8 Inflammatory Responses Acquired Following Environmental Exposures Are Involved in Pathogenesis of Musculoskeletal Pain

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INTRODUCTION

At first, one might not associate environmental exposures with pain. Warm climate versus cold causing pain? Humid climate versus dry causing pain? Urban versus rural? Pain isn't the first response a physician might necessarily associate with environmental exposures.

Contrast that perspective to environmental exposures to low molecular weight biotoxins and/or inflammagens made by one-celled creatures. Inflammation from innate immune responses dominates the illnesses created by biotoxins. Innate immunity can heal, but can also become the overwhelming source of illness caused by exuberant host responses. Add to the level of concern when exposures are to water-damaged buildings (WDB; schools, workplaces and residences), a problem seen in 50% of US buildings (NIOSH) [1]; or fresh water bodies hosting blooms of cyanobacteria (for example, Lake Erie [2]; or even just exposure to tick habitat, an increasing problem now extending well beyond the Northeast of the US. In the cohort of affected, environmentally exposed patients, pain syndromes are found in over 85% (Table 8.1).

Inflammation from innate immune mediators does not quickly come to mind thinking about "wear and tear" arthritis or overuse syndromes like rotator cuff injuries or tennis elbow.

Curiously, the same inflammatory mediators involved in response to adverse environmental exposures also play major roles in musculoskeletal pain, as will be discussed.

What we can't say is that environmental exposures are the sources of worn and torn, painful hips, knees and lower back; but we can say that understanding how to correct the pathogenic inflammatory responses to environmental exposures to toxins and inflammagens now has become fertile ground for development of therapies, both proteomically and genomically active, that control innate immune responses.

While one might speculate if new therapies would have been developed for musculoskeletal pain without learning how to heal illness caused by environmental exposures, one simply can conclude that as we learn more about innate immune responses, and transcriptomic changes associated with the immune responses, we learn how to think differently about common sources of pain. Said another way, there are few activities defined that are exempt from immune participation.

As we understand and catalogue the unknown biotoxin exposures we frequently encounter, such as consumption of fish contaminated by algal neurotoxins, we understand more about the importance of recording diverse environmental exposures in assessment of pain. Ciguatera, caused by ingestion of reef fish contaminated by ciguatoxins, a potent voltage gated sodium channel activator,

TABLE 8.1
Symptoms in Various CIRS Conditions

Symptoms	Controls	Cyano	WDB-1	WDB-2	WDB-3	PEAS	Ciguatera	Lyme
N=	239	10	156	288	21	42	100	352
Fatigue	6	100	89	83	100	70	91	94
Weak	<5	80	75	70	84	—	83	89
Ache	8	90	77	68	95	43	77	81
Cramp	<5	80	66	56	63	14	68	77
Unusual pains	<5	50	62	51	42	—	82	86
Ice pick pain	<5	40	49	41	—	—	45	82
Headache	9	90	78	66	84	73	78	88
Light sensitivity	<5	90	71	66	89	68	67	85
Red eyes	<5	50	52	48	63	68	48	61
Blurred vision	<5	40	61	56	63	—	53	66
Tearing	<5	30	41	48	63	—	28	55
SOB	11	60	78	63	74	57	63	77
Cough	7	50	72	53	53	43	62	71
Sinus congestion	8	60	79	65	74	41	70	68
Abdominal pain	<5	60	61	39	37	41	79	42
Diarrhea	<5	50	48	39	21	57	72	51
Joint pain	11	70	75	53	84	—	62	88
Morning stiffness	6	70	72	44	—	—	59	80
Memory impairment	<5	80	83	66	68	84	81	80
Difficulty concentrating	<5	70	81	62	53	35	83	82
Confusion	<5	40	75	57	26	24	66	72
Decreased word finding	<5	80	81	66	11	—	80	84
Decreased assimilation	<5	80	72	65	37	—	78	88
Disorientation	<5	30	51	40	11	—	28	33
Mood swings	<5	20	69	65	—	—	42	65
Appetite swings	<5	50	58	58	—	—	61	77
Sweats (night)	<5	50	61	54	—	—	42	68
Difficulty reg. body temp	<5	50	63	60	—	—	67	72
Excessive thirst	<5	60	69	54	—	—	59	71
Increased urinary frequency	<5	60	66	58	—	—	66	75
Increased susceptibility to static shocks	<5	40	41	44	—	—	38	32
Numbness	<5	40	48	44	37	—	74	66
Tingling	<5	40	61	51	47	—	78	71
Vertigo	<5	40	39	48	42	16	29	37
Metallic taste	<5	40	45	36	47	—	46	38

was marked early on [3, 4] as a source of a reversal of hot/cold sensation called "cold allodynia." In recent investigations, additional sources of this curious pain syndrome have been identified in other environmental, exposure-related syndromes [5] as having abnormalities related to a variety of sensory neurons with transient potential receptor vanilloid-1 ([TPRV-1]; 6). This singular finding of the link from biotoxin exposure to chronic pain has opened a flood of academic papers that show promise in bringing new approaches to management of chronic pain. We will return to an in-depth discussion of TPRV-1 and its family of receptors in the section "CGRP, SP and VIP: Pain Regulators". Ciguatera didn't have a prominent reputation for teaching us about chronic pain; that lack of notoriety no longer applies.

The underlying basis of pain syndromes from these different types of exposures to environmental biotoxins/inflammagens can be summed up in one word: inflammation. Inflammation is a time-honored cause of pain. From the first day of pathology class in medical school, physicians are trained to recognize heat, redness, swelling, loss of function and pain as manifestations of inflammation. What we learned in the 1970s about inflammation, however, was just a proverbial "drop in the bucket." In a seminal lecture at Cold Spring Harbor in 1989 [7], Charles Janeway foretold the future of inflammation research and therapies to come. Innate immune responses were in their infancy then; now it is routine to pick up an immunology journal that has at least half of its "state-of-the-art" papers either on innate immunity or transcriptomics.

The expanding world of inflammation has been unveiled over the past thirty years, beginning with publications on cytokines, expanding to receptors, cell-based immunity and more. Our current era of research shows us the incredible diversity – and speed – of genomic responses to environmental stimuli. When inflammatory responses become chronic, as abnormalities in immunity often will become, those who suffer with pain will surely have pain that becomes chronic as well.

There are two areas that will be highlighted in this chapter regarding musculoskeletal pain and environmental stimuli. First are manifestations of chronic inflammatory response syndromes (CIRS) in exposed and subsequently affected patients. This actually is an incredibly large subset of patients; most aren't diagnosed. Second are the associated effects of individual elements of CIRS, like TGF beta-1, VIP and microRNA, for example (definitions to follow) that appear by routes other than those acquired following environmental exposures. For example, we know that certain HLA haplotypes are associated with increased relative risk for CIRS [8] and we know that certain HLA haplotypes are also associated with ankylosing spondyloarthropathies (HLA B27). We will not discuss mechanisms of HLA association with epidemiologic risk so much as to say that the established body of evidence demands that we know to look for these associations now that we know those associations are not random occurrences.

We will look in detail at the lessons learned from transcriptomics [9, 10]. The central dogma of molecular biology states that genes produce transcripts that are translated into protein, at which point the protein can perform the task required by the cell. The genome is the ultimately the director of all cellular activity What's important to understand is in addition to the presence of any given gene in the genome is the *differential expression* of that same gene. Single nucleotide polymorphisms (SNP), may tell us about protein function but nothing about actual gene expression. If an SNP causes a 5% decrease in protein function, but that protein is expressed at an amount 5% greater than normal, the system likely suffers no effect. But of greater importance than expression of a single gene is differential gene expression of entire molecular pathways, as well as transcription factors, receptors, clusters of differentiation (CDs), pseudogenes, microRNA and long noncoding RNA, among others.

A second facet of pathway analysis of illness causation is readily observed by looking at genes that are overexpressed. What other unexpected adverse health effects follow? Since transforming growth factor beta-1 (TGF beta-1) is overexpressed in CIRS-WDB [11], and since TGF beta-1 turns on the processes involved in fibrosis, will there be more fibrotic lung tissue in CIRS-WDB, for example? The data that say yes are easily found but are anecdotal. The data are far less clear on matrix metalloproteinase-9 (MMP-9), also seen routinely to be a problem in CIRS-WDB. Can we show that herniated nucleus pulposus (HNP), a process involved with MMP9 is more common in CIRS-WDB patients? Or are TGF beta-1 and MMP9 simply reflecting redundancy of biological regulation of gene expression?

The argument regarding multiple sources of stimuli will be revisited when we discuss VIP and degenerative arthritis.

As an aside, this chapter will not focus on the cellular basis of nociception, the sensation of pain, so much as we will attempt to show that the diversities of inflammatory responses, ones that potentially can affect every cell in our body, are part of the daily maintenance of life itself. We suggest that pain, as part of what can be called disease, is dynamic, involving all regulatory and effector

arms of innate and adaptive immune defenses, though the role of adaptive immunity in pain will be covered in less detail.

We will begin by exploring CIRS and then return to musculoskeletal conditions beginning in the section "Herniated Nucleus Pulposus and Innate Immune Effectors".

WHAT IS PAIN?

Multiple attempts have been made to define pain. For the purposes of this chapter, the definition from the International Association of the Study of Pain is adopted (www.iasp-pain.org, accessed 7/2/2107). "Pain is the unpleasant sensory and emotional experience associated with actual or potential tissue damage."

