



Short communication

Advances in equine immunology: Havemeyer workshop reports from Santa Fe, New Mexico, and Hortobagy, Hungary

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Abstract

The horse has been human kind's most important partner throughout history. Similarly, in the field of immunology, many critical scientific advances have depended on the horse. Equine immunology today is an active and important field of study, with a focus on control of many common infectious diseases and immunopathologic conditions of broad comparative interest. In 2001 two major equine immunology workshops were held, in Santa Fe, USA, and in Hortobagy, Hungary, with major sponsorship from the Havemeyer Foundation. This report summarizes the scientific themes and foci of those meetings.

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1. Introduction

The horse played an important role in the early years of the modern immunological era. The diverse immunoglobulin isotypes and sub-isotypes of the horse were a common field of study for immunologists interested in immunoglobulin structure and diversity (Sandor et al., 1964; Weir et al., 1966), and equine anti-lymphocyte sera were commonly employed in early studies of cellular immunity

(Mosedale et al., 1968; Stewart and Bell, 1970). The advent of monoclonal antibodies removed the horse from the forefront of basic immunological investigation, but the horse continues to contribute important comparative models of diverse immunological phenomena ranging from combined immunodeficiency (McGuire and Poppie, 1973; Shin et al., 1997), to maternal tolerance of pregnancy (Baker et al., 1999), to immunity to lentivirus infection (McGuire et al., 2000; Mealey et al., 2001). Advances in equine immunological study have depended heavily on collaborative efforts organized as workshops, focusing on alloantigens (Lazary et al., 1988), and more recently on characterization of

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leukocyte differentiation antigens and immunoglobulins (Kydd et al., 1994; Lunn et al., 1998).

In 2001 two further workshops were held which provided a comprehensive overview of the state of equine immunological study with the aim of increasing collaborative ventures between equine and comparative immunological scientists. The first workshop, on Equine Immunology, held in January, 2001, in Santa Fe, New Mexico, USA, convened a group of equine scientists with a broad range of research interests which all had immunological investigations as a central theme. The program included presentations on infectious and inflammatory disease, new technologies and reagent development, and an extensive proceedings has been published (Lunn and Wade, 2001). The second workshop, the International Symposium on Allergic Diseases of the Horse, held in April 2001, in Hortobagy, Hungary, focused on immunological aspects of recurrent airway obstruction (RAO) and insect bite hypersensitivity (IBH). Presentations also addressed genetic aspects of these diseases, new reagents and technologies useful for the study of equine allergic diseases and potential new therapies. Because some topics were discussed at both meetings, the results of these two workshops are presented here as a single report, organized by scientific themes.

2. Infectious disease

The discussion of equine infectious disease included a plenary presentation by Travis McGuire (Pullman, USA), providing a detailed overview of the immune control of equine infectious anemia virus (EIAV). Recent adoptive transfer experiments to a foal suffering from severe combined immunodeficiency using lymphocytes from an infected and MHC class I matched donor have provided strong evidence for the importance of cytotoxic lymphocytes (CTLs) (Mealey et al., 2001). Limiting dilution analysis of CTLs specific for EIAV Env or Gag/Pr proteins indicate that memory CTLs are responsible for the control of viremia (McGuire et al., 1997). In the inapparent carrier state, when viremia is under control, most horses have memory CTL that recognize Gag matrix and capsid proteins. These CTL responses recognize a diverse set of epitopes, depending on

the MHC-1 haplotype of the horse. In addition, antigenic variation in these epitopes is present in different EIAV strains. Future vaccines will need to induce CTL to epitopes of various proteins in horses of different MHC-1 haplotypes (Ridgely and McGuire, 2002).

