

---

**BACTERIAL DISEASES (MYCOPLASMA)**

---

One theory suggests that illnesses in Gulf War veterans may result from infection with *Mycoplasma*—bacteria-like microorganisms that are the smallest free-living microorganisms. This chapter considers the *Mycoplasma* theory. It first discusses briefly the *Mycoplasma* species, including how they affect healthy persons and those with compromised immune systems. It then discusses hypotheses regarding how Gulf War veterans might have been infected with *Mycoplasma* and relates evidence for and against these hypotheses. It recounts the debate surrounding testing methods for *Mycoplasma* infection and provides preliminary data regarding response to antibiotic treatment of ill Gulf War veterans who test positive for *Mycoplasma*.

**BACKGROUND ON MYCOPLASMAS**

*Mycoplasmas* belong to the class Mollicutes. They are the smallest organisms capable of self-replication in cell-free media. (Lo, 1992). Like bacteria, they contain no nucleus but do contain DNA and RNA; however, unlike most bacteria, they have no cell wall (Lo, 1992; Marty, 1993). They cause serious disease in many animal species (as well as plants), where they may affect multiple organ systems or cause chronic disease. *Mycoplasmas* are often difficult to detect and to eradicate. They may elude the immune system, and they may alter it (inducing appearance of a lymphokine profile, or a set of signaling cells produced by those immune cells termed lymphocytes, that favors activation of B-lymphocytes that are involved in antibody production), possibly precipitating autoimmune disease (Baseman et al., 1996; Baseman and Tully, 1997). A *Mycoplasma* has been proposed as the most likely ancestor of the animal mitochondrion (Pollack, 1997; Karlin and Campbell, 1994). Mitochondria are elements within the cell that serve as the principal source of energy to the cell. *Mycoplasma* proteins are sufficiently similar to animal proteins (Baseman, 1996) that either the body's immune system may not recognize *Mycoplasmas* as foreign or they may cause the body to make autoantibodies that attack the host animal and produce autoimmune disease.

## **Mycoplasma and Disease in Those with Dysfunctional Immune Systems**

*Mycoplasmas* are commonly opportunistic organisms (pathogens) that cause illness principally in those whose immune systems are not fully functioning (e.g., persons with AIDS, genetic immune deficiency syndromes, or receiving chemotherapy for cancer or organ transplantation) (Lo, 1992). They have also been postulated to serve as cofactors in the development of AIDS in HIV-infected persons (Lo, 1992) (see below).

## **Mycoplasma and Disease in Those with Normal Immune Function**

*Mycoplasma pneumoniae* is the most common cause of pneumonia in normal young adults. *Mycoplasma genitalium* is considered a cause of nongonococcal urethritis (NGU, a sexually transmitted disease) in humans. Its presence in men with urethritis (inflammation of the urethra—the canal from the bladder that allows discharge of urine to outside the body—causing pain and discharge from the penis) is independent of the presence of the more commonly recognized urethritis-causing agent *Chlamydia trachomatis*. Using a technique called polymerase chain reaction (PCR), in which genetic material is amplified to improve organism detection, *M. genitalium* was found in urethral samples from 23 percent of 103 men with signs or symptoms of NGU but in only 6 percent of 53 men without NGU ( $p < .006$ ) (Marty, 1993). Response to treatment with the antibiotic doxycycline was at least as satisfactory in resolving symptoms in those with confirmed *M. genitalium* as in those with *C. trachomatis*, further supporting the role of *Mycoplasma* in causing the symptoms (doxycycline is effective against the *Mycoplasma*). *M. genitalium* has been implicated in pelvic inflammatory disease (PID), a serious consequence of sexually transmitted infection in women where the infection travels into the pelvis possibly resulting in infertility. Approximately 25 percent of infertile women have antibodies to *M. genitalium* (Marty, 1993), and this infection is increasingly considered a possible cause of male and female infertility. *M. genitalium* is also found in the respiratory tract and may cause disease in the respiratory tract independent of any genital disease (Baseman and Tully, 1997). *M. hominis* is also associated with PID and increased risk of preterm delivery. Fulminant respiratory distress syndrome and failure of multiple organ systems have been described with *M. fermentans* in immunocompetent individuals (Lo, 1992). Congenital infection (infection at birth in infants who acquired infection in utero) with *Ureaplasma urealyticum*, also a *Mycoplasma*, is associated with central nervous system damage, chronic lung disease of prematurity, neonatal bacteremia, pneumonia, meningitis, premature spontaneous labor and delivery, and possibly a condi-

tion termed “hydrops fetalis” (Marty, 1993) involving abnormal accumulation of fluid in fetal tissues.

