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## *Agrobacterium* & Morgellons Disease, A GM Connection?

*Preliminary findings suggest a link between Morgellons Disease and Agrobacterium, a soil bacterium extensively manipulated and used in making GM crops; has genetic engineering created a new epidemic?*

[Dr. Mae-Wan Ho](#) and [Prof. Joe Cummins](#)

### CDC launch investigation on Morgellons' Disease

The Centers for Disease Control (CDC) in the United States announced the launch of an investigation on 'Morgellons Disease' in January 2008 [1], after receiving thousands of complaints from people with this bewildering condition, which it describes as follows [2]: "Persons who suffer from this unexplained skin condition report a range of cutaneous (skin) symptoms including crawling, biting and stinging sensations; granules, threads, fibers, or black speck-like materials on or beneath the skin, and/or skin lesions (e.g., rashes or sores). In addition to skin manifestations, some sufferers also report fatigue, mental confusion, short term memory loss, joint pain, and changes in visions."

Morgellons Disease first became known in 2001, when Mary Leitao created a web site describing the illness in her young son, which she named after a 17th century medical study in France describing similar symptoms [3]. Until then, people with Morgellons Disease have been diagnosed as cases of "delusional parasitosis", in which the symptoms are deemed entirely imaginary, and lesions allegedly due to self-inflicted wounds.

Indeed, the debate over Morgellons Disease has continued in the pages of medical and scientific journals right up to the CDC's announcement [4-7]

Dr. Michele Pearson, principal investigator for the CDC said [1] that the primary goals of the study are "to learn more about who may be affected with this condition, the symptoms they experience and to look for clues about factors that might contribute to the condition," adding that the condition is "complex", and "may be due to multiple factors."

In response to questions from journalists at the CDC press conference, Pearson said:

" We are aware that many patients have suffered from this condition. And, I can tell you that here at CDC, we have really been seeing an increasing number of these reports over the past year or so."

CDC's investigation is to be carried out in conjunction with Kaiser Permanente's Northern California Division of Research and the US Armed Forces Institute of Pathology.

Dr. Joe Selby, Director of the Kaiser Permanente's Northern California Division of

Research, said the study would proceed in three stages. In the first stage, they will identify all members who may have seen a Kaiser Permanente physician with symptoms suggestive of this condition at any point during the 18 months between July 1 2006 and December 31, 2007, and determine whether they meet eligibility criteria for the study. In stage two, all eligible members will be invited to complete a comprehensive web based or telephone survey conducted by the CDC that examines the duration and severity of a variety of symptoms. And in stage three, those with active symptoms will be invited to the division of research for an extensive clinical examination including collection of skin biopsies, blood and urine samples.

In a paper [6] published in 2006, researchers from the Morgellons Research Foundation [3] identified the states of California, Texas and Florida as having the highest number of cases of Morgellons disease in the United States. Primary clusters were noted in Los Angeles and San Francisco (California) and Houston, Dallas and Austin (Texas). California accounted for 26 percent of cases in the US, but all 50 US states and 15 other nations, including Canada, the UK, Australia, and the Netherlands, have reported cases of Morgellons disease. The two main occupational groups reporting symptoms are nurses and teachers, with nurses outnumbering teachers three to one. The risk factor common to both groups is suspected to be the possibility of transmitted infectious agents.

