Immunoglobulin M and Immunoglobulin G Seronegative Q Fever: A Hypothesis for Veterans' Medically Unexplained Chronic Multi-symptom Illnesses

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ABSTRACT

We present Q fever as a credible hypothesis for Gulf War Veterans' Illnesses (GWVIs) and as a possible etiology for prevalent symptomologies affecting currently serving servicemembers. Q fever is caused by the bacteria *Coxiella burnetii,* which is endemic throughout the Middle East. Q fever may manifest in many forms of widely varying and often inconstant symptoms. Due to false-negative interpretations in current and past diagnostic testing, Q fever has not received appropriate consideration as a possible causative agent for medically unexplained veterans' illnesses. Review of current literature invites us to consider that a form of Q fever involving an incomplete immune response is a potential cause of these debilitating illnesses. We hypothesize *C. burnetii* infection coincidental to exposures suppressing antibody-specific immune response results in infection mediated by immunoglobulin D (IgD). Literature indicates that successful treatment for this form of Q fever requires the concurrent administration of doxycycline and hydroxychloroquine.[1,2]

Background

Q Fever is a zoonotic disease found worldwide, except in New Zealand, and is caused by infection with *Coxiella burnetii.*[3] While many species of mammals, birds, and ticks are reservoirs of *C. burnetii* humans are usually infected through bacteria shed into the environment by domestic animals.[3] Of concern to Special Operations Forces (SOF) medical professionals is the risk of infections associated with the provision of veterinary care and environmental exposure in areas where infected animals are or have congregated. *C. burnetii* contaminates the milk of infected livestock and is shed into the soil when cattle, sheep, goats, camels, and other species of mammals urinate, defecate, or give birth. The birth products in particular hold a staggering quantity of bacteria—up to a billion organisms per gram of aborted placenta.[4] *C. burnetii* is capable of lying dormant in the soil for months or even years. In one experiment it survived at room temperature for nearly 20 months.[5] Humans are most commonly infected by ingesting contaminated products or inhaling contaminated droplets or dust. Inhaling even a single spore is sufficient to cause illness.[4]

C. burnetii is a relatively obscure, difficult to study and subsequently, not well understood obligate intracellular organism.[6] Obligate intracellular bacteria only replicate inside of a host's own cells; while facultative intracellular bacteria may replicate within or outside of the host cell. *C. burnetii* is genetically distinct from other obligate intracellular bacteria and is the only species of the phylum *Coxiella.*[7] Consideration of aspects of intracellular bacteria more familiar to SOF medical professionals may provide some useful insight into aspects of *C. burnetii* lifecycle and pathogenicity (Table 1).

Relevant Aspects of the Immune Response to *C. burnetii*

Q fever in humans is usually asymptomatic or a mild disease with spontaneous recovery.[3] With effective humoral and cell-mediated immune responses most patients eliminate *C. burnetii* infection within three days.[10]

The main function of the humoral immune system is to produce antibodies and then introduce these antibodies into the serum. An antibody, also known as an immunoglobulin, is a large Y-shaped protein that identifies and neutralizes bacteria and other foreign targets which are commonly referred to as antigens. Each tip of the "Y" of an antibody contains a paratope—the "lock"—that is specific for one particular epitope on an antigen—the "key" –, allowing the immunoglobulin to bind with the antigen. Using this binding mechanism, immunoglobulins tag pathogens for attack by other immune components or neutralize them by, for example, blocking a structure or function essential for survival of the pathogen.

Antibody Classes

Immunoglobulin A (IgA) Found in mucosal areas such as the gut, respiratory tract, urogenital tract, IgA is also found in saliva, tears, and breast milk.

Table 1 Intracellular Pathogen Comparison

Disease	Pathogen	Human Cells Infected	Gram Stain Group	Obligate or Facultative Intracellular	Similarity to *C. burnetii*
IgM & IgG seronegative Q fever	*Coxiella burnetii*	Monocytes	Gram Negative	Obligate Intracellular	N/A
Brucellosis	*Brucella melitensis*	Monocytes	Gram Negative	Facultative Intracellular	Also survives in monocytes by disruption of phagosome/lysosome fusion[8]
Lyme	*Borrelia burgdorferi*	Not well described	Not Classified	Facultative Intracellular	Capable of causing similar chronic multi-symptom disorder
Rocky Mountain Spotted Fever	*Rickettsia rickettsii*	All cells	Gram Negative	Obligate Intracellular	Similarities in intracellular invasion, survival, and growth[9]

Immunoglobulin D (IgD) Discovered in 1965, immunoglobulin D is a unique immunoglobulin with a concentration in serum far below those of IgG, IgA, and IgM, but much higher than that of IgE. Despite studies extending for more than four decades, a specific role for serum IgD has not been defined.[11]

Immunoglobulin E (IgE) IgE binds to allergens and triggers histamine release from mast cells, basophils, and is involved in allergy.

