Moldy Buildings, CIRS, Sick People, and Damaged Brains
Part 1: Living in a Water-Damaged Building

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Imagine being trapped by your home, workplace or school. You can see the mold, smell the bacteria. You are living in a microbial dungeon. Deep down, you know the building is making you sick, but you can't leave when there is a lease, a mortgage, a job, or needed education dragging you back into the dangerous building. Trapped: you know that you can't stay either. Every day brings more symptoms, more brain fog and thoughts that living this kind of life just isn't worth it.

Finally, you leave your porous possessions behind and flee to a safe house (that is, one you thought was safe). But you don't get better. Removal does not bring resolution. Your illness isn't an allergy. Your chronic inflammatory response syndrome (CIRS) is based on never-ending dysregulation of gene transcription, both activation and suppression.1,2 Your genes relentlessly recruit more physiologic abnormalities: if the genes aren't fixed, you aren't fixed.

You seek out help, but what do you show the health care provider who tries to help you? How do you *prove* that you have fatigue, cognitive impairment, abdominal pain, and neurologic findings that change from day to day, in addition to twenty other symptoms? How do you show a third party your painful musculoskeletal problems when joints aren't red or warm, and sed rates are normal?

It doesn't take long to hear, "No one else has all these symptoms and all your standard tests are normal. Have you been under stress lately?" You kept searching to find a physician who might be able to know what is wrong with you. You had already been humiliated by a fistful of specialists who gave you meaningless diagnoses, others sold you worthless nostrums that had no chance of helping, and then there is the referral to a psychiatrist. And that guy didn't help you either. Yes, you are stressed, but so what? Who wouldn't be upset when your life, your brain, your marriage, or your job are lost? Not to mention the $50,000 you wasted on IVs and internet cures, all the while seeking your old self, the self you had before the wet building trapped you.

You bring the doctor photos of ugly-looking black patches on the walls or pictures of collapsed ceilings. "Here is the evidence," you exclaim. "OK, show me how these photos are transformed into causation of your illness?" Where is the irrefutable objective evidence? No one trying to help you knew that your evolutionarily conserved cell systems designed to make protein and create ATP for energy were

damaged by other descendants of evolutionary survivors whose ancestors waged war on other one-celled creatures 3 billion years ago.

Imagine a biological weapon that didn't kill people but disabled them instead. If the problem tragically is present in your child who attends a school where the custodial staff quickly put out buckets to catch the rainwater coming through the old flat roof Monday through Friday, what happens when you complain? The school district just cut funding for art classes to save money: there is nothing in the budget for a new roof this year. Besides, only a handful of kids say they are sick, and we think your home is the problem, not our school.

The trap gets worse, when you turn on the computer. Now you read breathless claims for cures for black mold, toxic black mold, spreading black toxic mold, and more; you risk being squeezed dry, bereft of your savings and hope. The Internet hype is just a sham. There is no science to be seen anywhere that confirms peer-review and evidence basis for magical cures of poultices, oils, diets, supplements and herbs. Yes, you were told to put some Petri dishes in your living room and let some mold settle from the air. Maybe something grows (so what?). Now breathe these special salt fumes (straight from Nepal!). Just look at these clear Petri dishes opened after the salt vapors are layered on! Just type in your credit card today!

You hear about lawsuits. Negligence caused the personal injury. The jury awarded millions! Except that *your lawyer* has no clue and wants a lot of money up front to open the case, and then lots more money to pay experts, commission reports, and schedule depositions. Be aware that the defense attorneys pool their experiences in countless mold cases; they will not be polite or gentle; get ready for more humiliation. Even if your case is heard in court and you win, you won't see any money until the attorney collects his fees and all his costs are paid.

It happens every day across the US. This scenario isn't written for the *Twilight Zone* or authored by Stephen King: it is happening as you read these words.

The Way Out of the Trap

In this special five-part series written for the *Townsend Letter*, I want to work with you as you start to learn from the published science:

1. What a chronic inflammatory response syndrome (CIRS) is and why you need to know a lot about your illness;
2. How you can confirm your illness is caused by exposure to a mixture of specific elements found inside a water-damaged building (WDB) and some non-specific elements too;
3. How you can use published, peer-reviewed protocols to define your illness and start to heal your damaged brain;
4. How you can quantitate physiologic parameters that demonstrate your deranged physiology and correct suppression of both ribosomal and nuclear

encoded mitochondrial genes (a) in support of the diagnosis of CIRS and (b) in support of ongoing salutary effects of treatment.

In 2019, we now use the "cure" word, albeit cautiously, but we can use it. After over 20 years of no cure, seeing a steady increase in number of cases return to normal is terrific. As yet, no one is guaranteed anything other than use of solid protocols, grounded in accepted science. Now that the magic of transcriptomics, differential gene activation, is available, we don't have to guess about treatment any longer. *And let all be informed: the problem is far more than mere mycotoxins.*

There is much you can do to help protect yourself. The most neglected expense needed for building health is maintenance. Even if WDBs aren't hurting you yet, exposure to damp indoor environments isn't healthy for anyone. If you are ill, then removal from exposure is job number one. You won't feel back to normal if you don't stop inflammation, the basic disease process of CIRS, from recruiting more genes to do bad things to your processes of antigen presentation, innate immune response, and metabolism.

First, begin by stopping assumptions. Second, stop guessing about what is wrong. Third, don't believe "expert" opinion in the absence of *rigorous* confirmation by published, peer-reviewed academic work, including case/control and prospective studies. Double-blinded, placebo-controlled studies would be nice.

No, your body and especially your brain can't afford any more inflammatory attacks made by an over-zealous innate immune system! The basic concept is that regaining your health begins by recognizing what objective biomarkers you have based on longstanding science, focus on correcting them, all the while casting out any false knowledge (with thanks to Aldous Huxley) that you might have picked up from the Wild, Wild West found on the Internet regarding the field of mold exposure and human health.

I want you to become familiar with the tools needed to show the skeptical physician a host of objective biomarkers that are found in cases of CIRS, but not in controls, that improve with therapy but not passage of time alone—tools that will demonstrate the transcriptomic basis of the illness and successful therapy. You will see what I mean when I say, "If you don't know the transcriptomics, you don't know the disease."

But first, what is a water-damaged building? Simply stated, water coming inside a building where it should not be, accompanied by amplified microbial growth, makes a building water damaged (WDB).

Water intrusion commonly affects buildings in the US. As many as 50% of our public buildings are water damaged.3 That is a huge number of buildings. Wet buildings are not safe buildings. What makes WDB unsafe is the growth of a group of single cell microbes invariably found in WDB that make specific compounds that can cause

inflammation (called inflammagens) or toxins made by bacteria (endotoxins or exotoxins), fungi (mycotoxins), mycobacteria (mycolactones) and actinomycetes. Add to the rogues' gallery of en-suite bad actors the very small cell wall fragments of fungi (beta glucans and mannans), fungal and bacterial enzymes/proteins (hemolysins, spirocyclic drimanes and proteinases), not to mention the result of each of these non-living elements acting synergistically, one with another. Is it any wonder that this indoor collection of inflammation causers can cause inflammation that hurts *some* of those exposed4?

I emphasize some because curiously, and fortunately, not everyone exposed becomes ill. And even more curiously, people with successfully treated illness will relapse with re-exposure unless they are protected by use of preventive medications. What is our protective antibody arm of the immune system doing? Sleeping? Could antigen detection and antigen presentation *both* be defective? These concepts will return in later discussion.

As we will discuss, it is inflammation that sets off more inflammation, uncontrolled like a runaway freight train without brakes, causing changes in gene activation (*NOT* allergy) that is the ultimate source of CIRS.

It is a basic tenet of real estate that landlords/building owners bear the responsibility to provide a safe indoor environment for users of that indoor space. Builders/sellers of buildings have a duty to provide buildings that are safe for new occupants. When an outdoor deck collapses, for example, throwing a group of wedding guests into a creek, an injured guest might claim that negligent construction caused the personal loss, pain, and suffering. Exchange of money might not make a person whole again, but the idea of compensation for caused injury applies.

Who is negligent if water gets indoors, foments growth of one-celled creatures, that then cause inflammation, creating a multisystem, multi-symptom illness that most docs don't know about? Who pays the plaintiff when causation of personal injury is confirmed in court?

As far as WDB goes, when water enters an enclosed space, and that space stays wet for as little as 48 hours, there will be microbial growth.5 While bacteria might be the first colonizers, fungi aren't far behind. Precisely what microbes grow is completely dependent on availability of moisture. We call this availability of water or water activity "A(w)." A(w) of 1.0 (or 100% relative humidity, RH) is open water compared to the water vapor pressure above the water. Bacteria and "wet" fungi, like *Chaetomium* and *Stachybotrys,* need a minimum A(w) of > 0.9 (>90% RH) to grow. "Medium wet" filamentous fungi, like *Aspergillus versicolor*, need A(w) of 0.8-0.9 to grow. "Drier" fungal organisms, including *Aspergillus penicillioides* need a minimum A(w) of 0.58-0.8 to grow. The dry (xerophilic) fungi, especially *Wallemia sebi*, like a range of A(w) that can go as low as 0.55 to 0.75,6 to grow.

Available water has relevance for all of us in the WDB field. Just look at your nasal mucus. It is full of water, yet mucus prevents growth of the vast percentage of potential pathogens that land in your nose as you breathe. Why doesn't every germ in the nose cause infection? Simple, the water needed for growth of bacteria and fungi *isn't bioavailable*. The mucopolysaccharide matrix prevents the water from nourishing fungi, for example. In the end, who cares if fungi are in nasal secretions? They won't make toxins or secondary metabolites without available water!
Cases of fungal sinusitis have 2.4 species of fungi cultured in mucus in their noses, but controls have 3.1 species cultured.7 Eighty-seven percent of all cases had positive cultures, but 91.3 % of controls also had positive cultures in a classic German report from 2003. Because "fungi can be identified in almost everybody's nose…when inhaled, these airborne fungi are only 'in transit' through the nose. Positive fungal cultures from nasal secretions have to be considered normal findings."7

If we only assay dust found in a WDB for fungal DNA, the presence of indicator fungal DNA tells us a lot about the building ecology as described by A(w). Just by looking at indicator DNA, we can get a solid idea what is abnormal in the ecology of a WDB that is making people sick.

Perhaps the most important organisms found in WDB are actinomycetes. No, not fungi, not mold, not black molds, not toxic black molds, but these filamentous bacteria are only recently becoming recognized in clinical dust samples as major players in adverse human health effects.8 If we add assays for endotoxins and actinomycetes to assays of fungal DNA, we can obtain a robust picture of the harmful microbes in a WDB.

