New Developments in Severe Combined Immunodeficiency Disease

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Severe combined immunodeficiency disease (SCID) is a fatal, inherited disease of Arabian foals. Studies characterizing the immunophenotype of lymphoid cells and the defect in V(D)J recombination in SCID foals has improved our understanding of the disease. A definitive test that identifies SCID foals and carrier horses is now available. Author's address: Dept. of Medical Sciences, University of Wisconsin—Madison, 2015 Linden Dr. West, Madison, WI 53706. © 1997 AAEP.

1. Introduction
Severe combined immunodeficiency disease (SCID) of Arabian foals is a fatal, autosomal recessive condition first reported in 1973.1 SCID results in the absence of both B and T lymphocytes, but neutrophils, macrophages, natural killer cells, and the complement system function normally.2,3 It has been estimated that 2–3% of Arabian foals have SCID, suggesting that 25% of Arabian horses are carriers of the recessive gene.4 Most SCID foals become ill by 1 month of age and die by 5 months of age. Affected foals suffer from a variety of infectious diseases that are unresponsive to appropriate antimicrobial therapy. These infections are often caused by agents that are rarely pathogenic in immunocompetent animals. In SCID foals, adenovirus pneumonia is common, and Pneumocystis carinii respiratory infections have also been reported.5 Gastrointestinal infections may be caused by uncommon pathogens such as coronavirus and cryptosporidia.6,7

The diagnosis of SCID has been based on three criteria: (a) persistent lymphopenia, (b) the absence of serum IgM in foals over 4 weeks of age, and (c) lymphoid hypoplasia. New findings into the pathogenesis of SCID have led to a better understanding of the disease plus a definitive antemortem diagnostic test.

2. Pathogenesis of SCID
A. Characterization of Lymphoid Cells and Tissue
The thymus and lymph nodes from foals with SCID are small or undetectable on gross postmortem examination. Histologically, the thymus consists of small hypocellular lobules, interspersed with adipose tissue. The lobules have no corticomedullary differentiation and contain a few widely dispersed lymphocytes. Lymph node follicles and germinal centers are absent. The cortices consist of a reticular framework with small accumulations of lymphocytes. Lymph node follicles and germinal centers are absent. The cortices consist of a reticular framework with small accumulations of lymphocytes. Functional tests indicate that SCID foals lack mature B and T lymphocyte activity. The few lymphocytes present in the circulation have natural killer cell activity. Prothymocytes are present in the thymus of SCID foals but do not mature into functional T lymphocytes. The successful immunological reconstitution of one SCID foal with a histo-
compatible bone-marrow transplant indicates that the architecture of the thymus and other lymphoid organs is intact. This supports the hypothesis that a biochemical defect in prothymocytes results in their inability to mature to B and T lymphocytes. Lymphocyte subsets can be defined by the expression of cell-specific surface markers. These cell surface markers can be characterized by using monoclonal antibodies. Thymic and lymph node tissues from two foals with a confirmed diagnosis of SCID were examined in an immunohistological study by using a panel of monoclonal antibodies that identify equine leukocyte differentiation antigens. The majority of the cells were leukocytes of lymphoid origin (EqCD11a/18, EqCD44, EqCD133). The predomi-
nant lymphoid cell phenotype in both foals was EqCD3-EqCD4-EqCD8+. This phenotype has not been previously described in horses. Helper T lymphocytes (EqCD3-EqCD4+) and cytotoxic T lymphocytes (EqCD3-EqCD8+) were absent. The unique phenotype, EqCD3-EqCD4-EqCD8+, could be imma-
ture lymphocytes or natural killer cells. This is the phenotype for natural killer cells in human beings and rats.

