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The immunopathogenesis of equine infectious anemia virus

Debra C. Sellon *, Frederick J. Fuller, Travis C. McGuire

NCSU College of Veterinary Medicine, 4700 Hillsborough Street, Raleigh, NC 27606, USA

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1. Historical overview

Horses with recurring cycles of fever, anemia, weight loss, and ventral edema were first described in the veterinary literature in France in 1843 (Lignee, 1843). In 1904 equine infectious anemia (EIA) became the first disease proven to be caused by a filterable animal virus (Carre et al., 1904; Carre et al., 1906). In the early twentieth century research lagged because of an inability to propagate the virus in vitro. In the 1960s Japanese investigators developed successful in vitro leukocyte culture systems (Kono et al., 1970). They were able to use this system to describe the distribution of virus in tissues of infected horses (Kono et al., 1971), provide the first detailed accounts of histopathological lesions (Konno et al., 1970), and demonstrate recrudescence of infection in inapparent carrier horses (Kono et al., 1976). In the early 1970s, Dr. Leroy Coggins developed a specific agar gel immunodiffusion test for detection of serum antibodies in infected horses (Coggins et al., 1970; Coggins et al., 1972). The Coggins test is still widely used, in conjunction with a competitive ELISA test, as the basis for state and federal disease control measures. Quarantine rules were based on epidemiologic and entomologic evidence of mechanical transmission of EIAV by hematophagous insects (Hawkins et al., 1973; Hawkins et al., 1976; Issel et al., 1984; Stein et al., 1942). In the 1970s researchers provided the initial evidence that sequential febrile episodes were associated with the emergence of novel antigenic strains of the virus (McGuire et al., 1987; Montelaro et al., 1984; Payne et al., 1984; Payne et al., 1987; Salinovich et al., 1986; Kono et al., 1973). Subsequently EIAV was the first lentivirus in which antigenic variation of neutralization-sensitive epitopes was

* Corresponding author. Fax: + 1 (919) 515-3044.

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documented (McGuire et al., 1987; Salinovich et al., 1986; Kono et al., 1973). Pathogenesis research revealed that many of the clinical signs of disease were a result of widespread activation of the immune system (McGuire et al., 1969; McGuire et al., 1971; McGuire et al., 1972). In the 1980's and 90's, biological and molecular clones of the virus have been developed by several researchers and the full nucleic acid sequence of many of these clones determined (Rushlow et al., 1986; Stephens et al., 1986; Yaniv et al., 1986; Kawakami et al., 1987; Whetter et al., 1990; Carpenter et al., 1991; Perry et al., 1992). A virulent molecular clone of the virus is now available, enabling research efforts to now be aimed at identification of specific molecular determinants of virulence (Fuller, unpublished data).

2. Clinical features

Horses infected with EIAV are usually described as developing one of three clinical syndromes. Although useful for the purposes of discussion, the distinctions between acute infection, chronic infection, and inapparent carriers are often blurred. In general, acute infections refer to those animals experiencing the first febrile episode of disease. Following exposure to a virulent strain of the virus, horses may develop a high titer viremia with clinical signs of fever, thrombocytopenia, and depression within 7–30 days. These signs are often mild and may be overlooked by other than the most vigilant owners. This febrile episode usually lasts 3–5 days. In some horses the febrile period is prolonged and the horse develops moderate to severe anemia, ventral edema, anorexia, and weight loss. Rarely, horses may die during their first febrile episode of disease. Those horses that do recover from the initial febrile episode may remain afebrile for a period of days to weeks and then experience another episode of clinical disease. The number and severity of febrile episodes varies widely; however, most episodes occur during the first year of infection (Kono, 1973a). If febrile episodes occur often and are severe, the horse develops classic clinical signs of disease: anemia, weight loss, and ventral edema (Issel and Coggins, 1979). If clinical episodes become less frequent and severe, the horse becomes an inapparent carrier of the virus. Many horses infected with EIAV never show any recognizable clinical signs of disease, and exist as inapparent carriers of the virus until serum antibody is detected during routine herd health evaluations (Issel and Coggins, 1979; Issel and Adams, 1979).

Like other lentiviruses, once infected with EIAV, a horse remains infected for life. Although the level of viremia in inapparent carriers is low compared to the acutely febrile horse, they can serve as a reservoir of infection, and can transmit disease, under field conditions, to uninfected horses (Issel and Coggins, 1979; Issel and Adams, 1979). Recrudescence of clinical signs may be stimulated experimentally by administration of exogenous corticosteroids, suggesting that environmental stress may induce clinical signs (Kono et al., 1976a). Occasionally EIAV infection has been associated with neurologic abnormalities such as ataxia. In these horses anti-EIAV antibody may be detectable by agar gel immunodiffusion of CSF (McClure et al., 1982).

The predominant means of viral transmission is via the intermittent feeding of hematophagous insects, especially of the family Tabanidae (deerflies and horseflies) (Hawkins et al., 1973; Hawkins et al., 1976; Issel and Foil, 1984; Stein et al., 1942; Kemen et al., 1978). The interrupted feeding of a single horsefly can transmit EIAV from an acutely infected horse to a susceptible horse. Because tabanids act only as mechanical vectors for the virus, and because the virus is viable only a short time on the mouthparts of these flies, a fly must be interrupted during its blood meal on an infected host and immediately finish feeding on an uninfected host in order to transmit the virus. Transmission is more likely to occur following tabanid feeding than mosquito feeding because of the large mouthparts of tabanids which inflict very painful bites and carry relatively large quantities of blood (up to 10 nl) (Foil, 1983; Foil et al., 1987a,b). Transmission may also occur vertically, although the exact mechanisms have not been determined. Transplacental transmission of EIAV is most likely to occur if the dam experiences an acute febrile episode and high titer viremia during gestation (Issel, et al., 1988). Experimental infection of equine fetuses on or before 203 days of gestation resulted in abortion between 21 and 64 days postinfection. If fetuses were infected later in gestation, foals were born seropositive but died within 60 days (Issel et al., 1992a). Experimentally, less than 10% of foals born to mares that were inapparent virus carriers were born virus- and antibody-positive (Burns 1974; Kemen and Coggins, 1972). Although the evidence is not conclusive, virus may be transmitted in colostrum and/or milk. Venereal transmission is possible but probably does not occur frequently. Subcutaneous inoculation of an uninfected horse with semen from an infected stallion will transmit the virus (Stein et al., 1944). Iatrogenic transmission via contaminated blood transfusions, hypodermic needles, or surgical instruments has largely been eliminated.