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Part 2: Building to avoid water damaged buildings

How Does Water Get Inside?

ROOFS: Have you ever put on a new roof or watched someone else do the work? How many thousand nails are required to shingle a 3000 square foot roof (4 nails/shingle in low wind areas; 320 nails per 100 square feet)? If one in a hundred nail heads are exposed, that means 10 exposed/300 square feet. Not much room for error! Remember, it takes fewer than 10 exposed nail heads to create a significant risk for a leak.

How long does it take to get out the binoculars and look for exposed nail heads peeking out from under your shingles reflecting light on a sunny day?
How about flat roofs with special membranes? How long is the shelf life of a man-made material used to prevent gravity from finding a way for water to seep in through a pinhole defect in that membrane? Oops, not 30 years.

And look at the "boots" woven under shingles around the ventilation pipes that take moisture out of bathrooms to exhaust to the outside world? They range in price from $25 to $75. The cheap ones might last 10 years until they leak. Newer boots are rated at 50 years (is that with 20 nights of 40 mph winds per year or just 15?) but cost $75 each. How many builders use the $25 boots? Take a trip to your attic. Look at the attic side of your boots. Are there any moisture stains? Make sure all vent pipes go through the roof deck too.

Look at your ventilating soffits, inside the attic and out (binoculars time again). Is air flowing as expected through the soffit and out the ridge vent? If you have a chimney, can you see daylight between the edge of the roof and the chimney stack? If you see light, there will be water coming down the outside of the chimney inside your home. Time to flash and counter-flash. Or how about the chimney that is off center in a gable end of a house? Water coming down from the higher pitched areas will be directed against the flashing around the chimney. Time for a mini-roof ("cricket") used to deflect water away from the pocket created by siding touching flashing.
And for cold weather folks, what are those little iron things sticking up and out of the roof near the fascia board? Those snow dogs will help prevent ice dams, but all snow dogs eventually leak. Inspect the dogs and the attic below where ice dams might form. How about your attic insulation? See anything discolored?

WINDOWS: Windows turn out to be tricky to install. It is not surprising to find moisture from leaking windows in wall cavities below windowsills, especially in new construction. As an aside, new construction ends up being far riskier for hidden construction defects than older construction. The reason might not be obvious. It can take a year or two to recognize construction problems and microbial growth, but water-damaged buildings (WDB) are usually shown to have moisture intrusion over time as one defect or another comes to light.

DOORS: Doors leak from above and below when they aren't installed right. Inset fan lights over doors must be caulked yearly. What makes your steel door warp and wood door swell?

GUTTERS: Don't forget to look (or hire someone else to look) at your gutters. Gutters that are high off the ground (second floor and above) require a long ladder to reach; they often are neglected. What a mistake! It doesn't take too many pine needles or decaying leaves to create a mat that will block flow of rainwater or melting snow from entering the downspout. If an overhanging tree is present, the obstruction problem is essentially guaranteed. As the gutter continues to fill, the water goes somewhere. It will usually overflow the front or the back. If there is a slight backward pitch of the gutter, water will impact against the fascia board. If this wide flat board is not tight, there is free entry for water behind the fascia into the attic or inside of the cladding. Once inside the attic, water can then run downhill, sometimes going a very long way along a rafter. When water meets a drop off point, maybe just a bow of the wood or a protruding nail head, gravity will direct the water downwards. I have seen homes with blocked gutters showing their leaks 40 feet away on the other side of the attic from a blocked gutter. It happens.

SIDING (CLADDING): Any siding materials can leak. Brick, stone, wood, vinyl, concrete, block, and stucco are just barriers. Wind-driven water can track uphill under edges of siding, through nail holes and cracks too. Where one expanse of fake stucco meets another, the seals often are open invitations for water intrusion. If porous oriented strand board (OSB, I call it oriented sponge board) has been used to protect the stud walls underneath the cladding on the inside of the house, the water coming from the leaks of the seals is an invitation to microbial growth that no one will suspect, as OSB is the nurturing sponge of life for microbes. Mold and bacteria, especially, will grow through the four-foot-by-eight-foot wood particle-chip-glue sheet rapidly, leading to microbial growth on *both sides* of the OSB. As the OSB continues to stay wet and fungi digest cellulose in the OSB, it doesn't take too long before additional water is dumped into a wall cavity creating the potential for massive problems. As particulates from microbial growth become airborne, even in wall cavities, they can find their way into the inner sanctum, traveling on air flowing around switch plates, receptacles, defective drywall joints, or even nails used to hang pictures. Actinomycetes get through to interior walls more often than fungi, but bacteria are the swiftest to penetrate.

The same problem of moisture penetration applies when people have **masonry exterior walls** as there is a space (void) behind bricks or stone in which air circulates. Bricks and stone are both porous, which means that moisture that hits the outside of the brick can migrate through to the inner side. At the inner surface it will then drain downward under the influence of gravity. At the bottom on the exterior wall, there should be an opening, called a "weep hole" that will let water leave the inner space and not create microbial disasters between the brick and plywood or OSB.

Vinyl siding goes up quickly, is inexpensive, and comes in many colors. It is no surprise that we have so many vinyl-sided homes in the US. While the vinyl itself is impervious to inflow of outside water, the junctions between pieces of vinyl or areas where the vinyl has been nailed create potential portals for water intrusion. Wind makes gaps in vinyl walls!

The nice green material growing on the outside of exterior walls is not mold but instead is **algae**. The algal growth can be so profuse such that a mat of algae can form in corners. Under the mat there can be an air space between overlapping pieces of siding that lets water wick underneath.

A word of caution regarding vinyl siding. Attempts to clean siding can create their own sets of problems. Power washing will remove algae (not so quickly!) but use of bleach solutions to clean siding can damage plant life below making it a high price to pay to clean up algae. In siding as in all things, sometimes when we use chemicals that kill living things, we sacrifice the overall good for temporary improvement!

Leaving the outside of the house, *the building envelope*, we then head downstairs into the **basement** or the crawlspace. Here is the source of microbial growth that is found in 95% of homes that have these subterranean structures. Having a walkout basement is no guarantee of safety in that the inground side of a walkout basement is subject to additional water pressure that can create a wet wall. Water pressure will overcome any temporary barrier created by tar solutions or fancy paints designed to be waterproof. Don't believe the manufacturers' claims!

Many people with a walkout basement will dig trenches on the inground side approximately 3-4 feet deep, installing perforated pipe covered by pebbles to create a "French drain." At each end of the building, pipes are connected to side pipes, making a right angle at the corners of the home that direct ground water away from the in-ground wall to the side of the home. The pipes extend beyond the downhill side of the house. When patients have done this kind of preventive maintenance work, they are surprised about how much water comes out of the drainpipes. It is an ideal source for making a year-round freshwater pond as part of the garden (and not an aquarium 30' by 40' in the basement).

To take care of moisture problems created by subterranean structures, some people will install sump pumps, using a chiseled notch or trench cut into the concrete slab

of the floor that leads to the sump pump and then the pump will move the water somewhere, hopefully outside of the basement. This approach sounds pretty good except when we recognize that the water that has just come in and is in a trench creates a microclimate of elevated A(w) that is perfect for bacterial growth. Sump pumps themselves are almost guaranteed to be sources of bacterial colonization and endotoxin. A better solution is not to have a basement!

In our coastal area of Maryland, basements are uncommon, but **crawl spaces** are common. Usually three-to-four cinder blocks high, crawls are the standard approach to lower cost on new home construction. A finished crawl space will often have several vents installed every 8-12 feet, as if making a portal of entry for hot humified air in summertime into the cool climate of a crawl space was a good idea. When the hot moist air hits the cool air over the exposed soil in the crawl (usually about 54 degrees), it doesn't quite rain under the house but close to it. The excessive moisture that comes from Mother Earth herself provides a continuous source of moisture to nourish micro-organisms growing in soil. Don't be confused by the dry appearance of the soil in a crawl space: it just means that soil water has evaporated over time. More is on the way from deeper soils for sure.

A simple approach to crawl spaces is to condition or seal off the crawl space such that the air in the crawl is never exposed to moisture from soil or moisture from the side walls. By putting 20-28-gauge plastic (pond liners are better than swimming pool liners; swimming pool liners are better than thick plastic) and then folding the edges of the liner upwards so that the liner can be attached to the board (sill plate) sitting on top of the foundation, we can create a water intrusion barrier that works. Meanwhile, any water coming from Mother Earth runs into the underside of the piece of plastic and stays with Mother Earth. There is no excessive moisture on the floor side of the liner available to nourish microbes that might be living opportunistically in the crawl space. The vents are sealed shut.

This conditioning idea sounds radical, understanding that many people feel that all crawl spaces should have vents (compare that to the newer approaches where no crawl spaces have vents); their moisture problem continues. Conditioning is defeated when someone hooks up a HVAC vent or a duct that will pump warm or cool air into the crawlspace. Now we are creating the opportunity to share indoor moisture with a sealed crawl space system, thereby defeating the purpose of making a moisture-tight sealed air chamber.

As always, if there is a basement or a crawl space, it makes sense to seal (using expandable foam) any holes that are made in the subfloor to permit passage of pipes, electrical wires, central vacs and the like. It is amazing how much air can come through 3/8" circle around each pipe penetration through the subfloor! Such spray foams will release VOCs, so seal well before occupying the building.

INTERNAL SOURCES of moisture are often overlooked. Outside humidity, transferred to the inside when an outside door is opened is a definite problem in

tropical areas. Two choices exist to prevent exposure. The first involves matching tonnage of HVAC (heating, ventilation, air conditioning) equipment to size of a building that will permit drying out of indoor air. The second approach is to increase ventilation (windows wide open, for starters). In the US, buildings often use HVAC to safeguard homes from tropical fungi (especially *A. penicillioides*).

COOKING creates the biggest source of moisture in the first floor of most homes, with bathrooms creating the source of moisture most commonly in the second floor. This is assuming of course that there are no sliding glass doors that leak, no sky lights that leak, no elaborate roof structures with valleys and pitches that are impossible to close off and no flat roofs that will leak regardless of what is done.

HUMAN SOURCES of moisture (think of breathing!) also contribute to the availability of water inside the home. Leaking shower pipes are notorious for having small pin hole leaks just above the sweat joint where a copper supply tube meets a plastic or PVC junction. These pin hole leaks rarely are visible in the bathroom side of a wall cavity but if there is a closet that abuts the back of the wall cavity, that is where you will see moisture and mold growth. If the closet is closed most of the time, expect to find *A. penicillioides*, an organism that does its damage by the company it keeps as opposed to being a horrible toxin former. *A. penicillioides* does not like to be ventilated. You will find it in vanities, behind refrigerators, at the end of hallways that are not ventilated or in closets. When you see shoes in a closet growing mold, be thinking about reduced ventilation. *A. penicillioides* is also found on carpets, soft furnishings, and drapes.

