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Inflammation as a Therapeutic Target for Diabetic Neuropathies

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Abstract

Diabetic neuropathies (DNs) are one of the most prevalent chronic complications of diabetes and a major cause of disability, high mortality, and poor quality of life. Given the complex anatomy of the peripheral nervous system and types of fiber dysfunction, DNs have a wide spectrum of clinical manifestations. The treatment of DNs continues to be challenging, likely due to the complex pathogenesis that involves an array of systemic and cellular imbalances in glucose and lipids metabolism. These lead to the activation of various biochemical pathways, including increased oxidative/nitrosative stress, activation of the polyol and protein kinase C pathways, activation of polyADP ribosylation, and activation of genes involved in neuronal damage, cyclooxygenase-2 activation, endothelial dysfunction, altered Na^+/K^+-ATPase pump function, impaired C-peptide-related signaling pathways, endoplasmic reticulum stress, and low-grade inflammation. This review summarizes current evidence regarding the role of low-grade inflammation as a potential therapeutic target for DNs.

Keywords

Diabetic neuropathy; Peripheral nerve dysfunction; Chronic inflammation; Inflammatory cytokines

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Conflict of Interest Rodica Pop-Busui, Lynn Ang, Crystal Holmes, Katherine Gallagher, and Eva L. Feldman declare that they have no conflict of interest.

Compliance with Ethical standards

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Introduction

Diabetic neuropathy (DN) ultimately affects up to 50 % of patients with type 1 diabetes (T1DM) and type 2 diabetes (T2DM) [1, 2]. The prevalence of diabetes and prediabetes in the USA and worldwide has reached epidemic proportions [2, 3]. For instance, between 2001 and 2009, there was a 23 % increase in the prevalence of T1DM among children in the USA [4, 5], and recent data from NHANES reported almost a doubling in the prevalence rates of total confirmed diabetes in the adult population [3]. As the prevalence of diabetes increases, so too will the prevalence and morbidity of DNs increase.

DNs have a wide spectrum of clinical forms and manifestations based on the type and anatomical distribution of the nerve fibers involved. Among the various forms of DNs, distal symmetrical polyneuropathy (DSPN) and cardiovascular autonomic neuropathy (CAN), a manifestation of autonomic neuropathy (AN), are by far the most prevalent [6•, 7].

DNs are a major cause of disability, high mortality, and poor quality of life [1, 2]. For instance, patients with DSPN have a 25 % cumulative risk of a lower extremity amputation. The 3-year survival in patients with DNs is 20 % less than in age- and sex-matched diabetic patients without DN [8]. Using a newly developed accelerated failure time model which includes alcohol consumption, proteinuria, race, retinopathy, sex, smoking, type of diabetes, body mass index (BMI), duration of diabetes, hemoglobin A1c (HbA1c), and DN, it was found that DN most significantly contributed to mortality [9]. CAN, although silent in earlier stages, is a significant cause of morbidity due to a high risk of cardiac arrhythmias, silent myocardial ischemia, and myocardial dysfunction [7, 10–15]. Strong evidence also demonstrates that CAN is an independent predictor of mortality [11]. Due to its high morbidity and mortality, the socioeconomic costs of DN and CAN are staggering and are estimated to be $22 billion/year in the USA.

Despite the high morbidity associated with DN and CAN, results from randomized clinical trials assessing the efficacy of various therapeutic agents in established DN and CAN have been disappointing. The Diabetes Control and Complications Trial (DCCT) demonstrated that intensive glucose control designed to achieve near-normal glycemia was essential in reducing the risk of DN and CAN in T1DM [16, 17]. Initial optimism over the DCCT results was tempered when long term follow-up of the cohort in the Epidemiology of Diabetes Interventions and Complications (EDIC) study revealed a high prevalence of DN and CAN 13–14 years after DCCT closeout in all subjects [18, 19]. These compelling results suggest that intensive glycemic control, although necessary, is insufficient to prevent adverse nervous system effects, justifying a critical need to identify new drug targets to treat DN and CAN early in their course.

