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Local Passive Immunization by Monoclonal Antibodies against Streptococcal Antigen I/II in the Prevention of Dental Caries

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Local passive immunization with monoclonal antibodies (Mc Ab) to *Streptococcus mutans* was attempted as an alternative approach to active systemic immunization. We prepared an immunoglobulin G class Mc Ab to the cell surface protein determinant of streptococcal antigen I/II and applied it repeatedly to the teeth of rhesus monkeys. This resulted in decreased colonization by *S. mutans* in fissures and smooth surfaces of teeth and no dental caries, unlike the results in control animals, which developed caries and showed a high proportion of *S. mutans* on their teeth. There was no significant difference in serum, salivary, or gingival fluid antibodies to *S. mutans* between the two groups of animals. Any objections raised over systemic immunization inducing cross-reactive antibodies are therefore overcome by local passive immunization. The mechanism of prevention of colonization has not been established, but we postulate that the Mc Ab which is directed against an important cell surface antigenic determinant of *S. mutans* (streptococcal antigen I/II) prevents adherence of *S. mutans* to the acquired pellicle on the tooth surface. *S. mutans* reacts with the Mc Ab and becomes opsonized, phagocytosed, and killed by the local gingival traffic of neutrophils.

Although systemic immunization against *Streptococcus mutans* is an effective means of preventing caries in subhuman primates (10, 11, 17), there is some evidence that immunization with *S. mutans* may induce cross-reactive tissue antibodies in rabbits (6, 22, 23) but not in subhuman primates (1). We have, therefore, explored an alternative approach of local passive immunization which would be devoid of systemic side effects. The rationale for local passive immunization was the finding that systemic passive immunization with immunoglobulin G (IgG) antibodies to *S. mutans* protects rhesus monkeys from dental caries (12). We then formally demonstrated that ^{125}I-labeled serum IgG administered intravenously passes through the gingival crevicular epithelium and is detectable in gingival fluid within 30 min of injection (3). Furthermore, specific IgG-class antibodies to *S. mutans* were demonstrated in the gingival fluid of animals which developed specific serum IgG antibodies after systemic immunization with *S. mutans* (20). We have, therefore, prepared an IgG-class monoclonal antibody (Mc Ab) to the protein determinant of streptococcal antigen (SA) I/II (21) and applied it repeatedly to the teeth of rhesus monkeys. This resulted in decreased colonization of the teeth by *S. mutans* and prevented the development of dental caries.

MATERIALS AND METHODS

Experimental animals and caries. Nine young rhesus monkeys had all their deciduous teeth, but none of the permanent teeth had erupted. They were offered a human-type diet consisting of sandwiches, eggs, bananas, oranges, and about 15% sugar (9). Monkeys develop indigenous *S. mutans* (serotype c) in the dental plaque, and this leads to the development of dental caries (9, 10). The teeth were examined at about 2-month intervals with a probe and mirror as well as by X rays. The mean (± standard error) number of carious lesions per animal is given as the caries score. The experiment was carried out over a period of about 1 year.

Immunization method. Four monkeys were immunized 12 times (indicated by the arrows in Fig. 1 and Fig. 2) with the Mc Ab to SA I/II by using whole ascites fluid (21). The teeth were dried with gauze, and about 100 μl (containing 1 mg of IgG) of Mc Ab was applied to the smooth and occlusal surfaces of all the teeth (about 5 μl of Mc Ab per tooth). To avoid dilution and washing away of the Mc Ab by saliva, we made individually prepared silicone rubber appliances with Optosil (Beyers Dental), fitted them to the teeth and gums, and maintained them with slight digital pressure for 5 min. Five control monkeys had only saline applied to their teeth.

Culturing of *S. mutans*. Dental plaque was collected separately from the smooth surfaces of the upper central incisors and from the fissures of the upper left second molar with sterile probes and placed into transport medium (2). The proportion of *S. mutans* grown on tryptone–yeast extract–L-cystine medium was determined (4) and expressed as the mean (± standard error) percentage of the total number of colonies grown on that medium.

