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Immunology of the Equine Lung

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Introduction

In a 24-hour period, 100,000 liters of air pass through the respiratory system of an adult horse [1]. As a result, both inert and infectious agents may find their way into the lung. Depending upon the environment this exposure may be minimal or quite severe. The ability to penetrate the respiratory tract and gain access to the lung is dependent upon the size of the particle and the status of the respiratory system. Particle size will determine both the depth of the penetration into the lung and the nature of the removal mechanism [2]. The nasal passages with their projecting turbinates provide the initial filtering mechanism (Fig. 1).

Those particles up to 10 microns in diameter which pass through the nasal passages contact the airway wall by inertial impaction at bends in the upper airway. Those in the 2 - 5 micron range will penetrate more deeply prior to sedimenting onto the walls of the airways. The smallest particles, < 1 micron reach the alveoli where they contact the epithelial surface by diffusion. The primary mechanism for removing these particles and maintaining the sterility of the lung is the mucociliary transport apparatus. The upper respiratory tract of the horse is lined by a mucus membrane that is contiguous with the wall of the trachea and is composed of mucosa, the lamina propria containing submucosal glands of both serous and mucus types, the cartilage plate and the adventitia. The respiratory mucosa is lined with columnar ciliated epithelium with large numbers of goblet cells at the beginning of the trachea and progressively fewer cells towards the bronchioles such that the terminal bronchioles that are covered only by the epithelium and a thin muscular layer. Goblet cells are responsible for the production and secretion of mucus into the airways and their number can vary depending upon the health status of the horse [3]. The ciliated cells are responsible for the transport of the mucus out of the lung. The cilia beat in a coordinated fashion in the sol layer of the mucus layer. This low viscosity fluid supports the gel layer of the mucus that protects the sol layer from desiccation and entraps the inhaled particles. The mucus also contains immunoglobulins that can neutralize invading bacteria and viruses.



Figure 1. Cross section of equine skull showing lymphoid tissues. 1-9 Nasal associated lymphoid tissue, 10-12 Laryngo-tracheal associated lymphoid tissue. (adapted from [17]). - To view this image in full size go to the IVIS website at www.ivis.org . -

Respiratory Immunoglobulins

The primary immunoglobulin isotype found in mucus is IgA. Most of this antibody is produced in the lamina propria and the dimeric IgA is transported across the epithelium. Influenza virus infection leads to the induction of a nasal IgA response that is associated with protection. By contrast vaccination with inactivated viral vaccines containing an alum adjuvant leads to predominantly IgG(T) response in the peripheral blood and no mucosal antibody response [4]. Infection with equine herpesvirus-1 likewise leads to a virus-specific mucosal IgA response, though IgG antibodies were also detected [5]. Mucosal IgA responses have also been noted against bacterial antigens, though IgG isotypes can be detected, as well [6-9]. Transudation of serum proteins including IgG and IgM likely occurs during infections and transudation might also explain the predominance of these isotypes in the lower lung [10]. The presence of IgE antibodies in the lung is associated with allergic airway diseases including "heaves" [11,12].

Alveolar Macrophages

The predominant innate cellular defense mechanism of the lung is the alveolar macrophage, a specialized population of phagocytic cells found in the distal airways and alveoli. By contrast, the presence of neutrophils in the airway is often pathognomonic for inflammatory airway disease. Particles deposited on the alveolar surface are cleared by alveolar macrophages (Fig. 2).

Macrophages are also present in the mucociliary system and in the lymphoid tissues associated with the airways. In contrast to other monocytes and macrophages, alveolar macrophages express low levels of major histocompatibility complex (MHC) class II antigens and accessory molecules (CD80 and CD86) and thus present antigen poorly to CD4⁺ T cells [13]. Alveolar macrophages also respond weakly to interferon-gamma (IFN- γ) in terms of the up-regulation of MHC Class II expression, though they are more responsive to other cytokines including granulocyte-macrophage colony stimulating factor (GM-CSF) [13]. We have observed similar low level expression of MHC Class II antigen on equine alveolar macrophages collected from disease-free horses and elevated levels of MHC Class II antigen on those macrophages collected from horses with inflammatory airway disease.

