Corticosteroid immunosuppression and monoclonal antibody-mediated CD5⁺ T lymphocyte depletion in normal and equine infectious anaemia virus-carrier horses

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The immune control of chronic equine infectious anaemia (EIA) lentiviral infection was investigated by specifically depleting CD5⁺ T lymphocytes in vivo with monoclonal antibody (MAb) or by immunosuppression with corticosteroids. MAb was given at 25 to 50 mg/day intravenously for 11 days. Murine IgG1 anti-equine CD2 MAb (n = 2 horses) or IgG1 (n = 2) and IgG2a control MAb (n = 2 normal; 2 EIA-infected) did not deplete CD2⁺ T lymphocytes in horses. Horses given murine IgG2a anti-CD5 MAb HB19A (n = 4 normal; 5 EIA-infected) did not develop CD5⁺ T lymphocyte depletion. These horses, however, maintained a residual population of CD2⁻ T lymphocytes [15 (± 3) % of pretreatment numbers] that did not express CD5 but expressed either CD4 or CD8. These antigenically modulated CD5⁻ T lymphocytes responded normally in vivo to intradermal inoculation with phytohaemagglutinin and in vitro to allogeneic leukocyte stimulation in one-way mixed lymphocyte reactions. EIA virus-infected horses (n = 5) did not develop recrudescent viraemia or disease following in vivo CD5⁺ T lymphocyte depletion. Immunosuppression of EIA virus-infected horses with corticosteroids (1 mg/kg body weight/day, intravenously for 9 days) resulted in detectable recrudescent EIA viraemia in 6/11 horses (55 %) and recrudescent disease in 9/11 horses (82 %). Normal horses (n = 3) treated with corticosteroids developed no clinical disease. These results demonstrate that the use of murine IgG2a MAbs to appropriate equine lymphocyte antigens will facilitate in vivo investigation of the role of T lymphocyte subpopulations in the control of EIA or other important equine diseases.

Introduction

The immunological control of lentiviral infections has received increased attention due to the world-wide human immunodeficiency virus (HIV) epidemic. Identification of an immune effector system capable of controlling lentiviral infection despite antigenic variation in viral proteins would be a significant contribution to lentiviral research. Subsequent targeting of this effector system with appropriate vaccination strategies may lead to the development of a successful lentiviral vaccine.

The asymptomatic carrier state of equine infectious anaemia (EIA), a natural lentiviral infection of horses, provides a system for the identification and study of immunological mechanisms capable of controlling persistent lentiviral infection. Although the acute stage of EIA is typified by recurrent episodes of plasma viraemia and associated clinical disease including fever, thrombocytopenia, anaemia and inflammatory lesions in the liver and other organs, most infected horses become asymptomatic EIA carriers in the apparent face of rapid antigenic variation of the virus (Kono et al., 1973; Cheevers & McGuire, 1985). Asymptomatic carriers are aviraemic in vitro tissue culture assay, have regression of the EIA-related inflammatory lesions, and are often resistant to homologous and heterologous viral challenge (Kono et al., 1973). The significance of these observations is underscored by the relatedness of EIA virus (EIAV) and HIV. EIAV and HIV are genetically (Stephens et al., 1986; Rushlow et al., 1986) and antigenically (Montelaro et al., 1988) similar, have at least one common cell target, the monocyte/macrophage (McGuire et al., 1971; Clabough-Sellon et al., 1992; Collman et al., 1990), and have a similar mechanism and pattern of antigenic variation in neutralization-sensitive epitopes present in...
their major viral envelope glycoproteins (Payne et al., 1987; Clements et al., 1988). Although it has been demonstrated that EIA viraemia is immunologically controlled in the acute and asymptomatic stages of infection, the immune mechanisms that are responsible for this control have not been identified (Kono et al., 1975; Perryman et al., 1988). When infected with a tissue culture-adapted strain of the virus, EIAV-WSU5, foals with severe combined immunodeficiency syndrome are incapable of controlling acute infection and terminating the initial episode of plasma viraemia, whereas age-matched normal foals terminate initial episodes of plasma viraemia (Perryman et al., 1988). The control of plasma viraemia in chronically infected and asymptomatic EIA carrier horses also appears to be immune-mediated. Horses that are chronically infected with wild-type virus (Wyoming or Goshun strains) develop recrudescent viraemia when treated with immunosuppressive drugs (Kono et al., 1975). Use of inactivated whole EIAV or subunit vaccines enriched with EIAV glycoproteins results in protection from homologous viral challenge but not from heterologous challenge (Issel et al., 1992). These vaccines induce cell-mediated immune responses and this protection correlates with an increase in serine esterase activity following challenge but occurs in the absence of detectable anti-EIAV-specific neutralizing antibody (Issel et al., 1992).