3. Pathologic features

If an EIAV-infected horse dies or is euthanatized during a febrile episode, necropsy lesions include generalized lymph node enlargement, hepatomegaly, splenomegaly, accentuated hepatic lobular structure, mucosal and visceral hemorrhages, ventral subcutaneous edema, and vessel thrombosis (Kono, 1973; Kemen and Coggins, 1972). Histopathologically there are accumulations of lymphocytes and macrophages in periportal areas of the liver, and in lymph nodes, adrenal gland, spleen, meninges, and lung (Ishii and Ishitani, 1975; McGuire, 1986; Henson and McGuire, 1971). These lymphoproliferative lesions have been hypothesized to be the result of spread of virus-reactive T-lymphocytes in an attempt to control infection. Fatty degeneration of the liver and hepatic cell necrosis may also be observed (Ishii and Ishitani, 1975). Kupffer cells are often swollen and may contain hemosiderin aggregates. Similarly, macrophages in lymph nodes, spleen, and bone marrow may show increased hemosiderin accumulations (Ishii and Ishitani, 1975; Henson and McGuire, 1971). Hemorrhage and edema have been attributed to vascular changes, especially in vascular mesenchymal tissues (Konno and Ya-

mamoto, 1970). Severe thrombocytopenia during febrile episodes may also contribute to petechial and ecchymotic hemorrhages (Clabough et al., 1991).

Necropsy of a horse that is an inapparent carrier of EIAV is often unremarkable (Konno and Yamamoto, 1970). In horses that become inapparent carriers of EIAV following an acute febrile episode, hepatic lesions regress and become morphologically undetectable, although they will return with the recurrence of clinical disease (Konno and Yamamoto, 1970; Henson and McGuire, 1971). There may be a glomerulitis with increased cellularity and thickening of glomerular tufts. Retinal depigmentations and prominent choroidal vessels have been observed in the eyes of some inapparent carrier horses. Histopathologically, there is evidence of chronic non-granulomatous choroiditis with foci of lymphocytic infiltrates (Tashjian, 1984).

Thrombocytopenia is the earliest and most consistent laboratory abnormality observed in febrile horses (Clabough et al., 1991). Platelet counts start to decline prior to any increase in rectal temperature. Thrombocytopenia recurs with subsequent febrile episodes, usually persists throughout the viremic period, and may become severe enough to precipitate hemorrhage, especially epistaxis or petechial hemorrhages of mucous membranes (Issel and Coggins, 1979; Clabough, 1990). Platelet counts will generally rebound rapidly following resolution of viremia. Severe anemia is usually observed in chronically infected horses, but decreases in packed cell volume and total erythrocyte count may become evident as early as 5–10 days post-infection (Perryman et al., 1988). A CBC will also often reveal leukopenia or leukocytosis and monocytosis (Issel and Coggins, 1979; Issel and Adams, 1979; McGuire et al., 1969b). Chronically infected horses develop hyperproteinemia due to a non-specific hypergammaglobulinemia (McGuire et al., 1971b).

4. Host: Virus interactions

The virus

EIAV is classified in the family Retroviridae, genus Lentivirus, based on its ultrastructure (Gonda et al., 1978), serologic cross-reactivity (Egberink et al., 1990; Henderson et al., 1987; Montagnier et al., 1984; Van der Maaten et al., 1972), reverse transcriptase activity (Archer et al., 1977; Charman et al., 1976), peptide composition (Ishizaki et al., 1978; Parekh et al., 1980), and genomic organization (Stephens et al., 1986). It is an enveloped RNA virus with an average diameter of 140 nm, covered with surface knobs, and containing a dense conically-shaped core (Matheka et al., 1976; Weiland et al., 1977). The surface knobs are formed by two virus-encoded glycosylated proteins, gp90 and the more hydrophobic gp45 (Parekh et al., 1980). The viral envelope is derived from host cell plasma membranes during the budding process essential to particle maturation (Gonda et al., 1978; Matheka et al., 1976; Weiland et al., 1977). The viral core contains a Mg^{2+}-dependent reverse transcriptase enzyme (Archer et al., 1977; Charman et al., 1976), a 5.5×10^6 Da RNA genome (Charman et al., 1976) consisting of two identical

subunits of single-stranded RNA (Cheevers et al., 1977), and four virus specific non-glycosylated core proteins (p15, p26, p11, and p9) (Henderson et al., 1987; Montelaro et al., 1982). About 1/3 of the core nucleic acid is thought to be tRNA (Charman et al., 1976). The viral genome encodes three major product groups. These are designated (in 5′ to 3′ order) *gag* (group associated antigen) encoding the major internal structural proteins; *pol* (polymerase) encoding the reverse transcriptase enzyme, protease, ribonuclease H, a dUTPase activity, and a DNA nicking enzyme to aid integration into the host cell genome; and *env* (envelope) encoding the envelope glycoproteins gp90 and gp45 (Kawakami et al., 1987; Threadgill et al., 1993).

In EIAV the process of reverse transcription is primed by binding of a specific cellular tRNA to a specific primer binding site (pbs) at the 5′ end of the viral genomic RNA. The EIAV pbs is complementary to lys_3 tRNA (Stephens et al., 1986). The details of reverse transcription and integration have not been determined for EIAV, but they most likely occur in a manner similar to that of other lentiviruses, such as HIV-1 (Haseltine, 1991; Whitcomb and Hughes, 1991).

Through the viral reverse transcriptase and ribonuclease H, cDNA is generated that contains 5′ and 3′ long terminal repeat (LTR) regions. The lentiviral LTR is important in control of viral transcription. The EIAV LTR contains U3, R, and U5 regions, similar to other retroviral LTRs. The transcriptional control elements associated with the U3 region of the EIAV LTR have not been extensively investigated, but several consensus motifs for binding of various cellular factors have been identified, including TATA, an octamer motif (ATTTGCAT) found in other cellular promoters and enhancers, a c-*fos* consensus sequence, and a core enhancer sequence (Carpenter et al., 1991; Payne et al., 1987a; Derse et al., 1993). A cell type-specific negative regulatory element is located between positions −76 and −111 and a potential enhancer region between −76 and −41 (Derse et al., 1987; Dorn and Derse, 1988). The EIAV LTR may be activated by treatment of HeLa cells with phorbol ester. This activation is mediated through an ets/PEA3 motif in the U3 region of the LTR (Carvalho and Derse, 1993). The extensive variability in LTR sequence may be associated with virulence through altered binding of cellular proteins that regulate viral replication.

Five (Noiman et al., 1990) or six (Stephens et al., 1990) viral transcripts have been isolated from cells infected in vitro with EIAV. The largest, 8.2 kilobases in length, is probably full-length viral genomic RNA. Each subgenomic RNA species shares a common 5′ splice donor originating from the 5′ LTR (exon 1). There is an 8 of 9 match with the consensus splice donor sequence 116 bases after the primer binding site in the EIAV LTR (Stephens et al., 1986). The 5.0 kb mRNA is difficult to consistently detect, but appears to result from a single splicing event linking the 5′ exon to 3′ *pol*. A 4.0-kb transcript is considered the *env* mRNA (Noiman et al., 1990; Stephens et al., 1990). The relative quantities and types of viral transcripts produced appears to vary in a cell-dependent fashion. Four spliced transcripts have been identified from liver tissue from an acutely infected horse. These include the *env* mRNA, the product of the S2 open reading frame, and a single transcript encoding both *tat* and *rev* (Beisel et al., 1993). In addition a novel

transcript was identified and found to encode a tat-TM fusion protein (*Ttm*). The *Ttm* protein is translated from the tat initiation codon and contains the carboxy-terminal portion of the envelope transmembrane protein (Beisel et al., 1993).