DECKS: Adding decks makes for enjoyable living, but if the deck ribbon board is screwed into the foundation board of the home, make sure that there is adequate flashing to protect the home from water coming from the deck itself through the deck understructure.

STILTS: A special circumstance applies to coastal living. There is a financial benefit to building homes on elevated pilings, usually 8-10 feet off the sand, in order to avoid flood damage. For some people, this 10-foot high space the size of the footprint of the building, is too tempting. Just look at this ground floor bonus room! Up go the walls and up go the fungal counts coming from the damp coastal soil. What we now have is an outdoor fungal growth chamber sitting outside of the entry door so that every time you go in or out there becomes a vortex of sick air entering the living space. Not a good idea; don't make extra storage space or living space and sacrifice your health. Ventilate it!

Part 3: Diagnosis

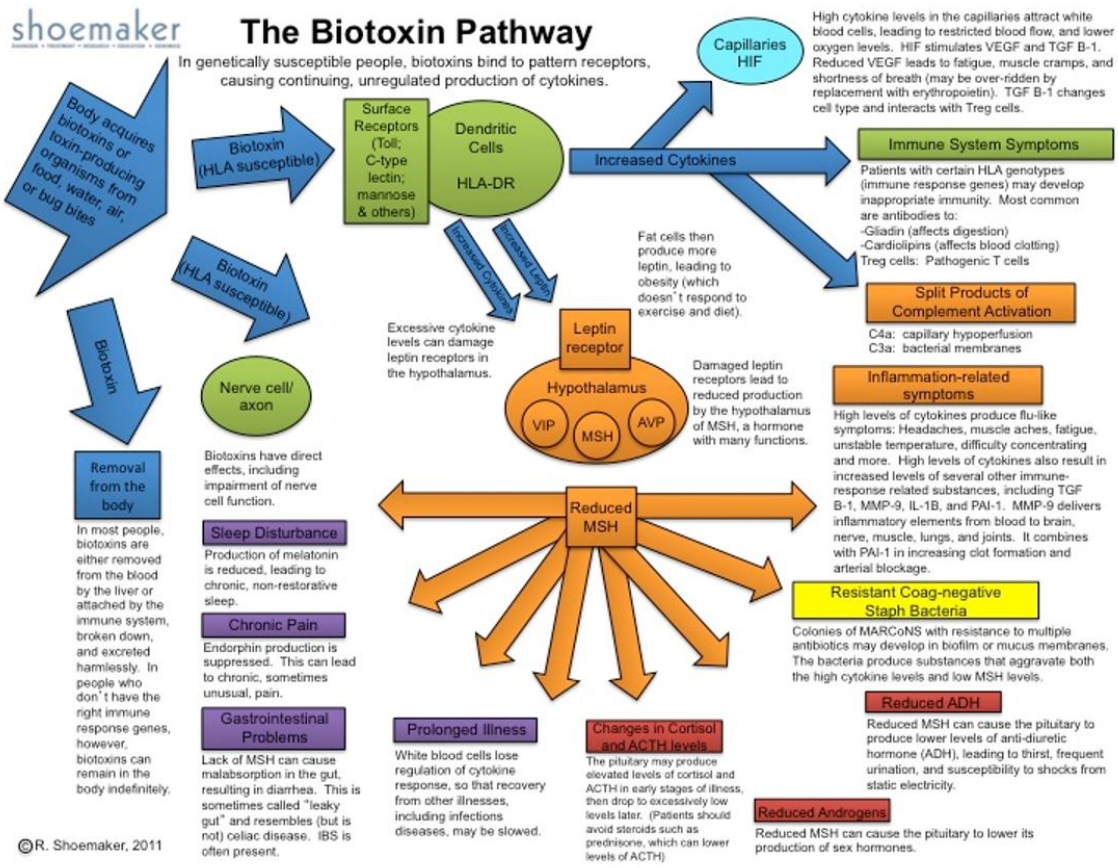
CIRS Diagnosis

When we think of chronic inflammatory response syndrome (CIRS) in 2019, our initial case definition has been expanded to include not only

1. abnormal proteomics;
2. abnormal regulation of immune functions and hormonal feedback loops;
3. loss of neuropeptide regulation of the above;
4. but also, abnormal transcriptomics;
5. together with suppression of ribosomal and nuclear encoded mitochondrial genes.

In the text that follows these terms will hopefully become clear and begin to act as your friend. Suffice to say, these CIRS illnesses are all around you; but of possible greater importance, the concepts of (6.) dysregulation of inflammation and (7.) dysregulation of gene transcription set the precedent for looking at underlying inflammatory bases for other illnesses, including autoimmune problems, diabetes, obesity, atherosclerosis, and neurodegenerative processes in a new light.

Clinical Case Definition: Chronic inflammatory response syndrome (CIRS) is a chronic illness acquired following the exposure to the interior environment of a water-damaged building (WDB) with resident microbes including, but not limited to, filamentous fungi, bacteria, including actinomycetes and mycobacteria; and their toxins and inflammagens, including, but not limited to, hemolysins, beta glucans, mannans, and spirocyclic drimanes. Cases with CIRS-WDB will have multisystem, multi-symptom illness. Presence of multiple reliable objective biomarkers, taken as a group but not individually, will aid in diagnosis and in monitoring therapy. Markers include genetic haplotypes, innate immune inflammatory elements, deficiency in neuroregulatory peptides or their receptors, dysregulation of pituitary and end organ endocrine factors, as well as clearly defined abnormalities in transcriptomics.



The Biotoxin Pathway

Perhaps of the greatest diagnostic and therapeutic importance is the finding of a marked suppression of 1) ribosomal; and 2) nuclear encoded mitochondrial genes in CIRS patients before treatment.

There are at least 30 entities found inside WDB that individually and collectively can set off innate immune responses.1 Indoor exposure will perpetuate these responses with eventual differential gene activation providing a mechanism for inflammatory compound production in the absence of ongoing exposure. Some of these inflammatory elements are well known. Endotoxins made by Gram-negative rod bacteria and actinomycetes, long overlooked but of marked importance and second only to endotoxins as activators of dysregulated gene activity, all cause differential gene activation.

We also know there is a pathogenic role for mycotoxins, albeit much smaller than thought just a few years ago. As additional research is becoming clear, mycolactones, made by mycobacteria, also have an inflammatory role. Of importance, are beta glucans, mannans, hemolysins, proteinases as well as cell wall fragments, hyphal fragments, and particulates found in reservoirs in air, including

small, fine and ultra-fine particulates. We have not yet defined adequately a pathogenic role for mVOCs (microbial volatile organic compounds), but consensus opinion supports mVOCs having some ill-defined role in creating adverse human health effects. References for these important entities are found in the 2015 Medical Consensus Report on the Surviving Mold website.

Differential Diagnosis, Other CIRS: Even though CIRS caused by exposure to the interior environment of WDB remains the most important source of CIRS, there are other important exposures that must be included in differential diagnosis. These include consumption of ciguatoxic fish found on tropical reefs. We must also look to see if there is exposure to freshwater bodies with resident blue green algae (cyanobacteria), found in every state of the Union. CIRS is also found in post-Lyme syndrome2 and the rarely observed bites from recluse spiders. Of interest, is the discussion coming from the chronic fatigue syndrome (CFS) community commenting on CFS as a CIRS as well. Traumatic brain injury, including concussion and repetitive head injury from either concussive force such as firing artillery weapons or athletic competition with head contact, can set off innate immune responses that appear similar to CIRS is some ways.

Federal Agency Case Definition*:* In 2008, the US GAO provided us with a federal agency approved case definition that includes (1) potential for exposure; (2) symptoms like those seen in published, peer-reviewed literature; (3) laboratory findings similar to those seen in published, peer-reviewed literature and (4) response of objective parameters to treatment. This case definition provides flexibility for the clinician but also demands a benchmark of documentation of objective parameters to be found before a person can be labeled as having CIRS or an illness caused by exposure to a wet building.

In **differential diagnosis**, a process that sorts illnesses from non-illnesses, we will see normal laboratory findings of ESR, CRP, CBC and CMP. In addition, CIRS usually has normal thyroid functions, ANA, total IgG, total IgE and IgM. Cholesterol will be normal, and serum protein electrophoresis is normal. Viral studies are often thought to be indicative of ongoing viral infection but according to transcriptomic data are just telling us about intercalation of viral DNA into our own DNA. CIRS cases will usually have a normal EKG, normal pulse oximetry, and normal chest x-ray. There are interstitial lung disease findings less commonly found in CIRS so that chest x-ray must be obtained and reviewed. Of possible interest is the finding that certain cancers are under-represented in CIRS compared to the normal population. These include colon cancer, adenocarcinoma of the lung, and breast cancer (unpublished clinic data).

Looking for biomarkers, VCS (visual contrast sensitivity) deficits are found in 92% of CIRS cases. Analysis of symptoms, using cluster analysis (see Table 1), shows unique findings of 8 of 13 clusters found in adults with CIRS. Children are less often found to have distinctive clusters but finding 6 of 13 is consistent with CIRS in pediatrics. As an aside, reliable peer-reviewed literature3 has shown that CIRS can

extend to single symptoms in children with an emphasis of chronic headache and chronic abdominal pain.

Table 1. Cluster Analysis of Symptoms

Individual categories:

1. Fatigue
2. Weak, assimilation, aching, headache, light sensitivity
3. Memory, word finding
4. Concentration
5. Joint, AM stiffness, cramps
6. Unusual skin sensations, tingling
7. Shortness of breath, sinus congestion
8. Cough, thirst, confusion
9. Appetite swings, body temperature regulation, urinary frequency
10. Red eyes, blurred vision, sweats, mood swings, icepick pains
11. Abdominal pain, diarrhea, numbness
12. Tearing, disorientation, metallic taste
13. Static shocks, vertigo

A positive cluster analysis for biotoxin illness is presence of 8 or more of 13 clusters.
Finding a combination of VCS deficits and positive cluster analysis results in a 98.5% accuracy shown for CIRS with a 1.5% total source of abnormalities in false positives plus false negatives.

On physical exam, it is common to find a resting tremor in cases with this finding best observed by having the patient hold their hands out straight with palms facing down to the floor, spreading fingers as wide as possible. Then a single sheet of paper is placed on the outstretched hands; the fine tremor is easy to see. Hypermobility is commonly seen as well, particularly in those with antigliadin antibodies and anticardiolipin antibodies.

