⊙ **PERIPHERAL NEUROPATHIES**

Schwann cell interactions with axons and microvessels in diabetic neuropathy

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Abstract | The prevalence of diabetes worldwide is at pandemic levels, with the number of patients increasing by 5% annually. The most common complication of diabetes is peripheral neuropathy, which has a prevalence as high as 50% and is characterized by damage to neurons, Schwann cells and blood vessels within the nerve. The pathogenic mechanisms of diabetic neuropathy remain poorly understood, impeding the development of targeted therapies to treat nerve degeneration and its most disruptive consequences of sensory loss and neuropathic pain. Involvement of Schwann cells has long been proposed, and new research techniques are beginning to unravel a complex interplay between these cells, axons and microvessels that is compromised during the development of diabetic neuropathy. In this Review, we discuss the evolving concept of Schwannopathy as an integral factor in the pathogenesis of diabetic neuropathy, and how disruption of the interactions between Schwann cells, axons and microvessels contribute to the disease.

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The incidence of diabetes mellitus has increased dramatically over the past two to three decades. According to the International Diabetes Federation Diabetes Atlas, 415 million people worldwide had diabetes in 2015, and this number is expected to grow by 5% annually, predominantly as a result of increasing prevalence of type 2 diabetes. Furthermore, an estimated 100 million Europeans and 80 million Americans have impaired glucose tolerance or prediabetes. Few people die from acute diabetes in countries with comprehensive health care systems, but the disease is inflicting a huge medical, social and economic burden on society owing to the need for lifelong treatment of its systemic consequences and the insidious development of multi-organ damage.

Peripheral neuropathy (BOXES 1,2) is a common but often neglected complication of long-term diabetes, and the lack of treatment options reflects an incomplete understanding of the pathogenic mechanisms. Hyperglycaemia is generally accepted as the primary pathogenic insult in type 1 and type 2 diabetic neuropathy, although roles are emerging for other factors, such as impaired insulin signalling, hypertension and dyslipidaemia (particularly for type 2 diabetes), which might precede overt hyperglycaemia[1]. Many preclinical studies, and occasional clinical studies, have indicated that diabetic neuropathy — like diabetic nephropathy and retinopathy — results from microvascular disease, with a focus on axonal degeneration as a consequence of ischaemia and/or hypoxia. This mechanism, however, is likely to be only one aspect of a more complex pathogenesis.

The earliest descriptions of pathology in diabetic neuropathy indicated that Schwannopathy accompanied axonal degeneration. The majority of clinical and basic research in diabetic neuropathy since then has focused on the effects on neurons. However, accumulating data from research into the development and regeneration of the PNS has identified Schwann cells as equally indispensable components that maintain neuronal structure and function, nourish axons, and promote survival and growth upon injury. The early reports from 1979 that demonstrated morphological changes in Schwann cells in human diabetic neuropathy[2] are now supported by an increased awareness of molecular alterations in Schwann cells during diabetes[3]. Schwann cells express a wide range of receptors and, when they sense insults or danger signals, they upregulate synthesis and secretion of factors that stimulate neuroprotection, regrowth and remyelination, or factors that aggravate disease phenotypes[4]. The most recent studies have demonstrated that Schwann cells regulate many aspects of axonal function, so that disruption of their metabolism by diabetes results in the accumulation of neurotoxic intermediates and compromises production of neuronal support factors, contributing to axonal degeneration, endothelial dysfunction and diabetic neuropathy.

REVIEWS

Key points

- Peripheral neuropathy is a serious but often neglected complication of diabetes mellitus

- Schwann cells support the structural and functional integrity of nerves, so their damage as a result of the metabolic consequences of diabetes adversely affects axons

- High polyol pathway flux, oxidative stress and inflammation are the main pathways activated in Schwann cells during diabetic neuropathy

- Disruption of Schwann cell metabolism by hyperglycaemia and/or dyslipidaemia results in accumulation of neurotoxic intermediates that confer axonal and vascular vulnerability to injury

- Microvascular changes within the endoneurium create a hypoxic environment that has the potential to disrupt Schwann cell function, promoting activation of inflammatory cascades that lead to neurodegeneration

Here, we review the interactions between Schwann cells, axons and microvessels that are known to contribute to the pathogenesis of diabetic neuropathy, with an emphasis on the mechanisms by which Schwannopathy might make axons and vessels vulnerable to injury.

Schwann cells and their interactions

Schwann cells are the most abundant cells in the PNS, and include two broad categories: myelinating and nonmyelinating Schwann cells. Together, the two types ensheath all axons of peripheral nerves. Myelinating Schwann cells individually wrap large-calibre axons (>1 µm diameter), leaving unmyelinated gaps (nodes of Ranvier) between adjacent Schwann cells that facilitate saltatory axonal conduction. Schwann cell myelin comprises various lipids and proteins compacted into a multilayer structure that provides electrical insulation. Nonmyelinating Schwann cells do not wrap axons, but small sensory axons (<1 µm diameter) are embedded in grooves in their membranes. Several small nociceptive axons can be embedded into the same Schwann cell, forming a Remak bundle[3].

Schwann cells derive from neural crest cells that first develop into Schwann cell precursors (SCPs) that associate with the first compact columns of peripheral axons. These proto-nerves are devoid of all other structural elements that characterize mature nerves, such as connective tissue and blood supply, and the SCPs connect with each other to envelop large numbers of axons[5]. During embryonic development, Schwann cells facilitate neuronal survival via neurotrophin secretion; to migrate and form myelin. Schwann cells require axonal secretion of neurotrophins and must express neurotrophin receptors[6,7]. Schwann cell proliferation is stimulated by secretion of vascular endothelial growth factor (VEGF) from ganglion neurons, which also stimulates axonal outgrowth and formation of microvessels[8]. The development of peripheral nerves, therefore, depends on signalling between Schwann cells, the axon and microvessels in a complex temporal and spatial manner.

During Schwann cell maturation and alignment, multiple receptors, ligands and adhesion molecules orchestrate neuronal sprouting and targeting. Among these factors, the neurotrophins β-nerve growth factor (NGF), brain-derived neurotrophic factor

(BDNF), NT-3 and NT-4/5 signal via two structurally unrelated types of receptors: the low-affinity neurotrophin receptor p75NTR and the tropomyosin-related kinase receptors TrkA, TrkB and TrkC. In the PNS, neurotrophins and their receptors are expressed by both Schwann cells and sensory neurons. Activation of the two receptor types can elicit opposing effects — survival and differentiation via Trks, or apoptosis via p75NTR[9,10]. Adding to the complexity, TrkA and TrkC can act as dependence receptors that elicit apoptosis when unstimulated[11], and the biological effect of secreted proneurotrophins might depend on the expression ratio of Trk receptors and p75NTR[12,13] and on expression of p75NTR co-receptors[14,15].

