DIARRHEA: THE NEMESIS OF THE ARTIFICIALLY REARED, EARLY WEANED PIGLET AND A STRATEGY FOR DEFENSE¹

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ABSTRACT

Rearing early weaned piglets artificially for the purposes of increasing the efficiency of the sow is an attractive management concept. However, high death losses resulting from diarrhea in artificially reared piglets have dampened enthusiasm for early weaning. Enterotoxigenic *Escherichia coli*, transmissible gastroenteritis virus and rotavirus are the three main enteropathogens responsible for causing the diarrhea. The enteropathogens infect the small intestine, which produces a secretory or malabsorptive diarrhea. In nature, the nursing piglet is protected from the enteropathogens by antibody bathing his gut. The source of the antibody is the dam's colostrum and milk. It should be possible to protect artificially reared, early weaned piglets from enteropathogens by feeding them diets that contain antibodies to putative enteropathogens.

(Key Words: Piglets, Diarrhea, *Escherichia coli*, Transmissible Gastroenteritis Virus, Rotavirus.)

Introduction

The sow is used inefficiently. Sows farrow about 11 piglets per litter and wean 7.5. This 20 to 30% loss, from birth to weaning, occurs mainly in the first few days postpartum. The sow has a limited milking capacity and this limited capacity often dictates the number of pigs that will survive to weaning. Because more piglets are already being born than reared, scientists have been reluctant to direct their research efforts towards increasing the number of piglets produced per litter.

Schemes that increase the number of piglets in a litter would be rewarding if there were a practical way for artificially rearing the extra piglets, i.e., those numbers of piglets beyond the rearing capacity of the sow. Artificial rearing of piglets for the purposes of increasing sow efficiency has been an attractive concept for years (Lecce, 1975). The major drawback to rearing pigs artificially has been the high death losses associated with artificially reared, early weaned piglets. Diarrhea, the nemesis of

the early weaned piglet, accounts for most of the losses, and it seems that the earlier a pig is weaned, the more difficult it is to rear (Bellis, 1957). This may be so because the younger the piglet is, the more susceptible his enterocytes in the small intestine are to enteropathogens (Kohler, 1972; Moon et al., 1975; Kirstein et al., 1985).

Enterotoxigenic *Escherichia coli* (ETEC), transmissible gastroenteritis virus (TGE) and rotavirus are the main etiological agents that cause piglet neonatal diarrhea. Enterotoxigenic *Escherichia coli* produce a secretory diarrhea and the viruses produce a malabsorptive diarrhea. The cell that is perturbed by ETEC or destroyed by virus is the enterocyte in the small intestines. Immunoglobulin in the sow's colostrum and milk protects the nursing piglet from enteropathogens by neutralizing pathogens in the lumen of the gut.

Systems for rearing piglets artificially should not ignore the evolutionary wisdom of nature. As such, it would seem prudent in devising strategies for defense to imitate nature by feeding artificially reared piglets liquid diets that contain antibodies to the putative enteropathogens.

For the purposes of this symposium, this report provides the uninitiated with a brief background in immunology and suggests how the immune response can be husband for the

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control of diarrhea in the neonatal, artificially reared piglet. Also, the major etiological agents and the means whereby they produce diarrhea are described.

Background

Evolving vertebrates developed defenses for regulating the growth of pathogenic microorganisms. The immunological response is one such defense. A complicated interaction between pathogen (antigen), leucocytes and lymphoid tissue is involved in this defense. The leucocytes of primary importance are B-lymphocytes, T-lymphocytes and macrophages. B-lymphocytes are educated in the bursa of Fabricius in birds and the "bursa equivalent" in mammals. T-lymphocytes are educated in the thymus. Macrophages are phagocytic cells that develop from blood-borne monocytes.

Upon initial exposure to a pathogen, the host's macrophages phagocytize the pathogen and are activated. Antigen-activated macrophages interface with specific B- and T-lymphocytes. This interaction stimulates the B- and T-lymphocytes to proliferate into a clone or population of cells that are specifically geared to do battle with the pathogen. Only those lymphocytes that are keyed to the antigens on the pathogen expand into a clone.

