diet had no direct effect on the oral microbiota of mice.

Oral hygiene and antimicrobial agents. Oral hygiene is one of the most important factors in the maintenance of oral homeostasis and oral health. The mechanical removal of plaque by tooth brushing and flossing can almost completely prevent caries and periodontal diseases (307, 460). The addition of antimicrobial agents to dentifrices, mouthwashes, and varnishes increases the effect of mechanical oral hygiene procedures. Antimicrobial agents may assist in protection by reducing bacteria adhesion to the tooth surface, by reducing the growth of microorganisms and plaque accumulation, by selectively inhibiting only those bacteria directly associated with oral diseases, or by inhibiting the expression of virulence determinants, such as acid production or protease activity (302). Fluoride is found in most toothpastes and mouth rinses and is well known for its anti-caries properties. The principal caries-preventive effect of fluoride is attributed to the formation of fluoroapatite and calcium fluoride, which lead to an increase in the resistance of enamel to demineralization (48). It can also inhibit bacterial growth by reducing the sugar transport, glycolytic activity, and acid tolerance of many gram-positive species (48, 301). Fluoride can help stabilize the composition of

the microflora by reducing the rate of acid production and the fall in pH during frequent carbohydrate intake (43). Other agents that have been formulated for commercial toothpastes and/or mouth rinses include chlorhexidine, quaternary ammonium compounds, plant extracts, metal ions, and phenolic compounds (97, 304). These antimicrobial agents have been shown to reduce dental plaque formation, caries, and gingivitis (97, 302).

Drugs and diseases. Salivary gland hypofunction and xerostemia may result from the intake of xerogenic medication, irradiation treatments for head and neck cancer, and Sjøgren's syndrome. Patients with xerostomia have a decrease capacity to eliminate sugars and buffer the acids found in plaque. In addition to suffering from the reduction in saliva protection, patients with xerostomia generally consume soft, high-sucrose diets and suck sour candies to keep their mouths moist (299). Patients suffering from xerostemia have higher levels of mutans streptococci, lactobacilli, staphylococci, and *Candida*, while the levels of *S. sanguis*, *Neisseria*, *Bacteroides*, and *Fusobacterium* are reduced compared with those in a healthy individual. They are also more susceptible to dental caries and candidiasis (63, 273).

Antibiotics that are given orally or systemically for the treatment of different infections may enter the oral cavity via saliva and gingival crevicular fluid and lead to a inbalance in the oral microbiota (416, 417). Antibiotics may suppress some resident bacterial populations which can result in overgrowth of antibiotic-resistant bacteria, infections by opportunist pathogens such as *Candida*, and colonization by exogenous potential pathogens such as yeasts and members of the *Enterobacteriaceae.*

Other factors. Many other external factors may affect the oral microbiota; these include the wearing of dentures or partial dentures (305), smoking, oral contraceptives usage (539), malnutrition (474), host macroenvironment (150, 294, 295), and various exposures to exogenous bacterial species (150, 396).

SECRETORY IgA SYSTEM

IgA Structure

SIgA is the principal immunoglobulin isotype found in saliva and all other secretions. It exists as a polymeric molecule composed of two (or more) IgA monomers (300,000 Da), a J (joining) chain (15,600 Da), and a secretory component (SC) (70,000 Da) (reviewed in references 54, 81, and 220) (Fig. 1). Each monomeric IgA is formed of four polypeptides, two α-heavy chains and two light chains (kappa or lambda) linked covalently by disulfide bonds. The J chain and SC are disulfide linked to the Fc region of the IgA molecule (220). The J chain is a polypeptide synthesized within plasma cells that is involved in initiating the polymerization of IgA. The SC is a heavily glycosylated protein produced by mucosal epithelial cells. The SC stabilizes the structure of polymeric IgA and protects the molecule from proteolytic attack in secretions (224). It is referred to as the polyimmunoglobulin receptor (PIgR) in its membrane-bound molecular form. It is present on basolateral epithelial cell membranes and acts as a receptor for transepithelial transport of polymeric IgA (and IgM) (81, 497).

In humans, there are two IgA subclasses, IgA1 and IgA2, which occur in similar proportions in saliva and other secretions. The IgA1 and IgA2 heavy chains differ in only 22 amino acids, predominantly due to a deletion of 13 amino acids in the hinge region of IgA2; these amino acids are present in IgA1 (220, 498, 501). This structural difference renders IgA2 resistant to the action of a number of bacterial proteases that specifically cleave IgA1 in the hinge region (220). These IgA1 proteases are produced by several mucosal pathogens as well as by a large number of resident bacteria of the oral cavity and are thought to interfere with most of the protective properties of IgA antibodies (224). Salivary IgA antibodies against proteins and carbohydrates of bacteria occur predominantly in the IgA1 subclass, and the antibodies against lipoteichoic acid and lipopolysaccharide are more prevalent in the IgA2 subclass (64).

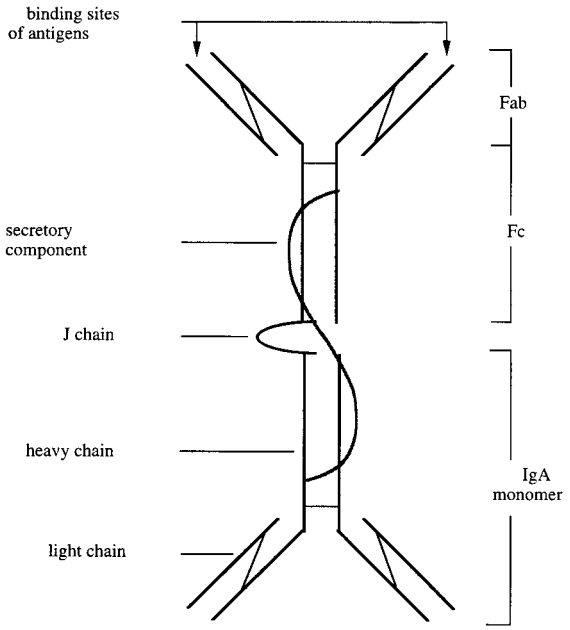

FIG. 1. Schematic representation of SIgA. SIgA consists of at least two IgA monomers linked to a J chain and a secretory component (SC). The J chain and SC are disulfide linked to the Fc region of the IgA molecule. Each IgA monomers consist of two α-heavy chains and two light chains linked covalently by disulfide bonds. The wavy line represents the SC.

Two subclasses of IgA, IgA1 and IgA2, similar to those in humans have been identified in chimpanzees, gorillas, and gibbons (215, 216). Generally, only one IgA isotype is found in other primates, rats, and mice, with its structure differing from IgA1 and IgA2 (215, 216, 395, 502). Only IgA1 from gorillas and chimpanzees and IgA from orangutans are susceptible to cleavage by IgA1 proteases (380, 384).

Synthesis and Transport of Salivary IgA

Salivary IgA is produced by plasma cells that are located adjacent to the duct and acini of salivary glands (243). IgA-secreting plasma cells predominate in the major and minor salivary glands over plasma cells producing other Ig isotypes (328). Polymeric IgA containing J chain, secreted by plasma cells, is specifically recognized by the PIgR located on the basolateral surface of the ductal and acinar cells (497). The polymeric IgA-PIgR complex is internalized into endocytic vesicles and transported to the apical surface of the epithelial cells. After fusion of the vesicles with the cell membrane, the PIgR is proteolytically cleaved, which releases a portion of PIgR, called SC, and polymeric IgA into the secretions as SIgA (81, 497). During the external translocation, disulfide bonds covalently link SC with polymeric IgA, which in turn stabilizes the IgA-SC complex (54).

Induction of Salivary IgA Response

The salivary IgA response against oral antigens may be induced by two mechanisms. First, oral antigens may stimulate the proliferation and differentiation of lymphoid cells locally in the salivary glands. Salivary glands contain lymphoid tissues consisting of macrophages, T cells, and B cells, which may be directly accessible to oral antigens (110, 346, 363, 364). Oral antigens may enter into the duct gland by natural retrograde flow and gain access to underlying immune system cells through endocytosis by ductal epithelium (92). The antigens may then be captured by macrophages and presented to T and B cells (364). The indirect evidence for the presence of a local immune system associated with salivary glands was derived from topical immunization studies. The instillation of antigens into the parotid duct of monkeys has been shown to induce salivary IgA antibodies (129, 133, 346, 517). In humans, topical application of glucosyltransferase from *S. sobrinus* to the labial mucosa results in a salivary IgA antibody response (443). The minor salivary glands have short ducts and are located superficially throughout the lamina propria of the lips, cheeks, and soft palate mucosa. They may be continuously exposed to oral antigens by natural retrograde flow (346). The presence of bacteria deep within the secretory ducts of labial salivary glands of primates suggests that antigens can naturally infiltrate these ducts (346). However, the duct length of the major salivary glands probably precludes retrograde stimulation under natural conditions.

The second important mechanism involves the migration of antigen-sensitized IgA precursor B cells from gut-associated lymphoid tissues (GALT) to salivary glands (320, 324, 328). The GALT, including numerous solitary lymphoid nodules and particularly Peyer's patches, are a rich source of precursor IgA B cells that have the potential to populate distant lymphoid tissues. These lymphoid follicules are covered by a specialized epithelium termed follicule-associated epithelial cells (FAE cells) or microfold cells (M cells) that take up and transport antigens from intestinal lumen into the underlying lymphoid tissue. Following antigen presentation by accessory cells, precursor IgA B cells and T cells leave the GALT via the efferent lymphatics and reach peripheral blood through the thoracic duct. The circulating B and T cells then migrate to the lamina propria of the intestine, the lungs, the genitourinary tract, and the secretory glands, where they are selectively retained (54, 81, 324). In these mucosal and glandular tissues, the precursor IgA B cells clonally expand and mature into IgA plasma cells under the influence of T cells. This cell distribution pathway from inductive tissues such as GALT to mucosal and glandular distant tissues has been termed the common mucosal immune system. Support for the existence of a common mucosal immune system is the finding of naturally specific IgA in secretions of glands not directly stimulated by antigens (10, 72, 123). Furthermore, experimental work with animal and humans has demonstrated that oral administration of bacterial antigens induces specific SIgA antibodies in saliva and other secretions (99, 101, 170, 325, 327, 333). Although GALT constitutes the major part of the mucosa-associated lymphoid tissues (MALT), the nasal mucosa-associated lymphoid tissues (NALT), which are represented by the tonsils and other organized lymphoid tissues of Waldeyer's pharyngeal ring, may also constitute an important IgA inductive site (54). SIgA antibodies against inhaled antigens have been detected in various secretions including saliva.

Thus, the salivary glands contain the lymphoid cells that are necessary for generating a SIgA response independent from MALT and for pursuing the last stage of differentiation of antigen-sensitized IgA precursor B cells arising from GALT or NALT. It is possible that the induction of SIgA antibodies in saliva involves an initial antigenic stimulation in major inductive sites (GALT and NALT) followed by further local antigenic stimulation in the salivary glands to expand the pool of plasma cells.

Biological Functions of Secretory IgA

SIgA is considered the first line of defense against pathogens which colonize and invade surfaces bathed by secretions (323, 328). It is known that SIgA plays an important role in protection against infections caused by enteropathogens and viruses both in human and in animal models (147, 323, 328, 334, 387). The intrinsic resistance of IgA to proteolysis, which is reinforced by the presence of the SC, preserves the biological functions of the molecule in secretions (320). The multivalence of SIgA enhances its potential to agglutinate bacteria and neutralize toxins, enzymes, and viruses (224, 320). The decreased ability of SIgA to activate complement and opsonize bacteria for phagocytosis may limit local inflammatory reactions and mucosal tissue damage (54).

Inhibition of bacterial adherence. The inhibition of bacterial adherence by SIgA is considered one of the most important defense mechanisms against mucosal bacterial invasion. In vitro, SIgA has been shown to limit the attachment of bacteria to epithelial cells isolated from buccal (525), intestinal (286, 530), urinary (463, 464), genital (499), nasal (250), and bronchial mucosa (461). SIgA antibodies to *Vibrio cholerae* reduce the adsorption of this bacterium to rabbit intestinal sections (286). Human salivary IgA inhibits the adherence of oral streptococci and *Candida albicans* to buccal epithelial cells (515, 525). Although SIgA may inhibit the attachment of oral streptococci to teeth (183, 222, 225), they may also promote the adsorption of some strains (222, 270). An explanation for these contradictory results will be given below. Evidence for the antiadherence properties of SIgA was obtained in studies involving passive immunization (32). Specific monoclonal SIgA antibodies directed against microbial surface components protected mice against intestinal infection caused by *Salmonella typhimurium* (334), *V. cholerae* (14, 528), and *Shigella flexneri* (373).

SIgA interferes with bacterial adherence to host surfaces by preventing both nonspecific and stereochemical interactions. The binding of SIgA to adhesins can reduce the negative surface charge and the hydrophobicity of bacteria, thus limiting the potential for ionic and hydrophobic interactions between bacteria and host receptors (283–285). The reduction of the hydrophobicity of bacteria is probably due to the heavy glycosylation of Fc and SC components, which confer hydrophilic properties to the SIgA molecule (224). Studies have also found that SIgA can sterically block the binding of adhesins to complementary surface receptors on host surfaces (183, 224). SIgA antibodies against fimbriae of gonococci and members of the *Enterobacteriaceae* reduced the adhesion of these bacteria to epithelial cells (464, 499). In addition, SIgA may impair bacterial adherence by agglutinating bacteria, thereby facilitating their clearance by secretions (59, 270, 286, 358). In vitro, the aggregation of *E. coli* by SIgA prevents its passage across intestinal tissue sections (9). The mechanisms involving aggregation of members of the *Enterobacteriaceae* by SIgA may be independent of antibody specificity. SIgA was shown to possess a carbohydrate receptor for the mannose-specific type 1 fimbrial lectin of *E. coli* (530).