The perception of pain, nociception, involves multiple layers in which inflammatory effects might be exerted: (1) signal transduction by sensory neuron receptors; (2) transmission of signal via neurons: (3) modulation in the dorsal horn of the spinal cord; (4) and perception in the thalamus (ref). Each of these main elements of the experience of pain will be investigated for possible association with CIRS and its innate immune effectors.

WHAT IS CIRS?

Chronic inflammatory response syndromes (CIRS) are multisystem, multi-symptom illnesses acquired following exposure to environmentally produced biotoxins [11–15].

CIRS has gone through an evolution of names over the years. Initially, in the 1990s, CIRS was called a neurotoxin-mediated illness [12, 13]. As more information developed, the term was changed to chronic biotoxin-associated illness (CBAI). The third change to CIRS occurred following development of a commercial assay for TGFβ1 in 2008, readily available for insurance, coverage then by the development of a commercial assay for acquired T regulatory cells in 2009. CIRS was confirmed to involve many arms of the immune response systems acting simultaneously and in combination.

CIRS itself is modeled after an acute systemic inflammatory response syndrome (SIRS), an acronym typically only used to describe an acute inflammatory illness, sepsis. In patients with sepsis there is simultaneous activation of Th1-, Th2- and Th17-immunity; coagulation factors; and complement in response to an overwhelming stimulus of infection and endotoxin present in the blood stream. In this regard illness becomes the host response as eloquently described by Thomas [16].

Survivors of sepsis have been well studied; they do not have the same immune reactivity after one month as they did before sepsis started. Survivors have a significant increase in interleukin 10 and go on to develop greater incidences of chronic fatiguing illnesses [17, 18].

If an ICD code is to be used for an affected survivor of sepsis, should we be calling their illness chronic sepsis survivor syndrome? We clearly would not be able to call the illness an acute systemic inflammatory response syndrome. What should we call it?

In 2008, followed by a publication in 2010 [19], members of the small "mold" medical community began using a jargon term, CIRS-WDB, to describe illness seen with the same activation of Th1, Th2, Th17, coagulation, complement activation and more. If the illness came from water-damaged buildings, we called it CIRS-WDB. If it came from Post Lyme Syndrome, we called it CIRS-PLS. If it came from ciguatera we called the syndrome CIRS-ciguatera. Theoretically, we could call the syndrome most anything; possibly the syndrome would be codified by a regulatory agency, much as the CDC changed the name, "Pfiesteria health illness syndrome (affecting) humans," PHISH, for acute and chronic illness caused by exposure to blooms of toxigenic, fish-killing dinoflagellates, including Pfiesteria, to "Possible estuarine-associated syndrome" (PEAS) [11].

CASE DEFINITION OF CIRS

In 2008, the US General Accountability Office (GAO) published an overview of publications from US agencies working on the problem of damp indoor buildings [20]. Fifty-four studies were noted, showing no coordination of efforts across agency lines. But for the first time, a Federal case definition for what has become CIRS-WDB was proposed.

1. There must be the potential for exposure to a damp indoor space.
2. There must be a multisystem, multi-symptom illness present with symptoms similar to those seen in peer-reviewed publications.
3. There must be laboratory testing results similar to those seen in peer-reviewed, published studies.
4. There must be documentation of response to therapy (symptoms correction alone wasn't enough).

This definition still is used even though there has been an explosion of publications in the CIRS community noting various objective parameters in wide clinical use now (SM consensus).

Symptoms are noted to be essentially identical in multiple sources of environmental exposures resulting in CIRS (see Table 8.1). Of interest is the appearance of clusters of symptoms among the 37 symptoms recorded in CIRS (Table 8.2). Presence of eight or more (of the 13) clusters is virtually diagnostic of an unspecified type of CIRS (US Patent #US 9,770,170 B2).

Returning to the multiple sources of TGF beta-1, and the association of TGF beta-1 with painful musculoskeletal syndromes, the clinician can use the case definition and cluster analysis to quickly rule out CIRS in initial evaluation of a patient with a painful shoulder or low back pain. If there is

TABLE 8.2
Cluster Analysis

Cluster Analysis of Symptoms

Individual categories:

- Fatigue
- Weakness, assimilation, aching, headache, light sensitivity
- Memory, word finding
- Concentration
- Joint, AM stiffness, cramps
- Unusual skin sensations, tingling
- Shortness of breath, sinus congestion
- Cough, thirst, confusion
- Appetite swings, body temperature regulation, urinary frequency
- Red eyes, blurred vision, sweats, mood swings, icepick pains
- Abdominal pain, diarrhea, numbness
- Tearing, disorientation, metallic taste
- Static shocks, vertigo

A positive cluster analysis for biotoxin illness is presence of eight or more of 13 clusters

When we use persistent health symptoms as a group of 37, recorded by a trained health care provider in a medical history (never use patient-completed checklists), we can collate individual symptoms into groups, called clusters. Statistically, these clusters of symptoms, 13 in number, yield a diagnostic capability to separate out CIRS from essentially all diseases. If you have eight or more clusters of symptoms, the likelihood of CIRS exceeds 95%.

When combined with VCS deficits, symptom clusters can yield an accuracy in diagnosis of 98.5% (that means the sum of false positives and false negatives is less than 2%).

no multisystem illness identified by a medical history taken by a licensed health professional (NB: patient-completed checklists have too much potential for reporting bias to be used), CIRS is not the problem. But if elements of CIRS are present in the absence of actual CIRS, they must be noted and treated to affect clinical outcome. Elevated levels of TGF beta-1 cannot be simply ignored in cases of neuropathic pain, for example.

In the experience of this author, most consultants looking at pain are surprised when a seemingly isolated problem, like a rotator cuff tear or an enthesopathy, like lateral epicondylitis, are present as a part of a constellation of twenty other symptoms! Who knew? The medical historian. If the "diffusely positive review of systems," is not recorded, CIRS will never be diagnosed.

SYMPTOMS OF PAIN IN CIRS

In a medical history, the physician is not using a check list. Symptoms questioning is perhaps better understood after observing how a skilled attorney will question a witness. Essentially, he will likely be asking for the same information from multiple different angles trying to have a clear idea of what the witness actually said or did. Similarly, a physician will follow the line of thought of a patient in discussing symptoms, but will circle back to pin down any possible vagaries that might be present. In this manner, a physician will learn to a reasonable degree of medical certainty, more likely than not, on a day-to-day basis, does a patient have muscle aching? Does a patient have muscle cramps? Are there unusual pains, sharp stabbing pains that seemingly come unexpectedly and lancinate in one area of the body only to disappear and reappear elsewhere the next day? This type of pain description is typical of what CIRS patients experience and is absolutely not confabulated-but will sound odd to the physician who is not used to recording unusual pain histories.

Ask about unusual posturing of fingers or toes, sometimes called "clawing." These involuntary spasms in small muscles of fingers and toes can be painful, can be quite unusual for those affected and are certainly unusual elements of history. Some patients will have their long and fourth finger split apart making a sign of a V as we saw so often from Mr. Spock in Star Trek. Sometimes there will be arching of the MCP and MTP joints as well. If you don't ask about clawing, you won't be told. Patients will recognize a careful historian who asks about unusual muscle spasm when they have clawing.

Muscle cramping is a common problem in athletes (and CIRS patients!). How many times have we seen a star basketball player clutching at his calves in the middle of the 3rd quarter of a heated basketball game? Cramps are disabling! He limps off the court; his team's fans hold their breath until he stretches the cramp out and returns to the 4th quarter to win the game.

The calf cramps CIRS patients often have are not related to heat or to exertion. They are far more commonly brought on by lying down in bed or arising from sleep. The cramp is a muscle spasm; CIRS patients quickly learn that the spasm experienced in the middle of the night can be severe, especially if they have been sleeping with their ankles extended. Simple dorsiflexion of the ankles, stretching, for example, is mandatory but sometimes the spasm is so severe the gastrocnemius muscle essentially twists into a knot, occasionally tearing. Just so you know, in the middle of the night, that pain is agonizing. If you don't ask about cramps like these you won't be told. Physicians just aren't taught to take CIRS spasm histories. The spasms are real.

Joint stiffness is what we see often in CIRS as well as in day-to-day musculoskeletal medical practice. Stretching upon arising is a common way to start the day for both types of patients. Shoulders, elbows, knees, low back, all will require some sort of attention. The rate of stiffening, however, with *cessation* of activity, called "gelling," is far faster in CIRS than it is for patients with wear and tear degenerative arthritis. If a patient tells you that he would prefer to stay standing after activity rather than sit down and rest that may be an indication of his awareness of his own rate of gelling.

Aching is perhaps the most common CIRS pain. Not from over-doing the work in the yard or trying to work out excessively, muscle aching can be vague until the aching occurs almost every day regardless of antecedent activity, but not in the same area and not with the same intensity. CIRS

aching won't respond to most meds, including NSAID. The aching often comes from areas of muscle insertion on tendons, raising the concern regarding enthesopathies. The enthesium has reduced blood supply and reduced capillary perfusion to begin with; inflammatory responses in CIRS make capillary hypoperfusion worse. Pain will be enhanced in the face of hypoperfusion. If one sees an enthesopathy but doesn't find a convincing history of overuse, spend a few minutes exploring the rest of the CIRS symptom roster with the patient.