Thus, the treatment of DNs, especially its reversion, continues to be very challenging, likely due to the complex pathogenesis of DNs which involves an array of systemic and cellular imbalances in glucose and lipid metabolism. These may lead to the activation of various biochemical pathways, including increased oxidative/nitrosative stress, activation of the polyol and protein kinase C pathways, activation of polyADP ribosylation, and activation of genes involved in neuronal damage, cyclooxygenase-2 activation, endothelial dysfunction,

altered Na+/K + −ATPase pump function, impaired C-peptide-related signaling pathways, endoplasmic reticulum (ER) stress, and low-grade inflammation [20, 21•].

In recent years, the DN research field has evolved from a glucocentric viewpoint to a more broad understanding that DN is a complex disorder secondary to multiple linked metabolic and inflammatory insults. Evidence that low-grade inflammation plays an important role in the pathogenesis of DN is emerging from both experimental and clinical studies.

Below, we summarize the current evidence regarding the role of low-grade inflammation as a potential therapeutic target for DNs.

Inflammation, Diabetes, and Vascular Complications

A potential link between inflammation and diabetes was first suggested more than a century ago based on observations that high doses of sodium salicylate (>5 grams/day) diminish glycosuria in diabetic patients having “the milder form of the disease,” presumably T2DM [22•]. More recently, several epidemiological studies found that increased levels of markers and mediators of inflammation and acute-phase reactants such as fibrinogen, C-reactive protein (CRP), interleukin (IL)-6, plasminogen activator inhibitor-1 (PAI-1), sialic acid, and white cell count correlate with incident T2DM [22•, 23–25]. Eventually, several groups of investigators demonstrated activation of distinct molecular pathways mediated by adipocyte-derived pro-inflammatory cytokines, including activation of the transcription factor nuclear factor-κB (NF-κB) and IκB kinase-β (IKKβ)/NF-κB axis [22•] and links to ER stress [26••]. The interplay between chronic inflammation, obesity, insulin resistance, and T2DM is now established, was amply discussed previously [22•, 26••], and is beyond the scope of this review. Targeting the NF-κB pathway with salsalate, a salicylate prodrug, in patients with T2DM, was initially shown in few proof-of-principle studies to have beneficial effects on blood glucose, triglyceride, free fatty acid (FFA), and C-reactive protein (CRP) concentrations and to improve glucose utilization [27]. Most recently, the NIH-funded targeting inflammation using salsalate in type 2 diabetes (TINSAL-T2D), a randomized, placebo-controlled parallel phase 3 clinical trial further confirmed these observations reporting a significant improvement in glycemia and decrease in mediators of inflammation in patients with T2DM after 48 weeks of treatment [28].

More than a decade ago, seminal work by Libby et al. [29••] also unveiled that chronic inflammation plays a critical role in the pathogenesis of atherosclerosis and vascular complications, participating in all stages of atherogenesis and endothelial dysfunction directly via multiple mechanisms. These mechanisms include activation and increased expression of vascular cell adhesion molecules (VCAM-1, ICAM-1) and E-selectins on the endothelial cells, recruitment and binding of leukocytes, particularly monocytes and T lymphocytes, further release of pro-inflammatory cytokines like tumor necrosis factor-α (TNF-α), IL-6, IL-1, and IL-18, migration of macrophages, wall stress augmentation, arterial smooth muscle cells proliferation, and lipid oxidation that further promote inflammatory responses [29••, 30]. Chronic inflammation may also reduce the local production of endothelium-derived nitric oxide (NO) and increase production of angiotensin II, PAI-1, free fatty acids (FFAs), and advanced glycation end products (AGEs) [29••, 30].

