Antibodies to Neuronal Structures

Innocent bystanders or neurotoxins?

AARON I. VINIK, MD, PHD1,2
DHARSHAN ANANDACOMARASWAMY, MD1
JAGDEESH ULLAL, MD, MS1

Diabetic neuropathies are a group of clinical syndromes that affect distinct regions of the nervous system, singly or combined, and markedly affect quality of life (1) and activities of daily living and increase morbidity and mortality (2). Therapy directed at the basic pathogenesis is sorely needed. Neurologic complications occur equally in type 1 and type 2 diabetes and additionally in various forms of acquired diabetes (3). Diabetic neuropathies may be diffuse somatic and involve proximal or distal nerves, occur as focal mononeuropathies, and involve the autonomic nervous system (4,5).

The pathogenesis of diabetic neuropathies is multifactorial. Hyperglycemia causes nerve damage by inducing the activation of the polyol, protein kinase C, and hexosamine pathways and the accumulation of advanced glycation end products. Hyperglycemia also induces oxidative stress by enhancement of mitochondrial respiration, redox alteration, and uncoupling proteins, which leads to elevated superoxide anions (6,7). Oxidative stress depletes nitric oxide within the peripheral nerves and endothelium of the microvasculature by reducing endothelial nitric oxide synthase; altering nerve perfusion (8). In addition, there is deficiency of or a poor response to neurotrophic factors (9). However, there is now increasing evidence to suggest that autoimmunity has a role in the development and progression of diabetic neuropathies.

Fifty years ago, Waksman and Adams (10) suggested an autoimmune etiology of peripheral neuropathy when they injected rabbits with neuronal components to produce what they called “allergic neuritis.” To be able to implicate autoimmunity as a causative factor of neuropathy, there would have to be a clear association between the antibody and the disease; neuropathy would have to be induced by introduction or development of antibodies, and there would have to be reversal of the disease with removal or neutralization of the antibodies. Peripheral nerves are normally protected against the immune system by tight capillary endothelial junctions and the perineurium. Nerves are also a rich source of glycoproteins, lipopolysaccharides, and other lipoproteins that can potentially form active antigenic material. In the autoimmune onslaught against nerves, there is active antigenic material. In the autoimmune assault against nerves, there is first damage to the protective sheath and then to the inner components. These can be brought on by viral or bacterial infections (e.g., polio, leprosy, Lyme’s disease), neoplasms, or connective tissue disorders, and often there is strong genetic predisposition, such as HLA DR-3 and -4, in type 1 diabetes. Table 1 illustrates the association of different types of antibodies with various neuropathy syndromes.