Serum, salivary, and gingival fluid antibodies. Blood was collected from the femoral vessels, whole saliva was allowed to flow into petri dishes after injection of 0.5 mg of pilocarpine per kg, and gingival crevicular fluid was collected by repeated washings with a Hamilton syringe as described previously (19). Serum and gingival fluid IgG antibodies were determined by a solid-phase radioimmunoassay (20) with SA I/II and ^{125}I-labeled affinity-purified rabbit anti-monkey IgG (Nordic Immunological Laboratories). Salivary IgA antibodies were assayed by an indirect method with rabbit anti-monkey IgA (Nordic), followed by ^{125}I-labeled affinity-purified goat anti-rabbit IgG (Tago Inc.). The bound ^{125}I-antiserum was counted in a gamma counter, and the results were expressed as the mean (± standard error) counts per minute, after the base-line value (i.e., the count before immunization at 0 weeks) was subtracted.

Preparation of Mc Ab. The Mc Ab was prepared by fusion

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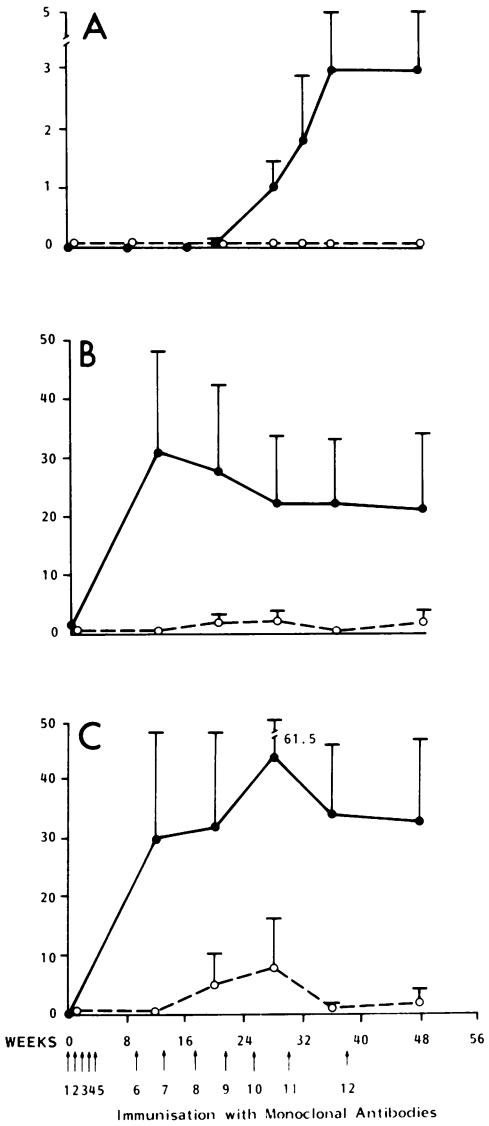

FIG. 1. Effect of local passive immunization with Mc Ab on the caries score (A) and the proportion (as a percentage) of *S. mutans* in fissures (B) and smooth surfaces (C) of teeth in five control animals (●) and four immunized animals (○).

of a mouse myeloma line with spleen cells (7) from a mouse which had been immunized with SA I/II derived from *S. mutans* serotype c (15, 16). The cells producing antibodies to SA I/II were cloned twice and injected into mice to produce ascites fluid (21). The Mc Ab was of the IgG2a class, as determined by double-diffusion precipitation in 1% agarose with rabbit anti-mouse IgG1, IgG2b, and IgG3 and goat anti-mouse IgG2a (Nordic) (21). A solid-phase radioimmunoassay for anti-SA I/II, I, II, and III (21) revealed a high antibody titer only to SA I/II (10^7). The specificity of the Mc Ab to SA I/II was established by inhibition with the four separated SA preparations. Serotype specificity was tested by direct binding of the Mc Ab with cells of the seven serotypes of *S. mutans* as well as *S. sanguis* in a fluid-phase radioimmunoassay (21). The results showed that the Mc Ab cross-reacted with serotypes c, e, and f but not serotypes a, b, d, and g and failed to react with *S. sanguis*.

