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Passive Immunization: Systemic and Mucosal

Chapter 46

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The passive transfer of maternal immunity is responsible for keeping all mammalian species alive. The process of evolution developed effective mechanisms for the passive transfer of both systemic and mucosal immunity from the mother to her offspring. Experimental passive transfer of systemic immunity via serum antibody is well established, but the experimental passive transfer of mucosal immunity has only recently been accomplished. This chapter addresses the contributions of both natural and experimental mechanisms to the study of passive immunization.

NATURAL PASSIVE IMMUNIZATION

Systemic immunity

The transfer of systemic immunity (IgG) from mother to offspring occurs prenatally via the placenta or yolk sac and after birth via the colostrum. Species vary in the contribution each route makes to the transfer of immunity (Waldman and Strober, 1969) and can be grouped into three categories: prenatal transfer only, combined prenatal and postnatal transfer, and postnatal transfer only.

Prenatal transfer only

This group includes primates, rabbits, and guinea pigs. Transport of IgG in primates occurs almost exclusively through the placenta. IgG transfer occurs via a receptor-mediated transcytosis across the syncytiotrophoblast and a transcellular pathway through the fetal endothelium (Leach *et al.,* 1990). Human placental transfer of protective IgG antibodies to a number of pathogens, including hepatitis B (Hockel and Kaufman, 1986), measles (Lennon and Black, 1986), and group B streptococcus (Baker *et al.,* 1988), has been reported. This process suggests an effective method of neonatal immunization, *i.e.,* immunization of the pregnant mother in order to protect the neonate. Prenatal transfer of IgG in the rabbit occurs via the yolk sac, and in the guinea pig, via both the yolk sac and fetal gut (Waldman and Strober, 1969).

Combined prenatal and postnatal transfer

This group includes rats, mice, cats, and dogs. Prenatal transmission occurs via the yolk sac/placenta and the fetal gut in the rat (Waldman and Strober, 1969). IgG is bound rapidly to receptors on the surface of the yolk sac membrane (Mucchielli *et al.,* 1983), is endocytosed in clathrin-coated vesicles, and, early in gestation, is stored in subapical vacuoles. By late gestation, the antibody has been hydrolyzed or transferred to fetal capillaries (Jollie, 1985). Prenatal transmission in mice occurs by a similar mechanism (Gardner, 1976).

Although placental transfer occurs, studies in rodents have shown that most transport of antibody occurs postnatally from colostrum or milk (Arango-Jaramillo *et al.,* 1988; Barthold *et al.,* 1988; Heiman and Weisman, 1989; Kohl and Loo, 1984; Nejamkis *et al.,* 1975; Oda *et al.,* 1983) over a period of 10 to 21 days, depending on the species. There is a gradual decrease in transmission over the last 3 days (Waldman and Strober, 1969), and transmission is limited to antibodies of the IgG class (Appleby and Catty, 1983; Hammerberg *et al.,* 1977). Transport is a receptor-mediated process (Simister and Rees, 1983). In rats, the receptor (FcRn) is found in enterocytes of the proximal intestine during the early postnatal period but is absent after weaning (Jakoi *et al.,* 1985). FcRn is specific for IgG and its Fc fragment and consists of two similar polypeptides of 48,000 to 52,000 daltons (p51) in association with β2 microglobulin (Jakoi *et al.,* 1985; Simister and Mostov, 1989). The Fc binding subunit (p51) has three extracellular domains and a transmembrane region that are all homologous to the corresponding domains of class I major histocompatibility complex (MHC) antigens (Simister and Mostov, 1989). Junghans and Anderson (1996) have shown that disruption of the FcRn in knockout mice also destroys the receptor (FcRp) necessary for the prolonged half-life of serum IgG in adults, suggesting that the same receptor protein that mediates transient IgG transport across the neonatal gut functions as the FcRp throughout life.

Postnatal transfer only

This group includes ruminants (cattle, sheep, goats), horses, and pigs (reviewed in Tizzard, 1987). Transport of colostral proteins from the lumen of the ileum in ruminants is largely nonspecific, but in the horse and the pig, IgG and IgM are preferentially absorbed. Proteins are actively taken up by epithelial cells through pinocytosis and passed through these cells into the lacteals and intestinal capillaries (Tizzard, 1987). Intestinal absorption occurs for only the first 24 to 48 hours after birth. Following this, the "open gut" closes down, and no further transfer from milk or colostrum occurs (Ellis *et al.,* 1986; Francis and Black, 1984; Tizzard, 1987; Waldman and Strober, 1969). Newborn piglets have also been shown to absorb colostral lymphoid cells during this period (Tuboly *et al.,* 1988). It is unclear whether these cells are fully functional and capable of immune processes such as the transfer of delayed-type hypersensitivity (DTH).