## MYCOPLASMA IN GULF WAR VETERANS

It has been proposed that *Mycoplasma* infection may have contributed to illnesses in Gulf War veterans, possibly through contaminated anthrax vaccines. Supporting this theory, two investigators have reported high rates of positive tests for *Mycoplasma* in ill Gulf War veterans (in one instance, using a new technique called nucleoprotein gene tracking that remains to be externally validated; and in the other, using PCR).<sup>1</sup> One reports that many of those who tested positive responded favorably to antibiotic treatment, often with resolution of long-standing severe symptoms.

Some members of the scientific community have criticized this theory, raising four objections. First, no significantly increased rates of conversion to *Mycoplasma*-antibody-positive status, from pre- to postdeployment, were found using stored blood from Gulf War veterans enrolled in a Gulf War health registry compared with those not so enrolled. Second, the newly devised test is itself new and unproven. Third, *Mycoplasma* could not plausibly grow in anthrax vaccine (one postulated mechanism of transmission). Finally, they observe that no controlled trials of treatment have been published (although such a trial is currently under way) (Duerksen, 2000).

Evidence supporting a connection between illness in Gulf War veterans and *Mycoplasma* derives almost exclusively from non-peer-reviewed sources. Such evidence cannot prove that *Mycoplasma* is a cause of illness in Gulf War veterans. However, this preliminary evidence provides a strong case for additional research into this putative mechanism.

Several distinct subhypotheses are implicit in the *Mycoplasma* hypothesis as articulated by its author, Garth Nicolson. These subhypotheses may be examined for their independent merits. Elimination or confirmation of one subhypothesis does not imply elimination or confirmation of the others. Furthermore, each subhypotheses engenders a set of questions. Table 3.1 presents each subhypothesis and the related questions.

## EVALUATING ELEMENTS OF THE MYCOPLASMA HYPOTHESIS

For each set of questions, the next section reviews available information.

---

<sup>1</sup>Recent news reports confirm that an ongoing federally funded study has also found similar high rates of positive tests for *Mycoplasma* in ill Gulf War veterans (Duerksen, 2000).

**Table 3.1**  
**Subhypotheses and Related Questions**

Subhypothesis	Related Question
Anthrax vaccine given to Gulf War veterans may have been contaminated with <i>Mycoplasma</i>	<b>Origin:</b> What is the evidence favoring and opposing anthrax vaccine as a source of <i>Mycoplasma</i> agents? What are other postulated origins of <i>Mycoplasma</i> as a cause of disease in Gulf War veterans?
Nucleoprotein gene tracking is a reliable and valid testing method for <i>Mycoplasma</i>	<b>Testing:</b> It has been reported that nucleoprotein gene tracking and Forensic PCR more reliably detect <i>Mycoplasma</i> infections than traditional PCR, and therefore reliably distinguish patients who carry, or are infected with, <i>Mycoplasma</i> from those who do not or are not. Others challenge the plausibility of these reports, questioning whether these tests are reproducible and if they really are superior to traditional PCR.
<i>Mycoplasma</i> is a plausible source of illness in ill Gulf War veterans	<b>Theoretical plausibility:</b> Irrespective of concerns about the origin of the <i>Mycoplasma</i> agent, is present knowledge of <i>Mycoplasma</i> characteristics theoretically consistent with the possibility that <i>Mycoplasma</i> could produce disease such as that seen in Gulf War veterans?
Data support the presence of <i>Mycoplasma</i> in ill Gulf War veterans	<b>Evidence:</b> What are the data regarding presence of, or infection with, <i>Mycoplasma</i> in ill Gulf War veterans?
Ill Gulf War veterans who test positive for <i>Mycoplasma</i> (using nucleoprotein gene tracking or PCR) respond to antibiotics	<b>Treatment:</b> Irrespective of whether <i>Mycoplasma</i> is the etiology, does treatment with stated antibiotics lead to symptom abatement or resolution in ill Gulf War veterans who do and do not test positive for <i>Mycoplasma</i> ?

## Origin

The theory regarding an anthrax vaccine origin for *Mycoplasma*-induced illness is speculative. The theory's author first considered *Mycoplasma* as a cause of illness in a subject who became chronically ill after volunteering for military medical experiments involving vaccinations and who tested positive for *Mycoplasma*. Subsequently, Nicolson saw ill Gulf War veterans in whom *Mycoplasma* was also detected, using methods he devised (Nicolson and Nicolson, 1997) (see below).<sup>2</sup>

The possibility of accidental contamination as a source of *Mycoplasma* in anthrax vaccines can be scrutinized in light of current understanding of *Mycoplasma*. *Mycoplasma* frequently contaminates mammalian (or, more generally, eukaryotic) cell lines and tissue cultures (Baseman and Tully, 1997), such

<sup>2</sup>G. Nicolson, letter, 1997.

as those in which viral vaccines are grown. For this reason, *Mycoplasmas* are tested for at the start of and at the end of the viral vaccine production process.