Neurons and pancreatic β-cells are neuroendocrine derivatives and therefore share common antigens, especially in the early stages of cellular evolution. Type 1 diabetes results from an autoimmune destruction of pancreatic β-cells. There also may be a direct destruction of neurons by the same autoimmune process in diabetes. The pancreatic islets of Langerhans are surrounded by a Schwann cell sheath. These cells form a tight cellular mantle that envelops the endocrine islet tissue. Components of the peri-islet Schwann cells include GAD (11). There is an early appearance of anti-GAD65-specific T-cells in type 1 diabetes. Anti-GAD65 antibody is a strong predictive marker for the onset of type 1 diabetes (12). Presence of this antibody in patients with recent-onset type 1 diabetes is associated with worse glycemic control and worse peripheral nerve function, suggesting a common mechanism for β-cell and neuronal damage (13). Patients with high GAD65 antibodies were shown to have positive correlation with motor nerve conduction velocities, F wave latencies, thermal threshold detection, and cardiovascular autonomic function (14). However, many studies have failed to show any significant relation of GAD antibodies to the development of neuropathy. These studies concluded that GAD antibodies had no effects on residual β-cell function or diabetic neuropathy (15). There is also no association between GAD antibodies or even islet-associated protein 2/islet cell antibody 512 with autoimmunity to nervous tissue structures or cardiac autonomic functions (16). Serum collected from type 1 diabetic patients is toxic to neuroblastoma cells of the N1E-115 cell line (17). About two-thirds of the toxicity is due to autoimmune serum factors. One of the components of this serum that mediates immune destruction of neuroblastoma cells in cultures was found to be Fas-specific IgG antibodies. These antibodies bind to Fas-ligand on the surface of N1E-115 neuroblastoma cells and induce apoptosis. Serum from patients with diabetic neuropathy contains an activator of Fas-regulated apoptosis that may contribute to the pathogenesis of diabetic neuropathy (18). There is no doubt that a variety of antibodies are present in the sera of diabetic patients with neuropathy and that the sera exert apoptotic effects on neurons grown in culture, but the missing link is the relation with clinical neuropathy and the potential for reversibility with immune therapy.
<table>
<thead>
<tr>
<th>Antibodies</th>
<th>Associated syndrome/medical condition/symptoms</th>
<th>Anatomical structure/target</th>
<th>Author or related article</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscarinic ganglionic AChR antibodies</td>
<td>Orthostatic hypotension without tachycardia, cholinergic dysautonomia, abnormal blood pressure and pulse rate response to valsala maneuver, dry eyes and mouth, abnormal pupillary response, upper and lower gastrointestinal symptoms, Sicca complex, neurogenic bladder, thymoma</td>
<td>Ganglionic AChR</td>
<td>Sandroni et al. (35), Goldstein et al. (38), Klein et al. (36), Vernino et al. (44)</td>
</tr>
<tr>
<td>Neuronal nicotinic AChR antibodies</td>
<td>Autonomic neuropathy, seizures, dementia, movement disorder, carcinomas, dementia, sensory neuropathy, gastrointestinal hypomotility, dilated pupils with impaired light response, distended bladder, subacute autonomic neuropathy and related syndromes, Eaton-Lambert myasthenic syndrome</td>
<td>Neuronal nicotinic AChR</td>
<td>Lennon et al. (37), Vernino et al. (34)</td>
</tr>
<tr>
<td>Antibodies against muscle AChR</td>
<td>Myasthenia gravis</td>
<td>Muscle AChR (all subjects)</td>
<td>Vernino et al. (44)</td>
</tr>
<tr>
<td>Neuronal AChR antibodies</td>
<td>Myasthenia gravis, thymoma</td>
<td>Brain, peripheral nerves, serum and cerebrospinal fluid, neuronal ganglionic AChRs</td>
<td>Bogousslavsky et al. (46), Vernino et al. (34)</td>
</tr>
<tr>
<td>Ganglionic receptor–binding antibodies</td>
<td>Decreased salivation, idiopathic gastrointestinal dysmotility and constipation; dry skin, orthostatic intolerance, diabetic, idiopathic, or paraneoplastic autonomic neuropathy, postural tachycardia syndrome</td>
<td>Ganglionic receptors</td>
<td>Vernino et al. (44)</td>
</tr>
<tr>
<td>Antibodies to ( \delta )-type calcium channel, P/Q-type ( Ca^{2+} ) channel antibodies, ( \alpha )-type ( Ca^{2+} ) channels, anti-VGCC antibodies</td>
<td>Type 1 diabetes, Eaton-Lambert myasthenic syndrome</td>
<td>Smooth muscle ( \delta )-type calcium channel at the dihydropyridine binding site, P/Q-type ( Ca^{2+} ) channel, ( \alpha )-type ( Ca^{2+} ) channel, solubilized calcium channel–( \omega )-conotoxin complexes, VGCC, small cells of the lung</td>
<td>Jackson et al. (41), O’Suilleabhain et al. (47), Lennon et al. (48), Kaiser (49)</td>
</tr>
<tr>
<td>Anti-CV2 antibodies</td>
<td>Paraneoplastic syndrome, sensory or sensory motor neuropathies</td>
<td>Peripheral nerves</td>
<td>Antoine and Camdessanche (50)</td>
</tr>
<tr>
<td>Anti-Hu antibodies</td>
<td>Subacute sensory neuropathy, demyelinating neuropathy, rapidly developing sensory neuropathy or peripheral neuropathy, early-onset dysautonomia, symptoms of Encephalomyelitis, Eaton-Lambert myasthenic syndrome</td>
<td>Type 1 antineuronal nuclear antibody, small-cell lung cancer, thymoma</td>
<td>Antoine and Camdessanche (50), O’Suilleabhain et al. (47), Camdessanche et al. (51), Winkler et al. (52), Lucchinetti et al. (53), Kusunoki and Kanazawa (54), Dalmau and Clouston (55), Anderson et al. (56), Vernino and Lennon (57)</td>
</tr>
</tbody>
</table>
Proximal neuropathies

