



NEW ANTIBODIES DISCOVERED IN GULF WAR SYNDROME PATIENTS

FOR IMMEDIATE RELEASE

New Orleans, January 31, 2000 - Autoimmune Technologies LLC, a New Orleans biomedical company, today announced the publication of a research study that found anti-squalene antibodies in a very high percentage of Gulf War Syndrome patients. The study, by Drs. P. B. Asa, Y. Cao and R. F. Garry, appears in the February 2000 issue of *Experimental and Molecular Pathology*.

The antibodies were discovered at Tulane University Medical Center. The blood test that detects the antibodies is called the Anti-Squalene Antibody Assay, or ASA Assay. Tulane has a patent pending on the ASA Assay, and Autoimmune Technologies has licensed the rights to the ASA Assay from Tulane.

Gulf War Syndrome, or GWS, is an illness that affects many veterans of the Persian Gulf war. Symptoms include muscle aches and pains, chronic fatigue, unexplained rashes, and other symptoms. The illness is not well understood, it is difficult to diagnose, and its cause is unknown. Until now, there has been no laboratory test that could serve as a biomarker for GWS. The authors of the study developed the ASA Assay in order to determine whether the presence of anti-squalene antibodies correlates with the presence of signs and symptoms of GWS.

The ASA Assay measures the binding of a human serum immunoglobulin known as IgG to squalene. Squalene is a lipid that occurs naturally in humans, animals and plants. Squalene is normally found in cell membranes in humans and is one of the building blocks for producing cholesterol. Squalene within cell membranes is not typically recognized by the human immune system because it is sequestered or hidden within the membrane. However, injuries to tissues and cells routinely produce free squalene. The released squalene may serve as a signal for tissue damage and may stimulate immune responses, but the release of squalene from damaged tissue apparently does not normally trigger the production of antibodies to squalene. Because it does have the ability to stimulate the immune system, squalene has been incorporated as an adjuvant in several experimental vaccines in an attempt to boost the immune response to a variety of weak vaccines, such as those for HIV.

The research now being published included both blinded and unblinded studies. In the blinded study, the ASA Assay was used to test blood samples from 56 individuals who were in active military service or who were civilian employees of the U.S. armed forces or their contractors during 1990-1991. Most, but not all, of the members of this group were actually deployed to the Persian Gulf theater of operations. The group comprised 38 deployed individuals who were ill, 12 deployed individuals who were healthy, and 6 non-deployed individuals who were ill. The results of the blinded study showed that 95% of the deployed sick individuals tested positive, none of the deployed healthy individuals tested positive, and 100% of the non-deployed sick individuals tested positive for anti-squalene antibodies. The study notes that all six of the sick non-deployed individuals had received the full complement of immunizations given to those who were deployed to the Gulf theater.

In the unblinded study, the ASA Assay was used as a screening tool to gather further data. Blood samples from 86 additional individuals who were in active military service or who were civilian employees of the U.S. armed forces or their contractors during 1990-1991, including healthy individuals, were tested, and 69% of them tested positive. Because squalene is used as an ingredient

in some cosmetics, 48 samples from blood banks were tested to see if the antibodies were present in a larger segment of the general population. Of these, only 5% tested positive. To see if the antibodies were a marker for other autoimmune disease processes, 40 samples from patients with systemic lupus erythematosus were tested. Of these, only 10% tested positive. Because patients with chronic fatigue syndrome have many symptoms similar to those of Gulf War Syndrome patients, 30 chronic fatigue patients were tested. Of these, only 15% were positive.

The research also included a small adjunct study in which two individuals who had previously volunteered to participate in a vaccine trial in which squalene was an adjuvant in the vaccine were tested for the presence of anti-squalene antibodies. Both subjects tested positive. These two were the only patients in the research group who had a known exposure to squalene from vaccines.

The research paper also discusses some of the work with anti-cholesterol antibodies that has been published by Dr. C. R. Alving of the Walter Reed Army Institute of Research.

The conclusion reached as a result of this research study is that most patients in the study groups who are ill with Gulf War Syndrome have serum antibodies to squalene while most other people do not. The clinical significance of the presence of the antibodies, however, is still not known, and while it is possible that the antibodies play a role in the disease process itself, the study does not explore the mechanisms involved in developing the antibodies.