Presentations on equine herpesvirus-1 (EHV-1), an important respiratory and abortigenic pathogen of horses which results in latent infection, identified important parallels to EIAV in terms of mechanisms of immunity. There is good evidence that CTLs play a key role in immunity (Slater, Cambridge, UK), and low levels of CTLs may predispose to infection as documented by limiting dilution analysis (Kydd, Newmarket, UK). Latent infection reactivates from CD8+ lymphocytes in draining lymph nodes and trigeminal ganglionic neurones. Latently infected cells do not express viral antigens, so that the immune system may only play a role in the face of reactivation. The targets of CTL responses remain unknown, and identifying these antigens and determining the role of CTLs in the respiratory epithelium lamina propria are important goals for vaccine development. An investigation of mucosal immunity to EHV-1 infection (Breathnach, Lexington, USA) demonstrated a strong EHV-1 specific IgA response after challenge infection, which was associated with protection from subsequent challenge (Breathnach et al., 2001).

Further presentations on infectious disease included a review of immunity to *Rhodococcus equi*, an important intracellular bacterial pathogen that results in pyogranulomatous pneumonia in 1–6-month-old foals (Giguere, Gainesville, USA). Virulent *R. equi* contain an 80–90 kb plasmid encoding a family of seven virulence associated proteins. Antibodies directed against two of these proteins, VapA and VapC, are at least partially protective (Hooper-McGrevy et al., 2001). The occurrence of *R. equi* pneumonia in foals suggests an immunologic susceptibility to disease, analogous to that seen in humans affected by human immunodeficiency virus. Investigations of cellular immunity in foals have demonstrated that animals infected with virulent plasmid-containing strains have a reduction in IFN-gamma mRNA expression and an increased IL-10 expression, consistent with down regulation of T-helper 1 (Th-1) responses (Giguere et al., 1999). Investigation of immunity to *R. equi*, and many other equine pathogens, has been hampered by a lack of reagents recognizing targets such as equine

cytokines. Steve Hines (Pullman, WA) reported the development of an anti-equine IFN- γ monoclonal antibody that can be used for intracellular staining in flow cytometric analysis, and early results of its use in the study of *R. equi* infection. Flow cytometric analysis demonstrated that in adult (immune) horses, clearance of an *R. equi* challenge infection was associated with an influx of CD4+/IFN- γ + cells into bronchoalveolar fluid. Availability of these new technologies in conjunction with new information about the virulence plasmid (Byrne et al., 2001) offer opportunities for gaining a better insight into protective immune responses.

3. Induction of mucosal immunity

Several presentations focused on mucosal immune responses in horses. The equine respiratory mucosal immune system has a key role in protection from several important equine pathogens, and has been extensively studied (Hannant, Newmarket, UK). An investigation of mucosal immunity to EHV-1 infection (Breathnach, Lexington, USA) demonstrated a strong EHV-1 specific IgA response after challenge infection, which was associated with protection from subsequent challenge. Both inactivated and modified live EHV-1 vaccines failed to induce an IgA response, or protective immunity. An extensive investigation of mucosal immunization strategies targeting *Streptococcus equi* infection was presented by John Timoney (Lexington, USA). Strategies investigated included microencapsulation of antigen, the use of a mucoadhesive compound (sucrose acetate isobutyrate—SAIB) (Nally et al., 2000), delivery of cholera toxin/antigen chimeric molecules, and the use of avirulent *Salmonella typhimurium* as a mucosal vector (Sheoran et al., 2001). Both SAIB and *S. typhimurium* produced very promising results. Gisela Soboll (Madison, USA) described the use of hemagglutinin (HA) gene DNA vaccination to protect horses from influenza virus infection. Co-administration of HA DNA and cholera toxin at mucosal surfaces produced a primary mucosal IgA response and an anamnestic response after challenge infection (Soboll et al., in press). These promising results suggest that practical mucosal vaccination strategies may be developed soon, and this will remain an important area of equine immunological study.