Perhaps the clearest link between autoimmunity and neuropathy has been the demonstration of an 11-fold increased likelihood of chronic inflammatory demyelinating polyneuropathy, multiple motor polyneuropathy, vasculitis, and monoclonal gammopathies in diabetes (19). These are proximal neuropathies presenting with pain in the buttocks and thighs, fasciculation, and weakness with inability to rise from the sitting position or when kneeling on the floor. They may be the presenting symptom in many autoimmune vasculitides and celiac disease, a multigenetic, T-cell–mediated autoimmune disorder that results from a loss of tolerance to gluten (20). In support of an autoimmune mechanism for proximal neuropathies is the salutary response to intravenous Ig and immunotherapy (21).

Somatic neuropathies

The situation with somatic neuropathies is less clear. Several different autoantibodies in human sera have been reported that can react with epitopes in neuronal cells. Prominent among them are the gangliosides, and antibodies to GD1a, GD1b, GM1, GM2, GaLNAC-GD1a, etc., are not uncommon. Other antibodies include anti-sulfatide, anti-myelin-associated glycoprotein, anti-Hu (associated with neuropathy in paraneoplastic syndromes), perinuclear anti-neutrophilic cytoplasmic antibodies, and cytoplasmic anti-neutrophilic cytoplasmic antibodies. We have reported a 12% incidence of a predominantly motor form of neuropathy in patients with diabetes associated with monosialoganglioside antibodies (22). Furthermore, we previously found that sera with high titers of phospholipase antibody inhibited the growth and differentiation of neuroblastoma cells in culture (23). Unfortunately, this is so commonplace that the issue has been raised that phospholipase antibodies do not directly contribute to nerve damage and that they are formed as a result of antigen release from tissue damage. Pitenger et al. (24) reported on neurotoxicity of sera from 39 patients with diabetic neuropathy. Neurotoxicity was assessed using the NIE neuronal cell line (adrenal medulla and ventral spinal cord 4.1, a motor cell line). Neurotoxicity correlated with vibration detection thresholds and sera from patients with motor neuropathy were highly toxic to the VSC 4.1 line, indicating that...
there was a relationship between the specific nerve fiber function and the type of neuronal cell killed by the serum factors. Unfortunately, there have been no trials on immunotherapy for somatic neuropathies to confirm or refute the importance of these findings.

Autoimmune to neuronal structures

Autoimmune to neuronal structures has historically been stronger than somatic neuropathies and was first suggested in the early 1980s with the report of coincident autoimmune iridocyclitis and diabetic autonomic neuropathy (25). Autoantibodies against autonomic structures are frequently found in diabetes, although rare in type 2 diabetes (26). However, whether these antibodies lead to autonomic dysfunction is not clearly known. Retesting of neural and adrenal antibodies in diabetic autonomic neuropathy demonstrated that once present, these antibodies normally persist in these individuals; most patients who were negative at the beginning remained negative. We have previously identified the frequent occurrence of phospholipid antibodies in diabetic patients and demonstrated the positive correlation of these antibodies to the extent of neuropathy (23). Furthermore, we also reported that autoimmune neuronal destruction may contribute to the development of autonomic neuropathy in type 1 diabetes (17). Anti-sympathetic and -parasympathetic antibodies are relatively specific for type 1 diabetes, and there is evidence to suggest that these antibodies can be associated with dysautonomia. At the same time, there are many reports that dispute this and claim that antibodies against autonomic nervous system antigens are an inconsistent feature of diabetes (27). Hypoglycemia unawareness in the presence of anti-adrenal medullary antibodies (28) and diminished catecholamine output with orthostasis (29) in individuals with anti-sympathetic nervous system antibodies provide some evidence in support of the pathogenic role of autoantibodies. Furthermore, it is believed that autoimmune nerve destruction may be involved in diabetic neuropathy, even in type 2 diabetic patients, as parasympathetic nerve antibodies were found to be related to the severity of parasympathetic neuropathy in these patients (30,31). Surprisingly, the frequency of sympathetic nerve antibodies was low in type 1 diabetic patients (31). The finding of similar frequencies of IgG binding to adrenal medulla in both type 1 and type 2 diabetic patients, as well as in normal control subjects, argues against specificity of these autoantibodies (32). No association was demonstrated between anti-vagus nerve, anti-sympathetic ganglion, and anti-adrenal autonomic antibodies with retinopathy, peripheral somatic neuropathy, or nephropathy, even though they were frequently present in type 1 diabetes (33).

