

Immunization Strategies for Dental Caries Vaccine Delivery.

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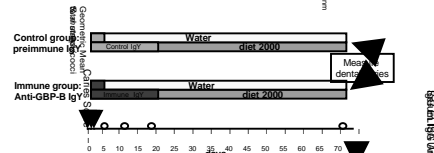
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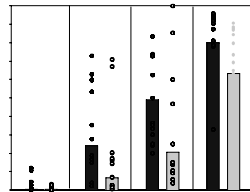


1. Protection via Passive Transfer of IgY AB to *S. mutans* GBP-B

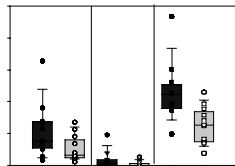
1a. Purpose and protocol: Active immunization by systemic and by mucosal routes with *S. mutans* glucan binding protein B (GBP-B) induces immune responses which protect rats from dental caries caused by experimental infection with mutans streptococci. The purpose of this experiment was to evaluate whether protection in the rat model could also be achieved by passive immunization in which antibody to GBP-B would be available in the diet during initial infection with cariogenic *S. mutans* accumulation and resulting dental caries in the rat model. IgY antibody was prepared by immunizing white leghorn hens subcutaneously with *S. mutans* GBP-B and purifying IgY from egg yolks. Pre-immune IgY was also prepared. Initially, 28-day old Sprague-Dawley rats were fed diet 2000 supplemented with 0.44% immune or control IgY as shown in the figure. Animals were also infected with 5×10^6 streptomycin-resistant *S. mutans* on days 0,1,2. Infection was periodically measured by systematic swabbing of animals. Dental caries on molar surfaces was measured 78 days after the start of the experiment (54 days after the removal of antibody).



1b. Passive Immunization - Bacterial Accumulation: Systematic swabbing of molar surfaces of control and antibody fed rats occurred 5, 13, 20 and 78 days after infection. In the following figure is shown the level of *S. mutans* accumulation as a percentage of the total streptococcal CFU. Mean % were always low in the GBP-IgY group and differences reached significance on days 13 and 78.



1c. Passive Immunization - Molar Caries: Dental caries was measured on all molar teeth of control and antibody-fed rats on day 78, both with respect to surface (figure below) and with respect to tooth. Antibody fed rats had significantly lower caries on occlusal surfaces and on eight of the 12 molars, compared to control IgY-fed rats

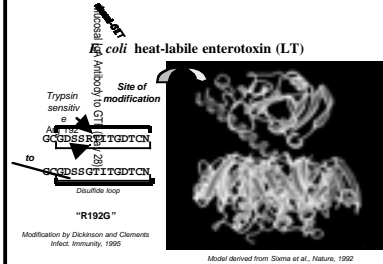


2. Mucosal Induction of Salivary IgA Antibody to Peptide Constructs

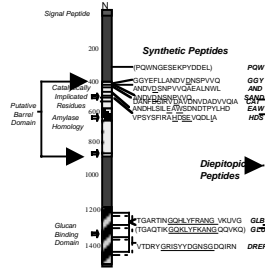
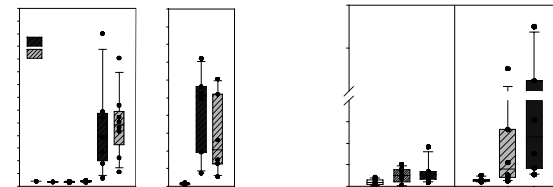
2a. Purpose and protocol: Synthetic peptide vaccines whose sequence is drawn from putative functional domains of mutans streptococcal GTF can induce protective immunity after subcutaneous injection in the salivary gland region. One of these peptides, HDS, associated with the α_7 strand in the catalytic barrel region, is particularly immunogenic (shown in bold in the figure).

Human caries vaccine applications are likely to use mucosal routes for salivary antibody induction. Since peptide constructs are poor mucosal immunogens, we used three approaches to enhance the formation of salivary antibody to the HDS epitope after intranasal administration.

Previous studies with intact GTF had showed that incorporation of GTF in PLGA microparticles induced long-lasting salivary antibody (Smith et al., Oral Microimmunol, 1999). Other studies indicated that combination of catalytic epitopes with the peptide, GLU, increased salivary antibody induction after sc immunization (Taubman et al., J Dent Res, 1998). The GLU peptide had been shown to contain strong B and T cell epitopes.