4. Immune reagents

Limited availability of equine-specific immunological reagents has frequently limited progress. In recent years an increasing list of monoclonal reagents has become available for characterization of leukocyte antigens and immunoglobulins (Lunn et al., 1998). Many current investigations are focused on cytokines, and an extensive range have been cloned (Horohov, Baton Rouge, USA). The development of various quantitative RT-PCR systems (Giguere), including real time methods (Horohov) has led to identification of putative equine Th-1 and Th-2 responses, and application of this technique to the study of infectious and inflammatory disease. Development of monoclonal antibody detection systems for equine cytokines, in addition to the reagent described above for IFN- γ (Hines), remains a key goal.

Leslie Nicolson (Glasgow, UK) described the production of biologically active recombinant IFN- γ , IL-12 and IL-18, and the preliminary characterization of polyclonal and monoclonal antibodies to some of these cytokines (McMonagle et al., 2001). Steinbach (Berlin, Germany) also cloned and expressed the equine interferons IFN- α , IFN- β and IFN- γ and investigated their functional activity (Steinbach et al., 2002). Equine IFN- α has a broad anti-viral activity and also inhibits proliferation of lymphocytes, while IFN- γ mainly displayed immune modulatory effects on monocytes, up-regulating MHC class II expression. The equine CC chemokines eotaxin, monocyte chemoattractant protein (MCP)-1, MCP-2 and MCP-4 have been cloned and an early induction of equine eotaxin and MCP-4 and up-regulation of MCP-1 by recombinant equine IL-4 in dermal fibroblasts have been reported (Cunningham, London, UK) (Benarfa et al., 2000).

Flow cytometric analysis is increasingly widely applied to clinical investigations. These include evaluation of complement fixation and opsonins (Gron-dahl, Uppsala, Sweden), phagocytosis and oxidative burst (Raidal, Perth, Australia; Flaminio, Ithaca, USA). The diagnosis by flow cytometry of immune-mediated thrombocytopenia and hemolytic anemia as well as lymphatic cancers by cell cycle analysis was also described (Rush, Manhattan, USA).

A website has been established which provides information about equine specific reagents (<http://www.vetmed.wisc.edu/research/eirh/>).

5. Immunogenetics

An important highlight of the meeting was an investigation of the equine immunoglobulin heavy chain gene loci, described by Bettina Wagner (Hanover, Germany) (Wagner et al., 2002). The equine IgH-loci consists of one μ , six γ , one ϵ , and one α genes (Overesch et al., 1998; Wagner et al., 1997, 1998). The six $c\gamma$ genes correspond to three of the four equine IgG sub-isotypes currently defined by monoclonal antibodies ($\gamma 1$ encodes IgGa, $\gamma 3$ encodes IgG(T), and $\gamma 4$ encodes IgGb), and there is serological evidence that the remaining γ genes are also expressed.

Expression of IgH loci as recombinant complete chimeric Ig molecules in mammalian cells is now possible, and this approach has led to the production of chimeric IgE containing murine light chains and VH domains, and equine constant heavy chain domains, which is biologically functional. This approach will considerably simplify production of monoclonal antibodies to immunoglobulins, such as IgE, which have previously proven difficult to characterize in this manner. The equine IgE receptor alpha chain has been cloned and expressed (McAleese, Edinburgh, UK) (McAleese et al., 2000). These reagents will provide useful tools to further investigate the involvement of IgE in hypersensitivity diseases of the horse.

Additional advances in equine immunogenetics are resulting from the current Horse Genome Project (<http://www.uky.edu/Ag/Horsemap/>), including identification of MHC, Ig and TCR regions (Antczak, Ithaca, USA).

