**Background**

Dental caries represents a health expenditure of several billion dollars per year in the United States, even though water fluoridation has reduced caries by one-half. Half the children (5-17 yo) in the United States experience caries, and about half are caries free -- testimony to the tremendous impact that preventive dental care has provided. Thus, although great progress has been made in preventive dentistry, dental caries is still a major health problem, afflicting about 50% of our children.

The National anticaries strategy has been four-pronged with goals to (1) to combat the microbial agent; (2) to increase tooth resistance; (3) to modify diet; and (4) to deliver anticaries measures to the public.

The first goal, combatting the microbial agent, is based on evidence that specific microorganisms are an important part of the pathology of dental caries. Therefore, we can target some, but not all, bacteria for immune regulation. In 1924, Clarke (1924. *Brit. J. Exp. Pathol.* 5: 141-147) isolated an organism that he felt to be from the earliest carious lesions in humans, *Streptococcus mutans*. Although bacteria were widely accepted as the cause of dental caries, it wasn’t until 1945-46 that McClure and Hewitt (1946. *J. Dent. Res.* 25: 441-443) showed that bacteria were indeed potential etiologic agents of dental caries. Using penicillin, rats, and *Lactobacillus acidophilus*, these workers demonstrated a positive correlation between microbial colonization and dental caries. Subsequently, Orland *et al.* (1954. *J. Dent. Res.* 33: 147-174) used *gnotobiotic* rats to prove that ingestion of a cariogenic diet alone was not enough to produce dental caries; and in order for caries to occur, the animals had to be infected by certain bacteria.

By the mid-1960s, the stage was set to combat cariogenic microbes specifically, when a consensus regarding the target organism as well as the target host defense system was reached. After languishing for a decade in the shadows of *Lactobacilli*, *Streptococcus mutans* re-emerged as the prime candidate for antimicrobial attack as a result of various epidemiological and etiological studies. As discussed in the preceding chapter, Thomas B. Tomasi and colleagues (1965. *J. Exp. Med.* 121: 10-24) provided an equally important demonstration that the IgA system was the primary specific immunological element in saliva. These two advances set the stage for dental vaccination approaches targeting a specific pathogen (*S. mutans*) and manipulating a specific humoral immune system (sIgA).

But unfortunately, the momentum behind caries vaccination has dissipated. It’s hard to determine why, except that advances in microbiology needed to be made before we could go forward with advanced vaccine strategies. There is reason for optimism, and this has recently been summarized:

“...preclinical application of modern methods of mucosal vaccine design and delivery has routinely resulted in protection from dental caries caused by *S. mutans* infection, using antigens involved in the sucrose-independent or sucrose-dependent mechanisms of infection by these cariogenic streptococci. Passive administration of antibody to functional epitopes of *S. mutans* virulence
antigens has also provided a degree of protection in preclinical studies and small-scale human investigations.


In this section on caries immunology, we will examine evidence that the host mucosal immune system -- and sIgA in particular -- can be protective against caries and discuss how HYPERimmunization (vaccination) may confer specific immunity against dental caries.

**Role of Innate Factors in Caries**

Dental caries is a multifactorial disease, as such, protection against dental caries involves a number of factors. The teeth are protected by the mucosal immune system discussed in Chapter 12, but for obvious reasons, lack some of the cellular components of that system. Thus, fluid phase factors secreted by salivary glands are thought to be the most important of the mucosal immune components. Persuasively, individuals with salivary hypofunction (especially, xerostomia) often exhibit rampant decay. Although this is usually attributed to water (and quite appropriately so), “nonspecific” innate factors also play a number of functions in protecting the exposed, calcified tissues of the tooth from dental caries (Mandel, 1979): this includes the buffering of bacterial acids, clearance of organic waste (such as bacterial acids and ingested substrates), reduction of surface free energy (resulting in a decrease in both physical and chemical reactivity), clearance of bacteria, secretion of nonspecific immune factors such as acidic proline-rich proteins, lactoferrin, lysozyme, mucins, histatins, and salivary peroxidase. Of these factors, we’ve mentioned all in the previous chapter. Herein, let us reconsider the proline-rich proteins, as an illustrative example of pleomorphism and dental caries.

**Human Salivary Proline-Rich Peptides**

The human salivary acidic proline-rich proteins (PRP) are 150-170 amino acid proteins comprising a major fraction of the protein in saliva. Importantly, these proteins maintain saliva in a supersaturated state with respect to calcium phosphate and constitute a significant fraction of the acquired enamel pellicle. These two concepts suggests that the PRP play an important role in mineralization processes near the surface of the tooth and may also modulate microbial adherence prior to plaque formation.

The PRP are encoded by two genes, *PRH1* and *PRH2*, which have been localized to the short arm of chromosome 12 (Mamula et al., 1985. *Cyto.genet. Cell Genet.* **39**: 279-284). *PRH1* encodes three main PRPs, including “Db, PIF, and Pa.” *PRH2* encodes two PRPs, including “PRP-1 and PRP-2.” Interestingly, some pleomorphism has been documented in the PRPs, and some variants may be associated with greater susceptibility to dental caries, although this is not clear (Hay et al., 1994. *J.Dent. Res.* **73**: 1717-1726). There was no strong association between pleomorphism of acidic proline-rich proteins and dental caries, and the same thing may be said about other nonspecific host factors and dental caries (including lysozyme, mucins, histatins, salivary peroxidase, and lactoferrin).

**Natural Development of sIgA Secretory Immunity**

**Fetal development.** As described in the previous chapter, the sIgA system may be divided into two functional sites, the inducer site where B-cells are exposed to antigen and develop IgA commitment, and the effector site, where B-cells differentiate into plasma cells and where epithelium is involved in the transcytosis of IgA into the secretion. During fetal development, inducer sites develop prior to effector sites. Peyers patches develop by week 11 of gestation.

Salivary gland epithelial cells possessing the polymeric Ig receptor are not
observed in the fetus until week 19. At this time, the B-cells in the salivary glands primarily form IgM, indicative of a naive condition. No sIgA is present in saliva at birth. Shortly after birth, both IgA and IgM immunoglobulins appear to be secreted in saliva.

**Predentate infants (16-28 weeks).** Secretory IgA antibodies to oral streptococci have detected in the saliva of children as young as 6 weeks of age. These antibodies are primarily directed against the “first wave” of streptococcal invaders, ie., *S. mitis* and *S. salivarius* (Smith and Taubman, 1992. *Crit. Rev. Oral Biol. Med.* 3: 109-133). These organisms initially colonize mucosal surfaces. No antibodies to *S. mutans* are detected.

**Dentate children.** As teeth erupt into the oral cavity, the microbiota undergoes a second wave of change. Tooth colonizers such as *S. sanguis* and *S. mutans* begin to establish. Antibodies against *S. mutans* are usually observed in 1 year old children. Antibody specificities are against the serotype-specific carbohydrate, protein I/II, glucosyltransferase, glucans, and teichoic acids, antigens of *S. mutans* which may be of future therapeutic significance (see below). Within 10 years, the child exhibits levels of IgA which are comparable to those of an adult. For the record, adult parotid saliva normally contains 30-160 µg/ml of IgA immunoglobulin.

