SYMPOSIUM IV:
Pathogenesis of different respiratory infections

ROLE OF *CHLAMYDIAE* AND *MYCOPLASMA* SPP. IN CHRONIC AIRWAY DISEASES IN HUMANS

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Chlamydophila and *Mycoplasma pneumoniae* (so called atypical respiratory pathogens) are known as pathogens causing bronchial or pulmonary infections, especially in outpatients. The prevalence of these pathogens is under discussion. Estimates for both pathogens range from 1 to more than 20 % of all pneumonia cases. These differences can be explained by variations in the appearance from year to year, and by difficulties in identification of atypical pathogens. Repeated serologic assessments, which had been used for a long time, overestimate the prevalence of infections, since the titres of antibodies remain high over longer periods. Newer, PCR based identification techniques are not standardised, and a real gold standard does not exist yet.
The German competence network CAPNETZ, in which more than 5,000 patients with community acquired pneumonia had been recruited and diagnosed since 2001, revealed a mean prevalence for *M. pneumoniae* of 4 %, and for *C. pneumoniae* of less than 1 %, measured with PCR. This does not exclude that infections may be much more frequent in regions with local outbreaks. It can be assumed that both pathogens cause mild pneumonia of short duration and early resolution. The mean mortality was lower than average, when atypicals had been found. Neither the German, nor the European guidelines for treatment of community acquired pneumonia (CAP) recommend macrolides or fluorochinolone antibiotics, the standard substances for atypicals, as first line treatment.

A much more dangerous role of *C. pneumoniae* is discussed for other diseases, like coronary artery disease, myocarditis, or depression. However, associations between the intracellular persistence of *C. pneumoniae* and manifestation of these diseases could not been proven. In respiratory medicine, associations between C. pneumophilia infection and exacerbation of COPD and status asthmaticus are discussed. Preliminary results form CAPNETZ show that even after complete resolution of *C. pneumoniae* infection, hyperreagibility of the airways persists for months, even in otherwise healthy persons. It has been assumed that these pathogens may cause long lasting neurogenic inflammation, explaining these observations. In a recent study, steroide standard treatment of status asthmaticus was extended for a ketolide, and a faster resolution of the asthma attack could be documented.
There might be further pathologic effects of the atypical respiratory pathogens, on the other hand, anti-inflammatory properties of macrolides and ketolide antibiotics have to be taken into account.
A role of *C. pneumoniae* in exacerbation of COPD is discussed, and more exacerbation results in faster progression of airway obstruction. However, no certain results are available yet. The sufficient therapeutic effect of fluorochinolones in exacerbation of COPD, even if atypicals could not been detected, is suggestive of effects beyond current concepts.

Chlamydophila and mycoplasma attack cells with important functions for the homeostasis of the airways. Resulting inflammatory reactions might be a significant factor for the progression of the underlying pulmonary disease. Further studies with improved diagnostic techniques, performed in animals as well as in humans, are urgently needed.

HOST CELL RESPONSES TO *CHLAMYDIA PNEUMONIAE* AND OUR UNDERSTANDING OF THE PATHOGENESIS OF RESPIRATORY INFECTIONS

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Introduction

Chlamydia pneumoniae is one of the most frequent respiratory pathogens in man. The seroprevalence in adults is more than 50 %. However, most of the infections are less symptomatic. Only in some cases of broncho-interstitial pneumonia *C. pneumoniae* can be identified as the causative agent. On the other hand, chronic infections are discussed to play a role in asthma, chronic obstructive airway disease (COPD), atherosclerosis, and neurological diseases (1,2). The hypothesis that *C. pneumoniae* may promote inflammatory processes in chronic diseases is based on the ability of this pathogen to survive in its host cells in a persistent state. Primary host cells of *C. pneumoniae* are epithelial cells and macrophages. Chlamydiae enter the cell as infectious elementary bodies (EBs) that differentiate into the metabolically active but noninfectious reticulate bodies (RBs). During the normal growth cycle these RBs multiply within the expanding endosome and reorganize into new elementary bodies that are released by host cell lysis or exocytosis. In contrast, intracellular persistence is characterized by the development of inclusions containing atypical RBs and only few EBs.

Cellular immunity with activation of Th1 lymphocytes and cytotoxic T lymphocytes is crucial in the immune response to chlamydial infection. In this relation the induction of a delayed type of hypersensitivity (DTH) reaction has been postulated to play a role in *Chlamydia*-associated diseases. As shown in murine lung infection models a Th1 type DTH associated with a predominant mononuclear cell infiltration may be responsible for protective immunity whereas a Th2 type DTH characterized by infiltration of eosinophils in addition to mononuclear cells leads to disease and tissue damage (3).

Research on the interaction of *C. pneumoniae* with its host cells has demonstrated that the response of infected cells may contribute to the initiation and maintenance of inflammation as well as to tissue remodeling and fibrosis.

Cytokine and chemokine production by host cells

Infection of epithelial cells and monocytes/macrophages by *C. pneumoniae* in vitro induces a cascade of cytokines and chemokines that can activate and recruit immune cells. In epithelial cells increased amounts of interleukin 6 (IL-6), IL-8, IL-11, and granulocyte macrophage colony stimulating factor (GM-CSF) are produced upon infection (4,5,6,7). *Chlamydia*-stimulated macrophages release IL-1β and tumor necrosis factor α (TNF-α) (8). The activation of p38-mitogen-activated protein kinase (MAPK), extracellular receptor kinase (ERK 1/2), and NFκB regulates the induction of these inflammatory mediators in response to host cell invasion (4,5). Furthermore, chlamydial antigens such as Hsp60 also activate macrophages and dendritic cells. The response of dendritic cells that includes the production of TNF-α and IL-12p40 depends on toll-like receptors (TLR) 2 and 4 (9). Because IL-12p40 induces NK and T cells to produce IFN-γ, the TLR-mediated activation of dendritic cells by chlamydial Hsp60 may represent an important mechanism of innate immunity in *C. pneumoniae* infection.

Establishment of intracellular persistence

IFN-γ is essential for an effective protective immune response to chlamydial infection. Although the effector mechanisms of IFN-γ-mediated control of an infection are not completely understood, it is established that IFN-γ inhibts the intracellular growth of *Chlamydia* through inducing indoleamine 2,3-dioxygenase (IDO), an enzyme that catalyzes the degradation of intracellular tryptophan (10). However, IFN-γ has also been shown to induce the development of persistent RBs of *C. pneumoniae* in monocytes and epithelial cells (11). It can be supposed that the induction of IDO represents a double-edged sword causing eradication or intracellular persistence of *C. pneumoniae.* The infection of bronchial smooth muscle cells by *C. pneumoniae* in vitro results in expression of IDO which is mediated by the induction of IFN-β acting in an autocrine and paracrine manner (12).

