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Passive Immunity for Protection against Mucosal Infections and Vaccination for Dental Caries

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I. Introduction

A variety of innate and acquired defense mechanisms exist that protect the host from potential pathogenic microorganisms. The outcome of a particular infection depends on interactions between the virulent capability of the pathogen to evade and damage the host as well as the degree of adaptive immune responses in the host. The adaptive immune response is quiescent until stimulated by immunizing events, usually infections. Vaccination is the intentional process that can stimulate adaptive resistance in the host by enhancing humoral immune responses. Since a variety of microbial infections occur at the mucosa or penetrate through mucosal surfaces of the body, induction of antibodies in the mucosa is desirable in vaccinations. Since it is frequently difficult to induce sufficient immunoglobulin levels for protecting the host following current immunization procedures, passive immunization may be considered as an alternative measure for controlling infectious diseases in humans and animals. Use of egg yolk antibodies from hens immunized by specific virulence factors or microorganisms may provide a novel approach to the control of infectious disease; this approach is reviewed in this chapter.

II. Concept of Passive Immunity

A. Basic Aspects of Passive Immunity

Empirical observations of the transfer of immunity from mother to offsprings represent perhaps the first observation for passive antibody protection. The factors conferring immunity not produced by the infants or the fetus could be provided by the mother who possessed antibodies directed against microbes present in her environment (Goldman *et al.*, 1985). It is now well known that IgG alone among the five immunoglobulin classes is actively transported across the placenta. This property provides passive immunity to the newborn baby. The IgG molecules are degraded with a half-life of about 1 month, which accounts for the decrease in the serum IgG concentration in the newborn over the first 3 months after birth. During the first 6 months of life, the rate of newly synthesized IgG by the infants overcomes the decrease of the IgG passively derived from the mother. IgM in infants reaches adult levels by 9 month of age; however, other immunoglobulins, i.e., IgA, IgE, and IgD, are not clearly demonstrated in the serum of infants (Lydyard and Grossi, 1989).

The first successful demonstration of passive immunity can be attributed to von Behring and Kitasato (1890). They clearly demonstrated that immunity to diphtheria and tetanus could be transferred passively to naive mice by the antisera produced in rabbits. This discovery revolutionized the concept of humoral immunity, revealing that serum antibodies were the active entity for protecting the host from infectious and/or toxemic diseases. Thus, recipient animals which had serum antitoxin antibodies became resistant to the challenge with the culture filtrate of *Corynebacterium diphtheriae* or *Clostridium tetani*, which contained an otherwise lethal dose of diphtheria or tetanus toxin, respectively. Their pioneering approach revealed that serum antibodies played a pivotal role in host defense against virulence factors of infectious agents. Passive immunization can confer immunity in the host very quickly. This prin-

ciple is practically applied to the treatment or prophylaxis of venoms from snake or spider bites, from tetanus toxin, or from the rabies virus that may result in otherwise lethal consequences (Renegar and Small, 1994).

B. Naturally Occurring Passive Immunity

Evidence indicates that colostrum and mother's milk sustain and even augment the protection of infants against infections in humans and in experimental animals prior to the development of gastrointestinal (GI) tract immunity. Epidemiological surveys also revealed that breast-fed infants induced fewer intestinal infections and hospital admissions than non-breast-fed control subjects (Goldman *et al.*, 1985). The class of immunoglobulins in milk and colostrum may vary with the animal species. In humans, secretory IgA (S-IgA) is the major antibody isotype found in milk and colostrum; however, the concentrations of IgG and IgM are as high as 600 mg/liter in colostrum and 50 mg/liter in milk (Mehta *et al.*, 1989). S-IgA is resistant to proteinases and gastric acid and remains active in the intestinal tract of infants. Thus, S-IgA and most probably other components in milk and colostrum provide local passive immunity in the urinary and intestinal tracts (Haneberg, 1974; Prentice, 1987; Glass *et al.*, 1983; Rolfe and Song, 1995).

In ungulates, the major antibody in their colostrum is IgG; however, during lactation, the concentration of S-IgA markedly increases, which of course is the opposite for levels of S-IgA in human colostrum and milk. For example, piglets receive almost all of their maternal antibody contribution from colostrum (i.e., IgG) during the first 24 hr after birth. Thus, piglets at birth obtain IgG from the colostrum of the dam, which is absorbed by the intestinal mucosa for up to 34 hr and is then transported to the circulation of the suckling pig (Yokoyama *et al.*, 1993). In calves, absorption of colostral antibodies is limited to the first few hours of life (Besser and Gay, 1994). Rodents, on the other hand, can actively take up IgG for about 2 weeks, and their protection may be supported either by the direct action of the milk suckled or by the maternal antibody being adsorbed into the blood stream and secretions of the offspring, or both (Shope and Schiemann, 1991). In summary, the mother's colostrum is an effective means by which the offspring can acquire passive immunity to infectious agents which are encountered from the environment.