EIAV *gag* and *pol* gene products are translated from a full-length, 8.0 kB viral message (Noiman et al., 1990). The *gag-pol* message is translated as a Gag precursor polyprotein with a molecular weight of 55,000 ($Pr55^{gag}$) (Henderson et al., 1987; Hussain et al., 1988a). This precursor polypeptide is cleaved, via several intermediate cleavage products, to yield the four major internal proteins of EIAV, p15 (124 residues), p26 (235 residues), p11 (76 residues), and p9 (51 residues) (Stephens et al., 1986; Hussain et al., 1988a). In $Pr55^{gag}$ the proteins exist as p15-p26-*-p11-p9 where the asterisk represents a pentapeptide removed during processing (Henderson et al., 1987).

The N terminus of p15, the first protein in the precursor, is predicted to have a sequence of met-gly-asp-pro-leu-thr (Stephens et al., 1986). When the protein is sequenced, however, the initiator met is missing, and there is a blocked or modified N terminus, that is not due to myristylation of the *gag* precursor (Henderson et al., 1987). Other lentiviruses, including HIV-1, have a myristic acid residue linked to the amino terminus of the capsid precursor protein that is thought to aid insertion of the protein into the cell membrane during virus assembly and release (Veronese et al., 1988). The p15 protein is variably phosphorylated at serine and threonine residues (Montelaro et al., 1982).

The major EIAV core protein is the group-specific antigen, p26, which constitutes about 40% of total virion protein. Because of its conserved nature among different viral strains, and because it is produced in vivo and in vitro in such high quantities and because infected horses consistently make antibodies to it, p26 is the major viral protein used in commercial diagnostic tests for detection of an EIAV antibody response in infected horses (Coggins and Norcross, 1970; Issel and Coggins, 1979; Parekh et al., 1980).

The small p11 protein is thought to be closely associated with viral RNA in the intact virion. It is highly basic, with 17% lysine and arginine residues, and has a relatively high glycine content (Montelaro et al., 1982). These amino acids are associated with the nucleic acid binding and flexible nature of ribonucleoproteins of other viruses and of histones (Montelaro et al., 1982; Oroszlan and Gilden, 1980). The ribonucleoprotein complex of EIAV contains all of the detectable virion-associated p11 and only small quantities of p26 and p15 (Montelaro et al., 1982). The smallest internal protein, p9 contains 28% acidic residues, glutamate and aspartate, and has an acidic isoelectric point of 5.0 (Montelaro et al., 1982). Its function is not known.

The *pol* gene encodes the viral reverse transcriptase, a protease for processing of precursor polypeptides, a dUTPase enzyme required for viral replication in macrophages, and an endonuclease to aid in viral integration into the host cell genome (Stephens, et al., 1986; Threadgill et al., 1993). Because the *pol* gene message is encoded in a different reading frame from *gag*, with a 251 base pair overlap, its translation is hypothesized to require a relatively infrequent frameshifting event (Stephens et al., 1986). A sequence suspected of being a frameshift site

exists 195 nucleotides from the 3′ end of the *gag-pol* overlap and shares structural homology with the *gag-pol* frameshift site of HIV (Jacks et al., 1988a,b).

Like the HIV *env* message, mRNA for the EIAV envelope proteins is a singly spliced product including a short segment from the 5′ LTR joined to a splice acceptor sequence 35 base pairs upstream of the *env* start site (Kawakami et al., 1987; Noiman et al., 1990). Both envelope glycoproteins of EIAV originate from a common mRNA that encodes a large precursor polypeptide 859 amino acids in length (Rushlow et al., 1986; Kawakami et al., 1987). The potential proteolytic cleavage site between gp90 and gp45 is located just upstream of a large hydrophobic domain (Kawakami et al., 1987).

The *env* gene products of EIAV share considerable structural homology, but little sequence homology, with the *env* proteins of visna virus and HIV-1 (Rushlow et al., 1986). The major surface protein of EIAV, gp90, was predicted to have an N-terminal amino acid of glu, based on the assumption that the initial 15 hydrophobic residues constitute a signal peptide removed during polyprotein processing (Kawakami et al., 1987; Ball et al., 1988). However, protein sequencing shows that the N-terminal residue is actually tyr, the seventh residue encoded by the *env* gene (Ball et al., 1988). There are 18 potential sites (asn-x-ser/thr) for N-linked glycosylation in the EIAV *env* sequence, 13 of which are located 5′ to the potential transmembrane region (Kawakami et al., 1987).

The transmembrane (TM) protein of a lentivirus anchors the major surface protein of the virus to the viral envelope and usually has two highly hydrophobic regions separated by a 110–160 amino acid stretch with at least two cysteine residues. Near the N terminus, one of these regions may function in fusing the viral envelope with the host cell membrane. The second hydrophobic region is thought to span the viral envelope (Rice et al., 1990).

Besides major gene products characteristic of all members of the Retroviridae family, the EIAV genome contains three short open reading frames that are hypothesized to encode viral regulatory proteins (Fig. 1). The first two of these sequences, designated S1 and S2 respectively, occur in the *pol-env* intergenic region, but do not overlap the 3′ *pol* sequence. S1 contains 50 codons, none of which is met, in the same reading frame as *pol* and *env* (Rushlow et al., 1986). S2 is in a separate reading frame, extending into the *env* gene, and contains 66 codons with a possible initiator met as the second codon (Rushlow et al., 1986). Both S1 and S2 contain about 17–18% basic residues (lys and arg) (Rushlow et al., 1986). S3 is located overlapping the 3′ *env* gene sequences, contains 135 codons, and lacks a met codon (Rushlow et al., 1986; Ball et al., 1988).

The EIAV genome encodes a gene for production of a transactivating (*tat*) protein that is capable of increasing LTR-directed viral transcription by both transcriptional and posttranscriptional enhancement (Derse et al., 1987; Sherman et al., 1988). The EIAV *tat* protein mRNA is comprised of three exons: the first two, located in the 5′ LTR and S1, encode this *tat* activity and the third, S3, may encode a protein similar to *rev* of HIV-1 (Dorn et al., 1990). A *tat* gene product has been identified in association with the primate and bovine lentiviruses, but not with feline, caprine, or ovine lentiviruses. In general, the lentiviral *tat* gene

