

# I-Cubed (Infection, Immunity, and Inflammation) and the Human Microbiome



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## KEYWORDS

- Infection • Immunity • Inflammation • I-Cubed • Human microbiome • Neurology
- Public health

## KEY POINTS

- It has been learned that human beings are superorganisms integrating the identity, function, and immunity of resident micro-organisms.
- Low-cost high-throughput sequencing of microbial communities comprising the human microbiome is unveiling the identity and function of unculturable microbes.
- Our own innate and adaptive immune systems have evolved to deal with invading organisms.
- Public health officials and neuroepidemiology researchers will be called on to guide the understanding of I-Cubed illnesses and the implications of the human microbiome.

## INTRODUCTION

The human microbiome, defined as the collection of micro-organisms that reside within our body, have coevolved over the history of mankind and have been overlooked as determinants of health and disease. Given the appearance of new microbial agents and the everyday occurrence of unexplained lethal neurologic syndromes of suspected infectious cause, scientists have begun to identify a plethora of microbial agents in our body and in the human genome. The true capability of such microbes residing in our body to cause human disease has become the focus of medical science. Postinfectious autoimmune illness is increasingly recognized because of resident and invasive microbial agents that have the capacity to trigger our immune system, turning it on and off at will. With differences in resident microbial niches,

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imperfect host defenses, and susceptibility to epidemic and endemic diseases in the environment, there are ever-increasing opportunities for infectious bacterial, virus, parasitic, and fungal exposures. The triumvirate of infection, immunity, and inflammation, termed I-Cubed, which posits that there are complex and often self-sustaining host adaptive immune responses to acute and chronic infection, forms the etiopathologic basis for diverse medical and neurologic disorders. Public health officials and neuroepidemiology researchers will be called on to guide the understanding of I-Cubed illnesses and the implications of the human microbiome for communicable and noncommunicable diseases, at times one leading to the other, as the natural history is appreciated and the responsiveness of given medical and neurologic disorder to a variety of medical approaches including strong antibiotics and immunomodulatory is established.

## BACKGROUND

Imagine the excitement of a scientist at a medical conference claiming to have discovered a new human organ which, like the immune system, contains collections of cells and 100 times more genes than the host. Not only is it tailored to the individual host, but it is modifiable by stress, diet, medications, exercise, and antibiotics. When lost, nearly all aspects of the host's normal physiologic function are altered. Although it has been known for some time that the human body is inhabited by resident flora in a factor greater than 10:1, most researchers have focused instead on a minority of disease-causing or "pathogenic" organisms with far fewer examining the benefits of the resident bacterial flora. The completion of the human genome sequence in 2001, which was the crowning achievement in biology, was incomplete because it did not look at the synergistic activities of humans and microbes living together. With many well-recognized neurologic diseases of likely infectious trigger yet unassigned to infectious microbes, such as most cases of aseptic meningitis, encephalitis, and cerebral vasculitis, there was a need for a second human genome project to provide a comprehensive inventory of microbial genes and genomes at major sites of microbial colonization in the human body. Many investigators envisioned that understanding the microbial contributions to inflammatory disease could be addressed effectively through a thoughtful integration of modern technologies and clinical insight.

The concept of the human microbiome or microbiota originated with the Rockefeller University scientist Joshua Lederberg,<sup>1</sup> as an ecological community of commensal, symbiotic, and pathogenic micro-organisms sharing our body space. It is estimated that 20% to 60% of the human-associated microbiome, depending on body site, is still resistant to conventional culture techniques, making it difficult to accurately estimate its true diversity. More recently, the human microbiome has been studied in different biological states using gene sequencing techniques. Scientists have used molecular tools to extract and compare bits of a particular kind of RNA, the products of DNA transcription and translation, to determine if previously known or new microbes were present in a particular human tissue such as blood. This technique, which is widely used as a biomarker for microbial disease, uses a particular kind of RNA known as 16S ribosomal RNA (rRNA).