Absorption of colostral immunoglobulin is normally extremely effective, supplying the newborn with serum immunoglobulin (particularly IgG) at a level approaching that found in adults (Tizzard, 1987); however, failure of passive transfer (FPT) can occur and, when it does, can pose a considerable problem in animal husbandry. About 25% of newborn foals fail to obtain sufficient quantities of immunoglobulin (McGuire *et al.,* 1975; Tizzard, 1987). In the McGuire study (1975), two of nine foals affected by FPT died of infections within a few days of birth, and five of the remaining seven developed nonfatal respiratory infections between 2 and 5 weeks of age. McGuire *et al.* (1976) also reported FPT in calves, finding that 85% of calves less than 3 weeks old dying from infectious diseases have significant hypogammaglobulinemia. Although adequate methods to diagnose and treat FPT are available (Bertone *et al.,* 1988; Tizzard, 1987), the phenomenon remains a significant veterinary problem.

Mucosal immunity

Mother's milk provides passive protection of the mucosal surfaces it contacts. This protection may be mediated either by specific immunity or by nonspecific factors found in milk, such as lactoferrin, lysozyme, fatty acids, and complement (reviewed in Goldman *et al.,* 1985). The antibody composition of milk differs from that of colostrum (Tizzard, 1987), and the class of protective antibody in milk varies with the species and the route of immunization of the mother. With the exception of IgG in rodents, these protective antibodies are not systemically absorbed by the suckling offspring, but exert their protective effect locally by neutralizing viruses or virulence factors and by binding to microbial pathogens and preventing their attachment to the mucosal surface (Goldman *et al.,* 1985). Secretory IgA (S-IgA) is especially suited to this protective role, because secretory component enhances its resistance to proteolytic enzymes and gastric acid (Kenny *et al.,* 1967; Lindh, 1975; Tomasi, 1970; Zikan *et al.,* 1972), providing extra antibody stability in mucosal secretions.

Milk antibody in rodents

Rodents have been a popular model for the study of passive transfer of maternal immunity via milk; however, this class of animals has a major drawback as a model for passive *mucosal* immunity. Both rats and mice can actively transport IgG from the gut into the serum for approximately 2 weeks (see Combined Prenatal and Postnatal Transfer earlier in this chapter); thus, observed protection could be due either to antibody in the milk bathing the mucosal surfaces or to maternal antibody being transported into the serum and secretions of the offspring. This caveat should be kept in mind during evaluation of the many reports of milk-borne protection in these species. Three rodent models in which protection of mucosal surfaces is due to milk-borne, not serum-derived, antibody are described next.

The predominant immunoglobulin in mouse milk is IgG, although significant levels of IgA can also be present (Ijaz *et al.,* 1987). Protection of infant mice from colonization with *Campylobacter jejuni* can be achieved by the consumption of immune milk at and after the time of bacterial challenge. Infant mice were not protected by prior consumption of colostrum, showing that milk antibody was required in the gut lumen for protection to be observed (Abmiku and Dolby, 1987). A similar requirement for antibodies active at the intestinal cell surface in immunity to primate rotavirus SA-11 was reported by Offit and Clark (1985).

Protection of rats against dental caries by milk can be due to either IgG or S-IgA antibodies, depending on the route of maternal immunization. Rat dams immunized intravenously with heat-killed *Streptococcus mutans* developed IgG antibodies in their colostrum, milk, and serum. Their offspring demonstrated significant protection against *S. mutans*–induced caries formation. Rat dams locally injected in the region of the mammary gland with heat-killed *S. mutans* or fed formalin-killed *S. mutans* developed S-IgA antibodies in their colostrum and milk. Their offspring were also protected against caries formation (Michalek and McGhee, 1977). Caries protection in suckling rats could theoretically be due to bathing of mucosal surfaces and/or leakage of antibody into the saliva from the serum. Nonimmune adult rats can be protected from *S. mutans*–induced caries by feeding on lyophilized immune bovine milk or on immune bovine whey containing specific IgG (Michalek *et al.,* 1978a; Michalek *et al.,* 1987). Since adult rats are unable to transport orally administered IgG into their serum, protection must be from milk-derived antibodies bathing the oral cavity.

Milk antibody in ungulates

In ruminants (sheep, cattle, goats), the predominant antibody in both colostrum and milk is IgG. The predominant antibody in the colostrum of pigs and horses is also IgG, but as lactation progresses and colostrum becomes milk, IgA predominates (Tizzard, 1987). Protection can be mediated by either antibody class. While bathing of the mucosal surfaces by milk-derived antibodies can provide passive immunity to some pathogens, the high rate of infections in FPT

foals and calves shows that milk (mucosal immunity) alone cannot provide complete protection to neonates.

In cattle and pigs, passive immunity against enteric infections with viruses such as rotaviruses and coronaviruses (transmissible gastroenteritis, or TGE) is dependent on the continual presence in the gut lumen of a protective level of specific antibodies (Crouch, 1985). Passive immunity against intestinal infection with the TGE virus is generally more complete in piglets ingesting IgA antibodies than in those ingesting IgG antibodies, although both classes of antibody are protective. The class of antibody present in the sow's milk depends on the route of immunization (Bohl and Saif, 1975). In cattle, passive immunity in calf scours (neonatal bovine colibacillosis caused by *Escherichia coli*) correlates with the level of specific IgA antibody in the mother's milk (Wilson and Jutila, 1976).