Neuronal acetylcholine receptor (ACHR) antibodies are considered a novel serologic marker of neurologic autoimmune to neuronal structures, but the pathogenicity of neuronal ACHR autoantibodies in autonomic neuropathy has not been established (34). It has been shown that patients with orthostatic hypotension and prominent cholinergic dysautonomia are most likely to be seropositive for ganglionic ACHR antibodies (35) and that higher antibody titers correlate with greater autonomic dysfunction and more frequent cholinergic dysautonomia (36). Immune responses driven by distinct neuronal ACHR (ganglionic nicotinic ACHR) subtypes expressed in small-cell carcinomas account for autoimmune autonomic neuropathy, as well as seizures, dementia, and movement disorders (37). Antibodies to this receptor can also interfere with ganglionic neurotransmission and produce autoimmune autonomic neuropathy (38).

Among other antibodies, autoantibodies against amphiphysin I and II have been associated with sensory motor neuropathy (39). The anti-Sc 170 and anti-U1snRNP antibodies are associated with esophageal motor dysfunction and cardiovascular autonomic neuropathy (40). Autoantibodies that activate smooth muscle 1-type calcium channels produced specifically by type 1 diabetic patients may mediate gastrointestinal and autonomic dysfunction in these patients (41). Autoantibodies to nerve growth factor may play a role in diabetic autonomic neuropathy and may be a feature of evolving but not established neuropathy (42). In this issue of Diabetes Care, Granberg et al. (43) suggest a predictive association. The ultimate proof of the relevance of circulating antibodies to neuronal structures will rest with identification of the specific antigen and reversal of diabetic neuropathies with neutralization of the antibody to the antigen.

Autoimmunity to neuronal structures has always been a bridesmaid but never a bride. The article by Granberg et al. (43) suggests a predictive association. The ultimate proof of the relevance of circulating antibodies to neuronal structures will rest with identification of the specific antigen and reversal of diabetic neuropathies with neutralization of the antibody to the antigen.