III. Experimental Approach for Mucosal Passive Immunization against Infections

A. Use of Specific Antibodies for Passive Immunization

Relatively large quantities of antibodies specific for targeted pathogenic microorganisms are required in order to accomplish passive transfer of systemic and local immunity. For this purpose, antibodies elaborated in other individuals of the same animal species or in some cases in other animal species are prepared from various sources. Polymeric antibodies have been isolated from serum, milk, and colostrum of immunized animals as well as egg yolks from immunized hens. Monospecific antibodies can be easily obtained from these sources if a highly purified antigen from a pathogen is used as an immunogen together with appropriate adjuvant. In addition to obtaining preformed antibodies from live animals or humans, antibodies can be prepared from culture supernatants of hybridoma cells producing monoclonal antibodies (mAb). Theoretically, mAbs can be continuously obtained without sacrificing animals, and is a good source for use in passive immunity (Zimmerman *et al.*, 1985).

There are various methods commonly used for preparation and purification of antibodies, mainly IgG, from serum of immunized animals or humans (Motin *et al.*, 1994; Wong *et al.*, 1994). Nonpurified antibody preparations can be used successfully for passive immunization; however, in most cases, antibodies are purified to various degrees by using salt precipitation, ion exchange column chromatography, and/or gel filtration (Marchalonis and Warr, 1982). Some examples of antibody-containing preparations other than antiserum for testing efficacy of passive immunization are summarized below.

1. Saliva and Mucosal Surface Wash

As has been suggested, S-IgA at the mucosal tissue surface may protect from colonization and invasion by pathogenic organisms. However, unlike serum antibodies it is not easy to prepare S-IgA in sufficient quantity to use for passive immunity.

Human saliva is but one of the sources used for this purpose. Pooled saliva cleared by ultracentrifugation can be applied to jacalin (a lectin with specificity for human IgA1)-immobilized agarose columns and eluted with melibiose solution (0.1 *M*), dialyzed, and concentrated. This unpure preparation is purified from a saliva sample containing S-IgA by passage over glutardialdehyde glass beads coupled with a protein antigen, which can be eluted with glycine-HCl buffer (0.1 *M*, pH 2.1), dialyzed, and concentrated. This preparation may be recognized as monospecific, purified salivary S-IgA. It was found that an affinity-purified S-IgA protected against group A streptococcal infection under conditions where serum derived IgG was not effective (Bessen and Fischetti, 1988).

Body fluids bathing the mucosal surfaces can be obtained by washing the surfaces with buffered saline. The nasal cavity and the upper respiratory tract from immunized mice were flushed with balanced salt solution as a source of mucosal antibodies (Tamura *et al.*, 1991). The washings were next concentrated, and applied to a

protein G–Sepharose column. The effluent could be further loaded on the affinity column using tresyl-activated Sepharose 4B coupled with goat anti-mouse α chain. IgA was eluted with an alkaline buffer, the pH neutralized and the column effluent concentrated. The purified IgA contained 20% monomeric and 80% polymeric IgA as revealed by fractionation profile on Sephacryl S-300 HR column. Intranasal administration of the purified IgA from respiratory tract washings of mice immunized with influenza A virus hemagglutinin (HA) protected mice from challenge with live influenza A virus.

2. *Colostrum and Milk Antibodies*

Bovine milk immunoglobulins given orally/passively have been shown to prevent infections by GI tract pathogenic microorganisms. Immunoglobulin concentrates were prepared from the colostrum of dairy cows immunized with a specific pathogen. The cows were vaccinated by intracutaneous and/or intramuscular injections with a vaccine at appropriate intervals until calving. Hyperimmune colostrum on the first 3 days (typically 5–10 liters/cow/day) after calving was collected, and fat was removed by a cream separator. Colostral nonfat milk which had been pasteurized could be used for oral passive administration to subjects. After casein was removed from the colostral nonfat milk, immunoglobulins were purified by salt fractionation and column chromatography. Both IgG and IgA were found to exhibit anti-pathogen activities (Michalek *et al.*, 1987; Ebina *et al.*, 1990; Petschow and Talbott, 1994).