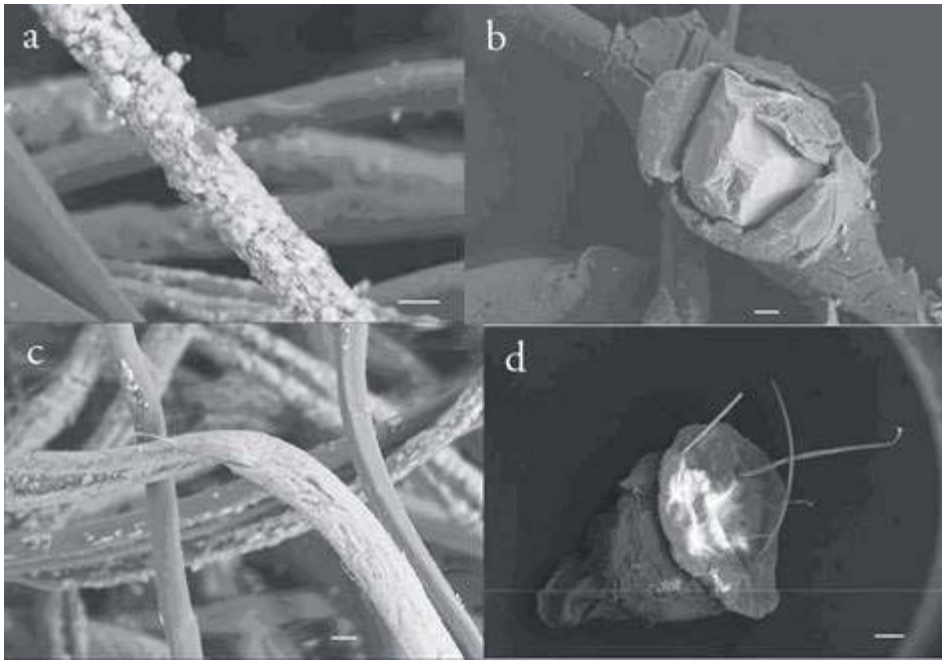
Skin lesions and fibres may not be readily apparent in all individuals with the disease, as family members of patients often report similar systemic disease symptoms without skin symptoms. Families in which all members are affected often have suspected simultaneous exposure to an inciting agent. Contact with soil or waste products appears to be associated with the disease. Cases have been reported in cats and dogs, as well as horses.

What finally prompted CDC to investigate the disease? The Morgellons Research Foundation [3] was set up in 2002 in honour of Mary Leitao, the Foundation's executive director. It publicises the plight of patients with similar conditions and operates a registry of afflicted families. The Foundation also funds scientific research. It has a Medical Advisory Board of seven with M.D. degree and two with nursing degrees. In addition, it has a Board of Nursing with five other nurses, and a Scientific Advisory Board of six scientists, all with Ph.D. degree; one of which is Vitaly Citovsky. It may have been Citovsky's discovery last year that finally persuaded the CDC to announce an investigation.

### The *Agrobacterium* connection

Vitaly Citovsky is a professor of molecular and cell biology at Stony Brook University in New York (SUNY). He is a world authority on the genetic modification of cells by *Agrobacterium*, a soil bacterium causing crown gall disease in plants, that has been widely used in creating genetically modified (GM) plants since the 1980s because of its ability to transfer a piece of its genetic material, the T-DNA on its tumour-inducing (Ti) plasmid to the plant genome (see later for details).

Citovsky's team took scanning electron microscope pictures of the fibres in or extruding from the skin of patients suffering from Morgellons disease, confirming that they are unlike any ordinary natural or synthetic fibres (see Fig. 1, assembled from Citovsky's website [8]).



*Figure 1. Scanning electron microscope images of fibres from skin biopsies of patients with Morgellons Disease - a, white fibre with calcite, scale bar 10  $\mu$ m; b, green fibre with alumina 'rock' protruding, scale bar 20  $\mu$ m; c, various ribbon-like, cylindrical and faceted fibres all coated with minerals, scale bar 10  $\mu$ m; d, skin lesion with fibres stabbing through the epidermis, scale bar 300  $\mu$ m*

They also analysed patients for *Agrobacterium* DNA. Skin biopsy samples from Morgellons patients were subjected to high-stringency polymerase chain reaction (PCR) tests for genes encoded by the *Agrobacterium* chromosome and also for *Agrobacterium* virulence (*vir*) genes and T-DNA on its Ti plasmid. They found that "all Morgellons patients screened to date have tested positive for the presence of *Agrobacterium*, whereas this microorganism has not been detected in any of the samples derived from the control, healthy individuals." Their preliminary conclusion is that "*Agrobacterium* may be involved in the etiology and/or progression" of Morgellons Disease.