While all of these pathways have been shown to critically affect the development of macrovascular disease, they may also underlie the link between inflammation and development of microvascular complications. For instance, in diabetic nephropathy, several experimental studies reported increased renal expression of several pro-inflammatory cytokines and adhesion molecules, such as IL-1, IL-6, IL-18, TNF-α, ICAM-1, and VCAM-1, with subsequent increased vascular endothelial cells permeability, dysregulation in the generation of hyaluronan, and initiation of glomerular hypercellularity [31]. Evidence for the role of chronic inflammation in the pathogenesis of diabetic nephropathy was also demonstrated in humans when a few cross-sectional trials in both T1DM and T2DM trials reported increased CRP and other cytokines in patients with nephropathy [30]. In the DCCT, baseline E-selectin and fibrinogen levels, but not CRP, independently predicted development of nephropathy in T1DM [32], while in a follow-up of a cohort with T1DM evaluating the natural history of diabetic nephropathy at the Joslin Diabetes Center, Krolewski et al. found that the presence of elevated urine levels in more than 2 inflammatory markers among IL-6, IL-8, monocyte chemoattractant protein-1, interferon-gamma-inducible protein, and macrophage inflammatory protein-1δ were more than five times as likely to have early progressive decline of renal function [33]. Most recently, data obtained in the same cohort showed that elevated serum concentrations of tumor necrosis factor receptors (TNFR) 1 or 2 are strongly associated with the risk of advanced stages of renal decline, such as chronic kidney disease stage 3 or end-stage renal disease and with the onset of renal decline itself [34, 35]. This supports the idea that inflammatory signals may drive disease in DN. In T2DM, the data is less clear; however, a few studies reported either that IL-18, a pro-inflammatory cytokine involved in both innate and acquired immune responses, independently predicted conversion from normoalbuminuria to microalbuminuria or reduced adiponectin levels in T2DM subjects with nephropathy independent of confounders such as body weight, insulin resistance, or glycemic control [30].

As with nephropathy, activation of pathways of inflammation, especially increased E-selectin and adhesion molecules and decreased adiponectin, was also linked to the pathogenesis of diabetic retinopathy [30].

Inflammation and Diabetic Neuropathy

Multiple pre-clinical [36–40] and clinical [15, 41, 42•, 43, 44] studies demonstrate a pathogenic role for inflammation, especially cytokine and chemokine production, in the DN and CAN disease course.

Although the inflammation process is quite complex, activation of the IKKβ/NF-κB axis plays a central role. NF-κB is a redox-sensitive transcriptional factor activated by a number of stimuli, including hyperglycemia, oxidative stress, and pro-inflammatory cytokines [22•, 36, 45].

Several lines of evidence obtained in experimental studies showed that activation of the NF-κB axis triggers inflammatory and immune responses that may lead to cellular injury and expression of adhesion molecules and cytokines [22•, 45–47]. In a model of streptozotocin (STZ)-induced diabetes, ischemia reperfusion injury induced NF-κB overexpression in the

diabetic sciatic endothelial cell and Schwann cell, with subsequent increased ICAM-1 expression and extensive infiltration of monocyte macrophages compared with controls, suggesting that the enhanced inflammatory response in diabetic nerves was mediated by NF-κB activation [48]. Similarly, the NF-κB axis also regulates the expression of many inflammatory genes and their downstream effects, and the contribution of genes including cyclooxygenase-2 (COX-2), NO-synthase, lipoxygenase, and endothelin-1 were directly tested in animal models of DN [38]. The NF-κB-derived cytokine TNF-α induces cyclooxygenase-2 (COX-2) overexpression and mitogen activated protein kinase (MAPK) activation [38, 39], two phenomena implicated in the diabetes-induced pro-inflammatory response and neuropathic changes [40, 49]. For instance, in two experimental models of T1DM neuropathy, our group found significantly increased levels of TNF-α in the sciatic nerve of diabetic rats and mice which were associated with both large and small nerve fiber dysfunction, as documented by reductions in the motor and sensory nerve conduction velocities (NCV) and in the intraepidermal nerve fiber density (IENFD) in the diabetic animals [40]. These changes were prevented by either COX-2 gene inactivation or treatment with a COX-2-selective inhibitor [40]. In another animal model of diabetes, 12/15 lipoxygenase inhibition improved motor and sensory NCV and mechanical allodynia, although it did not affect thermal hypoalgesia [38]. Our group has also reported upregulation of multiple inflammatory mediators, including COX2, iNOS, and TNF-α, in sensory neurons of db/db mice early in the course of DN, when the animals are experiencing pain [50]. Blocking the increase in these inflammatory markers blocked pain, suggesting that in an experimental model of T2DM DN, inflammation in sensory neurons can mediate pain [50]. However, the activation of the inflammatory cascade with downstream signaling and cytokines release may have dual effects. For instance, IL-6 is often co-released with TNF-α and is considered in general a pro-inflammatory cytokine [29••, 30]. However, a couple of experimental studies reported that treatment of diabetic rats with pharmacological doses of IL-6 improved motor and sensory NCV, corrected the altered thermal nociception and tactile allodynia, and promoted increased nerve blood flow [38, 51, 52]. Although a clear mechanism for these observations was not provided [38, 51, 52], these data would suggest a potentially opposing effect of IL-6, which is yet be confirmed by other studies, particularly in humans.