3. *Hen Egg Yolk Antibodies*

Hen egg yolk is another good source of IgG antibody for passive immunization (Bartz *et al.*, 1980; Yolken *et al.*, 1988; Ikemori *et al.*, 1992). Hens immunized intramuscularly or intradermally with adjuvant produce IgM and IgG antibodies in serum. Serum IgG is then transported into egg yolk, resulting in antibody titers in egg yolk which are similar to those seen in serum (Shimizu *et al.*, 1989; Horikoshi *et al.*, 1993). It is interesting to note that both IgM and IgA antibodies are found in egg white, but not in egg yolk. Thus, the latter is an excellent source of IgG (IgY) antibody for passive immunity (Rose *et al.*, 1974).

For purification of egg yolk antibodies (yAb), egg yolks separated from whites must first be delipidated by addition of saline and chloroform (Peralta *et al.*, 1994; Hamada *et al.*, 1991), propane-2-ol, and acetone (Bade and Stegemann, 1984), or by hydration and sedimentation (O'Farrelly *et al.*, 1992; Horikoshi *et al.*, 1993). After removing lipids, yAb present in the water-soluble fraction (WSF) were separated by differential precipitation with ethanol (Hamada *et al.*, 1991; Horikoshi *et al.*, 1993), ammonium sulfate precipitations (O'Farrelly *et al.*, 1992; Kuroki *et al.*, 1993), and other procedures. Highly purified antibodies can be obtained by DEAE-Sephacel and Sephacryl S-300 chromatography to give a single protein band with a molecular mass of 220 kDa (Fig. 1). This protein is dissociated into 76 kDa heavy (H) and 28 kDa light (L) chains after reduction with of 2-mercaptoethanol (2ME). On the other hand, serum antibodies give a 165-kDa protein band in SDS–polyacrylamide gel electrophoresis (PAGE). Since the 220-kDa yAb and 165-kDa serum antibodies were reactive with anti-hen IgG, it was established that both are of the IgG class. Although yAb have been described as IgY in the literature, these antibodies should be more correctly defined as the IgG class of antibodies (Hamada *et al.*, 1991; Horikoshi *et al.*, 1993).

Oral administration or feeding of egg yolk IgG (yIgG) with anti-pathogen specificity may provide a means for the prevention of oral and GI tract infectious diseases. Since ca. 100 mg yIgG can be obtained from hen eggs, yIgG offers strong advantages as a source of exogenous antibodies that can be used for passive immunization (Yolken *et al.*, 1988).

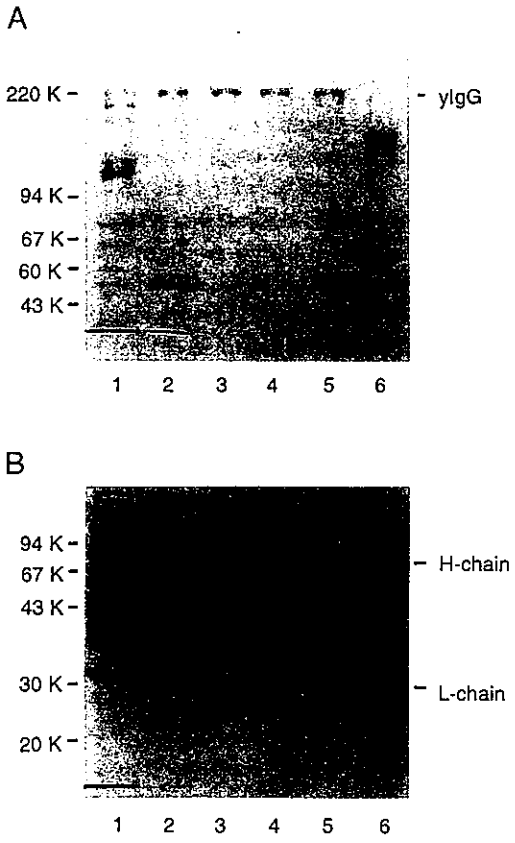

Figure 1. SDS–PAGE profile of various preparations of hen egg yolk (yIgG) antibodies. (A) Nonreducing gels without 2-mercaptoethanol (ME). Yolk proteins (1), WSF (2), chromatographically purified yIgG (3), ethanol-purified yIgG (4), purified hen serum IgG (5), purified rabbit serum IgG (6). (B) Reducing gel in the presence of 2ME. The same samples were applied to the gel as listed in (A). The location of heavy (H) and light (L) chains are indicated. Molecular sizes are shown in kDa. Adapted from Hamada *et al.* (1991) with permission.

4. *Monoclonal Antibodies*

The advantage of using mAb lies in the inherent homogeneity with respect to their immunological specificity, the class of immunoglobulins, and the theoretically limitless supply of antibodies. For production of mAb, hybridoma cells producing mAb are injected intraperitoneally into BALB/c mice that have been primed with pristane. Ascites fluid containing mAb can be taken from mice which exhibit tumor growth following inoculation of the hybridoma. The mAb can be purified by conventional methods (Renegar and Small, 1991a; Ma *et al.*, 1987; Mazenec *et al.*, 1992).