Additional laboratory findings include a distinctive HLA-basis of susceptibility with increased relative risk (RR >2.0); levels of MSH lower than 35 pg/ml; high levels of C4a, TGF beta-1 and MMP-9; with dysregulation of ACTH relationship to cortisol and antidiuretic hormone (ADH) relationship to osmolality. Commonly, there will be either low or high VEGF with one-third of cases each being under 31 or over 86. An abnormal von Willebrand's profile will be found in approximately 66% of patients. Nasal culture showing multiple antibiotic-resistant coagulase negative staph (MARCoNS) will be found in the deep aerobic nasal space in over 80% of cases with low MSH. Of these MARCoNS, over 60% will be resistant to methicillin.

An aside about mycotoxicosis: Since the advent of the shotgun use of antifungal medications to treat "mycotoxin illness," a re-run of what we saw in the ENT

literature of the early 2000s, there has been an explosion of creation of frightening antibiotic resistances found in these MARCoNS, known promiscuous exchangers of plasmids and circular DNA, especially when antibiotics and antifungals are used together. Our antibiotic, biofilm-busting nasal sprays that worked wonders with "pre-2015 MARCoNS," no longer worked when azoles were added to sprays. These resistances include resistance to vancomycin and gentamicin! There are multiple additional reasons ***to not use*** **anti–fungals indiscriminately**, as will be discussed.

Our concerns about unwarranted use of antifungals for mycotoxicosis go to more than the current explosion of deaths from multi-azole resistant *Candida auris*, called a "serious global health threat by the CDC" (CBS News 4/9/19); it goes to claims of illness using findings of mycotoxins in urine to diagnose the condition. Yet, there is no published control group data (PubMed search 4/9/2019) showing absence or simply a paucity of mycotoxins in urine in controls compared to cases. Moreover, when we look at the world's literature on urinary mycotoxins, we find scores of papers regarding healthy people with markedly abnormal levels of mycotoxins in urine.

As one reviews findings in CIRS beyond symptoms, VCS, and laboratory findings, depressed VO2 max and reduction reduced anaerobic threshold stand out. These findings, with low VO2 max thought to be related to ME/CFS,4 are typically found in all sources of CIRS. Simply stated, if one is given capillary hypoperfusion, as seen in CIRS, reduced VO2 max will invariably be found. One must also review the section below on hypometabolism, as our thinking on VO2 max has been radically modified by new data coming from transcriptomics.

"**Of all the things I have lost, I miss my brain the most.**"

We also see elevated pulmonary artery pressure at rest (≥ 30 mm Hg) in CIRS but more often we can confirm that in exercise, PASP pressure usually rises more than 8 mm over baseline in CIRS patients.5 This problem is called acquired pulmonary artery hypertension.

"Of all the things I have lost, I miss my brain the most." There is a uniquely abnormal fingerprint for CIRS-WDB found on NeuroQuant (NQ) with published evidence so strong that NeuroQuant is now one of the primary ancillary studies that should be done in all patients over age 7 and under age 92.6-8 We have come to conclude that NQ is uniquely able to demonstrate the dreaded complications of multinuclear grey matter atrophy.

Of significant concern is the increased incidence in CIRS of lateral ventricle enlargement suggesting normal pressure of hydrocephalus but also suggesting atrophy of cortical grey matter. This atrophy extends to grey matter nuclei, which will have a mean incidence of 2.4 out of 6 grey matter nuclei in CIRS-WDB. Post-Lyme syndrome patients show mean atrophy of 3.0/6.

For those patients who have been treated with antifungals, a total of 4.5/6 atrophic grey matter was confirmed.9 I don't understand taking medications that are optional at a cost of structural brain integrity.

With an eye towards defective antigen presentation, we know that there is no protection from other sources of CIRS provided by a given source of CIRS. Our concepts of an active and effective adaptive immune response don't necessarily apply to CIRS.

Transcriptomics: Perhaps of greatest diagnostic and therapeutic importance is the finding of a marked suppression of (1) ribosomal; and (2) nuclear encoded mitochondrial genes in CIRS patients before treatment. Recovery with use of a published, peer-reviewed protocol (the Shoemaker protocol) has brought new hope that we will finally be able to put aside the longstanding dogma that these illnesses are never cured.

Because of the common findings of CIRS, with over 50% of buildings in the US reportedly having water intrusion and microbial growth, it is a tautology that CIRS doesn't hide.

Unfortunately, CIRS is uncommonly diagnosed outside of the communities dedicated to CIRS. It is quite rare, however, to find patients or providers who don't know someone who is chronically ill from fibromyalgia or CFS. Those diagnoses are ones without objective diagnostic biomarkers; not to mention none that also guide therapy. Once physicians can fill in the CIRS gap that is missing from medical schools and post-graduate CME, they will rapidly learn just how simple diagnosis is and how effective treatments are.

Confirming CIRS: Homing in on the case definition, what does potential for exposure mean? Potential for exposure demands objective findings showing microbial amplification with (1) visible mold; (2i) or presence of musty smells; (3) or determination of the types of molds found by DNA testing. We now add measurements of endotoxins and actinomycetes in modern labs with top-flight molecular methods to diagnostic criteria as these sophisticated assays are now readily available. Remember that musty smells, usually stemming from geosmin made by actinomycetes and occasionally by bacteria, are often used (curiously) to support a diagnosis of a *mold* problem. Use of accurate mold specific QPCR testing is readily and inexpensively available.

Unfortunately, some practitioners still think that air sampling has a role in the medical work-up of CIRS patients. Use of air samples for diagnosis is worse than worthless in that sampling air for spores, at least three microns in diameter or greater ignores ***99.8% of the total amount*** of fragments that cause inflammatory responses! These fragments are so small they pass right through the spore trap devices. Spore trapping then can't possibly be used to look for disease-causing inflammation. Don't forget, exposure to small particles, really just biochemicals,

means that the bulk of bad actors in WDB are *not alive*. Please don't tell me to remediate a damp home by killing spores!

Even though spore sampling provides flawed information, that procedure is still widely used. Even worse than wrong-headed data generated by spore traps is the problem that occurs when people *believe that spore trapping makes sense* and that spore counts are indicative of something real in nature. Of note, air samples (1) only done for 5 or 10 minutes in a single-center location in a room do not tell us what has happened to bacteria or fungal spores that have settled out before the sampling; (2) don't tell us what particulates were missed by sampling in the center of a room and not in boundary layers on the bottom and sides of a room; (3) do not separate benign versus pathogenic species of both *Aspergillus* and *Penicillium* (these two very large genera are lumped as Asp/Pen!); (4) will rarely show presence of heavier particulates such as those made by *Stachybotrys*; (5) will never show presence of xerophilic organisms such as *Wallemia sebi*; and (6) without repetition of air sampling findings, multiple times per day in a given room for each of multiple days per week, multiple weeks per month and multiple months per year, the World Health Organization has declared that air sampling is of no benefit.10

One reason for the commonality of microbial findings in water-damaged buildings being similar in each of our states, together with foreign countries, is that the indoor ecosystem of a wet building is uniquely similar across all climates. There rarely is any wind, and there certainly is not any rain or frozen precipitation. There is only a narrow range of temperature in an occupied building and only limited diversity of visiting or exotic organisms will be found. Often there will be limited movement of fixed objects in a room, setting up areas of reduced ventilation ("still air"). Fixed walls (not to mention floors and ceilings) create boundary layers of both air and particulates.

MSQPCR (Mold Specific Quantitative Polymerase Chain Reaction) is a marker of presence of different species of filamentous fungi found inside homes, both water-

damaged and not. The fungal DNA present *tell us much about the activity of water* found inside the building. The EPA-developed ERMI (Environmental Relative Moldiness Index) purports to quantify an index of microbial contamination in a building from assessment of MSQPCR measured on dust samples. Initially done on vacuumed samples, ERMI was done later using Swiffer cloth wipe samplings, with comparable validity comparing Swiffer to vacuum.

Proper ERMI testing demands accurate use of high-quality probes and primers for detection and reporting. Laboratories that license the ERMI technology from the EPA must be able to show accurate and ongoing quality control. Never send a sample off to a lab that does not have ongoing EPA licensing, as errors are routinely seen with faulty use of primers or inexpensive, inadequate reagents. If a MSQPCR lab won't tell you the quality control methods they use over the phone, don't use it.

A lab test far more useful than ERMI and more accurate as a measure to show risk of recrudescence with re-exposure of CIRS patients to WDB is the Health Effects Roster of Type Specific (Formers) of Mycotoxins and Inflammagens, Version II (HERTSMI-2). HERTMSI-2 has been in increasingly wide use since its inception in 2011. Its accuracy is based on sorting health abnormalities associated with exposure to WDB to yield five species of fungi that (1) "span the globe," of A(w) from the driest to the wettest organisms; and (2) show the overwhelming increase in CIRS when these specified organisms are found. By relying on correlation of spore equivalents/mg dust with risk of acquisition of adverse human health effects, we finally have a measure that predicts safety (or not) for over 95% of CIRS patients entering schools, workplaces and residences.

We need to remember that MSQPCR will not report bacteria, endotoxins, mVOCs or any other of the inflammagens found in WDB.

The scoring system for HERTMSI-2 weighs severity of contamination from 0-10 points for given organisms. When we sort these organisms by A(w), we find that the drier-loving species of *Wallemia* and *Aspergillus penicillioides* are routinely found in A(w) of 0.65-0.8 but are rarely represented by air samples. The common species of *Aspergillus versicolor*, usually found in higher A(w) of 0.8 to 0.9, are reported by ERMI but are never reported at the species level by spore traps. Presence of *Chaetomium* and *Stachybotrys* reflect environmental conditions with an A(w) of 0.9-1.0 are only detected infrequently in air samples using spore traps.

A word regarding dust collection. Swiffer cloths and vacuum samples are equally useful. If you use a Swiffer cloth, routinely available in a grocery store, use a new Swiffer, one cloth per sample. Put a glove on your left hand and wipe in one direction, either left to right or right to left. Use one cloth for all the sampling. We want you to sample for dust on the back of a shelf and not on the front where it might be more commonly dusted. Don't use window sills or "public" areas, like hallways. I suggest avoiding shoes on closet floors, but closet shelves are fair game. Bathrooms are best avoided because of the role of water saturation following

showers or bathing. If there is evidence of obvious microbial growth, resist the temptation to swipe it, as sampling the black patch on the bedroom wall, for example, will skew your sample to render results less reliable. If there is a crawlspace or a sump pump or a basement or an indoor spa or areas in bathrooms that are hampered by low air flow with inadequate exhaust, be sure to test for endotoxins with the same sample used for HERTMSI-2. Testing for abundance of *Actinomycetes* species is of tremendous value, especially when *Aspergillus penicillioides* or *Wallemia a*re predominant. This is not to say that actinomycetes won't grow in wetter environments, but they are more commonly found in drier environments indoors. They also like more alkaline surfaces like concrete that is chronically moist.