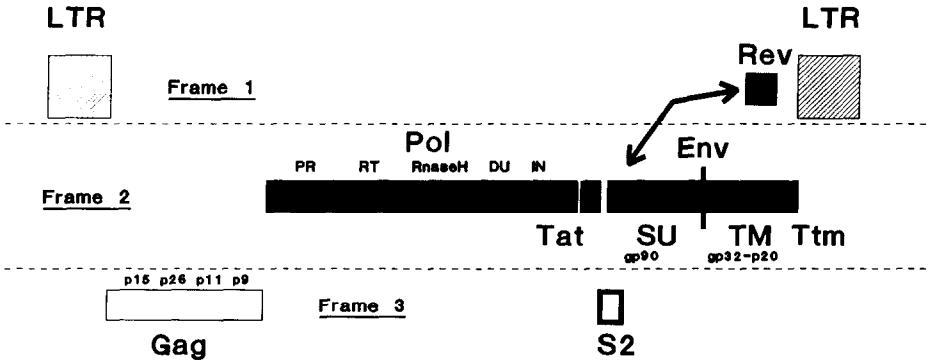

Fig. 1. Schematic diagram of the genomic organization of equine infectious anemia virus. LTR-long terminal repeats. Gag region; p15-matrix protein, p26-capsid protein, p11-nucleocapsid protein, p9-function unknown. Pol region; PR-protease, RT-reverse transcriptase, DU-deoxyuridine triphosphatase, IN-integrase. S2-function unknown. Env region; gp90-surface protein, gp32-transmembrane, p20-C-terminal end of transmembrane (function unknown), Ttm-truncated transmembrane protein.

products contain five important structural elements: the amino-terminal, cysteine-rich, core, basic, and carboxy-terminal regions (Dorn et al., 1990; Carroll et al., 1991). EIAV *tat* contains a highly conserved 16 amino acid core domain, similar to HIV-1, but not the cysteine-rich region present in HIV-1 *tat* (Dorn et al., 1990). The EIAV *tat* core domain is thought to be an essential part of the activation domain since removal of the four N-terminal amino acids of that domain abolish *tat* activity (Dorn et al., 1990; Carroll et al., 1991). The carboxy-terminal and basic regions of EIAV *tat* are critical for binding to the EIAV *tat* responsive element (TAR) located in the viral LTR (Dorn et al., 1990; Carroll et al., 1991; Carvalho and Derse, 1991). This basic domain may also be important in nuclear localization of the protein (Dorn et al., 1990). EIAV *tat* translation appears to initiate at a non-AUG codon in exon 1 of the mRNA (Stephens et al., 1990; Dorn et al., 1990).

Transactivation, but not promoter activity, is abolished by deletion of the R-U5 region of the EIAV LTR (Sherman et al., 1988). *Tat* protein increases the steady-state levels of viral transcription by interacting with the *cis*-acting *tat* responsive element (TAR) in the 5′ LTR (Carroll et al., 1991; Carvalho and Derse, 1991). Specific TAR sequences are downstream of the transcriptional start site (Dorn et al., 1988; Carvalho and Derse, 1991) in an hypothesized stem-loop structure involving nucleotides + 1 to + 25 (Carvalho and Derse, 1991). The EIAV TAR is similar in structure to the HIV-1 TAR (Dorn and Derse, 1988; Carvalho and Derse, 1991), but with a shorter stem than in HIV-1 and without the uridine bulge (Carvalho and Derse, 1991).

The putative EIAV *rev* protein is encoded primarily within S3 (Dorn and Derse, 1988; Saman et al., 1990), an open reading frame that overlaps the *env* gene but not the 3′ LTR (Kawakami et al., 1987). *Rev* potentially encodes a 135 amino acid polypeptide with a molecular weight of 15,990 (Kawakami et al., 1987), and contains stretches of basic residues that might function as nuclear localization

signals (Saman et al., 1990). The coding region begins with a splice acceptor site and no met codon is found in the sequence (Kawakami et al., 1987). Polyclonal antisera to S3 expressed in vitro recognizes a 20 kDa protein expressed in cells infected with EIAV (Saman et al., 1990). Sera from EIAV-infected horses specifically recognizes this protein, indicating that S3 is expressed in vivo in EIAV-infected animals (Saman et al., 1990). Truncation of the putative EIAV *rev* protein leads to upregulation of expression of multiply spliced viral messages (Stephens et al., 1990). This observation supports the hypothesis that this protein serves a function analogous to the HIV-1 *rev* protein.

Very little is known about EIAV maturation and release. The HIV-1 *gag* polyprotein precursor and *gag-pol* fusion protein assemble at the inner surface of the host cell membrane and insert themselves via a myristic acid residue attached to the amino terminus of the capsid precursor protein (Veronese et al., 1988). The EIAV *gag-pol* proteins lack this myristic acid residue, but the amino terminus of that protein is blocked, and whatever molecule is attached may serve a similar function (Henderson et al., 1987).

Cell culture characteristics and virulence of EIAV strains

A number of strains of EIAV have been described. They vary markedly in cell culture characteristics, in vivo infectivity, and virulence. A knowledge of these strains and their characteristics is essential to understanding many of the apparent discrepancies and inconsistencies of current and past EIAV literature (Table 1).

Most virulent EIAV strains cannot be propagated in tissue culture cell lines, but only in primary horse macrophage cultures (Carpenter and Chesebro, 1989;

Table 1
Commonly used field and laboratory strains of EIAV

Virus designation	Properties
Wyoming wild-type	Highly virulent field isolate of EIAV. Virust stocks are maintained by passage of serum in vivo (Malmquist et al., 1973)
Malmquist	Derived by repeated blind passage in vitro of the Wyoming wild-type strain of EIAV. Avirulent unless back-passaged in vivo (Malmquist et al. 1973)
F1LC	Malmquist strain of virus passaged in vivo. This strain retains the capacity to replicate in non-macrophage cell types and is moderately virulent when used to infect ponies (Payne et al., 1987a; Payne et al., 1987b)
CL22-V	Virus derived from first infectious molecular clone of the Malmquist strain of EIAV (avirulent) (Whetter et al., 1990)
pER-V	Virus derived from the infectious molecular clone pER. Similar to CL22 except that the U3 region is derived from the non-infectious clone 1369 (avirulent) (Perry et al., 1992)
Th-1	A field isolate of EIAV from Massachusetts (virulent) (Carpenter & Chesebro, 1989)
MA-1	Avirulent strain of EIAV (biologically cloned) adapted for replication in equine dermis cells by blind passage (Carpenter & Chesebro, 1989)

Kobayashi and Kono, 1967). Unfortunately, primary horse macrophage cultures are difficult to maintain, and relatively short lived (Kobayashi and Kono, 1967; Evans et al., 1984; Kono and Yokomizo, 1968). In vitro infection of primary horse macrophage cultures with EIAV induces a cytopathic effect which can be difficult to distinguish from normal cellular senescence (Kono et al., 1970; Kobayashi and Kono, 1967; Malmquist et al., 1973). The Wyoming strain of EIAV is a virulent, wild-type field isolate originating from a horse in Wyoming experiencing a febrile episode of EIA (Kemeny et al., 1971). In vitro the virus can only be grown in primary horse macrophage cultures. Virus stock is maintained in serum taken from an infected horse during a peak febrile response, and the titer of this stock is approximately 10^6 infectious doses/ml (as determined by horse inoculation assay). In primary horse leukocyte cultures infected in vitro with the Wyoming strain of EIAV, increases in virus titer can be detected as early as 24 h post-infection, with viral titer peaking at 48 to 72 h post-infection (Kono et al., 1970; Ushimi et al., 1972). Viral titers in culture supernatant are consistently higher than cell-associated titers (Kono et al., 1970; Ushimi et al., 1972) and electron microscopy confirms that progeny EIAV virions bud at the cell surface immediately upon maturation (Kono et al., 1970; Tajima et al., 1969). A cytopathic effect has been described as early as 72 h post-infection (Kono et al., 1970; Ushimi et al., 1972). Immunofluorescent staining of infected primary horse leukocyte cultures to detect viral antigen reveals only cytoplasmic fluorescence (Ushimi et al., 1972; Crawford et al., 1971; Ushimi et al., 1970).