Milk antibody in primates

In primates, IgA is the predominant immunoglobulin in both colostrum and milk (Tizzard, 1987). Both lysozyme and S-IgA in human milk remain functional in the digestive tract of the early infant (Eschenburg *et al.,* 1990). Human milk has been shown to contain S-IgA antibodies to at least five viral and nine bacterial pathogens, as well as to fungi, parasites, and food antigens (Goldman *et al.,* 1985). Mucosal immunity to rotavirus, for example, was shown to be transferred to the infant by the S-IgA in milk; there was a positive correlation between titers of secretory component (SC) in the mother's milk and the infant's feces vs. virus-specific IgA in the infant's fecal samples (Rahman *et al.,* 1987). In addition to providing passive mucosal immunity, human breast milk also stimulates the early local production of S-IgA in the urinary and gastrointestinal tracts, thereby accelerating the development of an active local host defense in the infant (Koutras and Vigorita, 1989; Prentice, 1987).

EXPERIMENTAL PASSIVE IMMUNIZATION

Since the original demonstration of transfer of immunity by the injection of serum (von Behring and Kitasato, 1890), passive transfer of humoral immunity has been intensively investigated. The use of specific serum antibody (IgG) to transfer protection to nonimmune individuals is now standard medical practice in, for example, the postexposure prophylaxis for rabies and tetanus and the treatment of snakebite (Arnold, 1982; Centers for Disease Control, 1991a, 1991b), while intravenous immunoglobulin (IVIg) treatment has been shown to lower the incidence of pneumonia in patients with common variable immunodeficiency (Busse *et al.,* 2002).

Local immunity has been correlated with the level of IgA antibody in various secretions (reviewed in Renegar and Small, 1993); however, direct demonstration of the mediation of local immunity by injected IgA could not occur until specific transport of passively administered IgA had been confirmed.

Transport of passively administered IgA to mucosal surfaces

Gastrointestinal tract

In rabbits, rats, and mice, polymeric IgA (pIgA) is efficiently transported from the circulation into the bile via the liver (Delacroix *et al.,* 1985; Koertge and Butler, 1986a; Mestecky and McGhee, 1987; Orlans *et al.,* 1978, 1983). These species express pIg receptor (pIgR) on their hepatocytes (Socken *et al.,* 1979) and, in addition, have pIgA as the primary molecular form in their serum (Heremans, 1974; Vaerman, 1973). Serum IgA is also efficiently transported into bile in cattle (Butler *et al.,* 1986). In fact, most IgA in ruminant bile may be of serum origin.

Transport of serum IgA into bile in humans has been reported (Delacroix *et al.,* 1982; Dooley *et al.,* 1982), although IgA transport is about 50-fold less efficient than in rats and rabbits. The human biliary IgA level is approximately 20% of the human serum IgA level and, under physiologic conditions, only 50% of human biliary IgA is derived from the serum (Vaerman and Delacroix, 1984). Even though transport is possible, passively administered IgA does not reach high levels in human bile. In one study, less than 3% of intravenously injected radiolabeled pIgA was found in human bile at 24 hours (Vaerman and Delacroix, 1984).

Saliva

Serum pIgA can be transported into saliva in dogs (Montgomery *et al.,* 1977), monkeys (Challacombe *et al.,* 1978), mice (Falero-Diaz *et al.,* 2000), and humans (Delacroix *et al.,* 1982; Kubagawa *et al.,* 1987). In humans the amount of IgA acquired from the plasma is low (only 2%) compared with the amount acquired from local production (Delacroix *et al.,* 1982). Transfer of pIgA from the plasma into canine or murine saliva is a selective process requiring the pIgR (Montgomery *et al.,* 1987; Falero-Diaz *et al.,* 2000), while transport into oral fluids in monkeys appears to be by leakage from the plasma into the crevicular spaces surrounding the deciduous molars (Challacombe *et al.,* 1978).

Milk

In sheep, active transport of IgA from the circulation into milk seems likely (Sheldrake *et al.,* 1984); however, studies on the transport of IgA into murine milk have produced conflicting results. Using radiolabeled IgA, Halsey *et al.* (1983) demonstrated that in the mouse, IgA can be transported from the circulation into milk during early lactation. Other investigators (Koertge and Butler, 1986b; Russell *et al.,* 1982), using assays based on antibody-binding activity, were unable to show transport of IgA into murine milk. Using radiolabeled IgA, Koertge and Butler (1986b) were able to show that the IgA present in milk was degraded and suggested that the previous study (Halsey *et al.,* 1983) detected only IgA fragments that had been transudated into the milk from the serum and not specifically transported IgA. Passively administered IgA is not transported into the milk of rats (Dahlgren *et al.,* 1981; Koertge and Butler, 1986b).

Respiratory tract

Only a limited number of studies on the transport of antibodies into respiratory secretions have been reported, but the results have shown that selective transport of passively administered serum IgA into the respiratory tract is possible in sheep and mice. Because of their importance as background to the experiments demonstrating the passive transfer of local immunity by IgA, these respiratory transport studies will be addressed in more detail.