As we will discuss in the section on transcriptomics, downregulation of nuclear encoded mitochondrial genes can be profound. Instead of postulating *intrinsic* mitochondrial disorders leading to abnormal mitochondrial metabolism of glucose, leading to lactic acid accumulation, a better approach is to recognize that nuclear transcription factors exert direct effect on mitochondrial function. By recording nuclear encoded mitochondrial gene activation, using Next Generation Sequencing, the physician can understand reduced energy delivery is abnormal due to aberrant genomic control. Still, whatever causes lactic acid accumulation, excessive lactic acid in capillary beds is a source of muscle pain, including aching.

Understanding that mitochondrial research is ongoing [21] looking at mitochondrial stress responses, we feel that a delineation of mitochondrial gene expression be examined as an upstream regulator of stress responses.

Headaches are not necessarily a musculoskeletal problem but can overlap with any pain syndrome. With CIRS we look for intravascular volume depletion and reduced antidiuretic hormone (ADH) levels for a given osmolality. If patients are troubled with headaches, especially if they have "migraine that lasts for more than 24 hours," think of ADH/osmolality and not an actual migraine.

Symptom variability is notoriously present in large segments of patient populations. Use of a physician history is necessary, as patient-completed questionnaires are unreliable. If someone presents a checklist of their health symptoms, the healthcare provider is obligated to review those symptoms to be sure whether they are present to a reasonable degree of medical certainty. Having said that, if a busy orthopedist needs to see eight patients per hour, he may need to delegate symptom recording to a staff member.

THE BIOTOXIN PATHWAY

Please refer to Figure 8.1 for a schematic representation of the series of events routinely seen in cases of CIRS. For a more detailed assessment of the elements of the Biotoxin Pathway, the reader is recommended to review information found on www.survivingmold.com.

The proteomic lab tests we rely on might be new to readers. We note abnormalities of a set of proteomic variables and have used proteomics to (1) aid in diagnosis of CIRS; and to (2) document systematic improvement with therapy, as self-healing won't occur in CIRS like it might in simple overuse syndromes.

The approach used to stratify lab abnormalities seen in CIRS encompasses a number of basic principles. First, there is a relationship between cases and controls that involves immune gene alleles on chromosome 6 (*HLA*). This relationship, called relative risk, looks at the incidence in cases for a given parameter, in this case the incidence of HLA-DR alleles in CIRS, divided by the incidence of the same parameter in controls. We have a data base of an approximately 10,000 patients for whom HLA has been recorded. What we see is the "susceptible" haplotypes of HLA, those with increased relative risk greater than 2.0, found in 95% of CIRS-WDB cases, are comprised of (1) 11-3-52B; (2) 4-3-53; (3) 7-2-53; (4) 13-6-52C; (5) 14-5-52B; and (6) 17-2-52A. The incidence of these haplotypes in control populations is 24% [8]. Of note, the HLA nomenclature has evolved, in particular the numbers used for haplotypes. At one time, we had approximately twenty identified haplotypes; now there are over 54, with subtyping extending HLA-DR to literally hundreds of additional descriptors. In an effort to keep things simple, we have maintained our registry of HLA based on what was current in 2000. It may be of use to look at a roster of HLA haplotypes and susceptibility by illness found in the Rosetta Stone appendix published in *Mold Warriors* in 2005 and in *Surviving Mold* in 2010 (Table 8.3).

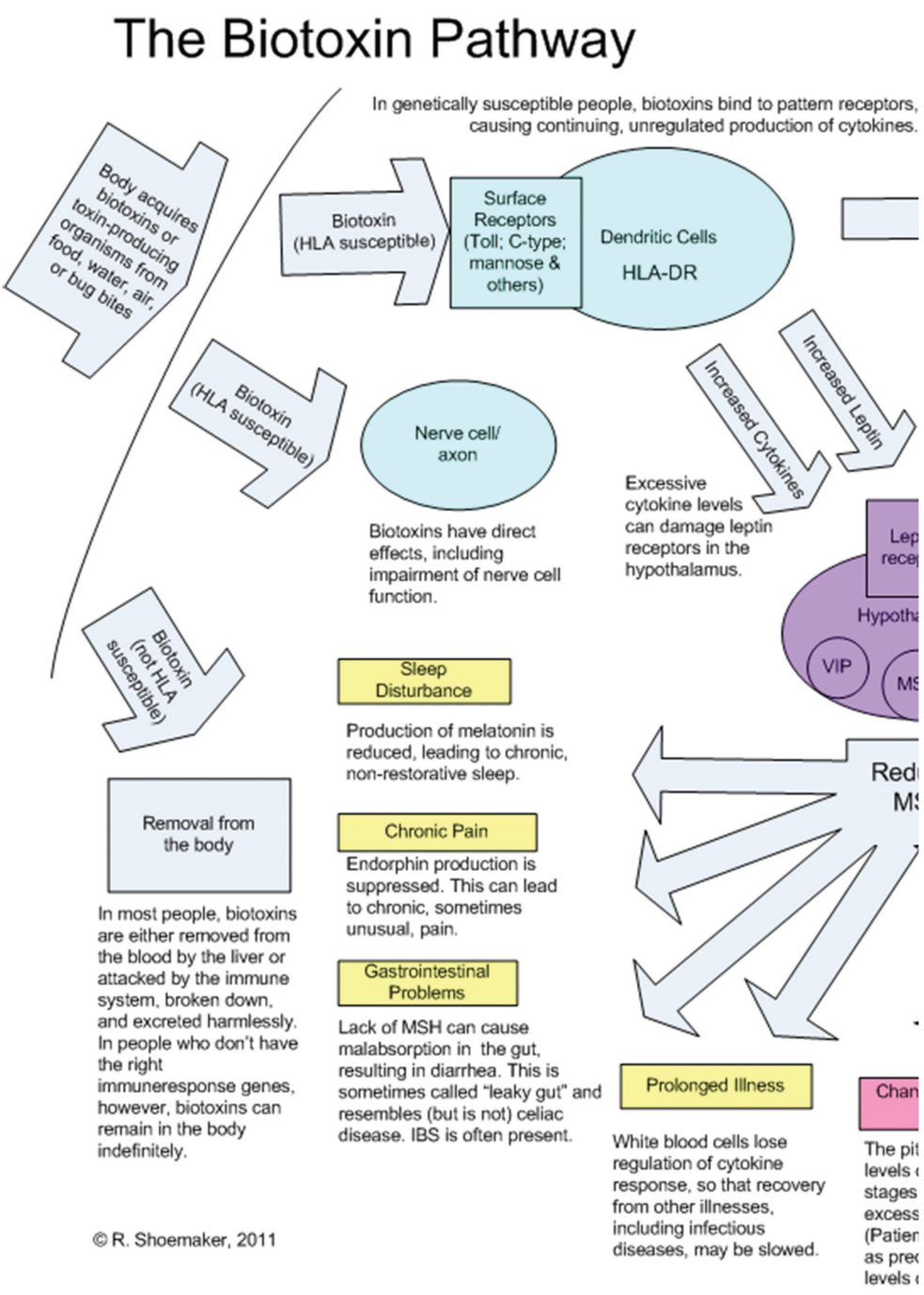
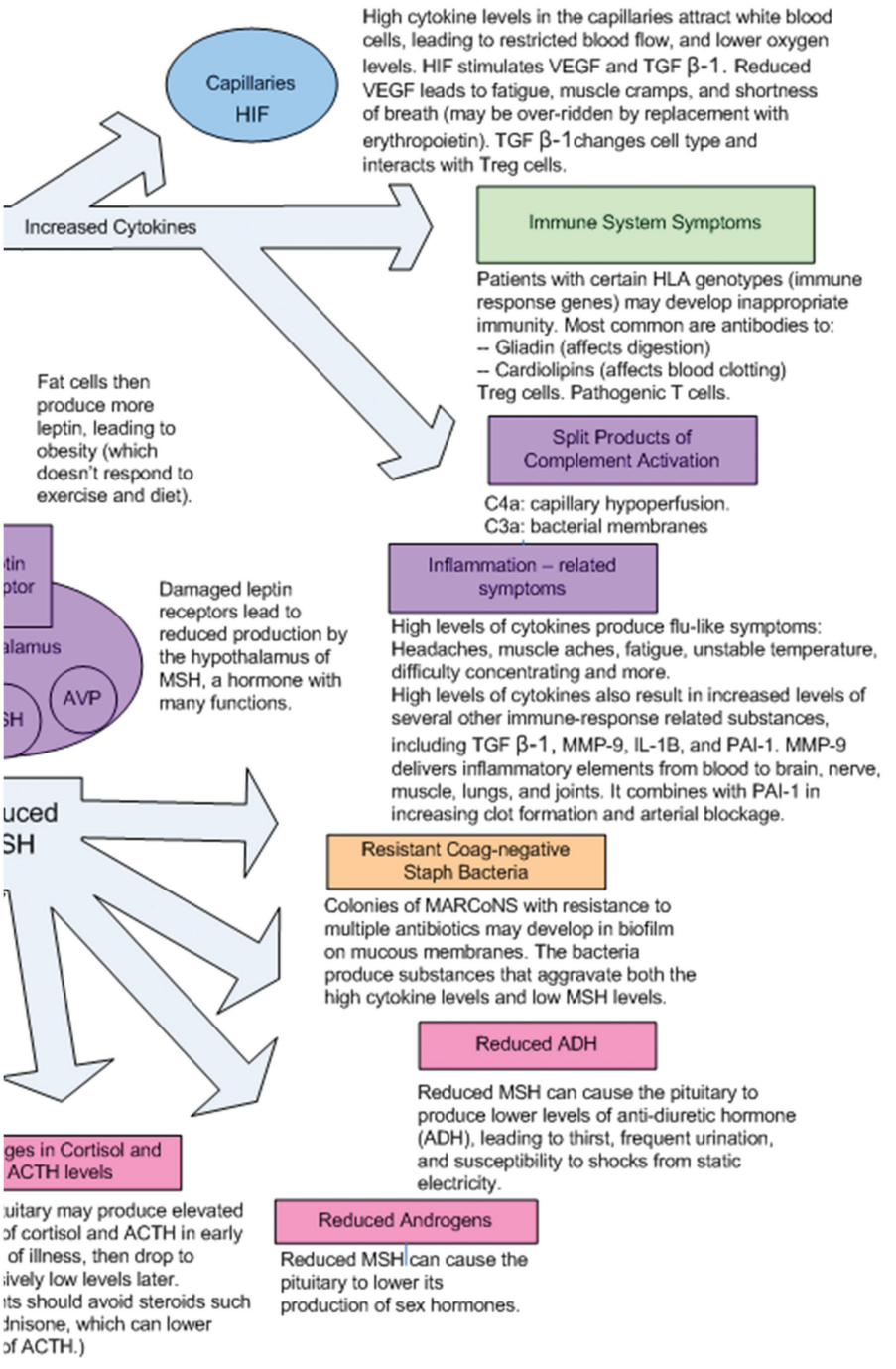