C. pneumoniae is able to inhibit apoptosis of infected cells. Anti-apoptotic effects of chlamydiae are characterized by the loss of BH3-only proteins resulting in the interrupt of the apoptotic pathway. It has been proposed that the chlamydial protease CPAF which is injected into the cytosol causes the degradation of BH3-only proteins (13). The resistance of infected cells against apoptotic stimuli may be essential for chlamydiae to complete their growth cycle but it may also promote intracellular persistence.

In cultures of HEp-2 epithelial cells persistently infected with *C. pneumoniae* IL-6 production is continuously up-regulated suggesting that a persistent infection may cause chronic inflammation (14).

Tissue remodeling

In chronic asthma and COPD the structural remodeling of the airway wall leads to irreversible airflow obstruction. These remodeling processes are characterized by smooth muscle cell hyperplasia and subepithelial fibrosis. *C. pneumoniae* stimulates the expression of connective tissue growth factor (CTGF) in epithelial cells and the production of basic fibroblast growth factor (bFGF) by bronchial smooth muscle cells (6,15). Because both CTGF and bFGF mediates smooth muscle cell proliferation, these data provide a mechanism by which chlamydial infection may promote airway remodeling. Moreover, the infection of smooth muscle cells by *C. pneumoniae* can also directly activate proliferative signals via up-regulation of the early growth response gene 1 (Egr-1) (16). On the other hand, *C.-pneumoniae*-infected smooth muscle cells secrete large amounts of prostaglandin E_2 (PGE_2) which may suppress smooth muscle cell growth in a paracrine manner (17).

C. pneumoniae infection of smooth muscle cells increases the production of matrix metalloproteinases 1 (MMP-1) and -3, and infected macrophages secrete MMP-1 and -9 (18,19). Because increased MMP activities have been linked to airway remodeling, these findings would provide an additional mechanism by which *C. pneumoniae* might contribute to obstructive airway diseases.

The thickening of the lamina reticularis, the lower part of the basement membrane area, is accompanied by a deposition of interstitial collagens. However, the infection of smooth muscle cells and fibroblasts by *C. pneumoniae* in vitro diminishes the synthesis of type I and III collagens (20). Furthermore, collagen expression by these cells was also found to be decreased after exposure to conditioned medium from infected epithelial cells.

TGF-β is one of the most important fibrogenic cytokines stimulating collagen expression but its role in respiratory *C. pneumoniae* infection is unknown.

Conclusion

It can be argued that the cytokine and chemokine response of host cells essentially contributes to inflammation in *C. pneumoniae* infection. However, there is only little knowledge of potential mechanisms explaining that *C. pneumoniae* may promote airway remodeling in asthma and COPD.

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MOLECULAR MECHANISMS INVOLVED IN THE PERSISTENT STATE OF *CHLAMYDIA*

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Chlamydophila (Cp.) psittaci, the causative agent of psittacosis/ornithosis in birds and humans, is capable of undergoing a morphologically altered state, the persistence state, induced by various intracellular environmental changes like deprivation of nutrients (amino acids, iron), application of antibiotics or exposure to cytokines (IFN-γ, TNF α). Chlamydial persistence has been associated with a number of chronic diseases, such as trachoma, reactive arthritis, chronic respiratory disease, cardiovascular disease, and pelvic inflammatory disease. Compared with acute infection, such chronic chlamydial infections are less susceptible to antibiotic treatment and the establishment of a persistent infection is thought to play a role in failure of therapy.
Differences in gene expression between acute and persistent infection were shown for *Chlamydia trachomatis*, *Chlamydophila pneumoniae* and *Chlamydophila psittaci* in several studies (Belland et al., 2004; Hogan et al., 2002; Polkinghorne et al., 2006; Goellner et al., 2006). In those studies, genes have been elucidated whose expression was specifically changed in the persistent state and can therefore be considered as "marker genes" for persistence. Furthermore, chlamydiae are capable to either induce or inhibit host cell apoptosis, which is an important feature for their reproduction within the host cell. As persistent chlamydiae establish a long-term relationship with the host cell and persistent aberrant chlamydial bodies are not released at the end of the normal acute developmental cycle, enduring inhibition of apoptosis during the persistent state is conceivable. Interestingly, members of the IAP (inhibitor of apoptosis) family as ciap1 and ciap 2 were found to be upregulated in persistent *Cp. psittaci* infection compared to uninfected cells. Therefore, inhibition of host cell apoptosis may facilitate colonization of the organism and seems to play a role in long-term survival of persistent *Cp. psittaci* within the host cell. Altogether, establishment of chlamydial persistence is accompanied by diverse molecular mechanisms which are not yet fully understood. Nevertheless, there is little doubt that the persistence stage plays an important role in the chlamydial development and may be considered as the third phase of the developmental cycle.

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DETECTION OF *CHLAMYDOPHILA PSITTACI* AND *ABORTUS* IN HUMANS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE AND HORSES WITH RECURRENT AIRWAY OBSTRUCTION

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Objective: Chronic obstructive pulmonary disease (COPD) in humans, with emphysema as the end stage of disease, and recurrent airway obstruction (RAO) in horses are regarded as multicausal diseases. Cigarette smoking or stable dust are the main factors. Infections with *Chlamydiaceae* had not been considered so far.
Methods: Tissue of humans with advanced alpha-1 antitrypsin deficiency (AATD) and non-AATD emphysema having undergone lung volume reduction surgery, or with giant bullous emphysema having undergone bullectomy, as well as sputum samples from patients with stable (S-COPD) and acute exacerbated COPD (AE-COPD) were investigated with light microscopy (LM), transmission electron microscopy (TEM), immunohistochemistry (IHC), immunofluorescence (IF), and PCR. 46 horses were examined for clinical signs of RAO, pathological lesions and infection by *Chlamydiaceae*. Horse lung tissue samples were examined by light microscopy, IHC and PCR.
Results: Bronchiolitis, interstitial inflammation and intra-alveolar accumulation of macrophages were seen histologically (LM) in a variable extent in all cases with human emphysema. In 100% (n=5) of AATD emphysema, 79.4% (n=27) of non-AATD and 73.3% (n=15) of giant bullous emphysema, persistent chlamydial infection was detected by ultrastructural examination (TEM). Chlamydial infection was seen in 83.3% (n=12) of AATD emphysema, 100% (n=50) of non-AATD and 93.75% (n=16) of giant bullous emphysema by IHC. Detection rates in macrophages, type 2 pneumocytes, bronchiolar epithelial cells, and lymphocytes revealed some differences between the three forms of emphysema. The presence of *Chlamydia (C.) psittaci* was demonstrated by PCR in lung tissue of four AATD (66.7%) vs. nine non-AATD emphysema patients (29.0%). Partial DNA sequencing of four positive samples confirmed the identity of the agent as *Chlamydophila (Cp.) psittaci*. In samples of induced sputum of patients with COPD IF revealed 69.6% *C. spp*. positive cases in AE-COPD and 50% in S-COPD. By PCR 42.4% of the samples were positive for *Cp. psittaci* in AE-COPD and 4.5% in S-COPD ($p<0.01$). Partial DNA sequencing of six positive samples confirmed the identity of the agent as *Cp. psittaci* in four and *Cp. abortus* in two patients.
In horses IHC revealed *C. psittaci* antigens in bronchiolar epithelial cells, type II pneumocytes and macrophages. Clinically healthy (n=20) and sick horses (n=26) could be separated by the total number of infected cells ($p<0.001$). But *C. psittaci* antigens were also detectable at low levels in healthy animals. PCR was positive in 54.3% of all horses, *omp*A sequencing identified *Cp. psittaci* (n=10) and *Cp. abortus* (n=13).
Conclusions: These data indicate a role of *Cp. psittaci* and *abortus* in human COPD and pulmonary emphysema as well as in equine RAO as trigger factors of inflammation or indicators of severe disease. But the abiotic factors smoking and stable dust seem to be of major relevance, probably facilitating chlamydial infection and replication by interfering with the pulmonary defense mechanisms. Further investigations on human and animal strains and on possible zoonotic transmission are necessary.

