# Anti–cell-associated glucosyltransferase immunoglobulin Y suppression of salivary mutans streptococci in healthy young adults

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*Streptococcus mutans* is a primary caries-inducing bacterial pathogen,[1] as well as a molecular target for dental caries vaccines.[2] Among other enzymes, *S. mutans* produces cell-associated glucosyltransferase (CA-gtf), which is a major virulence factor located on the bacterial surface and is essential for synthesis of sucrose-derived water-insoluble glucan. The formation of water-insoluble glucan is an important phase in initial dental plaque deposition and caries development.[1]

Because microbial adherence is an essential prerequisite for host colonization, any blockade of *S. mutans* surface structures before they make contact with a tooth surface can interfere with a key factor of dental plaque physiology. A common method for blocking microbial adherence is to administer a vaccine that is designed to induce antibodies against surface-localized microbial structures. The safety[3] of an *S. mutans* vaccine is controversial; issues regarding in vitro cross-reactivity between human heart tissue and certain *S. mutans* pro-

## ABSTRACT

JADA CE CONTINUING EDUCATION ARTICLE 3

**Background.** The authors evaluated the suppressive effects of lozenges containing egg yolk antibodies (that is, immunoglobulin Y [IgY]) against *Streptococcus mutans* cell-associated glucosyltransferase (CA-gtf) on oral colonization by mutans streptococci (MS) in healthy young adults.

**Methods.** In a five-day double-masked placebo-controlled trial, young adult participants self-administered lozenges containing anti–CA-gtf IgY (Ovalgen DC, GHEN, Gifu-City, Japan) or a placebo at prescribed times each day. On the basis of bacterial colony counts of saliva cultures, the authors analyzed the pretrial and posttrial differences in levels of MS and total anaerobic bacteria among participants in the treatment (anti–CA-gtf IgY) and placebo groups and a control group.

**Results.** Salivary MS scores in participants in the treatment group decreased significantly ($P < .001$), and the mean anaerobic bacterial count in the treatment group was not statistically different before and after the trial. In the placebo and control groups, posttrial changes in median MS scores and total salivary anaerobic bacterial counts were not statistically significant.

**Conclusions.** The results of the study show that lozenges containing anti–CA-gtf IgY can suppress oral colonization by MS in healthy young adults.

**Clinical Implications.** Lozenges containing anti–CA-gtf IgY may help reduce dental caries risk in humans.

**Key Words.** Egg antibody; glucosyltransferase; *Streptococcus mutans;* dental caries.

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teins[4-6] may linger. Although caries vaccines are considered to be effective in animal models, no caries vaccines are available commercially for human use to date, and few companies are willing to underwrite the investment for this type of vaccine development,[2] ostensibly owing to the cost considerations and the rigorous requirements for vaccine safety.

Given the hurdles involved in caries vaccine development, passive immunization has received attention. This strategy, which involves the introduction of exogenous antibodies to the oral cavity, has fewer safety issues than do parenteral caries vaccines.[7] The repackaging of antibodies as anti-infective agents in place of antibiotic agents has received critical scrutiny owing to the general trend in antimicrobial resistance by human and animal pathogens.[8] Depending on the targeted bacterial component, passive immunity against caries may be sufficient to retain all the functional mechanisms of antibodies generated during vaccination, including immunological interception,[9] bacterial agglutination and abrogation of dental plaque–building enzymes. With this approach, the results of earlier investigations have shown the potential value of using oral monoclonal and polyclonal antibodies to reduce the extent or frequency of carious lesions or decrease the level of oral *S. mutans* in rats or humans.[7,10-18]

Passive immunization against dental caries will not become a practical reality unless an inexpensive method for mass production of antibodies is available. To this end, poultry eggs have been found to be a convenient source of polyclonal antibodies in the form of egg yolk immunoglobulins (immunoglobulin Y [IgY]).[19] The results of passive immunization trials in rats in which IgY was used against different antigens from *S. mutans* showed varying degrees of success.[13,15,17,18] Among these antigens, CA-gtf is a target for immunological intervention in humans because antibodies to CA-gtf do not to cross-react with other human tissues.[20,21] The safety of the oral administration of IgY has been documented.[22]

Considering these findings, we conducted a study to determine whether anti–CA-gtf IgY (Ovalgen DC, GHEN, Gifu-City, Japan) administered via lozenges is effective in suppressing oral mutans streptococci (MS) colonization in humans.

## PARTICIPANTS, MATERIALS AND METHODS

**Purification of CA-gtf from *S. mutans*.** We used *S. mutans* strain MT 8148 to produce the CA-gtf enzyme. To isolate and purify CA-gtf from this strain, we followed a procedure described previously[23] and used ion exchange chromatography (DEAE Sephacel, GE Healthcare Bio-Sciences AB, Uppsala, Sweden) and hydroxylapatite (HA) chromatography. This purification process yielded a protein of approximately 156 kilodaltons as determined by means of sodium dodecyl sulfate polyacrylamide gel electrophoresis (data not shown).

**Production of IgY.** To produce anti–CA-gtf IgY, we administered immunizations to 18-week-old poultry hens intramuscularly by using an oil-in-water emulsion of purified CA-gtf[17] or, in cases of mock-immunized poultry, oil-in-water emulsion of phosphate buffered saline (PBS). We harvested all eggs laid by these hens between three and 10 weeks after the immunization, and we isolated the yolks. We then pooled and spray-dried the yolk in accordance with a method described previously.[24] The spray-dried egg yolk underwent further extraction by means of hexane and ethanol at 50°C followed by vacuum drying to produce egg yolk powder with less than 2 percent fat content. We designated the defatted egg yolk powder containing IgY derived from CA-gtf-immunized poultry (anti–CA-gtf IgY) as the treatment and the defatted egg yolk powder containing IgY from mock-immunized poultry as the placebo.

**Preparation of lozenges.** We mixed anti–CA-gtf IgY or the placebo IgY with tablet excipients and processed them into 9-millimeter lozenges by means of direct compression. Each treatment lozenge contained 72 milligrams of anti–CA-gtf IgY plus excipients (4 mg aspartame, 80 mg microcrystalline cellulose, 50 mg eggshell calcium, 16 mg calcium bicarbonate, 148 mg maltose, 6 mg silica, 8 mg fermented milk powder, 10 mg fruit powder and flavoring). Each placebo lozenge contained 72 mg of the placebo IgY plus the same excipients as in the anti–CA-gtf IgY lozenges. We stored the lozenges at room temperature in airtight amber glass bottles for one to two weeks until we used them for the clinical trial.

**Extraction and analysis of IgY.** To conduct in vitro reactivity assays, we extracted IgY from the anti–CA-gtf IgY or the placebo by dissolving 1 gram of the sample in 9 milliliters of PBS, mixing them with an equal volume of chloroform and incubating them at 20°C while stirring

**ABBREVIATION KEY. ATCC:** American Type Culture Collection. **BHI:** Brain heart infusion. **CA-gtf:** Cell-associated glucosyltransferase. **CFUs:** Colony-forming units. **ELISA:** Enzyme-linked immunosorbent assay. **HA:** Hydroxylapatite. **IgY:** Immunoglobulin Y. **MS:** Mutans streptococci. **PBS:** Phosphate buffered saline.