

PERSISTENT BLOOD-BORNE INFECTIONS AND COMPLEX DISEASE EXPRESSION

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INTRODUCTION

It is important for the clinician to remember that vector-transmitted bacteria and protozoa have a very long evolutionary history, which spans millions of years. Many vector-borne organisms, such as *Anaplasma*, *Babesia*, *Bartonella*, *Ehrlichia*, *Leishmania* and *Rickettsia* species have an intracellular and intravascular life style, which for certain organisms are obligate in nature.¹ Obviously, this means that the organism must reside within the cell of an arthropod, such as a louse, flea or tick, or within the cell of a mammalian host. As an example, *Rickettsia* has lost several critically important genes that are required for extracellular survival.² In the absence of these genes, *Rickettsia* species have developed mechanisms that allow intracellular survival by manipulating selected biochemical pathways within the mammalian cell. For the clinician, it is critical to recognize evolutionary adaptation has facilitated complex interactions between arthropod vectors, bacteria and protozoa and mammalian reservoir hosts.

BACTERIAL ADAPTATION AND COMPLEX DISEASE EXPRESSION

In some instances, disease expression occurs when a vector-borne bacteria is transmitted from a reservoir-adapted host such as a rodent to a non-reservoir host, such as dog or man.^{3,4}

Anaplasma phagocytophilum, *Borrelia burgdorferi*, and *Bartonella washoensis* are examples of organisms for which small mammals and rodents are seemingly healthy reservoirs, whereas transmission of these same organisms to dogs or humans can induce disease.^{3,4} In the case of *A. phagocytophilum*, cats, dogs, horses and human beings can all be infected following transmission by *Ixodes scapularis* or *Ixodes pacificus* in the northeastern, northcentral and western United States.^{5,6} Numerous wild and domestic animals also serve as reservoirs for specific host-adapted *Bartonella* species. Transmission to cats, dogs or humans can occur when a reservoir-adapted *Bartonella* species is introduced by a scratch, bite or arthropod vector into a non-reservoir host species.^{4,7} *Ehrlichia* and *Anaplasma* spp. inhibit apoptosis of monocytes and neutrophils respectively, so as to selectively prolong the natural life span of organism-infected cells.^{8,9}

Obviously, this ability to influence the rate of apoptosis would infer substantial benefit to a group of organisms that have the ability to persist within the host's immune cells for a prolonged period of time. Although as clinicians we perceive these organisms as pathogens that induce disease, bacteria and protozoa are merely attempting to protect and perpetuate their species through highly sophisticated adaptive mechanisms. These evolutionary relationships, as well as the requirement that the mammalian host serve as a reservoir for future generations of *Ehrlichia*, would suggest that it is not in the best interest of vector-transmitted organisms to induce immune recognition and disease (ehrlichiosis) in the host animal. In this context, disease develops when

the organism, the host, or perhaps the veterinarian (providing an antigenic challenge via vaccination or suppressing the immune system by administering corticosteroids) induces a state of immunological imbalance in the cat or dog that is persistently infected with one or more blood-borne organisms.

