Distribution of leucocyte antigens in Icelandic horses affected with summer eczema compared to non-affected horses

S. HALDÖRSDÓTTIR, S. LAZARY*, E. GUNNARSSON† and H. J. LARSEN
The Norwegian College of Veterinary Medicine, Department of Microbiology and Immunology, Box 8146, Dep., 0033 Oslo 1, Norway. *Institute for Animal Breeding, Division of Immunogenetics, University of Berne, Switzerland and †Institute for Experimental Pathology, University of Iceland, Reykjavik, Iceland.

Summary

Three hundred and three horses, exported from Iceland to Norway, Sweden, Denmark, Switzerland or Germany were tested for their distribution of leucocyte antigens. One hundred and thirty-six horses were affected with summer eczema. The panel of sera recognised the internationally accepted ELA-specificities A 1 to A10, and the nine work shop specificities W 11 to W 15 and W 18 to W 21. Also, some local specificities, characterised in Switzerland (Be I, Be III, Be 8, Be 25, Be 26, Be 27), and two non major histocompatibility complex (MHC)-linked antigens (Ely 1:1, Ely 2) were included. Only one antigen, Be 8, gave a statistically significant difference in distribution between the two populations: Relative risk = 2.5, $x^2 = 10.11$, corrected P < 0.01.

Introduction

SUMMER eczema is a recurrent seasonal dermatitis of horses. Similar conditions have been described worldwide under a variety of different names, although these do not necessarily have a common or related aetiology. A number of causes have been suggested, but growing evidence supports an allergic origin, resulting from hypersensitivity to bites of midges belonging to the genus Culicoides (Riek 1972; Mellor and McCaig 1974; Quinn, Baker and Morrow 1983; Larsen Bakke and Mehle 1988; Haldorsdottir and Larsen 1989). It has been assumed that some horses have a hereditary predisposition to the disease (Ishihara and Ueno 1957; McCaig 1975; Strothmann 1982). The condition has also been reported to be more common in certain families and breeds of ponies (McCaig 1975; Braverman et al 1983).

In man, many diseases have been linked to Human Leucocyte Antigens (HLA), and in most HLA-associated diseases, immunological processes are thought to play a major role in the pathogenesis. Some of the strongest associations involve diseases in which autoimmune responses may be involved. Associations of HLA with allergy and infectious diseases are relatively rare (Newman and Antczak 1983; Pollack and Rich 1985). The mechanism by which HLA-alleles are associated with disease susceptibility is unclear and remains the subject of many investigations. The study of the major histocompatibility complex (MHC) in animals is still in its infancy. In domestic animals, few conditions have been studied with regard to disease associations with MHC, and many of the results obtained require to be confirmed by further investigations. In chickens, a number of studies have established strong correlations of the red blood cells B-complex which constitute MHC in this species, with susceptibility to Marek's disease and Rous sarcoma (Hanson, Zandt and Law 1967; Håla, Boyd and Wick 1981). The results of Lazary, Gerber, Glatt and Straub (1985a) strongly suggest that the predisposition of horses to sarcoid, a cutaneous, fibroblastic tumour, is associated with or linked to MHC. Results of preliminary studies (Lazary et al 1982, 1985b) indicate that summer eczema in Icelandic horses may be associated with certain leucocyte antigens. The aim of the present work was to compare the distribution of leucocyte antigens in Icelandic horses affected with summer eczema with that in non-affected horses to reveal any differences which might exist.

Materials and methods

Horses

A total of 303 Icelandic horses, which had been exported from Iceland to Norway, Sweden, Denmark, Switzerland or Germany were tested (Table 1). The horses were five years old or more and of both sexes. Of the horses tested, 136 were suffering from summer eczema. This diagnosis was, for most horses, based on clinical examinations made when collecting blood samples. If the collection of blood samples was performed when few or no clinical signs of eczema were evident, the diagnosis was based on evaluation of the clinical signs described by the owner and confirmed by a veterinarian.

