Combined Immunodeficiency in Horses: Diagnosis, Treatment, and Significance

by Alan G. Brady*

Over the past 10 years, the discovery of a new immunodeficiency syndrome has been made in the Arabian horse breed. Designated as a combined immunodeficiency (CID) because affected foals show a deficiency in both cellular and humoral immunity, the syndrome has attracted much interest due to its seriousness (100% mortality in affected foals within 5 months of birth) and the prevalence of the syndrome in the Arabian breed (according to one study[1] 2.3% of all Arabian foals are affected).

The discovery of CID was the result of investigations into the causes of foal susceptibility to infection. Since 1967, researchers had been puzzled by a breed-specific susceptibility of Arabian foals to pneumonias, particularly adenoviral pneumonia. Johnston and Hutchins first described adenoviral bronchitis in an Arabian foal in that year.[2] In 1970 McChesney et al[3] published a retrospective case study of 24 foals that had died of pneumonia. Of the 10 Arabiaa foals in the group, 8 showed symptoms and lesions consistent with adenoviral pneumonia (nasal and ocular discharge, fever, adenoviral inclusions in the mucous membranes, glandular tissues and lungs). None of the non-Arabian foals had such symptoms and lesions. Of great interest in this study was the observation that five of the ten Arabian foals showed a "severe, absolute lymphopenia" when blood samples were taken during the course of the pneumonia. Although certain viral diseases, such as canine distemper and infectious canine hepatitis[17] are known to cause lymphopenia, this is the opposite of theusual lymphocytosis associated with a chronic viral infection. The

authors also described lesions of the reticuloendothelial system found in a detailed histopathologic study of one animal. They observed a decreased number of lymphoid follicles in the lymph nodes and an absence of splenic follicles in this animal.

Based on the available evidence, the authors suggested the following possible reasons for Arabian breed susceptibility to adenoviral infection: 1. Failure of the dam to produce circulating antibodies to adenovirus. 2. Failure of passive transfer of the dam's antibodies in the colostrum. 3. Inability of the foal to absorb adenoviral antibody. 4. Altered capability of the foal to produce an immune reaction.

McGuire and Poppie first proposed CID as the cause of the Arabian susceptibility in 1973.[4] Although the proposal was only based on two foals, it was followed in 1974[5] by a study of the immunologic competence of 30 sick, and 78 normal Arabian foals. In this group, 7 sick, and 2 healthy foals showed absence of germinal centers, lymphopenia, and absence of one or more of the immunoglobulins. These signs were given as evidence of a B cell defect (a defect in humoral immunity). Hypoplasia of the thymus and absence of T cells in the spleen was given as evidence of a T cell defect. These authors can also be credited with first identifying CID as a primary, genetic disorder, rather than secondarily connected with an infection. This was demonstrated in their identification of healthy foals with CID.

Diagnosis of CID

Since the discovery of CID, the pattern of a typical CID case has become well established: The foal is presented to the veterinarian at 2 to 65 days of age with some form of infectious

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disease (pneumonia, diarrhea, hepatitis, etc.). Massive antibiotic therapy may be tried, but it will have little effect. If the infection does regress, it will be followed by other infections until the foal dies. As noted above, no CID foal has been known to live longer than five months.[1] Because of the number of related disease conditons (described below) the disease course does not provide accurate diagnosis of CID. Accurate diagnosis requires the following evaluations:

1. *Differential leukocyte count.* Absolute numbers of lymphocytes have been found to be below 1000 cells per cubic millimeter in CID foals.[10] Normal equine blood contains between 1,050 and 7,000 lymphocytes per cubic millimeter.[17]

2. *Immunoglobulin M concentration.* Immunoglobulin M (IgM) is absent in the pre-suckle serum of CID foals.[10] As the CID foal absorbs colostral antibody, IgM levels will increase to a maximum level, then disappear within 36 days as the entirely maternal IgM is catabolized.