The unpublished findings have been posted on a website [8] since January 2007. They were further publicized in the "first ever" Morgellons conference in Austin Texas, attended by 100 in March 2008 [9]. A growing list of people are registered with Morgellons Disease, totalling 12 106 worldwide recorded by Morgellons Research Foundation [3], as of 12 April 2008.

San Francisco physician, Raphael Stricker, one of only a few doctors who believe the disease is real, said [9]. "There's almost always some history of exposure to dirt basically either from gardening or camping or something." He is one of the co-authors on the *Agrobacterium* research done in SUNY, which reported finding *Agrobacterium* DNA in all 5 Morgellons patients studied. Stricker suggests it is transmitted by ticks, like Lyme disease, and in a recent survey of 44 Morgellons patients in San Francisco, 43 of them also tested positive for the bacterium causing Lyme disease. Another factor consistent with *Agrobacterium* being a causative agent, if not *the* causative agent, is that when patients are treated with antibacterials for their Lyme disease, remission of Morgellons symptoms is seen in most of them [6].

Stricker also told his audience that *Agrobacterium* lives in the soil, and is known to cause infections in animals and human beings with compromised immune systems. It can cause skin lesions when injected into Swiss mice, a strain that is immune deficient, he said.

At this point, the findings on the *Agrobacterium* connection are still preliminary, as only seven patients have been studied. Nevertheless, the implications are far-reaching if this connection is confirmed, as existing evidence (reviewed below) suggests a link between *Agrobacterium* and genetic engineering in the creation of new disease agents, and it is paramount for the CDC investigation to include this aspect, if only to rule it out.

### *Agrobacterium* and the genetic engineering connection

*Agrobacterium* not only infects human and other animal cells, it also transfers genes into them. It was SUNY professor Citovsky and his team that made the discovery some years ago [10]. Until then, the genetic engineering community had assumed that *Agrobacterium* did not infect animal cells, and certainly would not transfer genes into them.

*Agrobacterium* was found to transfer T-DNA into the chromosomes of human cells.

In stably transformed HeLa cells, the integration occurred at the right border of the T-DNA, exactly as would happen when it is being transferred into a plant cell genome, suggesting that *Agrobacterium* transforms human cells by a mechanism similar to that involved in transforming plants cells (see Box 1). Human cancer cells, neurons and kidney cells were all transformed with the *Agrobacterium* T-DNA. Commenting on this research in 2001, Joe Cummins had warned of hazards to laboratory and farm workers [11] ([i-sis news11/12](#))

### The *Agrobacterium* vector system for gene transfer

Since the discovery in the 1970s that *Agrobacterium* can transfer genes into plants causing crown gall disease, the soil bacterium has been developed into a vector for inserting desirable genes into the plant genome to create transgenic (GM) plants [12].

*Agrobacterium* transfers T-DNA – a small region of approximately 5 to 10 percent of a resident tumour-inducing (Ti) or root-inducing (Ri) plasmid – into numerous species of plants; and as later turns out, also to fungi, algae, and even animal and human cells [13, 14] (see main text).

Transfer requires three major elements [13]: T-DNA border direct repeat sequences of 25 base pairs that flank the T-DNA and delineate the region transferred into the host, the virulence (*vir*) genes located on the Ti/Ri plasmid, and various genes on the bacterial chromosome. Plant genes are also involved in the successful integration of T-DNA [15]. The T-DNA contains oncogenes (cancer genes or gene for forming tumours) and genes for synthesizing opines; none of which is essential for T-DNA transfer, so they can be deleted and replaced with genes of interest and selectable markers.

Furthermore, the *vir* genes and T-DNA region need not be on the same replicating plasmid. This gave rise to the binary vector systems in which T-DNA

and the *vir* genes are located on separate replicating units. The T-DNA containing unit is the binary vector and contains also the origin(s) of replication that could function both in *E. coli* and *Agrobacterium tumefaciens*, and antibiotic resistance marker genes used to select for the presence of the binary vector in bacteria. The replicating unit containing the *vir* genes is the 'helper' plasmid. Strains of *Agrobacterium* harbouring the two separate units are considered 'disarmed' if they do not contain oncogenes that could be transferred to a plant.