Sheep. Using the intravenous injection of radioiodinated ovine immunoglobulin, Scicchitano *et al.* (1984) showed that 35% of the IgA in the mediastinal lymph of sheep is plasma-derived. It was further demonstrated (Scicchitano *et al.*, 1986) by the simultaneous intravenous injection of radiolabeled IgA and radiolabeled IgG_1 or IgG_2 that IgA is selectively transported into ovine respiratory secretions. Transport of IgA was approximately 4.5 times greater than transport of IgG, and the transported IgA was intact in the secretions. Biological activity of the transported IgA was not determined.

Mice. Mazanec *et al.* (1989) found that 4 to 5 hours after the intravenous injection of radiolabeled monomeric or polymeric IgA anti-Sendai virus monoclonal antibodies into mice, transport of pIgA into nasal secretions was three to seven times more efficient than transport of monomeric IgA (mIgA), while pIgA transport into bronchoalveolar lavages was only one to three times more efficient. This difference may reflect an increased contribution of serum antibody due to the transudation of IgG into alveolar fluids. Transport of pIgA into the gut was four to five times more efficient than the transport of mIgA, as expected. The agreement of the nasal secretion and gut transport indices suggests that transport at these two sites could occur by a similar mechanism. The investigators were unable to demonstrate the presence of functionally intact pIgA in the upper respiratory tract.

The pIgA transported into murine nasal secretions in the studies reported by Renegar and Small (1991a) was, in contrast, functionally intact. To avoid problems associated with the quantification of intact vs. degraded radiolabeled IgA in secretions (described by Koertge and Butler, 1986b), this study used an anti-influenza enzyme-linked immunosorbent assay (ELISA) to evaluate the transport of monomeric or polymeric IgA or IgG_1monoclonal anti-influenza antibodies into the nasal secretions of mice. Nonimmune mice were injected intravenously with influenza-specific mIgA, pIgA, or IgG_1, and sacrificed at varying times between 2 and 24 hours postinjection. The peak nasal wash pIgA titer was reached 4 hours after antibody injection and was approximately 35 times greater than the nasal wash titer of either monomeric immunoglobulin.

To determine whether pIgA was selectively transported relative to IgG_1, the investigators injected a mixture of the two monoclonals intravenously into nonimmune mice and calculated a selective transport index for nasal antibody for each mouse. Twenty-nine of the 31 mice studied showed selective transport of IgA relative to IgG. Using a similar model, Steinmetz *et al.* (1994) determined that passively administered monoclonal pIgA isotype-switch variants, generated from IgG hybridomas producing antibodies specific for bacterial respiratory tract pathogens, were selectively transported relative to IgG into both the upper and lower respiratory tract secretions of mice. In agreement with the results of Renegar and Small (1991a), Falero-Diaz *et al.* (2000) showed that, in mice, parenterally administered (either intravenously or by "backpack" tumor growth) monoclonal pIgA acquired SC as it was transported from the serum into nasal or vaginal secretions or into the bile, while similarly administered IgG did not, suggesting specific transport of the IgA but not the IgG. In contrast to Steinmetz *et al.* (1994) but in agreement with Mazanec *et al.* (1989), the Falero-Diaz group found efficient transmission of IgG but not of pIgA into the lungs of mice. In fact, they found that topical administration under light anesthesia of IgA as nosedrops was a more effective method for the delivery of IgA into the lungs than either parenteral method.

Thus, transport of serum IgA into nasal secretions is possible in some species. The relevance of this transport to the passive transfer of local immunity will be addressed in the following section.

Protection of mucosal surfaces by passively administered antibodies

Studies of the passive transfer of local immunity can be classified into two categories. In the first are those studies in which the antibody is introduced into the local secretions exogenously or mixed with the target pathogen prior to host challenge. The second category includes those studies in which systemically administered pIgA must be physiologically transported by a pIgR-mediated mechanism to its site of activity.

Exogenously administered antibody

The studies in this category have investigated the role of IgA in mucosal immunity by feeding antibody or instilling it intranasally or intravaginally and then challenging, or by administering antibody-pathogen mixtures intranasally.

Oral antibody. Offit and Clark (1985) demonstrated the ability of milk-derived IgG and IgA to protect the murine intestine from infection with primate rotavirus SA-11. Suckling mice were protected by milk from dams that had been orally immunized with SA-11 virus. This protective activity was detected in both the IgG and IgA fractions, but the IgA fraction was more potent *in vivo* than the IgG fraction. In newborn mice from immune dams foster-nursed on seronegative dams, the presence of circulating systemic antirotavirus antibodies in high titer did not protect against SA-11 viral infection. Thus, the specific antibody had to be present in the gut lumen to protect the intestinal cell surface from viral infection, and S-IgA could mediate this protection.

