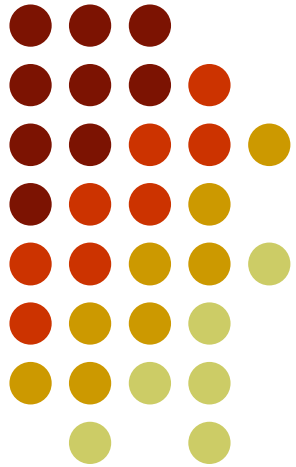
D Y PATIL DENTAL SCHOOL

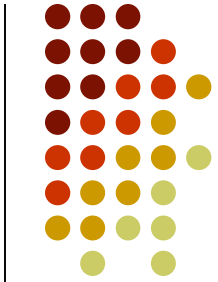
DEPARTMENT OF

PUBLIC HEALTH DENTISTRY



CARIES VACCINE

Contents



- Introduction
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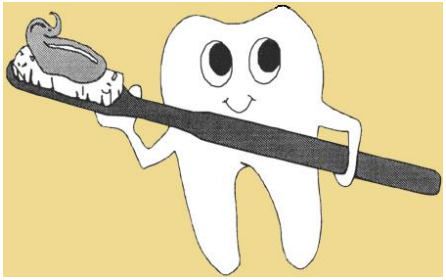
INTRODUCTION

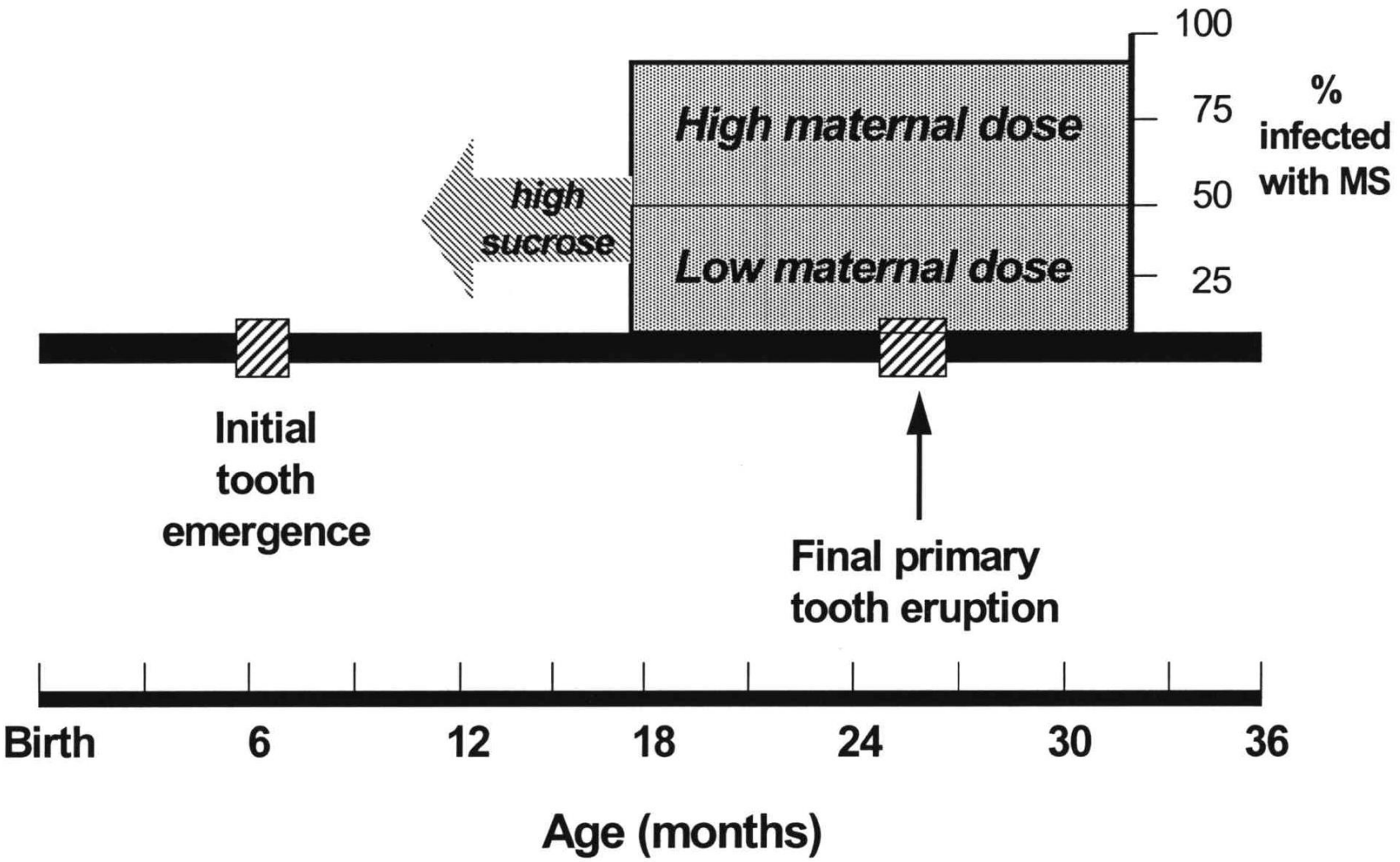
(1,2)

Dental caries

- ✓ One of the most widespread and infectious diseases.
- ✓ Epidemic proportions in developing countries.
- ✓ More effective public health measures are needed.
- ✓ **Window of infectivity** in children.



