Specific Fiber Deficits in Sensorimotor Diabetic Polyneuropathy Correspond to Cytotoxicity Against Neuroblastoma Cells of Sera From Patients With Diabetes

GARY L. PITTENGER, PHD
RAYAZ A. MALIK, MD, PHD
NICULINA BURCUS, MS
ANDREW J. BOULTON, MD
AARON I. VINIK, MD, PHD

OBJECTIVE — Neuropathy is the most common complication of diabetes, and toxic serum factors may contribute to its genesis.

RESEARCH DESIGN AND METHODS — We assessed neurotoxicity in the serum of 39 diabetic patients and correlated it with clinical measures of somatic and autonomic nerve fiber damage. Sera were applied to N1E-115 and VSC4.1 neuroblastoma cells in vitro as models of sensory/autonomic (S/A) and motor neurons, respectively. Neurotoxicity was measured as either complete or near-complete cell death (highly toxic), inhibited cell growth (moderately toxic), or normal cell proliferation (nontoxic) compared with pooled human serum controls during culture over 4 days.

RESULTS — There was an inverse correlation between neurotoxicity and vibration perception threshold ($P < 0.01$). Age ($P < 0.02$), duration of diabetes ($P < 0.02$), and HbA_{1c} ($P < 0.03$) correlated with neurotoxicity, suggesting that glycation may contribute to cytotoxicity in this model. S/A neurotoxicity occurred more frequently in the sera of patients with type 1 (19 of 25) than type 2 (5 of 14) diabetes ($P < 0.02$). None of the sera from either type 1 or type 2 diabetic patients displayed neurotoxicity on VSC4.1 cells, whereas sera from patients with motor neuropathy were highly toxic.

CONCLUSIONS — These studies indicate that there is a relationship between the specific nerve fiber dysfunction in the patient and the type of neuronal cell killed, not only for diabetic neuropathy but also for known forms of autoimmune neuropathies. Such toxic factors may contribute to diabetic neuropathy by acting in concert with hyperglycemia to damage sensory/autonomic neurons.

Diabetes Care 22:1839–1844, 1999

Neuropathy is a common and disabling complication of both type 1 and type 2 diabetes. The varied clinical presentation of diabetic neuropathy suggests an equally varied etiology. Thus, metabolic dysfunction, vascular dysfunction, alterations in neurotrophic support, and autoimmune destruction of nerve tissue may act in concert to result in nerve damage. Hyperglycemia is the predominant factor in the pathogenesis of diabetic neuropathy, in both type 1 (1) and type 2 (1–5) diabetes, although there is no indication from the Diabetes Control and Complications Trial (DCCT) data that once neuropathy has begun, tight glucose control is able to reverse the condition.

A number of investigators have described cytotoxic serum factors in diabetic neuropathy (6) and other peripheral neuropathies (7,8). In previous reports, we have demonstrated that the adrenergic, clonal N1E-115 neuroblastoma cell line is a useful in vitro model for assessing the role of circulating toxic elements in relation to proliferation and differentiation of neuronal cells in patients with type 1 diabetes and neuropathy (9,10). Neurotoxicity appears to induce apoptosis in a complement-dependent, immunoglobulin-mediated manner, through the *fas* signaling pathway (10,11). However, the relationship between serum toxicity on neuroblastoma cells and clinical measures of neuropathy has not been established.

The current studies sought to determine the relationship between clinical correlates of diabetes and diabetic neuropathy with serum toxicity in two different neuroblastoma cell lines representing sensory/autonomic (N1E-115 cells) and motor (VSC4.1 cells) neurons.

RESEARCH DESIGN AND METHODS

Patient information

Serum was collected at the Manchester Royal Infirmary from 25 patients with type 1 and 14 patients with type 2 diabetes (Table 1). The serum samples from these patients were supplied coded, and they were stored at −20°C for later blinded determination of cytotoxicity. No loss of toxicity from storage has previously been seen. All patients underwent a detailed clinical history and examination to rule out any other cause of neuropathy. The neuropathy symptom score (NSS) was assessed by a detailed questionnaire evaluating sensory symptoms such as burning, numbness, and tingling as well as aching and cramps, their distribution,

From the Department of Internal Medicine (G.L.P., N.B., A.I.V.), the Strelitz Diabetes Institutes, and Department of Pathology, Anatomy and Neurobiology (G.L.P., A.I.V.), Eastern Virginia Medical School, Norfolk, Virginia; and the Department of Medicine (R.A.M., A.J.B.), Manchester Royal Infirmary, Manchester, U.K.

