Elevation of cytokines associated with the thrombocytopenia of equine infectious anaemia

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Thrombocytopenia is a common finding in infection with equine infectious anaemia virus (EIAV), a lentivirus with some homology to human immunodeficiency virus (HIV). The thrombocytopenia of EIA, like that in some HIV patients, appears to have a multifactorial pathogenesis. To investigate the decreased platelet production seen in experimental EIA, the levels of three potential negative regulators of platelet production – tumour necrosis factor-α (TNF-α), transforming growth factor-β (TGF-β) and interferon-α (IFN-α) – were measured in serum and bone marrow of six severe combined immunodeficient (SCID) foals and ten immunocompetent EIAV-infected foals. Levels of cytokines in pre-infection foal sera and bone marrow were compared with levels observed during clinical EIA. Mean serum levels of TNF-α and IFN-α were significantly higher (P < 0.05) on days 4 to 0 of thrombocytopenia than before infection. Serum TGF-β was significantly elevated on all days except day 1 of thrombocytopenia. Bone marrow TNF-α levels were significantly increased in infected foals just before clinical thrombocytopenia. TGF-β activity was not different in pre-infection and pre-thrombocytopenia bone marrows, but levels of TGF-β protein as determined by immunohistochemical staining were significantly higher in pre-thrombocytopenia bone marrow. IFN-α activity in bone marrow increased just before thrombocytopenia, but the difference was not significant at P < 0.05. Serum TNF-α levels were 2–5 times higher in SCID foals on three of the days prior to thrombocytopenia than in immunocompetent foals. No significant differences were found between the levels in SCID and immunocompetent foals of serum and bone marrow TGF-β or IFN-α at any of the times examined.

Introduction

Thrombocytopenia is a common manifestation of infection with equine infectious anaemia virus (EIAV) (Clabough et al., 1991; Crawford et al., 1996), and is often seen in infection with human immunodeficiency virus (HIV), a related lentivirus (Stephens et al., 1986; Ballem et al., 1992), and in many acute, systemic viral infections (Young & Mortimer, 1984; Jandl, 1987). The mechanisms of thrombocytopenia remain controversial (Ballem et al., 1992; Zucker-Franklin, 1994). There is evidence both for immune-mediated platelet destruction (Karpatkin et al., 1988) and for viral infection of the megakaryocyte which could cause megakaryocyte destruction or interference with maturation (Zucker-Franklin & Cao, 1989; Louache et al., 1991). Immune-mediated platelet destruction has been proposed as the most likely aetiology of thrombocytopenia in EIA (Clabough et al., 1991), but low platelet counts occur following experimental infection even in the absence of specific immune responses (Crawford et al., 1996).

In a study using both normal, immunocompetent foals and severe combined immunodeficient (SCID) foals which lack functional B and T cells, Crawford et al. (1996) showed that both groups of foals became thrombocytopenic following EIAV infection, and that platelet production was suppressed by 44–89% in the combined group of foals, with no significant difference between SCID and immunocompetent foals. In addition, megakaryocyte numbers in bone marrow were not significantly different in pre-infection, post-infection and terminal samples (Wardrop et al., 1996) and no evidence of virus replication was observed in sections of purified megakaryocytes from infected foals using electron microscopy (Crawford et al., 1996). These findings indicated that a mechanism not mediated by specific immune responses suppressed platelet production in infected foals without destroying megakaryocytes. The close temporal association

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between viraemia and decline in platelet numbers and their rapid rebound upon resolution of viraemia also suggested that a transient, rapidly secreted factor(s) might be involved. Factors released by macrophages, the primary target cell for EIAV, could be considered as possible mediators of decreased platelet production since macrophages are present in normal numbers in SCID foals (Banks & McGuire, 1975).

