Old Herborn University Seminar Monograph 24:
Development of strategies to overcome barriers to effective
mucosal immunization of infants in developing countries.
Editors: Peter J. Heidt, Richard I. Walker, and Volker Rusch.
Old Herborn University Foundation, Herborn-Dill, Germany: 1-16 (2010).

MUCOSAL IMMUNE RESPONSES IN INFANCY AND EARLY CHILDHOOD: IMPLICATIONS IN SUCCESSFUL ORAL IMMUNIZATION

PEARAY L. OGRA

Department of Pediatrics, University at Buffalo,
State University of New York, Buffalo, NY, USA

INTRODUCTION

Since the introduction of routine childhood immunization nearly 4 decades ago, at least 10 major childhood infectious diseases have been either eliminated or effectively controlled with significant decline in associated mortality and morbidity in most developed parts of the world (Table 1). Yet, many vaccine preventable infections continue to pose major public health problems in many countries. In some disease situations, such as paralytic poliomyelitis, limited areas of endemic infection have continued to persist, often in spite of repeated immunization with otherwise highly effective vaccines. There is also evidence to suggest that current immunization approaches against several gastrointestinal infections, including cholera, polio, typhoid, rotavirus in the developing world and other economically under-privileged settings is either inferior or discernibly less effective in prevention of disease in children on re-exposure than observed in other, developed, countries (*Centers for Disease Control and Prevention (CDC)*, 1999).

The mucosal defences in the human and mammalian neonates, although quite competent at the time of birth of the neonate, continue to evolve and acquire functional maturity at varying intervals after birth. The mucosal barrier is a reflection of multiple host and environmental interactions. The major contributors to the mucosal barrier include a number of biophysical and non-immunologic factors, as well as specific soluble and cellular components representing both innate and adaptive immune function. Acquisition of mucosal microbial flora after birth serves a major role in the maturational process of mucosal defence. The functional maturation of the mucosal barrier is critically influenced by the gestational age at birth (pre-term vs. full-term birth), host genetic background, neuroanatomic components and intact epithelium (neuropeptide mast cells), quality and quantity (microbial load) of the mucosal microflora, and the development and nature of specific mucosal immune functions.

This brief review will focus on the nature and biologic characteristics of mucosal immune system and specific immune response in external mucosal surfaces in early childhood. The potential implication of these observations to the use of existing and the development of mucosal vaccines in the future will also be considered.

MUCOSAL IMMUNE SYSTEM: BASIC FRAMEWORK

Mature immunologic repertoire of mucosal surfaces, especially of the respiratory and gastrointestinal tracts constitutes the largest antibody-producing

Table 1: Provisional morbidity for certain infectious diseases in the United States in 1998 compared to peak morbidity prior to 1990, before universal use of vaccines in children (*Centers for Disease Control and Prevention [CDC]*, 1999)

Disease	Annual morbidity (Peak yr)	1998 Provisional morbidity	% Decrease
Smallpox	48,164 (1900)	0	100
Diphtheria	175,885 (1920)	1	100
Pertussis	147,271 (1922)	6,279	95.7
Tetanus	1,314 (1922)	34	97.4
Paralytic poliomyelitis	16,316 (1952)	0	100
Measles	503,282 (1958)	89	99.8
Mumps	152,209 (1968)	606	99.6
Rubella	47,745 (1967)	345	99.3
H. influenzae type B	20,000 (1985)	54	99.7

site in the human body. The important sites of the mucosal associated lymphoid tissue (MALT) include the gut (GALT), broncho-epithelium (BALT), and nasopharynx (NALT), sublingual tissue (SLLT), and possibly the skin (SALT), and larynx associated lymphoid tissues (LALT) (*Ogra* et al., 2008; *Thibeault* et al., 2009).

The lymphoid tissues of GALT, BALT, NALT and SLLT represent major inductive sites for mucosal responses. The GALT and the NALT possess a wealth of B cell follicles with well-defined T cell zones, and are repleted with dendritic cells and macrophages as the principal antigen presenting cells (APC). The mucosal lymphoid follicles do not possess afferent lymphatics. Over 80% of activated B cells in the human host reside in the gastrointestinal tract, and belong to IgA isotype, associated with J-chain, and secretory component (SC). The B cell differentiation in the mucosa is driven by a diverse spectrum of innate immune factors including pathogen recognition receptors (Toll-like receptors [TLR]), retinoic acid, cytokines, resident microflora, and other environmental macromolecules.

The mucosal sites destined for eventual expression of the effector functions of mucosal immune responses are the lamina propria of the gastrointestinal and respiratory tracts and sub-epithelial sites as well as other distant mucosal sites such as genital tract, mammary glands and products of lactation. This information has been extensively reviewed in several recent publications (*Ogra*, 2010; *Holmgren* and *Czerkinsky*, 2005; *Ogra* and *Welliver*, 2008).

Briefly, the functional activation of mucosal immune system is initiated by a cascade of events beginning with the exposure to an antigen and its sampling by the mucosal epithelial M cells. This is followed by participation of intra-epithelial lymphocyte (IEL), dendritic cells and regulatory T cells and several cytokines in the mucosal associated lymphoid tissue, such as IL-5, TGF-β, IL-6, IL-10, IL-23, IL-27, and retinoic acid. These events are followed by T cell activation and induction of regulatory T cells, and ultimately by the expansion of IgA plasma cell differentiation via IL-5, IL-6 and IL-10 by activation of the T-helper cell population in the specific mucosal tissue.