IMMUNE RECOGNITION AND DISEASE EXPRESSION

The development of disease in an animal infected with an obligate intracellular organism would seem to represent a strategic error on the part of the organism. Excessive immune recognition of an organism that can persist within erythrocytes (*B. canis* or *B. gibsoni*), the vascular endothelium (i.e. *Bartonella henselae*) or within circulating immune cells (i.e. *E. canis* in monocytes or *Anaplasma phagocytophilum* in granulocytes) would result in cellular damage, induction of auto-antibodies, excessive generation of immune complexes or elimination of the intravascular niche for the organism's perpetuation. In the context of infectious diseases, it has been suggested that circulating macrophages represent the "Trojan Horse" for a variety of intracellular pathogens.^{10,11} Once infected, monocytes disseminate organisms throughout the body. Differences in immune reactivity to various organisms also influences disease expression and severity.¹² In this regard, *R. rickettsii* frequently causes severe life-threatening disease in dogs and man, whereas other phylogenetically-related spotted group *Rickettsiae* such as *R. montana* or *R. rhipicephali* are not considered pathogenic in non-immunocompromised individuals.¹³ Following tick transmission, *Rickettsia rickettsii* is rapidly recognized by the innate, humoral and cell-mediated immune systems of the dog and man, resulting in an acute, febrile illness and rapid elimination of the rickettsiae, followed by long lasting (perhaps years) immunity.¹⁴ Regardless of the factors that induce alterations in immune recognition, the consequences in a chronically infected animal could include immune-mediated hemolytic anemia (babesiosis, bartonellosis), immune-mediated thrombocytopenia (anaplasmosis, babesiosis, bartonellosis, ehrlichiosis), vasoproliferative lesions such as peliosis hepatis secondary to *B. henselae* or glomerulonephritis secondary to immune complex deposition (borreliosis [Lyme nephropathy], ehrlichiosis, leishmaniasis).¹⁵⁻¹⁷

BLOOD AS A MICROBIAL ECOSYSTEM

Although counter intuitive, it is increasingly obvious that microorganisms, including *Anaplasma*, *Babesia*, *Bartonella*, *Borrelia*, *Chlamydia*, *Ehrlichia*, *Leishmania*, *Mycoplasma* species and retroviruses can persist in the blood or other tissues of animals for protracted periods of time (months to years). The same evolutionary adaptations that facilitate organism persistence also complicate clinical confirmation of disease causation. This is particularly true when assessing diagnostic test results for an individual patient residing in a highly endemic area for various blood borne infections. For example, *Ehrlichia canis* seroprevalence in *Rhipicephalus sanguineus* infested dogs from Texas can be extremely high (25-40%), whereas the *A. phagocytophilum* and *B. burgdorferi* seroprevalence in *I. scapularis* infested dogs from the north eastern or north central United States can approach 50-90% respectively.^{18,19} Using serology as a sole means of diagnosis could result in a substantial number of false positive diagnoses of ehrlichiosis, anaplasmosis or borreliosis in regions in which the tick vector and the organism are both highly prevalent and transmission to dogs is frequent and repeated. In many instances, the role of chronic and repeated infection with a spectrum blood-borne organisms is not

understood.²⁰

POLYMICROBIAL INFECTIONS AND COMPLEX DISEASE EXPRESSION

In most instances the influence of concurrent or sequential infection with multiple vector borne organisms on the host immune response is unknown. However, experimental infection of rodents with *B. burgdorferi* and *A. phagocytophilum* induces more severe immunopathology than infection with either organism alone.²¹⁻²² We have recently generated data to support an increased likelihood of disease in dogs co-infected with *B. burgdorferi* and *A. phagocytophilum*. Under natural exposure conditions, polymicrobial infection may be much more complex than an experimental infection in which two organisms are inoculated at a specific dose, to a specific inbred strain of mouse and administered at a single point in time. For example, while attempting to clarify an increase in unexplained deaths in a Walker hound kennel, we were able to amplify DNA of up to six different organisms (i.e. 6 different species from 4 different genera) from an EDTA-anti-coagulated blood sample, obtained at a single point in time.²³ PCR data from several dogs in this kennel identified complex patterns of co-infection with blood borne pathogens. The clinical implications of this study suggest that large breed hunting dogs with extensive tick, flea and louse exposure can be simultaneously infected (based upon detection of DNA) with multiple blood-borne organisms. Secondly, these working dogs appear to be “genetically” capable of compensating to a substantial degree for simultaneous blood-borne infections with bacteria, protozoa and rickettsiae. However, over half of the dogs in the kennel had ocular abnormalities, including anterior uveitis, ocular hemorrhage or retinitis, generally accompanied by thrombocytopenia, mild anemia and hyperglobulinemia.²³ Finally, age, sex, breed, nutritional status and variation in the type and number of blood borne organisms would influence the pattern of disease expression for each dog in this kennel. Presumably, these same factors complicate the clinical evaluation of individual cats and dogs presented to veterinarians on a daily basis which are infected with occult blood borne organisms.^{15,24-26} Although considered by the kennel owner to be functional working dogs for deer hunting, it is likely that these dogs were no longer in a state of immunological balance due to polymicrobial blood borne infection. If the limited spectrum of PCR testing utilized in our laboratory was able to detect six different species of bacteria or protozoa in a single dog, it is highly likely that other organisms were present within the blood ecosystem (kennel dogs) for which testing was not performed. Blood borne infections, such as retroviral infections (FIV and FeLV) in cats and *Dirofilaria immitis* infection in dogs are well recognized causes or co-factors in disease expression for which veterinarians screen on a routine basis in clinical practice. Although somewhat controversial, routine screening for tick borne infections may prove to be a rationale approach for the early detection and therapeutic elimination of blood borne organisms.