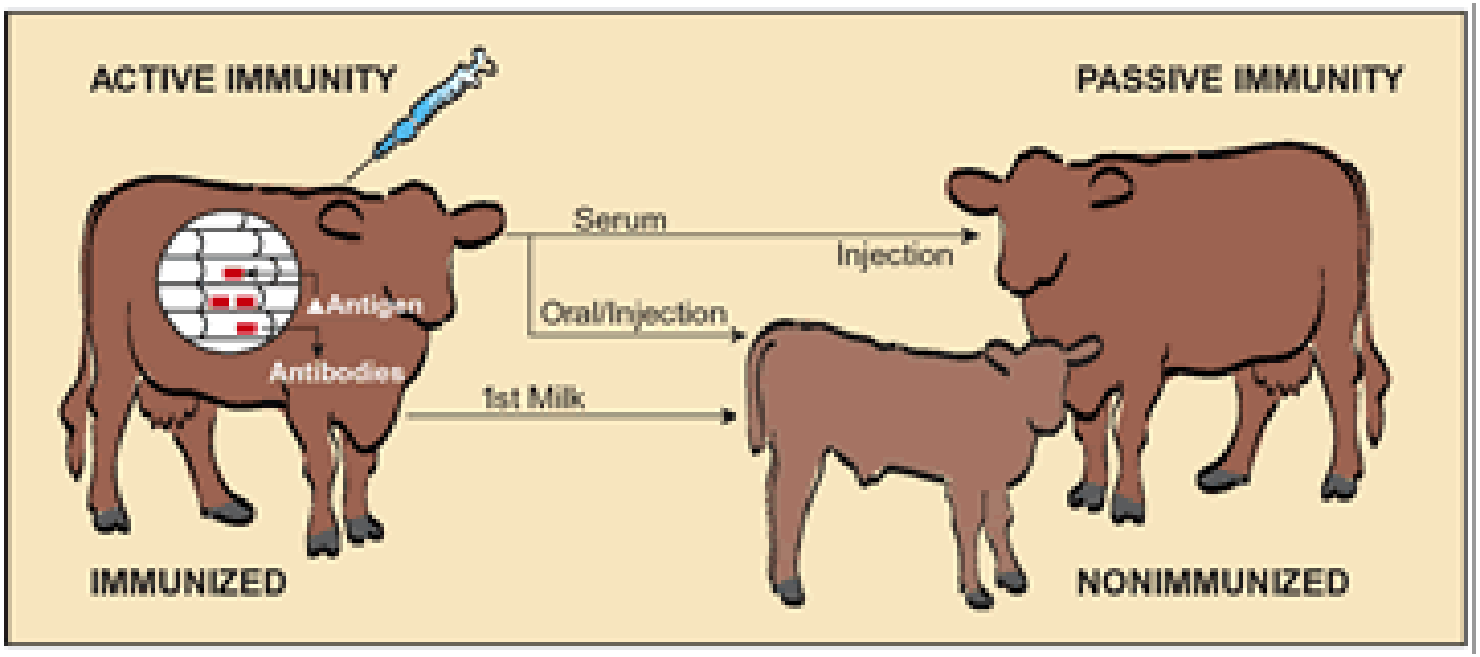
Mutans Streptococcal Colonization of Infants



Immunity –

Active

Passive



Vaccine

It is a immunobiological substance designed to produce specific protection against a given disease.

Types:

1. Live – BCG, Polio, Measles
2. Inactivated or killed vaccines - Cholera Vaccine
3. Toxoids – Diptheria, Tetanus
4. Cellular Fractions – Meningococchal Vaccine
5. Combination

Ontogeny of Mucosal immunity

Birth – Absent

⇩

1st month – dimeric IgA

⇩

6-9 months – Adult like

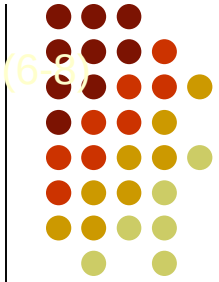
⇩

2-3 Year – MS antigen

- Salivary immune responses show significant individual characteristics. It may depend on:

- Extent of infection
- Age at the time of infection
- Inherent ability of the child to respond

Milestones in caries vaccine development



1. Canby in 1942 →The antigenic behaviour of Lactobacilli
2. Geller and Rovelstad in 1959 → Increased salivary gamma globulin with caries resistance.
3. Wagner (1967) 1st to successfully vaccinate rats against caries S. fecalis was used as immunogen.
4. Bowen (1969) successful vaccination of monkeys through the administration of S.mutans intravenously.

5. Sims in 1972 found no evidence that would modify the progress of dental caries.
6. Bowen 1976 - possibility of developing a vaccine against dental caries.
7. Bowen and Cole showed that lactoferrin & lactoperoxidase possess antibacterial effects in vitro.
8. Local passive immunization found to be protective by Lehner et al in 1985

Properties of caries vaccine

- Safe for relevant population.
- Cheap in comparison to current control measures.
- Stable under field condition
- Ease of delivery preferably single dose
- Possible combination with established vaccine
- Acceptable adjuvant to boost immune response.
- Simple, sensitive test for post vaccination immunity
- Synthetic polypeptide.

