observation of reduced vascular responsiveness of tumor-bearing mice. While we are aware that the presence of tumors may result in lowered immune function (*3*), our previous work has shown that LIA is essentially the result of a local graft-versus-host reaction and that the host's own immune system is not directly involved (*1*). Moreover, we have observed that immunosuppression of the host with cyclophosphamide does not interfere with the animal's responsiveness to LIA (*4*). In contrast, we have found that normal lymphocytes injected intravenously into irradiated C755 tumor-bearing animals are restricted in their ability to function in the humoral immune response to sheep red blood cells (*4*). The data from experiments with cancer patients are conflicting: Hattler and Amos (*5*) suggested that cancer patients had an impaired ability to react to allogeneic cells in the normal lymphocyte transfer reaction; Aisenberg (*6*) indicated that patients with Hodgkin's disease had an abnormally protracted response to such allogeneic cells (*7*); and Levin *et al.* (*8*) found that the response to allogeneic lymphocytes transferred into patients with lymphomas or other cancers did not differ significantly from the reaction of healthy persons in frequency, character, or intensity.

An alternative explanation of our results would be that tumor-bearing animals have a reduced number of circulating stimulator cells (*9*). We know such cells are required for activation of the donor lymphocytes in our system. A less likely possibility is that the tumor, having provided a continual source of angiogenesis-inducing factors (*10*), has already led to maximal activation of some target cell (such as the mast cell) population or of the host endothelial system itself, and that for this reason the host animal is incapable of responding to yet an additional stimulus for vascular differentiation and endothelial cell proliferation.

Whatever the basis for our observations, we should emphasize the importance of recognizing that tumor-bearing animals have a reduced responsiveness to immunocompetent lymphocytes, as measured by a deficient vascular reaction. That growing tumors can thwart this process must be considered a serious handicap in our efforts to harness the immune system for the control of malignancy.

Y. A. SIDKY
R. AUERBACH

Department of Zoology, University of Wisconsin, Madison 53706

References and Notes

1. Y. A. Sidky and R. Auerbach, *J. Exp. Med.* **141**, 1084 (1975).
2. R. Auerbach, L. Kubai, Y. A. Sidky, *Cancer Res.*, in press.
3. R. E. Ritts, Jr., and H. B. Neel, *Mayo Clin. Proc.* **49**, 118 (1974); R. T. McCluskey and S. Cohen, *Mechanisms of Cell Mediated Immunity* (Wiley, New York, 1974).
4. Y. A. Sidky and R. Auerbach, unpublished observations.
5. B. G. Hattler and D. B. Amos, *J. Natl. Cancer Inst.* **35**, 927 (1965).
6. A. C. Aisenberg, *J. Clin. Invest.* **44**, 555 (1965).
7. We cite (*5*) and (*6*) only because they appeared in major clinical journals, but cannot do so without expressing our grave concern over the ethics of this type of experimentation.
8. A. G. Levin, D. G. Miller, C. M. Southam, *Cancer* **22**, 500 (1968).
9. R. E. Billingham, *Cell. Immunol.* **2**, 1 (1971).
10. J. Folkman, *Adv. Cancer Res.* **19**, 331 (1974); J. Folkman and R. Cotran, *Int. Rev. Exp. Pathol.*, in press.
11. This work was supported by grants from NIH and NSF. We thank L. Kubai and L. Morrissey for technical assistance.

30 January 1976; revised 22 March 1976

Ingestion of *Streptococcus mutans* Induces Secretory Immunoglobulin A and Caries Immunity

Abstract. *Ingestion of killed cells of a highly cariogenic strain of* Streptococcus mutans *induced specific antibodies in both saliva and milk but not in serum of gnotobiotic rats. These antibodies were associated with the immunoglobulin A class. When infected with* Streptococcus mutans, *orally immunized animals developed significantly fewer carious lesions than nonimmunized infected controls.*

The bacterium *Streptococcus mutans* has been implicated as a principal etiologic agent of dental caries, an infectious disease that afflicts more than 95 percent of the world's population (*1*). The possibility of controlling this disease by active immunization is currently under intensive investigation (*2*).