Alloantisera

Over a period of three years, a total of 93 alloantisera, representing 29 different lymphocyte antigens, was tested. The sera were obtained from primiparous mares or produced by alloimmunisation with leucocytes. Twenty per cent of the sera were obtained from Icelandic horses and used with or without absorption (Lazary et al 1980). The panel of sera recognised the internationally accepted ELA specificities A 1 to A10 and the nine workshop specificities W 11 to W 15 and W 18 to W 21. These specificities are all (except

<table>
<thead>
<tr>
<th>TABLE 1: The distribution, according to country, of 303 Icelandic horses exported with or without summer eczema.</th>
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</thead>
<tbody>
<tr>
<td>Icelandic horses exported to</td>
</tr>
<tr>
<td>-----------------------------</td>
</tr>
<tr>
<td>Norway</td>
</tr>
<tr>
<td>Sweden</td>
</tr>
<tr>
<td>Denmark</td>
</tr>
<tr>
<td>Switzerland</td>
</tr>
<tr>
<td>Germany</td>
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<tr>
<td>Total</td>
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W11, W12, W13 and W21) known to be allelic gene products, coded for by one single locus. Also, some local specificities characterised in Switzerland (Be I, Be III, Be 8, Be 25, Be 26, Be 27), were recognised. Be I and Be III represent supertypic specificities, ie antigenic specificities shared by one or more allelic product of a single locus. Included in the panel of sera also were two non-MHC-linked membrane antigens; Ely 1:1 and Ely 2.

**Lymphocyte isolation**

Blood samples for lymphocyte isolation were collected from the jugular vein in 10 ml Vacutainer tubes containing 150 USP-U of sodium heparin. A lymphocyte suspension was obtained by gradient centrifugation at room temperature, as described by Bayum (1968) with some modifications: 10 ml of heparinised blood was incubated with a pinch of iron powder for 30 mins at 37°C with frequent mixing. The sample was allowed to sediment for the last 10 mins of this period, the leucocyte-rich plasma was then being removed and centrifuged at 118 g for 10 mins. Most of the supernatant was discarded and the remaining pellet was diluted in normal saline to a final volume of 6 ml and layered over 3 ml of Lymphoprep (Nyegaard and Co., Norway) adjusted to a density of 1084 g/ml with Na metrizoat (32.8 per cent w/v, Nyegaard & Co.). After centrifugation at 584 g at the interphase, for 10 mins, the lymphocytes were harvested from the interphase and washed twice in Hanks' Balanced Salt Solution (BSS), (Gibco, Scotland); 327 g for 10 mins and 118 g for 5 mins. The cells were re-suspended in RPMI 1640 (Gibco, Scotland) to a final concentration of 2 to 3 x 10^6 cells per ml.

**Complement**

Rabbit sera were pre-tested for the absence of cytotoxic antibodies, pooled and stored in aliquots at -70°C.

**Lymphocytotoxicity testing**

The testing was carried out in Berne and Oslo, over a period of three years. Differing with the time of testing, a serum panel recognising 25 to 31 different antigens was tested against each horse. A two-step lymphocyte cytotoxicity test was performed as described by Terasaki and McClelland (1964) with some modifications: Two microlitres of alloantiserum were placed in test wells in Terasaki microplates (Falcon) under an oil layer. The plates were stored at -20°C for a maximum of three months. After thawing at room temperature, 2 μl of the cell suspension (4000 cells) were added to each well. The serum and cell mixture were incubated for 30 mins at room temperature before 2 μl of complement were added. After a second incubation for 1 h at room temperature, 5 μl of eosin dye (5 per cent Eosin Y in distilled water) were added followed by 5 μl of fixative (approximately 40 per cent phosphate buffered formaldehyde). The plates were kept overnight and read the following day with an inverted phase-contrast microscope. The reactions were evaluated according to the following scale: 100 per cent dead cells = 4; 75 per cent dead cells = 3; 50 per cent removed and = 2; and 25 per cent dead cells = 1. A positive reaction was defined as one with a score of 2 or more.

**Statistical analyses**

Frequencies were calculated and association studies were performed on each of the 93 sera. This was done because the clustering of sera to characterise lymphocyte antigen specificities had previously been performed mainly on other breeds of horses. A cluster which represents a specificity in one breed of horses does not necessarily do so in another breed.

The significance of differences in distribution of antigens between horses affected with summer eczema and non-affected horses was calculated by Yates' chi-square test. P-values were corrected with the number of alleles known and tested within the different allelic systems +1.