**The Relationship Between Caries and sIgA**

In early studies, no consistent correlation between salivary IgA levels and resistance to caries was observed. Some hints were provided that suggested the potential for a protective effect of sIgA. For example, low titers of parotid sIgA appeared to correspond with higher rates of dental caries (Ørstavik and Brandtzaeg. 1975. *Arch. Oral Biol.* 20: 701-704). However, since the levels of IgA antibody rather than IgA immunoglobulin may be important, these studies were not definitive.

**IgA deficiency.** IgA deficiency is a relatively common disease afflicting 1:1000 individuals which has been associated with dental caries. Unfortunately, subjects with this condition suffer from chronic rhinitis and sinusitis (both infectious and allergic). This tends to increase habitual mouth breathing, use of sucrose-containing medicinal syrups, poor oral hygiene during acute infection, and bottle-feeding to help them sleep. Against this background, it is difficult to control these studies. With this caveat, it was found that subjects with IgA deficiency fell into two groups in terms of oral antibody: ie., those with compensatory IgM antibodies against *S. mutans* in saliva and those without. Only in the group without compensatory IgM was the caries activity significantly higher than age-sex matched controls (McGhee and Michalek.1981 *Ann. Rev. Microbiol.* 35: 595-638).

**Panhypo-or agammaglobulinemia.** These subjects are in worse shape than IgA deficiency. It is even more difficult to control these studies. However, cases of increased caries activity have been reported (which must be viewed as impressive given the antibiotic regimen that these individuals are often provided).

**Secretory IgA antibody against S. mutans.** Specific IgA antibodies against *S. mutans* have been measured and found to be significant in parotid saliva against all of the major serogroups of *S. mutans*. The protective effect of these antibodies has not been completely demonstrated. Recently, it has been shown that human parotid sIgA antibodies against surface antigen I/II (see below) of *S. mutans* could block *S. mutans* adhesion to saliva-coated hydroxyapatite (Hajishengallis et al., 1992. *Infect. Immun.* 60: 5057-5064), suggesting that there is a mechansim of protection available to the host against certain cariogenic bacteria. However, as yet, there has been no strong correlation between such antibodies in saliva and resistance to dental caries.

**Serum antibodies.** There are conflicting reports of the correlation between
serum antibody and caries resistance. Some would suggest that conflict results from (1) not testing the correct specificity, (2) not testing at the right time (i.e., after fillings or long after challenge has subsided), or more germane (3) that serum antibody is a non-protective response. Serum antibodies, intragingival antibodies, complement, and granulocytes are constantly extravasating from the periodontal crevice and into the oral environment. These components may confer modest protection to the tooth in the cervical area, but they are not likely to be significance in coronal portions of the teeth.

Specific Immunity
Against Dental Caries

Naturally-induced antibodies in children. As mentioned above, it is clear that infants and young children rapidly develop sIgA antibodies against many oral antigens, presumably by the enterosalivary pathway (Smith and Taubman. 1992. Crit. Rev. Oral Biol. Med. 3: 109-133). However, an association between these sIgA antibodies and resistance to dental infection by these pathogens has yet to be convincingly demonstrated. Some have observed neither salivary IgA nor crevicular IgG corresponds with colonization by cariogenic bacteria (Camling. 1991. Studies of naturally-occurring antibodies to mutans streptococci in humans. Dissertation. Dept. of Cariology. University of Göteborg.). The crevicular IgG antibody is produced locally and appears to reflect caries experience rather than protection. These results do not mean that naturally-induced antibodies are unable to interrupt the caries process. The student should maintain this perspective, although bacterial colonization was not impaired, the issue of bacterial metabolism and current caries activity was not addressed. Caries has been correlated with elevated sIgA antibodies and elevated serum IgM antibodies to S. mutans. This probably reflects the elevation of antibody which occurs during and after infections. As such, it is not surprising that it is difficult to make a case for a protective role for antibodies based upon cross-sectional data.

Caries vaccination. Naturally-induced immunity is not the same as artificially-induced “hyperimmunization,” as observed after vaccine administration. Hyperimmunization results in the elevation of antibody to therapeutic or preventive levels against a specific microorganism. Generally, the aim of a vaccine is to reduce the numbers of an offending pathogen or to interfere with its metabolic activity and pathogenic components. In order to use hyperimmunization, several important things must be considered: (1) What will be the microbial target (i.e., what is the offending pathogen?); (2) which component of the immune system should be targeted; and (3) prior to designing a vaccine, is there any evidence that hyperimmunization will work?

Offending Pathogens,
the Lactic Acid Bacteria

Criteria for cariogenicity. Focus has been placed upon the lactic acid bacteria as specific etiologic agents initiating dental caries: especially, the “mutans-streptococci,” Streptococcus mutans and S. sobrinus). Cariogenicity cannot be traced to any one property of these streptococci, but rather a combination of biological and biochemical properties. To be cariogenic, an organism must exhibit tropism for teeth and must be acidogenic and aciduric (Newbrun, E. 1983. Cariology, 2nd Ed. The William & Wilkins Company, Baltimore. pp50-85). Additionally, the organism should utilize refined sugar (sucrose, a disaccharide of glucose and fructose) as part of the disease process, in view of the direct (albeit, not necessarily linear) correlation between dietary sucrose consumption and caries experience (Newbrun, E. 1983. Cariology, 2nd Ed. pp86-121).

The Lactic Acid Bacteria as Prime Suspects. The lactic acid bacteria comprise a heterogenous family of microorganisms which possess the necessary biochemical characteristics to initiate and perpetuate the caries
Lactic acid bacteria are Gram-positive cocci and rods which include four genera: *Lactobacillus*, *Streptococcus*, *Leuconostoc*, and *Pediococcus* (Table 1). All of these organisms exhibit metabolic properties which may be classified as **indifferent facultative** (Loesche, 1975). In the process of generating energy, indifferent facultative organisms ferment hexoses and always utilize organic acids as terminal electron acceptors regardless of the presence or absence of oxygen (no oxidative phosphorylation). This is in distinction to true facultative organisms, which utilize oxygen when it is available, forming water and carbon dioxide, rather than acid. The indifferent facultative organism always produces acid (they are acidogenic). The predominant acid produced by most lactic acid bacteria is **lactic acid**, which exhibits a lower pKa and less volatility than most organic acids, and is therefore the most destructive to enamel. Lactic acid may also form chelates with calcium, which would facilitate the dimineralization of enamel.

The one other property peculiar to the lactic acid bacteria is their extracellular utilization of sucrose. Species representative of all four genera of the lactic acid bacteria form extracellular glucose polymers (glucans) from sucrose via a glucosyltransferase enzyme system that will be discussed in detail later. In general, extracellular carbohydrate polymers enable microorganisms to control their external environment. Glucose is a precious commodity. Therefore, expenditure of glucose to form glucans must be important to the lactic acid bacteria. Some of the lactic acid bacteria also form polyfructans from sucrose which may also participate in the caries process.