Because the genes for rRNA have changed little over millions of years as organisms have evolved, slight changes in their composition provide valuable clues to the very nature of microbial organisms located in the human body. The 16S rRNA gene is very short, just 1542 nucleotide bases, making it quickly and cheaply copied, sequenced, and then compared with libraries of stored 16S rRNA genes from

numerous known bacteria. The ones that match up perfectly are microbes that have been previously identified, whereas others that show differences may be previously unknown microbes. Such studies of gastrointestinal microbes at the 16S rRNA gene level have revealed significant diversity in the flora of individuals. An international meeting held in Paris in November 2005, hosted by the French National Institute for Agricultural Research, led to the recommendation of an initiative to precisely define the human intestinal microbiome in health and disease. Directly following the Paris meeting, the National Institutes of Health held discussions about the merits of a Human Microbiome Project, which soon became a roadmap for later biomedical research. Fast-forward to the present: we are presently at a public health crossroads, in a position to make gigantic gains in our knowledge to better understand how microbes impact on human health, transitioning from description to causality and microbial engineering. Underscoring this, 2 papers published simultaneously in the journals *Science*<sup>2</sup> and *Nature*<sup>3</sup> called for the establishment of a Unified [domestic] Microbiome Initiative and International Microbiome Initiative.

With more than 90% of cells in the human microbiome understood to be bacterial, viral, fungal, or otherwise nonhuman in nature, and human metabolism and immunity attributed to the molecular genetic contribution of microbial and human interaction, human beings are being referred to as superorganisms. In the last decade the United States, the Human Microbiome Project and European MetaHit, 2 large-scale genomic projects, along with several private efforts, have investigated the microbiota in a variety of human body niches using new molecular genetic tools. Although many sites such as the skin, oral and nasal cavities, and vagina are all relatively easy to access, most of the research in this area has focused on the gastrointestinal tract, in particular, the colon. The colon is where the greatest density and numbers of bacteria are found. Most of the data regarding the bacterial microbiota comes from fecal samples and tissue specimens lining of the lower intestine. Although the function of colonic microbiota is to efficiently degrade complex indigestible carbohydrates, the small intestine microbiome is shaped by its capacity for fast import and conversion of relatively small carbohydrates, and rapid adaptation to overall nutrient availability. The gut microbiota ferments carbohydrates in the upper colon, whereas other gut flora digest protein and amino acids, liberating short-chain fatty acids. The fermentation of short-chain fatty acids can lead to a range of potentially harmful compounds, some of which play a role in gut diseases such as colorectal cancer and inflammatory bowel disease. Studies in animals show that some compounds, like ammonia, phenols, p-cresol, certain amines, and hydrogen sulfide, play important roles in the initiation or progression of a leaky gut, inflammation, DNA damage, and cancer progression. High dietary fiber or intake of plant-based foods appears to inhibit this, highlighting the importance of maintaining vegetarian gut microbiome carbohydrate fermentation. The newly recognized axis of communication between the gut and brain has led to the recognition of a mind-gut connection, seeking to explain a spectrum of functional symptoms from anxiety and depression to irritable bowel syndrome. So recognized, customized food diets have emerged aimed at improving the gut by impacting on microbiota activities linked to systemic host physiology.

With 5 phyla representing most of the bacteria that comprise the gut microbiota, there are about 160 species in the large intestine alone of any individual, and very few of these are shared between individuals. The functions contributed by these species appear to be found in everybody's gastrointestinal tract, an observation that suggests that microbial function is more important than the identity of the species providing it. The understanding of human microbial biology first derived from pure cultures and genomic sequencing, has been limited by sampling bias toward 4 bacterial