The association of Morgellons Disease with dirt and soil where *Agrobacterium* lives, the widespread use of *Agrobacterium* in genetic engineering of plants, and the ability of *Agrobacterium* to infect human cells, all point towards a possible role of genetic engineering in the aetiology of Morgellans disease via *Agrobacterium*.

Extensive genetic manipulation of *Agrobacterium* does have the potential to transform it into an aggressive human pathogen. Genetic engineering is nothing if not enhanced and facilitated horizontal gene transfer and recombination, which is widely acknowledged to be the main route for creating new pathogens. Mae-Wan Ho was among an international panel of scientists have raised this very issue in 1998, calling for a public enquiry into the possible contributions of genetic engineering biotechnology to the aetiology of infectious diseases which has greatly increased since genetic engineering began in the 1970s [16].

The epidemiological data of Morgellons Disease are very incomplete, and the Morgellons Research Foundation's registry of more than 12 000 families afflicted worldwide is almost certainly only a fraction of the emerging epidemic. Still, it is significant that the majority of the cases are in the United States, the first country to release GM crops and remaining the top producer ever since.

There are other findings implicating *Agrobacterium* in transgenic plants released into the environment, particularly during the early years of field trials, when knowledge was poor and safety measures not as stringent as they may be today.

### *Agrobacterium* persists in transgenic plants and is a vehicle for gene escape

By the late 1990s, the *Agrobacterium* vector system became very widely used, and many GM crops created were commercially released.

Scientists at the Kinsealy Research and Development Centre in Dublin, Ireland, and the Scottish Crop Research Institute in Dundee, Scotland, were concerned that the inserted genes in plants would spread to wild populations by cross-pollination or by horizontal gene transfer to unrelated species, which was by then well-documented in the scientific literature.

They considered it "imperative" to address the risk posed in using *Agrobacterium* as a tool in genetic engineering [17], given its ability to transfer genes to plants. The transformation procedure involves inoculating the cells or tissue explants with *Agrobacterium* and co-cultivation the plant cells and bacterium for a short period, followed by the elimination of the bacterium with antibiotics.

However, if all the bacteria were not eliminated, then "release of these plants may also result in release of the *Agrobacterium* [with the foreign genes]", which will serve as a vehicle for further gene escape, at least to other *Agrobacterium*

strains naturally present in the soil.

Although various antibiotics have been used to eliminate *Agrobacterium* following transformation, the researchers stated that "very few authors actually test to ensure that the antibiotics succeed."

The difficulty is compounded because the bacterium can remain latent within the plant tissue. So putting transgenic plant material into culture medium without antibiotics and finding no *Agrobacterium* is no guarantee that the transgenic plant is free of the bacterium, as was often assumed.

In their study, they investigated the ability of antibiotics to eliminate *Agrobacterium tumefaciens* after transformation in three model systems: *Brassica* (mustard), *Solanum* (potato), and *Rubus* (raspberry). The antibiotics carbenicillin, cefataxime and ticarcillin were used respectively to eliminate the bacterium at four times the minimum bactericidal concentration, as recommended. They found that *none* of the antibiotic succeeded in eliminating *Agrobacterium*.

The contamination levels increased from 12 to 16 weeks to such an extent that transgenic *Solanum* cultures senesced and died. Contamination in shoot material decreased over 16 to 24 weeks possibly because only the apical node was used in further culture, but even that did not eliminate *Agrobacterium* from all the samples; 24 percent remained contaminated at 24 weeks.

The binary vector was also present under non-selective conditions up to 6 months after transformation, where approximately 50 percent of contaminated material still harboured bacterial cells with the binary vector at high levels of about  $10^7$  colony forming units per gram. The researchers pointed out: "Here is where the possibility of gene escape arises. The presence of the disarmed *Agrobacterium* in the tissue would not be a problem if the binary vector had been lost, but now its survival and spread are real possibilities." The binary vector contains the foreign genes as well as antibiotic resistance marker gene(s).