Tumour necrosis factor-α (TNF-α), transforming growth factor-β (TGF-β) and interferon-α (IFN-α) are primarily macrophage-derived factors with suppressive effects on megakaryocytopoiesis (Carlo-Stella et al., 1988; Chuncharunee et al., 1993; Maciejewski et al., 1994). Each of these cytokines has been shown to be elevated in many viral infections (Maury & Lahdevirta, 1990; Steinberg et al., 1991; Von Sydow et al., 1991; Lehmann et al., 1992) as well as in non-specific inflammation (Abbas et al., 1991). There are no reports describing levels of these cytokines in EIA or in SCID foals.

The purpose of this study was to test the hypothesis that increases in one or more of these cytokines during acute EIA occur at the time when platelet production appears to be inhibited. Elevations in circulating factors or in local bone marrow levels could suppress platelet production, contributing to thrombocytopenia. We report here that serum TNF-α, TGF-β and IFN-α and bone marrow TNF-α and TGF-β increased just prior to the onset of thrombocytopenia in EIAV-infected foals.

Methods

**Horses, viruses and sampling.** Foals infected in the study comprised six SCID and ten immunocompetent Arabian foals. With a single exception, all the foals were housed in isolation, weaned at 4 weeks of age and infected at 8 weeks of age. One immunocompetent foal was infected at 20 weeks of age. SCID foals were managed as previously described to minimize the likelihood of secondary infections (Perryman et al., 1978, 1988; Crawford et al., 1996).

Foals were infected intravenously with one of two strains of EIAV. All six SCID foals and two immunocompetent foals were infected with EIAV-WSU5, a fibroblast-adapted strain. SCID foals received 1 × 10⁶ TCID₅₀ of EIAV-WSU5. Immunocompetent foals were infected with 4 × 10⁵ TCID₅₀ of virus. Eight immunocompetent foals were infected with the more virulent horse-passaged Wyoming field strain of EIAV (EIAV-Wyo). These foals received plasma containing approximately 1 × 10⁸ horse infectious doses from an acutely ill pony.

Foals were examined daily and complete blood counts were performed every day after the first 5 days post-infection (p.i.). Blood was collected for plasma and serum which were separated promptly, aliquoted and stored at −80 °C. Bone marrow core samples were obtained by needle biopsy from the wing of the ilium following sedation with 0.5 mg/kg xylazine and local anaesthesia at the biopsy site. Bone marrow cores were stored at −80 °C. Bone marrow core biopsies were taken before infection and in the period 2–3 days just prior to the onset of clinical thrombocytopenia (Pre-Tp period). Necropsies were performed immediately following euthanasia and additional bone marrow samples were taken at that time.

**Platelet counts.** Platelet counts were performed within 90 min of blood collection. Platelet counts for the first nine foals were performed on platelet-rich plasma obtained from sodium citrate-collected blood, counted on a particle counter (Coulter Model ZBI-10) and the platelet-rich plasma values corrected to whole blood (Bull et al., 1965). Random samples were also counted in parallel on an automated cell counter (System 9000, Serono-Baker Diagnostics). The platelet counts were sufficiently similar such that the automated cell counter was used for all platelet counts on the last seven foals. The reference range for age-matched Arabian foals was established in this laboratory as 151000–437000 platelets/μl (Crawford et al., 1996).

**Detection of viraemia.** Viraemia was determined by RT–PCR on serum samples taken from each foal before infection, in the Pre-Tp period and on an intermediate day, 6–14 days before clinical thrombocytopenia.

For RT–PCR, virions were pelleted from 1-ml of serum by centrifugation at 47 000 × g for 1 h at 4 °C. Pellets were resuspended into 100 μl of lysis buffer (8 M guanidinium thiocyanate, 83 mM Tris–HCl pH 6.4, 36 mM EDTA and 2% Triton X-100). DNA was extracted with a phenol and guanidinium-based nucleic acid extraction reagent (Trizol Reagent, Gibco BRL) and phase separation was achieved with chloroform. The aqueous phase was precipitated with isopropyl alcohol and pelleted. Pellets were incubated with deoxyribonuclease I (DNase, Gibco BRL) to remove any virus-associated viral DNA or contaminating viral DNA from cells. Following inactivation of the DNase, 3 μl of each sample was used as template in a one-step RT–PCR reaction.