The Malmquist strain of EIAV was derived from the wild-type Wyoming strain by multiple blind passage adaptation of the wild-type virus in equine dermal fibroblast cells (Malmquist et al., 1973; Orrego et al., 1982). It can establish chronic infections in vitro in feline (FEA) and canine (Cf2Th) cells, as well as in equine dermal or kidney cell lines (Benton et al., 1981). Equine dermal fibroblasts infected with the Malmquist strain show no cytopathology or alterations in growth (Malmquist et al., 1973; Klevjer-Anderson et al., 1979). Virulence of the Malmquist strain is severely attenuated in vivo, but it can revert to virulence after serial backpassage in horses (Orrego et al., 1982; Gutekunst et al., 1979). This cell-adapted, virulent, prototype strain of EIAV maintains its ability to establish a persistent, non-cytopathic infection in fetal equine kidney cells in vitro. In fetal donkey dermis (FDD) cells, this same virus strain causes a cytopathic infection characterized by rounding and detachment of infected cells after 20–22 days in culture (Rasty et al., 1990). A FDD-adapted strain of EIAV will produce the same cytopathic effect after only 10–12 days in culture in FDD cells (Rasty et al., 1990).

A field isolate of EIAV originating from a Massachusetts horse and designated Th-1, has been adapted by blind serial passage to replicate in equine dermal cells. The virus stock collected after the fourth passage was designated MA-1/11, and used to biologically clone an equine dermal cell-adapted strain of EIAV, designated MA-1 (Carpenter and Chesebro, 1989). This EIAV isolate, selected for its ability to replicate in equine dermal cells, is no longer capable of replicating in primary horse macrophage cultures.

The first full-length molecular clone of EIAV was derived from *Eco*RI digested

DNA isolated from fetal equine kidney cells persistently infected with the Malmquist strain of EIAV (Stephens et al., 1986). The complete nucleotide sequence of this clone has been reported (Rushlow et al., 1986; Stephens et al., 1986).

An infectious molecular clone (CL22-V) was derived from Cf2Th cells infected with the Malmquist strain of EIAV (ATCC VR-778). An *Eco*RI digest of DNA from Cf2Th cells was cloned into an EMBL3 bacteriophage library (Whetter et al., 1990). One phage (CL22) produced infectious virus particles after transfection into equine dermis cells. Virus stock derived from this phage (CL22-V) is infectious but avirulent when inoculated into horses (Whetter et al., 1990). The entire nucleic acid sequence of CL22 has been determined (Perry et al., 1992).

A molecular clone was also derived from an avirulent equine dermal cell-adapted isolate (MA-1) of EIAV (Carpenter et al., 1991). Clonal cell lines were derived from Cf2th canine fetal thymus cells persistently infected with MA-1, and a full-length proviral clone obtained (Carpenter et al., 1991).

In vivo cell tropism

EIAV titers in the serum of infected horses fluctuate widely, but are highest during the initial acute febrile episode, decreasing during subsequent febrile episodes. Levels may decline to undetectable during the asymptomatic periods between acute attacks (Kono et al., 1971; Issel et al., 1979). Because equine leukocyte cultures are permissive for wild-type viral replication in vitro (Kobayashi and Kono, 1967; Kono and Kobayashi, 1967) and leukocyte cultures established from a horse acutely infected with EIAV contain viral antigen and particles in their cytoplasm (Evans et al., 1984; McConnell et al., 1977), it was hypothesized that virus infects and/or replicates in monocytes and/or macrophages (Cheevers and McGuire, 1985).

During the acute viremic phase of disease virus is detected mainly in the liver, spleen, and serum of infected horses (Kono et al., 1971). Fluorescent antibody studies of tissues from chronically infected horses indicate that viral antigen is present in the cytoplasm of macrophage-like cells in these tissues, especially the spleen (McGuire et al., 1971a). Viral antigen is also found in the lymph nodes, kidney, thymus, bone marrow, lung, adrenal, stomach, pancreas, cerebrum, cerebellum, pituitary gland, and circulating mononuclear cells (McGuire et al., 1971a). The amount of infectious virus in these tissues appears to decline rapidly once viremia subsides (Kono et al., 1971). Distribution of viral DNA in acutely infected horses closely parallels the distribution of viral antigen. Highest concentrations of viral DNA are in the liver, lymph nodes, bone marrow, and spleen, with lesser amounts in the kidney, choroid plexus, and peripheral blood leukocytes (Rice et al., 1989). Most of this DNA is not integrated into host genomic DNA, but exists in linear and circular unintegrated forms (Rice et al., 1989). Provirus is also detectable in peripheral blood cells of acutely infected horses (Rice et al., 1989; O'Rourke et al., 1991).

In situ hybridization of tissues from horses infected with the wild-type Wyoming strain of EIAV identified the liver, spleen, lymph nodes, kidney, lung, and adrenal

gland as the primary host tissue sites for active viral transcription during acute infection. (Sellon et al., 1992) Combined immunohistochemistry, with a monoclonal antibody recognizing a cytoplasmic antigen of equine mononuclear phagocytes, and in situ hybridization for viral RNA identified most infected cells as mature tissue macrophages. In contrast, in situ hybridization of adherent peripheral blood mononuclear cells collected from horses on various days during the first two weeks postinfection failed to detect any viral RNA in these cells (Sellon et al., 1992). These results suggest that mature tissue macrophages are the primary source of the high titer of viremia present during acute infection with EIAV. Like visna virus of sheep (Gendelman et al., 1985; Gendelman et al., 1986) and HIV-1 (Pauza et al., 1988), undifferentiated monocytes are permissive for virus infections, but viral replication appears restricted. Visna (Gendelman et al., 1985; Gendelman et al., 1986) and HIV (Pauza et al., 1988) demonstrate a surprisingly low level of production of infectious virion particles despite a high level of virus transcription. This posttranscriptional block does not appear to exist in horses acutely infected with EIAV. The high titer viremia observed in these horses suggests that active transcription results in the production of large numbers of infectious virions (Sellon et al., 1992).