The anthrax vaccine (the main “new” vaccine given in the Gulf War) is made in a sterile synthetic medium that would be presumed unfavorable to *Mycoplasma*. *Mycoplasmas* are fastidious organisms difficult to culture even from diseased tissue (Lo, 1992). They have complex nutritional requirements and depend on external supplies of biosynthetic precursors, including amino acids, nucleotides, fatty acids, and sterols (Baseman and Tully, 1997). Moreover, many tissue and blood components inhibit *Mycoplasma* growth (Lo, 1992). Experts suggest that if the *Mycoplasma* could survive, it would quickly be “outcompeted” by the much faster growing bacteria and die out. Moreover, both anthrax (AX) and botulinum toxoid (BT) vaccines (the BT vaccine being the other new vaccine) contain formaldehyde as a “cross-linker” in BT vaccine and as a stabilizer in AX vaccine. BT vaccine also contains thimerosal, a mercury compound, as a preservative and AX vaccine has “phemerol,” or benzethonium chloride, as a preservative. These preservatives would be “expected” (experts believe) to kill *Mycoplasma* unless very high *Mycoplasma* levels were introduced (Hardegree et al., 1997). At present no peer-reviewed studies have been found that studied the levels of the preservatives required to kill *Mycoplasma*.

A few have argued that *Mycoplasma* infection resulting from accidental *Mycoplasma* contamination of anthrax vaccines is unlikely or impossible (Food and Drug Administration (FDA), 1996). The DoD performed tests for *Mycoplasma* on anthrax vaccine batches and state that it found no contamination. A formal scientific-style report of this work, including full methods and results, has not yet been published in the peer-reviewed literature, nor has this finding been reproduced by DoD-independent scientists.

Nicolson and Nicolson (1997) reported that two ill British Gulf War veterans tested positive for *Mycoplasma*. Since the British anthrax vaccine differed from the U.S. vaccine in many respects, and was manufactured independently, this would seem to reduce the likelihood of contamination in the manufacturing process as a source of *Mycoplasma* infection in Gulf War veterans.

However, many sources of *Mycoplasma* contamination are possible (Baseman and Tully, 1997); therefore, the true likelihood of *Mycoplasma* contamination of vaccines is difficult to gauge. Both the FDA and the military have viewed *Mycoplasma* contamination of anthrax vaccines as very unlikely, based on information such as that described above (FDA, 1996). They have judged the possibility as low enough to merit no additional follow-up. However, the only reference identified in which anthrax vaccine was tested for *Mycoplasma* did report *Mycoplasma* contamination. *Mycoplasma* was cultured from Iraqi (local)

anthrax vaccine (Alshawe and Alkhateeb, 1987) although no Iraqi vaccine was used by the United States, and vaccine production methods in Iraq may differ substantially from production methods in the United States. (Direct information regarding Iraqi production methods is lacking.)

There is no direct or epidemiological evidence connecting *Mycoplasma* to the anthrax vaccine or to any other vaccines received by Gulf War veterans. However, the theory of mycoplasmal illness does not depend on the contamination of anthrax (or other) vaccines as a source; and vaccines are not the only possible source by which *Mycoplasma* infection might have emerged.

Other sources of pathogenic *Mycoplasma* have been postulated. For instance, there are suggestions that *Mycoplasma* may be endemic in the Middle East in sand or water, that the Iraqis may have used it as a biological weapon, or that it was dispersed as “blow-back” after their biological weapon stores were destroyed (Nicolson and Nicolson, 1996; Moehringer, 1997; Offley, 1996). Because of the difficulty growing *Mycoplasma*, many view *Mycoplasma* as an unlikely biological warfare agent. Some suggest that pathogenicity may have been enhanced by immune dysfunction resulting from other multiple vaccinations or other exposures or from breach of the blood-brain barrier (Nicolson and Nicolson, 1997), such as may have occurred with multiple chemical exposures or stress (see the companion report on pyridostigmine bromide (Golomb, 1999)). These theories do not involve a direct vaccine provenance for the *Mycoplasma*. Additionally, evidence suggests that immune system changes may occur as a consequence of exposure to acetylcholinesterase inhibitors (see Golomb, 1999, and the companion report on pesticides (Cecchine et al., 1999)), so that it is possible that individuals with these or other exposures may have enhanced susceptibility to infection with intracellular bacteria such as *Mycoplasma*.<sup>3</sup>

---

<sup>3</sup>Regarding the first of these hypotheses, that *Mycoplasma* is endemic in areas where Gulf War troops were deployed, discussions with Saudi, Kuwaiti, and Egyptian medical personnel indicate that symptoms of the type described in U.S. Gulf War veterans were not common in Saudi, Kuwaiti, or Egyptian troops or civilians. No local disease with these symptoms has been described. Thus, endemic *Mycoplasma* disease with these symptoms appears unlikely unless native immunity is present or illness reporting is poor. However, mycoplasmal disease cannot be absolutely excluded. Cultural differences may influence illness reporting. For example, cancers in these populations frequently present with quite advanced, highly visible disease. Local physicians may discount illness for which objective findings have not been isolated, complicating exclusion of such illness. Indigenous populations could have relative immunity to similar illness either through genetic selection or advantages produced by early exposure.