Of the lactic acid bacteria, two genera have been associated with caries (because they colonize teeth). These are *Lactobacillus* and *Streptococcus*. The former has been implicated in dentinal caries, whereas the latter has been associated with initiating the caries process in enamel.

Let us focus our discussion on the streptococci. There are many species or groups of streptococci inhabiting the mouth and the tooth surface, but the mutans-streptococci have been most closely associated with caries of dental smooth surfaces, pits, and fissures. There are six serotypes of *S. mutans* which have been described in man (table 2). In man, the most prevalent serotype of *S. mutans* associated with smooth surface dental caries is **serotype c**. In smooth surface caries, *S. mutans* **serotype c** is the predominant group associated with enamel caries. It is isolated in about 80-87% of cases in the United States, most of Europe, and Japan (Shklair and Keene, 1976. *In: H.M. Stiles, W.J. Loesche, and T.C. O'Brien (eds.) Microbial Aspects of Dental Caries*. Information Retrieval, Inc., Washington, D.C., pp 201-210; Perch et al, 1974. *Acta Pathol. Microbiol. Scand.* [B]. 82: 357-370; Hamada et al., 1976. *Jpn. J. Microbiol.* 20: 33-44; Bright et al., 1977. *J. Dent. Res.* 56: 1421). In Swedish children, 36% of all tooth surfaces harbored serotype c, and 54% **serotype d/g** (Brathall and Köhler, 1976. *J. Dent. Res.* 55: C15-C21).

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**Lactic Acid Bacteria are not bad guys!** These organisms are useful in the dairy industry (about $10^{23}$ lactobacilli per year are used globally for dairy products - Moineau et al., 2002. *ASM News* 68: 388-393), making pickles, as well as protecting us from terrible pathogens. The glucans from *Leuconostoc mesenteroides* have been used for extending blood (dextran) and for gel filtration chromatography (Sephadex).

Even in the mouth, they are normally beneficial. *Streptococcus sanguis* exerts antimicrobial effects against periodontal pathogens and *Candida albicans*.
Designing an Anticaries Vaccine

Targeted immune systems. Two immune systems were targeted for hyperimmunization: the secretory IgA system and the crevicular (serum and gingival) IgG-IgM-IgA system. "Cellular immune" mechanisms were not targeted for several reasons: first, cells would have difficulty functioning in the mouth and second, immunity against bacteria is usually not handled by cellular immune mechanisms unless they are chronic and persistent (usually meaning that the host is having a hard time handling the infection). Most bacterial infections are handled by secretory immunity (including secretory IgA) or the antibody (IgG)-complement-neutrophil axis. The neutrophil is not always necessary for the latter system to be effective.

Evidence that an anti-caries vaccine would be effective. A number of studies performed in the 1970s indicated that it is possible to protect laboratory animals against dental caries by using hyperimmunization. The results of one of these studies is shown in table 3. As you can see, hyperimmunization of rats (fed a cariogenic diet) lead to a high level of protection against smooth surface caries (buccal and proximal) but less than impressive protection against pit and fissure caries (sulcal). This data set is of interest because it not only shows the protective effects of hyperimmunization, but also the differential protective effects based upon location. That is, even if we could protect teeth by vaccination, such vaccination would not protect the pit and fissure locations. As future dentists, then, you realize that your patients would require additional protection against pit and fissure lesions (such as a sealant).

Why we cannot immunize parenterally with whole cells of S. mutans. Obviously, immunization against dental caries starts with the organism most tightly associated with caries, S. mutans. Unfortunately, S. mutans possesses antigens which are cross-reactive with heart muscle, especially, the cardiolipin of the sarcolemma sheaths. Although patient death is certainly one form of caries control, this means that whole cells of S. mutans are not likely to be viewed as acceptable parenteral antigens and they should be used with caution per os (orally administered). In monkeys receiving parenteral S. mutans, no heart muscle damage was noted. This may be just luck, since our species may respond differently to a cardiolipin challenge due to subtle differences in our immune response genes, especially MHC class II molecules and CD1. Therefore, it is of crucial importance to use an alternative means of vaccination. Several alternatives include (1) purification of the candidate antigens and use of a subunit vaccine or (2) using recombinant DNA
methods to place virulence factors from cariogenic organisms into a noncariogenic, non-crossreactive bacterium.

Candidate antigens have been selected because they are believed to play some role in the pathogenic activities of *S. mutans* and *S. sobrinus* (Figure 1). Extracellular protein targets include glucosyltransferases (GTF), dextranases, adhesins (such as Spa A or SA I/II), and glucan-binding protein. Other non-protein candidate antigens have also been proposed, including extracellular glucans and the serotype-defining antigen (Table 4). Let’s consider these antigen targets.

**Polyclonal B-cell activation.** Another reason why whole cells should not be used as an immunogen may be because the whole cells have mechanisms of immune evasion based upon polyclonal B-cell activation (not that I believe this, but you may be exposed to such information in the future). In polyclonal B-cell activation, many B-cells are stimulated regardless of antigen specificity. This may be a method to block specific responses against the pathogen, since it would lead to T-cell suppression. Thus, one of the virulence factors of *S. sobrinus* may be a polyclonal B-cell activator, which has been identified as NAD+ synthetase (Veiga-Malta *et al.*, 2004. *J Bacteriol.* 186:419-426).

### TABLE 4. LIST OF CANDIDATE ANTIGENS

<table>
<thead>
<tr>
<th>Class</th>
<th>Sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucosyltransferases</td>
<td>Enzymatic site; Glucan binding site</td>
</tr>
<tr>
<td>Adhesins</td>
<td>SA I/II, B, P1 (<em>S. mutans</em>)</td>
</tr>
<tr>
<td>Dextranase</td>
<td>SpaA (<em>S. sobrinus</em>)</td>
</tr>
<tr>
<td>Glucan-binding proteins</td>
<td>Glucan binding site; Glucan binding site</td>
</tr>
<tr>
<td>Surface polymers</td>
<td>Glucan-binding specific CHO Ag Lipoteichoic acid</td>
</tr>
</tbody>
</table>

**Glucosyltransferases**

At least two types of glucosyltransferases are known to exist in *S. mutans* (GTF-S and GTF-I), which synthesize water-soluble and water-insoluble glucans, respectively. In *S. sobrinus* there are three (GTF-S Pd, GTF-S Pi, and GTF-I). Pd refers to “primer-dependent” and Pi refers to “primer-independent.” A primer is a short glucose-oligosaccharide. Primer dependency simply means that these enzymes require a primer to catalyze glucose polymerization. Various molecular weights have been assigned to these enzymes, but in general, they have a molecular weight of about 160,000 kdal. The GTF enzymes may be evolutionarily related to both the glucan-binding protein and to fructosyltransferase. The GTF enzymes utilize the energy in the hemiacetal-hemiketal bond between the glucosyl and fructosyl sugars in sucrose, to form glucans (polymers of glucose in both α1-3 and α1-6 linkages). GTF enzymes also have lectin activity and feature a glucan binding domain. Antibodies which impede GTF function have been shown to be protective in animals.