Little is known about the cellular site of viral persistence in asymptomatic animals. Distribution of viral antigen is similar to that in the acutely infected animal, but the quantity is greatly decreased (McGuire et al., 1971a). Viral DNA is undetectable by Southern blot analysis of either blood or tissues from asymptomatic animals (Rice et al., 1989) and viral transcription is not detectable by in situ hybridization of tissues from these horses (our unpublished data). However, provirus is detectable at low levels in buffy coat cells from some asymptomatic animals following enzymatic DNA amplification (M. Flaherty, personal communication, 1991).

Anemia

The anemia of EIA is a result of both intravascular and extravascular hemolysis (McGuire et al., 1969), as well as an impaired bone marrow response (McGuire et al., 1969b). Erythrocyte life span in ponies with acute EIA varies between 28 and 87 days (normal is 136 days). Increased plasma hemoglobin and decreased plasma haptoglobin indicate intravascular hemolysis in these animals. In contrast, erythrocyte life span in chronically infected ponies varies between 89 and 113 days, but no changes in plasma hemoglobin or haptoglobin levels are detectable, indicating extravascular hemolysis (McGuire et al., 1969c). EIAV will hemagglutinate erythrocytes in vitro (Sentsui and Kono, 1976). This hemagglutination activity has been attributed to the surface glycoproteins of the virus (Nishimura and Najajima, 1984).

Complement-coated erythrocytes are present in EIAV-infected horses (McGuire et al., 1969a; Perryman et al., 1971). Although antibody cannot be detected with IgG, IgM, or IgG(T) specific direct Coombs tests (McGuire et al., 1969a), more sensitive elution studies have demonstrated that immunoglobulin is present in low quantities on the surface of erythrocytes from horses with EIA (Henson et

al., 1971). Immunofluorescent studies of bone marrow failed to find any EIAV antigen in erythrocyte precursors. It is possible that viral hemagglutinin, an activity attributed to surface glycoproteins (Sentsui and Kono, 1976; Nishimura and Nakajima, 1984), enables virus to attach to circulating erythrocytes and attract specific antibody (Henson and McGuire, 1971). Alternatively, circulating virus-antibody immune complexes (McGuire et al., 1972) may attach to erythrocytes via Fc or complement receptors. After virus or virus-antibody complexes adsorb to horse erythrocytes they activate complement via the classical pathway (Sentsui and Kono, 1987a; Sentsui and Kono, 1988), and complement-mediated (intravascular) hemolysis results. Alternatively, extravascular hemolysis may occur as complement-coated erythrocytes are phagocytized by macrophage-like cells and neutrophils (Sentsui and Kono, 1987b,1988). Erythrophagocytosis is prominent in bone marrow and splenic macrophages from acutely infected horses. Heinz bodies have been reported in some EIAV-infected horses (Matthias et al., 1956) but they are likely secondary to other red cell changes and not responsible for viral-induced hemolysis (McGuire et al., 1970).

Hemolytic anemia is exacerbated by an impaired bone marrow response, as evidenced by decreases in serum iron concentrations, percentage saturation of transferrin, and plasma iron turnover, as well as an increase in bone marrow myeloid:erythroid ratio (McGuire et al., 1969b). Arabian foals with combined immunodeficiency, experimentally infected with EIAV, become profoundly anemic despite their inability to mount a specific antibody or cell-mediated immune response (Perryman et al., 1988). Erythrocytes from EIAV-infected immunodeficient foals did not have complement on their surface, indicating that complement-coating is caused by immune responses.

Thrombocytopenia

A decrease in the number of circulating platelets is one of the earliest abnormalities detectable in horses infected with EIAV. Sequential febrile episodes are associated with cyclical thrombocytopenia in which the nadir of platelet counts coincides with peak febrile responses. During thrombocytopenia, circulating platelets have increased quantities of platelet-bound IgG and IgM (Clabough et al., 1991). Viral replication in bone marrow megakaryocytes is not detectable by in situ hybridization (Clabough et al., 1991). These results suggest that immune complexes are deposited on platelets, facilitating their removal from circulation by mononuclear phagocytes of the spleen and liver. This may help target infectious virus particles, in the form of immune complexes, to host cells most permissive for in vivo viral replication (Clabough et al., 1991). Arabian foals with CID lacking functional B- and T-lymphocytes become severely thrombocytopenic following infection with EIAV (Crawford et al., 1993). Endogenous metabolic labeling of platelets has shown that production is decreased significantly during the development of thrombocytopenia and, further, platelet survival is shortened by about 50% in EIAV-infected normal and CID foals (Crawford et al., 1993).

Immune-mediated glomerulitis

Glomerulitis with both mesangial and epithelial cell proliferation and basement membrane thickening is present in approximately 75% of EIAV-infected horses.

IgG and the third component of complement (C3) are present in small granular deposits along the glomerular basement membrane. Anti-EIAV antibodies can be eluted from isolated diseased glomeruli (Banks et al., 1972). Although deposition of virus-antibody-complement complexes in the glomeruli appears to be a common phenomena in EIAV infection, clinical proteinuria is rarely encountered (Banks et al., 1972).

Antigenic variation in vivo

Each febrile episode in the chronically infected horse is associated with the emergence of a novel antigenic variant of the virus (McGuire et al., 1987; Montelaro et al., 1984; Payne et al., 1984; Payne et al., 1987b; Salinovich et al., 1986; Kono et al., 1973b). It has been proposed that these novel antigenic variants emerge due to host immune pressure and are temporarily capable of escaping the host immune response (Kono et al., 1973a; Kono et al., 1973b), resulting in periodic febrile episodes. The majority of febrile episodes occur during the first year of infection. The eventual cessation of clinical disease in most horses may be the result of a host immune response against epitopes common to all variants of EIAV. Arabian foals with combined immunodeficiency, experimentally infected with virulent EIAV, were unable to clear virus from their plasma, confirming that viral clearance is a result of host B- and T-cell responses (Perryman et al., 1988). Antigenic variation appears to be important in the recurrence of viremic episodes and is a major obstacle to vaccine development.

Tryptic peptide and glycopeptide maps reveal alterations in the amino acid sequence and glycosylation patterns of both gp90 and gp45 (Nishimura and Nakajima, 1984). Oligonucleotide fingerprinting confirms that these changes are associated with changes in the viral *env* gene (Payne et al., 1984, 1987; Salinovich et al., 1986). In contrast, no variation is observed in the viral core proteins (Salinovich et al., 1986). Comparisons of peptide and genomic maps of virus isolates recovered from sequential febrile episodes reveal each isolate to be structurally unique, with variation occurring in a random, non-cumulative process (Payne et al., 1987b; Hussain et al., 1987). Rapid epitope alterations in gp90 and gp45 have been documented using neutralizing monoclonal antibodies against the virion surface glycoproteins (Hussain et al., 1987). Selective immune pressure has been hypothesized as the driving force behind the antigenic variation seen in clinical cases of EIA (Montelaro et al., 1984; Salinovich et al., 1986; Kono et al., 1973a,b). Alterations in the viral envelope may enable the new antigenic strain to escape neutralizing antibodies resulting in a new episode of viral replication and clinical disease. In clinical cases of EIA, several cycles of fever and viremia may occur prior to the detection of any neutralizing antibody in the serum (Kono et al., 1973a,b; Muesing et al., 1985). Rather than recognizing an altered epitope on the virion surface, the host immune system may recognize variant specific antigens on the surface of infected cells, and subsequently eliminate those infected cells (Carpenter et al., 1987).