Possibly consistent with these theories is the suggestion by U.S. medical personnel in Saudi Arabia that symptoms similar to those seen in Gulf War veterans may be common in Americans stationed in that area after, and possibly before, the Gulf War (E. McClure, personal communication to Beatrice Golomb, 1997; M. Kamel, personal communication to Beatrice Golomb, 1997). However, quantitative information on such reports of symptoms is not available. In one observational study of all encounters in one year in a Saudi Arabian primary care practice, 33.5 percent had chronic problems. Musculoskeletal and digestive disorders, among the most prominent symptoms in ill Gulf War veterans, accounted for 38 percent and 24 percent of encounters, respectively (Al-

## Testing of Veterans for Mycoplasma

Historically, testing for *Mycoplasma* has been problematic.<sup>4</sup> As mentioned, *Mycoplasmas* are fastidious and difficult to grow from diseased tissue. They may not provoke a marked antibody response, so that serological testing to detect antibodies to *Mycoplasma* is unreliable. The *Mycoplasma* particles, which occur in different forms (pleomorphic) and lack a tell-tale cell wall, are difficult to distinguish from fragments of extracellular cytoplasm or cell organelles released from degenerating cells.<sup>5</sup> A validated, readily available diagnostic test for *M. fermentans incognitus* has not been available for routine use.

Different investigators, using distinct testing methods, report dramatically different prevalence of *Mycoplasma* infection in ill Gulf War veterans (Table 3.2). Specifically, higher prevalence rates of *Mycoplasma* have been reported in ill Gulf War veterans than in controls by one investigator who employed nucleoprotein gene tracking and forensic PCR. Higher rates have also been reported in a small sample of ill Gulf War veterans than in a large sample of controls by another investigator using traditional PCR. A third investigator found no statistically significant increase in conversion to antibody positivity in Gulf War veterans who applied to a Gulf War registry compared with those who had not, and overall rates of positivity for *M. fermentans* were low using antibody tests. (As mentioned above, recent preliminary results from an ongoing large study support the possibility of high rates of positivity in ill Persian Gulf War veterans.)

Two factors may be responsible for discrepancies in the findings of the investigators, namely, testing differences and subject selection. Regarding testing methods, PCR and possibly nucleoprotein gene tracking may be more sensitive, whereas serological testing is insensitive and may miss true cases. As noted above, serological testing may not be reliable, because a significant antibody response may not be produced in response to *Mycoplasma*.<sup>6</sup>

Indeed, each testing method has been criticized. Skepticism regarding nucleoprotein gene tracking has been voiced by some who doubt that a technique (nucleoprotein gene tracking) that fails to involve amplification could be more sensitive than one that does (traditional PCR). Nucleoprotein gene tracking is a

---

Shammari and Nass, 1996). But the data collection and presentation strategy do not permit determination of whether any of these subjects had combinations of symptoms like those reported by ill Gulf War veterans.

<sup>4</sup>S. Lo, personal communication to Beatrice Golomb (1997).

<sup>5</sup>S. Lo, personal communication to Beatrice Golomb (1997).

<sup>6</sup>S. Lo, personal communication to Beatrice Golomb (1997).

**Table 3.2**  
***Mycoplasma* Test Results in Gulf War Veterans and Controls**

Investigator	% Gulf War Veterans Testing Positive	% Controls Testing Positive	Test	# Cases/ # Controls Tested	Gulf War Sample
Lo (1993) <sup>a</sup>	3 <sup>b</sup>	NA	Antibody—not sensitive	27/0	Registry participants
Lo (converters) (1994) <sup>a</sup>	2.6 <sup>c</sup>	1.3	Antibody—not sensitive	151/151	Registry participants
Nicolson, Nicolson, and Nasralla (1998)	45	5	Nucleoprotein gene tracking <sup>d</sup> —not validated	170/41	Ill Gulf War veterans
See (1997) <sup>e</sup>	70	7	PCR	20/>100	Ill Gulf War veterans

<sup>a</sup>As reported in Ribas (1996).