Inactivation of bacterial enzymes and toxins. SIgA can neutralize toxins by blocking their binding to cell receptors. SIgA

antibodies prevent the binding of cholera toxin to intestinal epithelial cells (286) and partially protect animals against diarrheal diseases (14, 505). SIgA can also inhibit a variety of enzymes, perhaps by blocking their binding to substrates or by destabilizing the enzyme-substrate complex (35, 148, 162, 171, 176, 233). SIgA directed against glucosyltransferases of streptococci has been shown to inhibit the synthesis of extracellular polysaccharides and reduce dental plaque accumulation (233). Purified colostral SIgA inhibits IgA1 proteases that are produced by *Neisseria meningitidis*, *N. gonorrhoeae*, and *Streptococcus sanguis* (162). If IgA1 proteases play a role in the infectious process, the ability to neutralize IgA1 proteases may constitute a defense mechanism against mucosal invasion by some pathogens (162).

Synergism with other defense mechanisms. SIgA may also act synergistically with innate immune factors present in secretions. It has been shown that the activity of the lactoperoxidase system against *S. mutans* was enhanced by the presence of SIgA (485). This improved activity was not dependent on the presence of specific antibodies against *S. mutans*. It has been suggested that the interaction between the Fc part of IgA and lactoperoxidase is responsible for stabilizing the enzymatic and antimicrobial activity of lactoperoxidase (224). SIgA may enhance the bacteriostatic effect of lactoferrin, and this effect can be inhibited by the addition of iron (149, 399). Although the mechanism by which SIgA increases the ability of lactoferrin to take iron from microorganisms is not clear, some evidence suggests that SIgA antibodies suppress the production of bacterial siderophores, which are able to remove iron from lactoferrin (149, 399). SIgA antibodies also appear to bind to lactoferrin and target the protein against microorganisms. The presence of complexes between SIgA and lactoferrin has been observed in secretions (224).

Some studies have suggested a synergistic action between SIgA and mucins. The binding of SIgA antibodies may render bacteria highly mucophilic, which facilitates their removal from the mucosa by the continuous renewal of the mucous layer (283). Furthermore, the low-molecular-weight salivary mucin, MG2, may participate in noncovalent interactions with SIgA. The binding of MG2 to *Pseudomonas aeruginosa* and *Staphylococcus aureus* is dependent on heterotypic complexing between MG2 and SIgA (33). The interaction occurs in solution but not when proteins are immobilized onto a solid surface. Thus, the complex MG2-SIgA does not promote bacterial attachment and can facilitate bacterial removal from the oral cavity by enhancing bacterial aggregation (33). The association between SIgA and agglutinins in saliva may also enhance bacterial aggregation (358, 406).

Virus neutralization. SIgA plays an important role in viral immunity because of its presence at the site of initial contact between virions and host cells (81). A protective effect of SIgA against respiratory and enteric viral infections has been demonstrated (348, 387, 483). Mice that had been passively immunized with SIgA antibodies against influenzae virus were protected against experimental nasal infection with this virus (387). In vitro, it has been demonstrated that human immunodeficiency virus type 1 can be neutralized by SIgA (66). The mechanisms involved in viral inactivation are complex and not fully understood. Traditionally, SIgA was thought to prevent the penetration of virions into epithelial cells by sterically blocking their adhesins. SIgA antibodies to hemagglutinin of the influenza virus reduce viral attachment to cells and penatration of virions into cells (483). However, SIgA antibodies enhance the binding of the gastroenteritis virus but block its subsequent penetration into the cells (348). It has been suggested that SIgA antibodies induce agglutination of the gastroenteritis virus by enhancing nonspecific viral attachment to epithelial cells (81, 224). Armstrong and Dimmock (15) provided evidence in support of different mechanisms for viral neutralization, according to the number of antibodies per virion. High concentrations of anti-hemagglutinin polymeric IgA inhibited the attachment and internalization of influenza virus virions to the tracheal epithelial monolayer, while lower concentrations of IgA did not prevent virus attachment and internalization but did inhibit the fusion and transcriptase activities of the virus. The mechanism of neutralization appears to involve conformational changes in the structure of the hemagglutinin following antibody binding, which subsequently transduces a signal that affects a crucial molecular function within the virion (15, 111, 484). Thus, dimeric IgA can neutralize influenza virus by the inhibition of fusion, internalization, and attachment as the ratio of IgA to virus increases (111).

Recent in vitro cell culture experiments suggested that dimeric IgA can neutralize virus within infected mucosal epithelial cells, if the epithelial cells express the polymeric Ig receptor (transmembrane secretory component) (310–312). The epithelial cells used were MDCK cells (derived from canine renal tubule cells) that have been transfected to express the rabbit polymeric Ig receptor and therefore were capable of endocytozing dimeric IgA. It was shown that dimeric IgA antibodies that were actively transported through epithelial cells by the polymeric Ig receptor, bind to viral proteins within the cells and prevent viral replication (253, 312). This mechanism of neutralization may also be effective during the transport of dimeric IgA into secretions in vivo (253). What remains to be determined is in which subcellular compartment this process takes place.

Complement activation. The ability of serum or SIgA to activate complement remains controversial. The use of different assays and different sources of IgA and complement have resulted in contradictory reports. Compared with IgG and IgM, IgA is a poor activator of complement, and it is not clear whether IgA may efficiently induce complement-mediated bacterial death via opsonization or cell lysis. There is general agreement that IgA does not activate the classical complement pathway in vitro (401, 408). Human serum or SIgA does not bind the C1q (193, 401) component, while rat polymeric and dimeric IgA can bind the C1q component but does not activate the C1 component (193).

There is evidence that IgA might activate the alternative complement pathway (191, 192, 372, 388, 389, 459). The ability of IgA to activate the alternative pathway in vitro has been demonstrated in experiments with aggregated SIgA and monoclonal IgA. It has also been shown that the alternative pathway can be activated, as revealed by C3 deposition and solubilization of the immune complex, by immune precipitates composed of mouse or rat monoclonal IgA and the corresponding antigen (191, 388, 389). Bohnsack et al. (37) reported that murine IgA monoclonal antibodies directed against group B streptococci mediated C3 deposition through the alternative pathway and enhanced phagocytosis of the bacteria. In patients with nephropathy, IgA deposition is frequently associated with the C3 component, suggesting that IgA may also activate the alternative pathway in vivo (31). In contrast, others have reported that all forms of human IgA antibodies bound to corresponding antigens fail to fix C3 component by the alternative pathway (93, 203, 349, 408). It has been postulated that denaturation of IgA might be responsible for its alternative-pathway-activating property (349, 408).

In general, studies have revealed that complement activation by IgA results in consumption of the C3 component but rarely

in consumption of the C5 component (220, 224, 372). Thus, the C5a component, a major anaphylatoxic and inflammatory peptide, is probably rarely released in vivo (224). Furthermore, the assembly of the membrane attack complex (C5 to C9) and bacteriolysis are probably rare events (220, 224). Several studies suggest that IgA antibodies inhibit the activation of the classical pathway by IgG and IgM antibodies recognizing the same antigens (178, 349, 411). IgA antibodies reduce IgG and IgM complement-mediated bacteriolysis in vitro and Arthus reactions in mice (414). This inhibition might be attributed to competition between IgA and other isotypes for antigen binding sites or to interference with the availability of the initial classical pathway components (193, 349, 411). Since it is known that SIgA is a poor activator of complement and that secretions are generally deficient in complement components, complement-mediated microbial killing is probably of limited immunological importance at mucosal surfaces.

IgA-dependent cell-mediated functions. Receptors for the Fc portion of IgA (FcαR) are expressed on several cell types including polymorphonuclear neutrophils (PMNs) (136), monocytes and macrophages (25, 136, 155), eosinophils (3), and lymphocytes (4, 200, 277). The expression of FcαR seems to be influenced by the level of IgA in the environment surrounding the cells. The proportion of PMNs and monocytes expressing FcαR cells is higher in the oral cavity than in the peripheral blood (25, 136). The expression of FcαR on blood neutrophils, monocytes, and lymphocytes can be enhanced by incubation with SIgA or myeloma IgA (136). However, the exact role of IgA as modulator of the leukocytes effector functions is still unclear. Some studies suggest that IgA is able to induce phagocytosis and a respiratory burst in monocytes, macrophages, and PMNs (25, 136, 167, 213, 429, 520, 537). Fanger et al. (136) observed phagocytosis of ox erythrocytes that had been opsonized with SIgA antibodies by PMNs from the oral cavity but not by peripheral blood PMNs. Human serum IgA and SIgA were found to enhance the phagocytosis of *Staphylococcus aureus* by PMNs and elicit a respiratory burst as measured by the release of H_2O_2 (167). The capability of IgA to induce antibody-dependent cellular cytotoxicity (ADCC) was also reported in experiments with monocytes (276) and T lymphocytes (470–472) as effector cells. However, these experiments have not been independently confirmed. Intraepithelial $CD4^+$ lymphocytes from the gut mucosa exerted antimicrobial activity against *Salmonella* and *Shigella* in the presence of specific SIgA antibodies (471). The number of T lymphocytes mediating IgA-dependent ADCC increased when individuals were orally immunized with *Salmonella* (470, 472).

In contrast, other studies have found that the interaction between IgA and FcαR leads to inhibition of phagocytosis, chemotaxis, and ADCC (415, 434, 513, 526, 531, 532). It was reported that SIgA and serum IgA inhibited the phagocytosis of *Staphylococcus epidermidis* and *Candida albicans* by PMNs (415, 526). It appears that IgA reduces PMN function by interfering with the binding of other opsonins such as IgG and the C3b component to their receptor (415, 531). In addition, other studies have suggested that IgA may directly inhibit phagocyte activation, induced by a variety of chemotactic stimuli (lipopolysaccharide, *Haemophilus influenzae* type B, and *n*-formyl-methionyl-leucyl-phenylalanine [fMLP]) without influencing receptor-ligand interaction. It has been shown that human serum IgA down-regulates the induction and release of inflammatory cytokines (tumor necrosis factor alpha and interleukin-6) in activated monocytes (531). Human serum IgA also inhibited the generation of respiratory burst and release of reactive oxygen intermediates, such as superoxide anion, H_2O_2, and the hydroxyl radical, in activated monocytes and PMNs (531). Thus, IgA has a dual effect on phagocytes which appears to be dependent on their state of activation (465, 531). Sybille et al. (465) demonstrated that the interaction of human IgA with FcαR on human PMNs stimulated chemotaxis and increased PMN migration in response to suboptimal concentrations of fMLP but decreased the PMN responses at optimal fMLP concentrations.

It is probable that SIgA-dependent cell-mediated activity does not constitute a primary line of defense at mucosal surfaces due to the small number of functional leukocytes in secretions and the large number of microorganisms that colonize mucosal surfaces (81, 224). However, epithelium-associated phagocytes and cytotoxic cells may act as a secondary line of defense against pathogens that invade intestinal epithelium, such as *Salmonella* and *Shigella* (470–472). Furthermore, by modulating the release of inflammatory mediators, IgA both in secretions and within the mucosa may interfere with the development of chronic inflammation initiated by microbial pathogens and antigens (54, 56, 531).

Immune exclusion. One of the major functions of SIgA is to perform immune exclusion, which consists of limiting the penetration of antigenic materials through the mucosal epithelium (54). This involves the binding of SIgA antibodies with antigens, which facilitates their removal from mucosal surfaces. Furthermore, polymeric IgA containing immune complexes formed within the mucosal lamina propria may bind to the polymeric IgA receptor and be transported across the epithelium by the same mechanism as free polymeric IgA (253, 312). This antigen-handling mechanism is particularly important in the intestinal tract, where components of food and microbiota may gain access to the lamina propria. It has been established that previous enteric exposure to foreign proteins diminishes subsequent intestinal adsorption of the same antigen in association with the production of specific SIgA antibodies (13, 518). It is also known that IgA-deficient subjects have an increased intestinal permeability to macromolecules that lead to the formation of circulating immune complexes (98, 188, 268). Since SIgA is relatively ineffective in activating complement and promoting phagocytosis, immune exclusion is primarily a noninflammatory mechanism (54). By competing with proinflammatory IgM, IgG, and IgE antibodies, SIgA at mucosal surfaces, as well as locally produced dimeric IgA and serum-derived IgA within the lamina propria, has been shown to control levels of inflammation in mucosal tissues (54, 253, 312). Experimental studies with rodent models suggest that an IgG or IgE immune response to antigens may damage intestinal mucosal tissues, rendering them more susceptible to penetration by a variety of macromolecules through mucosal epithelium (36, 496). A decrease in the number of IgA-producing cells and an increase in the number of IgG-producing cells have been observed in gastrointestinal inflammatory lesions (54, 106, 268). The mucosal integrity may be damaged by lysosomal enzymes and oxygen intermediates released from PMNs that are attracted by complement-activating IgG immune complexes. Some studies reported a higher incidence of inflammatory and autoimmune diseases in IgA-deficient patients (268).