References
2. Boulton AJ, Vinik AI, Arezzo JC, Bril V, Feldman EL, Freeman R, Malik RA, Maser RE, Sosenko JM, Ziegler D, the American Diabetes Association: Diabetic neuropa-
thies: a statement by the American Diabete
tes Association. Diabetes Care 28:956–
962, 2005
3. Dyck PJ, Kratz KM, Karnes JL, Litchy WJ,
Klein R, Pach JM, Wilson DM, O’Brien
PC, Melton LJ 3rd, Service FJ: The prevale
nce by staged severity of various types of
diabetic neuropathy, retinopathy, and ne-
phropathy in a population-based cohort:
the Rochester Diabetic Neuropathy Study.
Neurology 43:817–824, 1993
4. Watkins PJ: Progression of diabetic au-
tonomic neuropathy. Diabet Med 10 (Suppl.
2):775–785, 1993
5. Levit NS, Stansberry KB, Wychanski C,
Vinik AI: Natural progression of auton-
momic neuropathy and autonomic func-
tion tests in a cohort of IDDM. Diabetes
Care 19:751–754, 1996
6. Giugliano D, Ceriello A, Paolillo G: Oxi-
dative stress and diabetic vascular compli-
7. Hunt JV, Dean RT, Wolff SP: Hydroxyl
radical production and antioxidative gly-
cosylation: glucose autoxidation as the
cause of protein damage in the experi-
mental glycation model of diabetes mellis-
tus and ageing. Biochem J 256:205–212,
1988
8. Vinik A, Erbas T, Stansberry KB, Pittenger
G: Small fiber neuropathy and neurovas-
cular disturbances in diabetes mellitus.
Exp Clin Endocrinol Diabetes 109 (Suppl.
TS, Erbas T: Neurotrophic factors. In
Textbook of Diabetic Neuropathy. Stuttgart,
Germany, Georg Thiem Verlag, 2003, p.
129–169
10. Waksman BH, Adams RD: Allergic neur-
tis: an experimental disease of rabbits in-
duced by the injection of peripheral
nervous tissue and adjuvants. J Exp Med
102:213–236, 1955
11. Donev SR: Ultrastructural evidence for
the presence of a glial sheath investing the
islets of Langerhans in the pancreas of mam-
12. Winer S, Tsui H, Lau A, Song A, Li X,
Cheung RK, Sampson A, Affifiyan F, Elfl-
ord A, Jackowski G, Becker DJ, Santama-
rina P, Ohashi P, Dosch HM: Autoimmune
islet destruction in spontaneous type 1 di-
babetes is not beta-cell exclusive. Nat Med
13. Hoeldtke RD, Bryner KD, Horvath GG,
Byerly MR, Hobbs GR, Marcovina SM,
Lernmark A: Antibodies to glutamic acid
decarboxylase and per-
ipheral nerve function in type 1 diabetes.
J Clin Endocrinol Metab 85:3297–3308,
2000
14. Jaeger C, Allendörfer J, Hatzigelaki E,
Dyberg T, Bergs KH, Federlin K, Bretzel
RG: Persistent GAD 65 antibodies in long-
standing IDDM are not associated with
residual beta-cell function, neuropathy or
HLA-DR status. Horm Metab Res 29:510–
515, 1997
15. Zanone MM, Burchio S, Quadri R, Pi-
etropoli M, Sacchetti C, Rabbone I, Chi-
andussi L, Cerutti F, Peakman M: Auto-
nomic function and autotoxins to auto-
nomic nervous structures, glu-
tamic acid decarboxylase and islet ty-
rosine phosphatase in adolescent patients
with IDDM. J Neuroimmunol 87:1–10,
1998
16. Pittenger GL, Liu D, Vinik AI: The neu-
ronal toxic factor in serum of type 1 diabetic
patients is a complement-fixing auto-
17. Pittenger GL, Liu D, Vinik AI: The apo-
potic death of neuroblastoma cells caused
by serum from patients with insul-
in-dependent diabetes and neuropathy
may be Fas-mediated. J Neuroimmunol 76:
153–160, 1997
18. Sharma K, Cross J, Farrant O, Ayyar D,
Sheber R, Bradley W: Demyelinating neu-
ropathy in diabetes mellitus. Arch Neurol
59:758–765, 2002
19. Chin RL, Latov N: Peripheral neuropathy
and celiac disease. Curr Treat Options
Neurol 7:43–48, 2005
20. Sharma K, Cross J, Ayyar D, Martinez-
Arizala A, Bradley W: Diabetic demyeli-
nating polynephropathy responsive to
intravenous immunoglobulin therapy. Arch