One possible conclusion, derived from recent research, is that the mammalian body is an ecosystem that encompasses a highly developed immune system that interacts constantly with microorganisms on the skin, mucosal surfaces and within blood and other tissues. Imbalance in the blood ecosystem of dogs with extensive vector exposure is rarely due to a singular factor or event and is rarely precipitated by a single microorganism. Blood borne infections can induce disease in many organ systems because blood and infected blood cells (erythrocytes, neutrophils and monocytes) are disseminated throughout the body on a constant basis.

IMMUNOCOMPETENCE AND DISEASE EXPRESSION

There are important variations in genetic backgrounds, age, sex, nutrition, sanitation, vector exposure, drug history and potentially unrecognized contact with environmental toxins that will affect immune balance and disease expression, even when several individuals are acutely exposed to a single pathogenic microorganism. There does appear to be senescence of the immune system with advancing age.^{27,28} Therefore, geriatric patients will have a different immune response to a blood-borne organism than younger, healthy individuals. In many instances, defects in immune responsiveness are subtle and cannot be quantified with current tests of “immune competence.” Infection with *B. henselae* provides an excellent contemporary illustration of the direct relationship between a microorganism, disease pathology and the immunocompetence of the host.^{4,29} Following *B. henselae* transmission by the scratch of a cat, most healthy individuals will not develop obvious signs of illness, or will develop only an inoculation granuloma or an inoculation granuloma and regional lymphadenopathy (cat scratch disease). Children with incompletely developed immune systems, adults with senescence of the immune system, and individuals receiving immunosuppressive drugs for systemic lupus erythematosus or following organ transplantation are more likely to develop granulomatous inflammation involving parenchymal organs, such as the liver, spleen or lung (granulomatous hepatitis, splenitis or pneumonitis).²⁹ However, people infected with HIV develop unusual pathologic lesions, such as bacillary angiomatosis and bacillary peliosis, which in most instances resolve following appropriate anti-microbial therapy for *B. henselae*.³⁰ Therefore a major factor that determines disease expression is the immune competence of the host, yet as mentioned previously, there are no readily available tests to effectively establish immune function in a dog or cat in a practice setting.

CHRONIC INFECTION AND DISEASE CORRELATIONS

Clinicians frequently make a distinction between infection, autoimmune, immune-mediated and neoplastic diseases. From a clinical perspective, dogs with endocarditis, ehrlichiosis, systemic lupus erythematosus and cancer can share similar or identical disease features and laboratory test results. As is the case for any clinical data generated by a clinician for a patient, there are multiple interpretations for each diagnostic parameter in a sick individual. However, the overlapping occurrence of these parameters in association with a somewhat localized infectious process (endocarditis), a systemic vector-borne infection (ehrlichiosis), an idiopathic immune-mediated disease (SLE) and a plasma cell cancer illustrates the potential diagnostic complexity that might be encountered in the clinical setting. It also indirectly supports the possibility that the arbitrary divisions among disease categories are less than clear. It is now well recognized that babesiosis should be a differential diagnosis for dogs with immune-mediated hemolytic anemia.³¹⁻³³ It also appears that *B. henselae* and *B. vinsonii* (*berkhoffii*) can serve as infectious causes of IMHA and ITP in dogs.^{34,35} Although considered a hallmark for SLE, anti-nuclear antibodies have been described in dogs' with endocarditis, ehrlichiosis, leishmaniasis, bartonellosis and heartworm disease.³⁶ Anti-platelet antibodies have also been demonstrated in association with both acute (Gram-negative sepsis and Rocky Mountain spotted fever) and chronic (ehrlichiosis and babesiosis) infectious diseases.¹⁷ “Idiopathic immune-mediated” polyarthritis can be documented in dogs with chronic infections such as *B. burgdorferi*, *B. henselae*, *B. vinsonii* subspecies *berkhoffii*, *E. ewingii*, or *L. infantum*.^{19,34,35,37-39} Due to the