To delineate the role of T lymphocyte-mediated immune mechanisms in the control of EIA, we developed anti-equine T lymphocyte-specific monoclonal antibodies (MAbs) specifically to deplete or to inhibit T lymphocyte function in vivo. Efficacious in vivo depletion of T lymphocytes and their subsets with MAbs has been documented in mice, rats, non-human primates, humans, and most recently in cattle (Jonker et al., 1983; Cobbold et al., 1984; Waldmann, 1989; Howard et al., 1989).

Depletion is an effective method for establishing the role of T lymphocyte-mediated immunity in the control of a variety of infectious diseases (Titus et al., 1987; Weiss et al., 1988; Waldmann, 1989). The purposes of this paper are (i) to demonstrate specific in vivo T lymphocyte depletion mediated by anti-equine pan-T lymphocyte MAbs in horses, (ii) to verify that immunosuppression of EIAV carrier horses infected with EIAV-WSU5 results in recrudescent disease and (iii) to determine whether specific in vivo depletion of T lymphocytes with anti-equine pan-T lymphocyte MAbs results in recrudescent viraemia and disease in EIAV carriers.

Methods

Animals: Horses, mixed pony breeds that ranged in age from 2 to 15 years old, were seronegative for EIAV as determined by an agar gel immunodiffusion test (Coggins et al., 1972). Eleven horses were infected with 10⁶ TCID₅₀ of the WSU5 strain of EIAV by intravenous inoculation. EIAV-infected horses were examined for alterations in body temperature and packed cell volume and analysed for plasma viraemia three times per week. Infected horses became seropositive for EIAV capsid protein, p26, 20 days post-infection as detected by an agar gel immunodiffusion test (Coggins et al., 1972).

MAb production. MAbs HB19A, HB88A, HB61A, HT14A (Kyd & Antczak, 1991) and HT23A (Crump et al., 1988) were produced from hybridomas derived from splenocytes of BALB/c mice immunized with horse peripheral blood mononuclear cells (PBMC) and cloned once by limiting dilution (Davis et al., 1983). IgG1 and IgG2a isotype control MAbs that did not bind to equine PBMC were also used. MAb ascitic fluid was derived from pristane-primed BALB/c mice (Davis et al., 1983) and sterilized by passage through 0.8 μm and 0.2 μm filters (Nalge). The MAb concentration was determined using radial immunodiffusion plates (Tago) (Wyatt et al., 1988).

Preparation of cell populations. Blood was obtained from horses by jugular venepuncture in heparin or EDTA and PBMC were isolated by Ficoll-Hypaque (specific gravity 1.077, Sigma) gradient centrifugation of leukocyte-rich plasma as described (Wyatt et al., 1988). Peripheral blood lymphocytes (PBL) were distinguished from PBMC by their characteristic orthogonal and forward light-scatter properties as revealed by flow cytometry (Hoffman et al., 1980). A pure population of granulocytes was obtained from the resultant cell pellet by lysis of the erythrocytes with ammonium chloride solution (Banks et al., 1972) and determined by examination of smears stained with Wright's stain. Tissues for immunohistochemical analysis were collected from fresh equine cadavers, and then frozen in liquid nitrogen and stored at −70°C.

MAb specificity. The specificity of MAb HB19A for T lymphocytes was determined by two-colour immunofluorescent flow cytometry of PBMC (Wyatt et al., 1988). The reactivity of MAb HB19A for B lymphocytes, monocytes/macrophages and T lymphocytes was determined separately by reacting PBMC with MAb HB19A and with rabbit anti-equine IgM antibody (r-IgM), pan-granulocyte/monocyte-specific MAb DH59B (unpublished data) or anti-equine T lymphocyte MAb HBI9A (Crump et al., 1988) and HB88A (D. B. Tumas, A. L. Brassfield, A. S. Tavernor, M. T. Hines, W. C. Davies & T. C. McGuire, unpublished), respectively. Binding of the reagents to PBMC was demonstrated with fluorochrome-labelled goat anti-mouse IgG2a or IgG1 reagents (Caltag) as described (Wyatt et al., 1988). The preparation and characterization of these reagents was described elsewhere (Banks et al., 1972; Davis et al., 1983; Kydd & Antczak, 1991). MAb DH59B reacts with granulocytes and monocytes in cattle (Davis et al., 1987) and horses (D. B. Tumas, unpublished).