Exposure to specific antigens in different inductive sites has been shown to elicit a widespread but somewhat compartmentalized site-specific response in different effector sites. For example, oral administration of an antigen is associated with specific response in the intestine, genital tract and the mammary glands. Administration of antigen via sublingual or intranasal route has been shown to induce high levels of antibody response in the lung, upper airways as well as in genital tract. On the other hand, intra-rectal or intra-colonic, or intra-vaginal immunization appears to result in antigen-specific response restricted to the sites of immunization (*Ogra* and *Karzon*, 1969). The restricted nature of mucosal response in different effector sites appears to be related to the expression of specific homing ligands in activated B lymphocytes in different inductive sites. Lymphocyte trafficking and homing after respiratory tract immunization has been shown to be dependent on expression of α4β1 integrin and presence of VCAM-1, CCL28, and CCR10. On the other hand, homing after immunization in the GALT appears to be related to CCL25, CCR9, MAdCAM1 and expression of α4β7 integrin (*Kiyono* et al., 2008; *Holmgren* and *Czerkinsky*, 2005).

The principal functions of specific mucosal immune responses are:

1) barrier function, to prevent microbial translocation across mucosal surfaces and thus modulate antigen uptake via immune exclusion,
2) to regulate systemic immune responsiveness and limit uptake and presentation of dietary, microbial and other antigens by DCs to the regional lymph nodes (oral tolerance),
3) to facilitate development of regulatory cells (T-reg ($CD4^+$, $CD25^+$), and other regulatory cells in regional lymph nodes, and
4) to induce polyclonal T-cell-independent IgM responses against commensals, but not against pathogens, and thus provide a shield against specific adaptive immune responses to commensals and other established resident microflora in the mucosal surfaces (*Brandtzaeg*, 2005; *Ogra* and *Welliver*, 2008).

IMMUNE FUNCTIONS IN THE NEONATAL PERIOD AND EARLY CHILDHOOD

The development of immune function in the foetal, neonatal and early childhood years has also been discussed extensively in several recent reviews (*Levy*, 2007; *Adkins* et al., 2004; *Lewis* and *Wilson*, 2006). This information is briefly summarized here.

The neonatal period is characterized by reduced levels of innate mechanisms of defence, including reduced complement levels, lower number and function of leukocytes and macrophage, IFNγ, IL-10, and unpaired superoxide production. The neonate also exhibits reduced APC, CMI, altered antibody-producing patterns and enhanced eosinophilic responses (*Lewis* and *Wilson*, 2006).

Human neonates exhibit impaired Th1 T cell response, with a strong bias towards Th2 T cell responses, delayed maturation of IL-12 producing dendritic cells, reduced IFN-γ production by $CD4^+$ T cells, and NK cells, reduced $CD4^+$ T cells (but normal $CD8^+$ T cell responses), reduced DTH, but normal graft regulation, and reduced intracellular killing of cell associated organisms such as mycobacteria and DNA viruses. Recent studies have suggested

that neonates exhibit robust primary response with both Th1 and Th2 T cells. However, secondary responses are mostly of Th2 phenotype. Such shift to Th2 T cell response may be related to increased apoptosis of Th1 T cell by IL-4. Neutralization of IL-4 has been found to prevent apoptosis of Th1 cells and subsequent re-establishment of IFN-γ responses. Most T cells in the neonatal period are naive in phenotype and function (over 90% are $CDRA45^{+}$), and exhibit high activation threshold and co-stimulation dependence for IL-2 production. These cells initially express lower production levels of IFN-γ and IL-4, but these return to normal after activation-induced proliferation. In general neonates exhibit high overall T cell numbers for $CD4^{+}$ and $CD8^{+}$ cells than observed in older children and adults (*Holt*, 2003; *Zaghouani* et al., 2009). The unique nature of T cells and their functional characteristics in the neonatal period and early infancy may be the principal mechanisms underlying the delayed onset of T independent B cell response, delayed cell associated (HSV, CMV) viral specific $CD4^{+}$ response after perinatal infection, inverse temporal relationship between virus shedding and viral antigen load and CD4 specific response. However, Th1 specific responses have been observed in the neonatal and early infancy after immunization with BCG, whole cell pertussis (but not after cellular pertussis), and after neonatal HSV infection (*Wilson* and *Kollman*, 2008).

MUCOSAL IMMUNE RESPONSE TO VACCINES IN INFANCY AND CHILDHOOD

As pointed out above, the mucosal lymphoid tissue and the immunologic framework is well-developed and fairly competent at birth. However, there is significant lack of expression of effector function in the neonatal period and early childhood. Such functional maturation occurs after exposure to postnatal environment. It is now clear that the nature of early environmental exposure after birth is critical for specific programming and subsequent functional spectrum of mucosal lymphoid tissue as well as systemic immunoregulatory functions. This is exemplified by age related changes in the quantitative and qualitative aspect of Peyer's patches and GALT, NALT, IgA_1, and IgA_2, and expression of HLA-DR in respiratory mucosal tissue. Rudimentary lymphoid structures containing $HLA\text{-}DR^{+}$ and $CD4^{+}$ cells are seen in the intestine by 10-11 weeks of gestation as Peyer's patches. $CD8^{+}$ T cells $CD5^{+}$ IgA^{+} B cells are observed by 16-18 weeks of gestation. However, visible Peyer's patches in about 45-70 in number are first detected after 20 weeks of gestation. Subsequently, the number of Peyer's patches increase significantly between 24 hours and 6 weeks after birth, with significant expression of germinal centre only after exposure to external environment (*MacDonald* and *Spencer*, 1994; *Cornes*, 1985).