B. Passive Immunization for Protection against Mucosal Infections

1. *Passive Mucosal Protection from Bacterial Infections*

An earlier study reported that bovine milk antibodies obtained from lactating cows immunized with enteropathogenic *Escherichia coli* rapidly eradicated enteropathogenic *E. coli* from the intestine of infants suffering from diarrhea caused by this pathogen (Mietens *et al.*, 1979). The milk immunoglobulin concentrate used in this study was found to neutralize the action of *E. coli* enterotoxin by the rabbit ileal loop test and withstand proteolysis in the digestive tract to a considerable extent. Similar results were found in hamsters given bovine colostral IgG concentrate; bovine IgG specific for *Clostridium difficile* toxins A and B and other antigens protects against disease induced by *C. difficile* in a suckling hamster model of infection (Lyerly *et al.*, 1991).

Mouse IgG1 mAb to *E. coli* K99 pilus was also found to protect calves from experimental infections caused by *E. coli* K99. The mAb was given as ascites fluid at 10 hr of age and this treatment resulted in reduced weight loss, a shorter bout of diarrhea, and a lower mortality rate (Sherman *et al.*, 1983).

The offspring of mammals receive antibodies postnatally through colostrum and mother's milk, and these empirical observations have been experimentally confirmed. For example, foster mouse pups kept with mothers immunized orally by a virulent *Salmonella typhimurium* survived longer than control pups raised with naive mothers, suggesting that mucosal S-IgA antibodies prevent colonization by this pathogen. Since S-IgA is present predominantly in secretions, pooled human saliva is a good source for its isolation. Affinity-purified salivary S-IgA antibodies specific for type 6 M protein of group A *Streptococcus pyogenes* were given to mice intranasally, and it was found that passively administered S-IgA, but not serum IgG, protected mice from streptococcal infection at the mucosal surface. Of interest was the finding that S-IgA alone protected by preventing the initiation of the S. *pyogenes* infection (Bessen and Fischetti, 1988). In other studies, it was clearly demonstrated that mice given ascites containing IgA mAb specific for *Helicobacter felis* intragastrically were protected from *H. felis* infection of the gastric mucosa, although the IgA mAb did not contain secretory component-like serum IgA (Czinn *et al.*, 1993).

A unique model has been devised to provide continuous *in vivo* delivery of antigen-specific monoclonal S-IgA antibody passively into the small intestine of mice by using syngeneic "backpack" hybridoma tumors (Winner *et al.*, 1991). If hybridoma cells secreting IgA mAb specific for *Vibrio cholerae* LPS were injected subcutaneously into mice, the backpack tumors released specific mAb that resulted in increased appearance of this mAb in serum and in the lumen of the GI tract. On the other hand, IgG mAb to *V. cholerae* was not transported into the intestine, even though serum IgG mAb levels were increased. Neonatal mice are known to be highly susceptible to infection by *V. cholerae;* however, neonates injected with the IgA hybridoma cells were resistant to the challenge of *V. cholerae,* while control neonates became ill or died from a severe diarrhea during the same time interval. From these experimental results, it was concluded that the IgA hybridoma tumors backpacked in mice produce a single mAb that identifies a protective epitope and the IgA mAb could protect from mucosal infections by the same or related organisms (Winner *et al.*, 1991).

In this regard, it is essential to identify virulence factors of the pathogenic microorganisms in order to prepare protective antibodies in other animals. Recent progress in gene technology has made it possible to use cloned genes coding for a target virulence factor for production of molecular vaccines. Rabbit polyclonal IgG directed against a virulence factor protein (V antigen) of *Yersinia* species was prepared by using a fusion peptide, PAV. The IgG was found to provide excellent passive immunity in mice against *Yersinia pestis* and *Yersinia pseudotuberculosis,* but not *Y. enterocolitica* (Motin et al., 1994). Recombinant outer membrane proteins, OMPs, of *Pseudomonas aeruginosa* were also used to immunize rabbits to prepare protective serum antibodies. The antisera were found to protect severe combined immunodeficient mice, which do not have mature lymphocytes, against challenge with 1000 LD_{50} doses of *P. aeruginosa* (von Specht *et al.*, 1995).