ROLE OF SECRETORY IgA IN ORAL MICROBIAL ECOLOGY

It is well established that SIgA is the predominant Ig in whole saliva and is considered to be the main specific defense mechanism in the oral cavity. SIgA antibodies could help maintain the integrity of the oral surfaces by limiting microbial adherence to epithelial and tooth surfaces; by neutralizing

enzymes, toxins and virus; or by acting in synergy with other antibacterial factors such as lysozyme, lactoferrin, salivary peroxidase, and mucins. SIgA may also prevent the penetration of antigens in the oral mucosa. Since saliva is generally deficient in complement components and functional effector cells (monocytes, PMNs, and lymphocytes), it is unlikely that other functions associated with SIgA, such as complement activation, opsonization, and SIgA-dependent ADCC, occur in the supragingival environment. However, inflammatory cells and complement are present in the subgingival environment, and these functions may be accomplished by serum IgA.

One of the major intriguing questions concerning the role of SIgA in oral microbial ecology is whether it has an effect on indigenous bacteria. Despite the presence of the secretory immune system and high levels of SIgA in saliva, the resident microbiota still persists in the oral cavity. The survival of indigenous bacteria in the oral cavity has been attributed to their reduced susceptibility to and their ability to avoid immune mechanisms (30, 44, 87). Dubos et al. (114) postulated that autochthonous bacteria were not immunogenic for their host, perhaps because these microorganisms had achieved a state of symbiosis with their host over a long period of evolutionary adaptation. Other microorganisms of the resident microbiota that are potentially pathogenic would elicit a protective response and be eliminated or kept at low levels under normal circumstances. Several experimental studies support the hypothesis that the immune system is relatively tolerant to its autochthonous microbiota (28, 30, 74, 115, 141, 142, 281, 375). Germ-free mice monoassociated with indigenous *Escherichia coli* or *Bacteroides* isolated from conventional mice exhibited a lower systemic immune response than did germ-free mice monoassociated with *E. coli* and *B. fragilis* derived from human sources (30). Oral administration of *Salmonella paratyphi* to germ-free mice resulted in the development of germinal centers in Peyer's patches and serum antibody response, while oral administration of *Enterococcus faecalis*, a member of the resident microbiota of mice, failed to induce an immune response (375). Duchmann et al. (115) found that humans were tolerant to their own intestinal microbiota but responded to the intestinal microbiota of other individuals.

Tolerance could be the result of clonal elimination (cell death), clonal anergy (functional inactivation without cell death), or active suppression of antigen-reactive B and T cells (115, 146, 281, 427). It has also been suggested that indigenous bacteria could share surface antigens with host tissues (142) or become coated with host-derived molecules so as to not be recognized as foreign (44, 87). Although logical and appealing, these hypotheses have unfortunately often become part of established theories without the required support from a wide range of experiments. Furthermore, most of these studies compared the systemic immune response between indigenous and nonindigenous bacteria (28, 30, 141, 142) and only a few data reported on the mucosal IgA response (74, 375, 433). A comparison of germfree and conventional mice revealed that the presence of resident microbiota on mucosal surfaces is essential for the normal development of IgA-bearing plasma cells in Peyer's patches and lamina propria of the gut (95, 340, 507). Evidence indicated that the ability to induce the mucosal immune response may vary among indigenous bacterial species (340, 432, 433). In support of this view, Moreau et al. (340) reported that monoassociation of germfree mice with *Bacteroides* and *E. coli* elicited a higher development of intestinal IgA plasmocytes than did monoassociation with *Lactobacillus*, *Streptococcus*, *Actinobacillus*, *Eubacterium*, and *Clostridium*, while *Corynebacterium* and *Micrococcus* were not immunogenic. In that study, the specificities of the SIgA response were not tested.

Although indigenous bacteria may induce a lower mucosal immune response than allochthonous bacteria, many studies have revealed the presence of naturally occurring SIgA antibodies directed against a variety of resident bacteria in saliva and other secretions of humans (7, 10, 55, 59, 64, 151, 426, 436) and animals (108, 109, 293, 294, 296, 297). These antibodies have been detected against whole cells or purified antigens including polysaccharides, proteins, lipoteichoic acids, and glucosyltransferases. Oral bacteria are generally coated with SIgA, as demonstrated in both humans (55) and mice (108). Salivary IgA antibodies have also been detected in the acquired pellicle and dental plaque (361, 400, 477).

The development of the salivary IgA antibody response against oral indigenous bacteria has been investigated in humans. Smith and colleagues (153, 444, 452) found that the appearance of salivary IgA antibodies to oral streptococci in infants and children correlated well with the sequence of colonization of these bacteria in the oral cavity. Salivary IgA antibodies to antigens of *S. salivarius* and *S. mitis* were detected in infants as young as 5 weeks old, which reflects the early colonization of the mouth by these bacteria (444, 452). The specificities of the IgA antibodies were suggested by the absence of reaction against *S. mutans* and *S. sanguis*, which do not colonize predentate infants. Salivary IgA antibodies to glucosyltransferase of *S. sanguis* appeared only after 1 year of age, while salivary IgA antibody to glucosyltransferase of *S. mutans*, an organism that colonizes later than *S. sanguis*, was detected only at 3 to 4 years of age. The same study also found that salivary antibodies specificities to oral streptococci continued to increase in intensity and quantity with the length of exposure to these microorganisms (451). These findings suggest that the mucosal immune system is capable of responding to indigenous microorganisms early in life and that SIgA antibodies may influence the colonization patterns of the indigenous microbiota (441).

In contrast, other studies suggest that a major part of IgA antibodies directed against resident bacteria are generated by cross-reactive antigens from other bacteria (24). For example, lipoteichoic acids from a wide range of bacterial genera are cross-reactive (57, 413) and glucosyltransferases of oral streptococci are antigenically related (450). Some investigators have reported that salivary IgA antibodies react with *S. mutans* in saliva of infants without any detectable *S. mutans* in their mouths (24, 71, 390, 492). These antibodies may have resulted from a challenge with mutans streptococci that did not lead to permanent colonization. Alternatively, antibodies may have been induced by cross-reactive antigens from food or other indigenous oral or intestinal microorganisms. Cole et al. (91) found that adult conventional rats that were free of mutans streptococci exhibited salivary IgA antibodies reactive with whole cells, glucosyltransferase, and serotype carbohydrate (g) of *S. sobrinus*. Thus, the naturally occurring SIgA antibodies to indigenous bacteria may reflect a response to a number of different cell wall antigens, some of which are specific to bacteria and others of which are shared with other bacteria (24, 79, 522, 523).

It is thought that naturally occurring SIgA antibodies may play an important role in the homeostasis of oral resident microbiota and in the prevention against caries and periodontal diseases (328, 444). Naturally occurring SIgA antibodies have been detected against *S. mutans*, *A. actinomycetemcomitans*, and *P. gingivalis* which are strongly associated with oral diseases (64, 426). To gain insight into the role of SIgA in controlling the resident microbiota, we have reported a wide

range of studies evaluating the role of SIgA and bacterial IgA1 proteases in bacterial adherence to teeth, the association of natural SIgA antibodies with caries and periodontal diseases, the oral microbiota and oral health status in patients with IgA deficiency, and the effect of active and passive immunization on oral bacteria and oral diseases.

Role in Bacterial Adherence

The adherence to oral mucosa and teeth is the first important step for bacteria in colonizing the oral cavity. SIgA may interfere with this process by blocking adhesins, reducing hydrophobicity or aggregating bacteria. SIgA has been shown to inhibit the adherence of oral bacteria to oral epithelial cells (525). Salivary IgA may also reduce bacterial adhesion to hydroxyapatite and enamel and thus reduce the formation of the dental plaque. SIgA constitutes about 2% of the dry weight of human dental plaque. SIgA is found in the salivary pellicle in considerable quantities and in a biologically active state (270, 361, 477). Saliva-coated hydroxyapatite particles or beads are frequently used in model studies of dental research to mimic tooth surfaces (85). Studies in vitro have shown that SIgA and other salivary proteins are rapidly adsorbed by hydroxyapatite when this mineral is exposed to saliva (222, 360, 400). Studies have evaluated the adherence of bacteria to saliva-coated hydroxyapatite in the presence of various SIgA preparations, such as salivary IgA-containing fractions and colostrum SIgA (154, 183, 270, 383). The results were inconclusive and varied according to the strain tested (154, 183, 222, 270, 383). Although studies demonstrated that SIgA antibodies in solution inhibited the binding of some strains of oral streptococci (*S. sanguis* and *S. oralis*) to saliva-coated hydroxyapatite (183, 222, 291, 383), they enhanced the binding of other strains (222). Some investigators also found an inhibitory effect of IgA antibodies on *S. mutans* adherence (183, 256, 383), while others did not find such an effect (154, 222).

The difference in the size of bacterial aggregates formed by each bacterial strain may explain the contrasting results (269). It has been shown that the formation of large aggregates results in decreased numbers of adhered bacteria whereas the formation of small aggregates results in increased numbers (269). However, this parameter has not been measured in the above-mentioned studies. In most experiments, the antigen specificity of the antibodies was unknown and the lack of adherence-inhibiting effect could be explained by the lack of IgA antibodies against adhesins or by a difference in adhesins expressed on the surface of bacteria. One study demonstrated that only SIgA antibodies directed against antigen I/II inhibited the initial adherence of *S. mutans* to saliva-coated hydroxyapatite (183). The growth conditions of the bacteria prior to the adherence assay may influence antigen expression, reactivity with antibodies, and binding to hydroxyapatite. Kilian et al. (222) found that SIgA antibodies inhibited the binding of *S. mutans* only when they had been propagated in the absence of sucrose. The synthesis of glucan from sucrose may have enhanced adherence to hydroxyapatite. However, these results were not confirmed in a following study by the same research group (383). Under certain experimental conditions, bacteria may also release SIgA antibodies bound to cell surfaces and enhance their ability to adhere to the surface. It has been demonstrated that SIgA- and IgG-coated *S. mutans* may release bound antibodies in the form of an antibody-antigen immune complex, which reduces the inhibitory effect of antibodies on the adherence to salivary agglutinin-coated hydroxyapatite (256). The active release of bound antibodies may be a strategy used by bacteria to counteract the neutralizing effect of naturally occurring antibodies in the oral cavity.

Salivary IgA antibodies in the salivary pellicle may expose the Fab fragments, which can act as receptors for bacteria. The net effect of IgA antibodies on bacterial adherence to hydroxyapatite may be the result of the reaction of IgA antibodies in the fluid phase inhibiting bacterial adherence and those present in the salivary pellicle promoting bacterial binding (154, 222). The presence of SIgA antibodies adsorbed to hydroxyapatite enhances the sorption of some strains of *S. sanguis* (222, 270) but, in contrast, inhibits the adherence of *S. oralis* and *S. salivarius* and does not significantly influence the adherence of *S. mutans* (154, 222). The difference among bacterial species is difficult to explain and may be due to the antigenic specificity of antibodies bound to hydroxyapatite. The orientation of the SIgA molecule in the pellicle may allow the binding to only certain antigens. The active release of bound antibodies by bacteria may affect their desorption from hydroxyapatite, and this process may be specific only for certain antigens or bacteria. However, these hypotheses have not been verified.

Other salivary components, including mucins and other salivary agglutinins, are also important in inhibiting or promoting adhesion to hydroxyapatite. In most studies evaluating the effects of salivary IgA antibodies on bacterial adherence to saliva-coated hydroxyapatite, the bacteria were treated with IgA preparations before the adherence assay (183, 222, 256, 383). The effect of SIgA antibodies on bacterial adherence in relation to other salivary factors could be determined by treating bacteria with the different salivary fractions to which SIgA is added. Alternatively, SIgA could be added to saliva from IgA-deficient patients. Unfractionated parotid saliva and some salivary fractions with no detectable amounts of IgA have a more pronounced inhibitory effect on *S. mutans* adherence than SIgA-containing fractions (154). It cannot be excluded that in vivo, the influence of SIgA antibodies on initial bacterial adherence to hydroxyapatite is dependent on other adherence-promoting and adherence-inhibiting factors including salivary receptors, antimicrobial factors, bacterial antigen expression, active release of bound antibodies, and availability of sucrose.

Role of IgA Proteases in Bacterial Adherence

Several bacterial species that colonize mucosal and tooth surfaces produce IgA proteases. Most of these enzyme are known as IgA1 proteases, extracellular products that cleave serum IgA1 and secretory IgA1 in the hinge region and release intact Fab and Fc (or Fc.SC) fragments. The unique enzyme that is capable of cleaving both IgA1 and IgA2 of the A2m(1) allotype is produced by an intestinal isolate of *Clostridium ramosum*. The cleavage of SIgA1 may lead to a loss of protective properties of the antibody molecule. IgA1 proteases are produced by various mucosal pathogens including *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Neisseria meningitidis*, and *N. gonorrhoeae*, suggesting that they are important virulence factors. However, the production of IgA1 protease does not necessarily confer invasive properties to bacteria; other virulence factors such as capsules, toxins, and proteolytic activities are probably also required to cause diseases. IgA proteases are produced by some oral indigenous bacterial species including *S. sanguis*, *S. oralis*, and *S. mitis* biovar 1, which are particularly successful in initiating the colonization of oral mucosa and teeth (223, 225, 374). These enzymes are also produced by *Prevotella* and *Capnocytophaga* species, which may be involved in periodontal diseases (143, 144, 223). In addition,

suspected periodontopathogens, such as *Porphyromonas gingivalis* and *Prevotella intermedia*, produce proteases that are capable of completely degrading IgA1 and IgA2. However, this type of degradation does not appear to involve an initial cleavage in the hinge region (223).