Immunoglobulin G (IgG) In its four forms, IgG provides the majority of antibody-based immunity against invading pathogens.

Immunoglobulin M (IgM) IgM attacks pathogens in the early stages of humoral immunity before there is adequate IgG to eliminate infection. Additionally, changes in the concentration of IgM are the stimulus for antibody-producing cells to produce IgG.[12]

Intracellular pathogens such as *C. burnetii*, *B. melitensis*, *B. burgdorferi*, or *R. rickettsia* are protected by the wall of the host cell from circulating antibodies and are thus combated by the cell mediated immune (CMI) response instead of the humoral immune response. While many intracellular bacteria, such as *R. rickettsii*, replicate within a wide variety of host cell types; *C. burnetii* only replicate within monocytes, the cells key to the CMI response.

Monocytes normally overcome *C. burnetii* infection by phagocystizing, essentially devouring, the pathogen and subsequently presenting the *C. burnetii* antigens to immune system. In this process, called antigen presentation, the infected monocyte presents the surface proteins of the pathogen on the surface of the monocyte. This exposes these proteins (antigens) to the host's serum and circulating immunoglobulins. Then primary antibodies of the humoral immune response—IgM and the subsequently produced IgG—bind with the presented antigens and stimulate apoptosis; a programmed cell death of the infected monocyte. In patients with a complete immune response, this process effectively eliminates *C. burnetii* infection.[10]

Issues in Diagnosis

The current basis of testing for Q fever is the presence of *C. burnetii* specific IgM and IgG. The presence of these antibodies (seropositive) is considered by most to be diagnostically conclusive for *C. burnetii* infection. Unfortunately, current diagnostic criteria interpret the absence of *C. burnetii* specific IgM and IgG antibodies (seronegative) to rule out *C. burnetii* infection. This results in a false negative for any patient with IgM and IgG seronegative *C. burnetii* infection. Simply put, a lack of *C. burnetii* specific IgM and IgG does not accurately rule out Q fever.

Between 1982 and 1988, prominent Q fever researchers tested patients exhibiting clinical signs of Q fever for IgG, IgM, and IgA antibodies.[13] They identified infected patients who exhibited IgA "as the sole or main antibody response."[13] It was concluded these patients would have remained undiagnosed had they not been tested for *C. burnetii* specific IgA in addition to IgM and IgG.[13] Testing for all three antibodies was determined to be prudent criteria for diagnosis of acute Q fever. This identification of false negatives calls into question current practice of ruling out Q fever if a patient tests negative for *C. burnetii* by IgM and IgG alone. This finding also demonstrates the immune system is capable of producing other classes of *C. burnetii* specific immunoglobulins in the absence of IgM and IgG. Given IgD is a redundant isotype to IgM and IgD has antibody activity to specific antigens[11] an IgD mediated response to *C. burnetii* could occur in the absence of detectable levels of IgM.

Until very recently, researchers have been unable to culture *C. burnetii* outside of host cells.[13] As a result, *C. burnetii* is poorly studied in comparison to other intracellular pathogens and infectious diseases. No studies of chemical disruption of antibody production in Q fever have been identified in literature; however, studies of Lyme disease (*B. burgdorferi*) and Brucellosis (*B. melitensis*) have identified IgM and IgG seronegative disease when chemical disruption of antibody production occurs

early in infection. Patients with Brucellosis have failed to produce diagnostic levels of *Brucella sp.* antibodies after receiving early unspecified oral antibiotics.[15] IgM and IgG seronegative Lyme disease also occurs due to early chemical disruption of antibody production, most notably by antibiotics[16,17] and chemotherapy.[18] Other agents thought capable of disrupting antibody production include: the insecticide permethrin,[19] the nerve agent prophylaxis pyridostigmine bromide (PB),[20] N.N-diethyl-m-touluamide (DEET) insect repellent, and jet fuel.[21]

Antibody production in response to *C. burnetii* is likely to be exceptionally sensitive to chemical disruption. Bacterial lipopolysaccharides (LPS) are major outer surface membrane components present in almost all Gram-negative bacteria and act as potent stimulators of innate or natural immunity.[22] These outer surface membrane components are antigens the immune system recognizes and then responds to with antibody production. The LPS of virulent *C. burnetii* have been shown to have distinct structural features that permit the organism to avoid fully activating the adaptive immune response by blocking access of antibody to surface proteins.[13,23,24] The atypical capability of the *C. burnetii* bacterium to mask itself from immune recognition to this degree creates optimal conditions for seronegative responses when infection coincides with early exposure to an antibody production disrupting agent, such as antibiotics, DEET, permethrin, pyridostigmine bromide or jet fuel.

The IgM and IgG Seronegative Immune Response to *Coxiella burnetii*

The complexity of monocyte activity is poorly characterized by current medical investigation.[25] Despite this, enough is known to provide a framework to guide consideration of the possible effects of IgM and IgG seronegative immune response to Q fever (Figure 1).