2b. Mucosal induction of salivary IgA and serum IgG antibody to HDS. The following figures illustrate the ability of HDS in PLGA microparticles, dieptopic HDS-GLU constructs, or co-administration of HDS peptide constructs with the R192G LT mucosal adjuvant to induce salivary IgA antibody after 3 weekly intranasal administration. Only co-administration of HDS with R192G resulted in a detectable salivary IgA antibody response. Antibody was induced in all rats, levels were highest approximately one week after the last IN dose, and remained significantly higher than sham for at least 6 weeks. Primary or secondary (not shown) salivary IgA antibody responses resulting from co-administration of CT or R192G were comparable. Shown also are primary serum IgG responses to the HDS peptide construct were induced in all rats after co-administration with R192G. Significant increases in serum responses were achieved by boosting. Similar kinetics of serum IgG antibody reaction with the intact GTF were also observed. Serum from many HDS, R192G-IN immunized rats also inhibited the enzymatic activity of GTF to synthesize water-insoluble glucan (not shown).



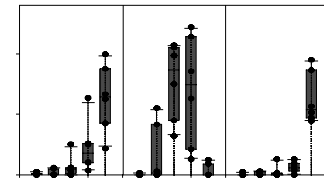
Cholera toxin (CT) and the very similar *E. coli* heat labile enterotoxin (LT-shown in picture) induce vigorous mucosal immune responses to co-administered antigens. Thus, a third approach to intranasal induction of salivary IgA antibody to HDS (and GTF) was to co-administer with the HDS peptide construct a mutated version of LT (R192G). The toxic properties of arg with gly at position 192 (arrow) by site-directed mutagenesis (Dickenson and Clements, Infect. Immunity, 1995). This mutant adjuvant has been repeatedly shown to retain its adjuvant properties.

3. Induction of Salivary IgA AB by Rectal Immunization.

3a. Purpose and protocol: Intranasal immunization with many antigens induces levels of salivary IgA antibody which are higher than oral antigen administration. Significant protection from experimental dental caries can be achieved using the IN route. Furthermore, this route has been used for attenuated influenza vaccine applications in humans. Thus, IN administration is the apparent mucosal route of choice for human dental caries vaccine. However, other mucosal routes of caries vaccine administration may be necessary since some members of the vaccine target population (12-24 month old children) may have respiratory issues precluding effective IN administration. In addition, side effects of some mucosal adjuvants may also preclude IN applications.

A significant feature of mucosal immunity is the appearance of antibody at sites remote from the site of induction of the immune response. The rectal mucosa contains inductive lymphoid tissue and many studies have shown that immunization by this route can result in nasal or salivary IgA antibody responses. We explored the rectal route for induction of salivary immune responses to GTF (four weekly doses) with and without mucosal adjuvants.

Group	Antigen	Adjuvant	Route
sham	unloaded PLGA	CT	rectal
rectal	PLGA/GTF	None	rectal
rectal CT	PLGA/GTF	CT	rectal
rectal d.LT	PLGA/GTF	R192G LT	rectal
nasal	PLGA/GTF	None	nasal



3b. Salivary, colo-rectal, and nasal IgA antibody responses. This figure illustrates the effect of rectal or nasal immunization with GTF on IgA antibody levels in nasal secretions, fecal extracts and saliva 28 days after immunization was begun. Colo-rectal immunization induced significant levels of IgA antibody in fecal extracts to GTF in 11/12 animals to whom CT or R192G LT was also given. The colo-rectal route also induced significant salivary IgA antibody in 7/12 rats, although levels were in most cases lower than salivary responses after IN instillation of GTF without adjuvant. A similar proportion of IN immunized rats developed low but detectable IgA antibody in fecal extracts. These data support the concept of compartmentalization within the common mucosal immune system, but also suggest that rectal immunization, using mucosal adjuvants, can induce potentially protective levels of salivary IgA antibody.

Summary and Conclusions

Short-term dietary administration of IgY antibody to *S. mutans* GBP-B can significantly reduce *S. mutans* accumulation in oral biofilms and subsequent dental caries.

Intranasal co-administration of Adetoox fed@ mucosal adjuvants (R192G LT) and GTF peptide constructs can induce significant salivary IgA (and serum IgG1 and IgG2a) immune responses to peptide epitopes and GTF.

Colo-rectal administration of GTF with R192G LT can result in the appearance of potentially protective levels of salivary IgA antibody to GTF.