**Adhesins**

**Surface Protein Antigen** (known as SA I/II, B, P1 in *S. mutans* and Spa A in *S. sobrinus*). Surface protein antigens are large proteins (185-210 kdal [in *S. mutans*] and 160-180 kdal [in *S. sobrinus*]) which constitute 35% of cell surface protein, and are thereby the predominant cell-surface protein. Interestingly, these are immunologically related to dextranase, an enzyme (below). The main function of these surface proteins appear to be sucrose-independent adherence (that is, the attachment of the bacterium to the tooth in the absence of sucrose). A “fuzzy coat” on the bacterial surface, observed by electron microscopy, is the ultrastructural appearance of these surface antigens. Mutants lacking SA I/II also lack a fuzzy coat and bind poorly to experimental pellicles (Harrington and Russell, 1993. *J. Bacteriol.* 175: 5925-5933). SA I/II also appears to mediate the saliva-induced aggregation of *S. mutans* (Koga *et
al., 1990. *Infect. Immun.* **58**: 289-296). Antibodies against the surface protein antigen are protective in monkeys. Refining these earlier observations is the more recent observation that SA I/II possesses a saliva binding region (SBR). Antibodies against SBR appear to protect against the colonization of *S. mutans* to teeth in mice (Huang *et al.*, 2001. *Infect Immun* **69**:2154-2161).

Dextranases

Dextranases are 160-175 kdal protein enzymes which break down polymers of glucose in α1-6 linkage (dextrans). These dextranases are probably used by oral streptococci to modify the glucan product of GTF, clipping away the α1-6 linked oligomers and thereby increasing the proportion of α1-3 linkages. Additionally, it is remotely possible that this may permit extracellular glucans to serve as energy stores. Dextranases may function in sucrose-independent adherence (via an SpaA-related epitope). Mutants lacking both dextranase and SpaA are avirulent.

Surface Carbohydrates

**Glucans** are tree-like homopolymers of glucose featuring gazillions of branches but only one trunk (Figure 2). The tips of the gazillion branches are called “non-reducing” and the tip of the trunk is called “reducing” (an arcane factoid). There are two physical types: Water-soluble and water-insoluble, and there are two main enzymes involved in their production (GTF-S and GTF-I, described above). Table 5 shows the results of methylation analysis of soluble and insoluble glucans. In methylation analysis, methyl groups are added to any un-reacted hydroxyl; thus, a 2,4,6-trimethyl ether of glucose would prove that those carbons had free hydroxyls. We can infer that sugar was linked at it’s 1 and 3 carbons. Inasmuch as you do not find any 1 glucosyl methyl ethers (well, there is one, the “reducing” terminus, it is heavily outnumbered), we are safe to say that the 1 carbon (the anomeric carbon) is always linked.

The results (Table 5) show that soluble and insoluble glucans derived from bacteria (or their cell-free fluids [CCF]) are quantitatively distinct, but not absolutely, with respect to linkage. Both contain α1-3 and α1-6 linkages in linear array, and both contain α1-3-6 linkage branch points. These results are reinforced in studies using (i) linkage-specific enzymes or (ii) anomeric proton-
NMR spectral shift analysis for nearest neighboring linkages (the NMR doublet of the anomeric proton shifts upward near \( \alpha 1-3 \) linkages).

There are somewhat more \( \alpha 1-6 \) (dextran) type linkages in the water-soluble form and more \( \alpha 1-3 \) (mutan) linkages in the water-insoluble form. The quantitative distinction increases with enzyme purity. For example, note the greater proportion of \( \alpha 1-3 \) (mutan) linkages in purified GTF-I compared to GTF-S from \( \textit{S. mutans} \) OMZ-176; but realize that we’re usually dealing with whole cells. Because we isolate soluble and insoluble glucans from cells, it is most likely that both enzymes participate in the production of both products. Another main difference between water-soluble and water-insoluble glucans appears to be size (water-soluble forms are usually 20,000-50,000 daltons, whereas water-insoluble forms are generally \( 10^6 \) - \( 10^7 \) daltons). Anyhow, I only mention this stuff since immunologists often tend to gloss over some really nice biochemistry when discussing carbohydrates.

**Can a carbohydrate be a good antigen?** Well, yes! Compare a polymer of hexose sugars with a polymer of amino acids. Two different amino acids can be linked in only two ways (ie., A---\( \rightarrow \)B or B---\( \rightarrow \)A). The immune system can distinguish such primary structure. Since we are talking about a homopolymer of glucose, let’s take a look at how homopolymers of glucose can convey information. One sugar will be linked to the other sugar via the hydroxyl of its anomeric carbon (C1) as either an \( \alpha \) or \( \beta \) anomer. That alone would provide two different structures, which the immune system can distinguish. Now, this \( \alpha \) or \( \beta \) anomer can form a linkage with the hydroxyl of the second, third, fourth, and sixth carbon (C2, C3, C4, or C6). Actually, there are instances where two anomeric carbons are linked to one another (ie., the \( \alpha \) or \( \beta \) anomer C1 can form a linkage with another \( \alpha \) or \( \beta \) anemic C1). We won’t even count that. Therefore, two identical sugars can produce at least eight different antigens (just think if we used one galactose and one glucose!).

Glucans function in plaque accumulation, act as molecular sieves and convection barriers, retain water, and although they do not play a role in the initial colonization of teeth by bacteria, they clearly may greatly strengthen the attachment of the producing organism to the tooth surface. In general, glucans enable the producing organism to control its microenvironment. The significance of this is clearly shown by the increasing proportion of mutans-streptococci in subjects with high dietary sucrose intake. Antibodies to dextran appear to prevent the binding of GTF to the bacterial cell surface and have been proposed as a possible means of conferring caries protection.

**Serotype-defining carbohydrate antigens** are complex carbo-hydrate heteropolymers containing galactose and glucose. There are eight “serotypes” of mutans-streptococci. These serotypes have been designated a-h. Only serotypes cef (\( \textit{S. mutans} \)) and dgh (\( \textit{S. sobrinus} \)) are important in man. These recognitive structures are probably used to specifically bind enzymes such as GTF to cell surface, and thus, they have been proposed as a target for a caries vaccine. Antibodies against the serotype-defining carbohydrate antigens are protective and appear to prevent the binding of GTF to cell.

**Lipoteichoic acids** are amphipathic molecules found on the surface of Gram-positive bacteria which are, in some ways, analogous to lipopolysaccarides of Gram-negative bacteria. They consist of a carbohydrate backbone of polyribose ± phosphate or polyglycerol ± phosphate. The carbohydrates are covalently coupled lipid. It has been proposed that these structures may be involved in adhesion by hydrophobic interaction (not likely). The problem with LTA as a candidate antigen is that they feature epitopes which may cross-react with host tissue antigens.