Dental caries develops in an oral milieu of secretions that contain locally produced immunoglobulin A (IgA) as the predominant antibody (*3*). Earlier unsuccessful attempts to protect experimental animals against caries by immunization can perhaps be partially explained by the limited induction of antibodies in saliva after systemic administration of antigen (*4*). Injection of *S. mutans* into either the oral mucosa, the salivary glands, or the parotid duct has stimulated salivary IgA antibodies (*5, 6*) and, in some instances, has protected against *S. mutans*–induced caries (*6*). However, these routes of immunization are not likely to be suitable for use in humans (*2*). Recent studies have indicated that specific antibodies in the IgA class can be induced in secretions of rabbit and human mammary glands by oral administration of antigen (*7*). We report here that ingestion of killed cells of *S. mutans* induces production of antibodies in the IgA class to *S. mutans* in saliva and milk, and provides significant protection against dental caries in gnotobiotic rats.

Young gnotobiotic rats (Charles River Laboratories) that were fed a purified caries-promoting diet were used in this study (*8*). Drinking water containing formalin-killed *S. mutans* strain 6715, mutant C211 (final concentration, 10^8 cells per milliliter) was provided freely to one group of weanling rats (group A) until the day they were killed; their littermates (groups B and C) were maintained in separate isolators and were provided sterile drinking water. At 45 days of age, rats in groups A and B were challenged with 50 μl of an 18-hour culture of *S. mutans* 6715, C211 containing 5.4×10^6 to 5.7×10^6 colony-forming units. Previous studies in this laboratory have shown this mutant to be highly virulent (*9*). Colonization of *S. mutans* was confirmed the day after challenge by culturing oral swab samples on Mitis-Salivarius agar (Difco Laboratories). Rats in group C served as nonimmunized, uninfected controls.

All rats were removed from the isolators at 90 days of age. Individual saliva samples were collected after pilocarpine stimulation (*6*). Animals were killed by cardiac exsanguination, and serum was collected. *Streptococcus mutans* 6715, C211 was reisolated from dental plaque of each infected animal (groups A and B), and no other bacteria were detected. Both mandibles from each animal were cleaned, stained, and then scored for caries by the Keyes procedure (*10*).

Table 1 gives the levels of immunoglobulins and mean agglutinin titers to *S. mutans* in the serum and saliva of 90-day-old gnotobiotic rats (*11*). Significant differences were not evident in the levels of either serum immunoglobulins or salivary immunoglobulin G (IgG) for the three groups of animals. No immunoglobulin M (IgM) was detected in saliva. Rats receiving *S. mutans* in their drinking water (group A) had significantly higher levels ($P \leq .01$) of IgA in their saliva than animals in either group B or group C. Low agglutinin activity was observed in

serum of rats from groups A and B, whereas no activity was seen in the rats of group C. Agglutinins could not be detected in saliva of nonimmunized animals (groups B and C), whereas rats in group A exhibited a mean $\log_2$ agglutinin titer of 4, which was enhanced to a titer of 6 by the addition of antiserum to IgA (*12*).

Examination of various tissues by the immunofluorescence technique (*13*) revealed that, in group A rats, numerous IgA- and some IgM- and IgG-producing cells were present in the salivary glands, lymph nodes, and the lamina propria of the small intestine. However, in the control animals from groups B and C, there were only a few or no cells that produced IgA, IgG, or IgM in any tissue examined. Immunoglobulin-producing cells were sparse in the spleens of all three groups.

To distinguish whether antistreptococcal antibodies in the IgA class were induced either by stimulating immunocompetent cells of major and minor salivary glands with penetrated antigen (*14*) or by homing of antigen-sensitized plasma cell precursors to these secretory glands from gut-associated lymphoid tissue (GALT), secretions of mammary glands that could not have been stimulated directly by *S. mutans* antigens were examined. Rats fed *S. mutans*, as before, were mated at 90 days. After parturition, each dam with her litter was removed from the isolator and housed in a sterile cage covered with a hood. A mechanical apparatus was used to milk each dam (*15*), and the whey samples were fractionated by molecular sieve chromatography (*16*). Fractions were pooled according to their immunoglobulin content and concentrated by negative pressure dialysis to the original whey volume. The level of anti–*S. mutans* agglutinin activity was determined by microtitration (*11*). Whey from rats receiving antigen in their drinking water exhibited high agglutinin titers ($\log_2 = 7$), whereas whey from group B rats (infected only) had low agglutinin titers ($\log_2 = 2$). Both agglutinin activity ($\log_2 = 8$) and IgA were present in the first fraction of whey (from group A dams) eluted from Sephadex G-200. The agglutinin activity could be enhanced by the addition of anti-IgA ($\log_2 = 11$), thereby further demonstrating the antibodies to be associated with the IgA class. No agglutinin activity was associated with the other fractions. Further, IgM could not be detected in rat whey; this was in agreement with findings of previous studies (*12*).