Radioimmunoprecipitation. Isolated equine PBMC were surface-labelled with ¹²⁵I by using the lactoperoxidase-catalysed reaction (Ledbetter et al., 1981) and then were immunoprecipitated (Suarez et al., 1991). Antigen was pre-adsorbed with 1 ml of packed Sepharose-Protein G beads (GammaBind Plus Sepharose, Pharmacia) for 1.5 h at room temperature to remove any labelled antigen binding to protein G alone. The Mᵦ of the immunoprecipitated antigen was determined by SDS-PAGE (Palmer & McGuire, 1984) and by comparison with ¹⁴Cmethylated protein standards (Amersham).

Tissue immunohistochemistry. Reactivity of MAb HB19A or an IgG2a isotype control MAb with sections of frozen equine spleen, peripheral lymph node and liver was determined by immunohistochemistry. A commercial kit (Vecostain ABC kit, Elite; Vector Laboratories) was used as described by the manufacturer.

Immunofluorescent flow cytometry. Immunofluorescent staining of equine PBMC for flow cytometry has been described elsewhere (Wyatt et al., 1988). MAbs used to monitor peripheral blood lymphocyte
populations included HB19A (anti-equine CD5), HT23A (anti-equine CD5) (Crump et al., 1988), HB88A (anti-equine CD2), HB61A (putative anti-equine CD4), HT14A (putative anti-equine CD8), r-XIgM (anti-B lymphocyte) (Banks et al., 1972), isotype control MAbs, and fluorescein-labelled goat anti-horse IgG (Chemicon). At the First International Symposium on Equine Leukocyte Antigens (1991), IgG1 MAbs HB61A and HT14A had the same reactivity as recently reported anti-equine CD4 and anti-equine CD8 MAbs, respectively (Kydd & Antczak, 1991; Lunn et al., 1991; Lunn, 1993). MAb HB88A reacted with the equine CD2 molecule on T lymphocytes (D. B. Tumas, A. L. Brassfield, A. S. Tavernor, M. T., Hines, W. C. Davis & T. C. McGuire, unpublished). The reactivity of the conjugate, fluorescein-labelled goat F(ab)2 anti-mouse IgG and IgM (Calltag) to PBMC alone was assessed to detect MAAb-coated cells. Analyses were performed with a FACScan equipped with a Consort 32 computer and LYSYS software (Becton Dickinson).

One-way mixed lymphocyte reaction (MLR). T lymphocyte function was assessed in vitro by a one-way MLR. The proliferative response of isolated equine PBMC to heterologous PBMC irradiated with 2000 rads from a 60Co source was determined by measuring the uptake of [3H]thymidine as described (Perryman & McGuire, 1978).

Intradermal phytohaemagglutinin (PHA) response. The response to intradermal inoculation of 50 μg of PHA (phytohaemagglutinin P, Difco Laboratories) in sterile saline, or sterile saline alone, was determined by evaluating skin thickness 24 h post-inoculation (Hodgin et al., 1978). The equine intradermal response to PHA is T lymphocyte-dependent and mimics a delayed-type hypersensitivity reaction (Hodgin et al., 1978).

MAb-mediated in vivo T lymphocyte depletion. Normal horses were given either MAb HB19A, MAb HB88A or isotype control MAb intravenously at a dose of 25 or 50 mg/day for 5 days (days 0 to 4) or 11 days (days 0 to 10). Horses that were chronically infected with EIAV were given either MAb HB19A or IgG2a control MAb at 25 mg/day intravenously for 11 days. MAbs in the form of ascites fluid filtered through 0.2 μm filters were diluted to 60 ml in sterile saline prior to administration. Horses were monitored by physical examination, rectal temperature, complete blood counts and by immunofluorescent flow cytometry of PBMC daily or every other day starting 7 days prior to the administration of MAb and for 14 to 21 days following the first administration of MAb. Additionally, T lymphocyte function assays were performed prior to and during MAb treatment and these included one-way MLR and intradermal inoculation of PHA.

Corticosteroid administration protocol. Dexamethasone (Azium, Schering Plough Animal Health Corp.) was given at a dose of 1 mg/kg body weight/day, intravenously, for 9 days (days 0 to 8) to three normal horses and 11 horses chronically infected with EIAV. Horses treated with corticosteroid were monitored and evaluated as described for the MAb-treated horses above.