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**Active Anticaries Immunization**

Recently, there has been a resurgent interest in the development of a
caries vaccine which would confer active immunity. As discussed above, whole, attenuated bacteria are not usually considered safe due to heart muscle cross-reactivity. However, this may be a false concern; heart muscle cross-reactivity may not be a problem if the vaccine is administered in an oral manner and stimulates the sIgA system rather than the IgG system. This is because IgA antibodies would tend to be beneficial and suppress inflammatory reactions by immune elimination at the mucosal surface (thereby decrease IgG responses) and if systemic IgA is elicited it could bind to antigen and prevent complement fixation.

**Historical.** The monoinfected gnotobiotic rat system has consistently demonstrated the efficacy of vaccines against caries when those vaccines stimulated sIgA. Less convincing (but generally favorable over a short term) results are obtained when an immunization route favoring responses by (but not limited to) the serum immunoglobulin system when conventional (not maintained gnotobiotically) rats and monkeys are used. Some believe that crevicular fluid IgG is elevated in gingival inflammation (pathotropic potentiation), and this can lead to opsonization and phagocytic clearance of pathogenic bacteria.

**Salient animal terminology.** As future cariologists, you may someday be asked to evaluate an animal study. As discussed briefly above, the results of the animal studies vary with how the animal was reared and maintained. Therefore, you need to know the terminology of animal studies. “Gnotobiosis” is a term derived from Greek, which means “known life;” ie., that the microflora of the animal is known. Gnotobiotic animals may be “germfree,” a condition referred to as “axenic” (ie., “axenic” means “germfree”). Germfree animals are animals originally derived by Caesarian section, and subsequently bred in a germfree environment. Not all gnotobiotic animals are germfree, nor do they need to start life as germfree animals. Gnotobiotic animals may also be “monoinfected,” ie., infected with a single microbe or multiply infected, provided that the microflora is defined. Colonies of animals can also harbor one or several microbes, thus the colony produces gnotobiotic offspring which begin their life infected rather than germfree. Axenic animals will not experience dental caries even when fed on an extremely cariogenic diet. Animals monoinfected with cariogenic bacteria will suffer much dental destruction on the same diet. Vaccines in the monoinfected model are always much more successful than vaccines in animals reared in a conventional manner. Thus, you should be wary of all such monoinfected studies. For this reason, some investigators attempted studies using “specific pathogen-free” (SPF) animals. This is a mealy-mouthed term indicating that the animals are raised under conditions in which a specific pathogen is eliminated. For example, in a caries vaccine study, it would be important to maintain an SPF colony in the absence of *S. mutans*, such that the pathogen can be introduced in a controlled manner. One problem with the SPF model is that investigators never really state with certainty that their SPF animals are indeed missing the the pathogen. Either germfree or SPF animals may be “Caesarian-obtained, barrier-sustained,” or COBS. Actually, any animal can be a COBS animal, so this is a bit of a useless term.

**Routes of vaccination.** Currently, there are two favored potential routes of vaccine administration: peroral (per os, p.o.) and intranasal (nasal) (Michalek et al., 2001. *BioDrugs* 15:501-508). Whole cells of *S. mutans* encapsulated within gelatin were used to immunize human volunteers p.o. The question was whether sIgA antibodies can be elicited by oral ingestion of whole *S. mutans*. *A priori*, this form of vaccination requires the activity of the “enteric pathway,” since gelatin capsules preclude intraoral immunization. It has been reported that peroral immunization by *S. mutans* can elevate sIgA antibodies (Gregory and Filler. 1987. *Infect. Immun.* 55: 2409-2415). Individuals were administered gelatin-capsules (10 consecutive days) containing killed *S. mutans* whole cells which was isolated from the volunteer themselves. SlgA, specific
against GTF and SA I/II were detected in all cases, and in each case, there was a reduction in viable \( S.\) \textit{mutans} isolated from dental plaque, but it was unclear whether this was of any value in terms of caries prevention. SIgA antibodies were detected in the saliva and tears, and in the colostrum/milk of mothers giving birth. The question which was not addressed was whether potentially harmful serum IgG antibody against LTA were elicited by this protocol. Further, other studies suggest that peroral immunization is not always this successful (Linzer \textit{et al.}, 1981. \textit{Infect. Immun.} \textit{31}:345-351; Cole \textit{et al.}, 1984. \textit{Infect. Immun.} \textit{46}: 702-709).

\textbf{Subunit vaccination.} There are several “twists” to the subunit vaccine approach which embellish the traditional methods of using purified bacterial antigens. Some of these are discussed below.

\textbf{Synthetic peptides.} It may also be possible to use only a piece of a large protein, such as GTF, as an immunogen (Smith \textit{et al.}, 1993. \textit{Infect. Immun.} \textit{61}: 2899-2905; Smith \textit{et al.}, 1994. \textit{Infect. Immun.} \textit{62}: 5470-5476). Synthetic peptides derived from a glucan-binding domain of glucosyltransferase, TGAQTIKGQKLYFKANGQQVKG, or a multimeric synthetic peptide derived from an amino-terminal sequence, DANFDSIRVADVNDADLLQ, have been used as potential immunogens. Antiserum against TGAQTIKGQKLYFKANGQQVKG raised in rats was found to inhibit GTF by 30\% (not that impressive), and a monoclonal antibody against DANFDSIRVADVNDADLLQ was 80\% inhibitory. By the way, you are not expected to know the primary sequences used in synthetic peptide vaccines, just that synthetic peptides offer an alternative.

\textbf{Molecular genetics and the enteric pathway.} Molecular genetic approaches now offer one of the most exciting means of delivering a “subunit” vaccine which would be cost effective. The problem with subunit vaccines has been the inability to maintain sufficiently high levels of antigen in the gut to stimulate antibody production in a cost-effective manner. Recently, candidate antigen genes have been introduced into “harmless” enteric bacteria (Figure 3). These bacteria proliferate for some time and exhibit considerable greater staying power in the gut than simple gelatin capsules filled with antigen. This method of immunization is currently under investigation. But think about it, no microbe which can colonize a human should be considered totally “harmless.” Also, some of the plasmid vectors used are marked with genes encoding antibiotic resistance (but this is just a minor problem).

\textbf{Gingival swabs and the “local pathway.”} The gingiva is an area in which local immune responses can be elicited. The swabbing of gingiva with a 3800 kdal low molecular weight component of \( S.\) \textit{mutans} has been found to elicit both increases in IgG in the crevicular fluid and sIgA in the saliva of monkeys (Lehner \textit{et al.}, 1986. \textit{Infect. Immun.} \textit{52}:682-687). From a didactic point of view, it is difficult to ascribe the sIgA response to local (gingival) immunization rather than the enteric pathway, since some antigen must be ingested. From a therapeutic point of view, the method itself may be useful: i.e., the swabbing was administered only ten times over a year period and resulted in a reduction in \( S.\) \textit{mutans} as well as caries.

\textbf{Liposomes.} Liposomes are artificial membrane vesicles which can be prepared to contain both aqueous-phase solutes internally or intramembranous
molecules within their membranes. Liposomes represent a relatively benign mechanism of increasing immune responses to antigens (ie., they are “adjuvants”). One method of increasing antibody responses by gingival immunization has been the sequestration of candidate antigens (GTF, in this case) into liposomes, permitting the liposomes to dessicate, and administering the dehydrated liposomes to humans. This resulted in salivary IgA2 antibodies against GTF, suggesting that dehydrated liposomes may be useful in generating specific salivary immunity against target antigens in the oral cavity (Childers et al., 1994. Oral Microbiol. Immunol. 9: 146-153).