Table 2 shows the mean caries scores observed in immunized and nonimmunized 90-day-old gnotobiotic rats. Significantly fewer carious lesions on all molar surfaces were found in rats given *S. mutans* in drinking water (group A) than in nonimmunized infected rats (group B). No marked differences were observed in the mean body weights of animals in the three experimental groups.

The foregoing results indicate that consumption of water containing whole *S. mutans* cells induces the formation of specific antibodies of the IgA class in saliva and milk. However, no significant antibody activity was detected in serum. The presence of this IgA antibody in saliva was reflected in a reduced incidence of *S. mutans*–induced caries.

A synthesis of available information suggests that ingested antigens selectively stimulate appropriate clones of IgA precursors that are located in GALT, for example, Peyer's patches and appendix covered with specialized pinocytotic epithelial cells. Leaving the GALT through draining intestinal lymphatics, these stimulated lymphoid cells enter the bloodstream and selectively home to distant secretory tissues, such as the salivary and mammary glands, bronchi, and intestine, where they differentiate into mature IgA-secreting plasma cells (*7, 17*). This hypothesis receives support from our investigation since we observed selective occurrence of antibodies to orally administered antigen in secretions of salivary and mammary glands, but not in serum. However, direct exposure of

Table 1. Immunoglobulin levels (in milligrams per milliliter) and agglutinin titers ($\log_2$) in serum and saliva of 90-day-old gnotobiotic rats. Values represent the mean ± standard error of 20 rats per group. Immunoglobulin was determined by radial immunodiffusion, and agglutinin titers by microtitration in which 2×10^8 *S. mutans* cells per milliliter were used. Antiserums to IgA and IgG were employed as previously described [McGhee *et al.* (*6*)].

Serum				Saliva					
Immunoglobulin			Agglutinin titer	Immunoglobulin			Agglutinin titer		
IgA	IgG	IgM		IgA	IgG	IgM	Untreated	Anti-IgA	Anti-IgG
Group A (immunized, infected)									
0.10 ± 0.007	0.76 ± 0.05	0.28 ± 0.02	1	0.21 ± 0.02	0.24 ± 0.02	< 0.002	4	6	4
Group B (infected only)									
0.05 ± 0.007	0.66 ± 0.07	0.27 ± 0.02	1	0.06 ± 0.007	0.18 ± 0.02	< 0.002	0	0	0
Group C (control, no infection)									
0.06 ± 0.005	0.67 ± 0.03	0.23 ± 0.05	0	0.09 ± 0.01	0.19 ± 0.01	< 0.002	0	0	0

Table 2. Mean caries scores of 90-day-old gnotobiotic rats. Rats in groups A and B were challenged with 50 μl of an 18-hour culture of *S. mutans* 6715, C211 which contained 5.4×10^6 to 5.7×10^6 colony-forming units. The mean caries scores were evaluated by the Keyes procedure (*10*); E, slight penetration into enamel; D_s, slight penetration into dentin; D_m, moderate penetration into dentin. Values represent the mean ± standard error of 20 rats per group. All values in group A were significantly less than those in group B ($P \leq .01$). The mean body weight is expressed in grams ± standard error of the mean.

Group	Mean caries scores						Mean body weight
	Buccal		Sulcal		Proximal		
	E	D_s	D_s	D_m	E	D_s	
A (immunized, infected)	12.8 ± 0.4	10.6 ± 0.5	12.4 ± 0.4	10.5 ± 0.7	3.6 ± 0.3	2.4 ± 0.4	156.2 ± 5.3
B (infected only)	25.5 ± 0.5	18.6 ± 0.8	21.7 ± 0.6	18.6 ± 0.7	7.6 ± 0.1	7.0 ± 0.2	150.7 ± 7.0
C (control, no infection)	0.0	0.0	0.0	0.0	0.0	0.0	165.1 ± 6.1

antigen to the salivary glands may contribute to the level of salivary antibody (*14*).

The occasional adverse reactions that accompany parenteral immunization may be circumvented by oral administration of antigen. This point is important in relation to prevention of dental caries in human beings. In previous studies, effective anticaries immunity was provided through immunization routes that are unlikely to be suitable for human use.