Tissue culture assay and titre of EIAV. Plasma samples from EIAV-infected horses were collected three times per week and stored at -70°C until use. Samples collected from 14 to 60 days post-infection and from 10 to 45 days before, during and 30 days after in vivo administration of either MAb or corticosteroids were tested for plasma viraemia by tissue culture assay (O'Rourke et al., 1988). Virus titres were determined by adding serial dilutions of plasma to equine kidney cell cultures, assaying endpoints by direct immunofluorescence and calculating the TCID50 by standard methods (Reed & Muench, 1938; O'Rourke et al., 1988).

In vitro rabbit complement activation assay. MAbs HB19A, HB88A and IgG1 and IgG2a control MAbs were tested for their ability to activate rabbit complement when bound to equine PBMC. One-hundred μg MAb was added to 5 ml Hanks' balanced salt solution (HBSS, Gibco) at pH 7.2, containing 5 x 105 PBMC, and then was incubated for 30 min at 37°C. The cells were then washed twice with 10 ml HBSS, at 4°C, and resuspended in 5 ml of HBSS. The suspension was warmed to 37°C and incubated for 30 min at 37°C, with an additional 0.5 ml of complement. The PBMC were washed twice with 10 ml HBSS and assessed for viability by trypan blue dye exclusion. Dead cells were removed by Ficoll-Hypaque density-gradient centrifugation and PBMC were evaluated by immunofluorescent flow cytometry for CD5+ and CD2+ T lymphocytes and for surface IgM+ B lymphocytes as described above.

Results

Binding of MAb HB19A to CD5+ T lymphocytes

The reactivity of MAb HB19A with equine erythrocytes, granulocytes, monocytes and lymphocytes was determined by immunofluorescent flow cytometry. MAb HB19A reacted with 75 (% 4) % of PBL (n = 15 normal horses) and in each horse the MAb and r-XIgM identified different lymphocyte populations (Fig. 1 and Table 1). The sum of the percentages of PBL identified by MAb HB19A and r-XIgM was 98 (% 3) % (n = 15 normal horses). The reactivity of MAb HB19A in sections of equine peripheral lymph node and spleen was restricted to T lymphocyte-specific areas in the horse (Wyatt et al.,...
Fig. 2. SDS-PAGE analysis of 125I-labelled equine PBMC surface proteins immunoprecipitated with Protein G (lane 1), IgG2a control MAb (lane 2) or MAb HB19A (lane 3) under reducing conditions. 14C-labelled Mr standards are shown on the left.

1988; Lunn et al., 1991) and other species (Poppema et al., 1981; Van Ewijk et al., 1981) and had the same pattern as anti-equine CD2 MAb HB88A. MAb HB19A reacted with the majority of lymphocytes in the peri-follicular cortical parenchyma, paracortex and medullary cords of lymph node and with the predominant population of lymphocytes present in peri-arteriolar lymphatic sheaths and the marginal zones of follicles in spleen. MAb HB19A reacted with only rare (< 2%) scattered cells within germinal centres of cortical follicles in lymph node or spleen and did not react with normal equine liver. MAb HB19A reacted with 84 (± 5)% of lymphocytes collected from 6- to 8-week old normal foals (n = 3). MAb HB19A did not bind to equine erythrocytes, granulocytes or monocytes (n = 5 normal ponies). Isotype control MAb did not react in the above assays with any of the tissues tested.

MAb HB19A immunoprecipitated a 62K PBMC cell-surface protein under reducing and non-reducing conditions (Fig. 2). This Mr is similar to that reported for human CD5 (Knapp et al., 1989) and putative equine CD5 (Crump et al., 1988; Lunn et al., 1991). MAb HB19A did not identify PBMC antigens by Western blot analysis (data not shown). Dual immunofluorescent flow cytometry demonstrated that MAb HB19A reacted with the same population of lymphocytes identified by anti-equine CD5 MAb HT23A and EqT3 (Crump et al., 1988; Wyatt et al., 1988) and MAb HB19A binding could be blocked by addition of these anti-CD5 MAbs (data not shown). MAb HB19A also reacted with the same lymphocyte population identified by an anti-equine CD2 pan-T lymphocyte MAb, HB88A (D. B. Tumas, A. L. Brassfield, A. S. Tavernor, M. T. Hines, W. C. Davis & T. C. McGuire, unpublished) in two-colour immunofluorescent flow cytometry (data not shown).