MOLECULAR PATHOGENESIS OF DENTAL CARIES

(6-10)

Cariogenic bacteria such as *S. mutans*

⇩

Hard surface/ teeth

⇩

Sustained colonization /accumulation

⇩

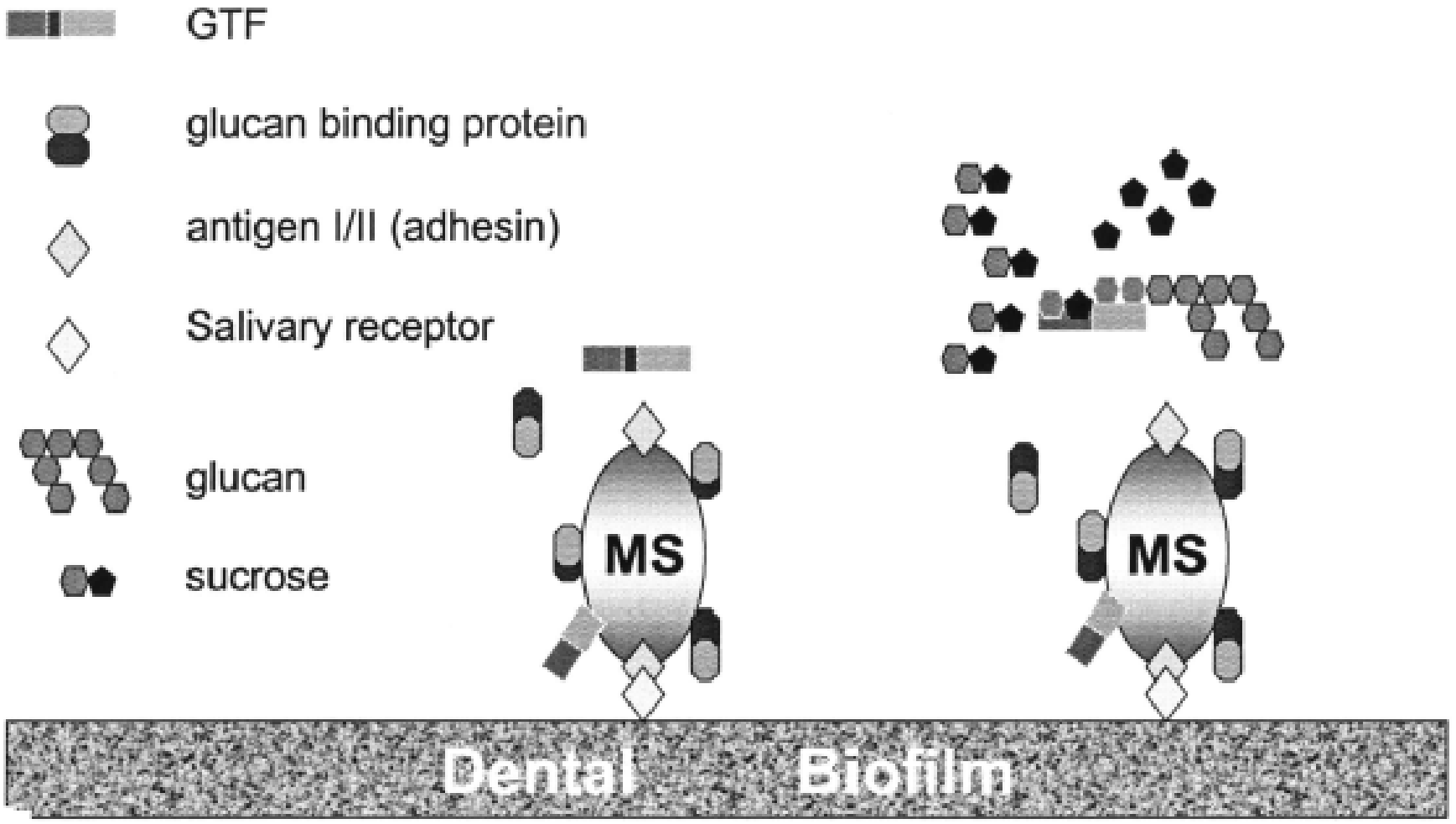
Dental Caries

Plaque formation Demineralization

- **Attachment: -** Adhesins (antigen I and II, Pac)
- **Accumulation : -** Extracellular glucosyltransferases