Physical or metabolic damage to adult peripheral nerves induces rapid and robust changes in the synthesis of neurotrophins in neurons and Schwann cells to guide and support regeneration[16,17]. For example, hypoxia upregulates VEGF production after nerve injury, initiating migration of Schwann cells[18]. VEGF also stimulates formation of endoneurial blood vessels into hypoxic areas, directing Schwann cells to bridge the gap between the proximal and distal nerve stumps[18]. However, studies have shown that diabetes reduces expression of the Schwann-cell-derived neurotrophic factors ciliary neuronotrophic factor (CNTF)[19] and sonic hedgehog[20], which act on neurons and endothelial cells, respectively. Moreover, Schwann cells that have been removed from mice with diabetes and maintained under hyperglycaemic conditions *in vitro* to model nerve injury also exhibit impaired production of NGF and NT-3 (REF. 21). The fact that Schwann cells ensheath the vast majority of axonal plasma membranes means that changes in their production of signalling molecules are likely to influence axons. Schwann cells are also in close contact with the basal lamina and the endoneurium, and receptor-mediated interactions between Schwann cells and these structures are known to affect several Schwann cell functions, including radial sorting and myelination[22]. Overall, these complex molecular interactions suggest that disruption of Schwann cell functions, including those triggered by diabetes, could have widespread consequences for peripheral nerve structure and function.

Schwann cells in diabetic neuropathy

Morphological changes. Nerve biopsy samples from patients with diabetes reveal overall fibre loss; degenerating fibres and clusters of regenerating axons are both present. Reduced axonal diameters, which suggest impaired maturation or atrophy, are frequently reported in rat models of diabetes, but are not a notable feature in many mouse models or humans with diabetes[23,24]. Whether axonopathy or Schwannopathy develops first in diabetic neuropathy[25] has long been debated, and a single nerve biopsy sample is not sufficient to determine whether Schwann cells undergo independent structural damage during diabetes or are responding to axonal degeneration. However, teased fibre sural nerve preparations from humans, cats and rodents with diabetes indicate morphological changes in the myelin sheath — ranging from thin myelin that

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Prediabetes
A condition in which blood glucose levels are higher than normal but not high enough to be considered as type 2 diabetes; individuals with prediabetes often develop diabetic neuropathy.

Dependence receptors
Proteins that mediate apoptosis by monitoring the absence of certain trophic factors.

Basal lamina
Sheets of extracellular matrix that are secreted by Schwann cells and surround a nerve.

Endoneurium
A layer of interstitial connective tissue that surrounds all axons, thereby separating individual nerve fibres.

Radial sorting
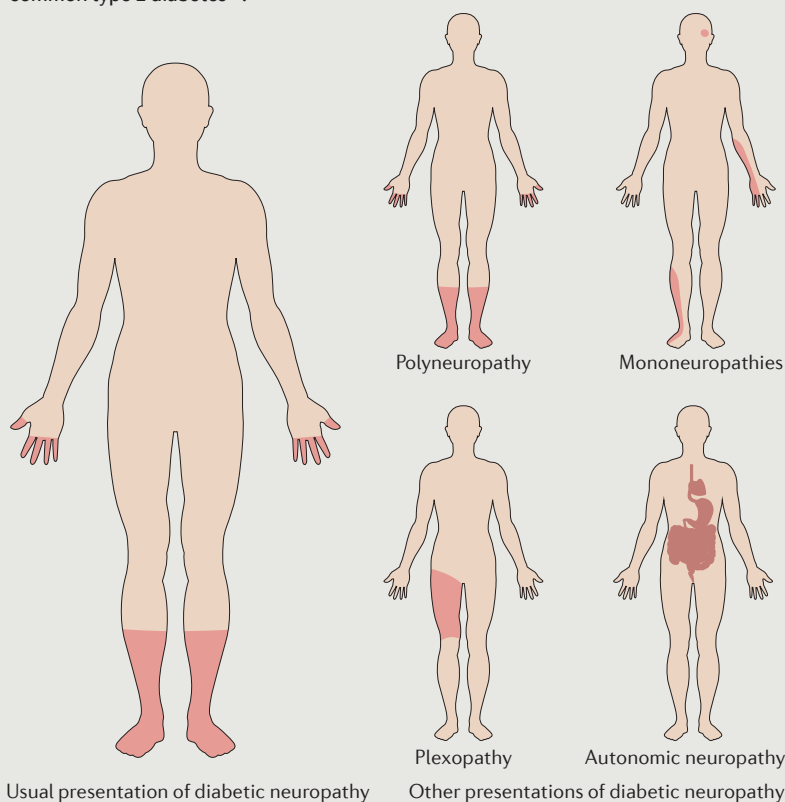The process that underlies selection of one axon for myelination by a Schwann cell during development.

suggests cycles of demyelination and remyelination, to full segmental demyelination — in the presence of an apparently normal axon, indicating that Schwannopathy can develop independently of axonopathy[2,3,26–28] (FIG. 1). Teased fibres from the same nerves can, however, exhibit predominantly axonal pathology, indicating that diabetes affects both cell types independently, as well as disrupting their interactions. Degenerative changes in nerve fibres are accompanied by the presence of enlarged mitochondria with effaced cristae and numerous

vacuoles in the Schwann cells[29], an observation that is consistent with emerging evidence of mitochondrial dysfunction and damage in axons and Schwann cells in animal models of diabetes[30]. Other diverse pathological markers, ranging from nonspecific signs of cellular stress through to overt cellular degeneration, are also present in Schwann cells from both animal models of diabetes and human nerve biopsy samples. Nonspecific Schwann cell changes include glycogen inclusions, lysosomal inclusions, accumulation of lipid droplets in the cytoplasm, cytoplasmic expansion, and an increased number of plasmalemmal vesicles[3,31]. The Schwann cell basal lamina also reduplicates and thickens[3,31]. In one study in a rat model of type 1 diabetes, levels of heparan sulfate in the basement membranes of Schwann cells in the dorsal root ganglia gradually decreased with increasing disease duration, with a reciprocal increase in deposited laminin. The authors of that study speculated that the early increase in heparan sulfate can exacerbate propagation of pain, whereas in advanced stages of the disease, basement membrane composition is maintained by overproduction of laminin in the Schwann cells, thereby blocking pain transmission and thermal neuronal stimuli; this hypothesis helps to explain the bimodal pain profile in diabetic neuropathy[32].

Polyol pathway flux and aldose reductase. Over the past 50 years, circumstantial[33] and experimental[34] evidence has established hyperglycaemia-driven increases in flux through the polyol pathway as the best-understood pathogenic mechanism of diabetic neuropathy. Numerous preclinical studies have demonstrated that inhibition of aldose reductase (the first enzyme in the polyol pathway) prevents almost all manifestations of neuropathy. Studies in which expression of enzymes involved in the polyol pathway has been manipulated have placed a particular focus on the metabolic consequences of excessive metabolism of glucose to sorbitol owing to high activity of aldose reductase[35–38].