In vitro rabbit complement activation

To determine the potential for MAbs to mediate in vivo depletion, HB19A, HB88A and isotype control MAbs were tested for their ability to activate rabbit complement. Only IgG2a MAb HB19A activated complement in vitro, killing 100% of T lymphocytes. The anti-equine CD2 MAb HB88A, an IgG1 isotype MAb, coated the targeted cell population but did not cause complement-mediated killing. Isotype control MAbs neither bound to PBMC nor resulted in complement-mediated killing.

MAb-mediated T lymphocyte depletion in normal horses

Isotype control and anti-equine T lymphocyte murine MAbs were administered to a total of 12 normal horses. Of those tested, only MAb HB19A caused depletion of the targeted cell population in vivo. In vivo administration of IgG1 isotype anti-CD2 MAb HB88A (n = 2 normal horses) resulted in the coating of all PBL with MAb for 12 to 14 days, but did not cause significant depletion of PBL (data not shown). MAb-coated T lymphocytes were recognized by fluorescein-labelled goat F(ab')2 anti-mouse IgG antibody and were dually positive with anti-CD2 MAb HB88A. IgG1 (n = 2 normal horses) or IgG2a (n = 4 normal horses) isotype control MAbs did not coat or deplete PBMC in horses. Injection of one mouse IgG2a control MAb (42/10.6.1) caused fatal anaphylaxis in two horses; however, another mouse IgG2a MAb (44/37.14.4) used as control (n = 2 horses) for subsequent in vivo depletion experiments did not cause adverse reactions.

A murine IgG2a anti-pan T lymphocyte MAb, HB19A (anti-CD5), was administered to four normal horses (25 or 50 mg/day for 10 days) and depleted CD5+ T lymphocytes from the peripheral blood within 30 min as determined by assay with both HB19A and HT23A anti-CD5 MAbs (Fig. 3). No PBL were recognized by goat F(ab')2 anti-mouse IgG or IgM antibody alone in horses treated with MAb HB19A, demonstrating that MAb-coated PBL were not present. In the four normal horses, MAb HB19A caused an 85 (± 3)% reduction in circulating T lymphocytes but produced no significant reduction of B lymphocytes, granulocytes, monocytes, platelets or erythrocytes. A moderate neutrophilia of 1 day duration occurred after intravenous administration of the first dose of MAb HB19A, but not of any other MAb. CD5+ T lymphocytes were depleted for the duration of MAb administration and for 1 to 2 days after treatment. A residual population of T lymphocytes
Fig. 3. In vivo depletion of peripheral blood T lymphocytes in normal and EIAV carrier horses treated with 25 mg/day of MAb HB19A (anti-CD5) intravenously for 11 days. Results represent the number of peripheral blood CD5⁺ (a) and CD2⁺ (b) T lymphocytes in two normal horses on the days specified. Results represent average values and standard deviations of the number of peripheral blood CD5⁺ (c) and CD2⁺ (d) T lymphocytes in treated EIAV-infected horses on the days specified.

[15 (± 3) % of pretreatment numbers] present after MAb HB19A treatment were CD2⁺ T lymphocytes that lacked surface expression of the CD5 protein (Fig. 3). This residual CD5⁻ T lymphocyte population was subdivided into CD4⁺ and CD8⁺ T lymphocyte populations present in an average ratio of 5:1 as determined by immunofluorescent flow cytometry. There was no significant change in the ratio of CD4⁺ to CD8⁺ T lymphocytes during in vivo MAb HB19A-mediated CD5⁺ T lymphocyte depletion.

PBMC collected from a normal horse during MAb HB19A-mediated in vivo T lymphocyte depletion were cultured in vitro in RPMI with 10% horse serum. Although only CD5⁻/CD2⁺ T lymphocytes were present at the onset of culture, all CD2⁺ lymphocytes regained surface expression of CD5 protein following 2 days of culture. This suggests that MAB HB19A both depleted T lymphocytes in vivo, as demonstrated by the significant reduction [85 (± 3) %] in PBL numbers, and caused modulation of the CD5 protein in a residual population of CD2⁺ T lymphocytes. These modulated CD2⁺/CD5⁻ T lymphocytes were refractory to anti-CD5 MAb depletion since they lacked expression of the targeted antigen.