AIRWAY OBSTRUCTION AND PULMONARY INFLAMMATION IN CLINICALLY LATENT RESPIRATORY INFECTION WITH CHLAMYDIAE IN CALVES

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Aim: In symptom-free calves, chlamydiae can be found quite regularly in samples obtained from the respiratory system. Whether this finding is related to functional and/or pathological changes within the respiratory tract has yet to be defined. This study evaluated the influence of chlamydial infections on pulmonary functions in conventionally raised calves.

Animals and Methods: Twenty five calves aged 20 ± 5 days (mean ± SD) were included. Group I (n = 12) was without any history of chlamydial infections, while Group II (n = 13) resulted from farms with known chlamydia-associated health problems. Multiple bacteriological, serological and PCR analyses confirmed that both groups differed significantly with respect to infection by *Chlamydiaceae*, but not for other confounding infections. All animals were examined between 2^{nd} – 7^{th} month of life clinically. In each animal, lung function was evaluated using the impulse oscillometry system (IOS). Twice per month, variables of ventilation (respiratory rate, tidal volume, minute volume) and respiratory mechanics (airway resistance, respiratory reactance) were measured. At the end of the study, broncho-alveolar lavage fluid (BALF) was obtained and lungs were examined histologically. Statistical analyses were conducted to clarify whether the presence of *Chlamydiaceae* had any significant influence on lung function and/or markers of inflammation in BALF.

Results: *Chlamydophila abortus* and *Chlamydophila pecorum* were the predominant chlamydial species found in nasal, faecal, and conjunctival swabs of Group II. Although there was no clinical illness in any group, mean rectal temperature was higher and average body weight was lower in calves of Group II compared to calves of Group I. In addition, pulmonary dysfunctions were observed in calves of Group II that were characterized by a significantly higher respiratory resistance in the frequency range 1 to 10 Hz (indicating peripheral airway obstruction) and significantly higher respiratory rates. In BALF samples of Group II, significantly higher concentrations of total protein and 8-iso-prostane (8-IP) as well as higher activities of matrix metalloprotease 2 (MMP 2) were measured. Histologically, markedly activated BALT that caused partial obstruction of bronchiolar lumina was found in the apical pulmonary lobes of calves of Group II.

In Conclusion, respiratory chlamydial infections appear to play a role in chronic inflammation of lung and airways. Despite changes noted on a sub-clinical level, pulmonary dysfunctions persisted in calves until the age of 7 months. Data obtained in this study provide new pathogenetic information about the impact of ubiquitous subclinical infection on the respiratory system.

THE INTRIGUING QUESTION OF CLINICALLY SILENT *CHLAMYDIA* INFECTIONS IN FARM ANIMALS

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Classical investigations on animal diseases induced by intracellular bacteria of the order *Chlamydiales* have revealed severe, but rare, disease manifestations such as pneumonia, enteritis, polyarthritis, sporadic encephalomyelitis, and abortion and fertility disorders (1, 2). Early investigators understood well that these worldwide sporadic appearances of acute diseases represented only the "tip of the iceberg" of a ubiquitous distribution of asymptomatic infections with these agents, and that only specific circumstances precipitated the clinical manifestation of disease (1). Later, the prevailing view was that animal chlamydial infection were a curiosity, significant as rare zoonosis, but not for animal health and production. Recent investigations using modern sensitive ELISA and PCR techniques, however, show a different picture of chlamydial infections in livestock. High seroprevalence, approaching 100%, and chlamydial genomic DNA prevalence as high as 50-70% is routinely found in many livestock species (3, 4, 5). Veterinary chlamydiologists are now faced with a high frequency of detectable chlamydial infections, but a lack of significant clinical disease manifestations. Demonstration of health effects of these widespread, low-level endemic chlamydial infections is a challenge. Some of the most interesting advances in veterinary chlamydiology come from production medicine studies in cattle that address the subtle health effects of these chlamydial infections.

Epidemiology of asymptomatic bovine chlamydial infections. Jee *at al.* (6) studied acquisition and prevalence of chlamydial infection in calves for a 12-week time period post-partum. This study showed that calves were born free of chlamydiae, but started to acquire both *Chlamydophila* (*Cp.*) *abortus* and *Cp. pecorum* infections within 2 weeks post partum. The contact group size of calves at any given time point in the study correlated in quadratic regression with chlamydial detection such that doubling of the contact group resulted in a four-fold increase in infection frequency and intensity. This investigation demonstrated the profound influence of population density (crowding) on prevalence and intensity of animal chlamydial infections.

Bovine fertility. DeGraves *et al*. (7) investigated the effects of controlled re-infection on the fertility of cattle naturally pre-exposed to *Cp. abortus*. Twenty virgin heifers were estrus synchronized with prostaglandin F2, artificially inseminated 2-3 days later, and challenged immediately by intra-uterine administration of 0, 10^4, 10^5, 10^6, or 10^8 inclusion forming units (IFU) of *Cp. abortus*. Ten heifers were estrus-synchronized, inseminated, and uterine-challenged 2 weeks later. These animals were also indirectly exposed to *Cp. abortus* infection (cohort challenged) by contact with their previously challenged cohorts. Pregnancy was determined by rectal palpation 42 days after insemination. All animal had prior serum antibodies against *Cp. abortus*, but showed no signs of clinical disease. One hundred percent, 83%, 50%, 66%, and 0% of heifers were pregnant after uterine challenge with 0, 10^4, 10^5, 10^6, or 10^8 IFU of *Cp. abortus*, respectively. Fifty percent and 65% of heifers were pregnant with or without cohort challenge, respectively. Uterine inoculum dose and cohort challenge, or alternatively a negative pregnancy outcome (infertility),

correlated highly significantly with a rise in post-challenge over pre-challenge anti-*Cp. abortus* IgM. Logistic regression significantly modeled that the uterine *C. abortus* inoculum causing infertility is 8.5-fold higher for heifers without cohort exposure and 17-fold higher for heifers with high IgM than for heifers with cohort exposure or with low IgM. This investigation demonstrated that an asymptomatic, circulating, non-sexually transmitted herd infection by *Cp. abortus* has a profound influence on the fertility of cattle bred at this time.