RESULTS

Dental caries. Application of the IgG class of Mc Ab to the deciduous teeth of rhesus monkeys prevented the development of caries over a period of 1 year, as compared with a mean (± standard error) of 3.0 (±2.0) carious lesions per control animal (Fig. 1). There were eight smooth-surface and seven fissure carious lesions in the control animals, so that both smooth-surface and fissure caries were prevented in the immunized animals. Caries was found in three of the control monkeys but in none of the passively immunized monkeys.

Colonization of *S. mutans*. Colonization of both smooth surfaces and fissures of teeth by *S. mutans* was significantly lower in those animals whose teeth were treated with Mc Ab than in untreated animals (Fig. 1). The proportion of *S. mutans* in the fissures of teeth of control monkeys was between 21 and 31%, as compared with 0 to 2% in immunized monkeys. The results for smooth-surface plaque were similar: 30 to 44% in control monkeys and 0 to 8% in immunized monkeys.

Serum, salivary, and gingival fluid antibodies. Serial studies of serum IgG, gingival fluid IgG, and salivary IgA antibodies to SA I/II failed to show significant differences in titers as compared with the controls (Fig. 2). Indeed, salivary IgA antibodies and, to a much lesser extent, serum and gingival IgG antibodies to SA I/II were slightly higher in control animals than in treated animals. The possibility that serum antibodies to the mouse Mc Ab might be induced was also explored by the radioimmunoassay, but a significant rise in antibodies was not found.

DISCUSSION

Local passive immunization by repeated application of anti-SA I/II Mc Ab to the deciduous teeth of rhesus monkeys prevented significant colonization of the fissures and smooth surfaces of the teeth by *S. mutans*. Dental caries failed to develop in the immunized animals over a period of 1 year, as compared with a caries score of 3.0 ± 2.0 in the control animals. A second experiment in irus monkeys was initiated, and mouse ascites fluid without any anti-SA I/II antibodies was used as a control. Preliminary results over 12 weeks showed that the proportions of *S. mutans* in fissures (62.2 ± 11.3%) and smooth surfaces (55.8 ± 15.2%) of teeth treated with the normal ascites fluid were much higher than in those treated with anti-SA I/II Mc Ab (fissures, 5.2 ± 2.7%; smooth surfaces, 4.0 ± 2.9%).

The colonization of teeth by *S. mutans* has been extensively studied and reviewed elsewhere (5). The mechanism of adherence of *S. mutans* to teeth involves two stages: (i) an initial, probably reversible, interaction between the organism and the saliva-coated acquired pellicle, and (ii) an irreversible stage which is mediated by insoluble glucan. Although the mechanism of action of local passive immunization with Mc Ab has not been established, we postulate three phases (Fig. 3). In phase 1, the Mc Ab adheres to the acquired pellicle on the tooth surface. In phase 2, *S. mutans* binds to the Mc Ab during the initial reversible interaction between the organism and the acquired pellicle. The Mc Ab reacts specifically with an important antigenic determinant (SA I/II) which is expressed on the cell surface of the organism (13, 14, 24), is hydrophobic, and may be an adhesin (13). In phase 3, *S. mutans* is opsonized by the Mc Ab to SA I/II, phagocytosed, killed, and removed by the local gingival neutrophils and complement (18, 19). This three-phase mechanism may operate during the reversible interaction between *S. mutans* and the pellicle on the tooth surface.

There is as yet no evidence that it may also function during the irreversible glucan-mediated stage.

It should be also noted that the relatively low incidence of caries in the control animals might be ascribed to dietary fluoride (45 ppm [μl/liter]), as was noted elsewhere (8). Hence, local passive immunization can almost completely prevent caries in animals which are partially protected by fluoride.