<sup>b</sup>Three positive for *M. fermentans* or *M. penetrans*.

<sup>c</sup>*M. fermentans*.

<sup>d</sup>Also forensic PCR.

<sup>e</sup>D. See, personal communication to Beatrice Golomb (1997).

new technique that has not yet been tested by outside groups, and reproducibility and validity remain to be demonstrated (testing is under way). Moreover, some researchers question whether forensic PCR (a PCR strategy developed for use when only small blood samples are available) would be more sensitive than traditional PCR when an adequate blood sample is available.<sup>7</sup> However, with traditional PCR, unless care is taken, the genetic material to be amplified may fail to be accessed.<sup>8</sup> Forensic PCR and nucleoprotein gene tracking are reportedly designed to help circumvent this problem.<sup>9</sup> Finally, antibody tests, although established, are thought to be insensitive and often fail to detect *Mycoplasma* infection. Other testing factors (e.g., differences in “blinding” of patient status during analysis of test results) could in principle contribute to the testing differences. In this scenario, reports of the high rates of *Mycoplasma* positivity in ill Gulf War veterans may be spurious results of bias in categorization.

The second factor that may help explain discrepancies in study results is differences in subject selection. The investigators who reported high rates of

<sup>7</sup>S. Lo, personal communication to Beatrice Golomb (1997); H. Watson, personal communication to Beatrice Golomb (1997).

<sup>8</sup>J. Baseman, personal communication to Beatrice Golomb (1997).

<sup>9</sup>G. Nicolson, personal communication to Beatrice Golomb (1997).

*Mycoplasma* positivity in ill Gulf War veterans may see particularly ill patients or patients whose primary symptoms are loosely consistent with chronic fatigue and fibromyalgia and who may have a different pathogenesis of disease. The investigator who found no difference in *Mycoplasma* prevalence between cases and controls defined as a case any patient enrolled in a Gulf War health registry, and defined as a healthy control any Gulf War veteran not enrolled. Yet many Gulf War veterans who report increased symptoms following the war have not elected to enroll in a Gulf War registry. Moreover, not all veterans enrolled in a registry report illness. “Misclassification bias” of ill and healthy veterans resulting from use of registry data would be expected to produce a bias toward the null,<sup>10</sup> that is, bias that would favor failure to detect a true difference, if there is one.

The reliability of nucleoprotein gene tracking and forensic PCR and their performance compared to traditional PCR are amply amenable to empiric evaluation. A large DoD-funded study is under way to investigate proposed and generally accepted diagnostic techniques for *Mycoplasma* and the validity of various testing techniques, including nucleoprotein gene tracking and forensic PCR.<sup>11</sup> Sixty ill Gulf War veterans (defined as having two of three of the following: six months fatigue, pain in more than one part of the body, and neurocognitive deficits) have been tested using Nicolson’s protocol. Of the 60, 30 are individuals not previously tested, 10 are individuals known to be nucleoprotein gene tracking negative, 10 are known to be forensic PCR negative, and 10 are known positive by conventional PCR. Each patient sample is to be tested four times, once by Baseman (University of Texas), once by Lo (Armed Forces Institute of Pathology), and twice by Nicolson (to ensure test-retest reliability). It is expected that preliminary data analysis will be forthcoming in the near future.

### **Theoretical Compatibility of Mycoplasma Infection with Symptoms in Gulf War Veterans**

Setting aside debates regarding the possible origin of alleged *Mycoplasma* infection in ill Gulf War veterans and the debate over testing methods, there is relatively more agreement among experts that a *Mycoplasma* could in principle

<sup>10</sup>Provided that the misclassification is “nondifferential.”

<sup>11</sup>S. Lo, personal communication to Beatrice Golomb (1997); G. Nicolson, personal communication to Beatrice Golomb (1997); C. Engels, personal communication to Beatrice Golomb and Lee Hilborne (1997).

cause the chronic symptoms seen. However, different levels of enthusiasm exist for this hypothesis.<sup>12</sup>

*Mycoplasmas* often localize to the joints, and some species produce arthritis in animals (Baseman et al., 1996; Cole and Ward, 1979).<sup>13</sup> *Mycoplasmas* are speculated to produce joint-related symptoms in humans;<sup>14</sup> such symptoms are prominent among Gulf War veterans (Joseph, 1997). Moreover, *Mycoplasmas* may localize to the mucous lining of the mouth, the respiratory tract, and the genital tract. Genital infection is postulated to cause infertility in men and women,<sup>15</sup> and *Mycoplasma* has been proposed as a source of endometritis and prostatitis.<sup>16</sup>