3. *Immunoglobulin A and G concentrations.* In the study cited[10] small amounts of IgG were found (.25 mg/ml as compared to 9.7 mg/ml found in the serum of normal foals) even when there was "CID and failure of transfer of Ig" in one foal. An earlier report by the same authors[9] states that CID foals are "incapable of synthesis" of IgG, IgA, and IgG (T). The presence of IgG in the foal described in the later study may be due to a diminished, rather than a complete failure of, colostral antibody transfer.

Although the above tests are considered sufficient for practical diagnosis of CID,[10] several other tests have been established to demonstrate the defect in cellular immunity (not shown by any of the above tests) and to further demonstrate defects in humoral immunity:

4. *Selective stimulation of T cells with phytohemagglutinin (PHA).* PHA stimulates proliferation of T lymphocytes. It has been used to differentiate selective immunoglobulin deficiencies from combined immunodeficiency, since only unimpaired T lymphocytes will proliferate under the influence of PHA.[6,9] This phenomena can be quantitated in lymphocyte cell cultures derived from suspect animals. A deficit in immunoglobulins

with a failure to respond to PHA would indicate combined immunodeficiency.

5. *Delayed hypersensitivity skin response.* This response is also mediated by T lymphocytes. Impairment of this response to an antigen to which the animal has been previously sensitized would also indicate a T cell deficit.[6]

6. *Production of specific antibodies.* This is another measure of the humoral immunity system. Antibody titers following antigenic stimulation, or the titers of the so-called "natural" antibodies (such as those to rabbit red blood cells) can be determined.[6] This provides a measure of the animal's ability to mount an immune response to a specific antigen. This is something that quantitation of immunoglobulins does not do.

7. *Histopathologic lesions.* Post-mortem diagnosis of CID could be of use in identifying carrier mares and stallions.[1,3,11] As noted above, CID foals show an absence of germinal centers and follicles in the lymph nodes, and absence of splenic follicles. Recent, more detailed studies[11] have also revealed an absence of periarteriolar sheaths, thymic hypoplasia, and fatty infiltration of the thymus in CID foals.

Other Immune Deficiencies That Can Be confused With CID

One reason for the number of tests developed for diagnosing CID is the existence of other, selective, immune deficiencies that give some, but not all, of the same results in diagnostic tests. The following are three such immune deficiencies that have been described in foals:

1. Failure of passive transfer of colostral antibodies (FPT). FPT is believed to be the most common immunodeficiency disorder in foals.[8] It has no breed specificity,[9] and may well be the predisposing factor in many foal deaths due to infection. This condition resembles CID in the foal's susceptibility to infection and low levels of immunoglobulins in affected foals before they are capable of synthesizing their own (within 24 days of birth[7]). FPT may be differentiated from CID by the normal lymphocyte count in FPT foals and normal function of B and T

lymphocytes. Since there is some foal production of IgM in utero,[8] this immunoglobulin would be present withour passive transfer (but would be deficient in pre-suckle samples from CID foals). Since FPT is treatable (by transfusion of dam's plasma), it is of prime importance that is be differentiated from combined immunodeficiency (which has not been successfully treated) when making a diagnosis.

2. *Immunoglobulin M Deficiency.* A selective IgM deficiency was reported in five foals early this year.[9] Radial immunodiffusion done on serum from these animals showed IgM concentrations close to or below the lower limit of test sensitivity (5 mg/ml). The deficient animals included 3 Arabian and 2 Quarter horse foals, all with some form of infectious disease. Although little is known about this condition, it can be differentiated from CID by the normal levels of other immunoglobulins, normal numbers of lymphocytes, unimpaired T cell function, and B cell function impaired only in the one class of immunoglobulins.