The highest number of Peyer's patches ranging between 185-325 is observed between 12-15 years of age. After 20 years of age the number of Peyer's patches begins to exhibit significant decline and only about 100 Peyer's patches are visualized after 70 years of age. Limited information is available about age related changes in BALT or NALT in humans. IgA and HLA-DR expression in tracheal wall tissue has been reported only after 1-month postnatal age. No IgA has been

detected at birth, but begins to appear in mucosal secretions after 1-2 weeks of age in over 90-95% of infants. However, adult concentrations of IgA in secretions are attained only after 5-7 years of age (*Cripps* et al., 2005).

Animal studies have suggested that while GALT exhibits involution in a manner similar to man as a function of aging, the NALT begins to develop only after birth and does not exhibit significant involution with ageing. In contrast to the development of Peyer's patches and GALT, the rodent NALT development appears to be independent of IL-7R and LTβR. It does not follow programmed inflammation model and cell development appears to be dependent only on Id2 gene expression (*Kiyono* and *Fukuyama*, 2004; *Kunisawa* et al., 2008).

Since the introduction of cowpox virus in 1798 for immunization against human smallpox, over 30 additional viral and bacterial vaccines have been introduced for routine immunization against many childhood infectious diseases (Table 2). Of these, only Sabin live oral poliovirus (OPV) reassortant rotavirus, attenuated influenza virus, adenovirus, cholera and typhoid vaccines are available for use by mucosal route of administration (Table 2).

Numerous observations over the past 30-40 years have amply demonstrated that mucosal administration of replicating or non-replicating microbial agents often result in the development of mucosal and frequently serum antibody and cell mediated immune responses. Induction of secretory IgA antibody responses provides microbial specific protection against many respiratory, enteric, genital and many systemic infections and reduces severity of clinical disease. Passive transfer of specific monoclonal IgA antibodies have been found to provide significant protection against re-infection challenge with influenza, rotavirus, respiratory syncytial virus, poliovirus, Salmonella, Helicobacter, and cholera in several experimental and human infection models (*Ogra* and *Welliver*, 2008).

Many earlier investigations have demonstrated that the development of mucosal antibody response is dependent on the route of vaccine administration, nature of vaccine antigens, age at the time of immunization and possibly the frequency of immunization. These studies employed priming and booster immunization with several bacterial and viral agents such as adenovirus, rubella, varicella-zoster, rotavirus cholera and polio (*Ogra*, 2984; *von Ginkel* et al., 2000; *Ogra* et al., 1989).

Many European countries and certain provinces in Canada in the past and more recently the U.S. have relied solely on the intramuscular use of Salk IPV or the more immunogenic enhanced potency IPV (EIPV). Carefully controlled studies with EIPV have demonstrated that intramuscular immunization with inactivated virus can provide sufficient protection against natural polio. The high degree of success with IPV has been largely attributed to the inclusion of entire populations in the primary vaccination programs and the ability to deliver booster immunization at regular intervals to large segments of the population. Such immunization programs have been found to maintain effective levels of specific circulating antibody over long periods.

In order to examine the nature of mucosal antibody responses after initial (primary) immunization by systemic or mucosal routes, groups of infants were immunized with trivalent OPV (TOPV) administered orally or trivalent IPV (TIPV) inoculated intramuscularly or intranasally. The subjects were two months of age when first immunized and received three doses of the vaccine

Table 2: Available vaccines listed by year of first vaccine development or licensure in the United States (1700-2009)

Period	Vaccine	Efficacy by recommended route of administration: Mucosal	Systemic
1700-1799	Smallpox	-	++
1800-1899	Rabies	-	++
	Typhoid	-	+
	Cholera	-	+
	Plague	-	++
1900-1959	Diphtheria	-	++
	Pertussis	-	++
	Tetanus	-	+++
	Tuberculosis	-	+
	Influenza	-	++
	Yellow fever	-	++
	Poliomyelitis (IPV)	-	+++
1960-2000	Poliomyelitis (OPV)	++	-
	Measles	-	++
	Mumps	-	++
	Rubella	-	++
	Anthrax	-	++
	Meningococcus (Aac)	-	++
	Streptococcus pneumoniae	-	+++
	Adenovirus[a]	++	-
	Hepatitis B	-	+++
	Haemophilus influenzae B	-	+++
	Japanese encephalitis	-	++
	Hepatitis A	-	++
	Varicella-zoster	-	++
	Lyme disease	-	±
	Rotavirus rhesus[b]	++	-
2001-2009	Typhoid[a]	++	-
	Cholera[a]	++	-
	Influenza A[c]	++	-
	HPV[c]	-	++
	Meningococcus	-	++
	Zoster (shingles) [c]	-	++
	Rotavirus[c]	++	-

[a]Not available for routine use in U.S.
[b]Discontinued
[c]Recently developed
+ to +++: Effective to highly effective

at monthly intervals. All subjects were subsequently immunized with a booster dose of the TIPV administered intramuscularly or intranasally or, with TOPV administered orally at 12 months of age. The IgG antibody in the serum and SIgA antibody responses in the nasopharynx were measured at various intervals (*Ogra*, 1984). The outcome of immunization relative to the route and type of primary vs. secondary immunization is summarized below.