Hyper IgD Syndrome and Q Fever

Hyper IgD Syndrome (HIDS), also known as intermittent fever syndrome, is a genetic disorder characterized by elevated levels of IgD (hyperimmunoglobulinemia D) and intermittent symptomology that may include fever, enlargement of the liver and spleen (hepatosplenomegaly), enlarged or abnormal lymph nodes (lymphadenopathy), abdominal symptoms, joint pain (arthralgias), and skin rashes.[32] HIDS represents a collection of symptoms not observed in other forms of Q fever, but which may be present in IgM and IgG seronegative Q fever (IgM and IgG SNQF). A comparison of the pathology for HIDS and IgM and IgG SNQF identifies contributing factors present in; elevated levels of IgD, decreased availability of cholesterols within monocytes, and defective apoptosis of monocytes.

HIDS results from a genetic disorder causing a deficiency of mevalonate kinase, an enzyme important for cholesterol synthesis.[33] Also known as mevalonic aciduria, melvalonate kinase deficiency is an inborn error of cholesterol biosynthesis. This deficiency decreases availability of cholesterols critical to the effective function of monocytes.[34] Similarly, *C. burnetii* also decreases cholesterol availability for monocytes, not by genetic defect, but by intercepting host cell cholesterol to develop a cholesterol rich parasitiphosphorous vacuole (PV), the intracellular compartment within which *C. burnetii* survives and replicates.[28]

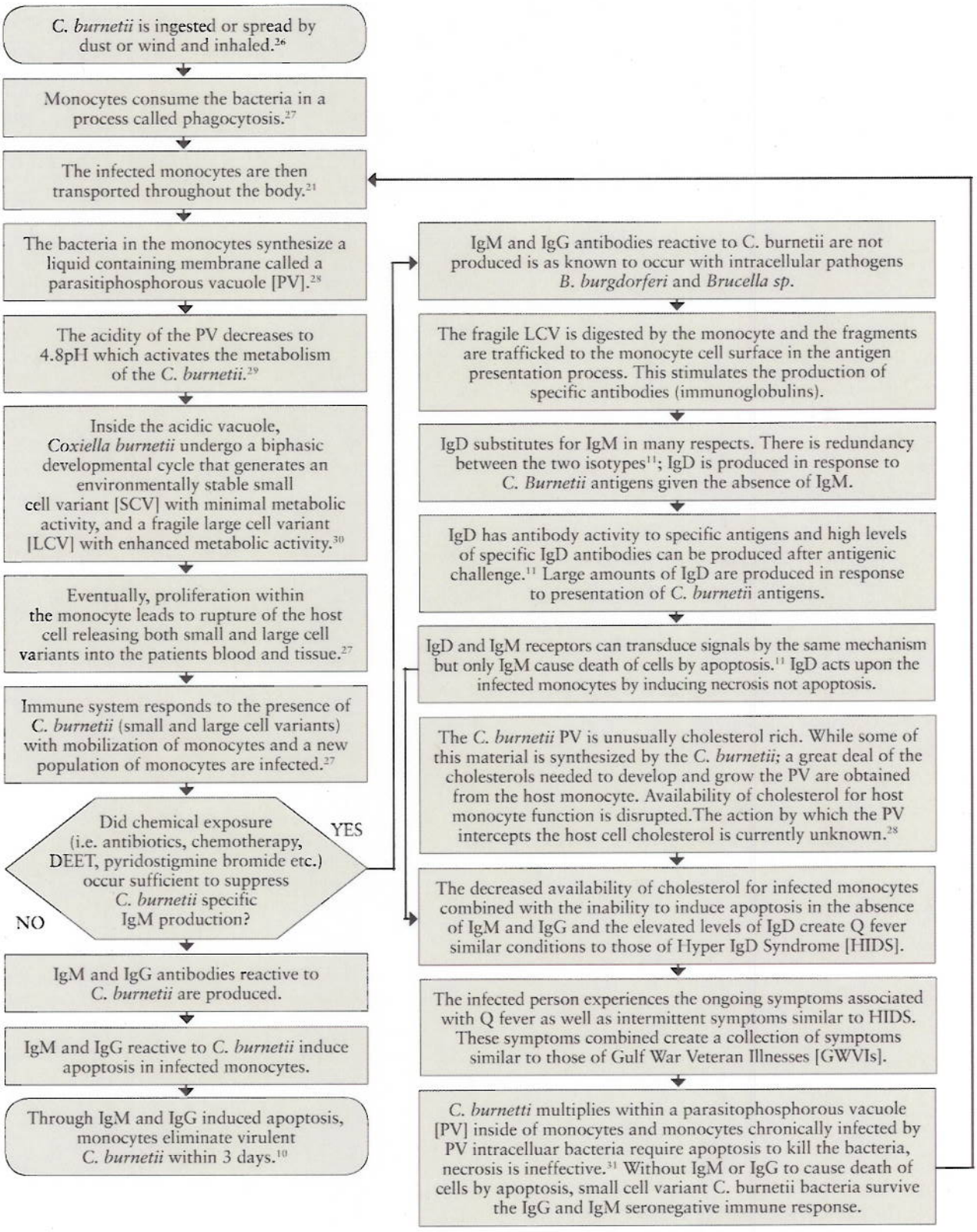
While the exact pathogenesis of HIDS remains unknown, insufficient cholesterol production is linked to dysfunction in the programmable cell deaths (apoptosis) of monocytes.[35] It has been proposed this ineffective apoptosis "may be central to the pathogenesis of HIDS."[35] Defective apoptosis is also present in IgM and IgG seronegative Q fever and results from the absence of IgM and IgG, which are the immunoglobulins capable of stimulating cell death of infected monocytes through apoptosis. As *C. burnetii* only survives inside of monocytes, apoptosis is the mechanism that eliminates infection.