6. Inflammation

Endotoxaemia is manifested in a number of important and common equine diseases, and was comprehensively reviewed by James Moore (Athens, USA). Equine gastrointestinal disease frequently results in absorption of endotoxic lipopolysaccharide (LPS) components of the outer membrane of enteric bacteria into the bloodstream. The resulting endotoxaemia can be detected in the plasma of approximately 40% of horses presented to veterinary college clinics with colic, and is typically associated with intestinal strangulation obstruction of severe inflammatory intestinal disease. In neonatal foal septicemia, LPS is detectable

in 50% of cases and is associated with derangements in hemostasis and fibrinolysis. In all these diseases survival is inversely correlated with the presence of LPS in the circulation. Under experimental conditions the onset of equine endotoxaemia results in peak elevations of TNF- α within 2 h, with consequent fever and leucopenia. Many of the effects of endotoxaemia in horses are mediated by thromboxane A_2 , and prostaglandins E_2 , $F_{2\alpha}$ and I_2 . A common sequel to endotoxaemia in horses is the development of laminitis, a severe sterile inflammatory condition of the laminar attachments of the hoof. There is substantial evidence that local digital hemodynamic alterations play a critical role in the development of laminitis (Moore, Baton Rouge, USA), resulting in increased capillary pressure due to increased venoconstriction, increased laminar interstitial pressure and oedema, thrombosis, and decreased digital blood flow. Ultimately this results in laminar ischemia and necrosis. There is evidence that this life-threatening condition may result from an imbalance between endothelium-derived vasodilator (nitric oxide) and vasoconstrictor (endothelin 1; ET-1) substances. The importance of ET-1 in the aetiopathogenesis of laminitis may indicate the potential of ET-1 antagonists as therapeutic agents.

Lameness and joint disease are common problems in horses leading to extensive suffering and loss of use. Wayne McIlwraith (Fort Collins, USA) described an extensive series of studies of the molecular basis of non-infectious equine arthritis. Rheumatoid arthritis has not been described in the horse, but joint inflammation consequent to traumatic injury is extremely common. Equine inflammatory mediators associated with the destruction of hyaluronan in synovial fluid and articular cartilage include the cytokines IL-1 and TNF- α , several MMP's, prostaglandin E_2 and free radicals. Conventional treatment has focused on symptomatic suppression of inflammation using corticosteroids or non-steroidal anti-inflammatory drugs. Therapy with corticosteroids is clinically effective, but can lead to steroid-induced arthropathy. In vitro studies demonstrate that corticosteroids reduce type II procollagen expression by articular chondrocytes in a dose-dependent manner (MacLeod, Ithaca, USA). The therapeutic activity of conventional therapeutic agents, including corticosteroids and polysulphated glycosaminoglycans may depend on pre-translational

regulation of the iNOS gene, as nitric oxide has a role in the pathogenesis of equine osteoarthritis. Recently a novel gene therapy has been successfully applied in an equine model of joint arthropathy, using an adenoviral vector for intra-articular delivery of the equine IL-1 receptor antagonist (Frisbie et al., 2002; Frisbie and McIlwraith, 2000). While this result was the first successful use of gene therapy for clinical treatment of joint disease, alternative gene delivery systems will be necessary for repeated treatment. Other cytokine-mediated therapies are under investigation including the use of anabolic cytokines such as insulin growth factor 1.

7. Hypersensitivity and airway diseases

Two presentations from the field of human allergy introduced the discussion of equine allergic diseases. Clémens Dahinden (Institute of Immunology and Allergology, Berne, Switzerland) highlighted the role of chemokines, chemokine receptors and basophils in inflammation and allergy. Reto Cramer (SIAF, Davos, Switzerland) demonstrated a new technology allowing the rapid cloning of IgE-binding molecules from complex allergenic sources like fungi, moulds or mites. Mould or mite extracts are complex mixtures of a large number of proteins and glycoproteins and the allergenic proteins represent only a small percentage of the total protein content. Furthermore, the composition of extracts varies between preparations, hampering a reproducible diagnosis as well as the study of the pathogenesis of mould-associated diseases. Pure recombinant (r)-allergens allow a much more sensitive and specific diagnosis of mould-associated diseases in humans. For example, r-*Aspergillus fumigatus* allergens allow the discrimination with serological methods between IgE-mediated asthma and life-threatening allergic bronchopulmonary aspergillosis (Cramer, 1998). Three r-*A. fumigatus* allergens were tested for their binding of equine IgE in ELISA. Two of these allergens were bound significantly more frequently by IgE from horses affected by RAO than from healthy control horses, suggesting that RAO-affected horses are partly sensitized against the same antigens as human patients with mould allergies (Eder et al., 2001).