The following formulae were used (Sveggaard et al 1984):

<table>
<thead>
<tr>
<th>Phenotype frequency for antigen A:</th>
<th>( f = \frac{n}{N} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( n ) = number of animals positive for antigen A</td>
<td></td>
</tr>
<tr>
<td>( N ) = total number of animals.</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Gene frequency for antigen A:</th>
<th>( g = \frac{1}{1-f} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative risk (RR) = ( \frac{a \times d}{b \times c} )</td>
<td></td>
</tr>
</tbody>
</table>

**Aetiological fraction (AF) = \( \frac{RR - 1}{RR} \)**

where

- \( a \) = antigen carrier - affected
- \( b \) = antigen absent - unaffected
- \( c \) = antigen carrier - unaffected
- \( d \) = antigen absent - affected

The relative risk (RR) indicates how many times more frequently a disease develops in individuals carrying the antigen compared with individuals in which it is lacking. The aetiological fraction (AF) indicates how much of a disease is "due to" the disease-associated factor at the population level.

**Results**

For the whole population of imported horses, six sera, characterising six different antigens, gave different gene frequencies in horses affected with summer eczema compared to non-affected horses (\( P < 0.05 \)). These sera recognised the internationally accepted specificity A1, the workshop specificity W13 and four local specificities; Be I, Be III, Be 8 and Be 27. The antigen representing A 2 showed a decreased frequency in horses affected with summer eczema compared with non-affected horses (Table 2). When correcting the obtained P values with the number of antigens tested within every allelic system, only one antigen, Be 8, gave a statistically significant difference in distribution between horses affected with summer eczema compared with non-affected horses (\( P < 0.01 \)). The antigen Be 8 showed a relative risk of 2.53 with an aetiological fraction of 0.186. The supertypic antigens Be I and Be III also showed a higher frequency in horses affected with summer eczema.

**Discussion**

The results of the present investigation showed that disease susceptibility of horses affected with summer eczema seems to be associated with a second locus ELA-antigen, Be 8. The antigen Be 8 represents a local Swiss specificity. It is not allelic to the internationally accepted ELA-specificities A 1 to A 10, but, as is shown by segregation studies in Thoroughbred horses, a product of a second locus of probably class I antigens. Four alleles are known to be coded for by this locus (Lazary, unpublished data). The AF of Be 8 (0.186) is not very high in accordance with the low frequency of this antigen in the population. Other known first or second locus alleles of class I antigens did not show any statistically significant difference in distribution in horses affected with summer eczema compared with non-affected horses. Whether yet unknown ELA-antigens play a larger role in the pathogenesis of the disease remains to be clarified. In preliminary studies made by Lazary et al (1982, 1985b), summer eczema seemed to be linked to, or associated with, the supertypic specificity Be 1. This finding was confirmed in the present investigation, but might be due to the fact that some of the horses investigated were included in the preliminary studies made by Lazary et al (1985b). The statistical significance of a difference in distribution of a supertypic specificity, possibly involving several
TABLE 2: ELA specificities showing different frequencies in horses affected with summer ezema compared to non-affected horses

<table>
<thead>
<tr>
<th>ELA</th>
<th>No. of animals tested</th>
<th>Affected (n)</th>
<th>Controls (n)</th>
<th>Antigen affected</th>
<th>Frequency (%) controls</th>
<th>RR</th>
<th>AF</th>
<th>X</th>
<th>P</th>
<th>cP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st locus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A 2</td>
<td>303</td>
<td>138</td>
<td>167</td>
<td>4.8</td>
<td>11.5</td>
<td>3.2</td>
<td>4.08</td>
<td>0.168</td>
<td>4.09</td>
<td>0.043</td>
</tr>
<tr>
<td>Be 27</td>
<td>125</td>
<td>78</td>
<td>47</td>
<td>11.5</td>
<td>11.5</td>
<td>3.2</td>
<td>4.08</td>
<td>0.168</td>
<td>4.09</td>
<td>0.043</td>
</tr>
</tbody>
</table>

2nd locus |                      |              |              |                  |                       |    |    |   |    |    |
| W 13    | 237                  | 110          | 127          | 9.4              | 4.3                   | 4.08 | 0.136 | 3.89 | 0.048 |
| B 8     | 303                  | 136          | 167          | 16.9             | 7.7                   | 2.53 | 0.186 | 10.11 | 0.0014 |

Supertypic |                |              |              |                  |                       |    |    |   |    |    |
| Be I    | 271                  | 117          | 154          | 45.2             | 33.9                  | 1.8 | 0.310 | 4.66 | 0.030 |
| Be III  | 162                  | 51           | 113          | 11.3             | 3.6                   | 3.54 | 0.150 | 5.64 | 0.0175 |

RR: relative risk; AF: aetiological fraction; cP: corrected P-value = P x (number of known alleles +1); ns: not significant

References


Acknowledgements

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