IgD, should it become the active class of immunoglobulin in the absence of IgM and IgG, is not capable of stimulating apoptosis of infected monocytes.[11] IgD, unlike IgM, is also not capable of stimulating antibody-producing cells to produce appreciable amounts of IgG.[36,37] IgG is capable of stimulating apoptosis of infected monocytes and is the immunoglobulin that provides long-term immunity against reemergence of disease in Q fever. While the exact role of defective apoptosis in HIDS is not well described, the similarities of the pathological conditions of HIDS and IgM and IgG seronegative Q fever provide useful insight into the potential conditions that could generate symptoms, which may be observed in IgM and IgG SNQF.

Cytokine Dysregulation in IgM and IgG Seronegative Q Fever

Immune system homeostasis is dependent on cytokines, which are the chemical messengers between immune cells. Cytokines are crucial to the mediation of inflammatory and immune responses.[38] Cytokine dysregulation is significant to IgM and IgG seronegative disease when one considers the consequences of IgD substitution for absent IgM. IgD replaces IgM in many respects; however, there are two aspects of IgD critical to the consideration of an IgD mediated immune response to *C. burnetii*. One aspect is the inability of IgD to induce apoptosis.[11] The other aspect is IgD, which is normally produced in very small amounts compared to IgM, is a potent inducer of cytokines with monocytes appearing to be the main

Figure 1 The Immune Response to C. *burnetii* Infection



Note: This figure diagrams the essential elements of an IgG and IgM seronegative immune response to C. *burnetii* infection.

production source of cytokines in the presence of IgD.[11] Therefore, the levels of IgD required to sustain an IgD mediated response to *C. burnetii* are the foundation for ongoing cytokine dysregulation. This is particularly detrimental to the patient because dysregulation of these powerful signaling chemicals contributes to depression, atherosclerosis, obesity, sleep disturbances, and metabolic syndrome.[39]

Antibiotics and *Coxiella burnetii*

The treatment regimen for non-acute forms of Q fever consists of doxycycline and hydroxychloroquine. Hydroxychloroquine serves as an alkalinizing agent to increase the effectiveness of the doxycycline. This combination has been shown to have a very high success rate for eliminating infection when administered for 18 months.[1] *C. burnetii* multiplies in monocytes within an acidic vacuole and most antibiotics are drastically inhibited by this acidic pH.[1] Alkalinization of the *C. burnetii*-containing vacuoles with a lysosomotropic agent such as hydroxychloroquine results in bacterial growth inhibition and improvement of the bactericidal activity of doxycycline.[1] The very stable *C. burnetii* small cell variant (SCV) maintains minimal metabolism until its parasitiphosphorous vacuole (PV) acidifies.[28] The action of the hyroxychloroquine prevents that acidification, thus, preventing replication of the *C. burnetii*. Therefore, the SCV *C. burnetii* being only minimally active, require a duration of treatment longer than that reasonably required for more metabolically active intracellular bacterial pathogens.

Current literature also indicates antibiotics, while not bactericidal to the SCV without the concurrent administration of an alkalinizing agent, could decrease the severity of symptoms in some cases of IgM and IgG seronegative Q fever. Howe and colleagues determined *C. burnetii* does not need to be replicating to develop the parasitiphosphorous vacuole (PV).[29] However, the stable non-replicating SCV has to actively synthesize the proteins required to develop the parasitiphosphorous vacuole (PV).[29] Many antimicrobials, while not bactericidal to the non-replicating SCV bacteria, may block the growth of the PV by disrupting the protein synthesis required for PV growth.[29] By blocking the growth of the PV, cholesterol available to the monocyte is increased, thus, mitigating a potential condition for HIDS type, symptoms in IgM and IgG SNQF.

Antibiotics also result in fewer organisms per cell,[29] presumably by disrupting the fragile and metabolically active large cell variant (LCV) *C. burnetii*. The LCV, not the SCV, is the *C. burnetii* variant capable of replication.[40] Stopping active *C. burnetii* replication by eliminating LCVs would be expected to contribute to temporary decreases in IgM and IgG SNQF symptom severity by decreasing the number of bacteria stimulating IgD production and reducing the severity of cytokine dysregulation associated with excessive IgD.