In mice, SCID results from a defective rearrange-
ment of T-cell receptor and immunoglobulin genes. EqCD3 antigens appear on the lymphocyte surface shortly after T-cell receptor rearrangement. The lack of EqCD3 expression on lymphocytes from SCID foals points to a possible defect in the T-cell receptor rearrangement. Practically, an immunohisto-
logical examination of lymphoid tissue could assist in the diagnosis of suspect SCID foals.

B. Defect in V(D)J Recombination

Adaptive immune responses arise when the recep-
tors of individual lymphocytes recognize foreign antigen. The ability of the immune system to recog-
nize a wide range of antigens in a highly specific manner is generated by the presence of immunoglobulins on B lymphocytes and T-cell receptors on T lymphocytes. These antigen-specific proteins are members of the same protein family. The structures of these proteins are uniquely adapted to their function. The constant regions of immunoglobulins and T-cell receptors are associated with universal functions such as cell binding and structural sup-
port. The variable regions are associated with anti-
gen recognition and binding and are so diverse that as many as hundreds of millions of antigens can be recog-
nized at any one time. The diversity of the structure of the antigen-binding variable regions of immunoglobulin and T-cell receptors is generated by rearrangement, recombination, splicing, or muta-
tion of a large number of genes. This process is called somatic recombination. The variable dom-
ains are encoded in three separate gene segments, namely the variable (V) region, diversity (D) region, and joining (J) region. There are multiple gene copies or segments in the V, D, and J regions. So-
matic recombination, also called V(D)J recombina-
tion, can join any V gene segment to any J or D gene segment. Lymphocytes fail to mature and eventu-
ally are eliminated if V(D)J recombination does not occur. The mechanisms by which V(D)J recombina-
tion occurs are not fully elucidated, but the process is largely enzyme dependent. Two sets of enzymes are used in V(D)J recombination: those encoded by the lymphoid-specific recombination activator genes and a general pathway of double-strand break repair that repairs broken DNA. At least three factors are shared between the lymphoid-specific and gen-
eral DNA repair pathways. One of these factors, the DNA-dependent protein kinase, has been shown to be deficient in SCID affected mice. The molecular defect in SCID mice has been located in the catalytic subunit of DNA-dependent protein kinase (DNA-PKCS). Through a series of elegant experiments, researchers have demonstrated that the factor defective in SCID Arabian foals is required for both V(D)J recombina-
tion and general double-strand break repair. Cell cultures from SCID foals lacked DNA-depend-
ent protein kinase activity, and DNA-PKCS concen-
trations were undetectable. These discoveries indicate that SCID foals, like SCID mice, probably suffer from a molecular defect in DNA-PKCS.

Recently the equine DNA-PKCS gene was cloned and sequenced by using cells lines from both normal horses and SCID foals. A five-nucleotide deletion was found that truncates the protein N terminus of the domain. This results in the complete absence of full-length DNA-PKCS and the lack of DNA-depend-
ent protein kinase activity. Labeled DNA frag-
ments, called DNA probes, that recognized either the normal DNA-PKCS or the suspected mutant SCID DNA-PKCS allele were used to screen genomic DNA from eight SCID foals and five phenotypically nor-
mal horses (four Arabian and one non-Arabian). The SCID allele probe hybridized strongly with all eight SCID foals, but the normal allele probe failed to hybridize, indicating they were homozygous for the SCID mutant allele. Three of the five phenotypically normal horses hybridized with the normal allele probe but not the SCID allele probe, indicating they did not possess the SCID allele. Two of the phenotypically normal horses strongly hybridized with both the SCID allele probe and the normal allele probe, demonstrating that these horses were heterozygotes or carriers of the mutant SCID allele. This confirms that most, if not all, Arabian foals with SCID suffer from this single five-nucleotide muta-
tion. Testing for both homozygous and heterozy-
gous horses is now possible.