Mucosal priming and mucosal challenge

Primary oral administration of TOPV resulted in the appearance of significant serum IgG and nasopharyngeal SIgA poliovirus antibody response in a predictable manner. Booster immunization with TOPV at 12 months of age (approximately eight months after the last dose of primary immunization) resulted in no significant change in the pre-existing serum IgG or nasopharyngeal SIgA activity. Primary intranasal administration of TIPV resulted only in the transient appearance of nasopharyngeal antibody activity, without any detectable antibody response in the serum. The level of pre-existing maternal IgG antibody continued to decline within its expected half-life. Booster or re-immunization with TIPV administered intranasally elicited the reappearance of SIgA antibody in the nasopharynx. Several subjects also manifested low levels of IgG antibody in the serum. Re-immunization with TOPV in subjects previously primed intranasally with TIPV manifested a booster effect for serum IgG as well as for nasopharyngeal SIgA. It should, however, be pointed out that the mean SIgA titres after mucosal challenge with TOPV in individuals previously primed with TIPV or TOPV by the mucosal route (intranasally) were remarkably similar.

Systemic priming and mucosal challenge

Primary immunization with TIPV administered IM resulted in high levels of poliovirus-specific serum IgG antibody response in all subjects studied. However, no SIgA response was observed in the nasopharynx. Re-immunization with TIPV via the intranasal route in such parenterally primed subjects resulted in the appearance of SIgA in the nasopharynx, but no significant booster effect on the SIgA response or on the levels of pre-existing serum IgG was observed. On the other hand, re-immunization challenge with TOPV in such individuals resulted in a significant boost of SIgA antibody in the nasopharynx and of pre-existing IgG antibody in the sera.

Other forms of immunization

Although no SIgA poliovirus antibody response was observed in the nasopharynx after primary IM administration of TIPV, re-immunization with TIPV administered IM elicited a modest SIgA activity and a predictable booster effect on pre-existing IgG in the serum. A similar booster effect on serum IgG antibody response was observed after IM challenge with TIPV in subjects who had received primary immunization with TOPV administered orally (*Ogra*, 1984).

No booster effect was observed for SIgA response after IM challenge with TIPV in subjects previously immunized with TOPV or intranasally with TIPV.

These observations suggest that mucosal priming may not significantly influence the outcome of specific SIgA immune response in the nasopharynx to subsequent challenge with antigen administered by the mucosal route. On the other hand, parenteral priming followed by parenteral challenge resulted in minimal enhancement of SIgA response in the nasopharynx. More sig-

nificantly, parenteral priming resulted in significant enhancement of poliovirus-specific SIgA response in the nasopharynx to subsequent oral administration of the vaccine virus. In subsequent more extensive studies on priming by mucosal vs. systemic routes, employing immunization with polio vaccines, serum neutralizing, nasopharyngeal neutralizing, and IgA antibodies were determined in 123 infants immunized with one of four schedules containing live oral vaccine (OPV), inactivated vaccine (IPV), or combinations of the two trivalent poliovirus vaccines: OPV-OPV-OPV, IPV-IPV-IPV, IPV-OPV-OPV, or IPV-IPV-OPV. Nearly 100% of individuals formed serum-neutralizing antibodies. The highest geometric mean titre (GMT) of antibody to polioviruses 1, 2, and 3 occurred in groups IPV-IPV-OPV, IPV-OPV-OPV, and IPV-IPV-IPV, respectively. Local neutralizing and IgA antibody responses were detected in 41%-88% and 75%-100%, respectively. Peak GMT of nasopharyngeal antibodies differed minimally between immunization groups. The data suggest that incorporation of at least one dose of IPV at the start of the immunization schedule tends to increase systemic as well as local antibody production. Over 70% of the subjects were monitored serologically over the subsequent 4 years and challenged with OPV at 5 years of age. Each of the immunization groups exhibited an initial 10- to 100-fold decline in neutralizing antibody to poliovirus types 1, 2, and 3 during the first 2 years of follow-up; thereafter antibody titres stabilized. The IPV-IPV-OPV group maintained the highest antibody levels throughout the observation period, including the response to OPV challenge at 5 years of age. These data suggest that immunization with OPV, IPV, and combinations of the two vaccines confer long-term immunity. Optimal systemic immunity was associated with two or more doses of IPV (*Faden* et al., 1990, 1993).

No discernable suppression of IgG response in the serum or SIgA response in the nasopharynx was observed with either the mucosal or the systemic form of administration in these children. However, studies by Svennerholm (*Svennerholm* et al., 1981) demonstrated a significant suppression of pre-existing SIgA activity in the milk after oral administration of OPV in women who were previously infected, presumably as a result of prior natural exposure to wild poliovirus. These studies were carried out in groups of lactating women in Sweden, a country with little or no wild poliovirus infection and in Pakistan, where poliovirus infection was endemic at that time. At the beginning of these studies, the Swedish women lacked significant titres of SIgA poliovirus antibody in the milk. Subsequent parenteral immunization with IPV in these women resulted in a low and a transient increase in the titres of SIgA activity in the milk. On the other hand, Pakistani women had significant SIgA titres in their milk before any active immunization was carried out. Parenteral immunization with IPV resulted in a significant increase in SIgA titres in the milk of 45% of the subjects tested. On the other hand following oral administration with OPV given in conjunction with subcutaneously administered killed *Vibrio cholerae* vaccine, the pre-existing titres of poliovirus antibody in the milk manifested a significant (as much as 40-fold) decline. However, when OPV was used alone, some subjects appeared to manifest a mild enhancement of SIgA titre, while other manifested a modest drop in pre-existing SIgA titres (*Svennerholm* et al., 1980).