Each reaction mixture contained a standard PCR buffer, 0.4 mM deoxyribonucleotide mix (PCR Nucleotide Mix, Boehringer Mannheim), 1 unit of RNase inhibitor (RNase-Inhibitor, Boehringer Mannheim), 20 units of Moloney murine leukaemia virus reverse transcriptase (SuperScript II, Gibco BRL), 1.5 units of Taq DNA polymerase (Gibco BRL) and 75 pmol each of forward primer (5'-GCC CGA ATG CGG CTG GAA ACA GAA ATT TTA) and reverse primer (5'-GGG CCG AGC TCA GGT TTT CCA ATC ACT ACT). These oligonucleotides prime both reverse transcription as well as the subsequent PCR reaction, and amplify a 448 bp segment of the highly conserved p26 capsid protein gene of EIAV. The identity of the amplicons as EIAV-p26 sequences has been confirmed previously by sequencing and restriction endonuclease digestion pattern (data not shown). The specificity of the assay for RNA was confirmed for positive samples by performing a duplicate reaction without reverse transcriptase.

Amplification was performed on a Perkin Elmer 2400-model thermal cycler starting with reverse transcription at 42 °C for 40 min, deoxyribonuclease I inactivation at 95 °C for 3 min, and 35 cycles of 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 30 s. Each RT–PCR run included positive and negative samples. PCR reaction products were analysed by electrophoresis through 1.5% agarose gels and visualized by ethidium bromide staining and ultraviolet light.

**Serum cytokine assays**

TNF-α. TNF-α activity was measured using L929-8 cells, a subclone of the murine fibroblastoid cell line, isolated by Branch et al. (1991). L929-8 cells in Dulbecco’s modified essential medium (DMEM) with 15% foetal bovine serum (FBS) and 2 μg/ml actinomycin D were seeded in 96-well plates with 4 × 10⁴ cells per well. Dilutions of foal sera and standards, including recombinant human TNF-α (R&D Systems) and lipopolysaccharide (LPS)-stimulated equine serum, were added to the plates. Distilled water was added to some wells for maximal cell killing (0% cell viability) and medium with no TNF-α was added for minimal cell killing (100% cell viability). After 20 h incubation at 40 °C, the plates were washed and stained with neutral red dye and the A₅₇₀ was determined. Percentage of cell lysis, as a measure of TNF-α activity was calculated by the following formula: [(A₅₇₀ of 100% cell viability control – A₅₇₀ of sample-containing wells)/A₅₇₀ of 100% cell viability control] × 100. To demonstrate specificity
of the assay, the equine TNF standard (LPS-stimulated equine serum) and selected foal sera were pre-incubated with an anti-equine TNF-α neutralizing monoclonal antibody (HL801, a gift from R. J. MacKay and J. Cargile, University of Florida, Gainesville, Fla., USA) prior to assay. Control wells received an equivalent amount of an irrelevant isotype-matched monoclonal antibody.

TGF-β. The activity of TGF-β was measured by its ability to inhibit growth of the mink lung epithelial cell line CCL64 (ATCC). Cells were plated in culture plates at 5 × 10^4 cells per well in minimum essential medium (MEM) with 10% FBS. After cells adhered for 2–4 h at 37 °C, dilutions of samples were added and plates were incubated at 37 °C. On day 4 of the assay, 1 μCi [³H]thymidine was added to each well. After another 24 h incubation, supernatants were removed, and cells were trypsinized and harvested onto filter paper with a microtitre plate cell harvester (Tomtec). Incorporation of [³H]thymidine into cells was measured using a liquid scintillation counter (Betaplate, Wallac). Recombinant human (rh)TGF-β1 (R&D Systems) was used as a standard and medium only was used in negative control wells. Percentage inhibition was calculated by the following formula: [1 – (sample c.p.m./negative control c.p.m.))] × 100.