In addition, more recent evidence links inflammation with dysregulation of several heat-shock proteins that function as molecular chaperones and protects cells against environmental stress. For instance, in vitro studies found that the 70-kDa heat-shock protein (HSP)70 bound with high affinity to the plasma membrane, activated NF-κB, and upregulated the expression of TNF-α, interleukin IL-1β, and IL-6 in human monocytes [53]. Dobrowsky et al. performed transcriptomic analysis of sensory neuron RNA obtained from diabetic wild-type and Hsp70 knock-out mice using RNA-sequencing and reported that diabetes strongly increased inflammatory pathways [54, 55]. In addition, modulation of Hsp70 and Hsp90 with targeted small-molecules ameliorated psychosensory, electrophysiologic, morphologic, and bioenergetic deficits of DPN in animal models of T1DM [54, 55]. It was also shown that the level of circulating HSP27, which has a role in cytoprotection and cell motility and is overexpressed in dorsal root ganglia in experimental

diabetes, perhaps as a compensatory mechanism, was associated with distal sensorimotor neuropathy [56].

A proposed cascade of events linking the chronic inflammation in diabetes to peripheral nerve fiber damage and loss is shown in Fig. 1. Briefly, hyperglycemia coupled with loss of insulin signaling and insulin resistance along with dysregulations in lipid metabolism and dyslipidemia lead to systemic inflammation and vicious cycles of oxidative/ nitrosative stress, endoplasmic and mitochondrial stress, and accumulating cellular damage [20, 21•, 57–61]. Glucotoxicity, insulinopenia, and lipotoxicity produce neuronal oxidative/ nitrosative stress and activate multiple downstream kinases, such as protein kinase C (PKC), MAPK, and jun N-terminal kinase (JNK), and redox-sensitive transcriptional factors, including NF-κB. These factors play a critical role in triggering a cascade of cytokine and chemokine production, including pro-inflammatory IL-1β, IL-2, IL-6, IL-8, TNF-α, chemokine (C-C motif) ligand 2 (CCL2), and chemokine (C-X-C motif) ligand 1 (CXCL1) [21•, 61]. Cytokines and chemokines not only enhance existing inflammatory and immune responses but also promote activation of the wide array of downstream cellular oxidative/ nitrosative stress, promoting even more neuronal damage [61].

Human studies support these pre-clinical findings. For instance, a study that included more than 150 participants with diabetes, with and without DN, and 55 healthy controls reported increased serum levels of inflammatory cytokines, including TNF-α and CRP, and markers of endothelial dysfunction in subjects with DN [41]. These markers were further increased in those with painful DN [41], data that are in concert with findings from another study that reported increased IL-2 in participants with neuropathic pain [62]. Duksal et al. recently reported that elevated serum levels of IL-6 and IL-10 in patients with prediabetes and T2DM correlated with markers of large nerve fiber sensory and motor axonal damage and with signs of motor nerve demyelination [63]. Since IL-10 is considered an anti-inflammatory cytokine, that negatively regulates TNF-α, one could speculate that the observed increased IL-10 levels in this study could have been a compensatory mechanism. In a cross-sectional sample of ~500 participants with T1DM from the EURODIAB Prospective Complications Study, there was an independent association between serum HSP27 levels and distal symmetrical polyneuropathy diagnosed based on the presence of one or more neuropathic symptoms, the absence of two or more ankle or knee reflexes, and abnormal vibration perception threshold [56]. A recent analysis of a large cohort of over 1000 participants in the population-based Cooperative Health Research in the Region of Augsburg (KORA) F4 study reported that serum concentrations of several inflammatory cytokines, including IL-1β and IL-6, were positively associated with measures of peripheral DN in age- and sex-adjusted analyses [64•].

Emerging evidence also demonstrates a role for inflammation in the development of autonomic dysfunction, particularly CAN in diabetes. In an earlier study, we reported significantly higher markers of inflammation, including higher levels of TNF-α, in patients with T1DM and CAN compared with patients with T1DM without CAN [44]. In a case–control study involving nondiabetic, newly diagnosed T2DM, and established T2DM obese subjects (15/group), Lieb et al. found that IL-6 correlated negatively with measures of CAN while the ratio of adiponectin to leptin correlated positively with measures of CAN [15].