A Case Study

This is a 41-year-old Caucasian male Special Operations officer who conducted engagements with tribesmen in the herding areas of rural eastern Iraq during 2007. His constellation of symptoms began in August 2007 following a tribal meal consisting of dairy and meat of unknown origin. Other members of team were also affected; however, this Soldier is the most ill of those who took part in the meal. This illness was treated with Levofloxacin 500mg by mouth for three days and symptoms resolved.

Several weeks following this episode the patient reports fatigue and extreme weakness, right ankle pain, and GI distress. These symptoms resolve following antibiotic treatment and recur within several weeks of completion of antibiotic treatment. Intermittent antibiotics are used to control symptoms for the remainder of the deployment.

Past Medical History/Surgical History and Family History

Non-contributory.

Diagnostics

Remote field conditions—limited to physical assessment. CBC, CMP, radiology, and other diagnostics are not immediately available.

ROS

Constitutional: Malaise and fatigue, no fever, or night sweats
Head: No ear pain, dizziness, nasal drainage, or sore throat
Respiratory: No cough, wheeze, or shortness of breath
Cardiac: No chest pain, dyspnea on exertion, or palpitations
Extremities: Pain in right ankle
Abdominal: Pain with diarrhea without blood

Objective

Vital signs: T 98.1, P 70, R 18, BP 145/92,
Pain Scale: 5/10, localization of the right ankle
General: Fatigued appearing middle-aged male in moderate distress, appears sick.
HEENT: Pupils equal round reactive to light. Extraocular muscles intact and sclera clear.
Neck: Supple without enlargements.
Lungs: Coarse bilaterally without wheeze or rales
Cardiac: Regular rate and rhythm without murmur, rub, or gallop

Abdomen: Discomfort on palpation, negative rebound tenderness, no fluid on ballottement, enlarged liver to percussion, 8cm inferior to the costal margin with what appears to be an enlarged/ prominent spleen by palpation
Skin: No rash, lesions, or breakdown
Back: Decreased ROM, generalized stiffness, and myofascial discomfort. No CVA tenderness.
Extremities: No clubbing or cyanosis, no evidence of anemia, right ankle shows joint effusion of the tibial-tarsal joint

Assessment
This patient has what appears to be an infectious condition that has the possibility of being either food borne and/or zoonotic in origin.

Plan
Referral to Primary Care provider upon return to United States.

Primary Care

May 2008: The patient presents with complaints of fatigue and extreme weakness, right ankle pain, GI distress, and insomnia.

Diagnostics
CBC, BMP, and LFTs WNL except ALT 60 (6–43) ANA, HLA B-27, bacteriology and parasitology negative, EBV negative for recent infection

Assessment
Symptoms follow a pattern of resolution following antibiotic treatment with gradual recurrence within several weeks of completion of antibiotic treatment.

Plan
Continue Ciprofloxacin and Rheumatology referral to rule out reactive arthritis

Rheumatology

November 2008: Following the discontinuation of Ciprofloxacin, the patient reports fatigue and generalized weakness along with right ankle pain, GI distress, insomnia, and unexplained rash.

Diagnostics
Radiology – No findings of significance. Chlamydia/GC, Tissue Transglutaminase IgA, and Anti-nuclear AB are negative

Assessment
Patient reports resolution of symptoms with the administration of Adalimumab (Humira) for a tentative diagnosis of psoriatic arthritis. Humira is an injectable protein that blocks the inflammatory effects of tumor necrosis factor alpha (TNF-α) in rheumatoid arthritis, psoriatic arthritis and ankylosing spondylitis. After eight months of Humira therapy, the patient notes resumption of fatigue and generalized weakness along with right ankle pain, GI distress and insomnia. Due to suspicion of a bacterial etiology, Trimethoprim and Sulfamethoxazole (Septra), a broad-spectrum antibiotic, was prescribed and an Infectious Diseases referral coordinated. The patient reported greatly diminished symptoms several days after beginning Septra.

Plan
Continue Septra and Infectious Diseases referral

Infectious Diseases

November 2009: Several weeks following the discontinuation of Septra, the patient reports fatigue, weakness, right ankle pain, GI distress, and insomnia.

Diagnostics
CT Abdomen/Pelvis – Diffuse fatty infiltration of the liver; T. whipplei – Small bowel biopsy negative; CRP, Leishmaniasis (Visceral), Brucella IgM and IgG, Proteus OX2/OX19/OXK, Salmonella, Tularemia, Lyme, and HIV1/2 are negative; Q Fever IgM and IgG is negative; Parvovirus B19, IgG 4.8, IgM 0.4;

R. Rickettsii IgG
24 February 2010 – 1:124, 05 Mar 2010 – 1:1024:
(See discussion for interpretation and significance)

TNF-α
August 2010 – 10.8 pg/ml, Feb 2011 – 6.5 pg/ml, Jul 2011 – 4.4 pg/ml (0.0–8.1)

Assessment
Presumptive diagnosis of IgG and IgM seronegative Q fever.