Specificity of the assay was determined by pre-incubation of the standard and selected foal sera with pan-specific TGF-β neutralizing antibody (R&D Systems) prior to assay. As a control, standard and samples were pre-incubated with an irrelevant antisera from the same species.

IFN-α. Activity of IFN-α was measured by its ability to prevent cytopathic effects of vesicular stomatitis virus (VSV) on equine dermal cells in culture. Equine dermal cells in MEM with 10% FBS were plated on culture plates at 5 × 10^4 cells per well. Dilutions of foal sera were acidified to pH 2–3 for 4 h, returned to pH 7.2 and added to wells. Standards tested in each assay included recombinant human IFN-α (Schering) and supernatant from poly(I:C)-stimulated equine peripheral blood mononuclear cells as an equine IFN standard. After 24 h incubation at 37 °C, 1 × 10^5 TCID₅₀ of VSV was added to each well and plates were returned to 37 °C for 24 h. Plates were scored visually, the end-point being the well that exhibited cytopathic effect equivalent to that of virus control wells (those to which no IFN standard nor sample was added). IFN-α units were determined as the reciprocal of the highest serum dilution inhibiting the VSV-induced cytopathic effect and titres expressed as units/ml.

- **Bone marrow cytokines**. Bone marrow cytokine activity was also measured using bioassays as described. Ten mg of each bone marrow core was minced finely in 1 ml of medium. The resulting suspension was spun at 400 x g for 15 min and the supernatant was assayed at three 2-fold dilutions. Controls were the same as described above for serum samples. In addition, some wells were spiked with a known amount of cytokine standard to test whether bone marrow supernatants contained factors that inhibited cytokine activity. Bone marrow activity was always tested in a pre-infection sample, a Pre-Tp period sample and a terminal sample.

- **Bone marrow TGF-β immunohistochemistry**. Immunohistochemical analysis of TGF-β was performed using the pan-specific polyclonal antibody to recombinant human TGF-β described in the previous section and an avidin–biotin–peroxidase kit (Vector Laboratories). Bone marrow biopsies were formalin-fixed and paraffin-embedded. Sections were deparaffinized, incubated with 0·3% hydrogen peroxide to block endogenous peroxidases, blocked with normal goat serum, incubated overnight at 4 °C with the primary (anti-TGF-β) antibody, and incubated with secondary antibody and avidin–biotin–peroxidase complex. After washing and colour development with an aminoethyl-carbazol substrate, sections were counterstained with haematoxylin.

Sections were evaluated using a 100-square eyepiece grid. The percentage of positive-staining cells was determined on 200 cells counted in a systematic manner on each section. Megakaryocytes were excluded from the count as they always stain highly positive for TGF-β. Two or three bone marrow sections from each foal pre-infection and Pre-Tp were counted.

- **Statistical analysis**. Results were analysed using analysis of variance based on a split-plot experimental design. Differences between pre-infection and Pre-Tp cytokine levels and differences between SCID and immunocompetent foals were detected using Fisher’s least significant difference for multiple comparison of means (Ott, 1988). Calculations were performed using statistical software (SAS software, SAS Institute, Cary, NC, USA). Correlation between mean platelet numbers and activity of each cytokine over time was calculated using the non-parametric Spearman Rank correlation (SPSS Version 7.0 software, Chicago, Ill., USA). A P value < 0·05 was considered significant in all analyses.

**Results**

**Platelet counts**

Thrombocytopenia was defined as a platelet count < 151 000/µl, based on reference ranges established from 250
platelet counts on uninfected SCID and immunocompetent foals (Crawford et al., 1996). There are no significant differences between platelet counts in uninfected SCID foals and those in age-matched, uninfected, immunocompetent foals (Crawford et al., 1996). The first day on which platelet counts dropped below 15,000/µl was designated as T(0).