Apropos of *Mycoplasma* as an illness etiology in Gulf War veterans, *Mycoplasma fermentans* has been postulated as a cause of rheumatoid arthritis, although data attempting to confirm a link have been inconsistent.<sup>17</sup> (However, preliminary data reported by Darryl See (personal communication, 1997, chronic fatigue syndrome and rheumatoid arthritis), a published abstract (Huang, See, and Tilles, 1997), and two reports published since this review appear to confirm high rates of mycoplasma positivity in both chronic fatigue syndrome (Huang, See, and Tilles, 1997; Nasralla, Haier, and Nicolson, 1999), and rheumatoid arthritis (Haier et al., 1999). Nonetheless, recent randomized treatment trials with antibiotics have confirmed a response to long-term (6–12 months) active antibiotic treatment (such as azithromycin or doxycycline, or recently minocycline (O'Dell et al., 1997)) significantly exceeding that of placebo, with arrest of progression of joint erosion and abatement of symptoms.<sup>18</sup> *Mycoplasma* cannot at this point be confirmed to be a cause of rheumatoid arthritis, but antibiotic treatment is effective regardless of the etiology.

### Evidence of *Mycoplasma* in Ill Gulf War Veterans

Sources differ to date in detection of *Mycoplasma* in ill Gulf War veterans, as shown in Table 3.2. In one study *Mycoplasma* species were reported in 45

<sup>12</sup>S. Lo, H. Watson, G. Nicolson, C. Engels, J. Tully, personal communication to Beatrice Golomb (1997).

<sup>13</sup>H. Watson, personal communication to Beatrice Golomb (1997).

<sup>14</sup>J. Tully, personal communication to Beatrice Golomb (1997).

<sup>15</sup>J. Tully, personal communication to Beatrice Golomb (1997).

<sup>16</sup>Genital symptoms, particularly painful intercourse in spouses of Gulf War veterans, were a common complaint in an informal review performed of a convenience sample of charts of family members of Gulf War veterans presenting to the San Diego Veterans Administration as part of the Gulf War evaluation.

<sup>17</sup>J. Tully, personal communication to Beatrice Golomb (1997).

<sup>18</sup>J. Tully, personal communication to Beatrice Golomb (1997).

percent of 170 tested Gulf War veterans using a novel testing technique, nucleoprotein gene tracking, that has not yet been evaluated for reproducibility, compared with approximately 5 percent of 41 healthy nonveteran adult controls (Nicolson and Nicolson, 1997). In a second study, the rate of *Mycoplasma fermentans* detection, using serological tests, was not significantly higher in Gulf War veterans who entered a registry than in those who had not, nor was the rate of serological conversion significantly higher. Preliminary data from a third unpublished study (see Tables 3.2 and 3.3) indicate positive tests for *Mycoplasma fermentans* in 70 percent of those ill Gulf War veterans who have been evaluated (14 of 20), compared with approximately 7 percent of “hundreds” of healthy controls (D. See, personal communication, 1997). The prevalence of *Mycoplasma fermentans incognitus* identified by PCR for ill Gulf War veterans is reportedly similar to that detected in patients with chronic fatigue syndrome with immune deficiency markers (CFIDS). Sixty-eight percent of CFIDS patients test positive for *Mycoplasma* (and a similar fraction for herpes-virus 6). Patients with chronic fatigue without immunodeficiency markers have a positivity rate similar to healthy controls. Nicolson and colleagues (1996) observed that symptoms in ill Gulf War veterans are similar to those in patients with CFIDS. Of note, *Mycoplasma* species were identified, using PCR, from the synovial fluid of about 60 percent of patients with rheumatoid arthritis.<sup>19</sup>

Table 3.3

*M. Fermentans Incognitus* by PCR, Using Primers Specific for a Hairpin Region of *M. Incognitus*, on Peripheral Blood Mononuclear Cells

Subjects	Published % <i>Mycoplasma</i> Positive	Published # <i>Mycoplasma</i> Positive	Revised % <i>Mycoplasma</i> Positive <sup>a</sup>
Healthy	20	6/30	5–10
Chronic fatigue syndrome without markers			~10
AIDS	40	12/30	40
Rheumatoid arthritis			60–70
Chronic fatigue syndrome with immune deficiency markers	68	57/84	68
Ill Gulf War veterans			70

SOURCES: D. See, personal communication with Beatrice Golomb (1997); Huang et al. (1997).

<sup>a</sup>Current numbers based on “hundreds of cases” for each category, except Gulf War n = 20 (Huang et al., 1997)

<sup>19</sup>D. See, personal communication to Beatrice Golomb (1997).