The objections raised against active systemic immunization, i.e., that cross-reactive tissue antibodies might be elicited (6, 22, 23), are now overcome, as neither serum nor salivary antibodies were induced (Fig. 2). Indeed, higher salivary IgA antibody titers to SA I/II were found in control monkeys than in immunized monkeys (Fig. 2); this may have been due to natural immunization elicited by the high proportion of *S. mutans* in the dental plaque of control animals. The possibility that antibodies might be induced to mouse serum was also formally excluded. Furthermore, there was no detectable clinical change in the gingiva over the experimental period. The frequency of application of Mc Ab to the teeth has not been established and needs to be further investigated.

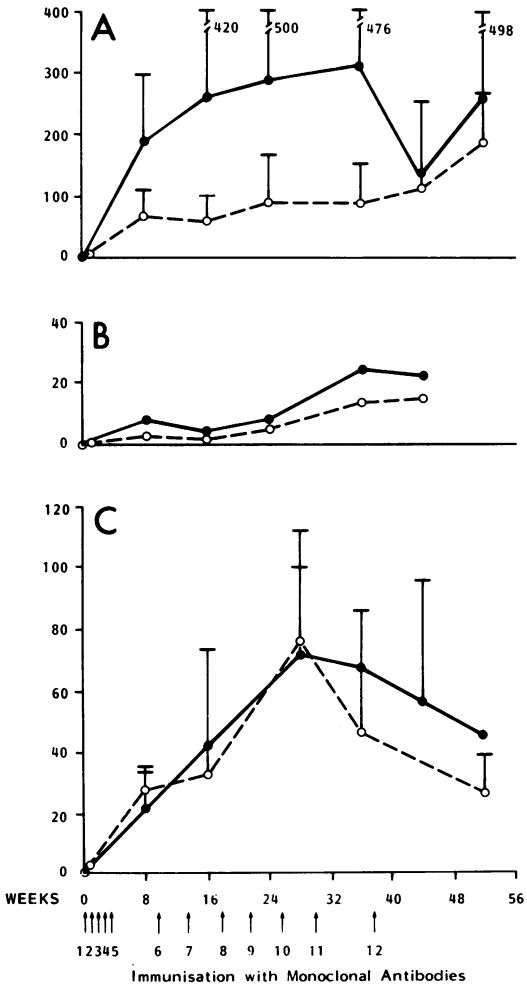

FIG. 2. Effect of local passive immunization with Mc Ab on serum IgG (C), gingival fluid IgG (B), and salivary IgA (A) antibodies to *S. mutans* in control animals (•) and immunized animals (○). The antibodies were assessed by a radioimmunoassay; data are reported as the binding of antibodies in counts per minute.

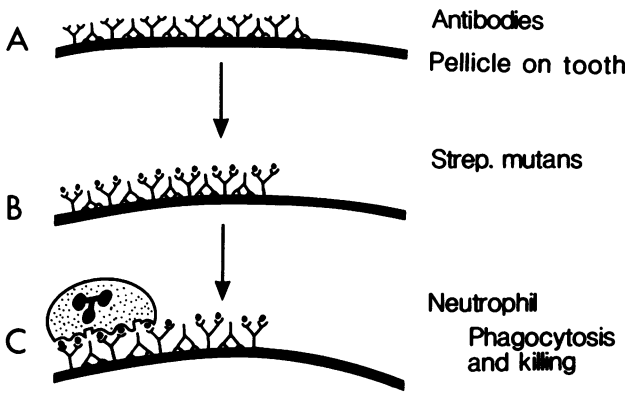

FIG. 3. Postulated three-phase mechanism of action of Mc Ab in preventing the adherence of *S. mutans* to the acquired pellicle on the tooth surface. (A) Phase 1; (B) phase 2; (C) phase 3.

It is possible that the principle of local passive immunization with Mc Ab might prove to be a significant means of prevention of other oral diseases, of which the most important is periodontal disease. Local passive immunization might also be applicable to microbial infections of other mucosal surfaces, such as the oropharynx and the respiratory, intestinal, and genital tracts.

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