On the basis of the involvement of the polyol pathway, the perception of diabetic neuropathy as a microvascular complication of diabetes has been underpinned by immunocytochemical studies that showed localization of aldose reductase to the endothelial cells of epineurial and dorsal root ganglion blood vessels and to perivascular sympathetic axons[39–41]. Indeed, recent data implicates aldose reductase activity in the production of endothelial proinflammatory and prothrombotic signals[42], a pathogenic cascade that is currently receiving close attention in the context of diabetic neuropathy[42] and is discussed in detail below (see the section 'Microvascular changes in Schwann cells'). However, within the endoneurium, aldose reductase is restricted to the myelinating Schwann cells of somatic nerves and to satellite glial cells in the dorsal root ganglia[39–41] (BOX 3). This localization implies that aldose reductase dysfunction does not only alter vascular function, although this observation has led to something of a conundrum with regard to the pathogenic role of the enzyme: demyelination can be a feature of human diabetic neuropathy[28,43], but diabetic rodents rarely exhibit marked Schwann cell pathology

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Box 1 | Diabetic neuropathy: presentation and treatment

Diabetic neuropathy classically presents as a sensory neuropathy that results from damage to both large and small fibres, which can cause negative symptoms, such as loss of sensation to touch, vibration, pinprick, hot and cold[177,178], and positive symptoms, such as paradoxical pain and hypersensitivity[179–183]. Negative and positive symptoms are both most pronounced distally, with a characteristic stocking–glove pattern (see the figure). The pathology is also time-dependent: early small fibre symptoms are followed by impairment of larger sensory afferent fibres and motor fibres, which causes muscle weakness and, ultimately, paralysis. The most common form of diabetic neuropathy is a distal symmetrical polyneuropathy, but diabetes can affect single nerves (mononeuropathy), multiple individual nerves (mononeuritis multiplex), a nerve plexus (plexopathy) or nerve roots (radiculopathy)[184–186]. The autonomic nervous system can also be affected, leading to a diabetic autonomic neuropathy that is characterized by multiple organ dysfunction.

A population-based study has indicated that neuropathy develops in as many as 50% of all patients with type 1 or type 2 diabetes[187]. Neuropathic pain develops in >30% of these patients, meaning that approximately one in six patients with diabetes develops a painful neuropathy[188–190]. Several drugs are approved for treatment of neuropathic pain in diabetes, but these drugs do not address the underlying pathogenic mechanisms. The only accepted approach to the prevention or slowing of progression in diabetic neuropathy is strict maintenance of euglycaemia, but clear evidence for the efficacy of this approach has only been documented in type 1 diabetes, and not in the more common type 2 diabetes[1,2].

Polyneuropathy   Mononeuropathies

Plexopathy   Autonomic neuropathy

Usual presentation of diabetic neuropathy   Other presentations of diabetic neuropathy

Box 2 | Sensory symptoms of diabetic neuropathy

Nerve fibre damage can cause positive and negative symptoms, which can coexist.

Positive symptoms
- Spontaneous pain
- Allodynia
- Hyperalgesia
- Dysaesthesia
- Paraesthesia

Negative symptoms
- Hypoaesthesia
- Anaesthesia
- Hypoalgesia
- Analgesia

or demyelination in peripheral nerves until long after nerve dysfunction is detected[44,45], unless neuropathy is accompanied by other stressors, such as hypertension[46]. Nevertheless, many rodent models of diabetes exhibit indicators of Schwann cell dysfunction, including reduced expression of myelin-associated proteins[47] and Schwann cell-derived trophic factors, such as CNTF[19] and desert hedgehog[20,48]. These perturbations have the potential to affect both neuronal[49] and vascular[48] function, and changes in CNTF expression are prevented by aldose reductase inhibition[50]. In addition, studies in cultured Schwann cells have suggested that increased flux through the polyol pathway drives Schwann cells towards an immature phenotype[51]. Furthermore, galactose intoxication, which increases polyol pathway activity[52], can produce Schwann cell pathology that parallels the pathology seen in human diabetic neuropathy[29,53], and can be prevented by inhibition of aldose reductase. This observation illustrates the cytotoxic capacity of increased flux through the aldose reductase component of the polyol pathway, and the fact that extreme stress is required to damage Schwann cells but does not necessarily evoke demyelination. Hyperglycaemia-induced increases in polyol pathway flux in Schwann cells might, therefore, be a primary pathogenic mechanism that impairs their structural, metabolic and trophic support of axons and blood vessels before (or without) precipitating frank demyelination (FIG. 2). Despite being highly controversial as a model for diabetic neuropathy, galactose intoxication elicits extreme osmotic forces and oedema, thereby leading to compression of the microvasculature and highlighting the importance of endoneurial hypoxia[54].

Aldose reductase inhibitors (ARIs) are highly effective in animal models of diabetic neuropathy[55,56], but their clinical development as a therapy in humans has largely stalled as a result of multiple inconclusive or negative studies[57]. Poor trial design and choices of end points, and limited drug potency have all been suggested as contributing factors in this translational failure[58]. Nevertheless, the regulatory approval of the ARI epalrestat in Japan remains the only example of a drug being licensed to treat diabetic neuropathy, and a recent trial

of an ARI in people with mild to moderate neuropathy has re-emphasized earlier findings[59] that this therapeutic approach can mitigate against some manifestations of diabetic neuropathy[60]. In the section 'Microvascular changes and Schwann cell function' below, we discuss polyol pathway activity and ARIs from the perspective of substrate availability for nerve energy metabolism.

Oxidative stress and mitochondrial dysfunction. A contribution of oxidative stress and mitochondrial disorders in Schwann cells to neuronal dysfunction during diabetes is becoming increasingly evident. Chronic hyperglycaemia is widely accepted as a trigger of excessive production of reactive oxygen species (ROS) in all cells, as is the fact that this toxic process is exacerbated by a concomitant reduction in endogenous antioxidant defences[61]. For example, genetic variations and polymorphisms in endogenous antioxidant enzymes have been associated with an increased susceptibility to diabetic neuropathy[62]. Potential sources of ROS in peripheral nerves include the polyol pathway, the mitochondrial electron transport chain, RAGE (receptor for advanced glycosylation end products) signalling, and NADPH oxidases and nitric oxide synthases[63–65]. Schwann cells are increasingly being recognized as an important site of ROS production, which, in this context, affects Schwann cell function and their interactions with other cell types within the nerve trunk.