FIGURE 8.1 The biotoxin pathway


Capillaries
HIF
High cytokine levels in the capillaries attract white blood cells, leading to restricted blood flow, and lower oxygen levels. HIF stimulates VEGF and TGF β-1. Reduced VEGF leads to fatigue, muscle cramps, and shortness of breath (may be over-ridden by replacement with erythropoietin). TGF β-1 changes cell type and interacts with Treg cells.
Increased Cytokines
Immune System Symptoms
Patients with certain HLA genotypes (immune response genes) may develop inappropriate immunity. Most common are antibodies to:
-- Gliadin (affects digestion)
-- Cardiolipins (affects blood clotting)
Treg cells. Pathogenic T cells.
Fat cells then produce more leptin, leading to obesity (which doesn't respond to exercise and diet).
Split Products of Complement Activation
C4a: capillary hypoperfusion.
C3a: bacterial membranes
Inflammation – related symptoms
High levels of cytokines produce flu-like symptoms: Headaches, muscle aches, fatigue, unstable temperature, difficulty concentrating and more.
High levels of cytokines also result in increased levels of several other immune-response related substances, including TGF β-1, MMP-9, IL-1B, and PAI-1. MMP-9 delivers inflammatory elements from blood to brain, nerve, muscle, lungs, and joints. It combines with PAI-1 in increasing clot formation and arterial blockage.
AVP
Damaged leptin receptors lead to reduced production by the hypothalamus of MSH, a hormone with many functions.
Resistant Coag-negative Staph Bacteria
Colonies of MARCoNS with resistance to multiple antibiotics may develop in biofilm on mucous membranes. The bacteria produce substances that aggravate both the high cytokine levels and low MSH levels.
Reduced ADH
Reduced MSH can cause the pituitary to produce lower levels of anti-diuretic hormone (ADH), leading to thirst, frequent urination, and susceptibility to shocks from static electricity.
Reduced Androgens
Reduced MSH can cause the pituitary to lower its production of sex hormones.

Another cardinal feature seen in CIRS is reduction of levels of alpha melanocyte stimulating hormone (*MSH*) below 35 pg/ml. LabCorp has been the primary provider for this test. For unknown reasons, that lab changed the normal range of MSH in September of 2006 from where it had been placed correctly at 35–81 pg/ml to 0–40. Levels of MSH <1 are not compatible with life, so a level of -0- is not logically considered to be normal. Control testing published in several publications [11, 22] show that the newer normal range is not accurate; the older normal range is.

MSH is vitally important in regulation of inflammation, regulation of energy balance, regulation of circadian rhythm, regulation of limbic system parameters as well as regulation of keratinocytes, local mucus membrane defenses, immune defenses in blood and immune defenses in skin. It is a ubiquitously found regulatory neuropeptide [23]. With MSH deficiency we see enhanced development of CIRS following a priming inflammatory, cytokine event. Patients are borne with a given HLA, but HLA-based susceptibility to development of CIRS occurs only after an acute inflammatory event.

MSH is produced in a variety of tissues, especially in the hypothalamus and arcuate nucleus. The mechanism of production begins with activation of the leptin receptor, a primordial gp-130 cytokine receptor. Signaling from this receptor activates transcription of the gene for proopiomelanocortin (POMC). This parent molecule is split into MSH and beta endorphin, the natural opiate of the brain. In the presence of defective production of MSH, due to cytokine blockage of the leptin receptor, there will be MSH deficiency. Such a deficiency will be associated with decreased production of beta endorphin.

Deficiency of MSH can lead to chronic fatigue; blockade of leptin receptors leads to leptin resistance (the mechanism is similar to insulin receptor resistance [24]) and refractory weight gain; and deficiency of beta endorphin creates chronic pain. Here we have the basis for a devastating illness: chronic fatigue superimposed on chronic weight problems superimposed on chronic pain. When the clinician sees even one of this poisoned triad, think to add MSH deficiency and CIRS to the differential diagnosis.

TABLE 8.3
Human Leukocyte Antigen (HLA) Sequences Associated With Chronic Inflammatory Response Syndrome

	DRB1	DQ	DRB3	DRB4	DRB5
Multisusceptible	4	3		53	
	11/12	3	52B		
	14	5	52B		
Mold	7	2/3		53	
	13	6	52A, B, C		
	17	2	52A		
	18	4	52A		
Borrelia, post Lyme Syndrome	15	6			51
	16	5			51
Dinoflagellates	4	7/8		53	
Multi Antibiotic Resistant Staph epidermidis	11	7	52B		
Low MSH+melanocyte stimulating hormone	1	5			
No recognized significance	8	3,4,6			
Low-risk mold	7	9		53	
	12	7	52B		
	9	3/9		53	

Along with disruption of regulation of MSH production, (see discussion of beta-endorphins, below) are the influences of MSH on other hormone systems. For people with MSH deficiency, dysregulation of ACTH/cortisol is found in approximately 70% of patients; dysregulation of ADH/osmolality is found in 80% of patients; and dysregulation of gonadotrophins and androgens is found in approximately 40% of patients. Curiously, TSH and thyroid hormones are spared MSH regulation abnormalities.

MSH also exerts a damping effect on production of matrix metalloproteinase 9 (*MMP-9*), a protein made following cleavage of MMP-14, which is induced by the effects of cytokines in endothelial cells and white blood cells. We use MMP-9 because of the inability to accurately measure the total burden of Th1 and Th2 cytokines. The problem here is that cells that produce Th1/Th2 cytokines can bind given cytokines (autocrine effects) or cells nearby can also bind the cytokine (paracrine effects). When we measure IL1 beta or IL6 in the blood, the result is only the endocrine function of those cytokines. MMP-9 has additional important effects in chronic pain, namely delivery from inflammatory elements out of blood across cell membrane of endothelial cells through the basement membrane into the sub-intimal space. This delivery mechanism can create havoc in lung, brain, muscle, tendon and nerve [25].

The role of complement with generation of split products of activation, *C3a* and *C4a*, is also widespread in CIRS. These "built in" innate immune response agents are released quickly following cleavage of parent molecules, C3 and C4, respectively. When challenged by antigens, including infectious agents, C3a and C4a are vitally important to innate immune inflammatory responses.

TGF beta-1 remains a cytokine of great interest in musculoskeletal issues, specifically pain, as well as delayed healing in tendinous structures. We will return to TGF beta-1 in greater detail.

Of perhaps singular importance are levels of vasoactive intestinal polypeptide (*VIP*). VIP is a potent anti-inflammatory, neuroregulatory peptide, similar in function to MSH. It acts on three separate receptors, two of which, VIPR-1 and VIPR-2, are richly endowed on synovial cells as well as in joint tissue [26]. VIP is highly correlated with lack of inflammation, as we see in MSH, but it also has shown the ability to correct genomic abnormalities [10] as well as correct proteomics [11] and gray matter nuclear atrophy [27]. VIP replacement has revolutionized care of patients with CIRS. Given its important role in maintenance of joint integrity, one wonders how quickly use of this well-established anti-inflammatory neuropeptide will become standard practice. As new information on VIP comes to clinical trials, published data will lead the way.

An additional factor important to musculoskeletal problems, also present in CIRS, is vascular endothelial growth factor (VEGF). VEGF will stimulate enhanced delivery of oxygen in the capillary beds, especially important if there is enhanced reduction of capillary perfusion due to cytokine presence. Lack of oxygen delivery will compromise aerobic metabolism, particularly in muscle beds, creating reduced exercise tolerance, increased pain and absence of normal efficiency of electron transport chains in mitochondrial function.

These fundamental elements of CIRS also have importance for musculoskeletal pain and musculoskeletal injury, both as a whole and as individual effectors. The example of VIP in arthritic joints is one issue; similarly, enhanced entraining of TGF beta-1 into crosslinked collagen can increase susceptibility of those with hypermobility to the inflammation in fibromyalgia.

Two unanswered questions remain. First, if an inflammatory element is involved in musculoskeletal pain, and then that element is increased as part of CIRS, will the musculoskeletal pain increase? If so, will therapies known to be successful in CIRS cross over to be successful in musculoskeletal pain syndromes?