It is apparent that the extent of serum and secretory immune responses

may be determined by the functional homeostasis of the regulatory T cell subsets, other immunoregulatory cells, immune complexes, histocompatibility, and the nature, physicochemical characteristics, and route of delivery of antigens. The possible synergism or antagonism of different organisms or antigens on the network of immunoregulatory mechanisms must be considered in the explanation of the diverse spectrum of changes in the systemic and SIgA immune responses with different antigens administered by different routes. The efficacy of IPV as well as OPV in the prevention and control of poliomyelitis has been conclusively demonstrated by their routine use over the past three decades, especially in the technologically developed countries (*Centers for Disease Control and Prevention [CDC]*, 2005; *Hayman*, 2004). However, serious concern has been raised about the effectiveness of immunization with OPV in the developing countries where paralytic poliomyelitis continues to exist in endemic albeit very small proportions. Even large-scale repeated immunization with OPV in these countries has been associated with a high rate of failure in several communities to seroconvert for poliovirus-specific antibody (*Mittal* and *Mathew*, 2007). In fact, several major outbreaks of paralytic poliomyelitis from community-acquired wild poliovirus infection have continued to exist in these countries in children previously immunized with high-potency OPV given in standard dosage schedules at appropriate intervals (*Chandrakant* and *Pradhan*, 2007). A number of possible explanations have been offered for this phenomenon. These include co-existing enteric viral infections interfering with implantation of vaccine virus, loss of potency of vaccine during transportation in the tropical heat, presence of other inhibitory factors such as interferon, or co-existing infection with wild polioviruses. However, none of these mechanisms can be clearly implicated in most if not all cases of OPV failure in such settings.

It is possible that the microbial ecology or specific environmental antigens in the alimentary tract of vaccinees in the developing countries may under certain situations have a profound influence on the activation of immunoregulatory mechanisms in the gut-associated lymphoid tissue, notably on functional activity of immunoregulatory T cell subsets. Such alterations may in turn determine the degree of systemic or secretory antibody response to vaccine-induced and, possibly, naturally acquired poliovirus infections (*Sack*, 2008).

MUCOSAL IMMUNIZATION AND ORAL TOLERANCE

Oral exposure to non-replicating antigens may significantly influence the outcome of systemic immune response to subsequent re-exposure to the same antigen. The phenomenon of systemic hypo-responsiveness observed following oral ingestion of an antigen gained scientific credence in the early 1940s with the classic experiments of Chase employing simple chemicals (Chase, 1946). Since then, oral sensitization with a number of non-infectious antigens has been observed to induce suppression of the systemic immune response to the homologous antigen following subsequent systemic challenge. These include picryl chloride, sheep red blood cells (SRBC), ovalbumin (OVA), ragweed antigen E, dinitrophenylated human gammaglobulin

Table 3: Effect of the route of priming on the outcome of immune response to subsequent challenge with soluble proteins and other non-infectious agents (*Ogra*, 1984)

Route of priming/challenge	Antigen	Effects on immune response: Systemic IgG	Mucosal SIgA
Mucosal/systemic	Picryl chloride	S	NA
	SRBC	S	NA
	OVA	S	NE
	Ragweed-E	S	NE
	DNP-HGG	S	NA
	Transplantation	S	NA
Mucosal/systemic	OVA	S	NE
	BSA	S	NE
Systemic/systemic	Haptens	S	NA
	Hapten-syngeneic cell complex	S	NA

SRBC = sheep red blood cells
OVA = ovalbumin
DNP-HGG = dinitrophenylated human γ-globulin
BSA = bovine serum albumin
SIgA = secretory IgA;
S = suppression (tolerance)
NE = no effect
NA = no available data

(DNP-HGG), and transplantation antigens as shown in Table 3 (*Ogra*, 1984). In addition, suppression of the systemic immune response has also been observed for OVA and bovine serum albumin (BSA) after mucosal challenge in animals previously primed via the mucosal route and for certain haptens after systemic sensitization. In virtually all experiments with such non-infectious antigens reported to date, no suppressive effect has been observed on the mucosal immune responses. On the basis of these observations subsequently Tomasi proposed the concept of "oral tolerance" as a mechanism of possible defence by which certain mucosally introduced antigens will result in systemic hypo-responsiveness, thus reducing the risk of the development of systemic immunologically mediated disease states.

While the observations on systemic tolerance with many soluble protein antigens and other macromolecules are clear-cut, an extreme degree of variation seems to exist for the induction of or the levels of systemic hypo-responsiveness to non replicating and possibly replicating infectious agents. The available data on the effects of mucosal vs. systemic priming on the outcome of subsequent re-exposure challenge with infectious organisms or specific microbial antigens are reviewed in Table 4. A careful examination of these observations suggests that with most infectious agents, the effect of mucosal or systemic priming on subsequent challenge is, in fact, in favour of a booster effect on the systemic immune response rather than tolerance. Similarly, the SIgA response to infectious agents does not manifest a consistent pattern

Table 4: Effect of route of priming on the outcome of immune response to subsequent challenge with infectious microorganisms or their antigens (*Ogra*, 1984; *Faden* et al., 1990; *Svennerholm* et al., 1981)