Another important approach is to develop more simplified and reliable ways for production of mAb or yAb. For this purpose, the technique of development of yAb has been studied extensively for prevention of bacterial infectious diseases during the past 4 years. For example, it was shown that powdered WSF containing yAb to pilus antigens (K88, K99, and 987P) of enterotoxigenic *E. coli* (ETEC) protected neonatal piglets against infection with each of the three strains of ETEC when administered orally (Yokoyama *et al.*, 1992). The

protection was found to be dose-dependent, and adsorption of yAb with the pilus antigen removed antibody reactivity and resulted in a significant reduction in the protective nature of the yAb preparation. The yAb were shown to inhibit the adherence of *E. coli* 987 to the intestinal mucosa of antibody-treated but not control piglets (Fig. 2). Similar results have been obtained in rabbits passively administered with yAb directed against heat-treated whole cells of *E. coli*. The rabbits given this yAb were protected from a diarrhea syndrome induced by ETEC, and remained well (O'Farrelly *et al.*, 1992; Ikemori *et al.*, 1992). Furthermore, when yAb specific for *Salmonella enteritidis* 14-kDa fimbriae were given orally to mice, the antibodies protected the host from a challenge with live *S. enteritidis* (2×10^{10} CFU/mouse). In contrast, control mice fed normal yAb manifested various clinical signs with high mortality rates. Thus, oral administration of yAb with specificity for *Salmonella* virulence determinants could serve as an effective tool for the control of intestinal colonization and disease manifestation caused by *Salmonella* infections (Peralta *et al.*, 1994).

2. *Passive Mucosal Protection from Viral Infections*

A large array of viruses infect the mucosal surfaces of the host, including those of the gastrointestinal and respiratory tracts, and are major causes of active infectious diseases on these surfaces, including rotaviruses and influenza viruses, respectively. The host's mucosal immune responses to these viral infections are transient in many cases, and therefore reinfection occurs frequently. Secretory IgA antibodies have been shown to protect mucosal surfaces from various viral infections. In addition to S-IgA, some evidence indicates that the transudation of antiviral IgG into mucosa secretions or passively transferred IgG antibodies can contribute to local mucosal immunity (Childers *et al.*, 1989).

Rotaviruses are ubiquitous in humans and infect animals worldwide. Thus, these viruses are the principal cause of acute infectious diarrhea in young infants both in developed and in third-world countries. Rotavirus infection may result in death from dehydration from the severe diarrhea and vomiting. Only *V. cholerae* infection can cause dehydration with a frequency equal to or greater than rotavirus gastroenteritis. No acceptable vaccine has been produced to date. Passive administration of exogenously produced antibodies elaborated against rotaviruses has been considered as an alternative to active vaccination for prevention of rotavirus infections and subsequent symptoms. It appears that passive immunity to rotavirus is acquired by the newborn from its mother through colostrum (DuPont, 1984; Hilpert *et al.*, 1987; Turner and Kelsey, 1993).

This problem has also been approached by use of passive yAb and it was shown that feeding of WSF containing yAb obtained from hens immunized with simian rotavirus protected 3-day-old mice from infection with murine rotavirus (Bartz *et al.*, 1980). This cross-protection could be achieved due to the antigenic relatedness of rotaviruses from various animal species. Further, passive anti-rotavirus colostral antibody to the human pathogen has been produced in immune cows (Ebina *et al.*, 1985; Tsunemitsu *et al.*, 1989) and from hen egg yolks (Yolken *et al.*, 1988; Ebina *et al.*, 1990). Oral administration of these colostral IgG antibodies prevented the development of diarrhea in infants (Ebina *et al.*, 1985; Turner and Kelsey, 1993). Infants given immune cow's milk concentrate with high rotavirus neutralizing activities in stools showed significantly shorter intervals of virus secretion (Hilpert *et al.*, 1987). yIgG to either homotypic or heterotypic strains of rotavirus prevented the development of gastroenteritis in a suckling mouse model (Yolken *et al.*, 1988; Ebina *et al.*, 1990; Kuroki *et al.*, 1993) as well as in calves challenged with virulent rotavirus (Kuroki *et al.*, 1994).