Chlamydial infection of the bovine mammary gland. Another approach at analyzing the effect of clinically inapparent chlamydial infections in cattle was taken by Uhe *et al.* (8). Mastitis is the economically most important disease in animal agriculture, affecting both milk quantity and quality. Most cases of mastitis in dairy cattle are clinically inapparent, and typical mastitis pathogens such as *Streptococcus agalactiae* are detected only in a fraction of the cases. Subclinical mastitis is nevertheless of major interest to "production medicine" because of the large impact on profit margins of dairy farms. Infections with *Cp. abortus* and *Cp. pecorum* are ubiquitous in cattle, and have been experimentally and clinically associated with bovine mastitis. In a prospective cohort study in a herd of 140 Holstein dairy cows they examined the influence of chlamydial infection detected by PCR on subclinical inflammation of the bovine mammary gland as characterized by elevated somatic cell counts (SCC) in milk. SCCs are a sensitive quantitative indicator of inflammation, and 10^5 somatic cells per ml milk are considered the upper limit for a healthy bovine mammary gland. All cows had serum antibodies against *Chlamydia*, and 49% of the cows were positive for *Cp. abortus* on day 0 on at least one PCR of a conjunctival or vaginal swab from day 0 of the experiment. *Chlamydia* infection and below-median anti-chlamydial serum antibody levels significantly associated with bovine subclincal mastitis in this investigation. An intervention approach by perturbation of the immune response to *Cp. abortus/Cp.pecorum* was used to further examine induction, and immune-mediated reduction, of mastitis caused by chlamydial infection. All dairy cows had established immunity to chlamydiae, and serologically- and/or PCR-demonstrated chlamydial infection. They received two doses of an inactivated Alum-Quil-A-based vaccine of *Cp. abortus/Cp. pecorum* elementary bodies (therapeutic vaccination) or a mock vaccine on days 0 and 35 of the investigation. This vaccination highly significantly reduced milk SCC (***Figure 1***), thus reduced bovine mastitis, and increased anti-chlamydial antibody levels, but did not reduce shedding of *Chlamydia* bacteria. *Chlamydia* vaccination also resulted in improved relative body condition of dairy cows after 10 weeks. The disease-protective effect was maximal 10 weeks after vaccination, and lasted for additional 4 weeks. This investigation demonstrated an etiological involvement of the ubiquitous chlamydial infections in bovine mastitis, a herd disease of critical importance for the dairy industry. Furthermore, it shows the potential for transient improvement of chlamydial disease by therapeutic vaccination.

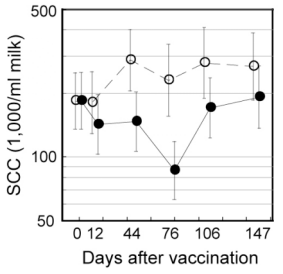

Figure 1. **Effect of *Chlamydia* vaccination on milk somatic cell counts (SCC).** ● *Chlamydia* vaccine, ○ mock vaccine, data were normalized for identical day-0 means of *Chlamydia*- and mock-vaccinated animals; means ± 95% confidence interval. *Chlamydia*-vaccinated cows (vaccine on days 0 and 35) have significantly lower milk SCC than mock-vaccinated cows ($p = 0.007$ for all combined time points after day 0, repeated measures ANOVA).

Respiratory chlamydial infections in calves. Reinhold *et al.* (9) studied the consequences of naturally acquired lung infections in calves infected with *Cp. abortus* and/or *Cp. pecorum* using impulse oscillometry. These clinically asymptomatic infections associated with significantly increased airway resistance as compared to calves without

PCR-detectable chlamydial infection. Therefore, sensitive lung function testing revealed that clinically asymptomatic chlamydial infection impedes lung function.

Mouse model respiratory *Cp. pneumoniae* infection. Murine model systems are well suited for examination of disease mechanisms. Wang *et al.* (10) analyzed how factors such as genetic background, immune status, or dietary protein affect disease outcome in a mouse model of chlamydial pneumonia. They found that the low-protein diet induced severe disease in C57BL/6 mice immune to *Cp. pneumoniae*, but not in immune A/J mice or in naïve mice of both strains. Transcript concentrations of immune- and inflammation-related genes were analyzed in infected lung tissue on day 3 after inoculation in order to identify potential mechanisms that precipitated exacerbated disease on day 10 in this murine model of chlamydial lung infection. Immune C57BL/6 mice on a low-protein diet showed a day-3 post challenge transcription profile of a suppressed Th1 immune and inflammatory response. In contrast, A/J mice on low-protein diet or C57BL/6 mice on high protein diet, both with low day-10 disease, showed an enhanced day-3 Th1 response. In confirmatory experiments that assessed functional aspects of Th1 immunity to the *Cp. pneumoniae* infection, disease prone C57BL/6 mice on the low-protein diet again showed highly reduced delayed-type hypersensitivity, lung lymphocyte and T cell numbers, and IFN-γ-secreting lung lymphocytes. Thus, in two comparative systems, a suppressed early Th1 immune response to the *Cp. pneumoniae* infection was associated with severe disease outcome. This investigation demonstrated the host-genetic and dietary protein restriction of disease outcome after chlamydial infection.

Conclusions. Recent data from epidemiological surveys indicate that chlamydial infection of farm animals is the rule rather than the exception. PCR detection typically indicates low numbers of the organisms, and the vast majority of these infections are without obvious clinical symptoms, suggesting endemic infections. Only if epidemiological risk factors coincide, such as a high-density host population, do these infections build up to become clinically manifest. Mouse model studies demonstrate that host genetics and nutrition strongly influence the disease outcome of chlamydial infection, thus most likely are also of epidemiological importance. Emerging data indicate that the asymptomatic chlamydial infections cause minor inflammatory reactions. These inapparent infections affect in subtle ways virtually every member of a farm animal population, and therefore are probably economically more important than severe chlamydial diseases.