relatively low percentage of dogs that develop polyarthritis or glomerulonephritis, when infected with any of these organisms, one might suspect that other factors, such as concurrent infection with other microorganisms (*Mycoplasma* spp. perhaps) should be considered in this subdivision of patients.⁴⁰⁻⁴¹

IMMUNOSUPPRESSIVE DRUG ADMINISTRATION AND BLOOD BORNE INFECTION

Corticosteroids and other immunosuppressive drugs are frequently administered to cats and dogs for the treatment of autoimmune or immune-mediated diseases. It is well recognized that the administration of corticosteroids or other immunosuppressive drugs can have devastating effects in animals with specific infections, such as histoplasmosis.⁴² However, few studies have addressed the influence of corticosteroids on the outcome of many infectious diseases. One exception to this statement is the use of high dose corticosteroids for the treatment of sepsis, where meta-analysis of human sepsis studies does not identify an increase in patient survival.⁴³ Needless to say, many dogs have died during experimental studies in an effort to prove efficacy for high dose corticosteroids for the management of sepsis in people. Currently, only physiological doses of corticosteroids are recommended for sepsis-induced hypoadrenalism.^{43,44} Obviously, it is comparatively easier to study the influence of immunosuppression on an acute infectious process, as compared to a chronic infectious process or a chronic polymicrobial blood-borne infectious disease. For example, administration of anti-inflammatory or immunosuppressive doses of prednisone in conjunction with doxycycline did not induce recognizable deleterious effects in dogs experimentally-infected with *R. rickettsii*.⁴⁵ This study was performed because our clinical experience suggested that dogs infected with *R. rickettsii* that received corticosteroids were more likely to develop gangrene. Although a deleterious effect was not found experimentally, this study does not prove that concurrent use of immunosuppression is warranted in dogs with Rocky Mountain spotted fever or that the use of corticosteroids will not result in an adverse outcome in selected individuals.

Of far greater complexity, the extent to which concurrent or sole use of immunosuppressive drugs influences the outcome of chronic, occult infections is unknown. It is currently our hypothesis that: "Clinicians unknowingly administer immunosuppressive drugs to animals with chronic infections in an effort to treat idiopathic immune-mediated diseases, such as IMHA, ITP and others." Support for this hypothesis has evolved from both clinical and research observations. Examples include finding *Ehrlichia ewingii* morulae in dogs with idiopathic, immune-mediated polyarthritis following immunosuppression,³⁸ isolation of *B. vinsonii berkhoffii* from a dog being immunosuppressed for a presumptive diagnosis of SLE,⁴⁶ isolation of a novel *B. vinsonii berkhoffii* type II from a dog being treated with immunosuppressive drugs for lymphoma⁴⁷ and the detection of *B. canis* by visualization or PCR in the blood of dogs being treated with immunosuppressive drugs for IMHA or ITP.²⁵ It is well established that dogs chronically-infected with *B. canis* will develop disease manifestations when subjected to hard work, pregnancy and lactation, stress or following administration of immunosuppressive drugs.⁴⁸ Recently, transmission of *Babesia gibsoni* has resulted in the development of IMHA following dog bites, particularly from chronically infected American pitbull terriers.⁴⁸ Occult blood-borne infection can persist in cats or dogs for prolonged periods in a state of immunological balance. A variety of factors can disrupt this state of balance and ultimately result in disease expression in

an individual animal.

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