TRANSCRIPTOMICS

The most sophisticated research laboratory tool available today for testing physiology is performed using DNA sequencing. What this means is that scientists can now look at differential activation for

all genes in the human genome using a Next Generation Sequencer. The Human Genome Project, completed in the early 2000s at the cost of billions of dollars, identified thousands of genes that code for proteins. What we saw then was the total genome structure, including duplicate copies called copy number variations (CNVs). Later, we learned that everyone had slight variations in most of their genes, called single nucleotide polymorphisms or SNPs. Many of these SNPs are now known to be important markers of disease because they can indicate a change in protein function or activity. However, these SNPs are fixed and do not change throughout your life. What may be the most impactful modulator of cellular activity is differential gene expression, since the amount of the gene expressed is ultimately in control of protein levels and cellular output. Based on current conditions, the genome will output a certain combination of genes, but when the conditions change, the gene output will change to best adapt to the new conditions or demands. This is generally what determines one's day-to-day, or even your morning-to-night, physiology.

What the first sequencing of human genome could not let us identify was this dynamic yet critical differential gene activity found over time.

We now know that the static genome is actively manipulated, constantly increasing production of some gene transcripts and decreasing others in response to its environment. Remarkably, environmental stimuli, and there are many, can cause gene activation in minutes, if not faster. Such rapid changes in gene activity provide incredibly precise adaptations of the host to a rapidly changing environment. Regulation is complex: nuclear transcription factors and newly discovered long noncoding RNAs, together with microRNAs and circular RNAs, as well as methylation and acetylation (don't forget demethylation and deacetylation!) can shut off and turn on gene function. If this sounds complicated, it is, though research into the interacting complexities of so many layers of regulation are only partially described.

We are at the dawn of a new era in science where we can use genomics and transcriptomics to our advantage in that we can find a distinct fingerprint for CIRS from water-damaged buildings, separating CIRS-WDB from ciguatera and from Post Lyme Syndrome. The application of genomics to complex human illness is in its early stages. Already, tremendous advances in medicine and science have resulted from transcriptomics.

HERNIATED NUCLEUS PULPOSUS AND INNATE IMMUNE EFFECTORS

Low back pain, discogenic back pain, slipped disc, ruptured disc, lumbar disc herniation, intervertebral disc degeneration/degradation are all names that basically imply pain and disability from back problems at some time in otherwise normal patients. The sufferers are many. Causes are relatively few, like repetitive microtrauma, overuse syndromes, improper ergonomics ("lift with your knees"), overweight, smoking, age ("time to use your brain, not your back"), malalignment, scoliosis, osteoporosis and more. Treatments are diverse but results are disheartening. Stretching, massage, physical therapy, disc injections and surgeries of all kinds are often recommended. Acupuncture, stress reduction and analgesics may have emerging roles.

What we rarely hear about is the benefits of reduction of inflammatory mediators in syndromes involving intervertebral discs [28]. Both disc cells and migrating macrophages release inflammatory cytokines in response to disc injury. We know that in injured discs, we see release of IL-1b, IL-6 and TNF as pro-inflammatory compounds and IL-8 as an anti-inflammatory compound, just like in CIRS. Enzymes that degrade extracellular matrix are induced by pro-inflammatory cytokines; matrix metalloproteinases worsen disc degeneration and pain [28].

Differential gene activation is observed in lumbar disc syndromes. From growth factors (VEGF and TGF beta-1, to platelet derived growth factors (PDGF)) the normal attempts to heal trauma include stimulation of new blood vessel growth to heal protruding discs; to intense pain from PDGF as part of the metabolic response to tissue healing [29]. If TGF beta-1 is activated, fibrosis won't be far behind.

It is possible that not all inflammatory effectors are going to cause pain in HNP. MMPs (there are many) and ADAM-4 ("a disintegrin and metalloproteinase with thrombospondin motifs") act synergistically and are associated with pain, but each may play a role in resorption of disc material after herniation is complete [30]. If we block MMP9 and ADAM-4, we might block pain acutely, but when do we stop as to not interfere with resorption of herniated fragments?

Perhaps transcriptomics can tell us [31] if we could see when tissue inhibitors of MMPs (TIMP) are activated. These effectors are increased in degenerated cells. Genetics plays a role, but so does the p38 mitogen-activated protein kinase pathway (MAP kinases), the same pathway turned on by exposure to trichothecene mycotoxins, especially deoxynivalenol [32]. For the 50% of the US work force (NIOSH) possibly employed in a WDB, do we know if their MAP kinases are actually helping them avoid ongoing pain?

To further confuse the issue of disc pain, look at comparisons of nucleus pulposus cells (NP) and articular chondrocytes in an animal model published by Ciu [33]. The cells look similar but gene profiling shows marked differences. Nucleus pulposus cells compared to chondrocytes are overproducers of MMP-2; MMP-14; ADAMS-1, -2, -17; as well as TIMP; but they are comparatively lower producers of MMP-1, -3, -7, -8, -10, -11, -13, -16, -19, -20, -21, -23, 24. 28; ADAMS-4, -5, -14, -18, -19; and TIMP-3. Chondrocytes expressed MMP-12 and MMP27, but NP cells did not. To confound these extraordinary results, we may ask what transcriptomic differences are created by being in cell culture instead of in vivo.

If genetics and activity, combined with overuse and obesity, remain underlying concerns about HNP, the same inflammatory mediators that we heard about in CIRS are supplying major loads of pain and disability. Does this mean that the salutary benefits from VIP apply to HNP independent of CIRS? Yes! (RCS, unpublished; 34).

OSTEOARTHRITIS AND VIP

The intriguing role of VIP in musculoskeletal pain is already well known [34]. Specific reasons for its benefits may simply relate to its presence in human synovial fibroblasts. Juarranz and colleagues report measurement of expression of VIP, VIP receptors, intracellular cAMP production and cell membrane adenylate cyclase [26]. VIP mRNA and VIP was detected in synoviocytes, with far less found in cells from patients with rheumatoid arthritis (RA) compared to cells from patients with osteoarthritis (OA). VIP receptor 1 (VIPR1/VPAC1) was more abundant on OA with VIP receptor 2 (VIPR2/VPAC2) in RA. Treatment of OA cells with tumor necrosis factor alpha (TNF) reproduced the findings of reduced VIP found in RA. VIP downregulated pro-inflammatory compounds IL-6, CCL2 and CXCL8 as did specific agonists of VPAC1 and VPAC2.

We can ask if the low VIP in RA was the result of pro-inflammatory compound generation, but we could also postulate either microRNA or long noncoding RNA blocking production of VIP. We know that in CIRS, VIP deficiency is associated with decreased production of Ikaros family transcription factors [10]. This transcriptomic work has not been done in RA to date.

Comparing VIP in human osteoarthritic cartilage to that of unaffected human controls was instructive. Jiang [35] published that VIP concentration in synovial cells from OA patients was much less than VIP from controls and that VIP was inversely correlated with progressive joint damage in OA.

In an excellent review article, Jang [34] notes the diversity of published benefits of VIP, though the correction of abnormal proteomics in CIRS by VIP is not referenced. VIP is downregulated in synovial fluid in OA; such downregulation leads to increases in pro-inflammatory cytokines. Moreover, VIP can prevent chronic joint cartilage damage and joint remodeling. Upregulation of VIP can counteract pro-inflammatory stimuli and *reduce pain*.

One must also consider the other sources of reduced pain from VIP. In mice lacking VIP, hypersensitivity apparently is mediated at a local mechanism in the spinal cord. This observation is

complicated by the additional observation that VIP-deficient mice also had enhanced inflammatory and greater glial reactivity compared to controls [36].

Could VIP be (1) decreasing local generation of painful stimuli and (2) reducing dorsal cord hypersensitivity? Or is VIP affecting thalamus modulation of pain inputs?

CYTOKINES AND OSTEOARTHRITIS

A review looking at the literature of pro-inflammatory (and anti-inflammatory!) cytokines shows an evolution of thought from 1990 to the present. Myers [37], writing in 1990, suggests that while synovitis is common in advanced osteoarthritis (OA), prevalence and severity of synovial inflammation is uncertain in early cases with early changes in synovial thickening unimpressive. Biopsy studies found an association between synovial mononuclear cell infiltration and thickness of the synovial lining of only 12% of patients. The severity of cartilage lesions was unrelated to severity of synovitis. Contrast that work with that of Mathiessen [38] who writes in 2017 that modern imaging studies show significant changes even before visible cartilage degeneration has occurred. Imaging studies and tissue evaluation has confirmed a high prevalence of synovial inflammation in all stages of OA with multiple studies confirming synovitis is related to pain, poor function and possible progression of structural injury.

And yet, the middle ground in this timeline from Fernandes [39] published in 2002, acknowledges that activated synovial sites upregulate MMP gene expression. This finding is no different than what we have seen in CIRS. The thought of Fernandes was to block cytokines using antagonists to receptors for interleukin-1 to reduce experimental injury from cytokines such as TNF-alpha. They were able to show in experimental dogs and rabbits that in vivo intraarticular injections of IL-R antagonist genes can prevent progression of destructive changes.

In 2008, Ray [40] discusses the evidence for abundant expression of inflammation-responsive transcription factors paralleling what we look at in transcriptomics today. This transcription factor, called SAF-1 regulates increased synthesis of MMP-9 together with VEGF. We are accustomed to hearing those acronyms in arthritis as well as with CIRS. The implication from Ray's work is that more important than the MMP-9 effect on cartilage erosion are the factors that regulate MMP-9 reduction. Those factors, related to the genes of MMP-14, which are related to pro-inflammatory cytokine release. Pro-inflammatory cytokine release in turn is under regulatory control of a nuclear transcription agent.