phyla, *Proteobacteria*, *Firmicutes*, *Actinobacteria*, and *Bacteroides*, of the 35 bacterial and 18 known archaeal phylum-level lineages. With roughly two-thirds of published microbiological research dedicated to only 8 bacterial genera, all of which grow well on agar culture plates, it is unlikely that they are representative of the 5000 or more species known to us. Phylogenetic molecular genetic methodologies using next generation sequencing have not only revolutionized our understanding of the origin and evolution of microbial organisms, but also have provided scientists with the means for identifying the types of organisms that occur in the environment and in the human microbiome. Molecular biologists using rRNA sequence have revealed a microbial taxonomy based on 1 of 3 aboriginal lines of descent separating bacterial organisms into 3 major kingdoms. The Eubacteria or modern *Eukaryotes* contain all of the typical bacteria-sharing 16S rRNA present in moderately large and varied collections of organisms and organelles of prokaryotic cells. The *Urkaryotes* sharing 18S rRNA comprise major ancestors of eukaryotic cells, including plant, fungal, and slime molds. The *Archaeobacteria* possess anaerobic metabolism based on reduction of carbon dioxide to methane, making them well suited for the type of environment presumed to exist on earth 3 to 4 billion years ago.

### INFECTION ACTIVATES IMMUNITY

Immunity was originally separated into 2 types based on the purported effects of immunization or vaccine against a given pathogen. The first type was the effect of immunization that resulted in definable changes in the cell-free bodily fluid or serum or humor, whereas another was the observed protective effect associated with multiplication of specific cells. Two primordial types of immune cells are now recognized, one lineage termed B cells that matures in the bone marrow and further differentiates into plasma cells and memory cells. Mature plasma cells are capable of producing antibodies capable of latching onto their target in a lock-and-key specific fashion when their surface antibody receptors recognize other cells displacing foreign antigens. Other B cells mature into memory B cells that circulate in the bloodstream. The other cell lineage of T cells, also derived in the bone marrow, instead passes through the thymus gland, where it achieves final immunoreactivity and is thought to be most protective in recognizing virus-infected cells. These cells participate in the defense against intracellular bacterial, fungal, and protozoan infections; cancers; and transplant rejection. Other aspects of enhanced cellular immunity includes the secretion of cell-signaling molecules termed cytokines that promote cell-to-cell communication in immune responses and stimulate the movement of cells toward sites of inflammation and infection.

Not surprisingly, major understandings of the pathophysiology of autoimmune diseases have been achieved through an appreciation of infectious triggers of the humoral and cell-mediated immune system. When Whipple disease was first identified as the causative agent of the neurologic disorder almost 25 years ago by Relman and co-workers,<sup>4</sup> it was unclear whether the uncultured bacillus *Tropheryma (T) whippelii* was a rare member of the normal human microbial flora and whether it might be associated with other human diseases. Whipple disease causes a systemic inflammatory disorder involving the gastrointestinal tract, heart, and brain. According to phylogenetic analysis, the isolated bacterium was a gram-positive actinomycetes not closely related to any known genus. A molecular genetic approach amplifying a 16S rRNA sequence directly from tissues of 5 unrelated patients determined its nucleotide sequence. A decade later, the same authors<sup>5</sup> performed ultrastructural studies of intestinal biopsy specimens from affected patients. These studies showed the location

of *T whippellii* rRNA to be most prevalent near the tips of the intestinal villi in the lamina propria just basal to the epithelial cells, located between cells and not intracellular, indicating that the bacillus grew outside cells and that it was not an obligate intracellular pathogen. Such studies ushered in a generation of molecular genetic technology used today in the study of resident human microbes.

Relman<sup>6</sup> later observed that molecular, cultivation-independent methods revealed that the distribution and diversity of micro-organisms in the world was far greater than previously appreciated. One particular molecular genetic technique compared human tissue-derived DNA sequences with those of known pathogenic and commensal bacterial, viral, fungal, and protozoan genomes in established expressed-sequence tag libraries. However, inefficient and cost-ineffective for screening large numbers of specimens in most laboratories, it revealed surprising findings of nonhuman genetic sequences that appeared to be an inherent feature of the human genome. It appears that all humans have human endogenous retrovirus sequences as an integral part of their genome.<sup>7</sup> At some time during the course of human evolution, exogenous progenitors of the human endogenous retrovirus inserted themselves into the human germ-line reproductive cells where they were replicated along with the host cellular genes. However, intact disease-producing retroviruses differ in the presence of at least one additional coding region, the envelope (*env*) gene that encodes viral membrane proteins that mediate the budding of virus particles to the cellular receptors enabling virus entry as the first step in the pathway to a new replication cycle and disease pathogenicity.