Several lines of evidence indicate that oxidative stress in Schwann cells contributes to nerve damage in diabetic neuropathy. Reports that ARI treatment prevents oxidative damage to lipids and DNA in nerves of rats with diabetes[66,67] indicate that increased flux through the polyol pathway contributes to oxidative stress, and implicates Schwann cells in causing this oxidative stress, as they express aldose reductase. Moreover, increased flux through the polyol pathway depletes cytosolic NADPH and subsequently reduces levels of glutathione (an important antioxidant), leaving neurons and Schwann cells vulnerable to toxic oxygen free radicals and peroxides[61]. Altered RAGE signalling in Schwann cells is another proposed mechanism of hyperglycaemia-induced free radical-mediated nerve damage. Excess formation of advanced glycation end-products (AGE) as a result of excess glucose activates RAGE and precipitates ROS formation[68] (FIG. 2). Sural nerve biopsy samples from patients with diabetes have revealed overexpression of RAGE in Schwann cells[69], and RAGE signalling has been implicated in several complications of diabetes[70]. Furthermore, AGE-induced modifications to key proteins (such as collagen), lipids and nucleic acids have the potential to alter the structure and function of Schwann cells, with detrimental effects on the axons they ensheath, leading to potentiation of diabetic neuropathy[71]. Finally, Schwann cells have been identified as the focus of injury induced by free radicals and oxidants and mediated by activation of poly(ADP-ribose) polymerase, a downstream effector of oxidative and nitrosative stress that also contributes to formation of superoxide anion radicals and peroxynitrite in diabetic peripheral nerves[72]. Hyperglycaemia can increase production of peroxynitrite in all nerve cells, and
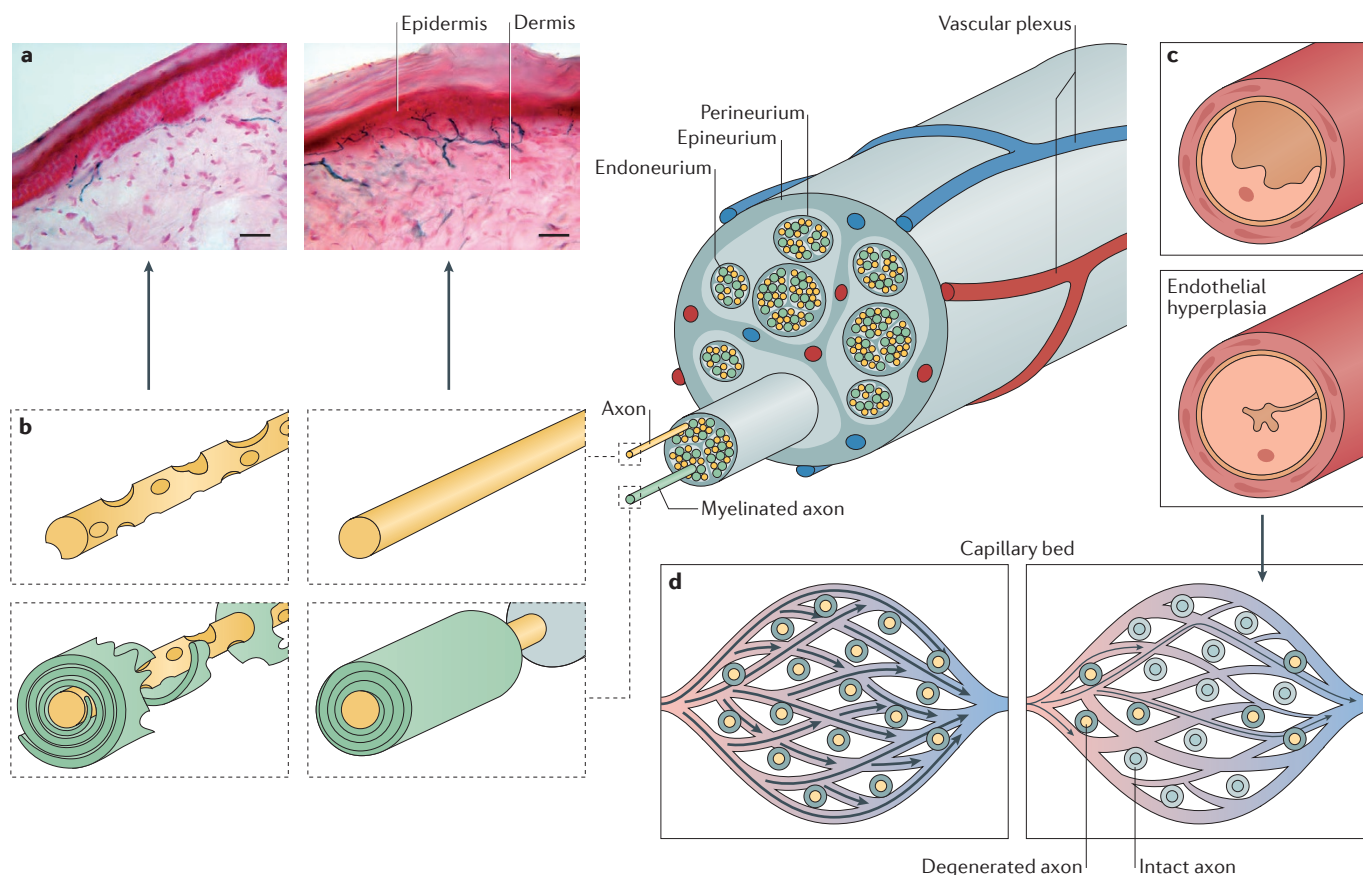
Figure 1 | **Pathogenesis of diabetic neuropathy.** Diabetic neuropathy has a complex pathogenesis involving interaction of axonopathy, schwannopathy and microvasculopathy. The figure shows the anatomical organization of myelinated and unmyelinated axons within nerve fascicles. Their nutrient supply is secured via endoneurial capillaries which, together with the perineurial membrane, form the blood–nerve barrier. **a** | Human skin biopsy samples immunostained with PGP9.5 to show progression of peripheral nerves from the dermis into the epidermis, where they exist as small unmyelinated C-fibres (scale bar 40 μm). Left panel shows loss of fibres in a patient with diabetic neuropathy and right panel shows fibres in a healthy individual. **b** | Changes in axons and myelin in diabetic neuropathy, showing degeneration of Schwann cells and nerve fibres, culminating in nerve and intraepidermal fibre loss. **c** | Endoneurial capillaries from patients with diabetes. Top panel shows a capillary from a patient without diabetic neuropathy, and bottom panel shows a capillary from a patient with neuropathy, in which endothelial cell hyperplasia and basement membrane thickening have reduced the size of the capillary lumen. **d** | Narrowing of individual capillaries might not prevent blood from passing through the endoneurial capillary bed per se, but the resulting increase in velocity of blood through endoneurial functional shunts or epineurial arteriovenous shunts prevents efficient oxygen extraction, causing hypoxia. Panel **a** courtesy of Dr Páll Karlsson, Danish Pain Research Center, Department of Clinical Medicine, Aarhus University, Denmark.

preoxynitrite in turn nitrates tyrosine residues to produce 3-nitrotyrosine, a fingerprint of nitrosative stress; levels of 3-nitrotyrosine and inducible nitric oxide synthase have been shown to be increased in Schwann cells[73–75].

Impaired mitochondrial function has been implicated in many complications of diabetes. Initial studies in cultured endothelial cells indicated that hyperglycaemia increased flux through the electron transport chain, leading to ROS formation[76], but these observations have been superseded by descriptions of impaired mitochondrial activity and bioenergetics in cultures of adult sensory neurons and in animal models of chronic diabetic neuropathy[77] and nephropathy[78]. Mitochondria do not seem to be a source of excess ROS in peripheral axons[79], but several findings have shifted the focus to Schwann cell mitochondria and their impact on axonal

function. Exposure of Schwann cells to high glucose levels *in vitro* causes oxidative stress that is accompanied by overactivation of caspase-9 and apoptosis regulator BAX, and decreased levels of apoptosis regulator Bcl-2 (REF. 80), changes that indicate mitochondrial internal stress. In an important study published in 2011, targeted disruption of Schwann cell mitochondria in a transgenic mouse model critically affected neuronal survival and produced a peripheral neuropathy resembling that caused by diabetes[81]. Subsequent work demonstrated that mitochondrial deficits activated a maladaptive stress response mediated by haem-regulated inhibitor kinase, and that acylcarnitines that were released from Schwann cells on mitochondrial disruption induced axonal degeneration[82], emphasizing the importance of Schwann cell mitochondrial homeostasis in axon–glia interactions[83].