Sandy [41] continues the approach looking at human genome-wide expression of inflammatory mediators in arthritis. This fascinating study looks at catabolic mediators in OA; discusses the prior focus of interleukin-1 beta; and shows that focus of interleukin-1 beta should be replaced by focus of soluble mediators such as IL17 or TGF beta-1 which are more likely to mimic disease in models of OA. Sandy concludes that early OA is related to the activity of soluble inflammatory mediators but late stage disease looks at the accumulation of biomechanical effects on the remaining cartilage.

The literature regarding cytokine inhibitors in arthritis is mixed. Calich [42] reviewed models of TNF neutralization through IL1Ra inhibitors in animals (NB: Sandy would substitute IL17 or/and TGF beta-1) but was not convinced there was adequate evidence that showed blocking TNF was beneficial in treatment of osteoarthritis in humans.

Kapoor [43] also reviewed the role of pro-inflammatory cytokines in the pathophysiology of osteoarthritis. The study defines osteoarthritis with its cartilage destruction, subchondral bone remodeling and inflammation of synovial membranes, and notes antecedent occurrences leading to degenerative changes. Kapoor also finds, (1) limited efficacy from blocking the effects of pro-inflammatory cytokines; and (2) little benefit in prevention of osteoarthritis in animal models.

It is likely that these studies looking at cytokines alone have overlooked the role of VIP and additional nuclear transcription factors, including Ikaros. Inflammatory mediators are invariably seen in a diverse group of pathologies with simultaneous activation of anti-inflammatory cytokines, Th-17

cells, neuroregulatory peptides and transcription-regulatory agents, making assessment of specific causation impossible.

TGF BETA-1 AND OSTEOARTHRITIS

Along with the progression of injury underlying the inflammatory bases of musculoskeletal pain is the increasing focus on transforming growth factor beta-1 (TGF beta-1) in osteoarthritis (OA). TGF beta-1 is defined as a "pleiotropic cytokine that is important in the regulation of joint homeostasis and disease." [44]. Of significance are both the direct effects of TGF beta-1 in immune signaling but also its indirect effects on differential gene transcription. Concentration of TGF beta-1 is normally low in healthy joints and high in osteoarthritic joints. This differential leads to enhanced activation of differential signaling pathways in joints themselves. There are indirect effects in TGF beta-1 including cartilage damage, osteophyte formation and synovial fibrosis as discussed earlier in this chapter but appear to be at least associated with, and possibly caused by, high levels of TGF beta-1. Interestingly, and consistent with the dual role of TGF beta-1, in younger patients, pathological changes are counteracted by TGF beta-1 but in joints of older patients those changes are enhanced. We know that TGF beta-1 acts in concert with T-regulatory cells, with those cells being anti-inflammatory in tissue, including joint, provided there is adequate presence of retinoic acid–related orphan receptor (ROR). In the absence of normal tissue levels ROR, TGF beta-1-driven T reg cells can be converted into T-effector cells, thereby enhancing tissue injury and releasing more TGF beta-1.

Although a significant literature on TGF beta-1 and OA has been published recently, there is reference to TGF beta overexpression contributing to experimental osteoarthritis as early as 2003 [45]. In experimental adenovirus transfection in an OA model in mice, TGF beta-1 had an important role in development of osteophytes in synovial thickening. These findings suggest that endogenous TGF beta-1 participates in pathogenesis of osteoarthritis.

Later work from 2006, [46] showed that lack of TGF beta-3 is associated with cartilage damage, suggesting loss of some protective effect and osteoarthritis progression.

Healthy cartilage is maintained by a dynamic balance of inflammatory effects on extracellular matrix and articular chondrocytes [47]. Cytokines and growth factors regulate the synthesis and degradation of extracellular matrix maintaining the stability and integrity of the joint. If the balance of synthesis/ degradation is disrupted, OA, a degenerative joint disorder characterized by destruction of articular cartilage, alterations of subchondral bone and synovial fibrosis, is likely to occur. According to Finnson, TGF beta-1 has emerged as an important regulator of osteoarthritis.

MICRORNA AND OSTEOARTHRITIS

Before leaving OA, one must return to the basic concepts of transcriptomics for added perspective. Our complement of protein-coding genes approximates 20,000. Many genes have been well-described and identified as part of discrete metabolic pathways. Another 30,000 genes are regulatory: they control gene transcription and translation. Not nearly enough is known about long noncoding RNA genes; this group of genes is a subject of intense research in 2017. Much more (though still not enough) is known about microRNA (miR). These small, regulatory RNA molecules fine-tune gene expression, participate in control of tissue development and homeostasis, with specific activities according to tissue and time [48]. In many instances, a single miR can influence multiple, different gene transcripts. Suffice to say, the layers of regulation exerted over mRNA translation are numerous and diverse.

As the reader has seen, we know OA is a dynamic interaction of cytokines, growth factors and regulatory neuropeptides and not just a wear and tear, degenerative process confined to overweight, inactive smokers. OA develops from aberrant regulatory control of effectors that act on synoviocytes, subchondral bone and inflammatory responses to the forces affecting abnormal control of

synthesis and degradation. As one might expect, there is a developing literature on miR involvement with disruption of the normal process of joint surface healing.

Again, one might expect the literature on miR and OA is newer, but in 2008, Yamasaki [49] identified miR-146a as a participant in apparent protection of cartilage from injury. The highest levels of miR-146a were found in cartilage with the least injury (using Mankin scale). Expression of miR 146a was induced by IL-1b stimulation of chondrocytes.

In 2017, Zhang [50] extends this research, but as opposed to Yamasaki, this paper showed that mice without miR-146a had far less cartilage degeneration. This paper also showed that miR 146a "aggravated pro-inflammatory cytokines induced suppressing the expression of cartilage matrix-associated genes," especially targeting two specific genes, Camk2d and Ppp3r2. More importantly, miR-146a has a crucial role in cartilage homeostasis: inhibitors ameliorated OA.

In 2011, Li [51] found a multi-level effect of miR-146a; (i) high levels suppressed extracellular matrix-associated proteins and regulated inflammatory cytokines found in human knee joints; (ii) low levels are found both in dorsal root ganglia and dorsal horn of spinal column in rats experiencing experimental OA-induced pain. Further, miR-146a modulated pain related molecules in human glial cells.

In 2009, Miyaki et al. [52] demonstrated that a different compound, miR-140, was expressed normally in normal articular cartilage but much lower levels were found in OA tissue. IL-1beta suppressed miR-140.

IL-1beta also induces production of cyclooxygenase-2 (COX-2). COX-2 is an important contributor to chronic pain and inflammation in OA [53]. Normal human articular cartilage expresses miR-558, with lower levels seen in OA. IL-1beta induced suppression of miR-558 and induced MAP kinases, compounds also activated by trichothecene mycotoxins [32]. Finally, overexpression of miR-558 downregulated MMPs.

Meng published work [53] on regulation of MMPs in OA, finding an important regulatory role for miR-320. As seen with miR-558, IL-1beta suppressed miR-320 and upregulated MMP-13.

Another miR, Hsa-miR-148a expression is decreased in OA [54]. Overexpression inhibits hypertrophy; increases Type II collagen, which is accompanied by increased retention of proteoglycans.

Finally, both miR-29a and miR-140 protect chondrocytes against adverse effects induced by IL-1 beta [55].

With so many protective miR, one might wonder what is happening in our society that we see so much degenerative joint disease. This author submits that environmental exposure to toxigens and inflammagens are more common than suspected. Now that transcriptomics is readily available, we are likely to understand far more about sources of pro-inflammatory injury in OA, beginning with disruption of normal regulation of DNA transcription and regulation of miR.

TGF BETA-1, TENDON INJURY AND REPAIR

Musculoskeletal tissues are quite diverse in makeup and physiology. We have seen differences between nucleus pulposus cells, synoviocytes and muscle, for example. Two additional tissues where inflammatory mediators have important effects are tendon and enthesium. These next two sections are devoted to these tissues. Tendon is defined as a "uniaxial connective tissue component of the musculoskeletal system. Tendon is involved in forced transmission between the muscle and bone" [56]. While tendon is not avascular, it is poorly vascularized. It is largely composed of Type I collagen fibrils organized in parallel fashion along the long axis of the tissue. Injury to a tendon involves injury to production and assembly of Type 1 collagen. Tendons establish specific connections between muscles, enthesium and the skeleton by transferring contraction forces from muscle to bone allowing organized body motion. Because of the reduction of blood flow in tendon, it is not surprising that tendon injuries heal slowly; surgery cannot restore a damaged tendon to its normal structural integrity and mechanical strength [57].

Sakabe, in a review article, discusses the current status of tendon treatment as well as cell-based therapies in degenerative medicine approaches. As he writes in 2011, tissue engineering for tendon

injury involves complex interactions among cellular sources, cytokines and gene delivery systems. This idea has arisen repeatedly in this area. Biological systems have limited means for diverse responses: the final common pathway of lab abnormalities in CIRS is paralleled by musculoskeletal tissue responses.

Nourissat and colleagues write in 2015 [59] that both growth factors and transcription factors involved in tenogenesis are emerging areas of focus in repair of tendon injury.