Hajjeh and coworkers<sup>8</sup> observed that unexplained deaths and critical illness possibly due to infectious causes in previously healthy persons occurred at an incidence rate of 0.5 per 100,000 per year from 1995 to 1998 among 7.7 million persons in 4 US Emergency Infectious Programs. However, only two-thirds were diagnosed by reference serologic tests, and the remaining one-third was diagnosed by polymer chain reaction (PCR)-based methods. These findings suggested the need for molecular genetic surveillance approaches to detect present and emerging infectious diseases. New molecular biological techniques have led to the identification of several previously unculturable infectious agents, such as non-A and non-B hepatitis, and hantavirus.<sup>9</sup> Real-time PCR methods with primers and a probe targeting conserved regions of the bacterial 16S rRNA revealed rRNA in blood specimens from healthy individuals, raising the possibility that there were normal populations of bacterial DNA sequences in the blood compartments previously considered sterile at least most of the time. Although persistent infection is a potential source of nonhuman sequences in normally sterile human anatomic sites, not all bona fide pathogens have been associated with abnormality.

The immunologic mechanisms and interactions between resident microbial agents and the human host have been studied at various body sites. The interaction between resident oral bacteria and human gingival epithelial cells in culture demonstrates their potential for virulence. The microbial agents frequently associated with periodontal diseases include *Bacteroides forsythus*, *Campylobacter curvus*, *Eikenella corrodens*, *Fusobacterium (F) nucleatum*, *Porphyromonas gingivalis*, and *Prevotella intermedia*. The effects of these bacteria on the production of interleukin-8, a proinflammatory chemokine, were also measured. *F nucleatum* adheres to and invades human gingival epithelial cells accompanied by high levels of interleukin 8 secretion from the epithelial cells.<sup>10</sup> By electron microscopy, this invasion occurs via a “zipping” mechanism that requires the active involvement of actins, microtubules, signal transduction, protein synthesis, and energy metabolism of the human gingival epithelial cells, as well as protein synthesis by *F nucleatum*.

Other investigators<sup>11</sup> noted a heightened risk of inflammatory bowel disease and colorectal cancer between diffusely adherent *Escherichia coli* and areas of dysplastic mucosa of the colon that made it easier for the bacterial pathogens to gain direct contact with the mucosal surface, a location that is relatively sterile in the normal colon. Such interactions between bacterial components and intrinsic T-cell receptors of the human mucosa with subsequent downstream protein signaling as the mechanism for early oncogenesis illustrating yet another molecular genetic property of the resident bacteria in their putative role in genotoxicity and human disease. If epithelial-associated bacteria play a causative role in inflammatory bowel disease and colorectal cancer, then dietary consumption of soluble plant fibers that prevent mucosal recruitment of bacteria may be protective against both conditions.

Postinfectious autoimmunity is a recognized phenomenon with several theories to explain its occurrence, including molecular mimicry, bystander activation, and viral persistence.<sup>12</sup> Alone or in combination, these mechanisms have been used to account for the immunopathology observed at the site of infection and in distant areas of the body. Molecular mimicry occurs when there are shared immunologic identities or epitopes between the microbe and host. One well-recognized example is rheumatic fever, a systemic autoimmune disease that occurs after group A  $\beta$ -hemolytic streptococci (GABHS) infection wherein affected patients develop and manifest circulating reactive antibodies to the bacterial organism reactive to the heart, joint, and brain, leading to the cardinal manifestations of rheumatic fever. Pediatric autoimmune neuropsychiatric disease associated with GABHS infection or PANDAS, is another example of bacterial-based molecular mimicry.