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PATHOGENESIS OF PULMONARY INFECTIONS WITH *PASTEURELLACEAE*

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Pneumonia occurs in numerous domestic animals due to infection with Gram-negative bacteria of the family *Pasteurellaceae.* The represented genera include *Pasteurella, Haemophilus, Actinobacillus,* and *Mannheimia*. Many of these bacteria are normal inhabitants of the nasopharyngeal mucosa that proliferate during stress and are inhaled into the lungs where they induce pneumonia. These bacteria produce virulence factors that allow them to establish infection and induce disease. The purpose of this presentation is to review some of the common and unique virulence factors that are present in pathogenically important members of this family and to review how those factors interact with the host to cause pneumonia. *Mannheimia haemolytica*-induced cattle pneumonia (Shipping Fever) will serve as the prototypic *Pasteurellaceae*-induced pneumonia in this presentation.

Important respiratory pathogens of *Pasteurellaceae*

BACTERIA	**DISEASE**	**ANIMAL SPECIES**
Actinobacillus pleuropneumoniae	Contagious pleuropneumonia	Swine
Pasteurella multocida	Enzootic pneumonia	Dairy Cattle
	Shipping Fever	Cattle
	"Snuffles"	Rabbits
	Fowl Cholera	Birds
	Bronchopneumonia (Serogroup A)	Swine
	Atrophic Rhinitis (Serogroup D)	Swine
Pasteurella trehalosi (formerly *P. haemolytica* T biotype)	Acute septicemia and pneumonia	Domestic & Bighorn Sheep
Mannheimia haemolytica (formerly *Pasteurella haemolytica* A Biotype)	Shipping Fever	Cattle, sheep, & goats
Histophilus somni (formerly *Haemophilus somnus*)	Shipping Fever	Cattle
Haemophilus influenzae	Bronchopneumonia (Community-acquired)	Humans

BACTERIAL VIRULENCE FACTORS

Phylogenetic similarities exist among these bacteria, and several virulence factors produced by them have marked similarities in both structure and function. Several important or potentially important factors are described below.

Lipopolysaccharide/lipooligosaccharide - Lipopolysaccharide (LPS) is a complex structure on the surface of all Gram-negative bacteria. The polysaccharide or oligosaccharide side chains are surface exposed and highly antigenic, whereas the lipid A

portion of the molecule, which is buried in the outer membrane, is responsible for endotoxic activity and important in the pathogenesis of respiratory and systemic disease. LPS binds to CD14 molecules on numerous cell membranes and stimulates the release of pro-inflammatory cytokines, prostanoids, and leukotrienes, thus resulting in intense inflammation.

RTX toxins - RTX toxins, named for their sequence repeats in the structural protein (Repeats in ToXin) are members of a family of pore-forming cytotoxins found in many Gram-negative bacteria. RTX toxins have been identified in *M. haemolytica, P. trehalosi,* and *A. pleuropneumoniae*. In vivo, RTX toxins often act on host leukocytes by inducing inflammatory mediators or exerting cytotoxic and cytolytic affects causing inflammation and cell death by apoptosis or necrosis.

Adhesin molecules - Adhesin molecules are surface exposed and allow the bacterium to adhere to mucosal surfaces. Numerous *Pasteurellaceae* adhesin molecules have been described with substantial variations among the bacteria. The polysaccharide capsule, LPS, various outer membrane proteins, and pili have all been described.

Excreted proteases – Within the culture media of several of the *Pasteurellaceae,* several proteases can be found that are specific for host cell proteins. These include sialoglycoprotease, neuraminidase, and IgG1-specific protease. Their actual role in causing lung disease has not been clearly demonstrated although there is evidence that they are produced *in vivo* during infection.

Immunoglobulin-binding protein (IBP) – IBPs have been best characterized in virulent (serum-resistant) strains of *H. somni.* These appear to bind the Fc portion of immunoglobulin possibly leading to steric hindrance of bacterial-specific antibodies. Sequence analyses of other members of *Pasteurellaceae* suggest that similar proteins may be present.

Iron-regulated outer membrane proteins (IROMPs) – For bacterial replication and virulence to occur, a source of iron is needed by the bacteria during infection. In the mammalian host, free iron is not readily available; therefore, bacteria have developed elaborate systems to extract iron from hemoglobin, transferrin, and lactoferrin. In iron-restricted conditions, bacteria upregulate genes that produce receptor surface proteins that bind hemoglobin or transferrin, thus allowing extraction and internalization of iron. Some of the *Pasteurellaceae* have also developed siderophore systems, whereas small siderophore molecules are released into the milieu and scavenge iron, which is then internalized through a complex system.

Polysaccharide capsule – *M. haemolytica, P. multocida*, *P. trehalosi, A. pleuropneumoniae,* and *H. influenzae* produce thick polysaccharide capsules that coat the surface of the bacteria. Encapsulated strains tend to be more virulent than unencapsulated strains, because they resist phagocytosis and killing by leukocytes.

HOST RESPONSES

Within the respiratory system, combinations of bacterial virulence factors tend to incite the release of proinflammatory cytokines, such as TNFα and IL-1, leukotrienes, and histamine. In addition, toxins enhance platelet aggregation, modify leukocyte function, and stimulate release of oxygen-free radicals. The end result is fluid, fibrin, and leukocyte exudation as well as thrombosis and necrosis. The end result is often consolidation of lungs with varying degrees of non-reparable damage to the pulmonary parenchyma.

LUNG INJURY CAUSED BY *MYCOPLASMA BOVIS* IN CATTLE: CURRENT KNOWLEDGE ON PATHOGENESIS

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Introduction

Mycoplasmas are unusual bacteria and are distinguished phenotypically from other bacteria by their minute size and total lack of a cell wall. Today, more than 100 species of the genus *Mycoplasma* are known. In general, mycoplasmas display a strict host and tissue specificity with a predilection for the respiratory and urogenital tract, the mammary gland and the joints (13). In man, *M. pneumoniae* is a well-recognised pulmonary pathogen inducing atypical pneumonia and other respiratory syndromes or fulminant pneumonia. There are several *Mycoplasma* species, which can cause acute and chronic diseases of the respiratory tract in domestic (farm) and laboratory animals (14). *M. pneumoniae* infection in hamsters and *M. pulmonis* infection in mice and rats are useful animal models of human respiratory mycoplasmal disease with clinical and pathological features resembling those in human respiratory mycoplasma disease. In domestic farm animals, respiratory mycoplasmoses of economic importance occur in ruminants and pigs (14). *M. mycoides* subsp. *mycoides* small colony (SC) variant causes contagious bovine pleuropneumonia (CBPP), one of the most serious and economically most costly diseases in cattle with fatal outcome (14).