Administration of either MAb HB19A, MAb HB88A or isotype control MAbs to normal horses (n = 4) did not result in significant reduction in the in vitro response to one-way MLR or the in vivo response to intradermal inoculation of PHA as determined by Student’s paired t-test (Fig. 4 and 5). Despite significant in vivo deletion and modulation of CD5⁺ T lymphocytes, MAb HB19A did not cause demonstrable suppression of T lymphocyte function.

MAb-mediated T lymphocytes depletion in horses with chronic EIA

Five horses infected with the WSU5 strain of EIAV for 12 months were treated with MAb HB19A to test whether CD5⁺ T lymphocyte depletion and modulation
Fig. 4. *In vitro* proliferative response of PBMC in one-way MLR. PBMC were collected pre- (Pre) and post- (Post) treatment with anti-CD5 MAb HB19A (25 mg/day, intravenously for 11 days), control MAb (25 mg/day, intravenously for 11 days) or corticosteroid (1 mg/kg/day, intravenously for 9 days). (a) Two normal horses treated with murine IgG2a control MAb; (b) two normal horses treated with MAb HB19A; (c) five EIAV carrier horses treated with MAb HB19A; (d) three normal horses treated with corticosteroid; (e) five EIAV carrier horses treated with corticosteroid.

Fig. 5. Response to intradermal inoculation of PHA in normal and EIAV carrier horses after treatment with control MAb (50 mg/day for five days or 25 mg/day for 11 days), anti-equine CD5 MAb HB19A (50 mg/day for five days or 25 mg/day for 11 days), or corticosteroid (1 mg/kg/day, intravenously for 9 days). (a) Two normal horses treated with murine IgG2a control MAb; (b) four normal horses treated with MAb HB19A; (c) five EIAV carrier horses treated with MAb HB19A; (d) three normal horses treated with corticosteroid; (e) 11 EIAV carrier horses treated with corticosteroid.

would cause recrudescence of EIA. The MAb caused immediate depletion of 100% of the peripheral blood CD5+ T lymphocytes in four EIAV-infected horses and caused delayed depletion in one infected horse (Fig. 3). By day 2, all horses were completely lacking CD5+ T lymphocytes and this depletion lasted for the duration of treatment (Fig. 3). MAb HB19A caused an average reduction of 75 (±5)% of CD2+ T lymphocytes in treated EIAV-infected horses. Maximum reduction of CD2+ T lymphocytes was an average of 86 (±3)% in these five horses. As was seen in normal horses treated with MAb HB19A, a residual antigenically modulated CD2+/CD5- T lymphocyte population remained in EIAV-infected horses during treatment which was refractory to depletion by MAb HB19A. This residual population consisted of CD4+ and CD8+ T lymphocytes and was functionally active as demonstrated by no significant reduction being found in the response to allogeneic leukocyte stimulation in one-way MLR or to intradermal inoculation with PHA (Fig. 4 and 5).

Depletion and modulation of CD5+ T lymphocytes by MAb HB19A did not cause recrudescence of disease or viraemia in the five horses chronically infected with EIAV-WSU5. Infected horses did not become febrile or develop significant reduction in numbers of circulating thrombocytes and remained aviraemic before and after treatment with MAb as tested by cell culture assay (Table 2).

Table 2. Recrudescent viraemia and disease in EIAV carrier horses following immunosuppression with corticosteroid

<table>
<thead>
<tr>
<th>EIA horse number</th>
<th>Fever*</th>
<th>Thrombocytopenia†</th>
<th>Viraemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>471</td>
<td>10-11†</td>
<td>None</td>
<td>Negative</td>
</tr>
<tr>
<td>475</td>
<td>None</td>
<td>None</td>
<td>Negative</td>
</tr>
<tr>
<td>477</td>
<td>10-13</td>
<td>13-20‡</td>
<td>6-102 (10³)§</td>
</tr>
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<td>11-13</td>
<td>0-20</td>
<td>4-16 (10³)</td>
</tr>
<tr>
<td>488</td>
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<td>10-22</td>
<td>Negative</td>
</tr>
<tr>
<td>500**</td>
<td>7-8</td>
<td>None</td>
<td>7-9 (10³)</td>
</tr>
</tbody>
</table>

* > 39 °C.  
† < 10⁴ platelets/μl blood.  
‡ Days after initiation of corticosteroid treatment.  
§ Numbers in parentheses are peak TCID⁵₀ of EIAV-WSU5 during viraemia.  
¶ Lowest values were less than 1 x 10⁴ platelets per μl blood.  
† Died on day 8.  
** Humanely killed on day 10 owing to severe disease.