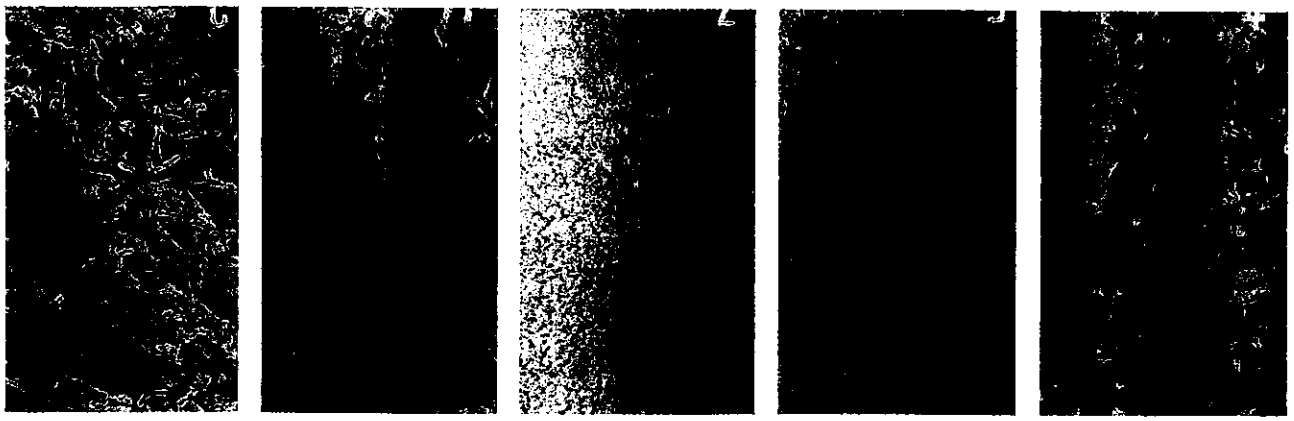

Figure 2. Scanning electron microscopic examination of cellular adherence of an ETEC strain in the ileum of piglets. Villi from the ileum of a nontreated piglet showing adherent enteric bacilli (C), villi of a piglet treated with yIgG to ETEC fimbriae at titers of 156 (1), 623 (2), and 2500(3). The ETEC-laden villus surface of the ileum from a piglet treated with yIgG absorbed with fimbriae. Bar, 10 μm. Adapted from Yokoyama *et al.* (1992) with permission.

Influenza A virus infection is restricted to the upper respiratory tract. Influenza A virus possesses surface glycoproteins, i.e. hemagglutinin (HA) and neuraminidase (NA), that may contribute to attachment and virus penetration during the initial phase of infection. Several major types of HA have been delineated; however, frequent antigenic drift and/or shift of HA occurs. Attachment of viruses to the host cell receptors involves interaction between the HA spike and a sialic acid moiety on epithelial cells lining the mucosal surface of the respiratory tract. Passive antibodies specific for the HA would be expected to prevent adsorption of the viruses. It appears logical to hypothesize that increased levels of anti-HA S-IgA secreted onto the respiratory tract mucosa would be responsible for protective immunity against influenza. Renegar and Small (1991a) demonstrated that intravenously injected polymeric IgA (pIgA) antibody to influenza virus HA was transported physiologically into nasal secretions. The IgA transported into the secretions was functional, as evidenced by antibody to the virus in an ELISA assay which protected mice from actual influenza infection. The protection was abrogated by the intranasal instillation of goat anti-mouse α-chain but not anti-mouse γ-chain, again providing evidence that the monoclonal IgA antibody was protective (Renegar and Small, 1991b).

Mice immunized intranasally with influenza virus A and cholera toxin B subunit (CT-B) induced mucosal S-IgA responses; HA-reactive S-IgA was recovered from the respiratory tract washings. Purified S-IgA from these samples clearly protected mice from the viral challenge when applied to the respiratory tract of naive mice subsequently challenged with virus (Tamura *et al.*, 1991). Purified goat IgG specific for influenza A virus was encapsulated within liposomes and was given to mice intranasally 24 hr before challenge with influenza viruses. These passively immunized mice were found to be fully protected. It was shown that the duration of protection offered by the liposome–antibody complex was relatively longer than of goat antibody not protected by liposomes. The liposome–antibody complex may be protected from *in vivo* dilution and degradation in the upper respiratory tract and lungs (Wong *et al.*, 1994).

Protection against other respiratory viruses with passively derived antibodies has also been reported. For example, it was found that IgA mAb against Sendai virus neutralized the viral activity *in vitro*, and prevented infection of mice challenged with this virus (Mazanec *et al.*, 1987). In a later study, IgG mAb to this virus was reported to be as protective as the IgA mAb in mice challenged intranasally with Sendai virus (Mazanec *et al.*, 1992). Infection of respiratory syncytial virus (RSV) in cotton rats was clearly suppressed by topical administration of human IgG; parenteral application of the IgG was much less effective (Prince *et al.*, 1987).