Address correspondence and reprint requests to Gary L. Pittenger, PhD, the Diabetes Institutes, 855 W. Brambleton Ave., Norfolk VA 23452. E-mail: pittengl@evms.edu.

Received for publication 4 March 1999 and accepted in revised form 2 July 1999.

Abbreviations: CF-ADM, complement-fixing anti-adrenal medulla antibodies; CF-SG, complement-fixing anti-sympathetic ganglion antibodies; DCCT, Diabetes Control and Complications Trial; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; MANOVA, multivariate analysis of variance; NDS, neuropathy disability score; NSS, neuropathy symptom score; R-R interval, beat-to-beat interval.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

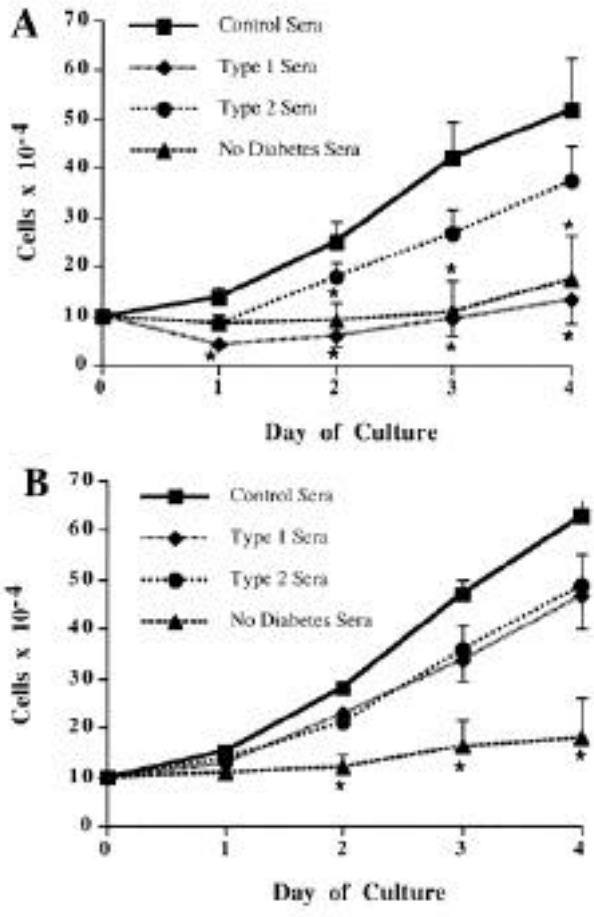

Figure 1—A: *N1E-115 sensory/autonomic cell growth curves for the four groups of patients show the growth inhibition, or toxicity, exhibited by serum from patients with either type 1 and neuropathy and those patients with other, nondiabetic autoimmune neuropathies. Sera from type 2 patients are not significantly different from controls.* B: *VSC4.1 motor cell growth curves for the four groups of patients show the growth inhibition, or toxicity, exhibited by serum from patients with nondiabetic neuropathy. In contrast, sera from type 1 or 2 diabetic patients are not significantly different from controls. In both panels, sera and days that are significantly different* ($P < 0.05$) *from control patients' sera by MANOVA and post hoc tests are indicated by an asterisk.*

and their time of maximal intensity as well as relieving and exacerbating factors; the score was graded 0–9 (12). The neuropathy disability score (NDS) was based on a clinical scoring system obtained from a neurologic examination that defined abnormalities of vibration perception using a tuning fork, pinprick perception, and temperature perception as well as the presence or absence of ankle reflexes, producing a score of 0–10 (12). Vibration perception was expressed as an average of three readings employing a biothesiometer applied to the 1st toe and medial malleoli of both lower limbs.