3. Diagnosis of Equine SCID

The diagnosis of SCID is made on the basis of three criteria: persistent lymphopenia, absence of IgM, and anatomical abnormalities consisting of thymic hypoplasia and absence of normal lymph node archi-
tecture. The first two criteria can be fulfilled ante-
mortem but surgical biopsies of thymus and lymph
nodes are very difficult to obtain because of the
organs’ small size and location. Therefore, a con-
firmed diagnosis of SCID is usually obtained only
after a postmortem histological examination of lym-
phoid tissue is completed. This results in prolonged
medical treatment of critically ill SCID foals while
enough evidence is obtained to recommend euthana-
sia. An antemortem test that definitively diag-
noses SCID would reduce the duration of animal
suffering and limit the owner’s financial expense.

Persistent lymphopenia (<1000 cells/µl) is a consis-
tent feature of this disease. Repeated lymphocyte
counts over several days are important because a
seriously ill immunocompetent foal can have a tran-
sient lymphopenia. For evaluation of the humoral
arm of the immune system, a serum IgM radioim-
mune diffusion assay can be performed before the
foal suckles colostrum or after 4 weeks of age. The
half-life of IgM is 4–5 days; thus by 21 days of age
>95% of maternally derived IgM has been elimi-
nated. Serum IgM concentrations should not be
used to diagnose SCID in foals. The half-life for IgG
is 15–21 days; thus 25–35% of maternally derived
IgG antibodies are still present in the serum of a
1-month-old foal. Maternally derived IgG antibod-
ies may be detected in foals 3–4 months of age.13

Another test for immunocompetence that assesses
T lymphocyte function is an intradermal phytohe-
magglutinin (PHA) test. Intradermal PHA causes a
delayed-type hypersensitivity reaction, resulting
in a skin swelling. A foal suffering from SCID fails
to respond to intradermal PHA. This test is not
affected by maternal antibodies so it can be used in
foals of all ages. Although simple to perform, PHA
is not readily available to most practitioners.14

Based on the previous immunohistochemical stud-
ies, lymphocytes from a foal suffering from SCID
should be negative for EqCD3 and EqCD4 antigens
but positive for EqCD8 antigens. A flow cytometric
analysis of peripheral blood lymphocytes stained
with monoclonal antibodies to EqCD3, EqCD4, and
EqCD8 could potentially be used to determine the
pheno

type(s) of the lymphocytes. Although flow cyto-
metric analysis in SCID foals has not been
reported, a similar procedure is commonly used in
human beings with acquired immune deficiency to
determine their helper T lymphocyte count (CD4+
lymphocytes).14

The recently discovered five-nucleotide genetic
defect in DNA-PKcs responsible for causing SCID in
Arabian foals provides the information needed to
develop a genetic test. VetGen,2 a company special-
izing in genetic testing of canine diseases, has ob-
tained the rights to the DNA test for equine SCID.
Whole blood and cheek mucosal swabs are the
samples recommended for testing. The test should
be commercially available by the fall of 1997. b
DNA
testing for SCID can be performed in horses of any
age. This test will definitively diagnose SCID foals
antemortem and identify horses that are carriers of
the SCID defect. This information could be used to
avoid the mating of two SCID carriers, used in a
prepurchase examination, or assist an owner’s deci-
sion if a horse should be used for breeding.

4. Conclusion
The diagnosis of SCID has been based on three
criteria: (a) persistent lymphopenia, (b) the ab-
sence of serum IgM in foals over 4 weeks of age, and
(c) lymphoid hypoplasia. Thus diagnosis is often
difficult, often takes a week or more, and usually
requires a postmortem examination. Breeding stock
that are carriers of the SCID defect can only be
identified by producing a SCID foal. Recent discov-
eries into the pathogenesis of equine SCID have led
to the development of a genetic test. This test will
enable practitioners to definitively diagnose clinical
cases of SCID antemortem. Arabian horse owners
will be able to identify SCID carrier animals, which
will assist their breeding and purchasing decisions.

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bJohn Duffendack, President of VetGen; Ann Arbor, MI 48108 (personal communication), March 1997.