Route of priming/challenge	Agent	Effects on immune response: Systemic IgG	Mucosal SIgA
Mucosal/systemic	OPV/IPV	B	NE, B(S)
	IPV/IPV	B/NE	NE, B
	Streptococcus mutans	S	NE
	Vibrio cholerae LPS	B	B, NE
Systemic/mucosal	IPV/OPV	B	B
	V. cholerae	B	(S)B
	V. cholerae toxoid	NA	S
	IPV	NE	NE
Mucosal/mucosal	IPV/OPV	NE	B
	OPV/OPV	B	B
	Live wild-type poliovirus/OPV	NE	S*
	RSV	B	B
	Rubella	B	B
	V. cholerae	NE, B	B
Systemic/systemic	IPV	B	NE
	V. cholerae LPS	B	(S)NE

OPV = live attenuated (oral) poliovirus vaccine
IPV = inactivated poliovirus vaccine
LPS = lipopolysaccharide
RSV = Respiratory syncytial virus
SIgA = secretory IgA
B = booster effect
NE = no effect
S = suppression (tolerance)
NA = no data available.

of suppression after mucosal or systemic priming and subsequent challenge. It would seem that the pre-existing SIgA responses to most replicating agents exhibit a booster effect or in certain situations no discernible change in pre-existing immunity.

CONCLUDING REMARKS

Possible approach to enhance mucosal immune response in childhood

Mucosal administration of vaccine antigens especially replicating agents and for organisms whose portal of entry are the external mucosal surfaces of the respiratory, enteric or genital mucosa in general mimic the development of immunity following natural infection. The observations summarized in the preceding sections of this review suggest that factors that favour devel-

Table 5: Status of existing non-replicating vaccines delivered by mucosal routes

- Antigen mass limited to the amount administered (no replication)
- Inactivated antigens used alone, not highly immunogenic
- Induce effective but transient secretory and little serum antibody and cellular immune response often exhibit:
  1. Lack of memory
  2. Require appropriate adjuvants, mucosal delivery formulations and immunogenic epitopes for effective immune responses and disease protection (cholera)
  3. Less efficient immune response and disease protection in the developing world
     - Minimal or no untoward side effects in vaccinees or in contacts
     - No community spread. Evidence of herd immunity with some? No potency loss in field setting

opment of mucosal antibody and cell-mediated immune response include mucosal route of immunization and the replicating nature of the vaccine antigen. However, to date, very few replicating vaccines have been available for mucosal immunization. The paucity of available mucosal vaccines is related in part to potential danger currently perceived with replicating agents, especially when the risk of continued vaccination may exceed the risk of disease following naturally acquired infection. This is best illustrated by the withdrawal of OPV from routine immunization from most of the developed world. In countries where wild poliovirus infection has been effectively eliminated, the reasons for limited use of other mucosal vaccines is related to the observations that it has also been difficult to induce mucosal protection consistently after mucosal administration of many candidate non-replicating antigens. The mechanisms underlying such poor mucosal responses include, poor antigenicity, rapid elimination, inactivation by mucosal enzymes or interference by existing mucosal environment, including gut microflora. Other potential limitations include lack of optimal contact of vaccine components with antigen presenting or processing mucosal cells including M cells, DC and mucosal macrophages (*Ogra* et al., 2001).

Based on the experience with existing vaccines, the development of mucosal immunity by administration of vaccines via the mucosal routes is clearly not a pre-requisite for the effective control of most infections. With the exception of oral rotavirus vaccine, cholera, and intranasal influenza virus vaccine, most newly developed vaccines (such as HPV, pneumococcal and meningococcal conjugates), and other vaccines currently under development, are designed solely for parenteral use. As the use of parenterally administered vaccines continues to remain single major option for new vaccine development; the average infant will have received over 25 vaccine doses by intra-dermal or intramuscular injections by 18-24 months of age of the infant. The availability of mucosally deliverable vaccines will provide simpler relatively painless approach for as frequent a delivery as necessary, and for multiple vaccine-antigens. The benefits and potential limitation encountered with currently available replicating and non-replicating vaccines are listed in Tables 5 and 6.

The possible approaches suggested to address the difficulties encountered in the development of effective mu-

Table 6: Status of Existing Replicating Vaccines Delivered by the Mucosal Route

- Induce amplification of antigen mass (based on level of replication).
- Induce of effective serum and secretory antibody and cellular (CTL) immune responses.
- Prolonged responses and induction of memory.
- Protection against both mucosal and systemic infection and or illness.
- Development of herd immunity and community spread, relative to the level of replication. However, immunization may be associated with:
  1. Development of untoward and sometime serious side effects in vaccinees and contacts
  2. Loss of potency in field settings
  3. Less efficient immune response and disease protection even after multiple immunization doses in many parts of the developing world

cosal vaccines are listed in Table 7. A number of approaches are being considered to reduce microbial virulence and enhance antigen load with replicating vaccines. These include use of recombinant protein and use of live vectors, subunit vaccines and use of specific antigen containing transgenic edible plants. The use of micro-particles, viral-like particles is being explored more extensively to improve delivery of antigens into the mucosa. Currently efforts are underway to employ safer mucosal adjuvants and, consider routes of mucosal immunization other than oral or intranasal. These include sublingual and trans-cutaneous routes to enhance development of effective mucosal response at desired target effector site (*Belyakov* et al., 2004; *Song* et al., 2008). Ample evidence has suggested that mucosally delivered vaccines could also be more effective in preventing systemic illness and mucosal infection during subsequent natural re-exposure to the virulent pathogen.