All foals became thrombocytopenic between 7 and 20 days p.i. Immunocompetent foals became thrombocytopenic significantly earlier (mean = 10±1 days p.i., range = 7–13 days) than SCID foals (mean = 18±5 days p.i., range = 16–20 days) ($P < 0.01$). Platelet counts in SCID foals continued to fall after the onset of thrombocytopenia, until these foals were euthanized within 2–3 days of becoming thrombocytopenic. Previous studies had shown that SCID foals became progressively and severely thrombocytopenic once the platelet numbers started to decline so foals in this study were euthanized before severe and debilitating clinical disease occurred (Crawford et al., 1996). Previous studies had also shown that platelet counts in immunocompetent foals consistently rebound to normal levels usually within 3–5 days of a decline in plasma viraemia (Clabough et al., 1991; Crawford et al., 1996). Early termination of immunocompetent foals precluded observation of this phenomenon in this study.

**Viraemia**

No viral genomic RNA was detected by RT–PCR in any of the foals’ pre-infection sera. Viral RNA was detectable in all serum samples taken during the Pre-Tp period (Fig. 1). These findings are consistent with earlier data generated by infectivity assays (Crawford et al., 1996). On the intermediate date (6–14 days before thrombocytopenia), no viral RNA was detected in foal sera except for a single band from one SCID foal 6 days before thrombocytopenia.

**Cytokine levels**

For each of the three cytokines, serum activity in pre-infection samples was compared with activity on the first day of thrombocytopenia T(0), and the 4 days just prior to the onset of thrombocytopenia, days T(−4) to T(−1). This time period was considered crucial with respect to megakaryocyte maturation and differentiation as the process takes approximately 5 days in the horse (Crawford et al., 1996).

To determine whether levels of these cytokines vary significantly in uninfected, age-matched foals, serum samples from two uninfected, immunocompetent Arab foals were analysed for serum TNF-α, TGF-β and IFN-α for a time period corresponding to the study period for infected foals. Although there was some variation in TNF-α and TGF-β levels (data not shown), they were never higher than those measured in pre-infection sera. Serum IFN-α was not detectable in uninfected foals.

**TNF-α.** Mean serum TNF-α level was significantly elevated on days T(−4) to T(0) in the group of all 16 foals and in the group of SCID foals (Fig. 2 A) when compared with the pre-infection level. Among immunocompetent foals, serum TNF-α was elevated only on day T(−1). Mean serum TNF-α levels from SCID foals were significantly higher than those in immunocompetent foals on days T(−3) and T(0).

The bone marrow TNF-α level also increased from a pre-infection mean of 10±9% lysis ($±$ 10±2) to a Pre-Tp mean of 39±3% lysis ($±$ 11±4) (Fig. 2 B). There were no significant differences between TNF-α levels in bone marrow from SCID and immunocompetent foals.
Elevated cytokines in EIA thrombocytopenia

Specificity of the assay was shown by complete neutralization of TNF-α activity in the equine standard and selected foal sera which were pre-incubated with an anti-equine TNF-α neutralizing monoclonal antibody, with no decrease in activity seen following pre-incubation with an irrelevant control antibody.

Mean foal serum TNF-α activity was inversely correlated with platelet numbers through the course of the study period, with a correlation coefficient of $-0.88$ ($P < 0.01$).

TGF-β. For the SCID foals, and the immunocompetent foals, and the combined group of all 16 foals, serum TGF-β levels were significantly higher than pre-infection levels on days $T(-4)$ to $T(-2)$ and $T(0)$ but not on $T(-1)$ (Fig. 3A). TGF-β activity in bone marrow was almost uniform in pre-infection and Pre-Tp samples with no significant differences between pre-infection and Pre-Tp levels (Fig. 3B). Mean serum TGF-β levels in SCID foals and immunocompetent foals were not significantly different at any of the times examined. Specificity of the assay was shown by complete neutralization of TGF-β inhibitory activity in the standard and foal sera when they were pre-incubated with pan-specific TGF-β neutralizing antibody, but not with irrelevant antisera.