We have seen that the causation of illness from water-damaged buildings is multi-factorial. We can't just rely on fungal DNA because in a way, what we are asking is, "Can we identify specific (only one thing causes the illness) causation of human illness found from water-damaged buildings?" The answer is NO!

Countless different measurements, mostly not available (think about spirocyclic drimanes, known to cause inflammation but for which there are no commercial test available), are needed for 100% surety. We are less able to measure beta glucans and mannans. Moreover, microbial V0Cs are showing great potential to be a biomarker for water-damage, but the human health effects data associated with mVOC exposure is not confirmed. Please note that transcriptomics is changing the "no specific causation" idea. Differential gene activation can tell us if it is likely that reactivity to mycotoxins, actinomycetes, or endotoxins has occurred.

Symptoms

When we look at specific symptoms in CIRS, using a list of 37 symptoms (the same symptoms as seen in Table I, but separated by organ system this time) that have been collated from charts of thousands of cases and each found in more than 30% of cases, we find fatigue and weakness together with headaches, aches, and muscle cramps, defining involvement of three separate organ symptoms. Unusual pains, sharp stabbing pains, clawing pains, electrical pains, comprise their own category (possibly due to activity of transient potential receptor vanilloid (TRPV) activity on sensory neurons). Here we will also find sensitivity to the light touch, especially from water droplets in a shower or rainstorm. Our ophthalmic findings (in addition to visual contrast sensitivity, VCS) are light sensitivity, redness, blurred vision, and tearing. Respiratory issues are shortness of breath, cough and sinus congestions; abdominal pains and secretory diarrhea comprise our abdominal findings. Musculoskeletal problems are quite common with joint pain, especially with morning stiffness, being routinely found in patients over age 25. Of note is that ESR is invariably low normal. Perhaps of greatest importance are the symptoms suggestive of brain injury with executive cognitive dysfunction represented by deficits in recent memory, concentration, difficulty with word finding, assimilation

of new knowledge, confusion, and disorientation leading the list. If present, think "brain fog," and get a NeuroQuant.

Hypothalamic symptoms include mood swings, appetite swings, unusual sweats, and difficulty with normal temperature regulation. Our renal findings are excessive thirst, frequent urination, and curiously, increased susceptibility to static shocks, but not just in dry environments. Neurologic findings of numbness, tingling, and taste abnormalities (metallic taste, especially) are common in CIRS. Additional neurologic problems include vertigo and skin tremor (get a NQ!).

Given that symptoms and VCS taken together (see cluster analysis, Table 1) are so accurate, one might reasonably ask, "Why do we need laboratory findings?" The answers are straightforward: (1) we must have an accurate ongoing differential diagnosis; (2) laboratory changes will show interval improvement with therapy or worsening with re-exposure (often not obvious!); (3) findings objectively demonstrate the physiology to interested third parties; and (4) provides opportunity for further study.

Figure 2 is the Treatment Pyramid. The Treatment Pyramid has also stood the test of time with a 12-step protocol providing predictable improvement as shown in at least 30 countries around the world and in all 50 United States.

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Part 4: Treatment

The Treatment Steps

Removal from exposure is the first step in treatment. This is followed by use of bile acid sequestrants, including cholestyramine or Welchol. Removal of biofilm-forming MARCoNS (multiple antibiotic-resistant coagulase negative staphylococci) is our third step.

(4) Correction of antigliadin anti-body positivity in non-celiac patients is key, with removal of dietary gluten indicated for those with the antigliadin antibodies. The role of MSH (alpha melanocyte stimulating hormone) deficiency is paramount in antigliadin antibody positivity and gluten sensitivity.

(5) Antidiuretic hormone (ADH) dysregulation of osmolality is shown routinely in over 80% of CIRS patients. Dysregulation can mean absolute high or low ADH; absolute high or low osmolality; or too little ADH for a given osmolality; or too much ADH for a given osmolality. These findings are paralleled by those that we see as abnormal for ACTH and cortisol. By looking at these two pairs of labs, we look at disruption of normal feedback relationships in MSH deficient patients.

(6) Androgen deficiency can be due to hypothalamic dysregulation of gonadotrophins, especially when MSH is low. A second mechanism for “Low T” is increased activity of *aromatase*. This enzyme converts androgens to estrone and then to estradiol. Don’t simply prescribe androgens!

(7) We must correct MMP-9, an indicator of pro-inflammatory cytokine effect on endothelial cells.

(8) We must also correct low (or high!) levels of VEGF. Interestingly, the effective use of omega-3s for correction of MMP-9 and VEGF has been a welcome addition to treatment.

(9) When C3a is elevated, there is presence of a bacterial membrane in blood. The membrane will serve as a source of attachment of the fragment of complement activation called C4bC2a that then provides a mechanism for attachment of C3 that will then lead to attachment of C5, 6, 7, 8 and 9. Complement puts a hole in bacterial cell, lysing the cell.

(10) C4a is probably the most important proinflammatory marker we have as part of the complement cascade. There is an enzyme, mannose binding protein associated serine protease-2 (MASP2), that can autoactivate. If that occurs, C4a will be produced even when *no stimulation* of MASP2 by antigen is occurring. This unique finding suggests "sicker quicker."

(11) Reduction of TGF beta-1 is mandatory, not just for its role as a pro-inflammatory cytokine but also for its role activating a whole series of metabolic pathways involved with T-regulatory cell function in tissue and reduction of autoimmunity.

The last step is use of vasoactive intestinal polypeptide (VIP). See below.

SAIIE

An adaptation of the treatment protocol permits use of a diagnostic, prospective re-exposure trial. Called "Sequential Activation of Innate Immune Effects (SAIIE)," this protocol is used when we must show causation. As opposed to a case/control study, one that lets us conclude an association of exposure with symptoms, visual contrast sensitivity (VCS) deficits, proteomics or transcriptomic abnormalities, a prospective study design can confirm the presence of the epidemiologic concept of risk and therefore, causation.

We take a known case, a patient who meets the GAO case definition, use the Shoemaker Protocol to correct symptoms, VCS, and proteomics. Before starting, we know that the building suspected to be making the patient ill is contaminated. We will also know that the building where the patient is staying is safe (using ERMI or HERTSMI-2).

The first step, after informed consent is obtained, is after RX 1 (AC1, usually beginning on Friday). Symptoms, VCS and selected labs (C4a, MMP9, leptin, VEGF and von Willebrand's profile) are recorded. All CIRS meds are stopped; the patient is kept away from the suspect building for three days, after having been exposed to "the ubiquitous fungi of the world." On Monday morning, having completed the prospective trial of no-known exposure to a water-damaged building (WDB), symptoms, VCS, and labs are repeated. This step, called Home Off Meds (HOC), ends when blood is drawn on Monday.

The patient then enters the suspect building each day for three days, with study measures done daily. On Tuesday AM, symptoms, VCS and labs are performed, showing us what happened on day 1 of re-exposure (BOC-1, Tuesday). The patient re-enters the building. Symptoms, VCS and labs are performed on Wednesday, telling us what happened on day 2 of exposure (BOC-2). The patient returns to the WDB a third time, with symptoms, VCS and labs done on Thursday (BOC-3) showing us what happened on Wednesday, day 3. If the building is causative, by BOC-3 VCS will fall, symptoms will increase to approximately 95% of initial levels; labs will show distinctive profiles sorted by day of trial. Because the lab changes are stereotyped, according to known physiology, a scoring system can be applied to not only quantitate recrudescence of symptoms, but recrudescence of objective parameters as well.

As an aside, this protocol readily shows absence of validity to alternative hypotheses regarding causation, such as presence of mycotoxins in urine1 reflecting fungal infection, for example.

Perspective on Medical Rx

In the course of 23 years working on CIRS, there have been relatively few novel therapies that have stood the test of time. The Shoemaker Protocol is one of those therapies; it is marked by one intervention at a time for 30 days in sequence, with monitoring of diagnostic biomarkers before and after any given intervention. This methodical approach lets us identify what is working with treatment and what is not.

Initially, when symptoms persisted beyond (a) removal from exposure and then (b) use of binders, it was almost a trial and error approach to patient-centered research that brought the protocol to its current status. The protocol is fluid in the sense that there are new approaches to treatment that are evolving as the disease itself evolves. A simple example is the emergence of multiple unusual bacterial resistances in MARCoNS, initially in users of anti-fungal medications. A previously effective nasal spray, used safely and effectively from 2002-2015, became useless in a span of weeks. Sharing plasmids for bacterial resistance, likely due to horizontal gene transfer, is what the promiscuous MARCoNS do! What happened? The organisms changed; our treatments changed to continue to be effective.

Perspective on Treatment of WDB

The treatment protocol begins with one step that has been called the hardest of all: removal from exposure. Removal from exposure can mean either literally moving away from a school, workplace or residence; or removing particulates from the air and elimination of reservoirs of potential particulates found inside WDBs. Remediation of a building can be expensive; alternatively, correction of particulate reservoirs can be agonizingly obsessive, especially when remediation isn't completed. The advantage of clearing the air and reservoirs of particulates is that

we are not talking about burning a house down or leaving all possessions, walking out with the clothes you have on. What we are talking about is correction of the source of inhalation of particulates, understanding we may not have eliminated all potential sources of contamination.

Remediation is a subject for many thousands of words on another day. A few concepts will be shared here. Follow the ABCs: **A**bate the water intrusion. **B**uilding materials that are contaminated must be removed (or be encapsulated if structurally irreplaceable). **C**lean reservoirs on possessions; **c**lean reservoirs in air; and **c**lean reservoirs on walls, floors and ceilings. Every room must be cleaned in a given building if air from one room could get into another. Even though many individuals aren't going to be affected by CIRS; cleaning must be performed assuming that all who enter the rooms in the future are CIRS patients. Finally, if you are talking with a remediator who doesn't use HERTSMI-2 to clear a building as cleaned, you can't assume the building is safe for a CIRS patient to re-occupy.
The Shoemaker Protocol is a methodical approach to treating chronic inflammatory response syndrome.

By taking another look at the section on definition of WDB, we can look at our check list for what has been found to be present and what is not present; stated another way, what is not safe about the house and what is safe about the house, for example. We know that there is no such thing as a safe basement even though people do all they can to make such structures safe. In treatment, there will be a dedicated air sanitation device running 24/7 in the basement. We look for maintenance of ambient humidity to be less than 55%. Sometimes that will require use of a dehumidifier with a transport mechanism combined with the dehumidifier to convey moisture removed from the air safely outside.