Proliferating B-lymphocytes develop into clones of plasma cells. These plasma cells secrete immunoglobulins that can bind specifically and with high affinity to the inducing antigen (analogous to a key fitting a lock). Binding of antigen to antibody utilizes every chemical bond except a covalent bond. Immunoglobulins (Ig) or antibodies can be separated into five isotypes; IgG, IgM, IgA, IgD and IgE. The sequence of events from invading pathogen to the secretion of specific antibody is called the humoral response.

While B-lymphocytes are proliferating and developing into plasma cells, antigen-specific T-lymphocytes also are expanding into clones of T-killer, T-helper and T-suppressor lymphocytes. Killer T's react directly with the invading cell or lesion, suppressor T's down-regulate the immune response, and helper T's augment the response. The antigen-induced expansion of specific T-cells is called the cell-mediated response. In most cases, both humoral and cell-mediated responses are involved in the defense of the host.

If the dose of pathogen is low, or if the

pathogen is attenuated or killed (vaccine), often the host can mount an immune response and have no clinical disease. Otherwise the issue will favor the pathogen and disease will follow. An initial contact with pathogen leaves the host's immune system with a memory for the pathogen. If the host contacts the pathogen again, then the immune system responds quickly, by expanding the pool of committed B- and T-lymphocytes and pouring out specific antibodies and T-cells. Immunity acquired in response to an antigen is called active immunity. Active immunity is long lived—sometimes as long as the natural life of the host.

Immunity can also be acquired passively. In the case of humoral immunity, this can be accomplished simply by injecting immunoglobulins from an actively immunized animal into an immunologically naive animal. Passive immunity is short-lived, e.g., the half-life of IgG is about 2 wk.

The Immunological Status of the Mammalian Neonate

At birth the neonate is an immunological virgin. That is, the neonate has produced no active immunity because the neonate was not exposed to antigens while residing within the sanctity of the uterus. However, if the neonate is a human infant, he will have circulating Ig acquired passively from his mother via the placenta. At the other extreme, if the neonate is a piglet, in addition to being born immunologically naive, he will be agammaglobulinemic (no circulating Ig). The piglet rapidly acquires Ig postnatally by nursing his dam's colostrum (Lecce, 1984). This first milk is rich in IgG and IgA. Passively acquired Ig, either via colostrum or placenta, has a spectrum of antibodies that reflect the immunological history of the dam (prior exposure to pathogens or vaccines).

Normally then, newborns have no active or long-termed immunity but they do have a passive, short-termed immunity that reflects the pathogens in the dam's environment. Nature's strategy for protecting the neonate from clinical disease is to convert short-termed, passive immunity, as it wanes, into long-termed, active immunity. This occurs when the neonate is exposed to a level of pathogen that does not produce disease, but, instead, results in active immunity as the passive immunity approaches a null point. Catastrophy interrupts this live-and-let-live scenario when the neonate

receives inadequate passive protection and(or) the level of pathogen is high in the environment, e.g., poor sanitation (Lecce et al., 1978).

Even though the piglet ceases absorbing Ig from his gut into his blood when he is about 36 h of age, the Ig in the dam's milk has continuing value to the piglet as a regulator of enteropathogens. If the piglet is nursing a dam that has been immunized against enteropathogens in the environment, then the antibody in her milk will neutralize those enteropathogens in the nursing piglet's gut. The piglet's passively acquired circulating antibody is not protective, because not much of this antibody reaches the gut lumen.

Figure 1 approximates the amount of Ig the sow presents to the gut of a nursing piglet in a day, the amount of Ig the piglet absorbs from the colostrum and the amount of Ig synthesized by the piglet. The assumption is that a nursing, day-old piglet receives about 200 ml colostrum from his dam, and a 4-d-old about 400 ml of milk (extrapolated from Lecce and Matrone, 1960; Miller et al., 1961; Morgan and Lecce, 1964 and Bourne, 1973). The synthesis of Ig produces active immunity as opposed to the passive immunity coming from the Ig in the sow's colostrum and milk.

The Defense

When devising strategies for rearing neonatal piglets artificially (early weaning) consider that: 1) enteric infections and the ensuing diarrhea account for most of the morbidity and mortality occurring in artificially reared, early weaned piglets; 2) the principal etiological agents producing the diarrhea are *Escherichia coli* (colibacillosis), transmissible gastroenteritis virus (TGE) and rotavirus; 3) artificially reared, early weaned piglets can be protected passively from enteric infections by feeding them diets containing antibody to enteropathogens (Lecce and King, 1981).