There is no limit to the foreign genes that can be inserted into the binary vector. A few years earlier, a research group in Israel had inserted a viroid that causes disease in citrus fruits into the disarmed Ti plasmid of *Agrobacterium* and used that to infect and transform several plant species including tomato (*Lycopersicon esculentum*) *Gynura aurantiaca*, avocado (*Persea americana*), and grapefruit (*Citrus paradisi*) grafted on Troyer citrange (*Pancirus trifoliata* x *C. sinensis*) [18]. Extracts prepared from tissues of the infected plants 38-90 days after inoculation were plated on selective media and found to contain large amounts of the engineered bacteria.

The researchers warned of "newly formed combinations of persistently transmitted viruses" coupled with "the opportunistic and systemically moving *Agrobacterium* vector infectious to a wide host range might eventually cause infection and damage to crop plants or natural vegetation" that are "not presently visited by the traditional vectors of the virus disease."

In other words, *Agrobacterium* persisting in transgenic plants released into the environment has the potential to spread new diseases, and to plants that normally would not be infected by the disease agents. At the time, the researchers did not know that *Agrobacterium* would also infect animals and humans, and could spread new diseases to them as well.

Have these warnings been heeded by other researchers? There is no evidence they have been taken on board. *Agrobacterium* has since been shown to transform at least 80 different non-plant species including yeasts and other fungi, algae, mammalian and human cells, also the gram positive bacterium *Streptomyces lividans*. In a recent review, the researchers stated [14]: "Future research has to show whether *Agrobacterium*-mediated transformation contributed to horizontal gene transfer between microorganisms in the rhizosphere."

But there is already evidence suggesting that *Agrobacterium* can indeed engage in horizontal gene transfer with a wide range of bacteria in the soil. (For more on horizontal gene transfer see [19] [Horizontal Gene Transfer from GMOs Does Happen](#), *SiS* 38)

### *Agrobacterium* gene transfer mechanisms similar to conjugation in bacteria

Ho first alerted regulators to the potential of *Agrobacterium* contaminating GM plants to facilitate the escape of transgenes in 2003 (see [Living with the Fluid Genome](#) [20] and [The Case for A GM-Free Sustainable World](#) [21] ISIS publications). By then, Gayle Ferguson and Jack Heinemann at the University of Canterbury, Christchurch, New Zealand, had already pointed out in a review that the process whereby *Agrobacterium* injects T-DNA into plant cells strongly resembles conjugation, the normal mating process between bacteria [22].

Conjugation, mediated by certain bacterial plasmids, depends on a sequence called the origin of transfer (*oriT*) on the DNA transferred. All other functions - called *tra* for trans-acting functions - can be supplied from unlinked sources. Thus, 'disabled' plasmids with no trans-acting functions, can nevertheless be transferred by helper plasmids, the same as the binary vector system of *Agrobacterium* (Box 1). The resemblance does not stop there.

The left and right borders of T-DNA are similar to *oriT* and can be replaced by it. Furthermore, the disarmed T-DNA binary vector, lacking oncogenes as well as virulence genes, *can be helped by similar genes belonging to many other pathogenic bacteria*. The trans-kingdom gene transfer apparatus of *Agrobacterium* and the conjugative systems of bacteria are both involved in transporting macromolecules, not just DNA but also protein.

Thus, transgenic plants with contaminating *Agrobacterium* [20] "have a ready route for horizontal gene escape, via *Agrobacterium*, helped by the ordinary conjugative mechanisms of many other bacteria that cause diseases, which are present in the environment." In the process, new and exotic disease agents could be created.

### Investigations on the role of *Agrobacterium* in Morgellons Disease urgently needed

The investigation launched by the CDC needs to clarify the role of *Agrobacterium* in the aetiology of Morgellons Disease as a matter of urgency. This should include:

- Molecular characterization of *Agrobacterium* DNA sequences in Morgellans

Disease patients

- Design of suitable probes for diagnostic purposes and for monitoring soil samples and other suspected sources of infection
- Introduction of stringent tests for *Agrobacterium* contamination for all transgenic plants already released or about to be released into the environment.

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