M. bovis is the second most pathogenic of the mycoplasma species isolated from cattle and infections have been reported throughout the world including most European countries. *M. bovis* is not only a major causative agent of pneumonia, but also of mastitis and arthritis in cattle (11). As many other *Mycoplasma* species, *M. bovis* often establishes chronic infections and infected cattle shed the organism via the respiratory tract for months or even years acting as reservoirs of infection. Histopathological examinations in calves naturally or experimentally infected with *M. bovis* reveal a spectrum of pulmonary lesions (4, 12).

Pathogenetic mechanisms

The virulence factors of *M. bovis* and mechanisms of pathogenicity are still incompletely understood. During the recent years, we had the opportunity to analyse the lung lesions developing in calves after intratracheal or aerosol infection with different *M. bovis* strains originating from various infection experiments, which have been carried out at different research institutions in Europe. Our studies reveal certain disease characteristics, from which conclusions concerning the possible pathogenetic mechanisms of acute and chronic pulmonary *M. bovis* disease can be drawn.

The most consistent feature during the first 2 to 7 days *post infection* (p.i.) is influx of neutrophils into the alveolar spaces and interalveolar septa, which is accompanied by increased numbers of alveolar macrophages. These findings resemble those reported for mycoplasma respiratory disease in rodents. Most likely, recruitment of mononuclear cells is mediated by certain β-chemokines, which are produced in the lungs of mice with *M. pulmonis* disease (15). Recruitment of neutrophils into lungs is a characteristic feature found in several pulmonary bacterial infections in man and animals. Neutrophils are thought to induce lung lesions by releasing oxygen radicals and proteolytic enzymes (9). During subacute to chronic stages (14-21 days p.i.) of *M. bovis* infection, different types of

lung lesions occur. Like in the acute postinfectious phase, accumulations of large numbers of macrophages are present and there is increasing proliferation of peribronchial lymphatic tissue. Often, suppurative bronchopneumonia occurs and in most of these cases, beside *M. bovis,* other bacteria can be isolated, which are thought to have a synergistic effect. The most severe lesion developing during chronic postinfectious stages is multifocal necrosis of lung tissue accompanied by severe fibrosis. The possible mechanisms by which *M. bovis* causes these severe necrotising lung lesions are not understood. Necrotic lesions are sometimes accompanied by obliterative bronchiolitis resulting from destruction of airway epithelium. Our findings in lungs from calves originating from different infection experiments strongly support the view of other investigators that certain agent-related factors (virulence and/or dose of the *M. bovis* strains involved), environmental factors (stress from overcrowding and transportation, poor ventilation in stables) and secondary bacterial infections influence the outcome of *M. bovis* infections.

Persistence of *M. bovis* variable antigens and organisms in the host

In vitro studies have shown that *Mycoplasma (M.) bovis* has two surface antigenic variation systems: a family of variable surface lipoproteins (Vsps) and an unrelated membrane surface protein (pMB67) (1, 2). *In vitro*, these antigens show high-frequency phase and size variations. Immunohistochemical studies with monoclonal antibodies to these variable surface antigens in lungs from calves necropsied at 21 days p.i. revealed that their expression occurs also *in vivo* and that they are widely distributed in infected lungs (5). Examination of lungs from calves at 21 days p.i. by *in situ* hybridization using Vsp-specific probes reveals persistence of *M. bovis* DNA, which can be found in over 90% of necrotic lung areas and also in other locations (7). These findings suggest that, most likely because of continuous antigen variation, the host is unable to eliminate the infectious agent and that this may enhance evasion of the immune defence systems of the host.

Interaction of *M. bovis* with the immune system of the host

Examinations of sera from infected animals with pneumonia show that the Vsp antigens are the predominant antigens recognised by the humoral immune response and that Vsp specific host antibodies can be detected for up to several months p.i. in infected animals (3). Serological and immunohistochemical analyses in experimentally infected cattle show that *M. bovis* stimulates increased production of antigen-specific IgG1 whilst only small amounts of IgG2 are produced (6, 17). These findings indicate that the infection of cattle with *M. bovis* results in a Th2-skewed immune response and that misregulation of Th2 cytokines may lead to lung pathology. Since IgG2, in comparison to IgG1, is the superior opsonin, the low IgG2 response may contribute to the chronicity of *M. bovis* infection (17). In pneumonic lungs, already beginning at 10 days p.i., a significant and continuous BALT proliferation develops. Immunohistochemical examinations show that mainly CD4 positive T lymphocytes are present, although lymphocyte activation studies reveal that not only CD4, but also CD8 and γδ-T cells are activated (6, 17). Beside neutrophils and lymphocytes, pulmonary alveolar macrophages (AM) participate in the inflammatory process during the acute and subacute postinfectious stages of *M. bovis* respiratory disease. Pulmonary AM, by their phagocytic capacity can exert potent antimicrobial effects and are known to be sources of several pro-inflammatory cytokines such as TNF-α, IFN-γ, IL-1 and IL-8 (8). Immunohistochemical studies in lungs of calves with chronic pulmonary lesions after experimental *M. bovis* infection reveal that there is a significant increase of MHC class II expressing DCs in the mucosa of bronchi and bronchioli (16). Airway DCs are specialised for antigen presentation and activation of T lymphocytes and, after recruitment into airway mucosa, are known to participate in the immunoregulation and pathogenesis of acute and chronic infectious airway diseases (8, 10).

Conclusions

The most consistent characteristic of *M. bovis* respiratory disease in calves during early postinfectious stages (2-7 days p.i.) is influx of numerous inflammatory cells, i.e. neutrophils, macrophages and lymphocytes. Further studies are needed to elucidate the role of pro-inflammatory cytokines, β-chemokines and other factors potentially released by these inflammatory cells and their possible involvement in the pathogenesis of pneumonic lesions. In calves with chronic pulmonary disease, both *M. bovis* Vsp antigens and DNA persist in the inflamed tissue despite specific humoral and cellular immune responses of the host. Several studies suggest that, because of continuous antigen variation and low IgG2 response the host is unable to eliminate the infectious agent and that this contributes to the chronicity of the disease.