SUZANNE M. MICHALEK
JERRY R. MCGHEE
JIRI MESTECKY
ROLAND R. ARNOLD
LOURENCO BOZZO
Institute of Dental Research and *Department of Microbiology, University of Alabama, Birmingham 35294*

References and Notes

1. H. W. Scherp, *Science* **173**, 1199 (1971); P. H. Keyes, *J. Am. Dent. Assoc.* **76**, 1357 (1969); W. J. Loesche, J. Rowan, L. H. Straffon, P. J. Loos, *Infect. Immunol.* **11**, 1252 (1975).
2. *Symposium on the Immunological Aspects of Dental Caries*, R. Genco, W. Bowen, T. Lehner, Eds., *J. Dent. Res.* **55**, C206 (1976), special issue; W. Bowen, R. Genco, T. O'Brien, Eds., *Immunologic Aspects of Dental Caries: Selection of Immunogens for a Caries Vaccine and Cross Reactivity of Antisera to Oral Microorganisms with Mammalian Tissues* (Government Printing Office, Washington, D.C., in press).
3. J. F. Heremans, in *The Antigens*, M. Sela, Ed. (Academic Press, New York, 1974), vol. 2, pp. 365–522.
4. B. Guggenheim, R. Mühlemann, B. Regolati, R. Schmid, in *Dental Plaque*, W. D. McHugh, Ed. (Thomson, London, 1970), pp. 287–296; J. M. Tanzer, G. J. Hageage, R. H. Larson, *Arch. Oral Biol.* **18**, 1425 (1973).
5. R. T. Evans, F. G. Emmings, R. J. Genco, *Infect. Immunol.* **12**, 293 (1975).
6. M. A. Taubman and D. J. Smith, *ibid.* **9**, 1079 (1974); J. R. McGhee, S. M. Michalek, J. Webb, J. M. Navia, A. F. R. Rahman, D. W. Legler, *J. Immunol.* **114**, 300 (1975).
7. P. C. Montgomery, J. Cohn, E. T. Lally, in *The Immunoglobulin A System*, J. Mestecky and A. R. Lawton, Eds. (Plenum, New York, 1974), pp. 453–462; R. M. Goldblum, S. Ahlstedt, B. Carlsson, L. Å. Hanson, U. Jodal, G. Lidin-Janson, Å. Sohl-Akerlund, *Nature (London)* **257**, 797 (1975).
8. Adult germfree rat dams were maintained in Trexler plastic isolators. Rat pups were weaned at 20 days of age, transferred to experimental isolators, and provided purified caries-promoting diet [S. M. Michalek, J. R. McGhee, J. M. Navia, *Infect. Immunol.* **12**, 69 (1975)].
9. S. M. Michalek, T. Shiota, T. Ikeda, J. M. Navia, J. R. McGhee, *Proc. Soc. Exp. Biol. Med.* **150**, 498 (1975).
10. P. H. Keyes, *J. Dent. Res.* **37**, 1088 (1958).
11. Immunoglobulin levels were determined by radial immunodiffusion as previously descrit → [J. R. McGhee, S. M. Michalek, V. K. Ghanta, *Immunochemistry* **12**, 817 (1975)]. Agglutinin antibody levels were determined by the microtitration technique as described [McGhee *et al.* (*6*)].
→ J. R. McGhee, S. M. Michalek, V. K. Ghanta, *Immunochemistry* **12**, 817 (1975).
13. Fresh tissues were frozen in liquid nitrogen and processed as described [F. W. Kraus and J. Mestecky, *Arch. Oral Biol.* **16**, 781 (1971)]. Fluorescein isothiocyanate–labeled goat antiserums to mouse μ, α, and γ chains (Meloy Laboratories) were applied to various sections. These antiserums were previously found to cross-react with the corresponding rat immunoglobulins. Sections were examined with a fluorescence microscope equipped with a vertical illuminator according to Ploem (Orthoplan, Leitz, Germany).
14. J. M. Crawford, M. A. Taubman, D. J. Smith, *Science* **190**, 1206 (1975); F. G. Emmings, R. T. Evans, R. J. Genco, *Infect. Immunol.* **12**, 281 (1975).
15. S. M. Michalek, J. R. McGhee, V. K. Ghanta, *J. Reticuloendothel. Soc.* **16**, 213 (1974).
16. After centrifugation and precipitation of casein [J. Mestecky, R. Kulhavy, F. W. Kraus, *J. Immunol.* **108**, 738 (1972)] the samples were chromatographed on a Sephadex G-200 column (1.6 by 100 cm) equilibrated in 1 percent ammonium bicarbonate buffer, *p*H 7.0. The distribution of immunoglobulin classes in fractions was determined by precipitation as described by McGhee *et al.* (*12*).
17. S. W. Craig and J. J. Cebra, *J. Exp. Med.* **134**, 188 (1971); J. G. Hall and M. E. Smith, *Nature (London)* **226**, 262 (1970); A. R. Moore and J. G. Hall, *ibid.* **239**, 161 (1972); D. Guygrand, C. Griscelli, P. Vassalli, *Eur. J. Immunol.* **4**, 435 (1974); M. D. Cooper, P. W. Kincade, D. P. Bockman, A. R. Lawton, in *The Immunoglobulin A System*, J. Mestecky and A. R. Lawton, Eds. (Plenum, New York, 1974), pp. 13–22; A. F. Williams and J. L. Gowans, *J. Exp. Med.* **141**, 335 (1975).
18. Supported by NIH grants DE 04217, DE 02670, and AI 10854. We thank Drs. M. D. Cooper, H. M. Fullmer, and F. W. Kraus for criticisms of this report; R. Kulhavy for excellent assistance; and C. A. Sims and J. Morris for editorial advice.