The Enteropathogens

Escherichia coli (colibacillosis). Infectious neonatal diarrhea has been the object of intensive study since the pioneer work of Jensen in 1891, followed by Smith and Orcutt in 1925 (see Barnum et al., 1967 for review). This area of research focused on the etiological significance of *E. coli*. It has been difficult to establish an exact causal role for coliforms in neonatal diarrhea. This difficulty stems from

the fact that coliforms are normal to the gut flora. Furthermore, classifying *E. coli* by enteropathogenic serotypes has not been particularly useful. However, recent research has revealed that coliforms should be viewed as "enterotoxigenic" rather than "enteropathogenic" (Wilson, 1981).

Enterotoxigenic strains of *E. coli* produce protein pili ("K" antigens) and polysaccharide capsules (another kind of K antigen). They also synthesize a heat-labile (LT) enterotoxin, a heat-stable enterotoxin (ST_a, ST_b) or both LT and ST (Runnells and Moon, 1984). The protein pili are fibrils located on the outer surface of the bacteria. There are four major antigenic types of pili (K99, K88, K987P and F41) on *E. coli* isolated from pigs. Doubtlessly, others will be discovered. Epitopes on pili and capsules are ligands that react specifically with binding sites on the brush borders of enterocytes, resulting in the adherence and colonization of the gut by the toxigenic strain of *E. coli* (Guerrant et al., 1973; Nagy et al., 1977; Dean and Isaacson, 1985). Figure 2 shows *E. coli* adhering to a villus from the small intestine of a piglet infected with rotavirus and hemolytic enterotoxigenic *E. coli* (Lecce et al., 1983).

Figure 1. Grams Ig in 100 ml piglet serum or 500 ml of sow's colostrum or milk. Solid line: approximate amount of dietary Ig consumed a day by a nursing piglet (passive gut immunity). White bars: amount of colostrum Ig absorbed into the piglet's serum (passive circulating immunity). Black bars: amount serum Ig synthesized by the piglet (active immunity). Note, weaning abruptly removes the piglet from a source of passive gut immunity (extrapolated from Lecce and Matrone, 1960; Miller et al., 1961; Morgan and Lecce, 1964 and Bourne, 1973).

Figure 2. Scanning electron micrograph from jejunum of a piglet infected with both rotavirus and hemolytic enterotoxigenic *E. coli*. A) Villus completely covered with *E. coli*. B) An enlargement of (A) showing the packing of adhering *E. coli*. (Lecce et al., 1983). Bar = 1 μ m.

Adhering enterotoxigenic *E. coli* then secrete enterotoxins in situ. LT resembles *Vibrio cholera* toxin in that it increases the level of cyclic adenosine monophosphate (cAMP) within the intestinal mucosal cell. An increase in cAMP inhibits NaCl and water absorption and stimulates an increase in the secretion of Cl^- and HCO_3^- by crypt cells. ST_a increases the level of cyclic guanosine monophosphate (cGMP), which in turn blocks NaCl absorption while Cl^- and HCO_3^- secretion is increased (Rubino and Guandalini, 1984). The means whereby ST_b affects gut cells is not well understood (Kennedy et al., 1984). Increased amounts of salts and water in the lumen can lead to diarrhea, dehydration and death. It is important to note that *E. coli* produce little damage to the mucosa (Kohler, 1972; Moon, 1974). Except for the temporary perturbation of salt and water absorption, enterocytes appear normal. And the mechanism for glucose driven Na^+ -coupled absorption of amino acids remains intact. For this reason, oral rehydration solutions using glucose and salts seem to be of therapeutic value (Bywater and Woode, 1980).