  - Glucan binding proteins
- **Metabolic Activities:** - Lactic acid

S. mutans in Oral Biofilms:
Colonization

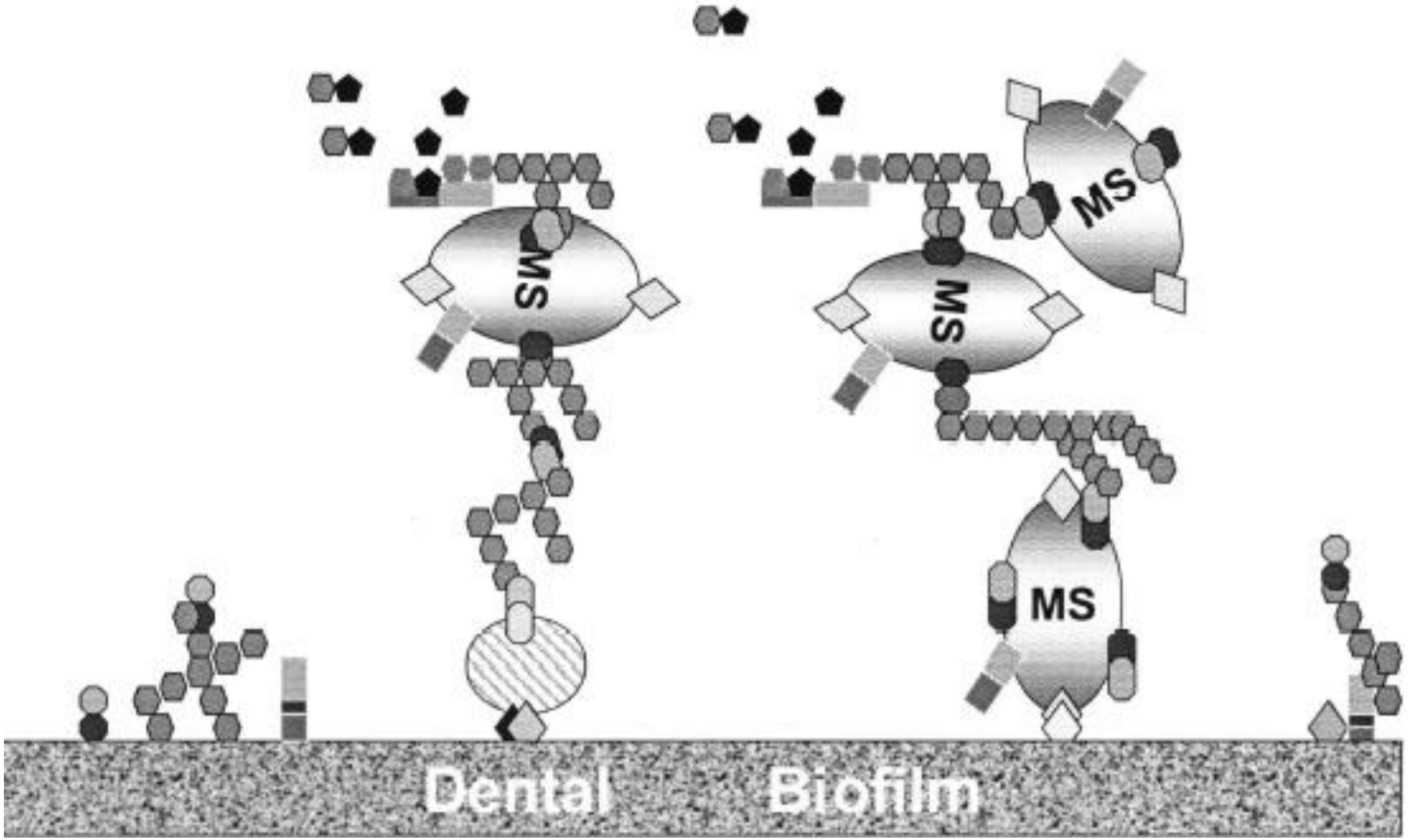


Initial Binding via Salivary Receptor

***in situ* Glucan Synthesis**

S. mutans in Oral Biofilms

Accumulation via Glucan-Related Binding



EFFECTIVE MOLECULAR TARGETS

- Micro-organisms can be cleared from the oral cavity by

✓ Antibody mediated aggregation

✓ Blocking receptors necessary for accumulation

✓ Inactivation of GTF enzymes

✓ Modification of metabolically important functions.

✓ Enhancement of antimicrobial activity of salivary IgA.

a) **Adhesins**:

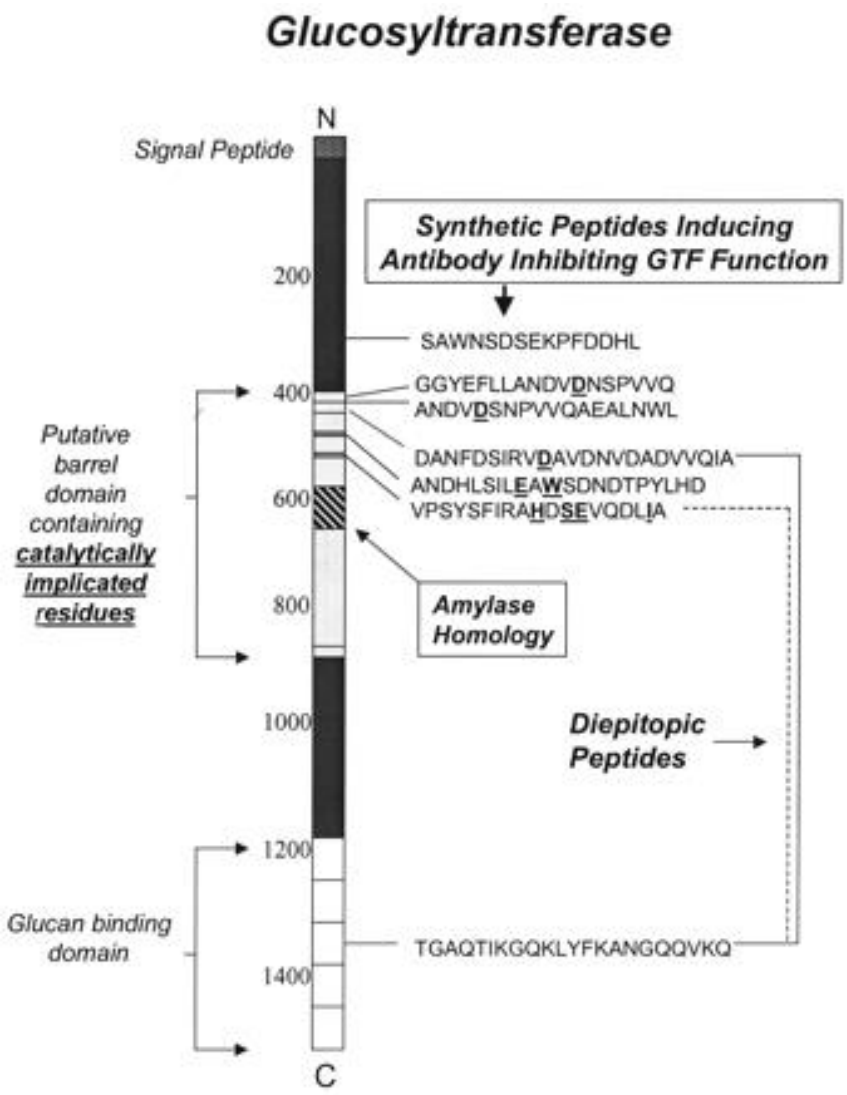
- S.mutans Ag I/II contains an alanine rich region in the N-terminal third and a proline rich region in the center of the molecule.
- Antibody directed towards this adhesin molecule block the adherence of S.mutans to saliva coated hydroxyapatite.

b) **Glucosyl transferases:**

The activity of GTF is mediated through

1) Catalytic site on GTF
2) Glucan Binding region on GTF

- gtfB, the gtfC, and the gtfD genes
- Mutational inactivation techniques.
- Antibody directed to catalytic or glucan binding functions


Glucosyltransferase
N
Signal Peptide
Synthetic Peptides Inducing Antibody Inhibiting GTF Function
200
SAWNSDSEKPFDDHL
400
GGYEFLLANDVDNSPVVQ
ANDVDSNPVVQAEALNWL
DANFDSIRVDAVDNVDADVVQIA
ANDHLSILEAWSDNDTPYLHD
VPSYSFIRAHDSEVQDLIA
Putative barrel domain containing catalytically implicated residues
600
800
Amylase Homology
1000
Diepitopic Peptides
1200
Glucan binding domain
TGAQTIKGQKLYFKANGQQVKQ
1400
C

C) Glucan binding proteins:

- GbpA, GbpB, GbpC

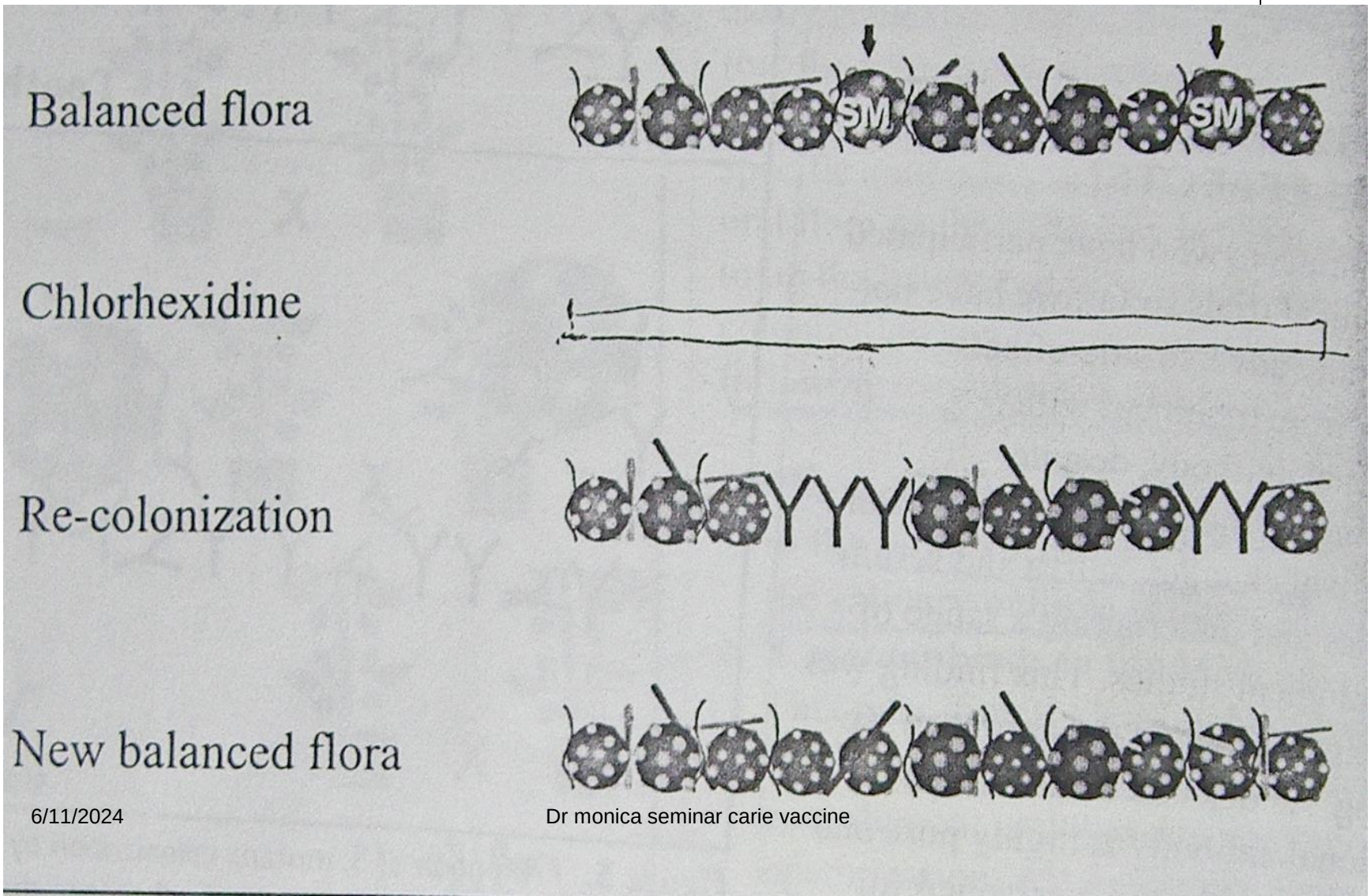
- GbpB has been shown to induce a protective immune response to experimental dental caries.

-Protection can be achieved by subcutaneous injection or by mucosal application by intranasal route.

MODE OF ACTION

- Monoclonal antibodies attach to the tooth surface by adhering to the tooth pellicle.
- S.mutans binds to antibody and may directly lead to killing or cause bacterial aggregation.
- Bacterial flora may be modified due to continued absence of S.mutans.

COMPETITIVE DISADVANTAGE FOR RECOLONIZATION

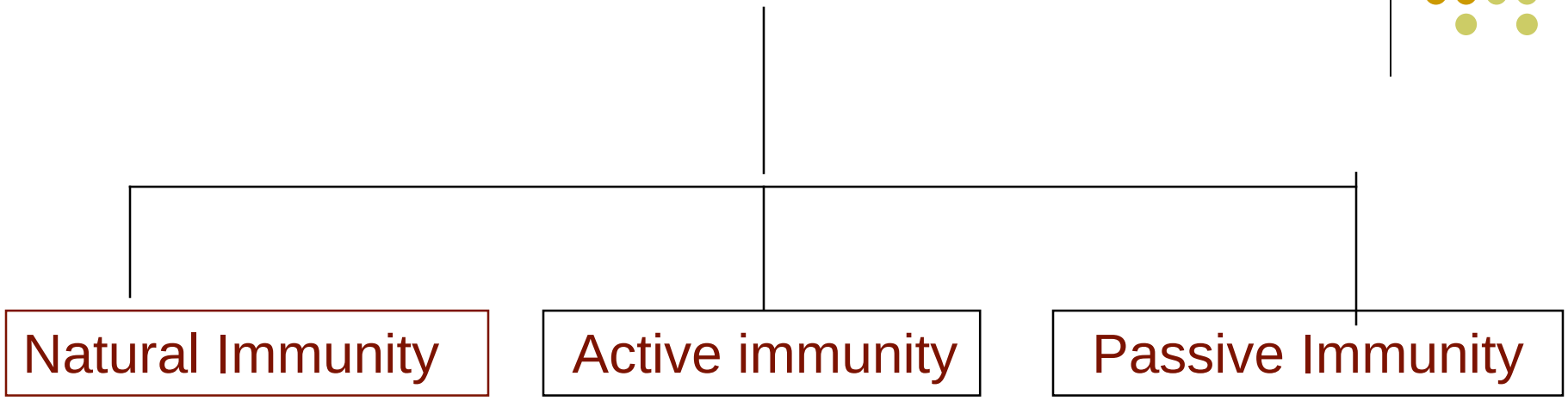


Antibodies to S.mutans and Mechanism of action :

Ig	Step in Caries Pathogenesis	Mode of Action	Antibody Specific
S-IgA	Adherence to salivary pellicle	a)Blocking of adhesive receptor interaction. b)Reduction of hydrophobicity c)Agglutination and clearance	Ag I / II Surface antigen
S-IgA	Binding to early colonizers	Blocking adhesion receptor clearance	AG I / II
S-IgA	Sucrose dependent accumulation	a)Inhibition of glucans production b)Inhibition of substrate binding c)Inhibition of polymer synthesis. d)Locking of adhesion	GTF Catalytic region Glucan binding region GTF and GBP
S-IgA	Acid production and other metabolic activities	Blocking glucose uptake synergism with a)Peroxidase (Inhibit acid production) b)Lactoferrin inhibit iron acquisition	Not Known
IgG	Colonization of crevicular tooth sites incision of dental tubules	Opsonization and phagocytosis Inhibitors of collagen binding	Ag I/II, Other surface Agr Ag-1 / 11