28 January 1976; revised 6 April 1976

Lekking Behavior in Kafue Lechwe

Abstract. *The Kafue lechwe (*Kobus leche kafuensis*) exhibits lekking behavior in which a number of males occupy small territories clustered together in discrete areas where females go for mating. Similar behavior in antelope is known only in the Uganda kob (*Kobus kob thomasi*). Lechwe lekking occurs only during a main rutting season although some breeding occurs throughout the year.*

Three subspecies of the lechwe antelope, *Kobus leche* Gray, are recognized in the Central African Republic of Zambia (*1*). The Kafue lechwe, *Kobus leche kafuensis* Haltenorth, exhibit territorial behavior on leks during their main rutting season. Although many African antelopes are territorial when they breed (*2*) and some features of lekking have been observed in the lechwe and other members of the genus *Kobus* (*3*), full-scale lekking has been reported only in the congeneric Uganda kob *Kobus kob thomasi*, which breeds on leks throughout the year (*4*).

The characteristic features of lekking behavior are known principally from a few species of ground-living birds (*5*). While satisfying the defining attributes of territorial behavior, that is, attachment to a limited area which is defended against conspecifics, lekking includes several additional features. A lek is a circumscribed area, often the same from year to year, in which a few adult males occupy a cluster of small, adjacent territories used solely for mating. The male occupants spend much of their time engaging in ritualized displays, regardless of whether females are present. Displays are enhanced by eye-catching morphological features usually found in males of lekking species (for example, vivid plumage). Females enter a lek to mate and choose a male for the purpose. The social status of males on a lek is unequal and it is usual to observe most or all females on a lek with a single male on a "prime" territory. Only a small percentage of adult males occupy a few scattered leks and, of these, only a small percentage do most of the breeding. It is likely that these features of lekking, in combination with its seasonality, explain why reports of lechwe behavior in the past have been contradictory with regard to the question of whether lechwe are aggressive or territorial (*3*, *6*).

The estimated population of Kafue lechwe in 1974 was about 110,000. It is centered on Lochinvar and Blue Lagoon National Parks, two small game reserves with a combined area of about 840 km^2 that are located on nearly opposite sides of the Kafue River, which drains central Zambia. Much of the park area falls within the Kafue Flats, a floodplain of the river located at about 15.5°S and 27° to 28°E. When possible, lechwe are usually found on the open floodplain and are often found grazing in or near water (*3*, *6*). Much smaller herds of zebra and wildebeest share the open plains with the lechwe. All natural predators were eliminated from the area years ago, and poaching has been the only major threat although hydroelectric development poses a major problem in the future (*7*). This report is based on ten field trips to Lochinvar at approximately 2-week intervals between August 1974 and February 1975, the first stage of an ongoing project.

The life of the lechwe is marked by seasonal fluctuations related to a marked annual flooding cycle on the Flats. Zambia has a single rainy season from about November until March, with a peak in December and January. There is a corresponding rise and fall in the water level on the Flats; the peak flood occurs from about April through June. This forces the lechwe herds into an annual cycle of movements and changes in population density related to the availability of grazing (*6*). Although mating and parturition occur to some extent throughout the year, there is a higher frequency of sexual activity and enlarged testes from mid-November until mid-February (*6*).