There was a generally inverse relationship between mean platelet numbers and serum TGF-β activity with a correlation coefficient of $-0.56$ which is significant at $P = 0.05$, but not significant at $P = 0.01$.

Based on immunohistochemical staining, there was an approximately 9-fold increase in staining for TGF-β in bone marrows taken from foals in the Pre-Tp period. The mean percentage of positively staining cells in bone marrows from pre-infection foals was $34.2 \pm 5.9\%$, which is significantly less than that in Pre-Tp bone marrows ($32.0 \pm 5.3\%$) (Fig. 4). Positive staining for TGF-β was found in a variety of cell types, including macrophages, neutrophils and fibroblasts.

IFN-α. Serum IFN-α levels were not significantly different in SCID and immunocompetent foals on all days analysed. Serum IFN-α levels were elevated on all days $T(-4)$ to $T(0)$ in the SCID foals, the immunocompetent foals, and the combined group of 16 foals (Fig. 5A). Bone marrow IFN-α appeared higher in Pre-Tp samples ($26.7 \pm 3.5 \text{ U/ml}$) than in pre-infection samples ($7.5 \pm 16.1 \text{ U/ml}$), but the difference was not significant at a $P < 0.05$ (Fig. 5B). Although IFN-α levels in Pre-Tp samples from SCID foals appeared higher than those from immunocompetent foals, the difference was not significant at $P < 0.05$.

The correlation between mean platelet numbers and serum IFN-α activity was the strongest of the three cytokines examined with a correlation coefficient of $-0.95$, which is significant at $P < 0.01$.

Discussion

These results show that the activities of TNF-α, TGF-β and IFN-α are increased in serum during acute EIA, just prior to and at the onset of thrombocytopenia. Similar elevations of these
cytokines are seen following infection with other lentiviruses (Maury & Lahdevirta, 1990; Allen et al., 1991; Lehmann et al., 1992), as well as non-retroviruses (Torre et al., 1994; Linderholm et al., 1996).

Systemic levels of TNF-α clearly increase during clinical EIA. The elevated TNF-α levels found in bone marrow from Pre-Tp foals may reflect this increased systemic activity, or it may represent increased activity produced within the local bone marrow environment.

TNF-α is produced primarily by activated macrophages, but may also be secreted by a number of other cell types (Abbas et al., 1991). Its effects are widespread and include signs of a host inflammatory response to microbial infection such as fever, anorexia and depression, which are hallmarks of EIA. Effects of TNF-α on the bone marrow, though complex, are predominantly suppressive. TNF-α inhibits megakaryocyte growth in vitro (Geissler et al., 1991) and is associated with thrombocytopenia in humans, rats and mice (Blick et al., 1987; Sherman et al., 1988; Johnson et al., 1989).

The availability of the SCID foals for this study provided an opportunity to compare cytokine responses to viral infection in foals lacking specific immune responses to those in immunocompetent foals. Two of the three cytokines examined were not significantly different in SCID and immunocompetent foal sera and bone marrow at the times examined. Serum TNF-α levels in SCID foals were significantly higher on days T(0), T(−2) and T(−3) than in normal foals on these days. The reason why TNF-α was higher in SCID foals than in immunocompetent foals during clinical EIA is not evident. There were no obvious clinical or clinicopathological differences between the two groups of foals. Regulation of TNF-α is complex and includes both positive and negative regulators, thus SCID foals may lack certain regulatory mechanisms, allowing abnormal expression of TNF-α.

Pre-Tp serum TGF-β activity was significantly increased at all times examined except for day T(−1). In bone marrow, TGF-β protein was present in significantly greater amounts Pre-Tp and terminally, relative to pre-infection, based on immunohistochemical staining. However, the measured biological activity of TGF-β was not significantly different in pre-infection and Pre-Tp bone marrows. This activity was abolished upon neutralization by anti-rhTGF-β antibody, indicating specificity for TGF-β. The measured activity also decreased linearly with successively higher dilutions of sample, indicating that saturation of the assay was not responsible for the uniform activity measured.