There are three mechanisms by which novel antigenic variants of EIAV may emerge. (1) The epitopic changes in sequential viral isolates from a single horse

may result from mutations generated during the viral replicative process. This is a mechanism common to retroviruses, due to errors in reverse transcription (Dougherty and Temin, 1988; Leider et al., 1988), and is most likely responsible for the in vivo antigenic variation observed with EIAV. (2) The initial EIAV inoculum is almost certainly a heterogeneous mixture of virus types. The sequential selection and multiplication of individual antigenic variants present in that initial inoculum cannot be discounted at this time. (3) Recombination between different viral strains may occur in vivo to generate novel antigenic variants, likewise aiding in evasion of the host immune response (Clavel et al., 1989). Until a pathogenic biological or molecular clone of EIA virus is developed to aid in the study of variant generation in a single animal after inoculation of a homogeneous viral population, the exact mechanism behind the antigenic variation of EIA virus will likely remain speculative.

Viral persistence

EIAV-seropositive horses are considered infected for life. Indirect evidence for viral persistence includes continuing anemia, hypergammaglobulinemia (McGuire et al., 1971b), decreased C3 levels (Perryman et al., 1971), deposition of gammaglobulin and C3 in gomeruli (Banks et al., 1972), and lymphoid hyperplasia (Henson et al., 1971). More direct evidence of viral persistence includes transmission of infection from inapparent carriers to susceptible animals (Issel et al., 1982), presence of infectious circulating immune complexes (McGuire et al., 1972), and induction of disease by immunosuppression (Kono et al., 1976). While antigenic variation undoubtedly contributes to recurrent viremias in the infected animal, it is unlikely to be the primary mechanism of persistence. Of primary importance to viral persistence is the ability of the virus to integrate in the host cell's genomic DNA and remain there in a latent or unexpressed condition. The subpopulation of cells carrying the viral genetic material, and the stimuli responsible for activation of viral replication, have not been conclusively identified. It is likely that cells of monocyte/macrophage lineage are the primary site of viral persistence.

A number of molecular and cellular events have been linked to lentivirus persistence. The integration and transcription patterns of EIAV proviral DNA differs between persistent and cytopathic infections of cultured cells (Rasty et al., 1990). In persistently infected cells only about 30% of EIAV cDNA exists in the integrated form, while the rest is equally divided between unintegrated linear and closed circular forms. In contrast, during cytopathic infection, integrated provirus ranges from 65 to more than 90% of the total proviral DNA. The total quantity of viral RNA is nearly 30-fold higher in these cells than in persistently infected cells (Rasty et al., 1990). These results differ from other studies of lentivirus cDNA (Harris et al., 1984; Muesing et al., 1985; Rice et al., 1978) which suggest that persistent, non-cytopathic infections are associated with a predominance of integrated proviral DNA, while cytopathic infections have a predominance of unintegrated proviral DNA. In vivo, cDNA from a horse acutely infected with virulent, cell-adapted EIAV existed mainly as unintegrated linear and closed circular forms (Rice et al., 1989).

Humoral immune response

The host reaction to EIAV infection involves both humoral and cell-mediated immune responses. A heterogeneous hypergammaglobulinemia is common in horses with EIA. In most horses IgG, IgG(T), and IgM are detectably increased approximately 60 days post-infection (McGuire et al., 1971b). Antibody to the p26 core protein is detectable in almost every infected horse by AGID by 45 days post-infection (Coggins et al., 1972). Lower frequency and lower titer antibody responses against the p11 and p9 core proteins are present in many animals (Charman et al., 1979).

A large humoral immune response is mounted against both type-specific and shared epitopes on the major envelope glycoproteins gp90 and gp45 (O'Rourke et al., 1988). Antibodies may or may not be virus neutralizing. Neutralizing antibodies are effective only against the specific viral strain that stimulated their production. Little or no cross-neutralization is observed between different isolates of EIAV even when obtained from successive clinical episodes in the same animal (Montelaro et al., 1984; Henson and McGuire, 1971; O'Rourke et al., 1989). This selective, type-specific neutralization is due to the rapid variation in neutralization epitopes on the viral envelope glycoproteins. Monoclonal antibodies that react with variable epitopes on gp90 or gp45 effectively neutralize the virus (Hussain et al., 1987; Payne et al., 1989). Non-neutralizing antibodies react primarily with variable epitopes of gp90 and gp45 (Hussain et al., 1987; Payne et al., 1989).

At least four immunogenic epitopes have been identified on gp90 using monoclonal antibodies (Hussain et al., 1988b). Of these, only two contribute to virus neutralization. The predominant and most consistent in vivo immune response against gp90 is directed against its carboxyl terminus (Payne et al., 1989). Immune sera from horses contain antibody reactive with both the carbohydrate and protein moieties of gp90, but most reactivity is directed against the protein epitopes (Montelaro et al., 1984). Indeed, serological cross-reactivity between HIV gp120 and immune sera from EIAV-infected horses is due to carbohydrate, and not protein, components of the viral glycoproteins (Montelaro et al., 1988). The predominant humoral immune response against gp45 in horses is directed against the amino terminus of that glycoprotein, between the hydrophobic fusion and the transmembrane functional domains (Payne et al., 1989; Chong et al., 1991b).

Despite the rapid generation of antigenic variants of EIAV during in vivo infection, most infected animals eventually suppress viral replication and become inapparent viral carriers (Issel et al., 1979b). This is thought to result from the eventual development of a broad spectrum of neutralizing antibodies that are capable of recognizing enough common viral epitopes to minimize viral replication (Rwambo et al., 1990) or from other effect or mechanisms which recognize conserved epitopes (Cheevers et al., 1985). This eventual suppression of viral replication and clinical disease is unique among lentiviruses.

Group-specific antibodies to the p26 core protein recognize all EIAV isolates, are not virus-neutralizing, and serve as the basis for current diagnostic tests (Coggins et al., 1970; Shane et al., 1984). The predominant humoral immune

response in horses is directed against a domain at the carboxy terminus of p26 (Chong et al., 1991a).

Serologic cross-reactivity between EIAV and human, feline, and bovine immunodeficiency viruses *gag* gene products have been documented (Egberink et al., 1990; Henderson et al., 1987; Montagnier et al., 1984; Van der Maaten et al., 1972; Goudsmit et al., 1986), but no cross-reactivity appears to exist between EIAV and the caprine and ovine lentiviruses (e.g., caprine arthritis encephalitis virus and visna/maedi virus of sheep) (Charman et al., 1976; Stowring et al., 1979).