REVIEWS

Box 3 | Satellite glial cells

Diabetic neuropathy is generally considered to be a length-dependent phenomenon, meaning that the longest nerve fibres are most at risk, with the most severe damage occurring in the terminal regions of long axons, but with other regions of the neuraxis also affected. For example, in the dorsal root ganglia, neuronal cell bodies are individually surrounded by satellite glial cells that, together with a thin layer of connective tissue, form an envelope around the neurons[16], and evidence over the past decade has established that satellite cells are important components of PNS functionality and post-injury responses, such as pain and nerve regeneration[191,192]. Like Schwann cells, satellite cells express aldose reductase[39], and the effect of diabetes on this enzyme leads to altered expression of several proteins in these cells[193,194]. In rodent models of both type 1 and type 2 diabetes, expression of glial fibrillary acidic protein is increased in satellite glial cells that surround sensory neurons; this increased expression has been proposed to be a reflection of satellite cell activation, which has been implicated in the genesis of neuropathic pain[195,196]. However, the biology and function of these cells during diabetes is essentially unexplored, and warrants further investigation.

Hyperglycaemia has also been shown to drive remodelling of the Schwann cell mitochondrial proteome that leads to increased expression of the α and β subunits of ATP synthase, and to cause suboptimal respiratory capacity by increasing the overall rate of oxygen consumption, suggesting that high glucose levels contribute to mitochondrial dysfunction and decrease the efficiency of oxidative phosphorylation in Schwann cells[84]. In a study published in 2016, mitochondrial damage, with upregulation of multiple subunits of complexes I, III, IV, V and of mitochondrial Rho GTPase 1, was described in a rodent model of type 1 diabetes[85]. These changes were observed only in peripheral nerves and not in the sensory or trigeminal ganglia, suggesting that they reflect disrupted Schwann cell metabolism.

Lipid metabolism and inflammation. Diabetes is associated with alterations in lipid levels in the circulation and nerves, and Schwann cell dysfunction has been linked to altered lipid metabolism and downstream inflammatory consequences. Accumulation of triglycerides, cholesterol and free fatty acids in blood plasma in diabetes seems to drive lipid-mediated neuropathology via mechanisms that are incompletely understood but might involve oxidative and inflammatory pathways in Schwann cells[86]. In rodents, a high-fat diet causes accumulation of oxidized lipids and activation of lipoxygenases in peripheral nerves, suggestive of a prediabetic condition[24]. Moreover, accumulation of oxidized LDLs in peripheral nerves promotes oxidative stress that has been associated with a low nerve conduction velocity and sensory deficits[87]. Cell culture studies suggest that Schwann cells are susceptible to lipotoxicity from free fatty acids, possibly as a result of lysosomal dysfunction[88,89]. Furthermore, evidence from a mouse model of peripheral neuropathy secondary to Schwann cell mitochondrial dysfunction suggests that disruption of Schwann cell mitochondria causes lipid metabolism to shift away from fatty acid synthesis toward lipid oxidation, resulting in early depletion of myelin lipid components and accumulation of acylcarnitine lipid intermediates, leading to axonal degeneration and neuropathy[83]. Perturbation of lipid metabolism has also been found in the peripheral nerves of mice with

streptozotocin (STZ)-induced diabetes; these perturbations include a reduction in short-chain triacylglycerols, changes in major structural and/or membrane lipids, and diminished levels of palmitic, stearic and eicosanoic fatty acids[85,90]. Exposure of human Schwann cells to high extracellular glucose levels reduced their synthesis of phospholipids, and this effect was counteracted by an ARI, implicating high rates of glucose metabolism via the polyol pathway in dysregulation of Schwann cell lipid metabolism[91].

In diabetes, lipoproteins circulating in the plasma are exposed to an oxidizing environment and can be modified by consequent glycation. Schwann cells express several Toll-like receptors (TLRs) and RAGE[92,93], to which the modified LDLs can bind and drive an inflammatory response[94]. After an injury, such as those triggered by hyperglycaemia or modified LDLs, intracellular cascades can be activated in Schwann cells, including nuclear translocation of NF-κB and consequent expression of various cytokines and chemokines, similar to the scenario after traumatic nerve damage. These events contribute to Wallerian degeneration and emphasize the potential involvement of Schwann cells in the subclinical inflammation that is observed in patients with type 2 diabetes and is detectable as increased circulating levels of C-reactive protein and IL-6, which are consistently associated with polyneuropathy[95]. Furthermore, via production of cytokines and chemokines, Schwann cells might contribute to immune cell recruitment[4,96] and nerve inflammation in diabetes.

Macrophage infiltration and proliferation have been observed in nerves from mouse models of diabetes[97,98], and upregulation of proinflammatory genes during activation of inflammatory pathways in the PNS[99–101] further implicates involvement of RAGE-mediated Schwann cell involvement in the inflammatory response. One upregulated gene encodes the S100 calcium binding protein A8/A9 (REF. 101), which is overexpressed in patients with type 2 diabetes[102]. This protein has been shown to stimulate a local inflammatory response via interaction with RAGE[103], leading to activation of mitogen-activated protein kinases (MAPKs), NF-κB and apoptosis[104,105]. Similarly, MAPKs (such as p38 MAPK, MAPK1, MAPK3, MAPK8 and MAPK10) are activated by high glucose levels in human Schwann cell cultures[106]; p38 is of particular interest, as it is a negative regulator of Schwann cell differentiation and myelination[107].

