

Evaluation of Adenosine Deaminase and Other Purine Salvage Pathway Enzymes in Horses with Combined Immunodeficiency

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Foals with combined immunodeficiency had normal levels of purine salvage pathway enzymes, including adenosine deaminase, nucleoside phosphorylase, and xanthine oxidase.

Functional studies of primary immunodeficiency disorders have increased our understanding of these conditions, but have not provided a molecular basis for the disorders. In one syndrome, severe combined immunodeficiency (CID), approximately half of the children examined lacked adenosine deaminase (ADA), which converts adenosine to inosine in a purine salvage pathway (reviewed in references 12 and 14). Most CID children with ADA deficiency had an autosomal recessive mode of inheritance, whereas normal ADA levels were associated with sex-linked CID (3, 5, 6, 12-15).

CID in young horses is similar to severe CID of children (9-11). Affected foals have lymphopenia, absence of serum immunoglobulin M, decrease of B lymphocytes, absence of specific antibody response to challenge, decreased lymphocyte proliferation to phytolectins, absence of organized lymphoid structures in the spleen and lymph nodes, and thymic hypoplasia. Af-

ected foals die before 4 to 5 months of age from a variety of infectious diseases, notably, though not exclusively, adenoviral and *Pneumocystis carinii* pneumonias. The defect occurs in Arabian and part-Arabian foals and appears to be inherited as an autosomal recessive trait. We felt it was important to determine whether ADA deficiency or deficiency of other enzymes (nucleoside phosphorylase [NP] or xanthine oxidase [XO]) of the same purine salvage pathway was associated with CID in foals.

Tissue (4), erythrocyte, lymphocyte (2), and platelet preparations were prepared from five CID foals (four males and one female) and five normal foals. Lymphocyte counts in the five CID foals ranged from 76 to 580/m³, whereas the normal range was 1,150 to 8,570/mm³. ADA was measured by adenosine conversion to inosine by recording the decrease in optical density at 265 nm (8). Another ADA assay was a coupled reaction requiring the addition of 70 µg of

TABLE 1. Amounts of ADA in tissues from normal and CID foals

Tissue	CID			Normal		
	No. examined	Mean	Range	No. examined	Mean	Range
Lymph node	5	93 ^a	43-153	3	80	49-111
Spleen	5	189	103-285	3	182	88-292
Thymus	3	60	12-102	3	41	26-66
Bone marrow	3	39	19-63	2	51	44-57
Intestine	4	48	26-93	2	27	13-41
Kidney	4	20	17-24	2	17	5-28
Lung	4	48	37-59	3	55	34-83
Liver	4	45	13-66	2	81	52-110
Heart	3	23	17-28	2	25	21-28
Brain	2	5	4-6	1	3	
Salivary gland	5	36	16-54	2	22	16-27

^a Amounts of ADA are expressed as micromoles of adenosine deaminated per minute per gram of wet weight × 10² and determined by appearance of uric acid using a coupled assay.

adenosine, 0.06 U of NP, and 0.05 U of XO (Sigma Chemical Co., St. Louis, Mo.). Uric acid appearance was measured at 293 nm (7). The NP assay consisted of measuring uric acid production after mixing 70 μg of inosine and 0.05 U of XO. To measure XO, 70 μg of inosine and 0.06 units of NP were mixed, and uric acid was measured. The approximate molecular weight of ADA in tissue extracts was determined by assaying fractions from a calibrated p-100 column (2.5 by 90 cm; Bio-Rad Laboratories, Richmond, California) (1).

No significant ADA activity could be detected by two assay methods in the erythrocyte lysates of 12 normal horses and five CID foals.

TABLE 2. Determinations of ADA in isolated lymphocytes from normal and CID horses

Horse no.	No. of determinations	$\mu\text{mol} \times 10^{-10}$ of adenosine deaminated/min per lymphocyte	
		Mean (range) as measured by appearance of uric acid	Mean (range) as measured by disappearance of adenosine
30 (adult)	4	0.93 (0.7-1.1)	1.15 (1.1-1.2)
31 (adult)	4	1.1 (1.0-1.2)	1.2 (0.8-1.5)
8 (foal)	3	1.5 (1.4-1.6)	1.7 (1.3-2.1)
9 (foal)	3	1.8 (1.6-2.0)	1.8 (1.5-2.1)
4 (CID foal)	2	3.4 (2.7-4.1)	ND ^a
5 (CID foal)	1	1.6	ND

^a ND, Not determined.

Mixtures of lysates from horse erythrocytes did not influence the ADA activity in lysates from normal human erythrocytes. Similar amounts of ADA were present in CID and normal foal tissues (Table 1). To confirm that ADA was present in progeny of CID bone marrow stem cells, lymphocyte preparations were examined (Table 2). Platelets from two CID foals deaminated 1.5×10^{-3} and 2.1×10^{-3} μmol of adenosine/min per μg of protein, whereas two normal foals had values of 0.6 and 2.3. The detectable ADA in two spleens, one lung, and one liver from both CID and normal foals had an approximate molecular weight of 37,000. The ADA in lymphocytes from parents of CID foals were compared with levels in lymphocytes from 11 other normal-appearing adult Arabian horses (it is unlikely that all of these horses were heterozygous for the CID trait) and from five adult Shetland ponies. The ADA levels were similar for all three groups. The levels of NP and XO in tissues from CID and normal foals were also similar (Table 3).

These studies reveal the following. (i) Normal and CID horses lack erythrocyte ADA in contrast to other species. (ii) Lymphoid and nonlymphoid tissue from CID foals contain normal levels of ADA, NP, and XO. The molecular weight of tissue ADA in CID and normal foals is approximately 37,000. (iii) ADA in lymphocytes and platelets from CID and normal foals are similar. (iv) Adult horses apparently lacking the CID trait have similar lymphocyte ADA levels to those of known heterozygotes for the CID trait.

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TABLE 3. Levels of XO and NP in normal and CID foals

Organ	Foal status	XO ^a			NP ^b		
		No. examined	Mean	Range	No. examined	Mean	Range
Liver	Normal	2	2.3	1.3-3.3	2	12	11-13
	CID	4	2.5	1.8-3.2	1	22	
Spleen	Normal	2	1.8	1.4-2.3	2	14	13-16
	CID	4	1.9	0.6-3.2	2	16	15-17
Lymph node	Normal	Not done			2	16	15-16
	CID	Not done			2	19	18-20

^a Levels of XO were measured by appearance of uric acid and expressed as micromoles per minute per gram of wet weight $\times 10^2$.

^b Levels of NP were measured by appearance of uric acid and expressed as micromoles per minute per gram of wet weight.

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