Several approaches using passive immune therapy for immunodeficiency virus infections have been reported (Zolla-Pazner and Gorny, 1992; Stein *et al.*, 1993). For example, investigators were able to prevent the infection of simian immunodeficiency virus (SIV) and human immunodeficiency virus (HIV) in monkeys as well as feline immunodeficiency virus (FIV) in cats with passive antibody administration (Putkonen *et al.*, 1991; Emini *et al.*, 1992; Hohdatsu *et al.*, 1993). It has been shown that immunization of chimpanzees with human recombinant soluble CD4 molecule elicits an anti-CD4 antibody response that inhibits HIV replication in chimpanzee and human lymphocytes (Watanabe *et al.*, 1992). The administration of serum IgG from AIDS patients which contained antibodies to HIV has been suggested to inhibit HIV replication and spread (Stein *et al.*, 1993). Interruption of the maternal–fetal transmission of HIV could be achieved by passive immunotherapies with human IgG exhibiting high titered antibodies to HIV structural proteins (Prince *et al.*, 1991; Lambert and Stiehm, 1993). These approaches may provide us with valuable insight into the pathogenetic nature of HIV infection (Karpas *et al.*, 1990; Marasco *et al.*, 1993). It should be noted, however, that results obtained by other investigators did not necessarily confer protection by passive antibody immunotherapy (Jacobson *et al.*, 1993; Kent *et al.*, 1994).

3. *Passive Mucosal Antibody Protection from Protozoan Infections*

Cryptosporidium is a protozoan parasite that infects the intestinal epithelium of a wide range of mammals including humans, and is a cause of gastroenteritis and diarrhea. This disease is basically self-limiting in normal hosts; however, it becomes persistent and life-threatening in immunocompromised hosts such as the AIDS patient. No efficacious vaccines or therapeutic agents are available for prevention or treatment of cryptosporidiosis (Fayer and Ungar, 1986). Attempts have been made to determine the efficacy of transfer of passive immunity to cryptosporidiosis through use of mAb, colostral antibodies, or yAb. All of these antibodies were found to neutralize infectivity of *Cryptosporidium parvum* sporozoites for mice. Immune bovine colostral whey was the most effective for neutralization of sporozoites and reduced infectivity in mice challenged with oocysts (Perryman *et al.*, 1990). Antibodies to *C. parvum* were prepared from egg yolks obtained from hyperimmunized hens. The yAb given by gastric gavage were found to reduce the number of parasites on villi of the neonatal mice orally infected with *C. parvum* oocytes (Cama and Sterling, 1991).

Hyperimmune bovine colostrum from pregnant cows immunized with oocytes of *C. parvum* provided protection of neonatal cows and mice against challenge with *C. parvum* oocytes (Fayer *et al.*, 1989a,b). It was

also shown that hyperimmune bovine colostrum suppressed infection of *Cryptosporidium* to monolayers of human intestinal epithelial cells (Flanigan *et al.*, 1991). Furthermore, remission of diarrhea in a child with hypogammaglobulinemia was obtained by treatment with hyperimmune bovine colostrum (Tzipori *et al.*, 1986). Similarly, administration of hyperimmune bovine colostrum to *Cryptosporidium* oocysts by direct duodenal infusion of an adult patient with AIDS and with severe symptoms of diarrhea resulted in both remission of symptoms and elimination of oocysts in stool of the patient (Ungar *et al.*, 1990). More studies, however, are required before this approach can be adapted to the general population (Nord *et al.*, 1990).

IV. Vaccination and Passive Immunization against Dental Caries

Dental caries is an infectious disease in which the principal causative agents are mutans streptococci including *Streptococcus mutans* and *Streptococcus sobrinus*. Based on the antigenic specificity of cell wall carbohydrate antigen expressed by these organisms, one can distinguish *S. mutans* by expression of serotypes *c*, *e*, or *f*, while *S. sobrinus* only expresses either serotype *d* or *g*. Mutans streptococci of serotypes *a*, *b*, or *h* have been isolated almost exclusively from animals and are not considered as human pathogens. Epidemiological surveys have revealed that serotype *c* organisms are most frequently isolated from human dental caries (Hamada and Slade, 1980). The abilities of mutans streptococci to adhere to the tooth surface in the presence of sucrose and to release acids (mainly lactic acid) from various dietary sugars are etiologically important. Therefore, these two properties, i.e., adherence and acid production, can be considered virulence factors for mutans stretptococci. Adherence of the organisms to the tooth surface is promoted by cell-surface protein antigen (PA) and glucosyltransferases (GTases) catalyzing insoluble, adherent glucan systhesis. Inhibition of cellular adherence by vaccination or passive introduction of preformed antibodies may confer protection against dental caries (Russell and Johnson, 1987; Michalek and Childers, 1990).