Some evidence also suggests that Schwann cells and T cells have reciprocal effects on each other in diabetes. A study published in 2013 suggested that recruitment of T cells into the nerves of patients with diabetic neuropathy is induced by production of the chemokines CXCL-9, CXCL-10 and CXCL-11 by glucose-stimulated Schwann cells[108]. Conversely, infiltrating CD8+ T cells have been shown to mediate Schwann cell cytotoxicity by activating apoptosis, thus contributing to progression of neuropathy[108]. Furthermore, as T cells found in diabetic nerves express high levels of CXCR3, the authors of this study suggest that this receptor is important for migration of CD8+ T cells into the peripheral nerve and, thus, offer a novel potential target for therapy.
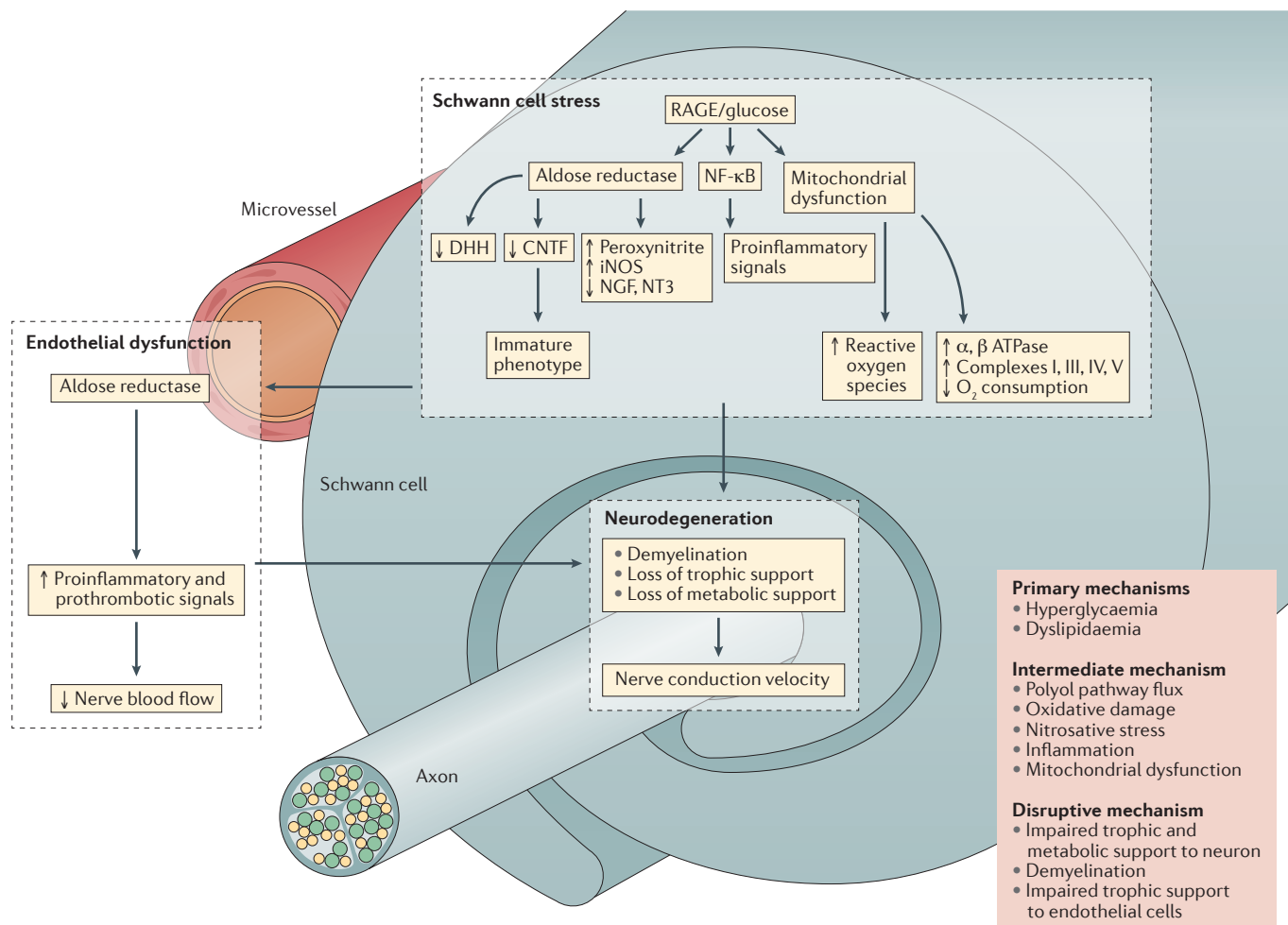
Figure 2 | **Hyperglycaemia-driven Schwann cell stress and neurodegeneration.** Hyperglycaemia and dyslipidaemia ultimately lead to reduction of neuronal support from Schwann cells and microvessels. In Schwann cells, RAGE (receptor for advanced glycosylation end products) signalling leads to increased glucose metabolism by aldose reductase, which generates local oxidative damage, causes inflammation and drives cells to an immature phenotype. It also affects mitochondrial function, which increases oxygen consumption, and reduces production of desert hedgehog (DHH), which affects endothelial cell function. Endothelial cells also express aldose reductase, and increased polyol pathway flux activates proinflammatory and prothrombotic pathways that reduce nerve blood flow. Disruption of neuronal support by Schwann cells and the vascular system contributes to neuropathy, in conjunction with the direct effects of diabetes on neurons themselves.

Additional factors in diabetes that promote immune cell infiltration of nerves and activation of Schwann cells are accumulation of AGE and the modification of myelin antigenicity via glycosylation of myelin proteins. Activation of Schwann cells in turn promotes their secretion of proinflammatory cytokines, thereby producing a positive feedback mechanism that perpetuates injury[109,110]. Inflammatory cytokines such as IL-1, tumour necrosis factor (TNF) and IL-17 can be produced by many cell types, including Schwann cells, and these factors sensitize Aδ-fibres and C-fibres, triggering neuropathic pain[111–113]. Indeed, blocking the TNF signalling pathway with a recombinant human TNF receptor–antibody fusion protein has been shown to be beneficial in animal models of diabetic neuropathy, leading to recovery of nerve conduction velocity and increased expression of myelin basic protein[109].

Schwann cells in the peripheral nerve express a variety of TLRs, including TLR4 (REF. 92), which might have a role in the pathogenesis of metabolic-induced retinal impairment and nephropathy[114,115]. Whether TLR4 in Schwann cells has a role in diabetic neuropathy, however, remains to be elucidated. Interestingly, an interrelationship between TLR4 and the p75NTR receptor has been demonstrated in immune cells. In lipopolysaccharide-stimulated dendritic cells, expression of p75NTR was increased via a TLR4-dependent mechanism. In TLR4-activated dendritic cells, NGF promotes secretion of cytokines, but silencing of p75NTR with small interfering RNA blocks this process[116]. These observations suggest that the increased expression of p75NTR in myelin sheaths around fibres that are susceptible to axonal degeneration in diabetic neuropathy[117] might be related to TLR4 signalling.

Microvascular changes and Schwann cells. Microvascular damage in diabetic neuropathy is likely to affect Schwann cell function by promoting inflammatory cascades such as those described above, and by disrupting their access to oxygen and glucose. The morphology and function of microvessels is altered in nerve biopsy samples from patients with diabetic neuropathy[118,119] (FIG. 1); these microvascular changes are particularly severe in endoneurial capillaries, and include capillary basement membrane thickening, loss of pericyte coverage, and endothelial hyperplasia[120]. Furthermore, microangiopathy can precede development of diabetic neuropathy[120,121], and the degree of microvascular changes correlates with the clinical severity of diabetic neuropathy[119,120].

Microvascular changes have been thought to elicit nerve damage by limiting nerve blood supply (ischaemia), but evidence now shows that endoneurial hypoxia can be caused by inefficient oxygen extraction alone via capillary dysfunction[122]. Tissue hypoxia, in turn, can activate the same cellular signalling pathways that inflammation activates[123]. These pathways can be activated in all cell types that are subjected to hypoxia, including Schwann cells. Low tissue oxygen tension upregulates the expression of hypoxia-inducible factor 1-α (HIF-1α) and NF-κB[123], a proinflammatory transcription factor that is present at high levels in peripheral nerves and dorsal root ganglia in experimental diabetic neuropathy[124]. HIF-1α causes upregulation of NADPH oxidase 2 levels[125], a major source of ROS in vessel walls[126]. NADPH-derived superoxides react with the vasodilator nitric oxide to produce peroxynitrite[127], which causes severe nitrosative tissue damage and inactivates tissue plasminogen activator (tPA)[128], creating a highly prothrombotic environment. Importantly, elevated tPA levels increase the formation of BDNF from proBDNF, and proBDNF is associated with apoptosis[129]; this observation is consistent with reports that the levels of BDNF in distal muscles negatively correlate with the severity of neuropathy in diabetic patients[130]. These mechanisms suggest that microvascular changes are likely to create a hypoxic endoneurial environment that exacerbates oxidative stress and inflammation, and leads to a loss of trophic support for Schwann cells and neurons.