Plan
Eighteen-month course of Doxycycline and Hydroxychloroquine in accordance with CDC treatment recommendations for chronic Q fever.

Discussion

This patient was referred to infectious disease specialist physicians with extensive experience treating individuals with unusual infections from a wide variety of remote areas of the world in addition to their academic research. The consensus of opinion is a presumptive diagnosis of IgM and IgG seronegative Q fever. This opinion has been most informed by the patient's history, high risk of both inhalation and ingestion *C. burnetii* exposure

and consideration of the concert of alleviating factors experienced by the patient over the course of his illness. Alleviating factors include;

- Antibiotics: Between September 2007 to March 2010, in response to periods of significantly increased symptoms, he was treated on 14 occasions for durations of three days to four months with varied agents and experienced remarkable relief of symptoms within three days of beginning each course of antibiotics.
- Adalimumab (Humira): The patient experienced relief of symptoms only three days after the initial injection. Adalimumab is "indistinguishable in structure and function from naturally occurring human IgG1"[41] and has been proven to induce apoptosis of activated monocytes.[42]
- Development of IgG antibodies for *Rickettsia rickettsii* in March 2010: Given the negative testing for *C. burnetii* IgM and IgG antibodies, it was concluded the *R. rickettsii* testing fortuitously coincided with an asymptomatic seroconversion. The patient resides on a family farm located in an area with one of the highest incidences of Rocky Mountain Spotted Fever (RMSF) in North Carolina, the state with the highest incidence of RMSF in the U.S.
  - In response to *R. rickettsii* seroconversion, the patient experienced decreased symptoms, which for the first time since symptoms began in 2007, occurred without medical intervention (administration of antibiotics or adalimumab).
  - Following *R. rickettsii* seroconversion, the patient noted increased symptoms occurred at intervals of about five to six weeks and lasted about five days before returning to a decreased level without medication. *R. rickettsii* is one of a very few bacterial pathogens that stimulate the production of antibodies known to exhibit serological cross reactivity with *C. burnetii*.[43]
  - The introduction of *R. ricketsii* IgM and IgG antibodies capable of stimulating apoptosis of monocytes activated by LCV *C. burnetii* would be expected to reduce symptoms in IgM and IgG seronegative Q fever.
- Doxycycline and hydroxychloroquine: The patient noted decreased weakness, fatigue, right ankle pain, and GI distress within one month of beginning this regimen in January 2011 and experienced a continued gradual decrease in all symptoms. Progressive decrease in TNF-α over the course of treatment is consistent with response to therapy.

IgM and IgG Seronegative Q Fever and Gulf War Veterans' Illnesses (GWVIs)

While Q fever was recognized in 1991 to have been an infectious disease endemic to the Persian Gulf area, there are no records of Q fever surveillance during the 1991 Gulf War.[44] This is not surprising due to the limited accessibility at the time of the testing required to conduct Q fever surveillance.[9] Even today, the U.S. military does not have field deployable capability to assay for Q fever.[45]

A study and analysis of deployment stressors associated with GWVIs completed by members of the National Center for Infectious Diseases of the Centers for Disease Control and Prevention (CDC) in 2000 identified severe to mild-moderate GWVIs were associated with chemical exposures to pyridostigmine bromide and insect repellents.[46] These findings were consistent with similar previous studies, namely Brandt and colleagues[47] and the Iowa Persian Gulf Study Group.[48] Pyridostigmine bromide (PB) is a medication which increases the availability of the neurotransmitter acetylcholine and was widely administered during the Gulf War to reduce the effects of nerve agent exposure. Beatrice Golomb's comprehensive review and meta-analysis of GWVI epidemiological studies identifies causal connections between pyridostigmine bromide and Gulf War Veterans' Illnesses.[49] She also found consistent linkage of exposure to pesticides and organophosphate nerve agents with GWVIs.[49]

Peden-Adams and colleagues found pyridostigmine bromide (PB), insect repellents, and JP-8 fuel have the potential to suppress antibody-specific IgM immune responses.[21] The dosage level for PB found to be suppressive of the IgM antibody response in the Peden-Adams laboratory study was well less than the total mg/kg dosage given Gulf War soldiers.[21,50] An incubation period for *C. burnetii* of between two and 48 days[51] provides ample opportunity for Gulf War veterans to have experienced coincidental exposures to immunosuppressive chemicals and to *C. burnetii*.