An analysis of the role of IgA1 proteases in bacterial adherence may indirectly provide information on the importance of salivary IgA in oral microbial ecology. The production of IgA1 proteases may counteract the adherence-inhibiting activity of SIgA antibodies and enhance the ability of indigenous bacteria to colonize oral surfaces. The effect of IgA1 proteases on bacterial adherence has been investigated in vitro by treating bacteria with SIgA alone or with SIgA and IgA proteases. In these studies, IgA1 proteases enhanced the adherence of oral streptococci, including *S. sanguis*, *S. oralis*, and *S. mutans*, to saliva coated-hydroxyapatite (183, 383). Although *S. mutans* does not synthesize IgA proteases, the production of this enzyme by primary colonizers may cleave SIgA1 antibodies directed against other bacteria and enhance their colonization to oral surfaces (183).

The cleavage of SIgA may interfere with the functions attributed to the Fc part of the molecule (224). As in the intact molecule, Fabα fragments retain their capacity to bind to antigens (291, 383). These fragments may bind to bacteria after the cleavage of SIgA1 molecules, or, alternatively, SIgA1 may bind to bacterial surfaces before being exposed to IgA protease, and Fabα fragments are left on bacterial surfaces (383). Although Fabα fragments retain their full antigen-binding capacity and may block adhesins (183, 291, 383), they are probably unable to aggregate or to reduce the hydrophobicity of bacteria. Fabα fragments are dominated by hydrophobic residues, whereas $(Fc\alpha)_2SC$ is more hydrophilic due to the large amount of carbohydrate within the Fcα region and SC (224). Furthermore, as hydrophobic interactions appear to be important in bacterial adherence to hydroxyapatite, Fabα fragments left on the bacterial surfaces may enhance bacterial adherence (183, 383). It has been demonstrated that Fabα fragments bind more readily than SIgA1 to saliva-coated hydroxyapatite (183). Although SIgA may be incorporated into the pellicle during its formation, it interacts poorly with preformed pellicle (183). Finally, Fabα fragments that are bound to surface antigens could protect bacteria from the mucosal immune system by blocking access to intact IgA antibodies (224).

Because IgA1 proteases exclusively cleave human, chimpanzee, orangutan, and gorilla IgA (88, 240), this precludes the use of animal models to study the role of these enzymes in oral microbial ecology. However, human studies may reveal the potential ecological significance of IgA1 proteases. The IgA proteases are not susceptible to physiological protease inhibitors, and some experiments suggest that the cleavage of SIgA occurs in saliva and dental plaque from humans (6, 201, 224). The detection of Fabα fragments on dental plaque bacteria suggests that IgA1 protease activity promotes the formation of dental plaque in humans (6). Other observations indirectly support the role of IgA proteases in bacterial colonization of the oral cavity. The IgA1 protease activity can be described as being associated almost exclusively with the streptococci that adhere to a clean tooth surface and to the oral mucosa, a shedding epithelial surface necessitating a constant recolonization by the bacteria (144, 225). The proportion of streptococci with IgA1 protease activity is much lower in mature supragingival and subgingival plaque, and on the tongue. Among the streptococci that initiate the colonization of clean dental enamel, nearly 90% have IgA1 protease activity compared with only 17% of the streptococci in mature dental plaque (144, 225). In support of these results, salivary SIgA antibodies that are reactive with oral streptococci or suspected streptococcal adhesins (proteins and glycoproteins) were found to be primarily of the IgA1 subclass (7, 64).

However, no difference in the proportions of IgA1 protease-producing streptococci was found between normal and IgA-deficient individuals (386). This finding suggests that evasion of the adherence-inhibiting effect of SIgA is not essential for the colonization of oral surfaces by members of the indigenous microbiota (144). Since IgA1 proteases are extracellular enzymes, they may not cleave IgA directed only against protease-producing bacteria (6). Thus, IgA1 proteases do not necessarily confer an ecological advantage to the protease-producing bacteria but may promote the colonization of all members of the oral indigenous microbiota (386). The equilibrium between the indigenous oral bacteria may depend on the balance between IgA-inhibiting adherence and IgA protease-promoting adherence activities, and the net effect of these factors may not be detectable in vivo. In addition to this complex interaction, there are salivary IgA antibodies directed against IgA1 proteases (162) and glycosidase activity which may increase or decrease the susceptibility of IgA1 antibodies to cleavage (385). As well, IgA proteases may have salivary substrates other than IgA which favor the colonization of oral streptococci to pellicle-coated enamel and oral mucosa (386). Salivary IgA antibodies and, subsequently, the degradation of these antibodies may not be important factors in the initial adherence of indigenous bacteria to oral surfaces. Other factors may also favor the colonization of IgA1 protease-producers such as the presence of large numbers of specific adhesion receptors on oral surfaces. In our research, it was shown that mouse IgA was resistant to IgA1 protease activity but that the ability to completely degrade IgA did not confer an ecological advantage to oral resident bacteria. Our studies showed that *Lactobacillus murinus*, the indigenous oral bacterial species with the lowest proteolytic activity against IgA and other salivary defense components, predominated in the oral cavity of mice (116).

Correlation between Secretory IgA and Oral Diseases

The role of salivary IgA in the protection against dental caries has been investigated in many studies both in children and adults. Earlier experiments attempted to correlate total salivary IgA concentrations with caries susceptibility, as recorded by an index of decayed, missing, filled teeth (DMFT) or surfaces (DMFS) (reviewed in reference 53). However, the results of these studies were variable, with positive, negative, or no correlation found between total salivary IgA and dental caries (53). Such correlation analyses are complicated by the high intrinsic variability in levels of salivary IgA between individuals and within the same individual over short and long periods (58, 109, 151). The level of salivary IgA may vary according to salivary flow rate (52), age (248, 486), hormonal factors (166, 521), smoking habits (27), emotional states (210), physical activity (494), and genetic background (179). Since the concentration of salivary IgA is inversely correlated with the salivary flow rate (52), experiments with uncontrolled salivary flow rate are subject to increased variability in levels of salivary IgA. Furthermore, this type of experiment does not take into account the antibody specificities of salivary IgA.

Since mutans streptococci are considered to be the primary agents of caries, other studies have examined the relationship between the level of naturally occurring salivary IgA antibodies against mutans streptococci (*S. mutans* and *S. sobrinus*) and the level of mutans streptococci or dental caries. Most of these studies were cross-sectional designs that attempted to correlate IgA antibodies with caries susceptibility (DMFT or DMFS) (1,

TABLE 2. Relationship between natural salivary IgA antibodies to mutans streptococci and caries experience

No. of subjects in study group (age [yr])	Sample	Antigens of mutans streptococci in immunoassay (serotype)	Correlation of salivary IgA antibodies with DMFS[a]	Protection against caries	Reference
17 (2.5–5.5)	Whole saliva	Whole cells (b, c)	−	Yes	134
289 (military recruits)	Whole saliva	Whole cells (c, g)	−	Yes	265
42 (22–39)	Whole and parotid saliva	Whole cells (c), antigen I/II, carbohydrate (c)	−	Yes	72
64 (3–6)	Whole saliva	Whole cells (c, d), antigen I/II, carbohydrate (c, d)	−	Yes	71
131 (17–44)	Parotid saliva	Whole cells (c), purified antigens	−	Yes	171
41 (7–11)	Whole and parotid saliva	Whole cells (c)	−	Yes	402
40 (dental school students)	Whole and parotid saliva	Whole cells (c), antigens	−	Yes	173
96 (18–24)	Parotid saliva	Whole cells (c)	+	No	75
100 (20–30)	Whole saliva	Whole cells (a, c, d)	No	No	135
63 (6–13)	Whole saliva	Whole cells (b–g)	No	No	390
50 (19–21)	Whole saliva	Whole cells (c)	No	No	169
67 (4–9)	Whole saliva	Whole cells (c)	No	No	1
59 (22–32)	Whole saliva	Whole cells (c)	No	No	491
43 (20–64)	Whole saliva	Whole cells (g), antigen I/II, carbohydrate (g)	No	No	195

[a] Negative (−), positive (+), or no correlation.

71, 72, 75, 134, 135, 169, 171, 173, 195, 265, 390, 402, 491) or the presence of active lesions (without considering past caries experience) (10, 38, 39, 72, 75, 202, 491). These studies also produced confusing and conflicting data. The correlation between salivary IgA antibodies and DMF index was found to be positive (75), negative (71, 72, 134, 171, 173, 265, 402), or not significant (1, 135, 169, 195, 390, 491) (Table 2). Similarly, studies found positive (202), negative (38, 39, 72, 75, 491), or no (1, 10, 169) correlation between salivary IgA antibodies to mutans streptococci and active caries (Table 3). A negative correlation indicated that low levels of salivary IgA antibodies were associated with high caries activity or caries susceptibility.

One major difficulty encountered in interpreting the results came from the fact that some studies attempted to correlate salivary IgA antibodies with DMF scores while others correlated salivary IgA antibodies with the presence of active caries. The correlation of salivary IgA antibodies with DMF scores is questionable. The DMF score provides information on present and past dental caries (missing and filled teeth) and does not specifically assess the dental health condition at the time of evaluation. It was found that subjects with large numbers of missing and filled teeth and active caries had lower levels of salivary IgA antibodies than did subjects with large numbers of missing and filled teeth without active lesions (75). It has therefore been recommended that subjects with present caries experience and those with previous caries experience be analyzed separately (71, 75). Furthermore, some studies measured a coefficient of correlation between salivary IgA antibodies and caries experience (DMF) (1, 134, 135, 491) while other studies compared salivary antibodies in caries-resistant (low DMF) and caries-susceptible (high DMF) subjects (71, 72, 75, 171, 173, 195, 265, 390, 402). The criteria used for separating subjects into caries-resistant and caries-susceptible groups were shown to vary among studies, and this may also have contributed to the contradictory reports. In general, the limit of DMFS scores for caries-resistant subjects was equal to zero or lower than 2 or 3, while the limit for caries-susceptible subjects was higher than 5 or 10.

The correlation of salivary IgA antibodies with active caries may also lead to erroneous conclusions. Investigators who observed a lower level of IgA antibodies to *S. mutans* in patients with initial or active lesions concluded that salivary IgA plays a protective role against caries (38, 39, 491). Another explanation is that the level of salivary IgA antibodies becomes de-

TABLE 3. Relationship between natural salivary IgA antibodies to mutans streptococci and the presence of active caries lesions

No. of subjects in study group (age [yr])	Sample	Antigens of mutans streptococci in immunoassay (serotype)	Correlation of salivary IgA antibodies with caries activity[a]	Protection against caries	Reference
100 (20–30)	Whole saliva	Lipoteichoic acid	−	Yes	38
53 (3–14)	Whole saliva	Whole cells (c), glucan, lipoteichoic acid	−	Yes	39
42 (22–39)	Whole and parotid saliva	Whole cells (c), antigen I/II, carbohydrate (c)	−	Yes	72
59 (22–32)	Whole saliva	Whole cells (c)	−	Yes	491
96 (18–24)	Parotid saliva	Whole cells (c)	−	No	75
55 (18–20)	Parotid saliva	Whole cells (b, c)	+	No	202
79 (mothers)	Whole saliva	Whole cells (c)	No	No	10
50 (19–21)	Whole saliva	Whole cells (c)	No	No	169
67 (4–9)	Whole saliva	Whole cells (c)	No	No	1

[a] Negative (−), positive (+), or no correlation.

TABLE 4. Relationship between natural salivary IgA antibodies to mutans streptococci and the level of mutans streptococci

No. of subjects in study group (age [yr])	Sample	Antigens of mutans streptococci for immunoassay (serotype)	Correlation of salivary IgA antibodies with the level of mutans streptococci[a]	Reference
? (children)	Whole saliva	Glucosyltransferase, antigen I/II, glucan-binding protein, 110-kDa antigen	−	453
10 (22–59)	Mouth rinses	Whole cells (c, d)	−[b]	246
12 (27–43)	Parotid saliva	Carbohydrate (a–g)	−[b]	177
20 (young adults)	Mouth rinses	Whole cells (c)	−[b]	357
55 (18–20)	Parotid saliva	Whole cells (c, d)	+	202
19 (adults)	Whole saliva	Whole cells (c)	+	266
21 (0.3–35)	Whole saliva	Glucosyltransferase, antigen I/II, glucan binding protein	+	451
12 (0.5–44)	Whole saliva	Whole cells	No	24
42 (22–39)	Whole and parotid saliva	Whole cells (c), antigen I/II, carbohydrate (c)	No	72
64 (3–6)	Whole saliva	Whole cells (c, d), antigen I/II, carbohydrate (c, d)	No	71
31 (0.8–3.8)	Whole saliva	Whole cells (c)	No	492
50 (19–21)	Whole saliva	Whole cells (c)	No	169
67 (4–9)	Whole saliva	Whole cells (c)	No	1
59 (22–32)	Whole saliva	Whole cells (c)	No	491

[a] Negative (−), positive (+), or no correlation.
[b] Negative correlation with implanted mutans streptococci.

pressed following the development of caries. An increase in the levels of IgG and IgM antibodies in serum has been observed during the development of caries, and immune complexes composed of serum antibodies and *S. mutans* antigens may suppress the stimulation of the mucosal immune system (75). This suggestion was supported by sequential studies which revealed that the treatment of caries was followed by an increase in the level of IgA antibodies to *S. mutans*, concurrent with a decrease in the levels of IgG and IgM antibodies in serum (75, 135).