For prevention of exposure I often will use "three machines" approach. This approach will use an air sanitizer as the "heavy lifter." I have no conflicts of interest to disclose in this regard, but I do use Air Oasis devices liberally, with a recent paper presented in January 2019, showing use of an Air Oasis device, the iAdaptAir, alone corrected transcriptomic abnormalities in a CIRS case,2 without causing adverse changes in a control.

The second machine is a HEPA filter. HEPA means high efficiency particulate air but it involves passing air in an indoor environment through a filter that is 0.3 microns in diameter when it starts, understanding that there will be reduction of pore size over time as particles can clog the filter (NB: clean filters regularly) with use of HEPA filters. In my cynical moments, I sometimes think that there is only one manufacturer of HEPA devices in the world, with a stack of different machine labels to be put on similar HEPA devices and shipped to the US. It seems that the less expensive devices perform equally as well as expensive devices if there is the 0.3-micron pore size filter. HEPA filters are often used one to a floor in a building, with one in the crawl space or the basement. Moving the HEPA units every twelve hours or so (but not between the basement and main house) helps deal with the boundary

layer problem. Finally, with an Air Oasis sanitizer in use which will remove particulates from the air by essentially making them so heavy that they will precipitate on the floor, we must have a mechanism to vacuum these particulates up and remove them from the indoor space. So, the three machines are an air sanitizer, HEPA filter, and a vacuum cleaner (HEPA is better).

I do not feel that we need to use expensive machines, we just need to use inexpensive machines regularly, moving them from spot to spot and room to room (twice a day each!) to disrupt boundary layers of air, keeping airborne particulates in circulation and available for removal.

Selected Aspects of Treatment

Once we have begun the process of removal from exposure then we need to verify that the case definition has been met. As the case definition is satisfied, use of bile acid sequestrants begins. The oldest and least well tolerated of these compounds is **cholestyramine** (CSM). CSM has a positively charged nitrogen side chain built into a polystyrene chain. This long molecule is not absorbed so that when it is swallowed there will be a location of binding action beyond the stomach, in the duodenum and jejunum where CSM will bind to bile acids and biotoxins. CSM has been used for years to bind hydrocarbons such as DDT and DDE as well as PCBs but also will bind unusual compounds such as the M protein of Arava, a rheumatoid arthritis drug, as well as dioxin. CSM is organic glue! Once glued, down the tubes!

A less gastrointestinal troublemaker is **Welchol** (colesevelam), which is another bile acid sequestrant. CSM is taken one scoop (4 grams of active ingredient) four times a day on an empty stomach, waiting 30 minutes before eating or taking medication. Welchol on the other hand, is taken with food already in the stomach to prevent mild symptoms of reflux. The dose of Welchol is 625 mg, two tablets taken three times a day with food.

Many people will want the better efficacy of CSM, which does have 25% more binding sites compared to Welchol. I often suggest a “combo program,” taking CSM first thing in the morning and at bedtime, with Welchol taken with lunch and supper. The combo causes far less disruption of daily activities.

Unfortunately, despite our concerted efforts, we could never show salutary changes in objective biomarkers after we used “natural” binders like clay (bentonite), charcoal, pectin, chitin or chitosan, chlorella and more. Added to the “confirmation of no-benefit” list was absence of improvement in VCS scores.

A word on reduction of **TGF beta-1**. This step is vital for adequate performance of the treatment protocol. TGF beta-1 is a cytokine that has both pro- and anti-inflammatory responses. The difference between the two is due to tissue receptors including retinoic acid orphan receptor (ROR). In order to determine the role of TGF

beta-1 in the complex issues of abnormalities in regulation of gene transcription, use of the test "Genomic Expression: Inflammation Explained" (GENIE) is recommended.

Treatment of elevated TGF beta-1 is accomplished by use of losartan (sold as Cozaar) a mild anti-hypertensive in the ARB class. If systolic blood pressure is less than 120, we can't use losartan due to the risk of hypotensive episodes. Patients are recommended to measure blood pressures at least twice a day for at least two weeks at home with a reliable blood pressure measuring device to know whether there is blood pressure present over 120 that would sustain use of losartan. If blood pressures stay at, say 130/90, then losartan can be initiated in adults. The adult dose is 12.5 mg at bed, increasing to 25 mg as tolerated. Losartan is the only ARB that has the breakdown product, named EXP 3179, that lowers TGF beta-1. The rights to use of this molecule belong to Merck. One would hope that sometime in the future we would see a pure anti-TGF beta-1 medication made available to the public.

VIP: The last step is step #12, that being use of vasoactive intestinal polypeptide (VIP) to (i) correct residual proteomic abnormalities; (ii) correct transcriptomic abnormalities; (iii) correct grey matter nuclear atrophy. Simply stated, VIP is a remarkably safe regulatory neuropeptide that has been used in humans as a nasal spray in the US since 2008. It has a much longer prior history of use in Europe. VIP is currently available as a compounded medication; two groups are actively working with the FDA to create an IND.

VIP, 50 mcg/0.1ml, is most often used one spray taken four times a day independent of fasting. It has shown the ability to reduce pulmonary hypertension and markedly improve exercise tolerance. Higher doses are used for correction of transcriptomic abnormalities and gray matter nuclear atrophy. Please review the module on use of VIP before prescribing it. The module is found on www.survivingmold.com.

VIP in MCS: Some patients who are markedly intolerant of foods, medications and organic substances will have difficulty using cholestyramine or Welchol. For these patients, "mini-dose" VIP has been used with excellent results. By dosing VIP at 1/100 dilution with a steadily increasing number of doses used per day ("ramping up"), up to six doses a day, can then lead to 1/10 dilution following the same increasing dose schedule. Once 1/100 and then 1/10 are tolerated, then full-strength VIP can be initiated for approximately three weeks at which time VIP can be stopped. At that time, medications can usually be taken without adverse effects.

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Part 5: Transcriptonomics

Transcriptomics

In the annals of medical history, there are advances that have changed both the art and science of the practice of medicine. A few we learned about in high school: Edward Jenner working to prevent smallpox with an inoculation with cowpox and sterilization from Joseph Lister come to mind. Louis Pasteur and the germ theory. Robert Koch and his proof of microbiologic causation, not to mention Semmelweis with his insights into prevention of child bed fever and maternal/fetal loss are revered (now) pioneers. Technical advances included use of radiation for x-ray machines, with CT and MRI scans to follow as the years went by. Certainly, automated blood chemistries, not to mention advances in development of antibiotics, beginning with penicillin and sulfa and extending to the modern armamentarium of effective oral and parenteral bacteria-killers, were great achievements. The new T-cell cancer therapies will soon be next (my opinion). And yet, even these advances pale in terms of scientific discovery to the work done in the early 2000s in the Human Genome Project. While Watson and Crick get credit for discovery of DNA and working out some of the structures of DNA, it was the ability to identify individual genes that has heralded the advances that we see now in the 21st century. Who knew that there would only be 50,000 genes (20,000 protein-coding and 30,000 non protein-coding)? There is so much complexity of protein interaction, the diversity of diseases, all of which essentially end up having their roots in genes and gene activity. Fifty thousand seems like a small number to me.

The initial 3-5 billion dollars that were spent to sequence the human genome work seems like an overwhelming hurdle that practitioners would have to clear before bringing use of manipulation of gene activity to primary care. In just 10 years, however, automated sequencing devices brought next generation sequencing and RNA Seq to practice—with entire human genome sequencing now costing $5000 and not $5 billion. What an achievement!

It is with the Human Genome Project as a back drop for the work of transcriptomists, including Dr. James Ryan, that has helped us take the next step in not only identifying diagnostic features of illness but also identifying objective biomarkers that will let us follow the results of interventions as a basis for modern therapies. Use of transcriptomics, differential gene activation, permits us to truly see the miracle of monitoring DNA activity. We now know that environmental stimuli rapidly cause a targeted but diverse transcriptomic response. That response is rich in information!

When I use the term “transcriptomics,” please recognize that this is a dynamic field of study with gene activity changing through a variety of mechanisms, including those regulated by non-coding RNA, by ribonuclear proteins, by microRNA, by

methylation and demethylation, as well as acetylation and deacetylation. All these controlling elements lead to the function of transcription factors that are doing the work to initiate the copying of individual genes from our DNA, onto another nucleotide backbone, called messenger RNA (mRNA), as a cornerstone of adaptation of our genetic material to metabolic needs.

Transcription factors are not one to one; indeed, a quick read of www.genecards.org will show that for a given gene there can be literally a hundred or more transcription factors that can cause that gene to be activated. By focusing on gene activation, we can then compare gene activity to control patients and understand what "normal" is supposed to be, with that insight also leading to the concept of what gene suppression is. Here we see less activity compared to controls. As an aside, we know that males and females have significant differences in gene activity more than seemingly would be based on reproductive functions alone, but we also see change in activity of genes through time of day and night. The more we learn, the more we don't know.

In the CIRS world, thanks to the work of Dr. Ryan, we were able to look at 50,000 genes in CIRS cases compared to controls with the discovery of approximately 2000 genes that showed significant differences between cases and controls, including both activation and suppression. For three years we used this 2000 gene registry to analyze complete human gene sequencing, looking at cases with CIRS and controls with RNA Seq.

This approach was unwieldy and used a long string of sample manipulations, increasing the likelihood of errors. By reducing the number of genes and using a simpler platform, we were able to create a diagnostic test called GENIE. GENIE involves less than 200 genes including some "housekeeping" genes designed to show stability of the test performance. The most important application in use of GENIE in CIRS patients as well as in other illnesses characterized by chronic fatigue had to do with Dr. Ryan's finding of "hypometabolism."

What we mean by hypometabolism is stunning in its evolutionary simplicity. Who knew that there were biological warfare elements that one-celled creatures used on other one-celled creatures 3 and 4 billion years ago? These biological warfare elements have different names such as mycotoxins, ribotoxins, ribosomal inhibitory proteins, endotoxins and others. As the names suggest, ribosomes, especially one structural element of the ribosome called the sarcin ricin loop, are attacked by ribotoxins disrupting cellular production of protein. Remember that ribosomes, found in the cytoplasm, can number in the low millions in a human cell. It's no wonder that ribosome production is one of the cells most energy intense operations. If a ribotoxin disrupts normal sarcin ricin loop functioning, which they do, the cost to a cell is such that it needs to suppress its metabolic rate to survive the attack.

All living creatures need to make protein; they all use ribosomal machinery to do so.