This low level expression of accessory antigens on alveolar macrophages likely accounts for the diminished antigen presentation capability of alveolar macrophages and may be the mechanism whereby immune responsiveness in the lung is minimized in order to avoid pathologic responses [14]. Though dendritic cells are thought to play a central role in the induction of specific immune responses in the lung [15], little is known regarding their function in the equine lung as the lack of suitable reagents for identifying equine dendritic cells have hampered such studies [16].

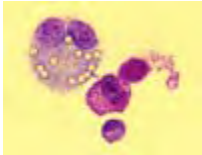


Figure 2. Alveolar macrophages with phagocytosed latex beads. A 3 μ m latex bead suspension was nebulized into pony lungs and a bronchoalveolar lavage was performed 24 h later yielding the pictured alveolar macrophage containing the latex beads. - To view this image in full size go to the IVIS website at www.ivis.org . -

Lymphoid Tissues

Lymphoid tissue within the respiratory tract can be divided into five different types; free luminal lymphocytes, intraepithelial lymphocytes, isolated lymphocytes in the lamina propria, dense infiltrates forming lymphoid tissue, and lymphoid nodules [17]. Lymphocytes and other leukocytes are present in the airway lumen of the normal lung and can become quite numerous in various disease states. The composition of lung leukocytes in horses has been studied principally in bronchoalveolar lavage (BAL) fluid. Unlike murine and human BAL fluid, in which lymphocytes are a small proportion of the recovered cells, BAL fluid from adult horses contains as many lymphocytes as macrophages [18,19], suggesting major differences in lung leukocyte composition between the two species. Younger horses and foals have fewer lymphocytes and more macrophages in their lungs. BAL fluid of adult horses yields a lymphocyte population containing more CD8⁺ cells than CD4⁺ cells [19].

Intraepithelial lymphocytes and lamina propria lymphocytes are present in all parts of the respiratory tract. The predominant intraepithelial lymphocyte in the equine lung is the CD3⁺ T cell, as it is in other species. While CD8⁺ cells are the predominant cells in the bronchial epithelium, these cells rarely express the $\gamma\delta$ version of the T cell receptor (TcR) [20]. This is in contrast to intestinal epithelial cells which are predominantly $\gamma\delta$ + CD8⁺ T cells. This has not been investigated in the horse, though it is likely there will be a similar distinction between intestinal and bronchial intraepithelial T cells. It is known that in the horse the density of T cells in the epithelia varies with both age and location. Thus the number of intraepithelial cells progressively decreases down the respiratory tract with the cell numbers in the lower airway being lower than in the trachea [16]. Likewise foals and younger horses have fewer T cells in their epithelium and in their BAL [16,21]. This age and site-specific difference in T cell numbers is likely the result of exposure to airborne antigens that provide the necessary stimulus for T-cell infiltration into the lung.

The predominant lymphocyte in the lamina propria is the plasma cell which presumably is producing the dimeric IgA which is transported across the epithelial cells to the lumen. Here again, lymphoid nodules are abundant in specific sites where inhaled particles are likely to impact with the largest populations in the larynx, nasopharynx and fewer in the nasal cavity, trachea and bronchi [22]. The abundance of these nodules in the upper respiratory tract constitute the nasal-associated lymphoid tissue (NALT) which is particularly well developed in the horse (Fig. 1) and may play an important role in protection against upper respiratory tract pathogens including influenza virus and *Streptococcus* sp. [23]. The lymphoid tissue and nodules of the equine respiratory tract resemble the bronchial associate lymphoid tissue (BALT) of other species, except that it extends even further distally in the horse. The nasopharyngeal tonsil is potentially an important mucosal immune induction site in the horse [24]. As in other species [25], the development of the BALT (and NALT) in the horse is presumably the result of microbial stimulation as it is more pronounced in older horses as compared to neonates and foals [22].