Dr. Russell B. Wilson, president of Autoimmune Technologies, said "We don't know what caused the immune system to produce anti-squalene antibodies in the Gulf War veterans, but this study shows that the antibodies are indeed there. Dr. Simon Wessely and his colleagues demonstrated last year (*Lancet* 1999; 353: 169-78) that vaccines may be involved with GWS, and it seems entirely possible that a vaccine could be associated in some way with the production of anti-squalene antibodies. The Department of Defense has said that squalene was not used as an adjuvant in any vaccines administered to the typical Gulf War veteran. Therefore I don't think that the antibodies are the result of reactions to squalene added to vaccines, though that possibility must still be formally ruled out. This then raises the question of how a vaccine that does not contain squalene might stimulate the production of anti-squalene antibodies. One very interesting possibility is that some component of one or more of the various vaccines administered to Gulf War veterans recruits naturally present squalene and causes the immune system to recognize it and generate antibodies to it. Dr. Alving's work studying the production of anti-cholesterol antibodies following vaccinations suggests that such a process may occur."

Dr. Alving demonstrated in a paper published in 1986 (*Chem. Phys. Lipids* 40: 303-314, 1986) that anti-lipid antibodies can be induced by vaccinating with the adjuvant "lipid A," a bacterially-derived lipid. Dr. Alving also showed that lipid A recruited naturally occurring cholesterol and stimulated the production of anti-cholesterol antibodies (*Crit. Rev. Immunol.* 10: 441-453, 1991). Squalene is structurally very similar to cholesterol and is used by the body to form cholesterol. Both of these substances are lipids. A vaccine made with whole bacteria would contain all parts of the bacteria, including the lipids and lipo-proteins, and it is possible that one or more of these may mimic the action of lipid A in stimulating the production of anti-lipid antibodies. "Dr. Alving's work points out the possibility that, through a process similar to the one involving the inducement of anti-cholesterol antibody production by lipid A, one or more of the components of a bacterially-derived vaccine might recruit endogenous squalene and induce the development of anti-squalene antibodies," Dr. Wilson said.

In interpreting the results of the research now being published, Dr. Wilson noted that anti-squalene antibodies apparently serve as a laboratory marker for Gulf War Syndrome.

In addition, Dr. Wilson noted that GWS exists in both deployed and non-deployed veterans and that in the research study the presence of anti-squalene antibodies correlated dramatically with the existence

of GWS regardless of whether the veterans with GWS had deployed or not. Thus the study strongly suggests that Gulf War Syndrome is not caused by something that took place only in the Persian Gulf geographical area. This data fits the vaccine theory advanced by Dr. Wessely and others.

Dr. Wilson also said that the human immune system normally works very hard - and very successfully - in determining which of the millions of substances within the body are "self" and "non-self" so that it doesn't begin to make antibodies to its own tissues. When the controls break down and the body does attack itself in this way, the resulting condition is usually called an autoimmune disease. Autoimmune diseases include lupus, rheumatoid arthritis, and others. "GWS has many of the characteristics of an autoimmune disease, and the presence of anti-squalene antibodies in GWS patients and the nature of the symptoms suggest that Gulf War Syndrome may in fact be an autoimmune disease," he said.

Autoimmune Technologies is Tulane's exclusive licensee for the ASA Assay. The company has not made the test available to the physicians of individual GWS patients in the past, and it plans to continue that policy for the time being. Michael D. Charbonnet, the firm's LLC Manager, said, "The scientific review of the importance of anti-squalene antibodies in Gulf War illness is just beginning, but we think it will proceed quickly. For example, we understand that the Department of Defense has already funded a study to further investigate the detection of anti-squalene antibodies in human serum. When the benefits of running the test become clear to everyone involved, we will immediately make it available."

For further information, please visit www.autoimmune.com.

Contact: Autoimmune Technologies, LLC, 1010 Common Street, Suite 1705, New Orleans, Louisiana 70112 USA, 504-529-9944. Russell B. Wilson, Ph.D., Chief Science Officer, RBW@autoimmune.com, or Michael D. Charbonnet, CEO, MDC@autoimmune.com.

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