The induction of tolerance is possibly an important limitation to the use of non-replicating antigens by the mucosal route especially in the absence of appropriate adjuvants. However development of mucosal tolerance has not been demonstrated for replicating or non-replicating microbial vaccine antigens in man. It is not known whether the failure to develop effective immunity against polio after repeated immunization with OPV described earlier in this review in some countries reflects induction of oral tolerance. One of the goals of vaccine delivery by the mucosal route should include approaches to examine the development of tolerance and to overcome such potential threats that may exist prior to exposure especially in the neonatal period or early infancy. Interestingly enough, in an earlier publication on immune response to Leishmania antigen in an experimental animal model infection, it was proposed that induction of tolerance to potentially harmful population of Leishmania antigens may permit development of protective immune response to other Leishmania antigens and thus prevent development of disease. The author proposed induction of oral tolerance may be a possible immunization approach in preventing disease with other cell associated pathogens such as Candida, Schistosoma and microflora (*McSorley* and *Gaside*, 1999).

Numerous recent observations have suggested that the acquisition and the nature of mucosal microbial flora in early childhood especially in the neonatal period is critical in later develop-

Table 7: Approaches to enhancement of mucosal immunity to vaccines (*Ogra* et al., 2001)

Goal	Approach
Reduce virulence and enhance antigen load	Recombinant proteins, live vectors Subunit vaccines DNA vaccines Transgenic edible plants
Improve delivery into the mucosa	Non-living micro-particle carriers VLP
Improve mucosal interaction with antigens	Adhesive antigens Adjuvants
Enhancement of immune response	Mucosal adjuvants Combination systemic-mucosal immunization Trans-cutaneous, sublingual and other routes of immunization

ment and regulation of the mucosal immune responses and their functions. Depending on the nature of microbial-host mucosal interactions, the functional nature of mucosal immune response is protective against disease producing microorganisms, and other environmental macromolecules. Such protection is mediated by colonization by commensals, development of protective B and T cell responses, possible activation of specific innate immune mechanisms and induction of tolerance to dietary antigens and other macromolecules. The nature of mucosal immune responses may also be pathogenic and facilitate development of immunologically mediated diseases and induction of autoimmunity. Such effects may be related to altered microbial colonization in early life, diet, use of antibiotics and failure to develop tolerance to dietary antigens and other external agents (*Ogra* and *Welliver*, 2008). The symbiotic relationship between the microbial flora and the host has evolved over millions of years of balanced co-existence, in which the host as well as the "normal" microbial flora contribute to each others functional integrity and survival. An exciting recent series of investigations has provided a new and unique dimension to the evolution of mammalian intestinal microbial flora. These studies have obtained convincing evidence to suggest that mammals, including the humans are "composed of not only their own gene pool, but also of all their associated microbes". Both the host diet and microbial phylogeny influence the nature of bacterial diversity in the mammalian gut (*Ley* et al., 2008). These observations open up new avenues for the development of effective mucosal vaccines against human microbial pathogens whose primary portal of entry represents the external mucosal surfaces.

LITERATURE

Adkins, B., Leclerc, C., and Marshall-Clarke, S.: Neonatal adaptive immunity comes of age. Nat. Rev. Immunol. 4, 553-564 (2004).

Belyakov, I.M., Hammand, S.A., Ahlers, J.D., Glenn, G.M., and Berzofsky, J.A.: Transcutaneous immunization induces mucosal CTLs and protective immunity by migration of primed skin dendritic cells. J. Clin. Invest. 113, 998-1007 (2004).

Brandtzaeg, P.: Mucosal adaptive immunity: Impact of exogenous stimuli and feeding. Euroconference: Nutrition, Immune Functions and Health, Paris, June 9-10, 2005.

Centers for Disease Control and Prevention (CDC): Impact of vaccines universally recommended for children--United States, 1900-1999. MMWR Morb. Mortal. Wkly Rep. 48, 241-243 (1999).

Centers for Disease Control and Prevention (CDC): Progress towards interruption of wild polio virus transmission worldwide. MMWR Morb. Mortal. Wkly Rep. 54, 408-412 (2005).

Chandrakant, L. and Pradhan, S.K.: Prospects of eradicating poliomyelitis by 2007: Compulsory vaccination may be a strategy. Indian J. Paediatr. 74, 61-63 (2007).

Cornes, J.S.: Number, size and distribution of Peyer's patches in the human small intestine. Part 1. The development of Peyer's patches. Gut 6 ,225-233 (1965).

Cripps, A.W. and Gleeson, M.: Ontogeny of mucosal immunity and aging. In: Mucosal immunology, 3rd edition (Mestecky, J., Lamm, M., McGhee, J., Bienenstock, J., Mayer, L., and Strober, W., Eds.). Elsevier Academic Press, New York, 305-321 (2005).

Faden, H., Duffy, L., Sun, M., and Shuff, C.: Long-term immunity to poliovirus in children immunized with live attenuated and enhanced-potency inactivated trivalent poliovirus vaccines. J. Infect. Dis. 168, 452-454 (1993).

Faden, H., Modlin, J.F., Thoms, M.L., McBean, A.M., Ferdon, M.B., and Ogra, P.L.: Comparative evaluation of immunization with live attenuated and enhanced-potency inactivated trivalent poliovirus vaccines in childhood: Systemic and local immune responses. J. Infect. Dis. 162, 1291-1297 (1990).

Heymann, D.L. and Aymarld, R.B.: Eradicating polio. N. Engl. J. Med. 351, 1275-1277 (2004).

Holmgren, J. and Czerkinsky, C.: Mucosal immunity and vaccines. Nature Medicine 11, S45-S53 (2005).