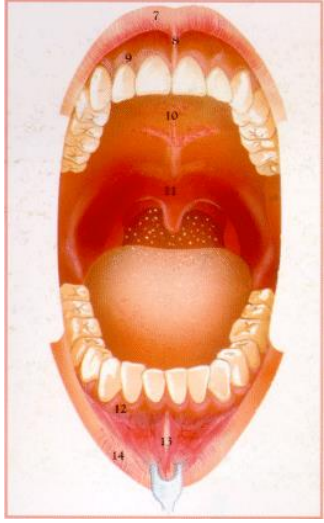
ROUTES TO PROTECTIVE RESPONSE

Caries Immunity



Natural Immunity to Dental Caries

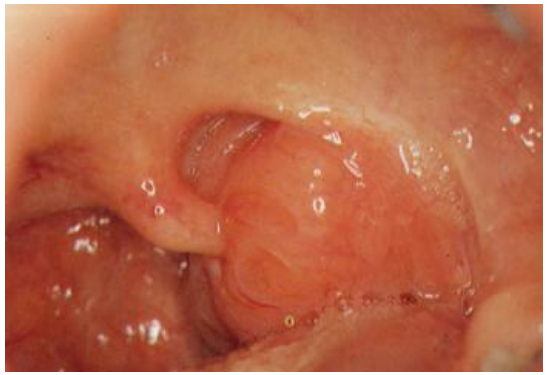
- Maternal Protection
- Ontogeny of Mucosal Immunity
- Natural Caries Immunity



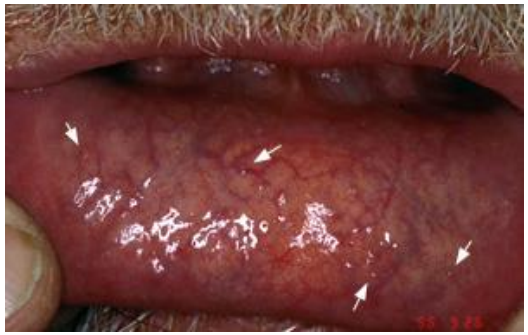
- **Oral route:**
- Oral induction of immunity in the gut associated lymphoid tissue to elicit protective antibody response.
- The route is not ideal mainly due to effects of acidity on stomach

- **Intranasal**
- Intranasal installation targets the nasal associated lymphoid tissue.
- Protective immunity could be achieved with antigen alone or combined with a mucosal adjuvant.

- **Tonsillar**
- Nasopharyngeal tonsils contribute precursor cells to mucosa effector sites.
- IgG response characteristics are dominant in this tssue.
- Repeated tonsillar application can induce the appearance of IgA antibody.



- **Minor salivary gland**
- Lymphatic tissue aggregates are found associated with these ducts.
- When administered topically onto lower lips significantly lower proportion of streptococcus have been found.



- **Rectal**
- This site has the highest concentration of lymphoid follicles.
- This route could be used to induce salivary IgA response.

Active Immunization

- It stimulates a protective immune response in the recipient, resulting in antibodies in the blood that would reach the gingival crevice and protect against S.mutans.
- A possibility of inducing heart cross-reactive antibody following immunization with components of S.mutans.

- Lehner et al, (1975)focussed on cell wall and surface proteins because immunization with their cell walls and with SA I/II resulted in considerable reduction of caries in monkeys.

Animal studies

ANIMAL	ROUTE	ANTIGEN	ADJUVANT	MUCOSAL	SYSTEMIC	EFFICACY
Monkeys	s.c	A I/II,(Pac)	FIA	ND	IgG>IgM >IgA	Decrease in SM, 70% reduction in caries
Monkeys	s.c	Antigens A, B	Al $(OH)_2$	ND	IgG	Decrease in SM, complete inhibition of caries
Monkeys	Intra oral	GTF	FIA	ND	ND	69% reduction of caries
Rats	oral	Ribosomal proteins	liposomes	IgA	IgG	reduction of SS, reduction in caries

Rats(gnotobiotic)	intranasal	Ag I/II	CTB	IgA	IgG>IgA>IgM	99% reduction in plaque, 73% reeduction in enamel proximal caries
Monkeys	topical. gingival	SA	None	IgA	gingival IgG	75% reduction of SM in soomth surface plaque, 92% reduction in caries
Monkeys	topical. gingival	SA	None	IgA	gingival IgG	Reduction of SM colonization
Rabbits	tonsillar	sobrinus cells	None	IgA	IgG	75% reduction of caries