Studies with cross-sectional designs may not reveal correlations between salivary IgA antibodies and caries. Salivary IgA antibodies are measured only at a single point in time, while the caries score measure a disease process that may take several months to develop. The level of salivary IgA antibodies varies considerably over time (59, 109, 151). The exact stage of development of the SIgA immune response in relation to an antigenic challenge at the moment of sampling is not known. The salivary IgA antibodies measured may reflect present or past stimulation by *S. mutans* antigens. The SIgA antibody response may be influenced by the age at which the challenge with *S. mutans* occurred and by the period of exposure to *S. mutans* (71, 444). Saliva of children recently colonized (<6 months) with *S. mutans* contained higher levels of IgA antibodies than did saliva of children who had carried *S. mutans* for longer periods (24 months) (71). These results may also reflect a short duration of the secretory immune system to antigenic challenges and may account for the difficulties in correlating salivary IgA antibody levels and caries. Although more expensive, a longitudinal study which could monitor levels of salivary antibodies directed against *S. mutans* as a function of caries development would better clarify the potential of salivary IgA antibodies to protect from caries.

Other studies did not demonstrate a decisive role of salivary IgA antibodies in the resistance against the initial colonization by indigenous *S. mutans* in children or in the control of the level of indigenous *S. mutans* at adult ages. Studies that attempted to find a correlation between the level of salivary IgA antibodies and the level of indigenous mutans streptococci led to variable results with positive (202, 266, 451), negative (453), or no (1, 24, 71, 72, 169, 491, 492) correlation (Table 4). In contrast, other investigators found that exogenous implanted mutans streptococci were more rapidly eliminated from the oral cavity of subjects with high salivary IgA antibody activities (177, 246, 357). However, indigenous mutans streptococci may be better adapted to environmental conditions in the oral cavity than are laboratory strains. It was demonstrated that cultivated strains were less hydrophobic than freshly isolated strains and adhered to teeth to a lesser extent (234, 315, 357).

One of the difficulties in correlating salivary IgA antibodies with the level of *S. mutans* and caries is the standardization of the measurement of salivary IgA antibodies. The type of sample analyzed varies between studies and includes unstimulated or stimulated whole and parotid saliva and mouth rinses. The use of whole saliva may be more relevant clinically, since it is the secretion that bathes the mouth (404). However, the bacteria in whole saliva (or mouth rinses) may adsorb antibodies prior to measurement, and this may lead to underestimated antibody values (72, 151, 265, 402). The sampling of parotid or submandibular saliva by placing a collector over the major salivary gland ducts provides an uncontaminated source of saliva, but the amount of salivary IgA from each gland may differ (522). Some investigators reported a lower level of antibodies to mutans streptococci in whole saliva of subjects with active caries or high caries history, but they did not find this relationship in the parotid saliva of the same subjects (72, 402). The level of salivary *S. mutans* was found to be higher in caries-susceptible subjects and may have adsorbed a greater amount of salivary IgA antibodies (72, 402). Another explanation could be that caries-resistant subjects produce more SIgA antibodies in minor, submandibular, or sublingual salivary glands than in the parotid (402).

In immunoassay methods, the use of whole cells of mutans streptococci as antigen measures antibodies against all surface components including cell wall carbohydrates, glucosyltransferases, highly immunogenic proteins (antigens I/II, I, II, III, C, and D) and other wall-associated proteins, glucans, glucan-binding proteins, and lipoteichoic acids (410). The reference strains used in immunoassays may be antigenically different from the indigenous strain present in the oral cavity. The

expression of cell surface protein antigens may vary according to the type of strains, the culture conditions, and repeated subculturing of microorganisms (234, 315, 523). Furthermore, the antibodies detected may be not specific to mutans streptococci and may also have been induced by cross-reactive antigens from other oral and intestinal bacteria (24, 71, 202). It has been reported that many salivary IgA antibodies to *S. mutans* were directed against lipoteichoic acids, which are found on all gram-positive microorganisms (57), or against glucans and polysaccharides, which are synthesized by a variety of oral microorganisms (24). Finally, the use of whole cells may not discriminate between protective antibodies directed against adhesins or enzymes from antibodies directed against all surface antigens (357).

A more accurate approach would be to determine which antigens may be responsible for the induction of the protective salivary IgA antibodies response against *S. mutans* (173, 195). However, studies in which purified antigens were used to measure antibodies also have led to contradictory results (Tables 2 through 4). The target antigens were primarily those considered to be involved in the adherence of mutans streptococci to tooth surfaces including glucosyltranferase, antigen I/II, glucan, and lipoteichoic acid but also serotype-specific cell wall carbohydrates. Bolton (38) reported a protective role of salivary IgA antibodies to lipoteichoic acid against dental caries in adult subjects. In contrast, Bolton and Hlava (39) observed that salivary IgA antibodies to lipoteichoic acid and glucan were lower in children free from caries, indicating no protective role for these antibodies. However, they observed that levels of salivary IgA antibodies to whole cells of *S. mutans* were higher in caries-free groups and concluded that surface antigens that induced a protective response remained to be identified. Gregory et al. (171) observed higher levels of IgA antibodies to glucosyltransferase, antigen I/II, and glucan in caries-resistant subjects than in caries-susceptible subjects and no difference in the level of IgA antibodies directed against antigen III, lipoteichoic acid, or carbohydrate serotype. In contrast, Camling and Köhler (71) found that salivary IgA antibody levels to the polysaccharide serotype antigen, but not the antigen I/II adhesin, were significantly higher in children without caries. The experiments of Hocini et al. (195) revealed no protective role for salivary IgA antibodies directed against carbohydrate serotype g and protein antigen I/II.

The sodium dodecyl sulfate-polyacrylamide gel electrophoresis/immunoblot technique has also been used to identify the specificities of salivary IgA antibodies directed against mutans streptococci antigens (173, 195, 522, 523). The immunoblot analyses have revealed that salivary IgA antibodies react with several antigens of mutans streptococci. More recently, some investigators used this method to correlate the reaction pattern of salivary IgA antibodies to *S. mutans* antigens with caries susceptibility or the degree of colonization by *S. mutans*. This approach seems to be more promising because the specificities of several salivary IgA antibodies are compared simultaneously, but more research is necessary before any conclusions can be reached. Smith et al. (453) observed in a longitudinal study that salivary antibodies of children not colonized by mutans streptococci were more frequently reactive with mutans streptococci components whose migration corresponded to antigen I/II, glucosyltransferase, glucan-binding protein, and a 110-kDa component, suggesting a regulatory role of these antibodies in the primary colonization of mutans streptococci. These results contradict other results by the same authors demonstrating that salivary IgA antibodies to *S. mutans* antigens were detected only in children colonized with *S. mutans* (444, 451). Gregory et al. (173) reported that caries-susceptible subjects had an impaired salivary IgA antibody response against the 94-, 40-, and 35-kDa *S. mutans* antigens. In contrast, Hocini et al. (195) showed no qualitative difference in the specificity of the IgA antibodies to *S. sobrinus* antigens between caries-susceptible and caries-resistant groups.

It has been postulated that SIgA2 antibodies are more efficient antibodies in secretion since they are resistant to IgA1 proteases. Gregory et al. (171) found higher levels of salivary IgA2 antibodies (but not IgA1) to whole cells of *S. mutans* in caries-resistant adult subjects than in caries-susceptible subjects. However, others reported no difference in the proportion of IgA2 antibodies directed against mutans streptococci between caries-susceptible and caries-resistant subjects (195, 402). Also, the avidity of IgA antibodies does not seem to play a major role in protection against caries (1, 171, 195, 265). Some studies reported no difference in avidity of salivary IgA antibodies between caries-susceptible and caries-resistant subjects (171, 195), while others observed higher levels of high-avidity IgA antibodies in caries-susceptible subjects (1, 265). Although the frequent contact of the secretory immune system with high doses of *S. mutans* in subjects with a long caries history may select for high-avidity antibodies, these antibodies do not seem to confer a higher protection level (265).

Little information exists on the role of salivary IgA in the development of gingivitis and periodontitis. It is more difficult to see how salivary IgA could control subgingival plaque, since secretory IgA antibodies do not penetrate the crevice or pocket. It is possible that salivary IgA antibodies, by modulating the accumulation of supragingival plaque, control the formation and composition of subgingival plaque and its potential for causing disease (527). Salivary IgA could prevent potential periodonthopathogens that inhabit the tongue from colonizing the gingival crevice. It could limit the spread of the disease by preventing bacterial transmission from infected to healthy gingival sites. Past studies have reported positive (181, 182, 189, 271, 362, 419) or no significant (80, 169, 298, 421, 426) correlations between total salivary IgA levels and periodontal diseases (Table 5). The increased release of serum IgA due to an increased permeability of the crevicular epithelium during gingival inflammation may contribute to the rise in IgA levels observed in whole saliva (181, 271, 381). However, higher SIgA levels have also been observed in parotid saliva of subjects with gingival inflammation (80, 189, 208, 209, 362). These studies suggest that an increased antigenic load from dental plaque induces an SIgA response (169, 209). In contrast, no direct correlation was found between the concentration of total salivary IgA and plaque accumulation (208, 209, 404, 435).

In a few studies, the level of salivary IgA antibodies to dental plaque bacteria was measured in relation to periodontal diseases. Salivary IgA antibodies to suspected periodonthopathogens were detected in both healthy subjects and subjects with periodontal diseases. Some studies observed no change in the levels of salivary IgA antibodies to *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans*, and *Bacteroides asaccharolyticus* between healthy subjects and subjects with periodontal diseases (182, 292). Other studies reported an increased level of IgA antibodies to *A. actinomycetemcomitans* in some patients with adult and juvenile periodontitis (124, 418, 445). Others observed that treatment of gingivitis and periodontitis by root scaling and polishing was accompanied by an increased level of salivary IgA antibodies not only against suspected pathogens such as *Actinomyces naeslundii* genospecies 2, *A. actinomycetemcomitans*, *P. gingivalis*, and *Treponema denticola* but also against *S. salivarius* (124, 418, 504). This treatment may remove large amounts of microorganisms that may induce a salivary SIgA response via minor salivary glands or

TABLE 5. Relationship between total salivary IgA and periodontal diseases

No. of subjects in study group (age [yr])	Sample	Correlation of total salivary IgA with periodontal diseases[a]	Reference
18	Whole saliva, parotid saliva	+[b]/+[c]	189
27 (20–33)	Parotid saliva	+	362
60 (19–63)	Whole saliva	+	181
66 (11–43)	Whole saliva	+	419
11	Saliva from different glands	+	182
19 (17–53)	Whole saliva, parotid saliva	+/No	271
50 (19–30)	Whole saliva, parotid saliva	+/No	381
66 (adults)	Parotid saliva	No	80
56 (23–57)	Whole saliva	No	298
40 (19–21)	Whole saliva	No	169
28 (15–38)	Whole saliva	No	421
12 (22–37)	Parotid saliva	No	426

[a] Positive (+) or no correlation.
[b] Correlation with salivary IgA in whole saliva.
[c] Correlation with salivary IgA in parotid saliva.

GALT. However, this does not explain the increase in the level of salivary IgA antibodies against *S. salivarius*, a microorganism which does not colonize teeth. This observation could, however, be explained by cross-reactivity between bacterial antigens. Experimental gingivitis may provide more meaningful results than cross-sectional studies because it is possible to measure the level of salivary IgA antibodies before the development of inflammation. In subjects who stopped using oral hygiene, the development of gingivitis was delayed when high levels of IgA antibody activities against *A. actinomycetemcomitans*, *P. gingivalis*, *Eubacterium saburreum*, and *S. mutans* were present in parotid saliva, suggesting a protective role of SIgA antibodies (426). However, the secretory immune system did not seem to respond to the increased oral antigenic stimulus that was provoked by plaque accumulation. In both groups, a slight significant decrease in the levels of SIgA antibodies to the bacteria tested was observed during the course of gingivitis. The level of antibodies in serum remained unchanged. The experiment should be repeated with more individuals and salivary IgA antibodies should be measured over a longer period after resumption of oral hygiene to verify if they will return to initial levels.

Caries and periodontal diseases are multifactorial diseases, and it is difficult to isolate the role of each factor separately. In studies that attempted to correlate SIgA levels with caries or periodontal diseases, other factors such as diet, oral hygiene, ethnic background, diseases, medications, tobacco, menstrual cycle, or salivary flow rate were often not controlled. As seen above, the level of salivary IgA is inversely correlated with salivary flow rate. In experiments that do not take into account the salivary flow rate, it is difficult to determine if the relationship with caries or periodontal diseases is caused by variations in salivary IgA or flow rate (403). Salivary flow alone has been negatively correlated with the *S. mutans* level and caries experience (169, 370). It should also be kept in mind that all antimicrobial factors in the oral cavity interact with each other (403, 404). A positive correlation has been reported between the levels of salivary IgA, lysozyme, and lactoferrin, and total protein in whole saliva (404). The analyses of many antimicrobial factors simultaneously may provide further information on the role of salivary IgA in the prevention of dental caries and periodontal diseases (169, 208, 209, 403, 404). A concomitant decrease in the level of salivary IgA to *S. mutans* and hypothiocyanite has been observed in patients with initial dental caries (491). Others have reported low levels of salivary IgA and serum IgG antibodies to *S. mutans* in subjects with high caries susceptibility (171, 173).