Viruses with cross-reactive epitopes to hepatitis B virus and myelin basic protein, a constituent of myelin, develop autoimmune experimental allergic encephalomyelitis (EAE) due to circulating T cells that preserve the memory of the virus and cross-react with myelin present in brain white matter of experimental mice. There is a form of postinfectious encephalitis named acute disseminated encephalomyelitis, an inflammatory demyelinating disorder of the brain in children that follows seemingly minor viral infection with a 2- to 30-day latency period that is thought to be postinfectious and autoimmune. It is thought that naissance of autoimmunity in such disorders originates when novel disease-inducing autoantigens are presented by specialized elements of the immune system in a trimolecular complex comprising antigen-presenting cells, major histocompatibility complex class II molecules, and autoreactive CD4<sup>+</sup> T cells.

Bystander activation and killing, a second mechanism that can also lead to autoimmune disease, has gained support through the use of experimental animal models mirroring some of the features of autoimmune disease, such as the nonobese diabetic mouse for type 1 diabetes (T1D) and EAE. It states that virus infections lead to significant activation of antigen-presenting cells that potentially activate preprimed autoreactive virus-specific T cells that migrate to areas of virus infection/antigen, such as the pancreas or brain. There, they encounter virus-infected cells presenting certain molecular tags, in turn releasing cytotoxic granules resulting in the killing or death of the infected cells. The dying cells, CD8<sup>+</sup> T cells, and inflammatory cells within such inflammatory foci release cytokines that lead to the demise of uninfected neighboring cells and additional immunopathology at sites of infection.

Persistent viral infection is a third mechanism of immune-mediated injury due to the constant presence of viral antigens that in turn drive the immune response. Yet unproven in humans, an example of this occurs in experimental mice who develop a condition termed Theiler murine encephalomyelitis,<sup>13</sup> in which persistent infection leads to a T-cell-mediated immunopathology in genetically susceptible animals. Susceptible

strains develop virus-specific delayed-type hypersensitivity responses, whereas resistant strains do not. This response has been proposed as the basis for flaccid paralysis that spreads rapidly to all 4 limbs after an incubation period of 7 to 30 days because of inflammation and demyelination in the brain and spinal cord.

## I-CUBED DISORDERS

With the preceding concepts in mind, this section considers 2 exemplary disorders, human leukocyte antigen (HLA) B27-related spondyloarthropathy and T1D and neuropathy, both of public health concern because of its pervasive occurrence in the population.

### *Spondyloarthropathy*

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The relationship between microbial infection and the gut, which has been known for decades as the basis for the spondyloarthritides (SpA)<sup>14</sup> has only recently been incorporated into the I-Cubed paradigm. SpA consists of diverse disorders of inflammatory arthritis. The reported incidence of 0.48 to 63/100,000 and prevalence of 0.01% to 2.5% for SpA diseases in the population<sup>15</sup> vary depending on the methodology and case definitions used for case ascertainment, and frequency of HLA-B27 in the population studied. Affected patients with symptoms referable to the vertebral column and limb joints may be seen by a variety of specialists including rheumatologists, neurologists, and general practitioners before the disorder is correctly diagnosed. Documentation of HLA B27 haplotype is a frequent associated feature.

Experimentally induced SpA occurs in mice with a striking resemblance to humans when HLA B27 components are introduced into genetically susceptible animals, establishing its central role in the human sickness.<sup>16</sup> Certain genetically prone mice develop colitis and later SpA when they are colonized with *Bacterioides* flora along with increased colonic cytokine expression compared with germ-free uncolonized animals.<sup>17</sup> The story, however, became more interesting when it was found that such animals also showed activation of Th17 helper cells<sup>18</sup> with HLA-B27 misfolding and a further heightened immune response to the unfolded protein associated with interleukin-23 production.<sup>19</sup>

Taken together, these findings suggested that genetically predisposed animals react to a microbial imbalance by altering their immune system in the intestinal compartment toward a more inflammatory state. The process is mediated by T-cell and interleukin production, which ultimately leads to local and systemic clinical disease manifested as aSpA-like human illness.<sup>20</sup> Such insights of the microbiomes have been used to advance therapy of SpA and other autoimmune arthritides. Empiric broad-spectrum antibiotics do not appear to have a therapeutic role and may select species with even more pathogenic potential. Bacterial modulation using alternative methods drawing from innate benefits of the microbiota,<sup>21</sup> such as fecal microbial transplantation, diet, and probiotics, have instead been used to restore a healthier intestinal microbiome.