We recently expanded our assessments of serum markers to include microarray analyses of sural nerves from patients with DN. Sural nerve gene expression signatures from subjects with progressive DN are highly functionally enriched in inflammatory and immune response pathways, and specific up-regulated genes include chemokines (CCL2, 5, CXCL1), cytokines (IL-1β, IL-2, IL-6), and complement [42•]. In contrast, gene expression signatures of sural nerves with anatomical evidence of regeneration from subjects with DN who have stable disease reveal significant downregulation of pathways and genes associated with inflammation and the immune response [43]. Table 1 lists 18 differentially expressed genes (DEGs) related to the “defense response,” including 14 “inflammatory response” DEGs.

Inflammation and Charcot Neuroarthropathy

Charcot neuropathic osteoarthropathy (CN) is a rare form of DN that causes significant morbidity and mortality to the affected patients [65]. The prevalence of CN varies between 0.1 and 0.9 % [66–68]. The clinical presentation of CN is characterized by an edematous, erythematous warm foot. CN often results in severe deformities and disfigurement that contribute to the development of ulcerations and lower extremity amputations. Although the pathogenesis of CN is still very poorly understood, recent evidence suggests that chronic inflammation in concert with the peripheral nerve neurogenic peptide dysfunction induced by diabetes play important roles in its development and progression [69]. The prodromal state of the disease that manifests with an acutely erythematous, edematous, and warm foot, all typical features of inflammation, supports this concept. Stimulation of the inflammatory cycle may directly stimulate increased bone turnover [70], which in concert with hyperglycemia leads to increased production of AGEs and upregulation of the receptors for AGEs (RAGE). The increase in RAGE stimulates elevated levels of receptor activator nuclear factor K ligand (RANKL), which promotes osteoclastogenesis [71]. The superimposed autonomic dysregulation with impaired sympathetic control documented in CN with subsequent increased perfusion further contributes, which can lead to weakened demineralized bone that is susceptible to fracture and dislocation [72]. Koeck et al. [73] also relate the combined components of neuropathy, microtrauma, and neurovascular effects resulting in a pro-inflammatory cytokine activity, including elevated TNF-α [74] and RANK-L [75]. Several human studies using infrared dermal thermometry in cohorts of patients presenting with unilateral acute CN found an average 8.8 ± 2.3 °F higher temperature on the affected joint, compared to the contralateral joint [76]. The temperature differences were found to correlate highly with radiographic changes [76] and with markers of bone turnover [77].

Chronic Inflammation and Impaired Wound Healing

The superimposed impaired cutaneous wound healing further complicates the complex clinical presentation and management of patients with DN. A third of the burden of the economic costs of diabetes is estimated to be due to peripheral wounds, a contributing factor to the high risk of lower extremity amputations in the USA and subsequent increase in 3-year mortality rates [78–80]. “Standard therapy” leaves 70 % of diabetic wounds unhealed; thus, development of more effective treatments is imperative. It is recognized that obesity, DN, and T2DM are linked and associated with chronic systemic inflammation [22•, 81–85].

Diabetic wounds are likewise characterized by a chronic inflammatory state maintained by imbalances between pro-and anti-inflammatory cytokines produced by immune cells [86–89]. Macrophages are the key immune cell that drives wound inflammation and are defined by the expression of specific surface markers and gene phenotypes [88, 90, 91]. Classically activated macrophages (M1) express a defined set of pro-inflammatory mediators, while alternatively activated macrophages (M2) display an anti-inflammatory phenotype [92–98]. In normal wound healing, first-responder macrophages mobilized from the circulation exhibit an M1 phenotype and secrete pro-inflammatory mediators, including IL-12, which participate in antimicrobial functions [99, 100]. This stage is followed by an M2 anti-inflammatory response that promotes tissue repair [101]. This M2 dominated wound repair phase appears to be markedly attenuated in diabetes [102, 103]. Given that monocyte/macrophages isolated from patients with diabetes constitutively secrete elevated levels of pro-inflammatory cytokines [104, 105], one approach to improve diabetic wound healing would be to reset the balance between M1 and M2 macrophage subsets. The production of these phenotypically distinct cell types facilitates the development of a tailored immune response to particular stimuli such as infection and injury.