Enriquez and Riggs (1998) developed a series of dimeric IgA monoclonal antibodies directed toward the sporozoite antigen P23 of *Cryptosporidium parvum,* an important diarrhea-causing protozoan parasite. When administered orally prior to parasitic challenge, these antibodies were able to

reduce the number of intestinal parasites in infected neonatal mice by up to 70%. These results extend the work of Albert *et al.* (1994), who successfully treated cryptosporidiosis in nude mice by the oral administration of rat bile containing *C. parvum*–specific IgA, while Czinn *et al.* (1993) protected germ-free mice from infection by *Helicobacter felis* by incubating the bacteria with specific IgA antibody prior to oral administration. The protective antibody was later shown to be directed against urease (Blanchard *et al.,* 1995).

A significant number of systemic infections in the human neonate originate from the gastrointestinal tract, especially in premature infants with immature gut barriers. Maxson *et al.* (1996) fed rabbit pups human S-IgA via intragastric gavage, then challenged them with *E. coli* K100. IgA-treated pups had significantly fewer bacteria translocated from the gut to the liver, spleen, and mesenteric lymph nodes. This neonatal rabbit model provides the first demonstration of control of bacterial translocation by IgA and suggests that oral supplementation with IgA may be beneficial for patients at risk for gut-origin sepsis.

Intranasal antibody. A number of studies have shown that exogenously administered IgA can protect against intranasal challenge with a pathogen. Bessen and Fischetti (1988) showed that S-IgA given by the intranasal route protected mice against streptococcal infection. Live streptococci were mixed with affinity-purified human salivary S-IgA or serum IgG antibodies directed toward the streptococcal M6 protein. The mixture was administered intranasally to mice. The S-IgA antibody protected against streptococcal infection, while the serum antibody had no effect. This study suggested that S-IgA alone is capable of protecting the mucosa against bacterial invasion.

Mazanec *et al.* (1987) demonstrated that IgA can protect mucosal surfaces against viral infection. Ascites containing IgA anti-Sendai virus monoclonal antibody was administered intranasally to lightly anesthetized mice before and after the mice were challenged intranasally with live virus. Three days later, mice were sacrificed and lung viral titers were determined. Animals treated with the specific monoclonal antibody were protected against viral infection. Further work from the same laboratory showed that local immunity to Sendai virus can also be mediated by intranasally administered IgG (Mazanec *et al.,* 1990). Tamura *et al.* (1991) purified anti-influenza S-IgA antibodies from the respiratory tracts of mice immunized with influenza hemagglutinin molecules. This IgA, when given intranasally, protected nonimmune mice from influenza infection. Protection was observed up to 3 days after antibody administration, was proportional to the amount of IgA administered, and was observed at IgA doses equivalent to naturally occurring antibody titers.

Intravaginal antibody. Zeitlin *et al.* (1998) were able to protect the mouse vagina from infection with herpes simplex virus 2 (HSV-2) by the topical administration of either IgG or IgA monoclonal antibody directed against glycoprotein D of HSV-2.

These studies show that topically administered local IgA or IgG can protect against viral or bacterial infection of the mucosa. They do not show that physiologically transported (secretory) IgA or serum-derived IgG actually does so. For that demonstration, antibody must be administered parenterally and transported into the mucosal secretions by a physiologic mechanism. The studies presented in the next section satisfy that criterion.

Systemically administered antibody

The definitive studies in this category have involved the respiratory and gastrointestinal tracts, although passive transfer of uterine immunity by pIgA has also been observed (Renegar and Small, 1993; Cotter *et al.* 1995; Pal *et al.* 1997), and Leher *et al.* (1999) demonstrated the protection of Chinese hamsters and pigs against *Acanthamoeba castellanii*–mediated keratitis by intraperitoneally administered antigen-specific monoclonal IgA. Work with the respiratory and gastrointestinal tracts will be presented in more detail.

Respiratory tract. The respiratory tract can be separated into an upper region (nose and trachea) and a lower region (lungs and bronchi) with immunity at each site involving different elements of the immune system. Numerous studies have shown that passively administered serum anti-influenza antibody (IgG) can prevent lethal viral pneumonia (Barber and Small, 1978; Kris *et al.,* 1988; Loosli *et al.,* 1953; Ramphal *et al.,* 1979; Palladino *et al.,* 1995). Serum antibody, however, does not prevent influenza infection of the upper respiratory tract (Barber and Small, 1978; Kris *et al.,* 1988; Ramphal *et al.,* 1979). Protection of the nose correlates with an increased nasal secretion IgA antibody level (reviewed in Renegar and Small, 1993), making influenza an excellent model in which to investigate the hypothesis that nasal immunity is mediated by S-IgA.

The first demonstration of the passive transfer of local immunity by physiologically transported S-IgA was reported by Renegar and Small (1991a) in the murine influenza model. They showed, as described above, that intravenously administered pIgA is transported into nasal secretions. To determine whether intravenously administered pIgA anti-influenza monoclonal antibody could mediate protection against local influenza virus challenge, passively immunized mice were challenged intranasally while awake with influenza virus. Twenty-four hours later the mice were sacrificed and the amount of virus shed in their nasal secretions was determined. Of the 24 saline-injected control mice, 23 shed virus into the nasal secretions, while only 5 of the 25 pIgA injected mice shed virus, and those 5 that did shed virus had a low titer. The observed protection was significant ($p < 0.001$). Passive immunization with influenza-specific pIgA therefore conferred complete protection against viral infection in 80% of the mice and partial protection in the remaining 20%. Serum IgG was found to confer only limited protection against nasal influenza infection (minimal reduction in viral shedding [$p < 0.02$], with only one of eight mice intravenously injected with influenza-specific IgG not shedding virus [$p < 0.5$]).

