Infectious Diseases of the Bovine Lower Gastrointestinal Tract and the Immune Response

J. Robert Duncan, *D.V.M.*
Animal Diseases Research Institute
Ottawa, Ontario, Canada

Of what significance is modern-day immunology with its T and B lymphocytes, various immunoglobulin classes and subclasses, transfer factor, suppressor cells, immunosuppressive factors, and immunodeficiency diseases to everyday bovine practice? The amount of talent being devoted to continuing education in this area might suggest that it is grossly over-rated. However, when we consider that the control, diagnosis and prevention of some of the major disease problems of cattle, namely, tuberculosis, brucellosis, blackleg, virus diarrhea, infectious bovine rhinotracheitis and vibriosis, to name a few, are being conducted principally with immunological tools, possibly it is not over-rated. If we further accept that a major portion of our practice problems are related to infectious diseases of epithelial surfaces, namely the respiratory, digestive, reproductive system and mammary gland; that failure of immunity in its broadest context, whether of animal origin or management-induced, plays an important role in infectious disease, the onus is on us as veterinarians to understand the immune system so that we may use it to our best advantage.

The problem we are focusing on this morning is immunity in relationship to infectious diseases of the gastrointestinal tract. To put this in its proper perspective let us look at the immune system of the gastrointestinal tract. First of all, lymphoid tissues associated with the digestive tract constitute some 70% of the entire lymphoid system of the body. This consists of organized lymphoid tissues such as the tonsils, peyers patches, mesenteric lymph nodes and the more diffusely distributed lymphoid cells which reside principally in the lamina propria of the mucosa. In addition, there are significant numbers of mast cells, eosinophils and macrophages in the lamina propria of the normal bovine intestine.

The lymphoid elements of the gastrointestinal tract are present for the purposes of: 1) the recognition of antigens for the induction of immune responses and 2) for effector functions such as the synthesis and secretion of immunoglobulins and lymphokines. The study of immunoglobulins and lymphoid cells of mucosal surfaces such as the gastrointestinal tract, respiratory tract, reproductive system and mammary gland resulted in the recognition of the principle of local immunity. However, the concept of local immunity was recognized and used diagnostically in veterinary medicine and in particular in the cow long before we had heard anything about IgA. For example, in bovine vibriosis it was recognized that antibodies in vaginal secretions served as a better index of infection than those in the serum. However, it has only been in recent years that we have re-examined this principle in regards to disease prevention in cattle.

The principle of local immunity simply stated is that the most effective induction of an immune response directed at a pathogen on a mucous membrane comes from the same site. In other words, if an antibody directed toward a pathogen at the luminal surface of the gastrointestinal tract is an effective means of disease control, whether we are talking about bacteriostasis or virus neutralization, then the antigen should be applied to the mucous surface. It also implies, particularly in the digestive tract, because of the digestive enzymes present and dose response relationships, that living vaccines will be more successful than killed vaccines. It further states that parenteral immunization, unless we go to massive antigen doses, will not only fail, but in some cases, such as the respiratory tract, may be harmful. There are exceptions to this principle such as the vaccination for bovine vibriosis which does not warrant discussion at this time.

The previous speaker has indicated that the immune system, namely that responsible for specific acquired immunity, is broadly broken into the humoral and cell-mediated immune system. Although they are closely linked in the induction of many immune responses and immunity, their distinction becomes important in daily practice as different infectious agents may induce one or the other preferentially or resistance may be more dependent on one than the other. If we consider briefly the division of immunity into cellular and humoral mechanisms, specifically in relationship to three important functions we serve as practitioners, namely, 1) diagnosis, 2) treatment, and 3) prophylaxis, this may become more apparent. In terms of diagnosis or assessment of protection, do we select a serological test or a measurement of cell-mediated immunity such as the delayed type hypersensitivity skin test? Bovine tuberculosis is an example of an infectious agent that classically induces a good delayed type hypersensitivity response and in turn the skin test appears to have been very successful in the eradication of this disease. Yet there is experimental evidence to indicate a serological test may be more efficient. By contrast, in brucellosis we have relied on serological tests for diagnosis. Yet the brucella organism is an intracellular parasite and induces a good cell-mediated immune response. Therefore, a reevaluation of our diagnostic approach to some problem cases of brucellosis may be useful.

In regard to treatment or what may be termed immunotherapy, should we attempt to augment the humoral or the cell-mediated immune mechanism? Passive transfer of immune serum has proven beneficial in the treatment of viral infections of the intestinal tract such as feline panleukopenia. Research in bovine coccidiosis would suggest that cellular immunity may play a very important role. With the recognition and hopefully practical application of transfer factor for the enhancement of cell-mediated immune responses this distinction will become more important.

In terms of prophylaxis, which antigen, in what dose, by which route and in which form do we optimize either humoral or cell-mediated immune responses?

Now, to put this into practice we will take a look at some of the diseases of the gastrointestinal tract which I have broken down into four categories: 1) bacterial, 2) viral, 3) parasitic, and 4) hypersensitivity. In the first category of bacterial infections of the intestinal tract, I have further subdivided them into an example of an extra-cellular non-invasive intestinal pathogen, *Escherichia coli,* and an invasive facultative intracellular parasite, *Mycobacterium paratuberculosis.* With regard to *E. coli* I will attempt to highlight from an immunological point of view those factors which I feel are important in the pathogenesis of the diarrhea induced by this agent. The entero-toxogenic, non-invasive form of enteric colibacillosis would appear to require adherence of the bacterium to the intestinal epithelium with the subsequent alteration of fluid and electrolyte transport of intestinal epithelial cells. In terms of what I have said before, this should ideally be a good example to which we may apply the concept of local humoral immunity. In other words, protection against this form of enteric disease requires the presence of antibody in the intestinal tract to prevent adherence of bacteria to the epithelium. We do not necessarily have to kill the organism nor do we want to induce an acute inflammatory reaction at this site. In swine there is good evidence to suggest that orally administered antibody is superior to the parenterally applied product. Once again it has been demonstrated in swine that the oral application of killed coliforms as part of the ration was both successful and superior to parenterally applied products in the reduction of post-weaning gastroenteritis.