It is also accepted that bone marrow (BM) hematopoietic stem cells (HSC) give rise to a number of multipotent progenitors which in turn generate common myeloid progenitors (CMP). CMP generate granulocyte-macrophage progenitors (GMP), which differentiate into monocytes/macrophages in the circulation and peripheral tissue, respectively [93, 106–108]. Thus, BM-derived monocytes in peripheral blood are mobilized to tissue in response to injury or inflammation [94, 109, 110]. Evidence suggests that epigenetic regulation (e.g., DNA methylation, histone modification) of gene expression plays a key role in influencing immune cell phenotypes [111]. Epigenetic modifications have been documented in inflammation in animal models, where chromatin modifications have been shown to regulate downstream immune-mediator expression in monocyte-derived macrophages [112, 113]. The notion that gene expression patterns can be maintained over a period of time and are heritable is due to the fact that the DNA is not completely stripped of its nucleosomes during replication, and hence, the remaining modified histones can act as templates to initiate identical modification during replication. Thus, any modifications can be transferred to daughter cells and more differentiated cells [114]. Presently, there is little data on epigenetic-based mechanisms that regulate macrophage phenotype in diabetic wounds. Work by Gallagher et al. demonstrates that epigenetic changes in the form of histone methylation may alter macrophages and skew them towards an inflammatory phenotype in the periphery. Although the concepts that chronic inflammation is due to changes in macrophage phenotype and is associated with impaired diabetic wound healing have been well accepted, no approach to date has been clinically effective in restoring the normal wound healing cascade in diabetic wounds. The current treatment in wound management is fundamentally passive, and despite significant advances in the medical treatment of diabetes, wound healing rates have not changed over the past 30 years.

Targeting Inflammation as a Potential DN Therapy

The findings described above demonstrate that elevated levels of inflammatory and endothelial dysfunction biomarkers accompany the development of DN in a manner

frequently independent of hyperglycemia alone. Although most of the data reports associative relationships only, some prospective studies suggest that inflammatory cytokines, and the NF-kB/ IKKβ axis in particular, are predictive of DN.

In vitro and in vivo studies and short-term human trials have shown that salicylate therapy markedly lowers circulating glucose, triglycerides, and FFAs, as well as CRP levels, effects mediated by inhibition of the IKKβ/NF-κB pathway [27, 115, 116]. Salicylates are also reported to have inhibitory effects on the production of chemokines, interleukins, and complement, all of which are upregulated in human sural nerves from subjects with progressive DN [117–119]. In a large observational cohort of patients with T1DM, serum lipids were independently associated with development of DN [120]. We reported that elevated triglycerides were the primary clinical parameter that correlated with a loss of myelinated fiber density, independent of disease duration, age, diabetes control, or other variables [121].

An agent that targets low-grade inflammation via modulation of the IKKβ/NF-κB pathway would therefore address several critical pathways involved in the pathogenesis of DN and of the DN associated pain.

Conclusions

A unifying hypothesis posits that inflammation occurs early in the development of diabetes, and in the presence of additional risk factors such as increased adiposity and insulin resistance, contributes to further metabolic deterioration and imbalance, and a complex inflammatory/endothelial dysfunction that targets both small and large nerve fibers to result in the peripheral nerve dysfunction characteristic of DN. Collectively, the data from experimental diabetes and from both serum and neural gene expression studies in patients with DN strongly support a role for inflammation in the onset and progression of DN. We contend that targeting inflammation is a mechanism-based strategy critically needed in the field of DN and it may be the missing link in finding a new viable therapy for DN.