### *Diabetes Mellitus and Neuropathy*

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#### *Type 1 diabetes*

T1D and type 2 diabetes (T2D) are both associated with diverse metabolic diseases that share the common feature of elevated blood glucose levels due to deficient insulin secretion or defective secretion or action, respectively. Both classes of patients are at perpetual risk for the development of nerve, kidney, and retinal disease. T1D usually develops before age 30 years, and such individuals need insulin injections for the

rest of their life. Their disease is caused by the gradual loss of insulin-producing  $\beta$  cells in the pancreas.<sup>22</sup> Patients with T2D are typically older, often obese, and at high risk for hypercholesterolemia and heart disease, with relative insulin resistance that perpetuates hyperglycemia. The epidemiology of diabetes is well known. In the United States alone, more than one million people are living with T1D and approximately 80 people per day, or 30,000 individuals per year, are newly diagnosed. The global incidence of T1D is increasing at a rate of approximately 3% to 4% per year, notably among younger children. These statistics highlight the need for both better T1D therapies and the continued push toward the prevention of T1D.

In the past several years, several lines of investigations have suggested the importance of environmental factors, including infectious diseases, making T1D an important candidate for an I-Cubed framework of understanding.

First, T1D appears to be caused by autoimmune mechanisms directed against the insulin-producing  $\beta$  cells of the pancreas,<sup>23</sup> with up to 90% of T1D patients harboring one or more autoantibodies.

Second, the pancreas of newly diagnosed T1D patients shows inflammation in the region of the insulin-producing  $\beta$  cells.<sup>24</sup>

Third, the possibility that the onset of T1D might be triggered in genetically predisposed individuals by a preceding infection inducing attack on islets by molecular mimicry was investigated in children with T1D who died prematurely. Their autopsy showed pancreatic islet cell, membrane-bound, superantigens, indicating integrated bacterial or viral genes. The genetic risk of T1D is strongly linked to HLA class II DR3 and DR4 haplotypes, with the highest risk in those with the DR3/DR4 genotype. The importance of HLA genes to T1D risk highlights the role of the adaptive immune system in the development of autoimmunity.

Fourth, T1D occurs with increased frequency in association with several other autoimmune disorders, including Grave disease, pernicious anemia, Hashimoto thyroiditis, myasthenia gravis, anti-phospholipid antibody syndrome, and Addison disease.

Fifth, there is an animal model of autoimmune diabetes in which T cells are strongly implicated in  $\beta$ -cell destruction, similar in nature to studies in humans in which primed autoreactive T cells recognize peptides common to both insulin and microbial antigens, suggesting that molecular mimicry may be the priming event in the destruction of  $\beta$ -islet cells in animals and humans.<sup>25</sup> Moreover, the response of T cells to homologous peptides derived from microbial antigens suggests that their initial priming could occur via molecular mimicry.

Sixth, it is hypothesized that perturbations in normal early microbiome development might predispose to disease whether through direct modulation of innate immunity or via alteration of intestinal permeability, with a downstream effect on adaptive immunity. The gut microbiome is both less diverse and protective in individuals with islet cell autoimmunity or recent onset T1D.<sup>26–28</sup> Whether this difference is causal to T1D in such patients is not known because multiple factors could affect the early intestinal microbiome, some of which also have been shown to correlate with risk of islet autoimmunity and T1D.<sup>29</sup> Nevertheless, increased intestinal permeability as a consequence of prolonged enteric intestinal infections could lead to increased susceptibility to T1D.<sup>22</sup> Viruses, with their potential to induce innate and adaptive immune responses and local inflammation in the pancreas and other organs, have been suspected of initiating these autoimmune processes. The etiologic link between T1D and viruses is based on epidemiologic, serologic, and histologic findings, as well as experimental *in vivo* and *in vitro* studies in DNA Herpesviruses and Parvoviruses, and RNA Togaviruses, Paramyxoviruses, Retroviruses, and Picornaviruses. A mechanism of molecular mimicry has been suggested on the observation that some



microbial/viral proteins and host proteins have sequence or structural homology and therefore go unrecognized as self-proteins stimulating immune response against the viral antigen, which becomes cross-reactive against the homologous sequence of the host  $\beta$ -cell proteins.<sup>12</sup> Another possible mechanism of infection-induced autoimmunity is bystander activation whereby the infection of neighboring  $\beta$  cells stimulates local inflammation with the appearance of T cells and other inflammatory cells that release inflammatory proteins that lead to bystander killing of  $\beta$  cells.<sup>30</sup>