In short, different investigators report markedly different prevalence of *Mycoplasma* in ill Gulf War veterans. These differences may result from differences in testing techniques and/or from differences in subject selection. Because two investigators report a high prevalence of *Mycoplasma* in ill Gulf War veterans and a third (using an insensitive technique and different criteria for case and control subjects) does not, further work should be done to evaluate and to standardize testing techniques, and to determine if high *Mycoplasma* positivity is confirmed in ill Gulf War veterans compared with healthy controls using sensitive, validated testing methods.

### Treatment of Veterans with Antibiotics

No controlled trials of antibiotics for illness in Gulf War veterans have been published to date. Nicolson and colleagues (1996) published a case series (uncontrolled) in which improvement of symptoms is reported in Gulf War veterans who were treated with antibiotics for a long time (doxycycline, azithromycin, ciprofloxacin, clarithromycin). There are few reports of success using other treatment strategies. Six-week treatment cycles with antibiotics have been used, reportedly because physicians had little experience with, and showed reluctance to treat with, longer courses of antibiotics. The data from Nicolson and Nicolson (1997) indicate essentially universal (100 percent) relapse after a single six-week course, with successively increasing recovery rates in subsequent cycles (Table 3.4). However, follow-up time after each cycle is not given, and it is possible that apparent “recovery” after later treatment cycles reflects insufficient follow-up time to detect the next “relapse.” Indeed the authors of this study speculated that the condition may not be cured but only controlled with antibiotics. *Mycoplasmas*, with their putative deep penetration into the cell (intracellular sightings have been made in eukaryotic cells from infected patients and tissue cultures using electron microscopy and anti-*Mycoplasma* antibodies (Baseman et al., 1995)) and their ability to evade host surveillance systems, are particularly difficult to eradicate. Concern exists that partial treatment may lead to antibiotic resistance.<sup>20</sup> Moreover, *Mycoplasmas* may reside in cells for six months despite antibiotic treatment.<sup>21</sup> Therefore, if *Mycoplasma* is confirmed to be present selectively in ill Gulf War veterans and if treatment trials are undertaken, longer treatment courses may be needed such as those used in successful antibiotic trials for rheumatoid arthritis (six months to a year).

---

<sup>20</sup>S. Lo, personal communication to Beatrice Golomb (1997).

<sup>21</sup>J. Baseman, personal communication to Beatrice Golomb (1997).

**Table 3.4**  
**Recovery of Ill Gulf War Veterans**  
**with Antibiotic Treatment**

# of Six-Week Antibiotic Treatment Cycles Completed	% Relapsing
1	100
2	89
3	73
4	50
5	27
6	19

SOURCE: Nicolson and Nicolson (1997).

Other physicians have also anecdotally reported benefit to ill veterans with antibiotic treatment (Ribas, 1996).<sup>22</sup> Many such veterans and their family members have reported marked resolution of chronic debilitating symptoms with antibiotic treatment.<sup>23</sup> To date, however, no controlled trials have been completed to confirm or refute the benefit of antibiotics. A large-scale, federally funded randomized controlled trial is presently under way and has enrolled all 450 subjects needed at the time of this printing.

### CORRELATION WITH GULF WAR ILLNESSES

Data are not available that conclusively support or refute *Mycoplasma* as a source of illness in Gulf War veterans. Factors favoring or consistent with infectious etiology of symptoms in ill Gulf War veterans from an organism such as *Mycoplasma* include variably delayed onset of illness, multiple organ system involvement, chronic symptomatology (*Mycoplasmas* are able to establish covert or overt chronic and persistent infections with concomitant activation of the immune system (Baseman and Tully, 1997)), putative communicability, purported response to antibiotics, and reported (but unsubstantiated and elsewhere contradicted (Asa et al., 2000)) worsening with steroid administration. If antibiotics were confirmed to produce marked attenuation of symptoms in even a portion of seriously ill veterans, this would constitute an important finding, since resolution of symptoms in ill veterans is the goal of greatest importance to these veterans and their families. For this reason, the *Mycoplasma* hypothesis merits serious scrutiny. (Note that symptom resolu-

<sup>22</sup>K. Leisure, personal communication to Beatrice Golomb (1997).

<sup>23</sup>Letters to Garth Nicolson from C. Hamden, P. Zalewski (1994); E. Geonetta, B. Harris, L. Millett, L. Roberts, R. Snyder, R. Toth (1996); Burnett and Burnett (n.d.).

tion does not confirm *Mycoplasma* as the etiologic agent; other infections responding to the antibiotic could theoretically be culpable.)

