Investigation of Novel Organism Implicated in Morgellons Disease

Introduction and Background:

Morgellons disease was first described 1674 and the term “Morgellons” was later reintroduced in 2002 to define an undiagnosed mysterious illness that afflicts tens or more likely hundreds of thousands of individuals in the United States and around the world. The manifestations of the disease are consistent between sufferers in different parts of the world with one uniform clinical sign and that is the appearance of fibers of different colors that protrude out of the skin in different parts of the body. In addition to the appearance of fibers, patients describe painful deep lesions under the skin, rashes or sores, chronic fatigue, mental confusion, brain fog, memory loss, crawling, biting and stinging sensations and other psychological symptoms that vary in severity between sufferers.

To this day, the medical community remains divided on the definition of Morgellons disease and many still believe that it is a mental illness or Delusions of Parasitosis despite the availability of substantial compelling evidence that it is a real devastating disease that afflicts unknown numbers of humans (and possibly animals) around the world. It was not until recently that the CDC acknowledged that this is a real disease with an unknown etiology. They stopped short of calling it a contagious disease because little is known about the epidemiology of the disease.

Some of Morgellons sufferers describe an environmental exposure incident that preceded the onset of symptoms and others describe exposure to an individual that has the disease. This disease is an infectious disease in every sense of the word and has been reported to spread in households. The mode of transmission is not yet confirmed although there are reported cases of transmission through blood transfusion and others through physical contact with someone showing symptoms.

The symptoms are debilitating and cause a great deal of morbidity. In many cases, patients become disabled because of this disease, unable to carry on with their lives, lose their jobs, homes and can no longer afford the basics that we take for granted. Even worse than material loss is the social impact, rejection, family breakdown and mistreatment these patients receive from some members of the society and medical community.

The Morgellons Foundation, founded by a scientist and a mother of an infected child and led by a number of experienced clinicians and professionals has been making a tremendous outreach effort to educate the public and medical research community about this debilitating disease. Today, there are more than 10,000 families worldwide registered on the Morgellons Foundation website.

A CDC task force was formed in 2006 to investigate the epidemiology of Morgellons, and a study protocol is being developed to launch the investigation. We learned about the disease a year ago from a sufferer and started a preliminary investigation in 2006. The descriptions of the disease indicate that this is a systemic disease that has pulmonary, urinary tract, digestive tract, skin and neurological involvement. The complexity of the symptoms and the lack of a consistent clinical profile among patients makes it very difficult to identify the causative agent(s) especially that no one, up to date was able to isolate, culture or identify the origin of the fibers that many have photographed. A sizable library of images collected over the years showing auto fluorescence under different wavelengths, electron microscopy images and other high resolution images of the

fibers and skin lesions is posted on the web on different websites. We have collected in our laboratory a number of images of unstained fibers showing auto fluorescences with both the blue and red filters of an epifluorescent microscope. It is clear that a thorough investigation is in order to first identify the causative agent of this disease and second to possibly study this organism *in vitro* if it can be cultured in a laboratory environment in order to determine drug effectiveness and a possible treatment strategy.

Research Approach:

Several investigators have been attempting to study the fibers isolated from Morgellons patients. Biochemical studies as well as extensive microscopy studies have been carried out. The information available today is limited and does not provide an answer to what the causative agent of Morgellons could be.

Although we truly believe that all efforts to investigate this disease are of significance, we take a straightforward approach to the problem and our focus is on identifying the organism using genetic methods and tools available in our laboratory.

Based on the structures that we observed microscopically from a number of Morgellons patients and the clinical profiles, we have reasons to believe that this organism is not a virus or bacteria. We hypothesize that this organism is a more complex fungus, algae or a novel parasite. The fibers are most likely feeding structures as they have strong resemblance to aerial hyphae observed in many fungal species. Our research is focused on genetic investigations of the DNA in lesions and fibers. Our experiments will include assays that attempt to amplify any bacterial sequences and identify them by DNA sequencing if present to rule out or confirm that the organism is a bacteria as other investigators have hypothesized.

We believe that progress can be made using a genetics approach. Specific Aims:

1. To identify this organism (or organisms) on a genetic level based on DNA sequence. Determining the genus and species would allow us to gain more insights into the biology of this organism and therefore would facilitate further steps in this investigation.

2. To establish a cDNA library from the fibers, clone the individual cDNAs into DNA vectors and sequence at least 1000 colonies to gather as much sequence information as possible. Once that aim is accomplished, the sequences can be analyzed against published sequences to establish an identity of this organism.

3. To culture this organism in the laboratory using enriched cell culture media under conditions that are used in standard cell culture laboratories. Once that is achieved, we will be able to study the morphology and learn more about biochemical processes involved in metabolism and toxicity to the host.

4. To investigate drug resistance. This can be accomplished using different drugs used for organisms in the same class as the causative agent of Morgellons disease.

Experimental approach to Specific Aims:

1. Clongen Labs use universal primers for identification of bacteria and fungi by DNA sequencing. These procedures are offered as a service to our clients and the assays are established and are performed on a regular basis. These primer sets amplify regions of the DNA that are conserved among eubacteria or fungi. Once a DNA product is obtained, we sequence it using our in house sequencers and blast the results against published sequences to obtain an identification of the organism. We have been able to successfully amplify a conserved fungal region from fibers obtained from a Morgellons patient and will sequence the product soon to gather more information about the possible cause of this disease. We have four different primer sets that amplify four known conserved regions in the fungal genomes and will use all four to attempt amplification of DNA extracted from Investigation of Novel Organism Implicated in Morgellon's fibers and lesions received from Morgellons Patients. Our goal is to test at least 15 samples from different patients and compare the findings.

2. Establishment of a cDNA library will be performed according to standard molecular biology protocols and using commercial kits that are optimized to produce the most comprehensive cDNA libraries. Cloning will be carried out into a small DNA vector using TA cloning. Clones will be analyzed separately for sequence of the inserts and sequences will be gathered and analyzed against published sequences on different public databases.

3. Using our cell culture facility, we will use different media formulations in a 24 well format to culture the organism from a fresh sample in the laboratory in a cell culture incubator in the presence of 5% CO2. If successful, *in vitro* studies of the structure, metabolism and cell toxicity of the organism will be possible.

4. Once the above aims have been accomplished, it will be possible to study drug resistance *in vitro* using different drug formulations available commercially.

Background on Clongen Labs.: The company was founded and registered in 1999 in San Francisco , CA . The laboratory operations started in 2004 in Germantown , MD when the current laboratory facility was built. We are a CLIA-certified laboratory and licensed by the Maryland Office of Health and Mental Hygiene as a Clinical Laboratory. Our business is divided into main divisions:

Clinical Diagnostics and Contract Research. We offer clinical testing to patients (hospitals, medical centers, private physicians and other reference labs) and we also work with the leading pharmaceutical companies in the US and Europe , government agencies, Universities, research institutions and other private organizations. We have extensive expertise in Molecular Biology research and our laboratory is equipped with state of the art equipment (6 real time PCR machines, 6 conventional PCR machines, gel imaging equipment, 14 incubators, 2 DNA sequencers, 5 microscopes of different configurations and microscope camera, and over 150 other pieces of equipment).

Dr. Kilani holds a B.S. in Medical Technology (JST, 83-88), M.S. in Clinical Science (SFSU, 88-91) Ph.D. in Infectious Diseases and Immunity from UC Berkeley (1994-1999) and did his postdoctoral training from 1999-2001 at Stanford University Medical School, Microbiology Program. He has been working in the field of Molecular Diagnostics since.