The exception is the nasopharyngeal tonsil and associated lymphoid tissue that regresses with age [24,26]. Lymph drainage from the lung is accomplished both via the deep lymphatics which drain toward the hilum and the superficial lymphatics which drain the visceral pleura through a plexus converging on the hilum.

Immune Response

In general the immunological status of the lung tends towards being immunosuppressive in nature, perhaps as a means of avoiding damage to the structural integrity of alveolar tissue [13]. This immunosuppressive environment is likely maintained by the presence of interleukin 10 (IL-10) an anti-inflammatory cytokine [27]. The introduction of harmless

antigens into the respiratory tract leads to a state of aerosol tolerance [28].

By contrast, the introduction of infectious agents results in the rapid movement of leukocytes from the lymphoid tissues into the luminal space. Infection of the horse with equine herpesvirus-1 resulted in profound and rapid fluctuations in bronchoalveolar leukocyte populations [19]. The initial acute inflammatory response consisted of a neutrophilia and a lymphopenia and was probably initiated within hours of the initial infection. Non-specific cytotoxic effector activity was also detected at this early time. A second, later influx of CD8⁺ lymphocytes coincides with the induction of antigen-specific cytotoxic T cell activity. These later cells are presumably involved in the elimination of the virus infected cells in the lung. While the precise mechanism responsible for this rapid response is unknown, it likely involves recognition of pathogen-associated molecular patterns by TOLL-like receptors on alveolar macrophages [29]. The signals provided through these receptors may enhance the ability of alveolar macrophages to present antigen and stimulate a specific immune response [27].

While an initial inflammatory response to an invading antigen is an important and necessary component of protective immunity, the inflammatory process in itself can be pathologic, particularly when chronic. A widely recognized, but poorly understood disease that is the apparent result of this chronic inflammatory response is "heaves" or recurrent airway obstruction (RAO) [30]. RAO of horses is similar to some forms of human asthma sharing characteristic features of airway hyperresponsiveness, airway inflammation, and episodes of obstruction of the airways that reverse either spontaneously or with treatment [31]. The chronic inflammation in human asthma is orchestrated by the cytokines IL-4, IL-5, and IL-13 derived from type 2 T-helper (TH2) lymphocytes [32]. Both IL-4 and IL-13 favor the IgE antibody production towards inhaled allergens, while IL-5 allows the differentiation, activation and survival of eosinophils [33].

The initial induction of immunoglobulin E (IgE)-mediated mast cell degranulation, up-regulation of adhesion molecules, and the production of inflammatory and chemotactic cytokines leads to the infiltration and activation of effector leukocytes (neutrophils and eosinophils) leading to pathological manifestations within the lung and airway [34,35]. A similar process is now thought to occur in equine RAO. Horses affected with RAO have IgE antibodies in their airways [11] and exhibit elevated levels of TH2 cytokine mRNA in bronchoalveolar lavage lymphocytes and peripheral blood mononuclear cells [36-38]. The most notable difference between human asthma and equine RAO is the cellular composition of the inflammatory response. Asthmatic patients have increased numbers of eosinophils in their airways whereas in horses with RAO the predominant cellular response is the neutrophil [39]. Bronchiolar neutrophilia may be the consequence of the over expression of cytokines chemotactic for neutrophil granulocytes, particularly IL-8 [40]. While the precise cellular source of the cytokine mRNA is not certain, RAO-affected horses exhibit elevated levels of CD4⁺ lymphocytes in the peripheral blood and CD3⁺ cells in pulmonary biopsy frozen tissue sections [18]. Though these findings support a hypothesis of immune dysregulation in the RAO-affected horses, they do not identify the underlying basis of this response. The underlying basis for asthma induction in humans is likewise unknown.

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