Transmissible Gastroenteritis Virus (TGEV). In 1946, Doyle and Hutchings discovered that a virus was capable of causing an

acute, rapid, fatal, transmissible gastroenteritis (TGE) in neonatal piglets. Later it was learned that TGE is caused by a corona virus. Transmissible gastroenteritis virus is an 80- to 120-nm pleomorphic, enveloped virus with knobby projections, which appear in electron micrographs like a crown or corona surrounding the viral particle. The nucleic acid in TGEV is a single strand of ribonucleic acid (RNA). Because TGEV is surrounded by a lipid membrane and fragile knobs, it is susceptible to solvents and extremes in environmental temperatures. Transmissible gastroenteritis virus replicates in the enterocytes of the piglet's small intestine, and destroys these cells. There is a massive loss of absorptive surface, producing a malabsorptive diarrhea. Figure 3 shows scanning electron micrographs of normal and blunted villi from piglet's jejunum. In the upper panel, villi are normal. In the lower panel, villi are in various stages of destruction. These piglets were infected with rotavirus, but piglets infected with TGEV have similar pathologic changes (Olson et al., 1973; Lecce and King, 1978). Piglets with severe lesions rapidly dehydrate and die. The disease tends to be self-limiting in that herd immunity develops as the gilts and sows become infected and they in turn become immunized and capable of protecting their

Figure 3. Scanning electron micrograph from the mid-jejunum of colostrum-deprived piglets. Upper panel of three: normal elongated villi from non-infected controls. Lower panel of three: blunted, shortened and fused villi from piglets infected with rotavirus (Lecce and King, 1978). Piglets infected with TGEV have similar changes in villi (Olson et al., 1973). (X 446)

progeny via colostrum and milk (Haelterman, 1972). Furthermore, since the virus is fragile it tends to disappear from the farm. There is some evidence that oral rehydration, glucose electrolyte solutions may be of values in malabsorption diarrhea, as well as in secretory diarrhea (Bywater and Woode, 1980).

Rotavirus. In 1973, Coalson and Lecce described an infectious diarrhea in artificially reared piglets that was neither TGE nor colibacillosis (Lecce et al., 1972; Coalson and Lecce, 1973; Lecce et al., 1976). The agent mimicked TGE in that it was a virus that destroyed enterocytes in the small intestine, just like TGEV. This reovirus-like agent was later classified as a rotavirus (Lecce et al., 1976; Lecce and King, 1978). Rotavirus was a heretofore unrecognized group of viruses that infect the intestines of all mammalian neonates. Rotavirus replicates within enterocytes and destroys the cells, causing a loss of absorptive surface. This loss results in a malabsorptive diarrhea, dehydration and death—much like

pathogenesis seen with TGEV (figure 4).

Rotavirus is an icosahedral 70-nm particle that is very stable to solvents and extremes in environmental temperatures. The virus resembles a wheel. Rota is Latin for wheel, hence, the name rotavirus (figure 4). The genetic material in the virus is made up of 11 segments of double-stranded RNA. Because rotavirus is so stable in the environment, sows are probably chronically infected and shedding the virus (Lecce and King, 1980). This ubiquitous virus acts as an opportunist. When management systems in the nursery increase the level of the virus beyond the protective capacity of the piglet, then diarrhea ensues (Lecce and King, 1978; Lecce et al., 1978).

Antibody to rotavirus in the sow's colostrum and milk will protect the nursing piglet. Again, for protection to be effective, the antibody must bathe the lumen of the gut. Feeding diets made in part with cow's colostrum also will protect neonatal piglets from porcine rotavirus (Lecce and King, 1981, 1982). Presumably, like

Figure 4. Electron micrograph of an aggregation of rotavirus. 70-nm icosahedral rotaviral particles. (X 60,000)

E. coli and TGEV infections, oral rehydration glucose electrolyte solutions have therapeutic value (Bywater and Woode, 1980). High nutrient intake will exacerbate the diarrhea (Lecce et al., 1983).

Summary

- 1) The nemeses of the artificially reared, early weaned piglets are enteric infections that produce diarrhea, dehydration and death.
- 2) The main etiological agents are enterotoxigenic *Escherichia coli* (ETEC), transmissible gastroenteritis virus (TGEV) and rotavirus.
- 3) All three pathogens affect enterocytes. Enterotoxigenic *Escherichia coli* produce a secretory diarrhea; TGEV and rotavirus produce a malabsorptive diarrhea.
- 4) Weaning abruptly removes the neonate from the protection of antibody in the colostrum or milk.
- 5) Specific antibody bathing the gut affords passive protection, either added to the diet or as a part of the normal mammary secretions.

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