A more recent approach is the use of multivariate and cluster analyses to define groups of subjects with similar profiles of salivary flow rate and antimicrobial factors (hypothiocyanite ions, lysozyme, lactoferrin, salivary peroxidase, and total protein) (208, 209, 403, 404). The subject clusters with similar salivary protein profiles are compared for short-term outcome variables such as incipient caries lesions, plaque accumulation, or microbial counts. Subjects with different profiles of antimicrobial protein concentrations may show different patterns of antimicrobial protein interactions and, consequently, different patterns of antimicrobial activity (208, 209, 403, 404). Jalil et al. (208, 209) found that although there was no straightforward correlation between salivary IgA levels and plaque accumulation, the plaque accumulation was greater in a group of individuals with both a low concentration of hypothiocyanite and a high concentration of total salivary IgA. It would also be interesting to include IgA antibodies to some oral bacteria, e.g., *S. sanguis*, *S. mutans*, *A. naeslundii* genospecies 2, *A. actinomycetemcomitans*, and *P. gingivalis*, as a variable in the cluster analysis. However, the number of antimicrobial factors assayed is probably limited by the volume of saliva harvested.

As an alternative to analyzing the concentrations of any antimicrobial factor in saliva, it has been proposed that studies be carried out on the functional aspects of all the specific and nonspecific defense systems together, such as the effect of saliva on the growth, agglutination, and adhesion of bacteria (490). The rate of plaque formation was found to be inversely related to the bacterial aggregation capacity of parotid saliva, but this could not be explained by the salivary content of IgA (435). Another study demonstrated that the capacity of saliva to support or inhibit the growth of mutans streptococci could not be explained by the levels of salivary IgA antibodies and other antimicrobial factors. In contrast, it has been reported that parotid saliva from caries-resistant subjects inhibited *S. mutans* growth, adherence, acid production, glucosyltransferase, and glucosephosphotransferase activities to a greater extent than did saliva from caries-susceptible individuals, and this difference was related to salivary IgA antibody activity (171). However, the interactions between antimicrobial factors as well as the content in salivary-promoting binding proteins and salivary nutrients were not considered.

Oral Health in Patients with IgA Deficiencies

In past studies, Ig-deficient patients have been used as potential models for elucidating the role of host immunity in the control of diseases. Selective IgA deficiency is the most common primary immunodeficiency and can occur at a frequency within a population varying from 1:300 to 1:3,000 depending on the screened population (268). Patients with antibody deficiencies show an increased susceptibility to microbial infections, more particularly in the intestinal and upper respiratory tracts (11, 106). Ig-deficient patients, especially those deficient in IgA, represent a useful model for studying the role of salivary IgA in oral health and diseases. If salivary IgA plays a major role in the maintenance of the homeostasis of the oral microbiota, Ig-deficient individuals should show an increased susceptibility to caries and periodontal diseases. Only a few stud-

TABLE 6. Oral health status in Ig-deficient patients compared with normal subjects

No. of subjects in study group (age [yr])	Plaque bacterial counts[a]		Clinical index score[a]				Reference(s)
	S. mutans	Suspected periodontal pathogens	Caries experience	Plaque accumulation	Gingival inflammation	Periodontal destruction	
23 (3–43)	Lower	Similar	Lower	Similar	Lower	Similar	62, 393, 394
25 (20–69)	NT[b]	Similar	Similar	Similar	Similar	Similar	102
45 (3–50)	NT	NT	Higher	Similar	Similar	Similar	257
22 (3–36)	Higher	NT	Higher	Similar	Similar	Similar	90
25 (4–36)	NT	NT	Higher	NT	NT	NT	19
12 (5–12)	Similar	NT	Lower	NT	NT	NT	137

[a] Given for Ig-deficient patients as a comparison with normal subjects.
[b] NT, not tested.

ies have attempted to correlate humoral immune deficiency with oral microbiological changes or oral diseases, because these conditions appear at low frequencies within a population and therefore a large number of patients may not always be available for statistical analysis (Table 6). Some investigators reported a higher frequency of harboring *S. mutans* and a greater susceptibility to dental caries in Ig-deficient patients (90, 257). In contrast, others found only minor changes in the oral microbiota of Ig-deficient individuals (62, 102, 351) and no increased susceptibility to caries and periodontal diseases (102, 393, 394). In a 2-year study of Ig-deficient patients, Robertson et al. (393, 394) reported a lower caries experience and gingival inflammation in Ig-deficient patients compared with normal individuals.

There are numerous variables which could account for the discrepancies between the different studies. Age, level of education, dietary habits, oral hygiene, dentist visit frequency, and fluoride exposure may influence the severity of the disease. Furthermore, individuals with recurrent infections may receive prolonged antibiotic therapy and Ig prophylaxis that may suppress microorganisms in dental plaque (102, 394). While not statistically significant, caries experience has been shown to be lower in immunodeficient patients with a history of antibiotic therapy (394). The relatively good oral health of IgA-deficient patients may also be attributed in part to compensatory IgM antibodies (16, 90, 137). An enhanced local synthesis of IgM at mucosal surfaces is frequently observed in patients with selective IgA deficiency (19, 352). It has been found that patients with selective IgA deficiency who compensate by producing salivary SIgM antibodies to *S. mutans* exhibit a caries susceptibility similar to that of normal individuals (16, 19, 90). However, other investigators did not find more caries or periodontal diseases in patients with agammaglobulinemia who were unable to compensate in this manner (393). The possibility of compensation by nonspecific defense factors must also be considered (226). Higher levels of lysozyme and lactoferrin have been reported in subjects with immune system dysfunction (19). Parotid saliva from IgA-deficient patients demonstrated greater agglutinating activity for *S. sanguis* and *S. mutans* than did saliva from control subjects. The compensatory increase in agglutination may be mediated by IgM antibodies but also by other salivary molecules such as parotid salivary agglutinins (288).

Immunodeficient rodents may represent a more simple model for studying the role of Igs in the control of oral microbiota, in part because external factors such as diet and host environment can be controlled more easily. Thymectomized and congenitally athymic nude rodents with thymic dysgenesis have been used as models for immunological studies of dental caries (119, 121, 458). The thymus is essential for the maturation of T lymphocytes, and the interaction between T and B cells is necessary for the generation of a normal humoral response. Consequently, nude and thymectomized rodents have a T-lymphocyte defect and a severely depressed mucosal and systemic antibody response (118, 119, 121, 481). Decreases in salivary and serum antibody responses in these animals have been associated with increased colonization by mutans streptococci and increased dental caries (121, 458). These results suggest that naturally occurring salivary IgA and IgG antibodies may reduce the colonization by allochthonous bacteria. We have also reported a change in the resident oral microbiota and a lower salivary IgA response to some indigenous bacteria in nude mice compared with phenotypically normal (*nu*/+) mice (294). Nude mice have a lower proportion of *Lactobacillus murinus* and a higher proportion of *Enterococcus faecalis* than normal mice. However, our results do not necessarily indicate that the modification of the oral microbiota of nude mice is due to the lower IgA antibody response. Nude mice are affected in so many immune functions including enhanced macrophage and natural killer cell activities. The differences in the oral microbiota detected between nude and heterozygous mice may also be attributed to stress. It was observed that the proportion of *Lactobacillus* spp. decreased in stressed mice (150, 424, 474). The skin of nude mice is defective in keratinization of hair, which results in hairlessness and thermoregulatory problems (197). At ambient temperature under the thermoneutral zone, nude mice must maintain their basal metabolism up-regulated, and this phenomenon may be associated with the release of hormones, physiological changes, and higher food and water intake (197, 519).

Since nude mice do not appear to be a good model for studying the effect of antibodies on the control of the indigenous microbiota, we have analyzed the oral and intestinal microbiota of B-cell-deficient knockout mice. These mice were produced by disrupting one of the genes encoding the membrane form of the μ chain of IgM (227). B-cell development in homozygous (μ*MT*/μ*MT*) mice is stopped at the stage of pre-B-cell maturation, while it is normal in heterozygous (μ*MT*/+) mice. The levels of salivary IgA and serum IgA and IgG were normal in μ*MT*/+ mice, while no Igs were detected in μ*MT*/μ*MT* mice (227, 295). The acquisition and proportions of the different species of the oral and intestinal indigenous bacterial populations were not significantly different between the two groups of mice (295). Our results thus suggest that SIgA and other Ig isotypes do not play a major role in the acquisition and the regulation of the indigenous microbiota of mice. The role of SIgA may be masked by more predominant factors such as bacterial interactions, environmental factors, host physiological factors, or other host defense mechanisms. The coadaptation between oral resident microbes and their host probably

TABLE 7. Active immunity against mutans streptococci in rodents

Immunization route	Antigens	Adjuvant or vehicule	Increase in:		Reduction in *S. mutans* counts	Protection against caries	Reference(s)
			Salivary IgA antibody levels	Salivary or serum IgG antibody levels			
Subcutaneous	Whole cells, glucosyltransferase	Complete Freund adjuvant	Yes	Yes	Yes	Yes	321, 448, 449, 479, 480
Topical	Whole cells	None	Yes	No	Yes	NT[a]	245
Intranasal	Antigen I/II	Cholera toxin	Yes	Yes	Yes	NT	214, 544
Oral	Whole cells, cell wall, ribosomal extract, antigen I/II, glucosyltransferase, anti-idiotype	Muramyldipeptide, peptidoglycan, concanavalin A, cholera toxin, liposome, aluminum phosphate, cloned *Salmonella*	Yes	Yes/no[b]	Yes	Yes	100, 175, 207, 331–333, 341, 382, 409, 449, 482, 516

[a] NT, not tested.
[b] In general, immunization with whole cells gives rise only to salivary IgA antibodies while immunization with soluble antigens and adjuvant, which render the antigens particulate, may also lead to an increase in the level of antibodies in serum.

begins immediately after birth, and the absence of one factor, such as SIgA, may be compensated for by other salivary defense factors (5, 403). No attempt was made to measure the level of innate defense factors in Ig-deficient ($\mu MT/\mu MT$) mice. On the other hand, the oral and intestinal microbiota of SPF mice is simple, and the effect of SIgA antibodies is perhaps observed only when the host is exposed to a more complex microbiota.

Active Immunization against Oral Diseases

Numerous studies have focused on the development of a vaccine that could induce salivary IgA antibodies and be effective in protecting against caries (reviewed in reference 328). These studies may also lead to new insights into the SIgA immune system and its ability to respond to antigenic challenges from oral bacteria and to control the equilibrium of the oral resident microbiota. Two major approaches have been used to induce the SIgA response to *S. mutans* in rodents, monkeys, and humans. The first approach consists of the induction of a local SIgA response in lymphoid tissues of salivary glands. Earlier studies attempted to elicit a salivary SIgA response in animal models by the injection of antigens in the vicinity of the major salivary glands, instillation of antigens into the parotid duct, or submucosal injections in the oral cavity. However, these immunizations may also induce serum antibodies that reach the oral cavity through the gingival crevicular fluid. Topical application of antigens to the oral mucosa also induces a local SIgA antibody response, probably via the entry of antigens to the salivary duct of minor salivary glands and stimulation of duct-associated lymphoid tissues (245, 247, 259, 260, 346, 443). The application of antigens to the gingival mucosa may also induce IgG antibodies in the crevicular gingival fluid (259, 260). It is difficult to determine the role of SIgA antibodies with immunization schedules which also give rise to serum and gingival crevicular fluid IgG antibodies. Studies with rodents and monkeys revealed that parenteral administration of *S. mutans*, which induces serum IgG but not SIgA antibodies, was protective against caries (46, 207, 261–263). The second approach to inducing salivary IgA antibodies originated from the evidence for a common mucosal immune system and consists of inducing a generalized secretory IgA response via the stimulation of GALT. Oral immunization elicits predominantly SIgA antibodies in saliva and other secretions with a poor serum antibody response. The induction of SIgA by oral immunization with nonviable antigens requires large and repeated doses of the antigens. This is probably due to enzymatic degradation of antigens in the stomach, poor adsorption of antigens by the intestinal mucosa, poor uptake by the GALT, or complexing of antigen with preexisting antibodies or mucins (324). Incorporation of antigens in liposomes or gelatin capsules may provide protection against digestive enzymes and result in a higher SIgA response (82, 89, 175, 325, 516). Animal studies demonstrated the ability of adjuvants such as muramyl dipeptide, concanavalin A, aluminum phosphate, or cholera toxin to increase the secretory immune system response when given orally with mutans streptococci antigens (332, 341, 409, 482). Cholera toxin has been shown to elicit a strong mucosal and systemic response against itself as well as against unrelated antigens (99, 100, 126). The adjuvant property of cholera toxin includes the ability of cholera toxin B subunit to bind specifically to a receptor on the surface of intestinal epithelial cells, to increase the permeability of the mucosal epithelia, to stimulate antigen presentation by enhancing major histocompatibility complex class II molecule expression and interleukin-1 production, and to promote B-cell isotype switch differentiation toward IgG1 and IgA (320).