The horse is commonly affected by two forms of chronic airway disease: RAO in middle-aged horses;

and inflammatory airway disease (IAD) in young performance horses (Robinson, 2001). RAO is a severe inflammatory disease of middle aged and older horses induced by exposure of susceptible horses to inhaled organic dust, generally from hay, although a summer pasture-associated form (SPAOPD) is also observed in the southern United States. As moldy hay exacerbated clinical signs in RAO-affected horses, it was postulated that RAO is a hypersensitivity reactions to moulds such as *A. fumigatus*, and *Faeni rectivirgula*. Removal of the hay dust by returning the horse to pasture leads to decreased inflammation within a few days. In RAO-susceptible horses, exposure to hay dust leads to invasion of the lungs and airways by neutrophils within 4–6 h and concurrent airway obstruction due to bronchospasm, inflammation, and increased mucus viscosity, which principally affect the bronchioles. RAO affected horses develop non-specific airway hyperresponsiveness, which is a bronchospasm in response to a wide variety of stimuli including inflammatory mediators and neurotransmitters. The importance of inflammation in RAO is demonstrated by the responsiveness of the condition to corticosteroid therapy (Robinson, East Lansing, MI, USA).

IAD affects approximately 30% of young horses in training. This condition has been associated with bacterial and viral infections, but in many horses no infectious aetiology is identified and allergic and environmental factors are implicated. The condition is typically associated with neutrophilic airway inflammation, although in some cases eosinophils or mast cell numbers are increased. Younger horses affected by IAD show high sensitivity to low levels of inhaled aerosols containing histamine in comparison to older RAO-affected animals (Viel, Guelph, Canada). There is presently no evidence that IAD can develop into RAO or whether these conditions are completely separate clinical entities. There is no way to predict in early life which horses will be affected by either condition. Horses affected by chronic non-infectious airway diseases, such as RAO and IAD, demonstrate increased histological lesions and worsening airway function with increasing age. In addition, significant histopathological changes are present before abnormal airway function can be detected. It is questionable whether mild airway inflammation in stabled horses is clinically significant, or is a normal

response to organic dust found in stable environments. It does not seem to impair dressage or show-jumping performance (Gerber, East Lansing, MI, USA). However, assessment of lung function (Hoffman, North Grafton, USA; Ohnesorge, Hanover, Germany) discriminates between healthy and IAD horses, suggesting that this mild inflammation can cause dysfunction of the lung.

There is evidence that the neutrophilic inflammation that is characteristic of chronic airway disease in horses is induced by IL-8 production, and IL-8 levels in BAL fluid are increased in RAO-affected horses (Franchini, Zürich, Switzerland) (Franchini et al., 2000). There is also a strong correlation between levels of airway MMP-9 and airway neutrophilia (Maisi, Helsinki, Finland) (Nevalainen et al., 2002) consistent with the importance of MMP-9 in neutrophil transmigration through the airway basement membrane (McGorum, Edinburgh, Scotland). When RAO susceptible horses are antigen challenged, peripheral blood and airway neutrophils are primed, as demonstrated by increased superoxide production and respiratory burst activity (Brazil, Edinburgh, Scotland). Neutrophil degranulation products are increased in airways, including neutrophil elastase and MMP-9. Much of the neutrophil elastase is inactive, while much of the MMP-9 is active (Raulo et al., 2001). However, in contrast to human chronic obstructive airway disease, airway remodeling in RAO-affected horses is minimal. This may be due to increased efficacy of the equine antiproteinase system. For example, equine alpha-1-proteinase inhibitor (API) is encoded by multiple alleles, in contrast to the single human locus, and BAL fluid levels are much higher in horses than in humans, in both health and disease. In addition, equine API is not a neutrophil chemoattractant. During the resolution of RAO, apoptosis of airway neutrophils is increased, and consequently neutrophil function and the resulting tissue damage are reduced (Brazil, Edinburgh, Scotland). In contrast, during exacerbations of RAO, neutrophil apoptosis is reduced possibly through cytokine mediated upregulation of NF- κ B. NF- κ B is highly activated in bronchial brushing samples (BBS) and BAL cells from RAO-affected horses compared with healthy horses and is highly correlated to the degree of residual lung dysfunction 3 weeks after antigen evicton (Bureau et al., 2000). Active NF- κ B complexes in BBS and