Human studies

Antigen	Route	Predominant antibody response (protective effect)
GTF	Oral	Increased salivary IgA antibody (delayed reaccumulation of indigenous *S. mutans*)
	topical (MSG)	(delayed reaccumulation of indigenous *S. mutans*)
GTF (+ AgI/II)	Oral	Increased salivary IgA2 antibody
	Nasal	Increased nasal IgA1, salivary IgA1 and IgA2 antibodies
	nasal or tonsillar (topical)	IgA1 nasal and salivary antibodies in nasal group
	nasal	Salivary IgA1 antibodies

Passive Immunisation

1) local

1)Enterically coated gelatin capsules.
2)Oral Immunization

2) systemic

1)Murine monoclonal antibodies
2)Immune bovine milk
3)Egg yolk antibody

Studies on passive immunization

SUBJECT	SPECIFICTY	APPLICATION PROCEDURE	EFFICACY
monkeys	AgI/II	Topical application	Decreased colonization by *S. mutans* in fissures and smooth-surfaces of teeth Compete inhibition of dental caries Development
rats	SpaA (antigen B)	Topical application	Reduction in the colonization level of implanted *S. sobrinus*
rats	*S. mutans*	Diet	60% Inhibition of total caries development by *S. mutans*
rats	GTF,	Diet	Inhibition of colonization by *S. mutans* and approximately 40% reduction of total caries development by *S. mutans* [158] Non-protection against caries in IgY to cell-free GTF and IgY to whole cells
rats	GLU	Diet, Drinking water	Inhibition of colonization by *S. mutans* Approximately half inhibition of caries development by *S. mutans*
Rats (gnotobiotic)	Mutans streptococci (serotypes a–g)	Diet	Inhibition of colonization by *S. mutans* Inhibition of caries development in rats monoinfected with *S. mutans* or *S. sobrinus*

Humans	Murine MAbs	AgI/II	Topical application	Decreased colonization by an exogenous streptomycin-resistant strain of S. mutans
Humans	Murine MAbs	AgI/II	Topical application	Inhibition of re-colonization by indigenous *S. mutans* (the long duration, about 1 year after application of MAb, of protection)
Humans	Plant SIgA/G	AgI/II	Topical application	Specific protection of re-colonization by indigenous *S. mutans* for at least 4 months
Humans	IgY	S. mutans	Topical application	Reduction in the ratio of *S. mutans* per total streptococci in saliva in the 4 h test using a mouth rinse containing 10% sucrose. A tendency for a reduction in the ratio in plaque but not in saliva in the 7-day test using a mouth rinse without sucrose
Humans	Bovine milk	S. mutans/S. sobrinus	Topical application	Reduction in the relative number of mutans streptococci in plaque
Humans	Bovine milk	PAcA-GB	Topical application	Inhibition of re-colonization by indigenous *S. mutans*

Subunit vaccines

- Increase the immunogenicity.
- Eliminate regions which may be unwanted
- They optimize immune response
- Target different functions to address variability in mucosal response.
- Inherent adjuvant potential

- Synthetic peptide vaccines
  - Monoclonal antibodies ⟶ AgI/II
  - Functionally relevant residues in GTF

- Recombinant/Attenuated expression vectors
  - Express larger portions of functional domains
  - Gene fusions

Conjugate vaccine

- To intercept more than one aspect of molecular pathogenesis, chemical conjugation of functionally associated protein/peptide components of bacterial polysaccharides.
- Provide multiple targets
- Enhance immunogenicity

STRATEGIES FOR ENHANCING MUCOSAL IMMUNE RESPONSES

Adjuvant enhances the antigenecity of the antigen by the following ways .

1. It acts as a deposit or reservoir, whereby the antigen can be released progressively.

2. The adjuvant is able to present the antigen directly to the immune competent cells.

3. Some adjuvant acts as chemical immune stimulators of lymphoid cells.

Types of adjuvants

1. Mucosal adjuvants - Cholera toxin (CT) etc., saponins, lections, derivatives of lipid A or muramyl dipeptide
2. Coupling to carriers - chemical conjugates, or genetically engineered
3. Microparticles/Microcapsules - Biodegradable polymers,
4. Liposomes, cochleates, multiple emulsions

What is an ideal caries vaccine?

- Broadest coverage to intercept infection
- Work with both low and high risk population
- Immunity lasts through critical infection periods.
- Can be given by various routes
- Inexpensive
- Delivered by individuals with little training.

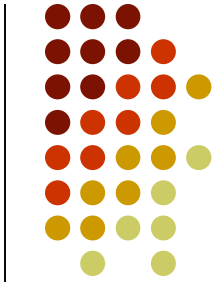
Problems associated with caries vaccine

- Caries is a multifactorial disease
- Vaccines have shown some cross reaction with heart muscles.
- Dental caries is not a life threatening diseases.
- Expensive
- Not all S.mutans cause caries.

Future prospects

- It will depend mainly on clinical trials aimed at establishing whether the findings from animal experiments can be transferred to humans.
- also determinig whether appropriate immune responses can be safely generated n humans, especially in susceptible age groups.

Conclusion



- Though caries vaccine are still in research state now, they will become a reality in managing, preventing and eradicating this disease.

- The present day dental practice is mainly concentrated on management of carious lesions.

• As caries vaccine and caries eradication are introduced in the clinical practice, in future, the work of the dentist will transform from caries management to mere caries prevention methods.

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