A. Vaccination against Dental Caries

Immunization of susceptible hosts with *S. mutans* or virulence-related substance(s) (i.e., a caries vaccine) should induce immune responses that might prevent the organisms from colonizing the tooth surface and/or inhibit biochemical processes/virulence factors of the bacterium. This rationale has been verified, at least experimentally, in rodents and monkeys, but a divergence of opinion exists regarding the determinants for induction of protection among research groups (Hamada and Slade, 1980; Russell and Johnson, 1987). In brief, local or oral immunization with whole cells or isolated antigens of *S. mutans* or *S. sobrinus* induced enhanced levels of salivary S-IgA antibodies specific for immunogen used, which eventually lead to reduced development of dental caries. A variety of antigens including GTase, cell wall lysates, serotype carbohydrate, surface protein antigens (e.g., antigen I/II or PAc, Spa A and 74K protein) and other components have been used (McGhee and Michalek, 1981; Krasse *et al.*, 1987). Oral and/or systemic administration of immunogen has been studied largely in the rat model. Oral administration of purified antigens with liposome–adjuvant complex to rats resulted in higher levels of IgA in saliva and greater protection against caries induction in rats infected with *S. mutans* or *S. sobrinus* (Michalek and Childers, 1990). Enhanced salivary IgA responses were noted in humans following oral administration of GTase derived from *S. sobrinus* (Smith and Taubman, 1987).

In a series of studies by Lehner's group, it was shown that systemic immunization of monkeys with *S. mutans* whole cells or purified protein antigen I/II plus adjuvant induced significant serum IgG responses which correlated with reductions in caries development (Lehner, 1992). It is thought that the serum IgG gains access to the tooth surface through the gingival crevice to exhibit anti-caries activity. However, the mechanism for this transport remains to be elucidated.

B. Passive Immunization against Dental Caries

Passive immunization has been carried out by several research groups in an effort to avoid possible systemic side effects which could result from active immunization. Systemic and local passive immunizations using IgG from immune monkeys or mAb to protein antigen I/II protected monkeys against infection of *S. mutans* and the subsequent development of dental caries in monkeys (Lehner *et al.*, 1992). In rats, passive transfer from dams to their offsprings via colostral IgA/IgG antibodies specific for *S. mutans* antigens resulted in caries reduction (Michalek and Childers, 1990). Good sources of antibodies other than those from the mother must be found for passive immunization. Milk obtained from cows hyperimmunized with *S. mutans* was shown to contain high levels of anti-*S. mutans* IgG1 antibodies. Rats monoinfected with *S. mutans* and given a diet containing the IgG1 exhibited lower plaque scores, numbers of *S. mutans* in plaque, and development of dental caries (Michalek *et al.*, 1987).

Successful trials of passive immunity in enteric infectious diseases using yIgG (*vide supra*) raised the

possibility of conferring protection against *S. mutans*-induced dental caries. Therefore, hens were immunized with various *S. mutans* antigens. Special attention was given to purified cell-associated GTase (CA-GTase) as immunogen, since the enzyme was critically important in synthesizing water-insoluble glucan from sucrose. Hyperimmune yIgG specific for CA-GTase and whole cells but not cell free GTase inhibited cell adherence to the glass surface. Only yIgG to CA-GTase reduced plaque accumulation and dental caries development, indicating that CA-GTase is a major virulence attribute of *S. mutans* (Hamada *et al.*, 1991). On the other hand, other investigators claimed that immune yolk powder from hens immunized with whole cells of *S. mutans* resulted in fewer caries lesions (Otake *et al.*, 1991). The immunological specificities of the yolk powder were not determined. In addition, our preliminary studies have indicated that yIgG specific for PAc showed less caries development; however, yIgG to CA-GTase was more efficacious than anti-PAc yIgG. Thus, egg yolks from hyperimmunized hens should provide a convenient and economical source of antibodies for passive immunization. For production of yIgG, it is not necessary to process antibodies from peripheral blood or ascites; thus the production is suited to current regulations for experimental animal protection.

It should be noted here that transgenic plants capable of generating functional S-IgA specific for antigen I/II of *S. mutans* have been developed. Transgenic plants may be suitable for a large-scale production of recombinant S-IgA for passive immunotherapy to infectious diseases (Ma *et al.*, 1995).

IV. Summary and Prospects

In this chapter, we have summarized recent progress for passive immunity against various infectious diseases which affect the mucosal surfaces in humans and in experimental animal models. Passive immunization may be meaningful under the current situation that many oral vaccines exhibit limited efficacy, although oral vaccines are more desirable than other administration routes in many respects. Large quantities of antibodies should be prepared in other animals or *ex vivo*. We have emphasized in this chapter here that hen egg yolk antibodies can be isolated and purified for use in passive immunization. yIgG antibodies have been demonstrated to be efficacious by providing passive immune protection against a variety of microbial diseases. yIgG are superior in various aspects to other antibodies including those from bovine colostrum and milk as well as monoclonal antibodies. The advances in biotechnology and genetic engineering should lead to the development of molecular vaccines that can be used for preparation of antibodies in other animals for passive immunization. In conclusion, existing experimental results suggest that passive immune vaccines may be a promising research area in the health sciences.

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