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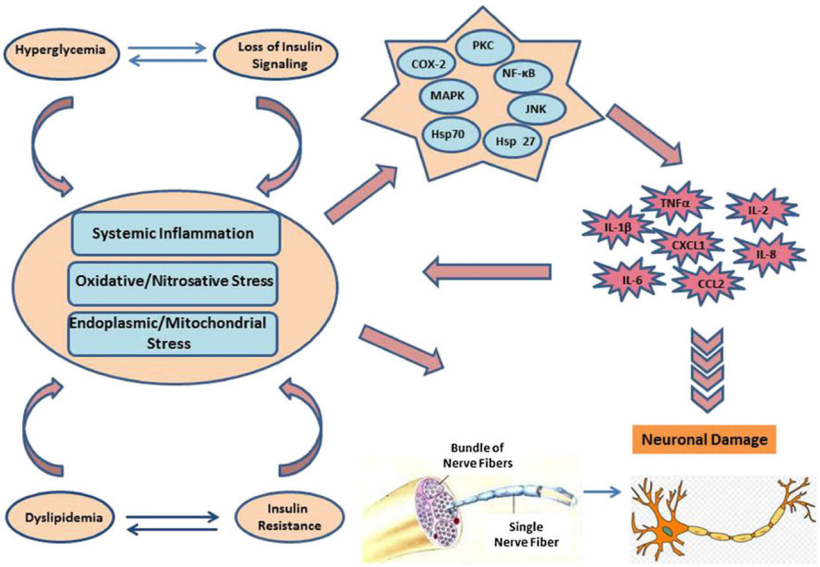

Fig. 1.

The link between chronic inflammation in diabetes and peripheral nerve fiber damage and loss. Hyperglycemia coupled with loss of insulin signaling and insulin resistance, along with dysregulation of lipid metabolism and dyslipidemia, lead to systemic inflammation and vicious cycles of oxidative/nitrosative stress, endoplasmic and mitochondrial stress, and accumulating cellular damage. Glucotoxicity, insulinopenia, and lipotoxicity produce neuronal oxidative/nitrosative stress and activate multiple downstream kinases such as protein kinase C (PKC), mitogen activated protein kinase (MAPK), and jun N-terminal kinase (JNK), as well as redox-sensitive transcriptional factors, including NF-κB. These factors play a critical role in triggering a cascade of cytokine and chemokine production, including pro-inflammatory interleukin-1β, interleukin-2, interleukin-6, interleukin-8 (IL-1β, IL-2, IL-6, IL-8), tumor necrosis factor-α (TNF-α), chemokine (C-C motif) ligand 2 (CCL2), and chemokine (C-X-C motif) ligand 1 (CXCL1). Cytokines and chemokines not only enhance existing inflammatory and immune responses but also promote activation of the wide array of downstream cellular oxidative/nitrosative stresses, promoting even more neuronal damage

Table 1

DEGs related to defense response and inflammatory response (upregulated genes in progressors)

		Entrez ID	Symbol	Description	*P* value	Fold-change
Defense response		136	ADORA2B	Adenosine A2b receptor	0.01	1.4
		2788	GNG7	Guanine nucleotide binding protein (G protein), gamma 7	0.03	1.2
		7033	TFF3	Trefoil factor 3 (intestinal)	0.01	1.5
		23601	CLEC5A	C-type lectin domain family 5, member A	0.02	1.7
		57817	HAMP	Hepcidin antimicrobial peptide	0.002	2.4
		81035	COLEC12	Collectin sub-family member 12	0.02	1.2
	Inflammatory response	140	ADORA3	Adenosine A3 receptor	0.03	1.3
		624	BDKRB2	Bradykinin receptor B2	0.03	1.2
		3075	CFH	Complement factor H	0.001	1.2
		4282	MIF	Macrophage migration inhibitory factor (glycosylation-inhibiting factor)	0.03	1.1
		4973	OLR1	Oxidized low density lipoprotein (lectin-like) receptor 1	0.0003	1.6
		7852	CXCR4	Chemokine (C-X-C motif) receptor 4	0.04	1.3
		10344	CCL26	Chemokine (C-C motif) ligand 26	0.0004	3.2
		10630	PDPN	Podoplanin	0.02	1.2
		25824	PRDX5	Peroxiredoxin 5	0.02	1.1
		53833	IL20RB	Interleukin 20 beta	0.02	1.3
		57834	CYP4F11	Cytochrome P450, family 4, sub-family F, polypeptide 11	0.03	1.4
		148022	TICAM1	Toll-like receptor adaptor molecule 1	0.03	1.2

(Modified with permission from Hur, J, et al. The identification of gene expression profiles associated with progression of human diabetic neuropathy. Brain, 2011. 134(Pt 11): p. 3222–35) [42•]