Neurol 59:751–757, 2002
21. Milicevic Z, Newlon PG, Pittenger GL,
Stansberry KB, Vinik AI: Anti-ganglioside
GM1 antibody and distal symmetric “dia-
betic polyneuropathy” with dominant
motor features. Diabetologia 40:1364–
1365, 1997
22. Vinik AI, Pittenger GL, Stansberry KB,
Powers A: Phospholipid and glutamic acid
decarboxylase autoantibodies in diabetic
neuropathy. Diabetes Care 18:1225–
1232, 1995
23. Pittenger GL, Burcus N, Malik R, Vinik AI:
Cytotoxicity of serum on sensory/auto-
nomic and motor neural cells in vitro pre-
dicts sensory neuropathy. (Abstract). Diabet
es 46 (Suppl. 1):125A, 1997
24. Guy RJC, Richards F, Edmonds ME,
Watkins PJ: Diabetic autonomic neu-
ropathy and iritis: an association suggesting
an immunological cause. Br Med J (Clin
25. Cachia MJ, Peetman M, Zanone M,
Sletten D, Low PA: The spectrum of auto-
toxins to glutamic acid decarboxylase and
adrenal medulla in type 1 and type 2 diabe-
tes mellitus: no evidence for an associa-
tion with autonomic neuropathy. J Intern
Med 239:139–146, 1996
26. Ejskæær N, Arlt S, Dodds W, Zanone MM,
Vergani D, Watkins PJ, Peakman M: Pre-
vulence of autoantibodies to autonomic
nervous tissue structures in type 1 dia-
betes mellitus. Diabet Med 16:544–549,
1999
27. Stroud CR, Heller SR, Ward JD, Hardisty
CA, Weetman AP: Analysis of antibodies
against components of the autonomic
nervous system in diabetes mellitus. QJM
80:77–81, 1997
28. De Riva C: Hypoglycaemia unawares-
ness in a young boy with insulin-dependent
diabetes mellitus and anti-adenal medul-
Iary antibodies. Diabetes Metab 23:528–
532, 1997
29. Brown FM, Brink SJ, Freeman R,
Rabinow SL: Anti-sympathetic nervous
system autoantibodies: diminished cat-
echolamines with orthostasis. Diabetes
38:938–941, 1989
30. Shugeta H, Yamaguchi M, Nakano K, Oba-
yashi H, Takemura R, Fukui M, Fuji M,
Yoshimori K, Hasegawa G, Nakamura N,
Kitagawa Y, Kondo M: Serum autoanti-
obodies against sulfatide and phospholipid
in NIDDM patients with diabetic neu-
31. Sundkvist G, Lind P, Bergstrom B, Lilja B,
Rabinowe SL: Autonomic nerve antibod-
ies and autonomic nerve function in type
1 and type 2 diabetic patients. J Intern Med
229:505–510, 1991
32. Husebye ES, Winqvist O, Sundkvist G,
Kampe O, Karlsson FA: Autoantibodies
against adrenal medulla in type 1 and type
2 diabetes mellitus: no evidence for an associa-
tion with autonomic neuropathy. J Intern
Med 239:139–146, 1996
33. Eyskæær N, Arlt S, Dodds W, Zanone MM,
Vergani D, Watkins PJ, Peakman M: Pre-
vulence of autoantibodies to autonomic
nervous tissue structures in type 1 dia-
betes mellitus. Diabetes Care 18:1225–
1232, 1995
34. Vernino S, Adamijs J, Kryzher TJ, Fealey
RD, Lennon VA: Neuronal nicotinic ACh
receptor antibody in subacute autonomic
neuropathy and cancer-related syn-
35. Sandroni P, Vernino S, Klein CM, Lennon
VA, Benrud-Larson L, Sletten D, Low PA:
Idiopathic autonomic neuropathy: com-
parison of cases seropositive and seroneg-
ative for ganglionic acetylcholine receptor
36. Klein CM, Vernino S, Lennon VA, San-
droni P, Fealey RD, Benrud-Larson L,
Sletten D, Low PA: The spectrum of auto-
nomic autonomic neuropathies. Ann Neurol
53:752–758, 2003
37. Lennon VA, Ermilov LG, Szurszewski JH,
Vernino S: Immunization with neuronal
nicotinic acetylcholine receptor induces
neurological autoimmune disease. J Clin
Invest 111:907–913, 2003
38. Goldstein DS, Holmes C, Dendi R, Li ST,
Brentzel S, Vernino S: Pandysautonomia
associated with impaired ganglionic neu-
rotransmission and circulating antibody
to the neuronal nicotinic receptor. Clin
Autoimmunity to neuronal structures


2072