There are two functional units that comprise the ribosome, called the large and small subunits, that wrap around a messenger RNA to then produce a protein by initiating and then elongating an amino acid chain that leads to a protein being created. *All known ribosomes of all creatures* carry the sarcin ricin loop, a structure that has been ultraconserved throughout evolution. Imagine, a vital piece of the mechanics of a cell not changing in 4 billion years.

Ribosomes are also found in mitochondria, the power house of the cell. Here we have proteins made that are needed for mitochondrial function. Were these "mitoribosomes" attacked by ribotoxins? You bet!

I hope that you have seen that disruption of protein synthetic machinery is a common result of a biological attack. The cell, under attack, has multiple feedback systems built in to help the cell survive. The cost is chronic fatigue and multisystem, multi-symptom illness in humans. Without taking evasive action, the cell would otherwise die.

Energy production systems are also subject to attack. When thinking of mitochondrial function, we think about electron transport chains and production of 36 molecules of ATP for each molecule of glucose, but those thoughts end up being just a small bit of what is involved. Mitochondria, speculated to be free-living bacteria before they were engulfed and kept alive inside the engulfing cell, provide energy to the engulfer. Mitochondria had their own genome. Over time, and 4 billion years is a long time, the mitochondrial genes have migrated (or been migrated) to the nucleus leaving only 37 behind in the mitochondria.

Monitoring gene expression, using transcriptomics, can show the effects of medical interventions, and give insights into a disease.

Going back to the idea that everything that happens in disease and illness is controlled by DNA, the *control of mitochondrial function now comes from nuclear DNA*. This point cannot be underestimated in that many have felt that treatment of mitochondria with one nostrum or another made sense and yet they were firing at the wrong place. *The therapeutic target was in the nucleus and not in the mitochondria itself.*

As nature would have it, energy production and protein production are protected in the search for life. By suppressing both mitochondrial gene activity and ribosomal gene activity, the cell can almost "go into hibernation," or torpor or reduced activity—call it what you want—to "lay low and be still." This state of reduced metabolism or hypometabolism permits the cell to survive by downregulating its gene activity.

The role of glucose metabolism in this whole concept of reduced cellular metabolism is less well defined. Normally, glucose will be delivered with insulin to bind to the insulin receptor, making a complex on the outside of the cell. That

complex will be internalized, surrounded by a cell membrane, in what is called an endosome, like a bubble. If there is insulin receptor substrate available, that bubble can be processed further, (1) releasing glucose to be used for fuel by mitochondria or (2) keeping glucose in cytoplasm where glycolysis, breakdown of sugar, provides a much smaller benefit of just two ATPs per glucose. It should not be a surprise that the role of sugar delivery becomes one of the feedback systems that guard against having too much sugar metabolism products present at a time since the mitochondria cannot handle sugar break-down products.

Said in a different way, instead of breaking down glucose into pyruvate, used for mitochondrial respiration, suppose that pyruvate *reduction* will help the cell survive biological attack. Remember that pyruvate is a three-carbon breakdown product of glycolysis; it can be converted to lactic acid, another three-carbon fragment, if pyruvate is not being taken up by the mitochondria. If mitochondria are being attacked and an endless supply of pyruvate supplied, there would be an endless supply of lactic acid made to either poison the cell or change pH-related activities in local tissue because glycolysis sent too much pyruvate towards the mitochondria. What the cell does is to reduce the impact of lactic acid production by preventing excessive amounts of pyruvate to be made in the cell. What an incredible feedback regulation system! Simply shut down glycolysis.

The complexity of these interactions of energy and protein are accentuated by mitochondrial translocases, proteins that are coded for by nuclear genes. These proteins provide a protein import system across the mitochondrial inner and outer membranes. If the translocase genes are suppressed, fewer necessary mitochondrial proteins will be transported into the mitochondria. By suppressing translocase activity, there is an additional mechanism to prevent excessive mitochondrial activation at a time of metabolic stress from external environmental attackers. There are other modalities of feedback interaction that are remarkable. Some seem so simplistic now that Dr. Ryan has identified what they are for us based on his review of next generation sequencing and review of existing literature. As Dr. Ryan would say, “I didn’t invent this, this was here, we have known all the time that these pathways were functioning in everyone’s body.”

Taking a step back, we see the complications that come from reduction of cellular metabolism. Where does chronic fatigue come from? Does this come from protein abnormalities or from energy abnormalities or both? Where does injury to grey matter nuclei that we see on NeuroQuant come from? Could there be a role for mitoribosomes in normal survival? What about in cardiac myositis? Could there be a role for reduction of activity of portions of cells that control contractility? You bet! As Dr. Ryan expands his work, he has quietly set the stage for a massive rethinking of what chronic fatiguing illnesses are, what we do to diagnose them, and what we do to correct them. Dr. Ryan has shown what I would like to call a “CIRS curve,” in which initial reductions of activity, gene suppression is a better term, for ribosomal RNA large and small subunits is evident. ATP synthesis (there are more new terms to come), mitoribosome large and small subunits, NAD-ubiquinone scaffolding for

electronic transport chain systems inside mitochondria, together with translocase functions of inner and outer mitochondrial membranes all are involved in hypometabolism. When these are all suppressed, as we see in patients who have not been treated, i.e., they are "naïve to treatment," with use of the first of the 11 steps of the Shoemaker protocol, there will be correction of these evidences of suppression of gene activity. Indeed, there is an *overshoot* to exceed control values at the end of the first eleven steps. This overshoot is corrected by use of VIP, leading to the last step of the Shoemaker pathway which is one of restoration and normalcy. Over time, this restoration will stay constant and there will be no change when patients go off VIP.

What the CIRS curve means is that we can look at patients, whether they began with post-Lyme syndrome or CIRS-WDB or ciguatera or possibly traumatic brain injury or many others, that will predictably lead to identify metabolic abnormalities that let us not only establish the stage of therapy where people are but also what is left to be done.

These are exciting times for those in the chronic fatigue world because we finally have reached an objective, testable abnormality that defines the illness for the first time ever. If one thinks that we have finally reached the "Holy Grail" of disease management, maybe so. I do too. But the reality is that our knowledge is woefully incomplete. Yet our duty is to make the ***best use of the best data available to help our patients***. So, let us enjoy the Holy Grail idea for today. Tomorrow is another day.

What We Learn from GENIE

The key indication for GENIE is to verify whether a patient has hypometabolism. Taken together, all the elements of abnormalities of nuclear encoded mitochondrial genes are the keys to mitochondrial functioning that can't be evaluated by any mechanism other than transcriptomics.

We have an additional series of biomarkers that involve immune functions as well. The first are **CIRS biomarkers**. These are genes that have importance for CIRS patients. These markers are used as guides for diagnosis and monitoring sequential therapy as we are looking for transcriptomic cure. Here the word "cure" means that the biomarkers would be returned by therapies to equal levels seen in controls. The vital importance of **apoptosis**, otherwise called programmed cell death, means that the cycle of cell life and death can go awry. When there is disruption of the enzymes that should be marking a cell to be killed by cytotoxic T-lymphocytes or natural killer cells, the cell is programmed to die but die safely. What can happen is that instead of dutifully packaging all of the intracellular contents before the cell is burst apart, when the cell enters *defective apoptosis* it will release into circulation free DNA as well as organelles such as Golgi body, endoplasmic reticulum, and mitochondria. These elements are intensely inflammatory, giving rise to the suggestion that defective apoptosis can be a form of ***endogenous inflammation***

added to exogenously induced inflammation in CIRS*.* We need more data to support this idea but right now the hypothesis remains tantalizing.

From ciguatera to mold to Lyme, **coagulation** abnormalities are routinely seen when transcriptomics are assessed. The coag abnormalities involve both suppression and activation of a series of genes that independently interact with platelet function together with coagulation pathways. We now know much more about coagulation problems in CIRS than just the well documented abnormalities in von Willebrand's profile. One of the observations for years has been the elevated levels of d-dimer in cases of CIRS as an unexplained finding in CIRS cases. I can't tell you in how many people I have chased after elevated d-dimer levels, looking for evidence of intravascular clotting, without finding a source.

Now that we have gene activity, we are looking at a different mechanism for this non-specific rise of d-dimer. At first glance, it seems odd that enhanced clotting would lead to enhanced bleeding. Missing is the role of enhanced lysis of sub-clinical clots, hence the increased d-dimer. With correction of inflammation, the transcriptomic basis for d-dimer formation, namely enhanced clotting, resolves. **Defensins** are substances made by white blood cells used non-specifically to combat ongoing bacterial or viral infection. With elevated defensins, there usually will be an infectious basis. Defensins are not activated without basis.

Granzymes are intimately involved with apoptosis. If granzymes are elevated, there will be activation of signaling for natural killer cells and cytotoxic T-lymphocytes to sort out and then kill the targeted cells. "No cell lives forever" is an old expression but has a lot to do with granzyme function.

For years, I have had wonderful Sunday afternoon discussions with clinicians regarding **methylation**. Methylation seems to be an active subject for discussion among many alternative providers. Epigenetics, anyone? The idea is that by putting a methyl group on a gene, there will be an effect on the gene activity. But that idea does not include the role of ***demethylation*** reversing the activation (or suppression) the methyl group could create. There is an additional role-modulating gene activity for a two-carbon chain attachment, that being ***acetylation*** and ***deacetylation***. This two-carbon subunit is more frequently attached to histones controlling the structure of the insulating proteins around DNA. We can't look at epigenetic change without thinking about methylation and demethylation—and acetylation and deacetylation. Some of these gene-changing properties of a single carbon group (or two-carbon group) can be long lasting, others are short lasting. It is another mechanism of regulation of gene activity.

Cytokine changes are very difficult to measure in blood. The reason for this oddity is that these pro-inflammatory humoral factors can be bound by the cell that makes them or by the adjacent cell. The only measure we see in blood test results is the so-called "endocrine" function of the cytokine floating unbound in blood. Assays, including the multiple cytokine assays, will tell us about the endocrine function of

cytokines but not the autocrine or paracrine. Gene activity from GENIE tells us activity immediately, without concern about false elevation or false suppression of endocrine values.

In all the discussion about what an objective test for **Lyme disease** is, NeuroQuant rises to the top in that it shows a distinctive pattern whether the patient has had Lyme for six months or six years. Finally, thanks to the work of Bouquet, et al,1 we also have an additional distinctive marker for Lyme, a transcriptomic series of genes that show us what occurs in Lyme before antibiotics and Lyme after antibiotics. We worked independently of Bouquet's group to now come up with a Post-CIRS marker for what we see in Lyme patients after antibiotics are done and CIRS therapy is initiated. These are exciting times for Lyme!