Capillary dysfunction affects the uptake of glucose and oxygen into tissue[131], and breakdown of glucose under hypoxic conditions generates far less ATP than does oxidative phosphorylation. When access to oxygen is impaired, therefore, Schwann cells and axons are likely to depend on alternative substrates or metabolic pathways to cover their metabolic needs. Glucose breakdown via the polyol pathway — despite its detrimental long-term effects — is an efficient source of ATP in these conditions. As a result, aldose reductase inhibitors could be detrimental in nerve fibres with severely affected microvessels, as they will limit this alternative source of energy[122].

The evidence discussed indicates that in diabetic neuropathy, capillary dysfunction disrupts the function of Schwann cells and axons — and vice versa — by altering the endoneurial microenvironment. In light of this hypothesis, creation of animal models of diabetes that include microvascular damage[46], or use of cell culture systems that mimic the diabetic endoneurial microenvironment, might increase the success of translational research.

Nodal disruption and ion channels. The tight association between Schwann cells and axons means that disturbances in Schwann cell function are likely to influence the excitability of large and small fibres and the propagation of action potentials. The resting membrane potential, depolarization and repolarization of axons are mediated by a series of voltage-gated sodium (Na_V) channels, potassium (K_V) channels, calcium channels and various ligand-gated channels (for example, transient receptor potential channels)[132]. In myelinated axons, the ion channels are clustered at the nodes of Ranvier to facilitate saltatory conduction, whereas in nonmyelinated axons, the channels are distributed diffusely and almost homogeneously along the axon[133,134]. Schwann cells are not excitable themselves, but do express ion channels that are involved in forming the nodal and paranodal regions where the neuronal ion channels are clustered, giving them the potential to influence axonal excitability.

The effects of Schwann cell dysfunction on neuronal ion channel function are not known and may be indirect. Impaired mitochondrial function and oxidative stress in Schwann cells (see above) could lead to changes in the distribution of Na_V and K_V channels at the nodes of Ranvier and the paranodal and juxtaparanodal regions, contributing to axonal damage. Mitochondrial dysfunction may also be a consequence of increased intracellular calcium[135]. A detailed discussion of the role of ion channels in diabetic neuropathy is beyond the scope of this Review, but abnormalities such as paranodal swelling and axon–glial disjunction have been described in diabetic neuropathy in humans, and were proposed as a cause of nodal ion channel redistribution[136]. Na_V channel expression in diabetes can be modified by various mechanisms, including neurotrophic factors[137] and methylglyoxal, a metabolite that is upregulated as a result of hyperglycaemia, and has been shown to change the distribution of Na_V1.8 (REF. 138). Together with changes in ion channel function and energy metabolism in the axon itself, such as impaired Na^+–K^+ ATPase activity in nodal regions of the axon, Schwann cell dysfunction could — owing to the normal function of these cells in saltatory conduction — contribute to the reduction in action potential propagation seen in diabetic neuropathy. In a study published in 2016, mathematical modelling was used to provide evidence that abnormalities in the excitability of sensory and motor axons in patients with type 1 diabetes were the result of reduced nodal Na^+ currents, reduced nodal and paranodal K^+ conductance, and Na^+–K^+ ATPase dysfunction[139]. Evidence also suggests that altered expression of Na_V channels, specifically Na_V1.7, Na_V1.8 and Na_V1.9, contributes to the neuronal hyperexcitability and allodynia seen in experimental and clinical diabetic neuropathy[52–55].

Capillary dysfunction
A state of dysregulated capillary blood flow patterns, in which oxygen, glucose and other diffusible molecules cannot be extracted efficiently by the tissue; uptake of these molecules can become critically impaired, although reduced tissue blood supply is not obvious.

Impact of schwannopathy on neurons

As discussed above, hyperglycaemia causes chronic Schwann cell dysfunction, and further evidence suggests that consequent transcriptional changes lead to persistent increases in glycolysis, ROS formation, cellular NADH depletion and altered DNA methylation that contribute to diabetic neuropathy[140]. This Schwann cell dysfunction has direct effects on neuronal function as a result of myelin disruption, demyelination, changes in axonal conduction, and impaired regeneration.

Myelination of nerve fibres by Schwann cells is essential for rapid saltatory conduction, and insults to the myelin sheath can lead to motor and sensory nerve dysfunction. Evidence indicates that the effects of hyperglycaemia on Schwann cells lead to disruption of the myelin sheath, which contributes to diabetic neuropathy. In one study, hyperglycaemia induced a progressive decrease of caveolin-1 in Schwann cells in culture and in STZ-induced type 1 diabetes in mice[141]. Caveolin-1 is a structural protein found in specialized sphingolipid–cholesterol microdomains called caveolae, which are thought to be important for Schwann cell physiology because cholesterol comprises ~25% of the total lipid content of myelin[142]. In this study, the reduction of caveolin-1 prolonged the kinetics of ErbB2 phosphorylation and enhanced the mitogenic response of Schwann cells to neuregulin1-β1; subsequent studies have demonstrated that changes in caveolin-1 expression in Schwann cells co-cultured with neurons leads to neuregulin-induced demyelination, and that interactions between caveolin-1 and ErbB2 signalling contribute to peripheral neuropathy in rodents with diabetes[143,144].

Studies have suggested that expression of the major myelin components myelin glycoprotein P0, myelin-associated glycoprotein and early growth response protein 2 in Schwann cells is reduced in diabetic neuropathy, and that these deficits might contribute to disorganization and loss of myelin[145–147]. In the case of P0, *in vitro* studies using glucose-stimulated primary Schwann cells showed that the reduced expression was apparently dependent on upregulated MAPK signalling, and was partially rescued by stimulation of Schwann cells with 10 nM insulin[147,148]. The importance of the MAPK pathway for nerve regeneration has been well documented[149], but evidence indicates that prolonged MAPK signalling also maintains Schwann cells in a dedifferentiated state, as myelinating Schwann cells in transgenic mice did not respond to pro-differentiation signals from axons while MAPK activation was maintained[150]. Studies have also shown that the MAPK p38 is similarly upregulated in diabetes and, together with increased flux in the polyol pathway, contributes to slowing of nerve conduction and generation of pain[35,151]. These observations emphasize the need for an appropriate signalling balance to ensure optimal cell proliferation and nerve fibre maintenance.

Deficits in large fibre conduction velocity are generally considered to be among the earliest functional markers of glucose neurotoxicity, as they typically present before decreases in axonal diameter or structural disruption of the myelin in rodents[152]. In the absence of structural pathology, impaired nodal biophysical properties, such as ion flux and current densities, that might involve Schwann cell dysfunction have been implicated[152,153]. A study of rats with STZ-induced diabetes has shown that reduced motor conduction velocity is associated with upregulation of the Rho–Rho-kinase signalling pathway and consequent misexpression and aberrant distribution of the myelin sheath adhesion molecules p120 catenin and epithelial cadherin, which are crucial for normal myelin formation to allow rapid propagation of action potentials[154].

Some evidence suggests that impaired Schwann cell function also affects peripheral axonal regeneration after injury has occurred. After traumatic injury to a nerve, reciprocal signalling normally occurs between the axon and glia, and this signalling is necessary for the reformation of the myelin–axon unit during regeneration. Compromised Schwann cell production of the neurotrophic factors NGF and NT-3 that are essential to nerve structure and function is linked to the loss of glia–axon associations and decreased neurite outgrowth, suggesting that reduced production of these factors by Schwann cells has an important role in impaired axonal regeneration[21,155,156]. Indeed, after experimental axotomy, Schwann cell growth was robust and extended into the superficial dermis in people without diabetic neuropathy, whereas people with injury as a result of diabetes exhibited atrophic Schwann tubes that were limited to the mid-dermis, and limited regenerative axonal sprouting[157].