With this background in mind, one of the concerns in tendon injury is avoidance of development of TGF-mediated fibrosis. Indeed, downregulation of TGF beta-1 genes were studies by Chen [59] in 2009. This study looked at the role of four separate microRNA that could effectively impact TGF beta-1 expression in chickens *in vitro*. They found that delivery of microRNA to the tendons substantially downregulated expression TGF beta-1 but did not affect expression of the collagen 1 gene. This is an encouraging study showing that collagen 1 repair could possibly proceed without fibrosis; additionally, collagen 3 gene expression was reduced by over 50%.

Wu and colleagues write in 2016 [60] that microRNA, which specifically inhibits function of TGF beta-1, holds promise for prevention of adhesion formation during tendon healing, with reduction of adhesion formation and improvement of tendon gliding in digital flexor tendon injury was noted. Unfortunately, the strength of tendon healing was adversely affected. Reduction of adhesions in fibrosis is one arm of treatment approach to tendon surgery but additional work remains to be done.

Enhanced tendon healing, again in the chicken model, showed microRNA limiting adhesions around tendons. In this study [61] we have additional strength for a model in chickens to improve outcomes in tendon injury.

These investigations were continued [62] in 2017 by the same authors looking at the effects of VEGF genes delivered to enhance healing of tendons. Since VEGF can increase new blood vessel formation, improving oxygen delivery, perhaps this novel approach would show benefit. The results of this investigation showed that reduction of adhesion was accomplished and the strength of healing tendons was significantly increased. In this study, Type 3 collagen expression was not suppressed as it had been with microRNA affecting TGF beta-1. Type 3 collagen expression was increased.

Application of molecular processes remain desperately needed as shown by two studies on surgical interventions. In 2013 Roche [63] discussed Achilles tendon disorders, separating them by non-insertional and insertional conditions. Non-insertional injuries rarely require surgery and procedures are minimally invasive if indicated. Rehabilitation protocols have been shown to be of benefit. Still, surgery is not an option for Achilles tendon repair as discussed by Li [64] who calls for improvement in basic approaches to Achilles tendon injuries.

The same type of discussion applies to rotator cuff injures as discussed by Isaac [65]. Isaac summarizes more than 70 papers with a focus on original research. There are a number of therapies involving molecular approaches including, growth factors, stem cells and tissue engineering added to augment classical surgical approaches to rotator cuff repairs. This again is an emerging field of evaluation; preliminary studies are promising.

Another paper by Nixon [66] underscores the rapid advances in cell and gene-based approaches to tendon regeneration. Nixon suggests the ability to use RNA gene therapy to provide the ability for tendons to reduce matrix metalloproteinase injury in degradation.

In two 2017 papers [67, 68] additional importance is presented showing that upregulation of TGF beta-1 expression in stem cells of rabbits can actually improve tendon to bone healing after ACL reconstruction. The TGF-MAPK signaling pathway is underscored. Similarly, substance P increases connective tissue growth factors (CCN2) as an adjunct to TGF beta-1 in tissue repair and fibrosis. Substance P itself is linked to collagen production. In an *in vitro* study cells express proteins typically seen as made by tenocytes with evidence of increased proliferation following exposure to substance P and TGF beta-1. Substance P induced TGF-1 expression in tenocytes. Further approaches in tendon injury are focused not just on stem cells but discrete stimulation of substance P which

can induce collagen Type 1, independent of the TGF beta-1 pathway. Taken together, the healing process is immediately involved with regulation of gene expression, controlled by microRNA and manipulation of TGF beta-1, VEGF and MMP-9. Understanding the importance of these inflammatory markers is vital to understanding excellent tendon healing and less than satisfactory tendon healing. Clearly, tendon injuries are an ongoing source of musculoskeletal pain.

ENTHESIUM

Between muscle and tendon lies tissue with its own unique construct, the enthesium. Here we see the emergence of increased amounts of Type 1 collagen suggestive of tendon structure intermingled with myocytes. As one looks closer to the tendon side of the enthesium, there is progressively less vascular supply and at the junction of the enthesium with tendons, the tissue becomes relatively avascular. As it so commonly happens in CIRS, there will be accumulation of inflammatory cells and mediators delivered to the end of capillary beds similar to what we see at the ends of fingers and at the ends of toes. There can also be accumulation of breakdown products of metabolism, including lactic acid. This combination of reduced blood flow and presence of inflammatory mediators from innate immune response leads to the increased incidence of extensor epicondylitis, patellar tendon pain and Achilles tendon pain at insertion sites together with plantar fascial pain. There are no reliable blood tests for inflammation of the enthesium. Fortunately, the painful syndrome of enthesitis usually responds to stretching and local heat. As opposed to classical overuse syndromes, in CIRS there is no therapeutic role for cessation of offending activity.

Of interest, is the appearance of psoriatic-related polyenthesitis and "classical" fibromyalgia. As noted previously, there are no reliable biomarkers for fibromyalgia. [69]

McGonagle [70] has looked in detail at psoriatic arthritis concluding that the arthritic pain in association with nail findings is actually clinically unrecognized enthesitis. He notes enthesitis is "associated with adjacent osteitis or bone and synovial inflammation." Normal insertion of tendons in McGonagle's view are associated with micro-damage and inflammatory change, strongly suggesting "that local tissue specific or what has been described as auto-inflammatory factors, may dictate disease expression."

In psoriatic arthritis, the diffuse inflammation involves the nail root and bed, but the nail itself is intimately related to entheses. McGonagle notes the extensor tendon of the DIP joint sends fibers from the bone that it envelopes the nail bed in an "interdigitation factor." His hypothesis is that "frequent micro-damage in tissue repair has resulted in a new model of pathogenesis which he calls auto-inflammation." Microtrauma leads to regional innate immune activation in persistent inflammation as an alternative to primary immunopathology driven by T and B cell abnormalities."

Similar findings are seen diabetes [71]. When studied by Ursini and his group, there was elevated prevalence of asymptomatic enthesopathic changes of the Achilles tendon insertions in Type 2 diabetes unrelated to peripheral neuropathy.

Approaches to treatment of enthesopathies have included conservative interventions as reviewed by the Cochrane Data Base [72]. The conclusion of this Cochrane Review, is that the available evidence from randomized trial is insufficient to advise on any specific conservative modality for treating exercise-related groin pain. Similarly, [73] Cochrane reviewers who looked at adhesive capsulitis or frozen shoulder, found that manual therapy and exercise were not convincingly shown to be of benefit. Further, injections of glucocorticoids provided short-term benefit. Finally, Page [74] looked at manual therapy and exercise from rotator cuff injury. Cochrane identified 60 studies but only one compared the combination of manual therapy and exercise to placebo. This study was judged to be high quality; no clinically important difference between groups were seen in any outcome. Cochrane calls for further trials of immunotherapy alone and exercise alone that would be needed to alter the opinions of the Cochrane Review. Compare that opinion to that of Moreno [75] who used intra-tissue electrolysis for enthesopathy of adductor longus in soccer players. These

authors show that EPI (electro-pulse electrolysis) treatment together with physical therapy insured a greater more rapid reduction of pain in soccer players. The benefit lasts at least six months.

In another study looking at interventions for enthesopathy, ultrasound was felt to show benefit [76] in extensor epicondylitis.

While the hypothesis of capillary hypoperfusion remains of interest regarding pain in the enthesium, to date, there is no sustained opinion regarding interventions. Simply stated, if there is an enthesopathy, stretch, stretch, stretch. Based on academic reports presented in this chapter, this author suspects that when funding is made available for evaluating the transcriptomics and inflammatory mediators of enthesopathy, we will see the same group of compounds like VIP, TGF beta-1, MMPs, VEGF, miR and nuclear transcription regulators as we have seen in OA and tendons.

TGF BETA-1, SMAD 2, SMAD-3, FIBROSIS AND SCARS

A theme of this chapter has been the ubiquitous involvement of particular cytokines and regulators of inflammation, in all their various forms, in illnesses as seemingly diverse as CIRS-WDB and OA. Consistent with this theme is the idea that blood flow bringing these compounds to tissues will result in tissue-specific changes, but also in a *systemic illness*. The only tissues not affected by blood-borne inflammatory mediators are ones without blood flow.

Perhaps no biological function is a better example of the "final common pathway of inflammation," than fibrosis. When one says TGF beta-1 from the podium during an academic lecture, some listeners can immediately visualize TGF beta-1 interacting with its cell membrane receptor (TGF beta-1R), a member of the serine/threonine kinase family of receptors. Kinases put phosphoryl groups on compounds; in the case of TGF beta-1, what is phosphorylated are Smad 2 and Smad 3 [77]. These activated compounds migrate to the nucleus to regulate transcription of a complex series of genes.

Smad3 has a greater role acting on epithelial cells and fibroblasts [78] but also activates other transcription factors [79], including connective tissue growth factor (CTGF). CTGF is a major factor implicated in formation of fibrous tissues. Inhibition of TGF beta-1 can be accomplished. Such an effort will help illnesses as diverse as interstitial lung disease to scleroderma; and from cirrhosis to burn healing.

As one might expect, miRs influence TGF beta-1 signaling. In a revealing paper, Guo [80] shows the inhibitory effect of miR-29b in mice on scar formation. miR-29b is downregulated in thermal injury. Treatment with mir-29b suppressed collagen deposition and fibrotic gene transcription. Specifically, miR-29b inhibited the TGF beta-1/Smad/CTGF pathway.

Another approach [81] uses a cytoplasmic protein, TRAP-1, to regulate Smad expression by phosphorylating Smad3. By regulating phosphorylation, TRAP-1 can regulate collagen synthesis in fibroblasts, possibly avoiding initiation of pathologic scar formation.