Holt, P.G.: Functionally mature virus-specific CD8+ T memory cells in congenitally infected newborns: Proof of principle for neonatal vaccination? J. Clin. Invest. 111, 1645-1647 (2003).

Kiyono, H. and Fukuyama, S.: NALT-versus Peyer's patch-mediated mucosal immunity. Nat. Rev. Immunol. 4, 699-710 (2004).

Kiyono, H., Kunisawa, J., McGhee, J.R., and Mestecky, J.: The mucosal immune system. In: Fundamental immunology, 5th edition (Paul, W.E., Ed.). Lippincott, Williams & Wilkins, Philadelphia, 983-1030 (2008).

Kunisawa, J., Nochi, T., and Kiyono, H.: Immunological commonalities and distinctions between airway and digestive immunity. Trends Immunol. 29, 505-513 (2008).

Levy, O.: Innate immunity of the newborn: Basic mechanisms and clinical correlates. Nat. Rev. Immunol. 7, 379-390 (2007).

Lewis, D.B. and Wilson, C.B.: Developmental immunology and role of host defenses in fetal and neonatal susceptibility to infection. In: Infectious diseases of the fetus and newborn infant, 6th edition (Remington, J., Klein, J., Wilson, C., and Baker, C. Eds.). Elsevier-Saunders, Philadelphia, 87-210 (2006).

Ley, R.E., Hamady, M., Lozupone, C., Turnbaugh, P.J., Ramey, R.R., Bircher, J.S., Schlegel, M.L., Tucker, T.A., Schrenzel, M.D., Knight, R., and Gordon, J.I.: Evolution of mammals and their gut microbes.

Science 320, 1647-1651 (2008).
MacDonald, T.T. and Spencer, J.: Ontogeny of the gut-associated lymphoid system in man. Acta Paediatr. Suppl. 83, 3-5 (1994).
McSorley, S.J. and Garside, P.: Vaccination by inducing oral tolerance. Immunol. Today 20, 555-560 (1999).
Mittal, S.K. and Mathewm J,L.: Polio eradication in India: The way forward. Indian J. Paediatr. 74, 153-160 (2007).
Ogra, P.L.: Mucosal immune response to poliovirus vaccines in childhood. Rev. Infect. Dis. 6, S361-368 (1984)
Ogra, P.L.: Ageing and its possible impact on mucosal immune responses. Ageing Res. Rev. 9, 101-106 (2010).
Ogra, P.L., Faden, H., and Welliver, R.C.: Vaccination strategies for mucosal immune response. Clin. Microbiol. Rev. 14, 430-445 (2001).
Ogra, P.L. and Karzon, D.T.: Distribution of poliovirus antibody in serum, nasopharynx and alimentary tract following segmental immunization of lower alimentary tract with poliovaccine. J. Immunol. 102, 1423-1430 (1969).
Ogra, P.L., Leibovitz, E.E., and Zhao-ri, G.: Oral immunization and secretory immunity to viruses. Curr. Top. Microbiol. Immunol. 146, 73-81 (1989).
Ogra, P.L. and Welliver, R.C.: Effects of early environment on mucosal immunologic homeostasis, subsequent immune responses and disease outcome. In: The window of opportunity: Pre-Pregnancy to 24 months age. Nestlé Nutrition Workshop Series Pediatric Program Vol. 61 (Barker, D., Bergman, R., and Ogra, P.L., Eds.). Karger AG, Basel, 145-181 (2008).
Sack, D.A., Qadri, F., and Svennerholm, A.M. Determinants of responses to oral vaccines in developing countries. Ann. Nestlé (Engl.) 66,71-79 (2008).
Song, J.H., Nguyen, H.H., Cuburu, N., Horimoto, T., Ko, S.Y., Park, S.H., Czerkinsky, C., and Kweon, M.N. Sublingual vaccination with influenza virus protects mice against lethal viral infection. Proc. Natl. Acad. Sci. USA 105, 1644-1649 (2008).
Svennerholm, A.M., Hanson, L.A., Holmgren, J., Jalil, F., Lindblad, B.S., Khan, S.R., Nilsson. A., and Svennerholm, B.: Antibody responses to live and killed poliovirus vaccines in the milk of Pakistani and Swedish women. J. Infect. Dis. 143, 707-711 (1981).
Svennerholm, A.M., Hanson, L.A., Holmgren, J., Lindblad, B.S., Nilsson, B., and Quereshi, F.: Different secretory immunoglobulin A antibody responses to cholera vaccination in Swedish and Pakistani women. Infect. Immun. 30, 427-430 (1980).
Thibeault, S.L., Rees, L., Pazmany, L., and Birchall, M.A.: At the crossroads: Mucosal immunology of the larynx. Mucosal Immunol. 2, 122-128 (2009).
van Ginkel, F.W., Nguyen, H.H., and McGhee, J.R.: Vaccines for mucosal immunity to combat emerging infectious diseases. Emerg. Infect. Dis. 6, 123-132 (2000).
Wilson, C.B. and Kollmann, T.R.: Induction of antigen-specific immunity in human neonates and infants. In: The window of opportunity: Pre-pregnancy to 24 months age. Nestlé Nutrition Workshop Series Pediatric Program Vol. 61 (Barker, D., Bergman, R., and Ogra, P.L., Eds.). Karger AG, Basel 61, 183-196 (2008).
Zaghouani, H., Hoeman, C.M., and Adkins, B.: Neonatal immunity: Faulty T-helpers and the shortcomings of dendritic cells. Trends Immunol. 30, 585-591 (2009).