ADVANCES IN IMMUNOLOGY LEAD TO NEW THERAPIES FOR HORSES

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SARAH STONEHAM considers the features of the equine immune system and looks at how advances in immunomodulators are being used in practice

EQUINE immunology is a rapidly expanding and increasingly complex field with considerable ongoing research.

As we understand more about the mechanisms by which the immune system successfully combats infectious disease, this knowledge can be applied to developing drugs that can modify the immune response to enhance protection against infectious challenge and treat disease, particularly in immunocompromised individuals.

This article will review the basic features of the immune system and immunomodulators used in clinical equine practice.

Innate immune response

The innate component of the immune system provides immediate, unconditional defence against infectious organisms. It provides nonspecific defence by mechanisms that pre-exist or are induced within a matter of hours by infectious challenge.

The innate response recognises molecular patterns (pathogen-associated molecular patterns; PAMPs) shared by large groups of pathogens, rather than the specific pathogenassociated

molecular patterns recognised by the adaptive immune system.

Recognition of PAMPs by pattern recognition receptors (PRRs) on phagocytic cells and dendritic cells leads to direct bacterial killing, enhanced phagocytosis and production of cytokines, for example, IL-1, IL-2 and tumour necrosis factor (TNF), which, in turn, initiate and maintain the inflammatory response. Costimulatory signals required for T cell activation are also induced following PAMP recognition. Other cytokines released by the innate response, for example, IL-4, interferon gamma, IL-10 and IL-12, influence the subsequent adaptive immune response.

Antigen-presenting cells, such as dendritic cells, act as a link between the innate and adaptive immune system. The recognition and binding of PAMPs to PRRs, such as tolllike receptors, on dendritic cells, results in activation of the dendritic cell that engulfs the pathogen, which is processed, and then antigen is presented via major histocompatibility complex (MHC) on the surface of the dendritic cell. These dendritic cells migrate to the lymph nodes where they activate T helper and cytotoxic T cells and B cells, so linking innate and adaptive systems.

Components of the innate immune system include the following.

- **Physical barriers**

Physical barriers include skin, mucosal surfaces and respiratory mucociliary escalator. They also include surface chemicals, such as lysozyme in tears and saliva, gastric acid and also the resident microflora of the gastrointestinal tract, skin, upper respiratory tract and vagina, which prevent colonisation by pathogens.

- **Cytokines**

Cytokines are low molecular weight proteins that are involved in cell-to-cell signalling, the messengers of the immune system. They are important in initiating and regulating both the immune and inflammatory response.

- **Complement**

The classic complement pathway is considered part of the adaptive immune system due to its reliance on antigen-antibody interaction – the alternative pathway occurs without this interaction. It is typically directly activated by foreign cells, pathogens and abnormal surfaces recognised as foreign.

- **Phagocytic cells**

Phagocytic cells include neutrophils, phagocytes and dendritic cells. Neutrophils are the first cells to reach the site of infection, followed a few hours later by inflammatory macrophages, which have

increased phagocytic and bacterial killing potential. Neutrophils and macrophages are involved in direct pathogen killing.

When antigens are bound to complement or immunoglobulin (opsonisation), phagocytosis by neutrophils and macrophages is enhanced.

Dendritic cells are specialised macrophages that act as antigen-presenting cells as part of the adaptive immune system, but also have an important role as messengers between the innate and adaptive immune systems.

• **Natural killer cells (NK cells)**

Natural killer cells are a small subset of lymphocytes that require no activation and destroy tumour cells, virusinfected cells and some intracellular bacterial pathogens.

Adaptive immune response

The adaptive immune response develops after the innate response and takes 48 to 96 hours to develop. The importance of the innate response in initiating and determining the type of adaptive response has recently become clear. The adaptive response is organismspecific and shows memory.

Adaptive immunity involves specific antibody response regulated by B-lymphocytes and the cell-mediated response mediated by T helper cells and cytotoxic T lymphocytes. The B and T cell responses are interdependent.

• **Antibody response**

B cells are responsible for the humoral adaptive response. Activation of B cells is antigenspecific and normally requires the assistance of activated T helper cells. Activated B cells may become plasma cells, which produce antibody, or Memory B cells, which are long-lived and able to respond rapidly to subsequent exposure to their particular antigen.

• **T cell response**

T cells are responsible for the cell-mediated immunity and coordinate the adaptive immune response. There are two major subsets of T cells – T helper cells that express CD4 on their surface, and the cytotoxic T cells that express CD8 on their surface. Antigen presentation (typically by a dendritic cell, but many other types of cell may present antigen as well) is particularly important for development of the T cell response.

The T cell response is both antigen-specific (due to antigenspecific receptors on the surface of T

cells) and has memory (when expanded populations of T cells remain in the body following infections, for example).

T helper cells produce cytokines and can be subdivided in type one (Th-1, playing a major role in intracellular infections) and type two (Th-2, playing a major role in extracellular infections), based on the cytokines they produce

- **Cytotoxic T cells**

Cytotoxic cells are involved in killing host cells that have been infected or have undergone neoplastic changes. Cytotoxic T cells recognise infected host cells using their antigen-specific receptor in conjunction with the CD8 on their cell surface that binds to MHC class one (found on almost all mammalian cells).