Live recombinant avirulent *Salmonella* spp. expressing cloned gene products of mutans streptococci or periodontopathogens have also been evaluated as an oral vaccine (117, 382). The selection of *Salmonella* as the carrier is based on the fact that this genus may persist in the intestinal tract and continuously invade the host via Peyer's patches of the GALT. More recently, it has been suggested that the mucosal immune system is not uniform and that intranasal immunization, which stimulates the NALT and tonsil-associated lymphoid tissues, is more effective than oral immunization in generating antibody responses in salivary glands (214, 412, 534).

Most studies on inducing salivary IgA against mutans streptococci to confer protection against dental caries have been performed in rodents (Table 7). Different antigen preparations were used for immunization and include whole cells (245, 321, 331, 333, 341, 392, 479), cell walls (331, 332), glucosyltransferase (448–450, 480, 482), surface proteins (100, 214, 382, 409, 516, 534), ribosomal preparations (172), synthetic peptides

TABLE 8. Active immunity against mutans streptococci in nonhuman primates

Immunization route	Antigens	Adjuvant or vehicle	Increase in:		Reduction in *S. mutans* counts	Protection against caries	Reference(s)
			Salivary IgA antibody levels	Salivary or serum IgG antibody level			
Subcutaneous	Whole cells	Complete Freund adjuvant	No	Yes	Yes	Yes	78, 261–264
Submucosal	Whole cells, glucosyltransferase	None	Yes	Yes	Yes/no[a]	Yes/no[a]	46
Intraductal	Whole cells, glucosyltransferase	None	Yes	Yes	Yes	NT[b]	129, 133, 272, 517
Topical	Antigen I/II	Dimethyl sulfoxide	Yes	Yes	Yes	NT	259, 260
Intranasal	Antigen I/II	Cholera toxin	Yes	Yes	NT	NT	412
Oral	Whole cells, antigen I/II	Capsule, aluminum phosphate, cholera toxin	Yes/no[c]	Yes/no[c]	No	No	78, 264, 272, 326, 517

[a] Immunization with whole cells was protective, but immunization with crude glucosyltransferase resulted in an increase in colonization by *S. mutans* and no protection against caries.
[b] NT, not tested.
[c] Slight or no increase in salivary and serum antibody levels.

(260), and anti-idiotypic antibodies (207). It has been demonstrated that germ-free and conventional rodents injected in the salivary gland region with mutans streptococcus whole cells or glucosyltransferase in complete Freund's adjuvant showed higher salivary IgA and IgG antibodies, lower mutans streptococcus colonization, and fewer carious lesions (321, 448, 449, 479, 480). The oral immunization of germ-free rodents with mutans streptococci whole cells or cell wall elicited salivary SIgA antibodies with no detectable serum antibodies (331–333, 341). Salivary IgA antibodies were directed against several cell antigens including glucan, serotype carbohydrate, and lipoteichoic acid (331, 332, 341). The rise in the level of salivary IgA antibodies correlated with a reduction in the level of mutans streptococcus colonization and dental caries, suggesting that SIgA antibodies alone are protective (333, 449). These studies demonstrated that a critical dose of orally administrated antigen was required to induce a secretory response. Also, the oral administration of *S. mutans* vaccine consisting of particulate antigens (whole cells or cell wall) seemed more effective in inducing salivary IgA antibodies and providing protection against caries than does administration of soluble forms (331, 332). Rodents that were given soluble antigens such as wall-associated antigens, antigen I/II, serotype carbohydrate, or anti-idiotypic antibodies exhibited only low salivary IgA antibody levels and caries protection (175, 207, 331, 332, 516). This is probably due to the intestinal digestion and lack of uptake by the GALT. The incorporation of soluble antigens in liposomes or the addition of adjuvant (muramyl dipeptide, aluminum phosphate), which renders the antigens particulate, resulted in higher salivary SIgA antibody levels and higher serum antibody response, which led to increased protection against caries (175, 207, 482, 516). Peroral immunization with the protein antigen I/II induced salivary IgA antibodies only when the antigen was conjugated with cholera toxin B subunit, which allowed binding to the host cellular membrane receptors (100, 184, 409). Oral immunization with a live avirulent recombinant *Salmonella typhimurium* expressing surface protein antigen A of *Streptococcus sobrinus* (analogous to antigen I/II of *S. mutans*) or glucosyltransferase elicited salivary and serum antibodies and reduced caries lesions in rodents (382). It was suggested that SIgA antibodies were also implicated in protection against caries in topical applications of whole *S. mutans* to the oral mucosa (245) or intranasal instillation of antigen I/II conjugated to cholera toxin B subunit (214).

A few investigations have studied the effects of stimulation of salivary IgA antibodies on the progress of periodontal bone loss in rodents. Germfree rats were immunized in the salivary gland region with whole cells of *Actinomyces naeslundii* genospecies 2 or *A. naeslundii* and then inoculated orally with the live homologous organism (96, 356). Crawford et al. (96) found that high salivary IgA and IgG responses limited the colonization of *A. naeslundii* and the development of periodontal bone loss but the immunization with *A. naeslundii* genospecies 2 increased periodontal loss. In another study, salivary antibodies were found to be capable of reducing the initial implantation of *A. naeslundii* genospecies 2 but had limited capability in suppressing the establishment of this organism (356). The injection of whole cells or fimbrial components of *Porphyromonas gingivalis* in the vicinity of submaxillary glands of germfree rats led to increased levels of salivary antibodies and protected against periodontal destruction (131, 132). The mechanism by which this mode of immunization provides protection is not known and may involve both humoral and cellular immunity. SIgA antibodies may have limited the adherence of *P. gingivalis*, but the level of *P. gingivalis* colonization was not measured. A mucosal immune response was also induced against *P. gingivalis* by oral immunization of rodents with liposomes containing adhesive fimbriae and adjuvant (117, 251, 355) or with attenuated *Salmonella* expressing a cloned *P. gingivalis* adhesin hemagglutinin (117). However, these studies did not evaluate the effect of salivary IgA antibodies on *P. gingivalis* colonization and the destruction of periodontal tissues.

Monkeys may be useful models of dental caries since they may develop caries similar to those of humans. However, monkeys rarely harbor mutans streptococci as part of their resident oral microbiota and do not usually develop caries in the wild (133). Caries can be produced in monkeys by infecting them with mutans streptococci and by feeding them with a cariogenic diet (133, 262). It was shown that macaque monkeys may also be naturally colonized by *S. mutans* (serotype c) when fed a carbohydrate-rich diet, but the origin of the bacteria was unknown (259, 260, 263). Furthermore, experiments on the effect of salivary SIgA antibodies on caries protection in nonhuman primates are limited in number (Table 8). Some studies demonstrated that the instillation of *S. mutans* whole cells or crude glucosyltransferase into the duct of parotid glands induced a salivary and serum antibody response (129, 133, 272, 517). The presence of salivary IgA antibodies correlated with a reduction in the colonization of implanted *S. sobrinus* (133), but changes in caries were not tested. Bowen et al. (46) re-

TABLE 9. Active immunity against mutans streptococci in humans

No. of subjects in study group	Immunization route	Antigens (serotype)	Vehicle or adjuvant	Increase in:		Decrease in the level of *S. mutans*	Reference
				Salivary IgA antibodies against *S. mutans*	Serum antibodies against *S. mutans*		
3	Topical (lower lips)	Whole cells (c)	Capsule	No	NT[a]	Yes[b]	247
11	Oral	Whole cells (d)	None	No	NT	Yes[b]	152
4	Oral	Whole cells (d)	None	No	No	Yes[b]	40
8	Oral	Whole cells (d)	Capsule	No	No	Yes[b]	89
5	Oral	Whole cells (indigenous)	Capsule	Yes	No	Yes	170
25	Oral	Glucosyltransferase (g)	Aluminum phosphate, capsule	Yes	No	Yes	442
23	Topical (lower lips)	Glucosyltransferase (g)	Aluminum phosphate	Yes	No	Yes	443
4	Oral	Whole cells (d)	Capsule	Yes	No	NT	325
5	Oral	Whole cells (c) (indigenous)	Capsule	Yes	No	NT	101
7	Oral	Glucosyltransferase (c)	Liposome	Yes	NT	NT	82

[a] NT, not tested.
[b] Decrease in implanted *S. mutans.*

ported that submucosal injection of whole cells or crude glucosyltransferase in the oral cavity of monkeys increased the level of salivary and serum antibodies. Animals that were intraorally immunized with whole cells were protected against caries, but animals that were immunized with crude glucosyltransferase had higher counts of *S. mutans* in dental plaque and were not protected. Topical gingival immunization by brushing live *S. mutans* cells onto the gingiva in rhesus monkeys failed to induce salivary antibodies or to prevent the development of caries (264). However, the application of antigen I/II in 50% dimethyl sulfoxide on monkey gingival tissues resulted in whole-saliva IgA antibodies, crevicular IgG antibodies, and a reduction in the proportion of *S. mutans* in plaque (259, 260). Since only whole saliva was examined, the source of IgA could not be determined. The IgA antibodies may have been produced locally by the gingival lymphoid cells or via the minor oral salivary glands that are scattered under the oral mucosa of the lips and cheeks (260). The protection of monkeys against dental caries has been principally related to IgG antibodies in serum (46, 78, 261, 263, 264), and it is difficult to estimate the importance of SIgA antibodies in these studies. The oral immunization of monkeys with mutans streptococcus whole cells or antigens failed to induce SIgA antibodies in saliva (272, 517) or induced only a short-lived SIgA response (78, 264, 326). The administration of live *S. mutans* in drinking water elicited low levels of salivary IgA antibodies, but no reduction in the level of colonized *S. mutans* or in caries frequency was observed (264).

The immunization studies in humans were conducted mainly by oral administration of whole cells of mutans streptococci (Table 9). Mestecky et al. (325, 330) were the first to demonstrate on SIgA response in the saliva of humans who ingested capsules containing whole cells of a laboratory strain of *S. sobrinus*. Subjects with no preexisting antibody activity to the strain before immunization were selected. Subsequent investigators used a similar oral immunization protocol and then challenged the subjects with the homologous streptomycin-resistant mutans streptococci strain. No increase in preexisting levels of antibodies to *S. mutans* was reported, but the number of implanted strains in saliva and dental plaque was reduced in immunized subjects (40, 89, 152, 247). Due to the high variability in the level of natural antibodies, the method may have not been sensitive enough to detect any change in the level of salivary IgA antibodies. Gahnberg and Krasse (152) observed no difference in the elimination of implanted bacteria after a second challenge 2 weeks after the last immunization, suggesting that if such a response exists, it is of very short duration. Furthermore, no difference in the level of indigenous mutans streptococci was observed during these experiments (89, 152). Czerkinsky et al. (101) observed an SIgA response in four subjects who were orally immunized with indigenous *S. mutans* whole cells but not in one subject who had high levels of SIgA antibodies against *S. mutans* before immunization. The presence of preexisting antibodies may interfere with the adsorption of antigens and with further stimulation of the secretory immune system. A few studies reported an effect of SIgA antibodies against indigenous *S. mutans*. The oral administration of glucosyltransferase from *S. sobrinus* with aluminum-based adjuvant led to an increased level of SIgA antibodies in parotid saliva and interfered with the reaccumulation of mutans streptococci after dental prophylaxis (442). A second series of immunization also increased the level of SIgA antibodies but did not affect the level of indigenous *S. mutans*. Gregory and Filler (170) reported an increase in the level of salivary IgA antibodies and a decrease in the level of indigenous *S. mutans* after both first and second oral immunizations with whole cells of *S. mutans* previously isolated from each individual. This finding suggests that the secretory immune system may respond to a greater indigenous antigenic challenge and that salivary SIgA antibodies may control the colonization of indigenous mutans streptococci and protect against dental caries. Topical application of glucosyltransferase in aluminum phosphate delayed the reaccumulation of indigenous mutans streptococci and was correlated with an increase in the level of SIgA antibody in the parotid but not the labial saliva. The rise in the level of parotid salivary IgA antibody was not likely to have resulted from ingestion of antigens or local immunization of the major salivary glands (443). The lymphoid cells stimulated in the minor salivary glands may have migrated to the parotid glands; however, this hypothesis has never been verified. The difficulty in collecting labial saliva and in measuring labial salivary flow rate may in part explain these results.