Moreover, if *Mycoplasma* (or other unidentified infectious bacterial disease) is construed as an unlikely source of illness in ill Gulf War veterans, testing is a fortiori important, because veterans are currently seeking treatment with antibiotics. If antibiotic treatment is ineffective, veterans should have the benefit of high-quality data demonstrating lack of efficacy. Because *Mycoplasma* infections are difficult to eradicate and *Mycoplasmas* survive long-term recommended antimicrobial treatment in humans and in tissue cultures (Baseman and Tully, 1997), consideration should be given to a long treatment trial to enhance likelihood of eradication and to minimize development of antibiotic resistance resulting from partial treatment. The postulated intracellular location of the organism and the extreme duration of therapy that led to initial success in rheumatoid arthritis cases might be construed as favoring quite a long duration of therapy, perhaps six months to one year, in a treatment trial. Consideration should be given, during such long-term treatment, to incorporating strategies to maintain intestinal flora and reduce risk of intestinal fungal overgrowth. (As mentioned above, since this report was reviewed and this recommendation generated, a multisite randomized trial of long-term antibiotic versus placebo in ill Gulf War veterans testing positive for *Mycoplasma* has been initiated and is now well under way.)

## SUMMARY

*Mycoplasma* has been postulated as an etiology for illness in Gulf War veterans. Although some have proposed anthrax vaccine contamination, this appears unlikely, because *Mycoplasma* growth requirements may be incompatible with the sterile vaccine medium and the preservatives (formaldehyde and benzethonium chloride) included in the vaccine, although the possibility of vaccine contamination cannot be excluded definitively. Investigators agree that *Mycoplasma* can be responsible for chronic multisystem symptoms. Although investigators disagree on whether ill Gulf War veterans have increased incidence of positive tests for *Mycoplasma*, more sensitive testing methods do appear to show increased rates. Experts agree in principle that *Mycoplasma* could be responsible for chronic symptoms such as those seen in ill Gulf War veterans, although other infections or noninfectious etiologies could be responsible instead or in addition. Definitive studies are currently under way to evaluate testing strategies to detect possible *Mycoplasma* infection.

Table 3.5  
Synopsis of *Mycoplasma* Studies in Gulf War Veterans

Author	Test System	Results	Considerations	Conclusions
Lo (1993) <sup>a</sup>	Antibodies to <i>M. fermentans</i> or <i>M. penetrans</i> , <i>M. genitalium</i> (27 sera tested)	1 positive for <i>M. fermentans</i> , 1 positive for <i>M. penetrans</i> , 5 positive for <i>M. genitalium</i>	Small sample size, identification of cases and controls problematic, lack of peer review, and methodology relatively insensitive	<i>M. fermentans</i> and <i>M. penetrans</i> are unlikely to play an etiologic role in Gulf War illness
Lo (1994) <sup>a</sup>	Antibodies to <i>M. fermentans</i> or <i>M. penetrans</i> , <i>M. genitalium</i> (151 cases from CCEP registry with non-CCEP Gulf War veterans as matched controls) using pre- and postdeployment stored sera. Positive antibody confirmed by Western Blot	<i>M. fermentans</i> : 10 cases and 10 controls positive pre- and post-deployment; 4 cases and 2 controls seroconverted. Adjusted OR = 2.3 (95% CI 0.4–13.1); <i>M. genitalium</i> : 56 cases and 56 controls positive pre- and postdeployment; 9 cases, 13 controls seroconverted; adjusted OR = 0.6 (95% CI 0.2–1.7); <i>M. penetrans</i> : 1 case and 1 control seroconverted	Not peer-reviewed; CCEP registration not a good classification; methodology relatively insensitive tests; cryopreserved specimens; pre- and postdeployment data provide distinct advantage; sample size may be inadequate because of estimates of positivity in population	No significant association between ELISA/Western Blot positivity for tested <i>Mycoplasma</i> species and being a CCEP participant
Nicolson, Nicolson, and Nasralla (1998)	Nucleoprotein gene tracking (NGT) used to test for <i>Mycoplasma</i> in ill Gulf War veterans	NGT positive lymphocytes in 45% (76/170) of those with Gulf War illness who "loosely fit clinical criteria for Chronic Fatigue Syndrome with Fibromyalgia"; nondeployed healthy controls positive in less than 5% (2/41)	Cases and controls poorly defined, NGT not yet shown to be reliable or valid, trial is nonrandomized, unblinded	Treated patients recovered following several 6-week cycles and reverted to <i>Mycoplasma</i> negative; multiple treatment cycles required for response to treatment
See (1997) <sup>b</sup>	PCR used to test for <i>Mycoplasma</i> in ill Gulf War veterans	14/20 (70%) tested positive for <i>Mycoplasma</i> genetic material	Unpublished, non-peer-reviewed data, limited sample size, PCR is a sensitive and proven method for detection	Possible high rate of positivity for <i>Mycoplasma</i> using PCR

<sup>a</sup>As reported in Ribas (1996). <sup>b</sup>D. See, personal communication to Beatrice Golomb (1997). OR = odds ratio. CI = confidence interval.