Immunomodulation

Immunomodulators are drugs that modify both the innate and acquired immune response in a non-antigen-specific way. They are biological response modifiers and can either stimulate or suppress the immune system (Rush, 2000).

They are widely used in human medicine as an adjunct to other therapies in treating chronic bacterial and viral infections, neoplasia, autoimmune diseases, and secondary immunodeficiencies, and as adjuvant in vaccines. In human medicine, they are often chemically defined drugs, such as recombinant cytokines used for their particular effect on a select component of the immune system.

Commonly used immunosuppressants include glucocorticoids, azathioprine and anti-proliferative drugs. These are used in small animal dermatology and treatment of autoimmune and immune-mediated disease (Griffin, 2006). Cyclosporin was originally considered an immunosuppressant, but is now regarded as an immunomodulator, due to its lack of bone marrow suppression and its more selective effects on the immune system.

An immunostimulant is an agent that activates immune cells and enhances the release of cytokines. This results in activation of the immune response, in particular, enhancing phagocytosis, intracellular killing of organisms, antigen presentation, cytotoxic and antiviral T cell function and antibody production (Flaminio, 2003). In part, the effectiveness of an immune stimulant will depend on the animal’s ability to produce cytokines. This article focuses on immune stimulants.

Veterinary immunomodulators are less specific than many of those used in human medicine, and come from bacterial, viral or plant sources. The substances stimulate immune cells to produce cytokines such as Interferon, IL-1, IL-6 and TNF. These cytokines activate both humoral and cell-mediated immunity. Transient pyrexia, anorexia and lethargy are commonly seen side effects. These are not adverse effects as they are clinical signs associated with cytokine release, the

desired effect of the immunomodulator.

Immunomodulators in equine practice

• Levamisole

Levamisole is a synthetic anthelmintic used in cattle. Its immune stimulatory effects were first recognised in mice. It has been shown to stimulate depressed cell-mediated immunity and enhance neutrophil mobility in immunocompromised animals, although its mode of action is not fully understood. It has also been shown to have no effect on the immune system of healthy individuals. Its clinical effects have only been observed when used as an adjunct to other therapies.

Evidence for its efficacy in horses is principally anecdotal. However, one study showed levamisole administered to pregnant mares for four to six weeks prior to parturition, increased colostral IgG and IgG (T) levels when compared to controls (Krakowski et al, 1999).

Levamisole administered orally has been used as an adjunct to treatment of chronic viral infections, equine protozoal myelitis and chronic obstructive pulmonary disease.

There are reports (Ho et al, 2009; Barker, 2009) that aminorex and rexamino (amphetamine-like compounds) have been found in blood and plasma of horses following normal administration of levamisole. It is possible to differentiate aminorex detected following levamisole administration from that found following synthetic racemic aminorex administration, but withdrawal times should be considered when using levamisole in competition horses or racehorses.

• Imiquimod

Imiquimod is a topical immune response modifier with potent antiviral and anti-tumour activity. In humans, it has been shown to induce the release of pro-inflammatory cytokines and activation of antigenpresenting cells that, in turn, trigger a marked T helper cell (Th-1 biased) anti-tumour cellular immune response (Schon, 2007).

It has been used in treatment of aural plaques in horses. One study followed removal of thick crusting of the plaques using five per cent imiquimod cream (Aldara, 3M, US) applied three times a week every other week. Duration of therapy ranged from one-and-a-half months to eight months, and the authors reported long-term resolution in 87.5 per cent cases (Torres et al, 2010).

It has also been used in the treatment of sarcoids. In a pilot study, five per cent imiquimod cream was applied three times a week for 32 weeks or until the tumour had resolved, whichever occurred first. In 80 per cent of cases there was a 75 per cent or greater reduction in tumour size, with resolution in 60 per cent of cases (Nogueira et al, 2006). Publication of further studies would be useful to corroborate these initial findings.

• **BCG vaccine (attenuated *Mycobacterium bovis)***

BCG vaccine has been used for many years against tuberculosis, and is considered to be a highly potent stimulator of macrophage function, resulting in release of IL-1 and TNF and colony-stimulating factors.

Live BCG vaccine is not recommended for use in horses due to the potential for systemic infection and risk of adverse reactions. Whole inactivated BCG and modified purified mycobacterium cell wall fractions are used in horses.

In the US, Equimune IV (Bioniche Animal Health, Canada), an intravenous purified *Mycobacterium* cell wall extract preparation, is licensed for treatment of equine viral and bacterial respiratory disease. In a randomised, double blind clinical study in horses, a single intravenous dose appeared to shorten the recovery period from naturally occurring respiratory disease in 83 per cent of horses (Flaminio, 2003). There are anecdotal reports of its use as an adjunct to therapy for equine protozoal myelitis (EPM). Severe adverse side effects have been reported, including severe inflammation of the respiratory tract, such as interstitial pneumonia and multifocal pulmonary granulomas.