Q Fever and Gulf War Veterans' Illnesses (GWVIs) Comparison of Symptoms

The manifestation of Q fever most similar to IgM and IgG seronegative Q fever is Q fever syndrome (QFS). Q fever syndrome, which is IgM and IgG seropositive, occurs in some patients following acute infection.[52] Literature indicates Q fever syndrome occurs due to significant immunogenetic differences between QFS patients and patients who recover from acute Q fever; specifically, increased frequency of a specific gene and genetic variations affecting interferon-gamma (IFNγ).[52,53] IFNγ, like TNF-α, is one the powerful signaling cytokines which are critical for the initiation and effectiveness of the human immune response.

IgM and IgG seronegative Q fever results from a similar set of conditions as Q Fever Syndrome (QFS) (dysfunction of cell mediated immunity) albeit due to a different

initiating mechanism (chemical v. genetic). This warrants a consideration of QFS symptoms, which in addition to the symptoms of HIDS, presents an array of symptoms comparable to those described for GWVIs (Figure 2).

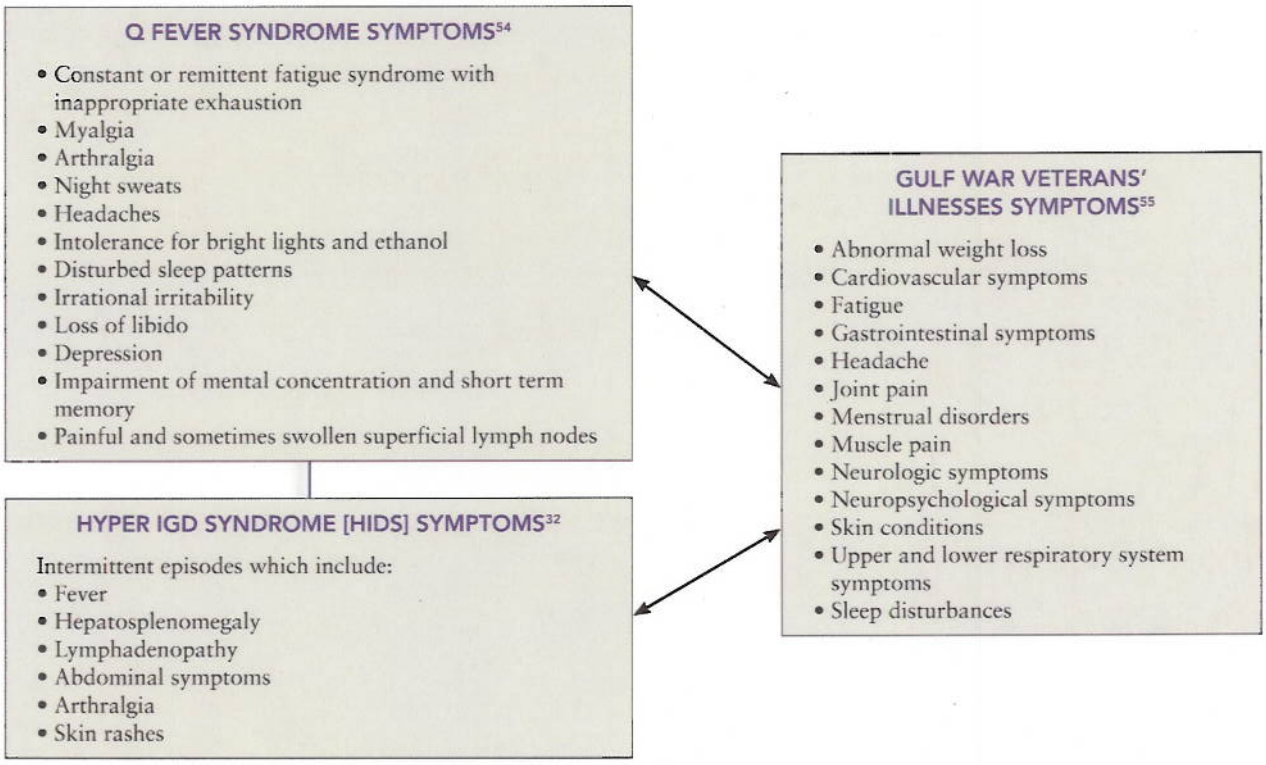
Gulf War Veterans' Illnesses (GWVIs) and Cytokine Dysregulation

While the specific symptoms and degree of severity of GWVIs vary between individuals; veterans with multi-symptom illness have been distinguished from asymptomatic veterans by demonstrating a "low grade, ongoing immune activation."[56] Skowera and colleagues also note, "despite the fact that our analyses were performed nine years after the original conflict (Gulf War), and several years after symptoms arose, we were able to detect significant differences in immune activation in symptomatic Gulf War veterans, compared with their well counterparts."[56] They conclude veterans with GWVIs experience elevated levels of the cytokine interleukin(IL)—10 production as a result of some internal or external exposure.[56] This finding is consistent with an ongoing IgD mediated immune response considering the cytokine dysfunction induced by elevated IgD would include elevated release of IL-6, IL-10, and leukemia inhibitory factor from peripheral blood mononuclear cells.[11]