BAL cells from RAO-affected horses are mainly p65 heterodimers. This finding could offer an explanation for why RAO-associated inflammation is neutrophilic and eosinophil numbers are usually not elevated in BAL from RAO-affected horses. Expression of p65 homodimers induces IL-8, a potent neutrophilic chemoattractant, but not other chemoattractants such as eotaxin, an eosinophilic chemoattractant which is under p65-p50 control (Lekeux, Liège, Belgium). Recent studies suggest that the presence of highly activated NF- κ B in BBS and BAL cells from RAO-affected horses may be due to failure of deactivation of by I κ B- β . This is due to an imbalance between high levels of IL-1 β and TNF- α mediated I κ B- β degradation and low levels of I κ B- β synthesis.

Bronchospasm in horses with RAO results from facilitation of parasympathetically mediated smooth muscle contraction by inflammatory mediators. In vitro studies have shown that activated neutrophils do not affect cholinergic responses in equine airways, but mediators from mast cell, such as histamine, serotonin, and leucotriene D4 greatly facilitate smooth muscle contraction and could be responsible for the cholinergic mediated bronchospasm in RAO (Olszewski, East Lansing, MI, USA) (Olszewski et al., 1999).

Inhalation challenges with endotoxin (Pirie et al., 2001), or a hay dust suspension (Pirie et al., 2002), have revealed that airborne endotoxins present in relatively high concentrations in dusty stable environment (McGorum et al., 1998), contribute to pulmonary inflammation and dysfunction in RAO. However, other components of the hay dust suspension, especially particulates, are required to induce lung inflammation and dysfunction in RAO-susceptible horses comparable to that of the natural disease (Pirie, Edinburgh, UK). Interestingly, inhaled endotoxins have been shown to be involved in human organic dust induced pulmonary disease and RAO shares some features with this human lung disease.

The immunological basis of RAO remains poorly elucidated. While IgE levels are increased in bronchoalveolar (BAL) fluid of RAO-affected horses, consistent with a type-1 hypersensitivity, the immediate onset of airway obstruction typical of a type-1 reaction to exposure to allergens is not observed. In addition, intradermal tests with various allergen extracts correlate poorly with the clinical diagnosis (Jose-Cunilleras

et al., 2001). Grünig (New York, NY) reviewed the role of T cell subsets and of Th1, Th2 and immune regulatory cytokines in human chronic inflammatory lung disease and in a mouse model of asthma. Although a polarization of T helper cells into predominantly Th1 and Th2 subsets has not been conclusively documented in the horse, studies suggest that equine Th1 and Th2-like immune responses do occur. Results of initial studies on the type of immune response associated with RAO have been inconsistent. Using in situ hybridization, BAL cells from horses with RAO chronically exposed to dusty hay had increased expression of IL-4 and IL-5 mRNA and decreased IFN- γ mRNA expression compared to controls (Lavoie et al., 2001). However, in other preliminary studies using RT-PCR on BAL cells no consistent findings relating to cytokine mRNA expression in RAO-affected animals could be found (Lavoie, Montréal, Canada). A quantitative RT-PCR analysis of horses affected with SPAOPD demonstrated elevated levels of mRNA for IL-4 and IL-13 during the summer, while control animals exhibited a bias towards IFN- γ mRNA production (Horohov, Baton Rouge, LA) (Beadle et al., 2002). A full understanding of the immunological basis of equine chronic airway diseases will require improved immunological tools, such as antibody detection systems for cytokines and IgE, and the development of equine models for these conditions (Bowles et al., 2002).