The passive protection studies showed that IgA *can* mediate local immunity. To confirm that IgA *is* the mediator of

local immunity, mice passively immunized with pIgA were given nose drops of anti-IgA antibody 10 minutes before and 6 hours after they were challenged intranasally with influenza virus suspended in anti-IgA antiserum. Anti-IgA treatment abrogated IgA-mediated protection in the passively immunized mice (Renegar and Small, 1991a). To show that the passive transfer of local immunity by IgA was a reflection of the natural situation, the abrogation technique was extended to mice convalescent from influenza infection (Renegar and Small, 1991b). Nonimmune mice and convalescent mice, *i.e.,* mice that had recovered from an influenza virus infection 4 to 6 weeks earlier and were therefore naturally immune, were treated intranasally with antiserum to IgA or IgG or with a mixture of antisera to IgG and IgM and then challenged while awake with influenza virus mixed with antiserum. Intranasal administration of antiserum was continued at intervals for 24 hours. One day after viral challenge, the mice were killed and their nasal washes were assayed for virus shedding. Nonimmune mice all became infected, regardless of whether the virus was administered in saline, normal rabbit serum, or anti-immunoglobulin antiserum. Convalescent mice, as expected, were protected from viral infection. Administration of influenza virus in either anti-IgG or a mixture of anti-IgG and anti-IgM antisera did not affect protection, *i.e.,* the convalescent mice were still immune. Administration of virus in anti-IgA antiserum, however, abrogated convalescent immunity. These results demonstrate that IgA is a major mediator of murine nasal immunity and suggest that passive immunization mimics the role S-IgA plays in natural immunity. These observations were extended by the work of Philipon *et al.* (1995). Using the backpack method of monoclonal antibody administration, they demonstrated that monoclonal IgA antibody directed against *Shigella flexneri* serotype 5a lipopolysaccharide protects mice against intranasal challenge with *S. flexneri.*

Mbawuicke *et al.* (1999), however, have used the IgA knockout mouse model to challenge these findings. Mice unable to produce IgA antibodies were able to generate a protective anti-influenza response and to transport passively administered anti-influenza antibodies into both the upper and lower respiratory tracts. Knockout mice, however, may not be the best model in which to study the role of IgA in nasal immunity, because, with the congenital loss of IgA, production of other classes of antibody such as IgG may be increased as a compensatory mechanism. The data of Mbawuicke *et al.* suggest that this may be the case, as influenza-specific IgG levels were higher in immune knockout mice than in normal mice, and an altered IgG subclass distribution in response to influenza infection was observed (Harriman *et al.,* 1999; Zhang *et al.,* 2002). Furthermore, perturbation of mucosal IgA transport in the pIgR knockout mouse (Johansen *et al.,* 1999) led to increased mucosal leakiness and increased serum total IgG levels, indicating a defect in the mucosal barriers. It is known (Renegar *et al.,* 2004) that at a dose high enough to give a serum titer seven times the normal anti-influenza IgG titer of convalescent mice, intravenously administered influenza-specific IgG can lower or eliminate the nasal secretion viral load; however, scanning electron microscopy has revealed that even this high IgG dose does not prevent infection of the nasal epithelium. Thus, the lowered nasal secretion viral titers reported in IgG-protected mice may be due to neutralization of newly replicated virus by serum antibody leaking through the virally damaged epithelium. Furthermore, the serum anti-influenza IgG antibody titer has to be several times higher than that normally observed for the IgG effect to be observed, because serum antibody at a level comparable to that of normal convalescent mice neither depresses viral shedding nor prevents viral pathology in the nasal epithelium.

Since the publication of the IgA knockout mouse work, a model has been reported in which mucosal IgA levels are depressed in genetically normal mice while serum IgG remains unaffected (Renegar *et al.,* 2001a, 2001b). In mice, total parenteral nutrition (TPN) depressed nasal mucosal immunity to influenza virus, resulting in increased viral shedding from the noses of TPN-fed immune mice **(Fig. 46.1A)**. In these mice, nasal influenza–specific IgA was severely depressed **(Fig. 46.1B)**, while the serum anti-influenza IgG titer was unaffected **(Fig. 46.1C)**. Thus, in genetically normal ICR mice, serum IgG alone is not capable of preventing viral infection of the nose. Protection could be restored by the intravenous administration of influenza-specific pIgA monoclonal antibody **(Fig. 46.1D)**. This work strongly suggests that IgA is required for the prevention of influenza virus infection in the noses of normal mice.