Li published a provocative paper [82] showing efficacy of a plant-based treatment for uninhibited TGF beta-1/Smad signaling. Identification of a flavonoid polyphenol, kaempferol, downregulated Smad2/Smad3 phosphorylation in a dose-dependent manner. The mechanism apparently was selective binding of kaempferol to TGF beta-1R.

BETA ENDORPHINS AND PAIN

As discussed in the MSH section, deficiency of beta endorphins has long been associated with chronic pain. Hartwig writes in 1991 [83] that beta endorphin circulates in blood following production. Pain is blocked by inhibiting activation of peripheral somatosensory neurons. Unfortunately, exogenous opioids suppress production of beta endorphin. This 25-year-old concept has direct application to the explosion of opiate-related deaths currently afflicting the US. Even in 1991, beta endorphin deficiency was a recognized factor in multiple pain syndromes, including trigeminal

neuralgia, migraines and rheumatoid arthritis (RA). The reader may be reminded of the occurrence of facial pain with MARCoNS; migraine diagnoses made in the face of ADH/osmolality dysregulation; and MSH deficiency as a typical finding in RA.

We can simply ask if pain perception from low back (for example) sources is worse in those with CIRS or MSH deficiency from another source. Bruehl [84] tells us that "endogenous opioid antinociceptive system dysfunction may contribute to elevated acute and chronic pain sensitivity among more disabled chronic pain patients."

By 2014, Bruehl added measurement of plasma beta endorphin to his approach [85]. Paradoxically, his group published that elevated resting levels of beta endorphins may be a biomarker for reduced analgesic capacity.

The disparity between reports that beta endorphin deficiency increased pain responses from one group and decreased pain responses from another suggests that additional mechanisms are involved. In 2011, we find reports of differential effects of mediators of chronic pain, namely neuropeptides substance P (SP), calcitonin gene-related peptide (CGRP) and VIP [86] in migraine. One may wonder if these neuropeptides have a role in pain from joint abnormalities as well.

CGRP, SP AND VIP: PAIN REGULATORS

Rapp [87] looked at differential effects of sensory neurons releasing SP and CGRP impacting cytokine release. Comparing fibroblasts in cell culture for patients with rheumatoid arthritis (RA) to those with osteoarthritis (OA), for example, RA fibroblasts increased release of IL-6 and IL-8 when treated with CGRP but not with SP. For OA fibroblasts, SP caused release of IL-8. When RA fibroblasts were treated with sympathetic nervous system mediators, adenosine and norepinephrine suppressed release of IL-6 and IL-8. The same treatment for OA caused release of both IL-6 and IL-8. Finally, Rapp presented evidenced that beta endorphin inhibited IL-8 secretion only at concentrations 1,000 times higher in RA compared to OA.

Moving from sensory neurons to dorsal root ganglia shows us more differential effects of SP, CGRP and VIP. Shadiack [88] showed that axotomy of superior cervical ganglia increased mRNA for VIP and SP. Axotomy of lumbar dorsal root ganglia increased mRNA for VIP but decreased mRNA for SP and CGRP. Use of an antiserum against nerve growth factor increased levels of VIP in cervical and lumbar dorsal root ganglia but decreased SP and CGRP when applied to sensory neurons.

Dallos [89] in 2006 provided some clarity by showing that neuropeptides SP, CGRP and VIP released from cutaneous nerves after an injury upregulated IL-1alpha and IL-8. The effects of SP, CGRP and VIP were to markedly increase release of nerve growth factor.

Two additional lines of inquiry support diversity of sources that affect pain in this complicated field. Dirmeier et al. published a paper [90] that possibly streamlines the understanding of SP and CGRP. Simply stated, SP is pro-inflammatory; CGRP is anti-inflammatory. Sensory nerve fibers carry both neuropeptides. OA had a greater density of CGRP nerve fibers; RA had more SP nerve fibers.

In a state-of-the-art paper from 2017, Grässel and Muschter [91] emphasize the crucial trophic effects from both sensory and sympathetic fibers required for normal growth of joint tissue and bone. The neurons are the source of neuropeptides including SP, CGRP and VIP. Given that neuropeptides are involved with inflammation in RA, it might be counter-intuitive to also see inflammation in OA controlled by neuropeptides. Given the massive role that OA plays in morbidity worldwide, therapies that enhance CGRP or VIP are likely to play important roles soon. As soon as clinicians have access to transcriptomic identification of abnormalities that are corrected by VIP, a shortcut to finding relief for OA sufferers may appear.

Finally, two papers from Lerner [92, 93] identify the role of SP, VIP and CGRP in regulation of bone formation and resorption. The working hypothesis is that the bone/joint link is regulated by neuropeptides. The burden of osteoporosis and arthritis, each a part of a system of tissues is due to

systemic and local effects of neuropeptides. Therapies that address dysregulation of neuropeptides, at least as far as VIP is concerned, have a reasonable likelihood of benefit.

TRANSIENT RECEPTOR POTENTIAL RECEPTORS, INCLUDING TRPV1

At one time substance P was called capsaicin; its receptor TRPV1, is a nociceptive-specific ion channel [94]. This ion channel is activated by thermal injury and acidification as well. Other TRP ion channels are TRPV2, TRPV3, TRPV4, TRPM8 and TRPA1.Toxins [95] known to induce TRPV1 activation include scorpion venom, botulinum neurotoxin, spider toxin (NB: species not identified), ciguatoxin and brevetoxins. Vanillotoxins from a tarantula activate TRPV1 "via interaction with a region of TRPV1 that is homologous to voltage dependent ion channels."

Guilak and Liedtke [96] in 2010 suggests that TRPV4 is the "sixth sense," as it is activated by heat, cold, mechanical loading, osmolality, physical and chemical stimuli. TRPV4 is particularly relevant to musculoskeletal systems as it is expressed in articular cartilage and bone, reacting to osmotic stress. This paper suggests that TRPV4 exerts a regulatory role for a sensory channel in musculoskeletal tissues. Disruption of regulation by biotoxins is a recurrent theme in biotoxin medicine.

Liedtke, in 2016 [97], notes TRPV4 functions in peripheral neurons, and central nervous system cells (astrocytes and microglia) in physiologic and pathologic conditions. They are involved in pain and inflammation.

Cortright discusses TRP channels and pain in 2009 [98]. TRP channel inhibitors block pain by blocking receptors where pain signals are generated. Xu published a report [99] on benefits of an antagonist of TRPV1 (called SB-366791) that dramatically reduced abdominal pain. Similarly, Zhu also published [100] on correction of pancreatic pain in animals by blockade of nerve growth factor. In these animals, density of TRPV1 on pancreatic sensory neurons was reduced.

To no surprise, TGF beta-1increases pancreatic pain [101] in the same rat model presented earlier. This pain was associated with upregulation of TGF beta-1 receptors in dorsal root ganglion cells and was blocked by TGF beta-1 receptor 1 antagonist SB431542.

To add to the concept of an organized hierarchy of layers of pain perception, intrathecal injection of TGF beta-1 reproduced abdominal pain in rats. One can speculate regarding what is happening in unusual pain syndromes in CIRS where blood-brain barrier defenses against transport of TGF beta-1 are compromised.

The role of TRP in pain continues to be unveiled. Jardin [102] focuses on TRPV1, noting that the TRP super-family now has 28 known isoforms in mammals. TRPV1 is expressed in sensory neurons and dorsal root ganglion neurons.

Returning to the role of regulation of transcriptomics by miRNA, decreased expression of miR-199, found in irritable bowel disease [102] leads to enhanced expression of TRPV1 and greater abdominal pain.

CONCLUSIONS

Advances in molecular biological approaches to long-established medical problems have expanded diagnostics and therapeutics. CIRS is an example of state-of-the-art application of differential diagnosis, proteomics and transcriptomics to diagnosis and treatment of complex, chronic fatiguing illnesses acquired following exposure to biologically produced toxins and inflammagens. The disease entity is not just one abnormality; understanding the pathophysiology of CIRS comes from use of a systems or landscape approach. Treatment involves correction of abnormal regulation of inflammatory effectors, regulatory neuropeptides and multiple layers of control exerted on the results of gene transcription.

Applications of molecular methods to diagnosis have validated CIRS and shown the overlap of innate immune responses in musculoskeletal disorders, including pathogenesis of

osteoarthritis (OA). Use of a systems approach to (1) innate immune activation and (2) dysregulation of neuropeptide control of inflammation in OA and musculoskeletal pain opens a new window of treatment, one that is focused on the effects of inflammation on neuropeptide release by sensory neurons, differential regulation of activity of TRP channels and possibly dorsal root ganglia cells.

What we are missing at this time are reliable assays for transcriptomics of neural tissues involved in initiation of painful stimuli and propagation to the brain. It is unlikely that the whole blood analysis used successfully in defining abnormalities and successful correction of gene abnormalities in CIRS can be used without study of actual neural cell genomic function. Despite that need, now that we have data supporting regulatory roles for anti-inflammatory neuropeptides VIP and CGRP; and inflammatory roles for substance P, MMP9, VEGF, TGF beta-1 in generation of acute and chronic pain, we may be able to use methods used in delineation of the biological subtleties of CIRS to help unravel the complexities of chronic pain.

We are confident that increased use of transcriptomics and regulation of gene expression will enable us to understand pathophysiology of pain and disability in ways not seen before. Perhaps musculoskeletal pain will become a window on chronic pain syndromes. If so, innate immune inflammatory processes are likely to hold the keys to defeating pain.

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