8. Insect bite hypersensitivity

Equine IBH, also known as sweet-itch or summer eczema, is a recurrent, seasonal pruritic dermatitis of the horse (Marti, Berne, Switzerland). As shown by skin tests, IBH results from hypersensitivity reactions to insect bites, mainly from midges (*Culicoides* spp.) and sometimes from black flies (*Simulium* spp.). IBH occurs worldwide in areas where these insects occur with a prevalence of 3–5%, although this can be much higher in some horse families and particularly in Icelandic horses imported from Iceland (where these insects do not occur) to Europe or North America (see below). Horses with IBH suffer from severe pruritic dermatosis affecting the mane and the tail area and sometimes also the ventral midline. Histopathology of lesional skin biopsies reveals a perivascular dermatitis

with eosinophilic and lymphocytic infiltrates. In chronic lesions, there is usually a perivascular lymphocytic infiltration with or without a small number of eosinophils. Currently, treatment of IBH depends on avoidance of contact with insects and the use of corticosteroids. The efficacy of specific immunotherapy with *Culicoides* whole body extracts is controversial and is only rarely attempted.

Intradermal injection of *Culicoides* extract (Cunningham, London, UK) leads to a time-dependent accumulation of eosinophils and mononuclear cells in the skin of in sweet-itch affected horses, consisting of CD3-positive (+) lymphocytes, most of which are CD4+ T cells (McKelvie et al., 1999). *Culicoides* antigen(s) causes a concentration-dependent proliferation of PBMCs which is higher in sweet-itch ponies than in controls during the active phase of the disease. Additionally, PBMC stimulated with *Culicoides* extract release a heat labile factor that causes eosinophil adherence (McKelvie et al., 2001). Equine eotaxin mRNA has been detected in the clinically normal skin of ponies with IBH but not in the skin of unaffected animals. The amount of eotaxin mRNA in lesional skin was increased compared to that in normal skin, suggesting that this chemokine may play a part in *Culicoides*-induced eosinophil recruitment to the skin of sweet-itch ponies.

A series of presentations demonstrated that IgE-mediated immune reactions are probably involved in the pathogenesis of IBH. Lesional skin biopsies from horses with IBH contain significantly more IgE, IgE-mRNA positive cells and tryptase positive mast cells than skin biopsies from healthy control horses (van der Haegen et al., 2001). Two presentations described the release of mediators (histamine and sulfidoleukotrienes) from peripheral blood leukocytes after in vitro stimulation with allergen extracts or antibodies. Leukocytes from horses with IBH release significantly more sulfidoleukotrienes (Marti, Berne, Switzerland) or histamine (Leibold, Hanover, Germany) when incubated with a *Culicoides* extract than leukocytes from healthy controls, and there is a strong positive correlation between histamine and sLT release (Marti et al., 1999). Simuliids also induce significant histamine and sulfidoleukotrienes release in some horses affected with IBH. Leibold also showed that histamine release can be induced in some foals with a polyclonal anti-IgG heavy + light chain or with *Culicoides* extract,

although these foals had no clinical signs of IBH. These findings suggest that sensitization can occur early in life in the horse.

The presence of antibodies in horse sera binding to *Culicoides* salivary glands was demonstrated by immunohistological staining (Harwood, Bristol, UK). IgE antibodies could only be detected in sera from horses with clinical signs of IBH, but not in sera from healthy controls or in sera from horses with a history of IBH but in remission at the time of sampling. Anti-salivary gland IgG antibodies were detected in sera from both IBH-affected and healthy horses exposed to *Culicoides* spp, but not in sera from unexposed horses (Wilson et al., 2001).