Gastrointestinal tract. Additional evidence that S-IgA is the mediator of local immunity comes from studies of the gastrointestinal tract. Polymeric IgA hybridomas against *Vibrio cholerae* were generated and the resulting monoclonal antibodies were used to determine whether IgA can mediate immunity toward a bacterial pathogen in the gut (Winner *et al.*, 1991). The investigators selected a clone that produced dimeric monoclonal IgA antibodies directed against an Ogawa-specific lipopolysaccaride carbohydrate antigen exposed on the bacterial surface. These antibodies were able to cross-link bacterial organisms *in vitro,* suggesting that they might be effective in preventing mucosal colonization by the pathogen *in vivo.* To provide continuous physiologic (*i.e.,* secretory-component–mediated) transport of specific antibody into the gut, hybridoma cells were injected subcutaneously into the backs of adult BALB/c mice.

These "backpack" tumors released monoclonal IgA into the circulation, and the plasma IgA was transported into the gut lumen. Neonatal mice bearing these backpack tumors survived challenge with *V. cholerae,* while neonatal mice bearing backpack tumors of unrelated IgA hybridomas and non-tumor-bearing neonatal mice died. This ingenious model provided the first evidence that S-IgA alone can mediate mucosal immunity to a bacterial pathogen. Michetti *et al.* (1992) have since demonstrated that pathogen-specific monoclonal S-IgA can protect mice from infection by *Salmonella typhimurium* following oral challenge.

The backpack model has also been used to treat rotavirus infections of the gastrointestinal tract of mice (Burns *et al.*,

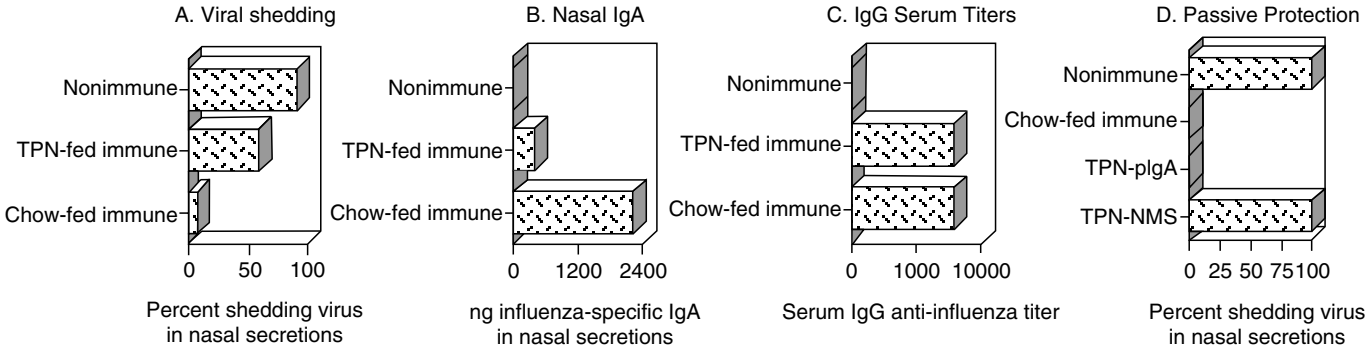

Fig. 46.1. Influenza immunity in total parenteral nutrition (TPN)–fed mice. **A,** Viral shedding: Following the surgical placement of intravenous catheters, influenza immune mice were fed mouse chow or intravenous TPN solution (IV-TPN). Nonimmune mice were fed chow. Following 5 days on their respective protocols, mice were challenged intranasally while awake (i.n.) with influenza virus and were assayed 42 hours later for the shedding of virus in their nasal secretions. This figure represents the pooled data from 17 experiments. Nonparametric statistical analysis: chow-fed immune vs. IV-TPN–fed immune, $p < 0.0001$; chow-fed immune vs. nonimmune, $p < 0.0001$. **B,** Nasal IgA: Fifteen mice were infected i.n. while awake with PR8 (H1N1) influenza virus. Three weeks later, they underwent surgical instrumentation and were randomized to IV TPN feeding ($n = 8$) or chow feeding ($n = 7$). Following 5 days on protocol, the mice were sacrificed. Levels of influenza-specific IgA in nasal secretions were determined by ELISA and normalized to ng-specific IgA/100 µg nasal protein (chow vs IV TPN, $P < 0.05$, ANOVA). Influenza-specific IgA was undetectable in nonimmune mice. **C,** Influenza-specific serum IgG titers: Immune mice ($n = 11$) underwent instrumentation and were randomized to IV TPN feeding ($n = 6$) or chow feeding ($n = 5$). Following 5 days on protocol, mice were sacrificed and serum IgG anti-influenza titers and nasal viral shedding were determined. Antibody titers were the serum dilution with an ELISA absorbance reading of 0.200 or greater. The IgG titers were comparable in chow-fed and TPN-fed immune mice. All of the TPN-fed mice shed virus in their nasal secretions, while none of the chow-fed mice did so. **D,** Passive protection: Immune mice were fed on mouse chow or IV-TPN. Following 5 days on their respective protocols, the IV-TPN mice were divided into two groups. Nonimmune controls, ($n = 5$), the chow-fed immune mice ($n = 5$), and one of the IV-TPN groups (TPN-NMS, $n = 4$) received 400µl ascites fluid containing MOPC 315 (does not recognize influenza) pIgA monoclonal Ab IV. The second IV-TPN group (TPN-pIgA, $n = 4$) received 400 µl ascites fluid containing pIgA anti-influenza monoclonal Ab. Four hours later, all mice were challenged i.n. with PR8 influenza virus. After 48 hours, the mice were euthanized and their nasal secretions assayed for viral shedding. Nonparametric statistical analysis: nonimmune vs. TPN-NMS, p = NS; immune vs. TPN-pIgA, p = NS; nonimmune vs. immune chow-fed, $p < 0.0001$; nonimmune vs. TPN-pIgA, $p < 0.0001$. Panels **A–C** modified from Renegar *et al.*, 2001b. Panel **D** from Renegar *et al.,* 2001a)