Corticosteroid-mediated immunosuppression of EIAV-infected horses

Three normal horses and 11 with chronic EIA were treated for 9 days with dexamethasone (1 mg/kg body weight/day) to test whether immunosuppression would cause reactivation/recrudescence of WSU-5. Five EIAV-infected horses previously treated with MAb HB19A
of EIAV infection

Fig. 6. Average effect of intravenous administration of corticosteroid on peripheral blood T and B lymphocytes in three normal (a and b, respectively) and in eight EIAV carrier horses (c and d, respectively).

(described above) were included in the group of 11 infected horses. None of these infected horses had fever, anaemia or plasma viraemia for 60 to 120 days prior to administration of corticosteroid. Treatment with dexamethasone caused profound reduction in both T and B lymphocyte numbers in the peripheral blood in eight infected horses as tested by flow cytometry (Fig. 6). Statistically significant T and B lymphocyte depletion started at day 4 after initiating treatment and lasted for 8 days. In both normal and EIAV-infected horses, dexamethasone administration produced immunosuppression that was statistically significant as demonstrated by the reduction in the response to one-way MLR and intradermal inoculation of PHA on day 8 after the initial corticosteroid treatment (Fig. 4 and Fig. 5).

Of the 11 EIAV-infected horses immunosuppressed with dexamethasone, nine (82%) developed fever (> 39 °C), 5/10 (50%) developed thrombocytopenia (< 10^5 platelets/μl blood) and 6/11 (55%) developed recrudescent viraemia detectable by cell culture assay (Table 2). Although recrudescent EIA viraemia could be documented in only 6/11 (55%) of the infected horses, four of the five remaining infected horses developed significant fever and/or thrombocytopenia during the same time period and one animal died from severe haemorrhage. Three uninfected normal horses immunosuppressed with the corticosteroids did not develop fever, anaemia, thrombocytopenia or other abnormalities as determined by physical examination. This suggests that the recrudescent disease seen in immunosuppressed EIAV-infected horses without demonstrable plasma viraemia was EIA-related.

In the nine EIAV-infected horses that developed recrudescence, fever began 6 to 10 days after initiation of corticosteroid treatment and lasted for an average of 4 days. Thrombocytopenia (< 10^5 platelets/μl blood) began on day 10 to 12 and lasted for an average of 9 days in five horses. Thrombocytopenia reached minimum levels, of less than 10^4 platelets/μl blood, during recrudescence in two infected horses. Significant thrombocytopenia occurred during recrudescent disease in EIAV carriers in spite of immunosuppression suggesting that
EIA-associated thrombocytopenia can be mediated by non-immune mechanisms such as by a direct effect of the virus on platelet production.

Two EIAV-infected horses developed severe depression and dyspnoea during the recrudescence associated with immunosuppression. Horse 497 was aviraemic and asymptomatic until day 8 of corticosteroid treatment at which point it developed respiratory failure and died. Samples were not obtained on day 8 for this animal and thus it was not possible to determine whether viraemia occurred on this day of apparent recrudescent disease. EIAV-infected horse 506 was killed humanely on day 11 since it showed symptoms of severe depression, respiratory distress and persistent lateral recumbency. Post-mortem examination of these two horses revealed severe multifocal ecchymotic pulmonary haemorrhage.

Discussion

MAb HB19A identified an equine pan-T lymphocyte-specific cell surface molecule having an Mr, lymphocyte specificity and tissue distribution that were similar to those of the CD5 molecule in humans (Knapp et al., 1989). Furthermore, IgG2a MAb HB19A showed the same reactivity with equine PBMC as three reported murine IgG1 anti-equine CD5 MAbs, EQT3, HT23A and CVS5 (Wyatt et al., 1988; Crump et al., 1988; Lunn et al., 1991), as demonstrated in this paper and at the First International Workshop on Equine Leukocyte Antigens (Kydd & Antczak, 1991). This workshop corroborated that MAb HB19A and anti-equine CD5 MAb HT23A identify the same lymphocyte population and that peripheral blood T lymphocytes are dually positive for MAbs HB19A (anti-CD5) and HB88A (anti-CD2).