The saliva of hematophagous insects contains a diverse range of factors that assist in successful blood-feeding. Few salivary glands proteins from *Culicoides* have been identified (Perez de Leon and Tabachnick, 1996), and their role as potential allergen is unknown. Presently only crude full body extracts from *Culicoides* spp. and *Simulium* spp. are available commercially. These crude extracts, where the allergens only represent a minute amount of the total extract, hamper studies of the pathogenesis of IBH as well as efficient-specific immunotherapy. One solution could be the production of recombinant *Culicoides* allergens. This work has started with the construction of a *Culicoides* cDNA library which will be screened with sera from IBH affected horses (Harwood, Bristol, UK).

IBH does not occur in horses born and living in Iceland because *Culicoides* spp. are not present there. However, when Icelandic horses are imported from Iceland to Europe (where *Culicoides* spp. are present) 50% of adults suffer from IBH if more than 2 years have passed since importation (Svansson, Reykjavik, Iceland). Onset of the disease takes place between 1 and 8 years after importation from Iceland, with a mean of 2.4 years. In contrast, only 7–10% of the Icelandic horses born in Europe suffer from IBH (Brostrom et al., 1987; Halldorsdottir and Larsen, 1991). The reasons for this phenomenon are unknown. Studies are underway to investigate whether genetic factors (i.e. lack of selection against IBH in Iceland) contribute to the high prevalence of IBH in horses imported from Iceland to *Culicoides* populated areas.

The therapeutic potential of DNA vaccination in equine hypersensitivity was summarized by Lunn

(Madison, WI, USA). There is evidence that TH2-type immune responses may contribute to allergic disease in the horse. Switching this immunological response to a TH1 phenotype is the base of immunotherapy for allergic diseases (Janeway et al., 2002). DNA vaccination offers an attractive technique for attempting this form of therapy, as DNA vaccination typically results in a TH1 immune response. In the horse successful DNA vaccination against infectious diseases has been performed using the gene gun (Lunn et al., 1999). To test the potential of DNA immunization as a therapy against allergic diseases of the horse, a model comparing the equine immune response after immunization with human serum albumin protein (HSA) and plasmid HSA-DNA is being established (Torsteinsdottir, Reykjavik, Iceland).

9. Hypersensitivity and immunogenetics

The genetic basis of human allergic diseases has been demonstrated in numerous studies and polymorphisms in several candidate genes show association with allergic conditions in humans (Barnes and Marsh, 1998). Matthew Binns (Newmarket, UK) explained how comparative genetics, through testing of candidate genes identified in human allergy, may allow identification of genetic markers for equine allergic diseases. The horse genome map (<http://locus-jouy.inra.fr>; <http://www.uky.edu/ag/horsemap>) has now reached the stage where it can start being used for finding genetic markers for different traits in the horse and in particular also for IBH and RAO. A genetic basis for these two conditions has previously been established (Marti et al., 1991, 1992). Curik (Zagreb, Croatia) showed that specific serum IgE levels in the horse are influenced by genetic factors although, as can be expected, the environment also exerts a significant effect (Eder et al., 2001). Curik also demonstrated that the ability to produce IgE against some pure recombinant mould allergens may be influenced by the equine MHC. Significant associations between equine leukocyte antigens or markers closely linked to the MHC and IgE levels against recombinant *A. fumigatus* allergens were found in Lipizzaner horses. The search for the other genes influencing IgE levels will be a first step towards identifying genetic markers for allergic diseases in the horse.

10. Conclusions

Equine immunological research remains at the scientific forefront of veterinary medicine, and the small but active community of equine immunologists have been quick to adopt the latest techniques to advance their work. Nevertheless, the principal limitations in this field remain the lack of key reagents, such as for the study of cytokines, the characterization of MHC and TCR diversity, and the identification of antigen-specific immune responses. To realize our goals of controlling infectious and allergic disease in the horse it will be essential to address these challenges. The rewards of these efforts will benefit not only the horse, but can provide unique opportunities for comparative immunological studies of disease.

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