1996). Non-neutralizing monoclonal IgA antibodies directed against VP6, a major inner capsid viral protein, were capable of both preventing primary and resolving chronic murine rotavirus infections. These findings are consistent with the hypothesis that *in vivo* intracellular viral inactivation by pIgA during transcytosis is a mechanism of host defense against rotavirus infection.

CLINICAL APPLICATIONS

The general approach of passive parenteral transfer of mucosal immunity has proven to be a useful research tool for determining the role of S-IgA in protection against various mucosal pathogens. The possibility of therapy by passively administered IgA antibody is more problematic. Passive protection by injection is highly speculative because of both the questionable efficiency of transport to the targeted mucosal surface and the potential adverse effects of intravenous IgA antibody. Serum IgA has been associated with both decreased complement activation (Russell *et al.*, 1989) and decreased immune lysis (Griffiss and Goroff, 1983). Systematically administered pIgA may also be suppressive of both specific humoral and cellular responses (Renger and Small, unpublished observations). A more thorough knowledge of the role IgA can play in regulating the immune response is needed before intravenous passive mucosal immunization can become an acceptable means of therapy in man; however, the direct oral or respiratory application of antibodies to the mucosal surfaces is both practical and acceptable.

Control of dental carries is a feasible target for topical IgA administration. One study is particularly intriguing. Ma *et al.* (1998) generated a monoclonal secretory antibody in transgenic plants and showed that it survived up to 3 days in the human oral cavity; furthermore, this antibody afforded specific protection against oral streptococcal colonization for at least 4 months.

The literature contains a number of reports of the passive transfer of immunity against gastrointestinal pathogens in humans (reviewed in Bogstedt *et al.*, 1996, and in Hammarström *et al.* 1994). This immunity has been provided by the oral administration of purified human IgG or serum IgA and has been used as both a prophylactic and a therapeutic measure against rotavirus infection in children (Barnes *et al.* 1982; Guarino *et al.*, 1994) and as a therapeutic measure against bacterial diarrheas (Tjellstrom *et al.*, 1993; Hammarström *et al.*, 1993). Oral administration of bovine antibodies has also been used successfully in the prophylaxis of bacterial and rotaviral diarrheas in man and in the treatment of human rotavirus infections (Bogstedt *et al.*, 1996; Mitra *et al.*, 1995). Chicken egg yolk antibody (IgY) protects calves against bovine rotavirus (Kuroki *et al.*, 1994).

Provision of passive immunity by the intranasal administration of antibodies has been reported in both human and

nonhuman primate models. Human gamma globulin administered intranasally showed promise in challenge experiments with influenza or coxsackie A-21 viruses (Fruchtman *et al.*, 1972; Buthala *et al.*, 1970). IgA antibodies (IgAbulin), given as a nasal spray to the Swedish ski team during the Albertville Winter Olympic Games, significantly reduced the level of upper respiratory tract infections (Hammarström *et al.*, 1994); however, IgAbulin treatment had no effect on the frequency of upper respiratory tract symptoms in elite canoeists studied during hard and moderate training regimens (Lindberg and Berglund, 1996). IgAbulin nosedrop treatment of variable immunodeficiency patients who were chronic nasopharyngeal carriers of nonencapsulated *Haemophilus influenzae* eliminated the carrier state in 2 of 5 patients and alleviated coughing in all 5 (Lindberg *et al.*, 1993). Heikkinen *et al.* (1998) treated 40 children aged 1 to 4 years intranasally with IgA or a placebo and found a 42% reduction in rhinitis in the IgA-treated group. Thus, intranasal instillation of antibodies is feasible and may prove to be effective in the management of immunosuppressed patients.

Intranasal administration of specific antibodies may prove to be even more exciting. Weltzin *et al.* (1996) treated rhesus monkeys with nose drops containing mouse monoclonal IgA antibody against respiratory syncytial virus (RSV). Treated monkeys had reduced viral shedding in the nose, throat, and lungs and developed neutralizing serum antibody to RSV, even in the absence of detectible viral replication. RSV is a major cause of lower respiratory tract disease in infants and young children, producing severe disease in children with underlying conditions of the heart or lungs. These results suggest that prophylactic administration of monoclonal antibody nose drops could provide effective protection against RSV infection in at-risk human infants.

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