**Coupling.** Another method for enhancing immune responses to antigens is to a “poor” antigen is to couple the “poor” antigen to a “good” antigen. For example, polysaccharide antigens are usually poor antigens: they tend to be T-independent and therefore, sustain primary immune response characteristics (without T-cells, you usually can’t get isotype switching or hypermutation). To circumvent this problem, polysaccharide antigens may be coupled to a protein (proteins are T-dependent, usually). This will result in increased specificity and isotype switching. Intragastric administration of liposomes containing polysaccharides of S. mutans coupled to a protein has been used in the rat model (Wachsmann, D. et al, 1986. Infect. Immun. 52:408-413).

**Antiidiotype vaccine.** One potential method for eliciting antibodies against any target includes the use of an antiidiotypic vaccine. In this approach, antibodies which possess an idiotope that resemble a bacterial epitope (ie., “internal image” antibodies) are injected into a host. If these antibodies are the same allotype as the host, the host will form antibodies against only the internal image. These antibodies against the internal image can then stimulate antiidiotypic antibodies which also can bind to bacteria (Jackson et al., 1990. Infect. Immun. 58: 1932-1936). Interestingly, the study cited also used liposomes as a mechanism of delivery of the antiidiotypic antibodies by gastric intubation. The antiidiotypic vaccine lead to greatly reduced caries in the gnotobiotic rat model.

**Adjuvants.** Many of the peptide antigens described above would be poorly immunogenic were it not for the use of adjuvants. The dentist should be aware that many traditional adjuvants used in animals (such as complete Freund’s adjuvant, a mixture of mycobacterial components and mineral oil) are too toxic for human use. An inexpensive adjuvant approved for use in humans is “alum,” an inorganic salt of aluminum. Liposomes, mentioned above, may offer an attractive adjuvant system.

The most promising adjuvant stimulating mucosal slgA responses appears to be cholera toxin, which is under intense investigation by Michael Russell’s group at the University of Alabama. It appears to stimulate persistently high levels of slgA after a single boost (Hajishengallis et al., 1996. Infect. Immun. 64:665-667). Cholera toxin is a heterodimer featuring a toxic CTA-subunit and a nontoxic CTB-subunit. Adjuvanticity is associated with the nontoxic CTB-subunit, and a clever approach has been to replace the CTA-subunit with antigens -- such as SA l/II -- derived from S. mutans (Wu and Russell, 1993. Infect. Immun. 61:314-322; Katz et al., 1993. Infect. Immun. 61: 1964-1971; Toida et al., 1997. Infect. Immun. 65: 909-915). And indeed, as you may have predicted, they have even constructed an enteric bacterial clone which expresses SAI/II-CTA2/CTB. The enteric bacterium selected for this was an ‘avirulent’ strain of Salmonella typhimurium (Harokopakis et al., 1997. Infect. Immun. 65:1445-1454).

Also of great interest to you budding clinical dentists out there is that the intragastric administration of fluoride, at concentrations attainable from inadvertant ingestion of fluoride gels or prescribed fluoride supplements, has been shown to be a potent adjuvant of mucosal immunity in rats (Butler et al., 1990. Immunol Lett. 26:217-220). Intragastric NaF caused increases in the size
and cellularity of the Peyer's patches and mesenteric lymph nodes as well as the number of plasma cells secreting IgG and IgA antibodies to various antigens concurrently administered in the drinking water. The frequency of CD4+ T cells in these lymphoid tissues was elevated while that of CD8+ T cells was significantly decreased. How fluoride does this has never been elucidated, nevertheless, it raises the possibility that one protective affect of fluoride administration is the stimulation of immunity to concurrently ingested oral bacteria, and argues in favor of administration of fluoride as part of a caries vaccine program.

Potential for a Passive Immunization Approach

When antibodies are passively administered to monoinfected gnotobiotic animals, as expected, a reduction in disease occurs. Monoclonal antibodies against S. mutans can also prevent the colonization of human teeth by S. mutans (Ma et al., 1987. Infect. Immun. 55: 1274-1278). Thus, passive immune approaches may reasonably be expected to be effective. However, "cost effectiveness" is another issue. Dental scientists have developed a number of fairly clever strategies which may see future application.

Maternal immunization. Passive immunization can occur by oral immunization (secretory IgA is stimulated) of pregnant rats. The milk from immunized rat mothers confers protection to the weanlings. It is possible that any mammal can be protected in this fashion.

Xenogeneic immunization. It has been shown that cows can be immunized against cariogenic bacteria and that antibodies against those bacteria appear in the cow's milk (Figure 4). The cow's milk (or whey) can then confer protection immunity in a passive manner. This type of immunization is shown in the diagram. The antibodies were of the IgG1 subclass, indicative of the parenteral immunization used. In cows milk and colostrum, IgG1 is the major secreted immunoglobulin isotype. Both S. mutans and caries scores were reduced (Michalek et al., 1987. Infect. Immun. 55:2341-2347) in gnotobiotic rats. Of course, gnotobiotic rats are easy to protect compared to conventional animals and humans; however, whey from immunized cows, used as a mouthrinse, appeared to decrease S. mutans in volunteers. Recently, a Finnish group has initiated studies on the potential therapeutic application of bovine whey IgG1 and report that such IgG1 interferes with the intake of carbohydrate, formation of glucans, and adherence of S. mutans (Loimaranta et al., 1997. Vaccine 15: 1261-1268) and a Japanese group has found that mouth-rinse with Holstein cow milk (funny, huh?) immunized with a fusion protein of S. mutans (a piece of a “cell surface protein antigen [probably SAI/II]” fused with the glucan-binding domain of GTF-1) has been effective in preventing the recolonization of eight human volunteers by S. mutans (Shimazaki et al., 2001. Clin Diagn Lab Immunol 8:1136-1139).

One other source of edible xenogeneic antibodies is also under investigation -- chicken eggs. A Japanese group working with Susan Michalek has begun to explore the potential therapeutic capacity of chicken egg IgY in a mouthrinse (Hatta et al., 1997. Caries Res. 31: 268-274). Rocky Balboa has been experimenting eating dozens of raw chicken eggs. Evidence would suggest that anti-glucosyltransferase antibodies from eggs can be protective in rats (Kruger et al., 2004. Caries Res. 38:9-14).