In the UK, mycobacterium cell wall products are used for the treatment of periocular sarcoids. A large study (Knottenbelt and Kelly, 2000) found 69 per cent resolution of periocular fibroblastic and nodular sarcoids treated with intralesional BCG. Its effect on verrucose and occult sarcoids was poor. A risk of anaphylaxis following repeated injection has been reported.

• ***Propionibacterium acnes***

The antiviral and antitumour activities of the Gram-positive bacterium *Propionibacterium acnes* have been recognised in man and mice for many years. It has been shown to activate macrophages, enhance NK cell activity, increase CD8-plus T lymphocyte expression of interferon gamma, inhibit tumour growth and increase non-specific resistance to infectious challenge.

In the US, an inactivated *P acnes* product (Eqstim, Neogen, Lexington, US) is licensed as an adjunct to conventional therapy for chronic and stressrelated respiratory infection in horses. It is an intravenous preparation, administered three times on day zero, day three to four and day seven.

In a blind randomised clinical trial, Eqstim was administered to horses with naturally occurring respiratory disease. It improved the speed of regression of clinical signs within 14 days of treatment in 96 per cent of horses compared to 35 per cent in controls (Vail et al, 1990).

Production of gamma interferon is associated with resistance to intracellular bacteria, including *Rhodococcus equi.* Foals with *R equi* infection have been shown to have lower levels of interferon gamma. As a result, research is ongoing into the potential of immunostimulants in neonatal foals to

enhance the immune response in order to increase resistance to *R equi* (Sturgill et al, 2011). *P acnes* was shown to reduce intracellular proliferation of *R equi* within monocyte-derived macrophages when compared to control foals. However, lymphoproliferative responses and IFN gamma induction were not significantly different between the treated and control groups (Ryan et al, 2010). Anecdotal reports mention its use in treating endometritis in mares.

• Parapoxvirus ovis

Observations of improvement in viral diseases and tumour regression following the use of poxvirus vaccination in the smallpox eradication programme triggered interest in poxvirus as a potential immunostimulant.

Although its mode of action is not fully understood, it is thought components in the viral envelope act as immune stimulants independent of viral replication. It has a strong influence on cytokine secretion in human immune cells upregulating inflammatory and Th-1-related cytokines, for example, interferon gamma, as well as anti-inflammatory Th-2-related cytokines. In humans, it is thought the balance between Th-1 and Th-2-related cytokine release may account for it being well tolerated and promote a more natural response than antiviral therapies using single cytokines (Friebe et al, 2004).

In horses, parapoxvirus ovis (PPVO) has been shown to increase interferon gamma production 24 hours after the first dose, but it declined thereafter. It also induces some of the regulatory cytokines, and further investigation of this feature is ongoing (Horohov et al, 2008).

Zylexis (Pfizer Animal Health, UK) has been licensed for use in horses in the UK to stimulate immune response in order to increase resistance to upper respiratory tract disease (such as EHV1). It is licensed for use prior to or, at the latest, on the day of “stress”, such as transportation, weaning or co-mingling. This product has been available in the US for some time (Baypamun HK, Bayer AG, Germany).

A randomised controlled clinical trial was carried out on 53, four to 10-month-old weanlings stressed by weaning, transport and co-mingling. The treated group was given three doses of Baypamun HK, on day of co-mingling, day two and day nine. The incidence of respiratory disease was high, with 50 per cent of horses seroconverting to EHV1 and EHV4.

Scores for clinical signs of respiratory disease were reduced by 40 per cent in the treated group compared to the placebo group. Fifty per cent of the treated group showed no purulent nasal discharge compared to 14.8 per cent of the placebo-treated group (Ziebell et al, 1997).

Understanding of the equine immune system is rapidly developing and, as a consequence, these findings are being applied to investigate substances that can modify the immune response to assist in prevention or treatment of disease.

As resistance of organisms to conventional therapy becomes an increasing issue, therapies that increase an animal's own immune defence mechanisms are likely to become increasingly important. Over the next few years this may help us reduce the incidence of disease such as *R equi* in foals, *Lawsonia intracellularis* in weanlings and respiratory disease in weanlings, competition horses and young racehorses.

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KEY FEATURES OF INNATE COMPONENT OF THE IMMUNE SYSTEM

• First line of defence

• Immediate and non-specific

• No prior exposure needed, but no memory

• Receptors that recognise pathogen-associated molecular patterns (PAMPs) on invading organisms

• Limits pathogen replication until adaptive component kicks in

• Innate response important in initiation and outcome of adaptive response

KEY FEATURES OF THE ADAPTIVE COMPONENT OF THE IMMUNE SYSTEM

• Targeted against specific organisms

• Prior exposure needed

• Response amplified with repeat exposure

• B lymphocytes antibody production

• T lymphocytes – cytotoxic cells and T helper cells

• Appropriate antigen presentation by dendritic cells and MHC necessary for development of T cell response, which is restricted by MHC:

– MHC class one presentation – cytotoxic T cell response

– MHC class two presentation – T helper cell responses