Gulf War Veterans' Illnesses and Response to Antibiotics

The results of the seminal study of GWVIs and antibiotics were published by Donta and colleagues in 2004. Veterans with GWVI were administered doxycycline in an 18 month, randomized, double-blind, placebo-controlled clinical trial. The researchers theorized Gulf War veterans' symptoms potentially resulted from intracellular bacterial infection and could be mitigated by long-term treatment with doxycycline. They found some veterans had improvement in symptoms but, even though their symptoms decreased, they still experienced symptoms of illness.[57] Another significant finding was veterans receiving doxycycline as part of the study were significantly less likely to use any other antibiotics during this time.[57] These findings are consistent with the findings one would expect for IgM and IgG seronegative Q fever given the potential of doxycycline to decrease the number of *C. burnetii* LCV and block the formation of parasitiphosphorous vacuoles (PVs) but not affect the stable and metabolically inactive small cell variant *C. burnetii*. The reduction in the use of antibiotics other than the study medication is also consistent with the assumption that veterans with IgM and IgG seronegative Q fever who take doxycycline would not seek additional medication to mitigate increased symptoms associated

Figure 2 Symptom Comparison



with HIDS conditions or more severe cytokine dysfunction. Donta and colleagues concluded that "doxycycline may have had limited effectiveness in treating GWVIs because there was no underlying infection or the GWVIs may have been sequelae of previous infections."[57] Consideration of IgM and IgG seronegative Q fever offers a third conclusion: doxycycline may have had limited effectiveness because without co-administration of an alkalizing agent, such as hydroxychloroquine, the doxycycline was neutralized in the acidic environment of the *C. burnetii* parasitiphosphorous vacuole and therefore, only bactericidal to *C. burnetii* large cell variants—not the small cell variants that perpetuate IgM and IgG seronegative Q fever.

IgM and IgG Seronegative Q Fever and Veterans of Iraq and Afghanistan

The aforementioned case study involved an Operation Iraqi Freedom (OIF) veteran, which demonstrates that significant risk of IgM and IgG seronegative Q fever is not confined to Gulf War veterans. A recent seroepidemological survey for *C. burnetii* among hospitalized troops deployed to Iraq found that of those diagnosed with unspecified pneumonia, unspecified viral illnesses and unspecified fever, 13%, 12% and 20% respectively tested positive for *C. burnetii* IgM and IgG .[58] As only *C. burnetii* specific IgM and IgG were tested, the prevalence of IgM and IgG seronegative Q fever among this group remains unknown. However, given the prevalence of seropositive illnesses, Anderson and colleagues recognize Q fever as a "significant infectious disease threat to troops deployed to this region."[58] With changes in U.S. Department of Defense PB administration policy effectively eliminating widespread distribution of the immunosuppressant pyridostigmine bromide (PB) in OIF and Operation Enduring Freedom (OEF),[59] one would expect decreased incidence rates for IgM and IgG seronegative Q fever in OIF/OEF veterans when compared to Gulf War veterans. However, many OIF/OEF veterans were still exposed to agents such as DEET, antibiotics, JP-8 fuels and other potential immunosuppressants.[60] Some of these exposures are certain to occur coincidentally with *C. burnetii* infection possibly resulting in a meaningful percentage of OIF/OEF veterans at risk of IgM and IgG seronegative Q fever.

Limitations and Caveats

IgM and IgG seronegative Q fever represents a new and credible hypothesis for veterans' medically unexplained chronic multisymptom illnesses of the Gulf War, Operations Enduring and Iraqi Freedom. Review of the available clinical literature supports the hypothesis that *C. burnetii* infection mediated by IgD may occur in patients who do not produce IgM and IgG in response to *C. burnetii* exposure. In light of the striking similarities between IgM and IgG seronegative Q fever and GWVIs, an IgD mediated response represents a tentative explanation for GWVIs. The authors recognize IgM & IgG SNQF and its potential role in medically unexplained multisymptom veterans' illnesses is a hypothesis and therefore, not more than a tentative explanation that can be tested through investigation. To test this tentative explanation through investigation, further research into the role of IgD in Q fever is imperative.

Conclusion

The risks of *C. burnetii* exposure in the Middle East coupled with exposures to pyridostigmine bromide and other immunosuppressant agents are epidemiologically consistent with GWVIs. The symptoms expected in an IgD mediated immune response to *C. burnetii* are consistent with the symptoms reported for GWVIs. The response of GWVIs to certain antibiotics is consistent with the expected response to the same in IgM and IgG seronegative Q fever. Van der Hoek and colleagues have found the current wide variation of serological and PCR testing for *C. burnetii* infection implies the diagnosis of chronic Q fever must be based primarily on clinical presentations.[61] These same diagnostic limitations imply the diagnosis of IgM and IgG seronegative Q fever must also be based primarily on clinical grounds. We recommend veterans with clinical findings consistent with IgM and IgG seronegative Q fever be provided the opportunity for a trial of doxycycline and hydroxychloroquine should they so choose.

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