Did you ever want to know about cow’s milk? Milk, as you budding dentists may know, consists of the following ingredients (in order of
weight/volume amount): water; lactose (4-O-β-D-galactopyranosyl-D-glucose); triacylglycerols; phosphoglycoproteins (α1, α2, β and κ-casein); β-lactoglobulin; α-lactalbumin; proteose-peptone; immunoglobulins; serum albumin; K+; Na+; Ca++; phosphorus; Cl−; diglycerides; cholesterol; phototidylcholine; phosphotidylethanolamine; sphingomyelin; monoacylglycerols; carboxylic acids; enzymes; hormones; and other lipids, proteins, carbohydrates, and minerals (Partridge. 1997. Dept. Food Sci. & Human Nutr.). Before getting too bored with that list, remember how we dentists are always extolling the virtues of milk calcium -- well, there it is, right after sodium. The caseins (phosphoglycoproteins) are the major ingredients of cheese, and fractionate as the main structural component of curds during the production of cheese. Whey is the fluid part of milk which separates from the casein-enriched curds. Within the whey is the lactose sugar (1/7 as sweet as sucrose), once considered waste but now quite useful as a carrier in pharmaceuticals, soups, and spices. The whey proteins (β-lactoglobulin, α-lactalbumin, proteose-peptone, immunoglobulins, serum albumin and other various minor proteins) are those components of milk which are of interest to us.

**Pasteurization.** Bovine milk is heat-pasteurized in two ways: (i) a standard holder method (63.5 degrees C for 30 min) and (ii) a high-temperature, short-time (HTST) method (71.7 degrees C for 15 s). An experimental method of “pasteurization” avoids heat altogether, and instead puts milk under extremely high pressures, drops the pressure and forces the milk through a tiny orifice at the speed of sound. Milk processed in this manner tastes better and can last for months at room temperature in unopened containers (this is actually better than standard pasteurization protocols). At any rate, heat pasteurization probably will not affect most immunoglobulins (except for IgE), and if it did, there are other experimental pasteurization methods on the horizon.

**Cows are different.** With respect to immunoglobulin levels, cow’s milk is rather different than human milk. The main immunoglobulin in bovine milkwhey, as mentioned above, is IgG1 (0.5 mg/mL), followed by IgA, IgM, and IgG2 (0.08, 0.08, and 0.06 mg/mL, respectively). The precision of these measurements has been questioned a bit, since some IgA and IgM may associate with fat and be underestimated, especially after refrigeration (Honkanen-Buzalsk and Sandholm, 1981. Comp. Immun. Microbiol. Infect. Dis. 4: 329-342). From the perspective of the cow, it would appear that chronic inflammatory immunoglobulins (IgG1, IgM, IgG2) are an important part of a defense axis which also may include secreted macrophages, which are the predominant cell type found in bovine milk (there are about 25 times more macrophages than neutrophils) and which possess Fc receptors for both IgG1 and IgG2 (Lee et al., 1980. J. Dairy Res. 47: 39-50). Anyhow, I just wanted you to sense that other mammals have found other defenses more useful than the IgA system to defend some secretions.

**Why are cows different?** Well, this could be because other animals may need to defend both their infants and their udders (fairly unique structures) against different types of enemies and in different ways. Additionally, in animals in which placental transfer of IgG does not occur -- such as sheep, cows, pigs, and horses -- milk is the only source of maternal antibodies. Unlike humans, such animals have specific mechanisms for the absorption of IgG in colostrum and milk within the intestines (Jensen et al., 2001. J. Nutr. 131: 3259-3265). Finally, the observation that there is nothing sacred about IgA in the secretions of other animals lends credence to the notion that other immunoglobulins may be able to compensate in IgA deficiency in humans.

**Other Potential Xenogeneic Passive Immunization Strategies**

**Monoclonal antibodies.** Monoclonal antibodies (MAb), are antibodies of a single specificity which are produced by cells derived from a single B-cell
clone. In most cases, the Mab are derived from mice (therefore, they are xenogeneic). Fusion of a normal mouse plasma cells and myeloma cells results in the formation of “hybridomas” with the antibody-forming properties of the plasma cell and the proliferative properties of the myeloma cell. When grown in tissue culture, hybridomas are capable of almost unlimited production of MAb. At present, MAb are used diagnostically to assess immunocompetence, to identify infectious diseases, and to monitor the concentrations of hormones and chemotherapeutic agents in the plasma. Some are used as immunosuppressive agents. Their exquisite specificity also make them ideal guidance systems as immunotoxins.

**Chimaeric MAb.** The most important factor which limits the therapeutic potential of MAb is that they are xenogeneic, and clinical testing of these reagents has led to some disappointment. One approach to circumvent this problem has been the combination of antigen-specific portions of MAb with human constant or framework domains. Chimaeric MAb represent the second generation of MAb which partially circumvents this problem by genetic engineering. Chimaeric MAb graft rodent immunoglobulin V regions to human immunoglobulin C regions (Figure 5). V-genes are cloned from hybridoma mRNA using the polymerase chain reaction and linked to preformed expression vectors containing human heavy and light chain C-regions. Because the C region of an immunoglobulin also confers function to an antibody, chimaeric MAb permit the engineering of functional attributes. For example, a chimaeric MAb possessing an IgG1 isotype C region will be most effective in complement activation and antibody-dependent cell-mediated cytotoxicity; whereas a chimaeric antibody of the IgA subclass may exhibit anti-inflammatory effects.

**CDR-grafted MAb.** Although chimaeric MAb seem rather exotic, even they are being replaced by a third generation MAb, the complementarity-defining region (CDR)-grafted MAb. CDR refers to those areas of an antibody which bind to an antigen. The variable region of immunoglobulin light and heavy chains actually contains 3-4 hypervariable regions and intervening framework regions. In general, the hypervariable regions are equivalent to the CDR. A CDR-grafted MAb contains rodent hypervariable sequences, human framework sequences, and human constant regions. There is some loss of affinity in CDR-grafted MAb, but it is usually less than one order of magnitude, and minor tweaking of the framework regions can often minimize loss of affinity. CDR-grafted MAb have already been used in therapy in organ transplant immune suppression (targetting CD3, CD4, or the IL-2 receptor), rheumatoid arthritis (CD4 and CDw52), Crohn’s Disease (CD4), systemic vasculitis (CDw52), leukemia and lymphomas (CDw52 and the IL-2 receptor), septic shock (TNFα), neoplasm (Lewis-Y, p185HER2 [human epidermal growth factor receptor 2], placental alkaline phosphatase, carcinoembryonic antigen), and viral infection (HIV, herpes simplex virus) (Winter and Harris, 1993. **Immunol. Today** 14:243).

**Xenomic mice.** A rather exotic form of *allogeneic* antibody therapy has
been developed against a xenogeneic background. In this case, xenomic mice are genetically engineered which make human immunoglobulins (Lewin. 1997. J. NIH Res. 9: 31-33). I guess in some ways, animal rights advocates would be pleased that xenomic mice do not suffer from tumors, but on the other hand, may be somewhat upset that xenomic mice need to be chronically exposed to antigen (immunized, usually with inflammatory consequences). It just doesn’t pay to be a mouse. And of course there is some hysterical concern regarding the exchange of genetic material from humans to mice. These considerations aside, presently, there are several xenomic mice. One group possesses genes for many human variable germline genes (66 out of 95 for the heavy chain locus and 32 out of 76 for the k light chain locus), and another has fewer variable region genes but all the constant region genes. In the latter case, purely human immunoglobulins can be produced in mice. The advantage of xenomic mice is that, theoretically, one strain of mice may be able to make polyclonal human antibodies against any number of antigenic challenges, thus circumventing the need to constantly form new hybridomas to combat new antigens and providing polyclonal specificity, which may have functional advantages over monoclonal specificity.