3. *Immunoglobulin deficiency (agammaglobulinemia)* with functional T lymphocytes. This condition has been identified in one Thoroughbred foal that lived to 17 months of age.[6] Tests on this foal showed absence or low levels of IgM, IgA, IgG, and IgG(T), but presence of delayed hypersensitivity response, normal numbers of lymphocytes, and normal proliferation of lymphocyte cultures when stimulated with phytohemagglutinin. The differences in these tests to those of typical CID foals should be noted, along with the age of the animal at death (much older than CID foals).

Eradication of CID

Since CID is a genetic trait, the only logical means of eradication is by identification of carrier mares and stallions. Severl authors[1,3] have stated the opinion that CID is an autosomal recessive trait, and one study,[1] involving 257 Arabian foals, supports this conclusion. In this study,[1] 6 foals (2.3%) were found to have combined immunodeficiency. These foals were born of normal parents (thus the conclusion that the trait is recessive). In a larger study, CID foals were found to be approximately evenly divided between males and females; thus, the conclusion that the trait is linked to an autosomal, rather than a sex chromosome. Because of the prevalence of CID, it has also been suggested that the trait might be related to some other trait that breeders select for.

No published attempt has been made to trace the CID trait through specific lines of inheritance ('blood lines") in the Arabian breed. Although such a study would be hindered by the number of undiagnosed cases of CID, it will become more practical as more veterinarians become aware of the condition. Such a study would be important if eradication of this trait is to be attempted.

Treatment of CID

Immunotherapy in two foals with CID has been attempted[13] and, if human patients are any indication, provides the only available means of therapy in these cases. Long term antibiotic therapy has been reported as being successful in Arabian foal adenoviral infection,[12] but these were probably not CID foals. Of the two foals given immunotheryapy, one received a bone marrow transplant from the most histocompatible sibling that could be found. Because of the absence of a marrow donor in the second case, a fetal thymus transplant was attempted. Both transplant experiments ended with the death of the foals. Evidence in the case of the thymus transplant foal was given for a graft versus host reaction, a result which points to some of the problems in CID immunotherapy.

The two main problems in CID transplant therapy are whether the host will accept the transplant, and whether the transplant will accept the host. The ability of the host to reject the transplant is weakened by the condition being treated, thus the graft versus host reaction assumes the greatest importance. It is a paradox that the very cells being transplanted probably represent the greatest threat in such a rejection.

Successful immune cell transplants have been made in human CID patients,[15] the greatest success being made with bone marrow transplants from histocompatible donors. In the absence of donors, a fetal thymus transplant has been tried with success

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in one patient (this transplant only restored cell mediated immunity). Injections of dialyzed transfer factor preparation have also had some very limited success. As seen in the foal marrow and thymus transplants, graft versus host reactions are frequent and mortality is high even under the best of conditions.

CID as a Model for Human Severe Combined Immunodeficiency

Comparisons between CID in foals and human CID are not limited to methods of treatment. CID in foals has been suggested as a possible animal model for human severe combined immunodeficiency (SCID).[16] Although one form of SCID is sex linked, another form is believed to show autosomal recessive inheritance. Both conditions are defects in both B and T lymphocyte function and both show similar results in immunologic tests, symptoms and post-mortem lesions. Unlike the horse, human patients have been maintained for long periods of time in germ-free environments. The authors of this animal model proposal suggest that CID foals may have use in experimenting with new, potentially dangerous methods of treatment for SCID before they are tried on human patients. Studies on CID foals might also be used to determine the specific lymphocyte defect(s) that cause immunodeficiency.

Conclusion

Much still remains to be learned about CID and other immune deficiencies in foals. The following are some questions that remain unanswered in CID research:

1. What is the root defect in the hematopoetic system that prevents the production of normal lymphocytes? Identification of this defect might open the way to new methods of treating CID in man and animals.

2. Is the agammaglobulinemia associated with CID due to an absence of B cells or merely a defect in Ig synthesis? Lack of germinal centers in the lymph nodes would seem to indicate an absence of B cells, but this has not been confirmed.

3. In what animals did the trait originage? Who are the present carriers of the CID

trait? Eradication of CID will depend on finding this information.

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