### **Diabetic neuropathy**

Peripheral neuropathy occurring in association with T1D and T2D affecting large somatic and small pain-sensitive and autonomic fibers may be similarly influenced by inflammatory autoimmune factors. Historically, early investigations of diabetic neuropathy used nerve trunks obtained from diseased limbs obtained at surgical amputation and postmortem examination; such patients had longstanding diabetes that tended to increase the likelihood of arteriosclerosis. However, one early case showed clinicopathologic features of mononeuritis multiplex and vascular thrombosis of arteriae nervorum, suggesting a vascular inflammatory pathogenesis of neuropathy.<sup>31</sup> Decades later, investigations of peripheral nerve microvessels using vital stains to examine endoneurial blood vessels showed thickening of their walls<sup>32</sup> that was subsequently found to be reduplication of the basal lamina, a change also common to retinopathy and nephropathy. Unable to validate the correlation between so-called microangiopathy and neuropathy, attention focused on metabolic alterations in nerve elements, but attempts to correlate chronic hyperglycemia with metabolic derangements and alterations of intrinsic nerve lipids, alcoholic sugars, and a series of biochemical consequences leading to altered protein synthesis, abnormal glycosylation, slowed axon transport, axoglia dysjunction, osmotic swelling, or thickening of axolemmal and endoneurial basement membranes was often inconsistent.

In a series of 2 articles spanning a decade in 107 patients with diabetic neuropathy, Younger and colleagues<sup>33,34</sup> noted a more pervasive contribution of inflammatory and immune-mediated damage to the pathogenesis of diabetic neuropathy than had ever been previously imagined. Inflammatory lesions in diabetic nerves stained by immunoperoxidase comprised primarily CD8<sup>+</sup> and CD4<sup>+</sup> T cells of varying severity situated around endoneurial and small epineurial arteries or veins measuring 70  $\mu$ m, leading to perivasculitis so noted in 23% of cases, and transmural inflammation of the vessel wall termed microvasculitis or frank vasculitis each in 3%. Associated immunologic alterations included complement deposition along vessel walls, not simply at sites of vascular inflammation, with expression of various interleukins and tumor necrosis factor $\alpha$ .

Two disorders of known autoimmune etiopathogenesis, chronic inflammatory demyelinating polyneuropathy (CIDP) and lumbosacral radiculoplexus neuropathy (LSRPN), were described in patients with T1D and T2D. Stewart and colleagues<sup>35</sup> and Haq and coworkers<sup>36</sup> described the clinical, electrophysiological, and histopathologic findings of a small series of diabetic patients meeting formal criteria for CIDP, the associated features of which did not discriminate diabetics from nondiabetics. Dyck and colleagues<sup>37</sup> compared 57 patients with LSRPN alone or associated with diabetes in 33 other patients, with regard to natural history variables, electrophysiological features, quantitative sensory and autonomic analysis, histopathology, and outcome, noting no differences in the indices between diabetic and nondiabetic patients.

More recently, Younger<sup>38</sup> described a living patient with diabetic LRPN in whom nerve biopsy showed necrotizing arteritis prompting combination immunosuppressive

therapy, who at postmortem examination had perivascular epineurial and endoneurial inflammation in the extradural lumbar plexus, sciatic, and femoral nerve tissue without evidence of systemic vasculitis. Painful small-fiber neuropathy confirmed by intraepidermal nerve fiber analysis also occurs in patients with T1D and T2D as well as in association with diverse connective tissue and infectious and autoimmune neurologic disorders.<sup>39</sup>

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