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DOES BREATHING COLD AIR GIVE YOU THE FLU? EFFECTS OF COLD AIR ON AIRWAY FUNCTION

Michael S. Davis

Ambient Temperature, Minute Ventilation, and Conditioning of Inspired Air

Except for tropical climates, inspired air is usually cooler than body temperature and relative humidity of the air is less than 100%. As a result, thermal and water vapor gradients are present between the inspired air and the moist surfaces of the respiratory mucosa. Heat and water are transferred from the airway mucosa to the inspired air, with subsequent cooling of the mucosal surface. Transfer of heat and water vapor from the respiratory mucosa to the inspired air is relatively rapid, and thus the process is usually completed by the time the inspired air reaches the lower airways. However, humidification of the air is limited by the warming of the air, and kinetics of heat transfer are sufficiently slow as to allow unconditioned air (air that has not been completely warmed and humidified) to enter the lower respiratory tract under conditions of increased ventilation, decreased inspired air temperature, or a combination of the two conditions. The subsequent cooling of the airways is a result of heat loss through warming of the air and, more importantly, the vaporization of water during humidification. To raise the temperature of 1 liter of air from 0^{o}C to 37^{o}C requires 110 calories of heat, and to fully humidify the same volume of air (assuming an initial relative humidity of 0% and a final temperature of 37^{o}C) requires 270 calories of heat. In fact, even if the inspired air were fully humidified at 0^{o}C, it would still require 230 calories to reestablish 100% humidity once the air was warmed to body temperature. Furthermore, completely humidified air at body temperature contains approximately 44 mg of water per liter of air, thus with a minute ventilation of approximately 60 l/min (during exercise, for example), the respiratory mucosa must contribute up to 2600 mg/min of water if inspired air was completely dry (regardless of inspired temperature). The direction of heat and water transfer reverses during exhalation as fully humidified air passes over cooled airway mucosa during exhalation. The efficiency of the airways to recover the heat and water transferred to the inspired air is not high, and it is estimated that approximately 50% of the respiratory heat transferred during inhalation is lost (29). It is probable that a larger percentage of the water transferred is lost, since the relative humidity of the expired air is typically higher than the inspired air. Thus, a considerable amount of heat and water is lost from the surface of the airways while breathing cold air, resulting in airway cooling and desiccation.

Heat and water loss from human intrapulmonary airways has been measured during strenuous exercise while breathing air chilled to -19^{o}C (18; 30), with central airways cooling to 33^{o}C. More proximal airways experience a greater magnitude of cooling. Similar magnitudes of airway cooling have been measured in horses under less severe conditions (4^{o}C inspired air, moderate exercise) (8), and changes in airway barrier function consistent with airway cooling have also been reported in cattle being shipped during cold weather (10). Similar findings of airway mucosal damage secondary to airway cooling and drying have been reported in normal animals, demonstrating the conserved nature of this response (8; 17). Larger animals (i.e., horses, cattle, and humans) are likely more susceptible to airway cooling during exercise due to their low conducting airway surface area (proportional to body surface area) relative to the volume of inspired air (proportional to body mass), resulting in high demand for heat and water transfer to the inspired air per unit of conducting airway surface.

Deleterious Effects of Pulmonary Airway Cooling and Desiccation

Airway cooling and desiccation triggers a variety of responses in both normal subjects and subjects with pre-existing airway disease, including mucosal damage, airway obstruction, and induction of inflammation. The most commonly studied phenomenon is Exercise Induced Asthma (EIA), a syndrome in humans characterized by acute bronchoconstriction after strenuous exercise. The acute phase bronchoconstriction in response to airway cooling and drying is typically self-limiting, but there is evidence that subjects concurrently experience airway mucosal injury and induction of inflammation (cold air-induced airway injury) (6). Crimi et al (7) reported increased numbers of airway epithelial cells in sputum from subjects with EIA after exercise, a finding recently confirmed by Hallstrand et al (20; 21). Morici has demonstrated a similar occurrence in nonasthmatic rowers after maximal exercise (31), suggesting that although subjects with pre-existing disease appear to have more fragile airway mucosa, the airway epithelium is susceptible to damage by hyperventilation even in the absence of disease. These findings were confirmed in 6 Thoroughbred horses in race training. Bronchoalveolar lavage was performed at rest and within 2 hr after a routine workout. The conditions of the workout were similar to that described in the above study documenting airway cooling: inspired air of approximately 4^{o}C and exercise intensity of approximately 75% of maximum capacity. The sequence of these lavages was random, and the paired lavages for a given horse were collected at least 2 weeks apart. Analysis of bronchoalveolar lavage fluid (BALF) cell profiles revealed that the percentages of airway epithelial cells recovered from equine peripheral airways were increased immediately after strenuous exercise (8). As increased airway epithelial cell recovery in BALF is strongly associated in other species with airway damage and dysfunction after challenge with unconditioned air (16), this study demonstrated not only the failure of upper airway conditioning of inspired air, but also that both the stimulus and initial response was similar between humans and horses.

The fidelity between the equine and human responses to exercise while breathing cold air extend to alterations in airway mechanical properties and airway leukocyte populations. Exercise while breathing cold air reliably produces bronchoconstriction in human subjects with pre-existing airway hyperreactivity, a response termed hyperpnea-induced bronchoconstriction. This response is typically transient, and will spontaneously resolve within 30-60 min. The occurrence of a second phase of bronchoconstriction (termed a late phase response due to its similarity to the late-phase response to allergen exposure in subjects with allergic asthma) is less reliably detected in humans, due to variability in timing of the response, interference by diurnal variation, and the difficulty in restricting potentially confounding medications for a prolonged period. Nevertheless, carefully controlled studies have shown that the late fall in 1-second forced expiratory volume (FEV_1, an indicator of airway obstruction) after exercise was greater than the spontaneous decay of lung function at the corresponding clocktime on the non-exercise control day in subjects with pre-existing asthma (6). In the analogous equine studies, horses were exercised while breathing either warm air (as a control) or chilled air (-5^{o}C) in random order, with a 2 week washout between challenges. Pulmonary function was measured using impulse oscillometry in unsedated horses before exercise, and 5 hr, 24 hr, and 48 hr after exercise. Breathing cold air during a single exercise challenge resulted in significant peripheral (3 Hz) and central (5 Hz) airway obstruction compared to the identical exercise challenge while breathing warm air. The obstruction was slow to develop, finally reaching statistical significance 48 hr after the exercise challenge.

Numerous studies have also demonstrated the pro-inflammatory effects of exercise on airways and, like the evidence of bronchoconstriction and mucosal injury, the airway pro-inflammatory effects of exercise are not limited to subjects with pre-existing disease.

Exercise causes an increase in plasma neutrophil chemotactic activity (27) and increased concentration of neutrophils in airway sputum (20; 21) in asthmatic subjects, as well as nonasthmatic subjects (2; 3). Alterations of equine intrapulmonary cell populations and cytokine production were examined using bronchoalveolar lavage fluid recovered 5, 24, and 48 hr post-exercise. Cytological changes 5 hr after challenge were limited to an increase in airway epithelial cells, similar to our previous study in horses as well as published studies in dogs and humans. There was a modest, but statistically significant increase in neutrophil concentration 24 hr after exercise while breathing cold air (3.3% vs 1.9 % in BALF from horses breathing warm air during exercise), as well as a trend towards increased mononuclear giant cells in the BALF. No differences were found in cellular components of the BALF 48 hr after exercise. These findings correspond well with data from humans exposed to airway cooling and desiccation during exercise, further reinforcing our belief that pulmonary airway cooling and desiccation, and the responses to this stimulus, are shared across large mammals.