Schwann cell proliferation after physical nerve injury can be studied in culture to investigate discrete pathogenic mechanisms. In this context, exposure to high levels of glucose reduces the number of Schwann cells, and cell loss occurs by apoptosis[158]. The remaining cells look thin and short, and fail to adequately extend processes[75,147], and chromatin becomes increasingly condensed with shrinkage of the Schwann cell cytosol[158,159]. When co-cultured with neurons, hyperglycaemia-stressed Schwann cells are dysfunctional[143], and a study from 2014 suggests that the stress causes them to produce VEGF, which subsequently impairs neurite outgrowth from co-cultured sensory neurons[160]. These observations indicate that impaired nerve regeneration after injury in diabetes is, at least in part, secondary to disruption of Schwann cell metabolism.

Several therapeutic strategies are being pursued to manipulate nerve plasticity and neurite outgrowth in diabetes, with the aim of encouraging axonal repair[161–163]. A study published in 2016 identified p75NTR as a novel therapeutic target in type 2 diabetes, on the basis of results using an adipocyte-specific conditional null mouse model shown to be resilient to high-fat-diet-induced obesity and insulin resistance[164]. p75NTR signalling increased adipocyte lipolysis via cyclic AMP and the protein kinase A pathway, thereby regulating energy balance[164]. The findings highlight the importance of this neurotrophin receptor for obesity and the metabolic syndrome. In patients with diabetes, high p75NTR expression has been observed in myelin sheaths around fibres

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that are susceptible to axonal degeneration, indicating a role for this receptor in axonopathy[117]. Furthermore, results obtained from rodent models of peripheral nerve injury indicate that p75NTR is upregulated in neurons and Schwann cells, and can induce growth cone collapse, dependent on its interactions with myelin-associated proteins, or regrowth, by forming neurotrophin chemoattractant gradients[165,166]. *In vitro* studies have demonstrated that interactions between p75NTR and NGF inhibit Schwann cell apoptosis[159]. These observations suggest that neuronal or glial synthesis of p75NTR might have an important role in the progression of diabetic neuropathy, but further investigation is required to determine the precise role of p75NTR.

Towards a new understanding

The fact that primary axonopathy is a major pathological feature of diabetic neuropathy is inescapable. Indeed, diabetic neuropathy is usually identified by clinical and neurophysiological examinations to detect indications of axonopathy, namely, reduced conduction velocity and amplitude in large fibres, and abnormal thresholds to sensory stimuli. The focus on axonopathy is highlighted by a growing interest in the detection of early degeneration of small sensory fibres by measuring intraepidermal nerve fibre density in skin biopsy samples or corneal nerve density by confocal microscopy[167] (FIG. 1). Nevertheless, we have presented a case that the direct effects of diabetes on Schwann cells can in turn affect the vasculature and axons, and distinguishing the precise contribution of Schwann cell dysfunction to that of axonopathy could provide valuable new insights.

One appealing approach to separating the roles of axonopathy and schwannopathy in diabetic neuropathy is to identify differences in the primary pathogenic mechanisms. *In vitro*, Schwann cells are damaged by hyperglycaemia[158] and, as the sole site of the polyol pathway in the endoneurium, are a source of glucotoxicity. Glia require relatively low energy under normal conditions; they derive sufficient ATP from glycolysis and supply excess pyruvate to axons[168]. Schwann cells might not, therefore, be well adapted to metabolize excess glucose. By contrast, neurons consume high amounts of energy and use the full capacity of oxidative phosphorylation, such that primary sensory neurons in culture thrive in conditions that mimic physiological hyperglycaemia. Consequently, hyperglycaemia is perhaps less of a direct threat to axons, suggesting that axonopathy in diabetes reflects other primary pathogenic insults, such as loss of trophic support[169].

The hypothesis that axonopathy in diabetic neuropathy results from a loss of trophic support is reinforced by the effects of insulin. Adult sensory neurons express insulin receptors, and addition of insulin or insulin-like growth factor 1 (IGF-1) to cultured adult sensory neurons drives a dose-dependent increase in neurite outgrowth[170]. Similarly, direct local injections of insulin into the skin at concentrations that do not modulate systemic glycaemia promote axonal growth in the epidermis[171]. Moreover, in animal models of type 2 diabetes, sensory neurons develop impaired insulin signalling[172]. Thus, it is becoming increasingly recognized that diabetes-associated loss of trophic support from insulin, and perhaps IGF-1 and C-peptide, represents a primary insult to neurons in both type 1 and type 2 diabetes[173].

Differences between the effects of diabetes on mitochondria in Schwann cells and neurons might also provide insight into the pathogenic mechanisms. Evidence indicates that in neurons, diabetes decreases the expression of mitochondrial proteins[174], the inner membrane potential[175] and the spare respiratory capacity[77] of otherwise functional mitochondria as a result of disruption to the AMPK–PGC1 pathway, which serves as a nutrient sensor and mitochondrial regulator[176]. These effects leave the neuron energetically viable but restricted in its capacity to respond to the increased energy demand of regrowth after physical injury, and to survive disease-mediated stress. This suppression of neuronal mitochondrial function in diabetes is in stark contrast to the impact on Schwann cell mitochondria, in which hyperglycaemia increases expression of proteins involved in the tricarboxylic acid cycle and oxidative phosphorylation, and increases overall oxygen consumption while decreasing coupled respiration[84]. The fundamental differences between Schwann cells and neurons in normal glucose metabolism[168] and the handling of hyperglycaemia might underlie the complex pathogenesis of diabetic neuropathy.

Conclusions

Primary Schwann cell damage is a largely forgotten aspect of diabetic neuropathy, partly as a result of the major historical focus on microvascular disease and axonopathy. Studies showing that injury to Schwann cells affects both the vasculature and axons have reignited interest in schwannopathy as a primary cause of diabetic neuropathy. In light of these findings, now is the time to rethink therapeutic strategies that account for both primary axonopathy and primary schwannopathy in the setting of ongoing stress that arises from vascular hypoxia, hyperglycaemia, impaired trophic support, dyslipidaemia, mitochondrial dysfunction, oxidative stress, and activation of inflammatory pathways.

An increased mechanistic understanding of the Schwann cell response to diabetes could unravel novel molecular mechanisms that can ultimately be targeted to ameliorate disease. A particularly pertinent approach might be to expand on the recent development of conditional knockout mouse models to understand the specific role of genes expressed by Schwann cells in the pathophysiology of diabetic neuropathy, and the relative contributions of axonopathy and schwannopathy. Our current understanding that a diverse range of signalling pathways are disrupted in diabetic neuropathy presents substantial challenges for drug development. Nevertheless, we believe that innovative drug delivery systems, improvements in clinical trial design and the generation of new biomarkers will combine to generate a multimodal approach that ultimately proves useful for treating diabetic neuropathy.

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The authors declare no competing interests.

FURTHER INFORMATION
International Diabetes Federation Diabetes Atlas:
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