Cell-mediated immune response

Cell-mediated immune responses to EIAV have been less well characterized than antibody responses. T-lymphocytes which proliferate in the presence of EIAV antigen are present in the peripheral blood of infected horses 143, Kono et al., 1976) and may be involved in the lymphoproliferative lesions seen in the liver, lymph nodes and spleen (Shively et al., 1982). Evaluation of horses with persistent EIAV infection found no defect in B- and T-lymphocytes numbers or ability to proliferate in response to in vitro stimuli (Banks et al., 1973). In contrast, horses with recent EIAV infections had suppressed responses in both mitogen-induced and antigen-induced lymphocyte proliferation assays (Newman et al., 1991). Suppression of the levels of the IgG(T) isotype occurs during persistent EIAV infection, although the significance of this suppression is unknown (McGuire, 1976). MHC restricted cytotoxic T-lymphocytes have not been convincingly demonstrated in EIAV-infected horses. However, non-MHC restricted cytotoxicity of unknown mechanism was shown against EIAV infected cells while antibody dependent cell-mediated cytotoxicity could not be demonstrated (Fujimiya et al., 1979). In another experiment, both MHC-restricted cell-mediated cytotoxicity and antibody dependent cell-mediated cytotoxicity were demonstrated against targets consisting of homologous peripheral blood lymphocytes stimulated with phytohemagglutinin (Gerencer et al., 1989). However, in the later experiments no verification of EIAV in the target was demonstrated, making interpretation difficult.

A peripheral monocytosis is seen in many horses infected with EIAV (Issel et al., 1979b; Banks et al., 1975). These monocytes show an increased binding to autologous erythrocytes, and exhibit decreased in vitro migration compared to monocytes from uninfected horses (Banks et al., 1975). These findings suggest an in vivo activation of monocytes and macrophages in infected horses. The significance of monocyte activation has not been investigated. However, tissue macrophages are suspected to be the major site of viral replication in vivo, and these cells are also known to remove complement-coated erythrocytes from the circulation of infected horses. Activation could occur concurrent with either or both of these activities (Banks et al., 1975).

Immune-mediated enhancement of infection

Monocyte/macrophage tropism is common for a number of viruses. Because the macrophage is an integral component of specific immune responses, infection

of the macrophage can have a variety of effects on disease progression, either enhancing or inhibiting viral infectivity. Because the macrophage is an actively phagocytic cell, it may be able to internalize virus in a non-specific manner. The Fc (Daughaday et al., 1981; Schlesinger and Brandriss, 1981) or complement (Cardosa et al., 1983; Cardosa et al., 1986) receptors of macrophages may bind circulating infectious immune complexes, internalize these ligands through clathrin coated pits, and then release intact virion cores from lysosomal structures (Gollins and Porterfield, 1984, 1985). Antibody dependent enhancement of infection of macrophages or macrophage-like cell lines has been demonstrated for flaviviruses (Schlesinger et al., 1981; Gollins and Porterfield, 1984; Cecilia et al., 1988), herpesviruses (Inada et al., 1985), retroviruses (Homsy et al., 1989; Jolly et al., 1989; Matsuda et al., 1989; Montefiori et al., 1990; Robinson et al., 1988; Tremblay et al., 1990), orthomyxoviruses (Ochiaia et al., 1988), togaviruses (Chanas et al., 1982), reoviruses (Burstin et al., 1983), and coronaviruses (Weiss and Scott, 1981a).

Dengue virus infection of humans (a flavivirus) and feline infectious peritonitis virus infection of cats (FIPV; a coronavirus) are the best-studied examples of in vivo and in vitro enhancement of disease severity by the presence of antiviral antibody (Weiss and Scott, 1981a; Halstead et al., 1988, 1979; Halstead and O'Rourke, 1977; Peiris and Porterfield, 1979; Vennema et al., 1990). These viral infections share many characteristics with EIAV infection: (1) Virus is found primarily in tissue mononuclear phagocytes during in vivo infection and grows in vitro in adherent blood leukocyte cultures (Weiss et al., 1981; Halstead et al., 1988); (2) Pathogenesis of clinical signs is considered primarily immune-complex-mediated and is characterized by the presence of circulating immune complexes, decreased C3 concentrations, depositions of complement, immunoglobulin, and antigen in glomeruli, and thrombocytopenia (Jolly et al., 1989; Weiss and Scott, 1981a–c; Halstead et al., 1988; Jacobse-Geels et al., 1982; Weiss et al., 1980); (3) clinical disease is more severe in patients with preexisting serum non-neutralizing antibody (Weiss and Scott, 1981a; Halstead, 1988). Direct evidence of this latter characteristic is not available for EIAV, but vaccination of ponies with a subunit vaccine of EIAV envelope glycoproteins enhanced the disease severity observed following challenge with wild-type EIAV, consistent with immune-mediated enhancement of disease (Issel et al., 1992b).

HIV-1 shares many of the characteristics of FIP, dengue viruses, and EIAV. It infects and replicates in a variety of tissue mononuclear phagocytes. Disease may be associated with circulating immune complexes, decreased circulating C3 concentrations, and thrombocytopenia. In vitro infectivity of HIV-1 may be enhanced in the presence of nonneutralizing antibody in an Fc and/or complement-mediated mechanism.

Comparison with other lentiviruses

Equine infectious anemia virus provides a unique model for investigation of several aspects of lentivirus pathogenesis. Its genomic organization is more simple than that of most other lentiviruses, consisting of only three short open reading

frames in addition to the gag, pol, and env genes found in all replication competent retroviruses. The S1 and S3 open reading frames appear to correspond structurally and functionally with the tat and rev genes of HIV-1, respectively. Like the primate lentiviruses EIAV uses differential splicing to generate various mRNA species from which viral proteins are translated. The availability of a variety of molecular clones of the virus, including at least one virulent clone, provides a powerful tool for manipulating the genetic elements of a lentivirus to identify specific sequences responsible for virulence.

EIAV is an especially powerful model of the events occurring during acute lentivirus infection. The incubation period between exposure to the virus and the initial development of clinical signs is measured in days to weeks rather than years. In addition, the high titer viremia that follows initial infection is profound and magnifies viral effects, making it easier to identify and define the mechanism of specific virus related effects. Because the virus replicates only in macrophages, not in lymphocytes, the effects of specific lentivirus/macrophage interactions can be more easily investigated.

EIAV also represents an intriguing model for investigation of the characterization of antigenic changes occurring following infection of the host with a lentivirus. Emergence of novel antigenic strains of the virus is associated with recurrence of fever and thrombocytopenia. Infection of experimental horses with a virulent molecular clone of the virus and sequencing of sequential isolates will allow delineation of patterns of antigenic change and changes that are associated with renewed virulence. This will aid in identification of antigenic domains associated with evasion of immune surveillance.

Perhaps the most intriguing aspect of EIAV pathogenesis is the ability of most horses to eventually control the replication of the virus. Unlike other lentiviruses, chronic infection is associated with long-term suppression of viral expression and a normal life-span for the host. Identification of the immune mechanisms responsible for this control of viral replication may have implications for vaccine design for all lentiviruses.

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