For all routes of immunization tested, the SIgA response was generally of short duration and rarely exceeded 2 to 3 months in humans (101, 170, 325, 443) and animals (78, 129, 133, 264, 272, 326). The antibody level dropped quickly to the

preimmune level when immunization stopped, even though the oral cavity had been infected by the immunizing strain (129, 264, 272). Only Hajishengallis et al. (184, 185) were able to induce a SIgA response that persisted for at least 11 months by oral immunization of mice with a chimeric protein in which the toxic A1 subunit of cholera toxin had been genetically replaced by a 42-kDa segment representing the saliva-binding region of antigen I/II. In most of the studies, a second immunization procedure resulted in a faster but generally not in a greater or longer response than did the primary immunization (78, 129, 185, 262, 272, 391, 392, 409, 443, 534). Only a few studies demonstrated an anamnestic SIgA response against mutans streptococcal antigens in rodents (516) and humans (170, 325). These findings suggest that there is little or no evidence of immune memory from the mucosal immune system and that a persistent antigenic challenge seems necessary to maintain salivary IgA antibody levels. The difficulty in inducing long-lasting and anamnestic responses may be due to interference with antigen adsorption by antibodies that are synthesized in response to the preceding stimulation (13), or it may be due to the induction of suppressor cells (125, 200, 319). Animals that were fed protein antigens were shown to develop a state of systemic unresponsiveness or oral tolerance which would have been mediated by suppressor cells in GALT and other lymphoid tissues. Some studies suggested that these cells do not inhibit the mucosal SIgA response and demonstrated that protein antigen feeding led simultaneously to the induction of SIgA antibodies and systemic unresponsiveness to these antigens (76, 77). In contrast, other results indicated that oral feeding of antigens may also lead to the emergence of T suppressor cells that inhibit the SIgA response (4, 125, 127, 200, 319). The development of unresponsiveness of the mucosal immune system to specific bacterial antigens has not been extensively studied (115, 281, 432, 433). However, studies with rodents showed that the intestinal microbiota may induce nonspecific suppressive cells, in lymphoid tissues, that regulate the secretory and systemic immune response (22, 309, 318). These nonspecific suppressor cells are not found in germfree rats and mice unless they are colonized by bacteria. Suppression appears to be mediated by macrophage-like cells or by T suppressor cells. Lipopolysaccharides from resident gram-negative bacteria may be involved in the maturation of precursor suppressor cells in GALT which are subsequently stimulated by antigens to become the antigen-specific suppressor cells that mediate tolerance (125, 229, 329). The phenomenon of oral tolerance is a complex and controversial area which needs more research. Its application to indigenous microbiota is largely unknown. The oral ecosystem could be an interesting ecosystem for such studies.

Most of the studies on vaccines against caries were performed in germfree animals or in animal models that did not harbour mutans streptococci as part of the resident oral microbiota and were infected with a human strain. The infection with *S. mutans* can be induced rapidly by a high-sucrose diet, and dental caries develop within 2 months. Under these conditions, a short SIgA antibody response (2 to 3 months) may limit the colonization of *S. mutans* and reduce caries. Although these experiments are valuable for the development of a vaccine, they do not provide additional information about the role of SIgA in the control of indigenous microbiota. Experiments with animals do not reflect the situation in humans, where dental caries are caused by indigenous bacteria and may take many months to develop. The persistence of resident bacteria in the oral cavity may result from a long-term adaptation between the host and the bacteria. As stated earlier, there is evidence that the host is more tolerant to autochthonous than allochthonous bacteria (115, 281). The chronic exposure of the host immune system to resident bacteria may result in the suppression of the antibody response (391, 392, 433). Riviere et al. (391, 392) demonstrated that primary oral immunization with *S. mutans* may induce SIgA antibodies in rats but that continued exposure to *S. mutans* whole cells led to progressive suppression of the SIgA response and the level of salivary IgA antibodies returned to baseline (391, 392). The acquired suppression of the response to mutans streptococci was long-lasting and could not be reversed by restimulation with antigens (392). The effect of long-term oral immunization on *S. mutans* colonization has not been studied in animals. The role of the mucosal immune system in long-lasting protection against caries and periodontal diseases could be studied by using rodents that are orally immunized with recombinant bacteria that persist in the gut expressing gene products from mutans streptococci or periodontopathogens.

Studies with our mouse model suggest that salivary IgA antibodies play a minor role in controlling the indigenous oral microbiota. In these studies, SPF BALB/c mice that naturally harbored a strain of streptococcus (*Streptococcus* sp. strain TG) exhibited a fourfold-lower salivary IgA antibody response than did another mouse colony that was inoculated with this bacterium at adult age (296). However, the increase in the level of salivary IgA antibodies in the inoculated group did not affect the colonization and persistence of *Streptococcus* sp. strain TG. In both the inoculated and naturally colonized groups, the proportion of *Streptococcus* sp. strain TG among the total cultivable microbiota reached similar levels (296). In another study, we found that the oral immunization of SPF mice with an indigenous bacterium (*Lactobacillus murinus*) elicited SIgA antibodies to whole bacteria in 50% of the mice and did not affect the proportion of *L. murinus* among the oral bacterial populations of these mice (297). It is known that indigenous bacteria may evade SIgA immune control by continually changing their antigenic composition (59, 196), by masking their epitopes with host molecules, or by releasing IgA-coated antigens (256). It has been shown that pathogenic bacteria may persist in the host through molecular mimicry and antigenic variations, but this phenomenon has not been widely studied for indigenous bacteria (65, 86). Only three immunization studies of humans suggest that salivary IgA antibodies may limit the colonization of indigenous *S. mutans* (170, 442, 443). A second immunization regimen was shown to affect the proportion of *S. mutans* in one study (170) but not in another (442). Other experiments should be performed with humans, using more individuals and over longer periods to evaluate if a protective secretory IgA response may be maintained against indigenous mutans streptococci and potential periodontopathogens. Until now, the short duration of salivary IgA response has failed to support the argument that the SIgA system plays an important role in maintaining the homeostasis of the oral resident microbiota.

The progress in vaccine research has been limited primarily by the difficulty in inducing an SIgA response. It is thus necessary to develop new immunization strategies for inducing SIgA antibodies over long periods (184, 382), e.g., by immunizing against synthetic peptide vaccines including epitopes that induce the protective response (B-cell, adhesion, and T-helper cell epitopes) but not the epitopes that induce the suppressive response (217). Other approaches may be used to prevent caries and periodontal diseases such as systemic immunization or passive immunization with monoclonal antibodies (41, 46, 261–263, 279, 280). These modes of immunization avoid the difficulties encountered in stimulating the secretory immune system. However, systemic immunization is difficult to

justify because of its undesirable side effects. There is evidence for cross-reacting antigens between *S. mutans* and human heart tissues. Furthermore, systemic immunization induces mainly IgG antibodies, which may promote an inflammatory response at the gingival margin, where plaque accumulates (410). Local oral passive immunization against caries and periodontal diseases presents a lower risk and seems more promising (138, 279, 280). It has been demonstrated that the topical application of IgG monoclonal antibodies onto human teeth prevented the recolonization of resident *S. mutans* following elimination of plaque with chlorhexidine. Protection against colonization by *S. mutans*, lasting up to 2 years, was observed in immunized subjects, although IgG monoclonal antibodies was applied over a period of only 3 weeks (280). These results are surprising considering the complexity of the oral ecosystem. It has been suggested that this long-term protection could have been due to a shift in the equilibrium of the oral microbiota which prevented recolonization by *S. mutans*. In the presence of monoclonal antibodies to *S. mutans*, *S. mutans* colonization is prevented and other bacteria take over the niche that is vacated by *S. mutans*. However, a shift in the proportions of the oral bacterial populations has not been demonstrated. Long-term evaluations of immunized subjects will demonstrate if the elimination of *S. mutans* prevents the initiation of caries.

Caries and periodontal diseases are multifactorial diseases that appear following a disequilibrium of the oral microbiota. It is possible that the induction of antibodies against only the principal etiological agents will not be sufficient to reverse the disease process. Diseases may result from other bacteria that will fill the niche vacated by the target species. This is principally the case in periodontal diseases, which seem to result from the microbial activity of a mixture of microorganisms (obligately anaerobic gram-negative rods).

Passive Immunity with Milk Secretory IgA

The oral cavity and the gastrointestinal tract are colonized immediately after birth. Breast milk is thought to play an important role in the protection of mucosa of breast-fed infants. The severity of gastrointestinal and respiratory infections is much lower in breast-fed infants than in bottle-fed infants (187). Breast milk is characterized by a complex host defense system that includes Igs, lactoferrin, lactoperoxidase, lysozyme, leukocytes, anti-adherent oligosaccharides, antiviral lipids, and anti-inflammatory agents (165). SIgA levels are low in saliva during the first few months, and stable levels of SIgA have seldom been found before 12 to 24 months (8, 68). Before weaning, the potentially protective effect of SIgA would be provided by mother's milk, which is a rich source of SIgA. Infants who are breast fed may get around 0.5 g of SIgA per day via the milk (187). Milk SIgA antibodies are directed against microbes and food proteins to which the mother has been exposed and to which the infants will also be exposed (187). These antibodies appear in milk mainly via the antigenic exposure of lymphoid tissues associated with gut and upper respiratory tract followed by the migration of antigen-sensitized lymphocytes to mammary glands. It is generally agreed that milk IgA antibodies protect infants against microbial infections and food allergies (431). Milk IgA antibodies have been detected against pathogens from the gastrointestinal tract (enterotoxigenic *E. coli*, *Shigella*, *Vibrio cholerae*, and *Salmonella*) and the respiratory tract (pneumococci, *Haemophilus influenzae*, respiratory syncytial virus). The protection against cholera in the breast-fed baby could be related to the level of the mother's milk IgA antibodies against cholera toxin and lipopolysaccharide (163).

Colostrum and milk also contain antibodies that are directed against resident oral streptococci (*S. mitis*, *S. salivarius*, *S. mutans*, and *S. sanguis*), including those that colonize the oral cavity of neonates, as well as mutans streptococci (10, 72, 123). It has been found that breast-fed infants are less caries susceptible than are bottle-fed infants (473). Although *S. mutans* colonizes the oral cavity only after teeth eruption and after breast feeding has been discontinued, milk IgA antibodies may interfere with the colonization of pioneer streptococci and subsequently with the colonization of other oral resident bacteria. Breast feeding may also influence the maturation of the infant mucosal immune system and the infant SIgA response against indigenous bacteria, but this remains a subject of controversy (140, 164, 244). To our knowledge, no study has yet compared the development of the oral microbiota between breast-fed and bottle-fed human infants. However, the development of the intestinal microbiota was found to be different between these two groups (538). In rodents, teeth are present before weaning, and this model proved to be useful for demonstrating the role of milk IgA antibodies in colonization of teeth. Rat dams that were fed *S. mutans* antigen exhibited IgA antibodies in their colostrum and milk which conferred protection against caries to their offspring infected with the *S. mutans* homologous strain (327). In contrast, we did not observe any differences in the changes of oral resident microbiota between pups from Ig-deficient (μMT) mothers and pups from phenotypically normal mothers (295). In addition to Igs, other properties of milk could influence the resident microbiota, including its nutrient contents, buffering capacity, and presence of other defense mechanisms (538). In the intestinal tract, the poor buffering capacity of breast milk could favor the growth of *Lactobacillus* and *Bifidobacterium*, which lead to conditions that are unfavorable for the growth of the *Enterobacteriaceae* (538).

CONCLUSION

The oral microbiota is controlled by several factors. At present, experiments have not succeeded in demonstrating a significant role for SIgA in the control of the indigenous microbiota. In vitro, SIgA antibodies reduce the colonization of bacteria to the oral mucosa and teeth, but attempts to demonstrate a role for SIgA in vivo have led to contradictory reports. Salivary IgA is part of a complex and interdependent immune defense system. Immunodeficient subjects do not seem to be more susceptible to oral diseases, and the absence of SIgA may be compensated by other defense factors. SIgA antibodies may play a major role in the homeostasis of the oral microbiota and reverse the disease process only if the mucosal immune system responds to an increased bacterial antigenic load by producing higher levels of IgA antibodies. However, correlation studies in humans have not demonstrated that a higher level of *S. mutans* is associated with higher levels of naturally occurring SIgA antibodies. Local and oral immunization experiments have revealed that high antigen doses are necessary to induce a secretory IgA response and that this response is relatively short-lasting. Only longitudinal studies in humans can show that SIgA antibodies may help to reestablish the initial equilibrium of the oral microbiota and prevent oral diseases. If a change in the bacterial population is not associated with a corresponding change in salivary IgA antibodies, the salivary IgA plays a no more important role than other salivary glycoproteins in the prevention of oral diseases.

It has been demonstrated in animal models that a protective

SIgA antibody response may be induced against allochthonous (nonresident) bacteria. However, only a few studies have been able to induce a protective SIgA responses against resident bacteria. Indigenous bacteria might survive in the oral cavity because they are less susceptible to or are able to avoid immune mechanisms (30, 44, 87, 142). Although immune responses against true pathogens consist of a rapid and protective SIgA antibody response, hyporesponsiveness seems to develop against the ever-present resident bacteria (147). There is much to be learned about the mechanisms by which resident bacteria may persist in the oral cavity. Fortunately, investigators have recently demonstrated an interest in understanding the regulation of the IgA response to resident bacteria and the cellular and molecular interactions leading to hyporesponsiveness to chronically present bacteria (115, 217, 433). It will also be necessary to gain further insights into the possible mechanisms by which resident bacteria can avoid reacting with SIgA antibodies (256). This information may help in the selection of target antigens for immunization and for the manipulation of the mucosal immune system in order to provide a long-term immunity against the principal agents of oral diseases.

The main role of SIgA antibodies in the oral cavity might be to prevent the colonization of pathogenic microorganisms. Resident bacteria and the host may have developed mechanisms to live together in a relatively commensal interrelationship. It must be kept in mind that the SIgA system not only is a mechanism for eliminating bacteria but also acts as a selective force in the interaction between the host and indigenous bacteria (30). SIgA antibodies may select for antigenic variants of bacteria with low antigenicity and pathogenicity. The great complexity of the oral microbiota makes it difficult to detect such subtle changes.

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