Cold Air, Hyperpnea, and Respiratory Immunosuppression

Although it is clear that strenuous exercise can predispose the subjects to infection (34), it is less clear why the predominant location of infection is the respiratory tract (33; 35; 36). Certainly the respiratory tract is an important interface with the environment, and a weakened immune system is likely to be challenged at the respiratory surface. However, the gastrointestinal tract is constantly exposed to potential pathogens, yet there is a paucity of reports in the literature on increased gastrointestinal infections secondary to strenuous exercise. What causes the respiratory tract to be the weakest point? Recent evidence suggests that the hyperpnea of exercise (and the resulting airway cooling and desiccation) is the key element in the preferential weakening of respiratory immune capacity following exercise, and this phenomenon may not be exclusive to exercise. Airway cooling and desiccation is believed to produce local hyperosmolarity, and thus cells found locally that are osmotically sensitive are likely to be stimulated. Both mast cells (11; 12) and airway epithelial cells (22) are osmotically-sensitive, and are activated by local airway hyperosmolarity that may occur during exercise while breathing cold air. Mast cells are rich sources of cytokines (4; 5), and although there are no studies specifically detailing cytokine production by osmotically activated mast cells, it has been previously shown that mast cells degranulate in response to hyperosmolar stimuli both *in vitro* (11; 12) and *in vivo* (17; 37). Futhermore, mast cell products are increased in blood after strenuous exercise while breathing cold air (25; 26), and drugs that inhibit mast cells are useful in blocking exercise-induced bronchoconstriction (13-15). Mast cells are an important source of T_H2 cytokines (IL-4, IL-5, and IL-10), and recent studies in our laboratory have identified preferential upregulation of T_H2 cytokines after exercise while breathing cold air (9). Thus, it is likely that mast cell activation is a key cellular event leading from airway cooling and desiccation to the local expression of T_H2 cytokines.

It is possible that locally enhanced production of T_H2 cytokines (in particular increased expression of IL-10 and suppression of interferon-gamma (IFNγ)) is responsible for increased susceptibility to respiratory viruses seen in athletes and people who perform strenuous exercise. Viral infection of airways in naïve subjects begins with entry of virus into susceptible cells, typically airway epithelial cells but also potentially resident macrophages as in the case of influenza (23). All infected cells produce Type 1 interferons (interferon alpha and beta, IFN-α and IFN-β respectively) as a key first step in the efforts to eliminate the infection. Type 1 interferons have two basic anti-viral functions: Impair the capacity of the virus to replicate within infected cells (through direct inhibition of viral replication or by increasing the likelihood of detection by NK cells through increased Major Histocompatibility Complex expression); and upregulate the activation state of the

resident leukocyte populations (19). Knock-out mice in which the Type 1 interferon signaling system has been eliminated are highly susceptible to viruses compared to normal controls (32), demonstrating the importance of these cytokines on resistance to viral infection.

Macrophages are a primary effector cell for Type 1 interferons in addition to being an important source of the cytokines. Of the airway cells typically infected by viruses, epithelial cells are relatively poor producers of Type 1 interferons. However, macrophages produce large amounts of Type 1 interferons (24), and macrophage production of these compounds is substantially increased by exposure to Type 1 interferons, both in an autocrine and paracrine manner (1). Type 1 interferons promote monocyte differentiation, increase antigen presentation capabilities, and increase antibody-dependent cytotoxicity activity (1). Interferon alpha increases the release of IL-1, -2, -6, -8, tumor necrosis factor (TNF) and interferon gamma (39). These cytokines in turn play important roles in amplification and maturation of the immune response to the viral invader, including activation of NK cells, maturation and activation of viral-specific cytotoxic T-lymphocytes, and recruitment of more macrophages. Thus, macrophages play multiple critical roles in amplifying the initial anti-viral response in the airways.

Interleukin 10 (IL-10) is an immunomodulatory cytokine with diverse and sometimes contradictory functions. IL-10 is a potent inhibitor of monocyte/macrophage activation. IL-10 is produced by apoptotic cells to reduce macrophage response to cellular debris, as well as by T_H2 lymphocytes to inhibit macrophage-driven differentiation of T-lymphcytes to the T_H1 phenotype (28). Most importantly with regard to immunity, IL-10 reduces production of Type 1 interferons by monocytes in response to viral challenge (38; 40). In fact, a number of viral pathogens actually have analogs of their host species' IL-10 encoded in the viral genome, and expression of this IL-10 analog is a critical first step in producing persistent infection in cases of Epstein-Barr virus, human respiratory syncytial virus, and equine herpes virus Type 2. On the other hand, there are scattered reports of IL-10 expression being necessary for activation of natural killer cells and some animal experiments have suggested that in some cases, IL-10 is necessary to limit immune-mediated pathology due to exuberant cell-mediated immunity. Although such a role for IL-10 is appropriate in the face of aggressive and vigorous stimulation of the immune response, overexpression of IL-10 in the absence of amplified immune system activity is likely to result in immunosuppression.

Dramatic alterations in cytokine mRNA expression have been reported following exercise while breathing cold air in horses, with cytokines displaying variable temporal relationships to the cold air exercise. The initial response is best characterized as a T_H2 profile (increased IL-4, IL-5, and IL-10), which is the profile not only associated with preferential production of antibodies and downregulation of cell-mediated immunity but is also characteristic of asthma (41). A more generalized inflammatory profile (IL-1, IL-6, IL-8, and TNFα) occurs 24 hr after exercise with cold air, and helps explain the cellular changes (neutrophil influx, activation of alveolar macrophages) also found at that time. The T_H2 profile tended to disappear within 24 hr except for expression of IL-10 mRNA, which maintained 5-8-fold greater concentrations compared to control airways through 48 hr post-exercise challenge. Given that IL-10 is strongly linked to suppression of cell-mediated immunity and that cell-mediated immunity is critical for resistance to most respiratory viral infections (such as influenza), the persistent increase in IL-10 message raises the possibility that exposure of pulmonary airways to cold air could result in prolonged susceptibility to pulmonary infection. These data are consistent with recent investigations into the prevalence of infectious respiratory disease in humans exercising in cold conditions. Published reports have cited disease prevalence in military populations

ranging from 43% in personnel participating in a South American training exercise to 66% in French commando trainees. A recent study of U.S. Marines found an 80% prevalence of respiratory tract disease while undergoing training at the Marine Corps Mountain Warfare Training Center (MCMWTC) in Bridgeport, CA. Baseline prevalence of respiratory disease (<20%) was obtained while the same subjects trained at 29 Palms, CA. Furthermore, when the Marines were transferred out of the cold training environment at MCMWTC, the prevalence of respiratory disease returned to baseline values.

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