Mother Nature has provided us with a passive protective mechanism in the form of antibody in colostrum and milk. In this case we are not considering what is absorbed into the systemic circulation but that which acts bacteriostatically in the intestinal lumen. It is important to recognize that milk taken well after parturition contains antibody which may have significant effect on potential enteric pathogens. It is well recognized that breast-fed human infants have a lower incidence of digestive tract disease in contrast to those which are not breast-fed. Although we recognize the beneficial effect of milk, not enough effort has been devoted to its potential prophylactic and therapeutic applications in contrast to that of other antimicrobial agents such as antibiotics. Furthermore, there is evidence to indicate that antibody in the lumen of the gastrointestinal tract is more effective as a bacteriostatic agent in the presence of normal flora, thus contraindicating the use of chemotherapeutic agents which will alter normal flora.

Johne's disease or paratuberculosis, in contrast to coliform enteritis, presents itself as a very different problem from an immunological point of view. First of all, it is an invasive parasite and resides primarily inside macrophages. As a result, cell-mediated immunity would predictably be very important in resistance and potentially important in diagnosis. If we concern ourselves with the diagnostic aspects of Johne's disease from an immunological point of view, why have we not made more progress with a disease which has been recognized for some seventy years? To answer this, let us start with the premise that paratuberculosis is a spectral disease and examine the currently used diagnostic tests for Johne's disease in this respect.

Table 1
Comparison of Current Diagnostic Procedures Across the Clinical Spectrum of Johne's Disease

	Clinical Signs		
Test	Sub-clinical	Mild or Early	Advanced
Delayed type skin test	+	+	±
Complement Fixation test	±	±	+
Direct fecal culture	–	±	+

Table 1 suggest four things: 1) skin testing procedures are most likely to be successful in subclinical cases of Johne's disease; 2) serological responses are most likely to be useful in more advanced clinical forms of the disease; 3) both tests should be used concurrently in the diagnosis of Johne's disease as we cannot always predict which form of the immune response will be paramount; and 4) fecal culture will most likely be successful when some signs of clinical disease are apparent. The spectral aspect of Johne's disease is further complicated by the fact that the organism is composed of a complex number of antigens, many of which cross-react antigenically with other mycobacteria and non-mycobacterial species. Due to the long incubation period of Johne's disease, individual differences in animal response to the complex number of antigens is expressed and therefore the selection of the appropriate diagnostic reagent may vary with the individual animal. What advances are then to be made in the terms of diagnosis of Johne's disease utilizing immunological tools? The advent of *in vitro* tests for cell-mediated immunity such as the lymphocyte transformation test would appear to be a sensitive assay for a sensitization to mycobacterial antigens. It compares favourably to currently used techniques but requires further refinements in terms of standardized conditions under which the tests must be conducted and standardization of antigens used to overcome cross-reactivity presumtively due to environmental antigens. Therefore, at the moment, the direct fecal-culture technique is the most reliable indicator of infection.

Bovine virus diarrhea is a significant cause of acute and chronic diarrheal disease in cattle as a result of intestinal epithelial and lymphoid cell necrosis. Both vaccines and passively administered antiserum appear to be beneficial prophylactically. A chronic form of BVD would appear to be related to some immunological deficit as passive immunotherapy (whole blood from immunized cattle) has produced a significant but temporary remission of clinical disease. The specific nature of the defect has not been defined, but this may be the precise type of disease where immunotherapy could be of practical benefit to the bovine industry.

Coccidiosis is a common intestinal parasite of cattle and is frequently observed in the intestinal mucosa in feces without clinical disease. However, in confined and crowded cattle it will frequently produce an acute hemorrhagic enteritis, with recovered animals developing immunity. Recent experimental work with bovine transfer factor indicates that cell-mediated immunity plays a significant role in protection against bovine coccidiosis which indicates that immunological intervention prophylactically and therapeutically as opposed to chemotherapy may soon be a practical consideration.

Hypersensitivity as the basis for enteric disease in cattle should also be considered. Some investigators have indicated that the intestinal and abomasal lesions seen in some forms of coliform disease of calves may be a form of protracted anaphylaxis. Hypersensitivity to dietary substances should be considered in neo-natal diarrheal disease where infectious agents cannot be incriminated. Finally, even the grossly observable lesions of Johne's disease should not be accepted as the cause of diarrhea. The diarrhea observed in this disease is frequently intermittent, it can be induced by the intravenous inoculation of Johnin and is responsive to antihistamines. Therefore, the suggested mechanisms in at least some stages of the disease is an immediate or Type I hypersensitivity rather than a maladsorption phenomenon related to the mucosal infiltration of bacterial laden macrophages.

The science of immunology is rather complex as is the etiology of enteric disease of cattle. However, out of this complexity comes a few significant pathogens and some rather basic immunological concepts such as the working division of the immune system and the concept of local immunity. With a better understanding of these concepts we can hopefully take a more informed approach to diagnosis, treatment and prophylaxis of enteric disease.