Pathogenesis. We have discussed primarily the pathogenesis and host defense of pit/fissure and smooth surface caries. The organisms associated with cervical caries includes the Streptococcus mutans, but is much more significantly associated with Gram-positive filamentous organisms, such as Actinomyces viscosus, A. naeslundii, A. odontolyticus, A. eriksonii, and Rothia dentocariosa (reviewed in Newbrun E. 1983. Cariology, 2nd Ed. William & Wilkins. Baltimore, pp50-85).

Neutropenia. Some evidence suggests that at least partially, cervical caries are controlled by the immune system. As one may suspect, given the gingival localization of these lesions, it is not the slgA system, but instead the complement-IgG-neutrophil axis. The most suggestive evidence that a different arm of the immune system is involved in the protection against cervical caries is the rather sporadic observation that root surface caries may be associated with neutropenia (Mishkin et al., 1976. O. Surg. O. Med. O. Pathol., 42:738-745; Pernu et al., 1996. J. Periodontal 67:454-459). If this is true, then is it justifiable to focus on the slgA system in caries vaccinations? The answer is “yes,” since root surface caries is not a major problem in the USA (especially for children... and really, who cares whether ‘gramps’ gets root surface caries anyway?).

Summary

In this chapter, there are several things which the student should try to learn. First, innate immunity (including mainly secreted factors) probably plays an important role in dictating caries resistance; however, pleomorphisms in no one factor have been associated with dental caries. Second, specific secretory antibodies conferring a “natural immunity” against dental bacteria follows a clear time course in the developing infant, with several waves of antibody production whose specificities match the changes which occur within the mouth, most notably, the eruption of teeth. Third, we identified the bacteria which were associated with dental caries, and found that they belonged to an extended family of “lactic acid bacteria.” S. mutans and S. sobrinus are those bacteria which are most often associated with smooth surface and pit and fissure lesions. These organisms possess two attributes which make them pathogenic in the presence of dietary sucrose -- formation of lactic acid and formation of extracellular glucans.

Fourth, we examined the potential of hyperimmunization against these cariogenic bacteria. In general, we noted that vaccination resulting in elevation of IgA may be effective against smooth surface caries, but probably provide little
effectiveness against pit and fissure lesions. We also noted that root surface
caries may be protected by the IgG-neutrophil mechanism, not the secretory
IgA system.

Fifth, the two broad categories of immunization were “active” and “passive.”
Active immunization protocols may require subunit vaccines, in order to avoid the
problem of heart muscle cross-reactivity. Potential subunits were identified and
included glucosyltransferases, adhesins, dextranases, and extracellular
carbohydrate polymers. Oligomeric components (i.e., peptides) refine the
specificity of the subunit vaccines. Since peptides are poorly immunogenic, one
aspect of the caries vaccine program has been to examine various adjuvant
strategies (liposomes, for example). Two forms of passive immunization which
may prove useful include maternal and xenogeneic immunization. These
xenogeneic antibodies can be administered to the child as food (mother’s or
cow’s milk, or raw chicken eggs...er, not that I would recommend raw chicken
eggs.... I used to eat raw chicken eggs, though... so it is possible!). Other
xenogenic antibodies include rather exotic “chimaeric” or “CDR-grafted
antibodies”.

SELF-HELP QUESTIONS

1. Sucrose is utilized in an extracellular fashion by certain bacteria to form glucans. Glucans:
   a. Contain α1-3 linkages at branch points
   b. are formed by "glucosyltransferases"
   c. Contain α1-3 linkages in linear array
   d. All of the above answers are true
   e. Only b and c are true

2. A caries vaccine must be both safe and effective. Therefore, a potentially acceptable vaccine is:
   a. Streptococcus mutans in attenuated form (a mutant, for example)
   b. Leuconostoc mesenteroides in attenuated form
   c. surface antigen I/II
   d. lipoteichoic acid of Streptococcus mutans
   e. recombinant SpA in whole cells of Streptococcus mutans serotype c.

3. The lactic acid bacteria possess the following characteristic(s):
   a. All are indifferent facultative bacteria
   b. All utilize an organic acid as a terminal electron acceptor
   c. All four genera contain species capable of forming extracellular glucans
   d. All of the above
   e. Only b and c of the above

4. Why can’t we use dead or attenuated Streptococcus mutans in a parenteral vaccine?
   a. Such a vaccine may lead to severe anaphylactic responses
   b. Such a vaccine would contain lipoteichoic acid
   c. Such a vaccine would contain endotoxin (lipopolysaccharide)
   d. Such a vaccine would not have a long enough shelf life
   e. None of the above

5. The predominant antigenic surface protein in Streptococcus sobrinus is:
   a. Spa A
   b. Glucosyltransferase
   c. Dextranase
   d. Serotype antigen
   e. Lipoteichoic acid

6. A woman is actively immunized (shows both elevated IgG and IgA antibodies) against Streptococcus mutans. She has
   a child. What protects the neonate against dental caries?
a. The baby will be actively immune against *S. mutans*
b. The baby will actively secrete IgA against *S. mutans*
c. The baby will be passively protected by maternal sIgA and serum IgG
d. The baby will not have teeth
e. None of the above

7. One strategy for vaccinating an individual against the organisms believed to be responsible for dental caries involves placing a gene for Spa A into a "harmless" enteric microorganism and administering the recombinant microbe *per os*. What will that do?
   a. Lead to sIgA antibodies against Spa A
   b. Lead to IgG antibodies against Spa A
   c. Lead to IgG against human heart muscle
   d. Lead to cytotoxic T-cells which destroy *S. sobrinus*
e. None of the above

8. In the ontogenesis of the sIgA response, the following is true:
   a. Salivary IgA levels are very high at birth
   b. Antibody specific for *S. mutans* are detected in the secretions of the neonate
   c. Peyers Patches can be observed prior to the presence of the plgR
   d. sIgA transcytosis is common at 10 weeks gestation
   e. Antibody specific for *S. mutans* are detected in the serum of the neonate

9. Why does immunization of a cow with a cariogenic organism potentially protect a human child against dental caries?
   a. Because cariogenic bacteria are often borne by animal vectors
   b. Because the cow's milk may contain protective antibodies
   c. Because the cow learns to avoid cariogenic foods
   d. Because the child stops drinking cow's milk
   e. Because the cow becomes lactose intolerant

10. Below is a list of possible routes and forms of active immunization against dental caries. Which would presently be considered acceptable?
    a. To administer a parenteral whole-cell vaccine of *S. mutans* to a child
    b. To administer a *per os* whole-cell vaccine of *S. mutans* to a child’s Mom
    c. To administer a *per os* whole-cell vaccine of *S. mutans* to a child
    d. To administer a parenteral whole-cell vaccine of *S. mutans* to a child’s Mom
    e. None of the above

Answers: 1d, 2c, 3d, 4b, 5a, 6d, 7a, 8c, 9b, 10e