TGF-β is a highly pleiotropic cytokine synthesized in a latent form by a number of cell types and released in biologically active form by activated macrophages and by T and B lymphocytes (Abbas et al., 1991; Su et al., 1993). Depending on its concentration and other factors, TGF-β exhibits either suppressive or stimulatory effects on growth (Jacobsen et al., 1993). It is a potent inhibitor of the proliferation and differentiation of megakaryocyte progenitor cells both in vivo and in vitro (Ishibashi et al., 1987; Zauli et al., 1992; Jacobsen et al., 1993). Elevated TGF-β levels are present in acute EIA at a time when suppression of megakaryocyte proliferation could contribute to the decrease in platelet production previously described (Crawford et al., 1996).

No significant difference in TGF-β levels was found between SCID and immunocompetent foals. This is consistent with several reports of apparently normal TGF-β production in SCID mice in response to infection with other organisms (Hunter et al., 1993).

The elevated serum activity of IFN-α in the viraemic foals in this study was not surprising since viral infection is a strong inducer of IFN-α, an important natural antiviral host response. A ‘virus inhibitory substance’, presumed to be interferon, has been reported in serum of EIAV-infected horses, but not identified (Ley et al., 1970). Of the three cytokines studied, the strongest inverse correlation between platelet numbers and
serum cytokine activity was seen with IFN-α; this may actually reflect a strong association between virus replication and IFN-α production.

Bone marrow IFN-α activity was elevated in Pre-Tp and terminal bone marrows, but the increase was not statistically significant at a $P < 0.05$. The importance of local production of IFN-α in the bone marrow is not known. Bone marrow immunohistochemistry was not performed, since available anti-IFN-α antibodies did not bind equine IFN-α.

IFN-α is produced primarily by activated macrophages, but T cells, B cells and natural killer cells may also secrete IFN-α under certain conditions (Tizard, 1995). Among the most prominent effects of IFN-α are inhibition of viral replication and inhibition of cell proliferation (Abbas et al., 1991). The antiproliferative effects of IFN-α on haematopoietic cells are well known and have been used therapeutically to suppress production of platelets in essential thrombocythaemia (Gugliotta et al., 1989; Wadenvik et al., 1991) in humans. Suppression of megakaryocytopoiesis by IFN-α in vitro has also been well-documented (Ganser et al., 1987; Wadenvik et al., 1991).

SCID foals are deficient in IFN-γ, but not in IFN-β production (Yilma et al., 1982). Serum IFN-α activity in EIAV-infected SCID foals has not been previously reported. The lack of significant differences between SCID and immunocompetent foals in this study confirms that SCID foals are not deficient in synthesis of IFN-α in response to EIAV infection.

The results presented here indicate that serum levels of TNF-α, TGF-β and IFN-α are elevated during acute EIA and thus could be important in the pathogenesis of EIAV-induced haematopoietic abnormalities. Whether any or all of these factors actually cause suppression of platelet production during clinical EIA has not yet been demonstrated. Experiments using neutralizing antibodies to block these cytokines in infected foals would be necessary to determine their contributions to thrombocytopenia in vivo. As the process of thrombocytopenia is controlled by positive regulators such as thrombopoietin, as well as negative regulators, it is most likely that virus-induced thrombocytopenia occurs as a result of complex interactions between factors.

In vitro megakaryocytopoiesis is suppressed by soluble factors in plasma from EIAV-infected, Pre-Tp foals and this suppression is partially reversed upon neutralization of TNF-α and TGF-β (Tornquist et al., 1996; Tornquist & Crawford, 1997). Collectively, the results of this and other studies from this laboratory show that the thrombocytopenia of EIA is caused, in part, by suppression of platelet production during a period of elevated levels of factors known to inhibit megakaryocytopoiesis (Crawford et al., 1996; Wardrop et al., 1996). Further elucidation of this mechanism of thrombocytopenia in EIA should provide insight into the pathogenesis of thrombocytopenia in other viral diseases.

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