As briefly mentioned previously, for years I have looked at HLA being the marker for who has increased relative risk for CIRS illness. The number 24% for total at-risk HLA in the US population has come up repeatedly both in my practice as well2 as in practices of many other physicians. We know that HLA has a lot to do with antigen presentation and immune response based on its location of chromosome 6. The idea has been that if there is a problem with antigen presentation, represented by HLA, then there will be a defect in antibody formation. No antigen presentation, no antibody. No antibody, no protection for repeat illness with re-exposure.

Absence of protection from relapse with re-exposure is a cardinal finding in CIRS. We now have an additional marker for defective antigen presentation, namely the gene abnormality seen in the **T-cell synapse** with antigen presenting cells. What this means is that when a pathogen is taken into an antigen presenting cell, the professional antigen presenting cell (1) breaks down the pathogen into small fragments in the lysosome; (2) is then processed through the endoplasmic reticulum; and (3) loaded onto a major histocompatibility (HLA) receptor; (4) which is then taken to the cell membrane, creating a signal that permits a naïve T-cell to home in and attach to this tasty morsel of this antigen ready for recognition and T-cell processing.

The first step for antigen presentation, after antigen processing, is formation of a synapse between the dendritic cell and the T-cell. The genes involved in this vitally important synapse are ones that are routinely found to be *suppressed* in untreated CIRS. Fortunately, treatment, especially with VIP, corrects the T-cell synapse abnormalities. The problem is not just HLA!

We know that the gene expression for **complement** remains important in many elements. By including a gene marker, we can look at the 33-member protein system in a different fashion compared to trying to make sense of changes in individual proteins. Complement interactions can be numbingly complicated!

In the world of **PTSD**, while we think we have identified a biomarker in NeuroQuant, we just don't have an adequate number of cases. There is a gene reported to be

involved with ACTH and cortisol metabolism that has an association reported in the literature suggesting that it is important in recognition of PTSD. This gene is part of GENIE. Initial results are promising; data is in its infancy.

The **cytoskeleton** of a cell is based on microtubular formation. These microtubules are dependent on their genes called tubulins. If there are tubulin abnormalities, like what we see in genes found in plants where benomyl (an azole anti-fungal) had been used, abnormalities can tell us about microtubule problems in day-to-day life of the patient.

There are additional genes as part of GENIE, including those looking at insulin signaling; those looking at anti-inflammatory nuclear transcription factors; those looking at activation of MAP kinases as well as B-cell markers for the synapse between T-cells and B-cells.

Taken together, the information we glean from GENIE cannot be accessed by any diagnostic mechanism. GENIE, therefore, is providing us with information about abnormalities of physiology based on gene activation. CIRS remains the teacher for other illnesses to follow. We have identified the origins of abnormalities in transcriptomics, followed through with abnormalities of proteomics, followed through with hormonal disruption and multiple layers of dysregulation of proteomic activity.

All these changes can be shown to be related to *(1) dysregulation of (2) dysregulation of (3) gene regulation*. That's right; at least three layers of defective regulation of gene activity are found in illnesses such as CIRS.

From where I sit today, there is no limit to what questions we can ask of the transcriptomic findings accumulated to date. Sometimes the more important features of a new paradigm aren't simply what is newly found to be true, but what was incorrect about older ideas. We return to Aldous Huxley telling us, "The key to understanding is casting out false knowledge." We are "casting out" every day, it seems. Let us not forget that only a few ideas in sciences survive the passage of time. Simple applications of the "casting out" from transcriptomics let us see that viral reactivation is not likely to be a root cause of CFS, despite antibody testing that appears significant. Another is the diagnosis of "mycotoxin illness," already been exposed as flawed earlier. With the ability to define the expected differential gene activation associated with mycotoxin exposure from the literature, we can flesh out what is likely associated with pathologic changes after mycotoxin exposure and what is not. Remember that in CIRS-WDB we see suspected endotoxin effects in over 50% of cases, closely followed in suspected incidence by actinomycetes. Mycotoxin findings are a distant third.

The enormity of importance of hypometabolism in assessment of a unified cause of fatigue and a transcriptomic mechanism to show correction of that cause brings hope to those searching for answers to fundamental questions, such as "When will I

get my life back?" Or, "When can I walk into a new restaurant without fear that the restaurant was once a WDB from a prior flood indoors?" Or, "Are my children condemned to this kind of life due to my HLA?"

We must also consider the role of several compensatory metabolic mechanisms once hypometabolism has been initiated. If mitochondrial injury from a ribotoxin attack on mitochondrial ribosomes (mitoribosomes) is present, the cell won't be able to shuttle its normal amount of the fuel source pyruvate, created by glycolysis, into mitochondria. Excess pyruvate not taken into the mitochondria would otherwise be converted to lactic acid, an intracellular poison. How does the cell avoid dying from lactic acid? Simple, reduce glycolysis! Curiously, in the presence of interferon gamma, one of the enzymes that does the work in glycolysis (GAPDH) also interacts with ribosomal protein L13a and a transfer RNA (EPRS) to form a protein complex called GAIT. The GAIT complex will bind to a specific set of messenger RNA in the cell to curb inflammation. "Surviving hypometabolism" is getting complicated. And there is more.

We are building a database to attempt to show what role the insulin receptor has in hypometabolism. We have interesting findings on insulin receptor substrate 2; the data show great promise.

The sustained finding of genes that predispose to defective apoptosis also holds great promise. We see one particular gene repeatedly in patients with abnormalities in the caspase-driven mechanism of programmed cell death. If the dying cell, programmed to be lysed by natural killer cells and cytotoxic T cells, fails to safely "package" its intracellular materials that are intensely inflammagenic before lysis, bad things will happen. Face it, if cellular contents, especially DNA, are released freely into circulation, we will have an endogenous source of inflammatory response. As Pogo would tell us, "We have found the enemy and he is us." Upcoming investigations are focused on correlation of abnormal NeuroQuant findings with early dementia. By looking at tau in spinal fluid and simultaneous transcriptomics, we hope to bridge the gap between unknown gene activity in brain tissue and known activity in blood cells. We can't use brain tissue for gene expression studies, but we may have a biomarker in blood to correlate to NeuroQuant abnormalities and cognitive decline, as one of the genes on our GENIE, found overexpressed in CIRS patients, is also found in beta amyloid plaques. The commonly found differential activation of coagulation genes that are responsive to VIP provides another reachable window for intervention. Stay tuned on this topic!

I would be remiss if I didn't mention treatment of dilated cardiomyopathy in a patient with at least four documented bouts with acute Lyme disease. As her ejection fraction by MUGA bottomed out at 11%, a referral to a cardiac transplant unit at the University of Maryland was made. RNA Seq using whole blood, not cardiac cells, showed a complex pattern of gene disruption, including adrenoceptors and those involved with the calcium/sodium pump, the basis for contractility. Addition of high dose VIP after antibiotics and CIRS RX resulted in correction of

heart failure, reduction of LVIDD from 9.2 centimeters to 6.8 in less than one year. I was elated but Dr. Ryan quietly reminded me that a N=1 study, as this one is, must be shown in many others because we can't confirm that cardiac myocyte findings would parallel the WBC findings of correction of genes involved in contractility. My N=1 case success is one more than anything I have seen from the Lyme-dilated cardiomyopathy world.

Even still, the patient is fully active now, without symptoms of heart failure, off all meds. An unexpected spin off in this trial was confirmation of negative effects from carvedilol that followed a dose/response of adrenoceptors. This beta blocker is widely used in heart failure, sometimes with serious adverse effects. Transcriptomics showed us the way in this case; future titration of carvedilol dose to adrenoceptor expression makes sense if the WBC genes hold up as a model for cardiac myocytes.

Summary

The story of CIRS could fit into Thomas Kuhn's *Structure of a Scientific Revolution*. What began as an *anomaly,* an isolated observation of fish kills and human illness from exposure to *Pfiesteria*, a dinoflagellate, has expanded over the past twenty-three years to an integrated *paradigm* of an entirely new illness concept that for the first time provides a supported, evidence-based explanation for countless chronic fatiguing illnesses. It is possible that there is no "modern illness" paradigm that has *more supporting biomarkers* than CIRS. Beginning with exposure assessments, especially use of HERTSMI-2 for CIRS-WDB, cluster analysis of symptoms, and labs ranging to proteomics and transcriptomics, and including volumetric studies of brain injury, stress echocardiogram measurements and VO2 max, the diagnosis and treatment is buttressed by association studies, prospective re-exposure trials using a published protocol (SAIIE), and randomized clinical trials. Using published protocols, we have corrected proteomics, transcriptomics, and grey matter nuclear atrophy.

What this density of objective biomarkers provides is confirmation of diagnosis and treatment, backed by nearly 40 published papers and clinical use by thousands of physicians. As the research basis of CIRS continues to expand, we feel that CIRS will provide the basis to look for new approaches to inflammatory illnesses of our era, especially atherosclerosis, obesity, diabetes, and chronic pain.

And one parting thought: hope for cure is here. Hope now rests on hard clinical trials that show us the way to help those trapped by WDB, among other causes of CIRS. The answers to causes of chronic fatigue are apparent; effective gene-based therapies also are apparent.

In data we will find our answers for today's hope and tomorrow's standard of care.

References

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Ritchie C. Shoemaker, MD, remains active in the field of biotoxin-associated illnesses, the focus of his practice since 1997. At that time, an outbreak of unexplained human illness, associated with exposure to blooms of a dinoflagellate, Pfiesteria piscicida, attracted his attention and interest. Pfiesteria was the first example of an acute and then chronic biotoxin-associated illness recognized and published in peer reviewed literature. Shoemaker's two papers on diagnosis and then treatment were the first in the world's literature on acquisition of illness from Pfiesteria in the wild. Since that time, other sources of biotoxin associated illnesses have come forward including other dinoflagellates, cyanobacteria and, most importantly, organisms resident in water damaged buildings. Shoemaker has spent the last 22 years treating patients and conducting research that unveils the extraordinary complexity of these illnesses, now called chronic inflammatory response syndromes (CIRS). Starting with no biomarkers and now progressing to over 25, CIRS has been shown to have abnormalities in proteomics and transcriptomics with differential gene activation, the final ultimate pathway of disease production in the world of chronic fatigue.

His collaboration with Dr. James C Ryan, transcriptomist, has led to multiple publications that have application, not just to chronic fatiguing illnesses but to the inflammatory illnesses of the 21st century including atherosclerosis, diabetes, obesity, and autoimmune illness.

As Shoemaker's work has progressed on the complex problems of grey matter nuclear atrophy, a small but growing cohort of patients with multinuclear atrophy

and cognitive impairment have led to improvements that may have application to illnesses such as Alzheimer's disease.