Murine IgG2a MAb HB19A caused in vivo depletion and modulation of CD5+ T lymphocytes, but IgG1 mouse anti-CD2 lymphocyte MAb did not. The ability of these anti-equine pan-T lymphocyte MAbs to deplete cells in vivo correlated with an ability to activate rabbit complement in vitro. Although IgG2a anti-CD5 MAb HB19A caused complement-mediated lysis of all T lymphocytes in vitro, when used in vivo a residual antigenically modulated T lymphocyte population [15 (±3)% of pretreatment numbers] remained which lacked surface CD5 but maintained other T lymphocyte markers including CD2 and CD4 or CD8. Two days of in vitro culture of this antigenically modulated population resulted in the return of CD5 expression in all of the CD2+ T lymphocytes.

A similar pattern of T lymphocyte depletion and surface marker modulation occurs during in vivo depletion of CD3+ T lymphocytes in humans with MAb OKT3 (Chatenoud et al., 1982). Treatment with this depletes CD3+ T lymphocytes but also produces a residual population of CD3+ T lymphocytes which express CD2 and CD4 or CD8 (Chatenoud et al., 1982). In this case however, antigenically modulated CD3+ T lymphocytes were not functional and did not mediate allograft rejection. The addition of MAb OKT3 blocks in vitro T lymphocyte cytotoxic killing and the response to MLR (Chatenoud et al., 1982). Surface expression of CD3 is apparently required for these functions.

The residual modulated CD5+/CD2+ T lymphocytes that were produced in response to MAb HB19A administration comprised CD4+ and CD8+ populations. These cells had normal T lymphocyte function as demonstrated by in vitro response to one-way MLR and in vivo response to intradermal PHA. CD5 expression was apparently not required for a T lymphocyte proliferative response to these stimuli. Although depletion and modulation of CD5+ T lymphocytes did not cause immunosuppression in treated horses, immunosuppression has been reported in mice when CD5+ T lymphocytes were depleted by anti-Lyt1 MAb (Michaeldes et al., 1981).

Since the mechanism of immune control of EIAV is unknown, we tested whether depletion and modulation of CD5+ T lymphocytes by MAb HB19A in vivo would result in recrudescent disease and viraemia in chronic EIAV infection. Use of a cell culture-adapted EIAV strain for infecting horses allowed for ease in assay for viraemia and will simplify future studies to evaluate cytotoxic T lymphocytes to EIAV-infected cells in vitro. Although the addition of MAb HB19A produced a significant reduction in peripheral blood T lymphocytes and complete depletion and modulation of CD5+ T lymphocytes in EIAV-infected horses, no recrudescence of disease or viraemia was observed. The role of T lymphocytes in the maintenance of the EIAV carrier state could not be determined because a reduced but functional population of CD5+/CD2+ T lymphocytes comprising CD4+ and CD8+ populations remained. We believe that production and use of anti-CD4 and anti-CD8 MAb of the IgG2a isotype will provide optimal in vivo T lymphocyte depletion in future experiments and allow critical assessment to be made of the role of these populations in the control of EIAV infection.

We have demonstrated that immunosuppression and loss of T lymphocyte function can cause recrudescence or reactivation of infection with a tissue culture-adapted strain of EIAV, and this is similar to a previous finding of induction of recrudescence of wild-type EIAV infection (Kono et al., 1975). Eleven EIAV carriers infected with the WSU5 strain that were aviraemic and free of EIA-associated disease for 60 to 120 days were immunosuppressed by corticosteroid treatment. Recrudescent disease, defined as fever (>39°C) and/or thrombo-
cytopenia (< 10^5 platelets/µg blood), occurred in nine of these horses (Table 2) within 6 to 11 days following the initiation of corticosteroid treatment. The results suggest that corticosteroid-induced recrudescence could provide a system for investigating the pathogenesis of thrombocytopenia associated with EIA. Reactivation of EIAV infected by immunosuppression implies that a negative-selection strategy can be utilized in vivo to identify the lymphocyte population required for maintenance of the EIAV carrier state. Specific in vivo depletion of this critical lymphocyte population with MAb in EIAV carriers should result in recrudescence.

The IgG2a anti-T lymphocyte MAb depleted 86 (±3)% of T lymphocytes and modulated the CD5 molecule on the remaining CD2^+ T lymphocytes in horses. This result suggests that murine IgG2a MAbs against other equine cell surface antigens will cause targeted cell depletion in vivo, particularly if the targeted cells do not undergo cell surface antigen modulation as a result of MAb binding. Further studies involving the production of IgG2a anti-equine CD4 and CD8 MAbs and their use to deplete T lymphocyte subpopulations in vivo in EIAV carrier horses will be important in determining the role of these lymphocyte populations in the control of EIAV infection.

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