Autonomic function was tested from the electrocardiographic beat-to-beat (R-R) interval variation during 1 min of six maximal expirations and inspirations (deep breathing test) to calculate the expiration/inspiration (E/I) difference, the mean maximal R-R interval during expiration subtracted by the mean shortest interval during inspiration, reflecting parasympathetic, vagal nerve function. The lying-standing 30:15 ratio was also determined by assessing the ratio of the R-R intervals at the 15th and 30th beat after standing, reflecting sympathetic nerve function. Electrophysiologic assessment of the dominant lower limb was performed with a Dantec Counterpoint EMG system using surface electrodes. The skin temperature was kept above 32°C using a surface heater.

Sera were also collected from healthy control subjects (n = 17) who were free of diabetes, autoimmune disease, and neuropathy and a group of patients with various autoimmune neuropathies unrelated to diabetes at the Diabetes Institutes (Norfolk, VA). These 18 patients included 3 with chronic inflammatory demyelinating polyneuropathy (CIDP), 2 with paraneoplastic syndrome, 3 with monoclonal gammopathies, 1 with POEMS (polyneuropathy, organomegaly, endocrinopathy, M-protein spike, and skin changes), 4 with inflammatory polyneuropathies (similar to CIDP but lacking electromyogram [EMG] abnormalities, histologic evidence, or elevated protein in the cerebrospinal fluid), 1 with Sjogren's syndrome, 2 with antisulfatide antibody, 1 with mononeuritis multiplex, and 1 with autoimmune vasculitis (inflammatory infiltrate of the vasa nervorum on biopsy). Fifteen of the patients had motor neuropathy, alone or in combination with either sensory neuropathy (13 patients) or autonomic neuropathy (2 patients, both accompanied by sensorimotor neuropathy), and 3 patients had pure sensory neuropathy. These patients were evaluated identically to the patients seen in Manchester with the following exceptions. The NSSs and NDSs at the Diabetes Institutes were graded on tests with a range of score of 0–19 and 0–288 (36 measures of sensory and motor function graded 1–4 for left and right sides), respectively. No statistical comparisons to the scores for the diabetic patients were made with these measures. Autonomic testing was only performed on six patients in the nondiabetic group, limited to those who exhibited possible symptoms of autonomic neuropathy on first examination. Two of the six were positive for autonomic neuropathy.

Cell growth studies

The murine adrenergic neuroblastoma cell line N1E-115 was cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37°C, 5% CO_2, and 95% relative humidity. In the presence of serum, there is no significant differentiation of N1E-115 cells. Cells were used between passage 9 and passage 25. The hybrid neuroblastoma/motor neuron cell line VSC4.1 (provided by Dr. Stanley Appel, Houston, TX) was cultured in DMEM supplemented with 2% FBS at 37°C, 5% CO_2, and 95% relative humidity.

N1E-115 cells were grown for assay of neurotoxicity of test sera as previously described (9,10). FBS and pooled human serum (Sigma, St. Louis, MO) were used as standardized treatments in all assays to verify cell viability. On each of 4 days, aliquots of the cells were diluted 1:1 in

0.4% trypan blue and counted in a hemocytometer using trypan blue exclusion criteria, and the total number of cells in the dish was calculated.

For the VSC4.1 cells, the toxicity studies were performed in a similar fashion, with the exception that the cells are normally cultured with 2% FBS, and therefore test sera were applied at 2% in normal media.

Statistics

We have previously shown significant differences ($P < 0.05$) between type 1 diabetic patients with neuropathy and controls (9,10). Thus, it was estimated that with 39 patients we would have power to detect differences from controls at the 5% level in both the type 1 ($n = 14$) and type 2 ($n = 25$) populations tested in these studies. Multivariate analysis of variance (MANOVA) was performed on the data, using cell numbers on each day as repeated measures of the dependent variables and clinical measures as the independent variables. Post-hoc contrast tests were only conducted on those effects determined to be significant. Figures showing linear regressions on the 4th day of culture, estimated by least-squares regression, are provided only for illustration of the relationship of clinical measures found significant by MANOVA with the type of diabetes the sera represent.

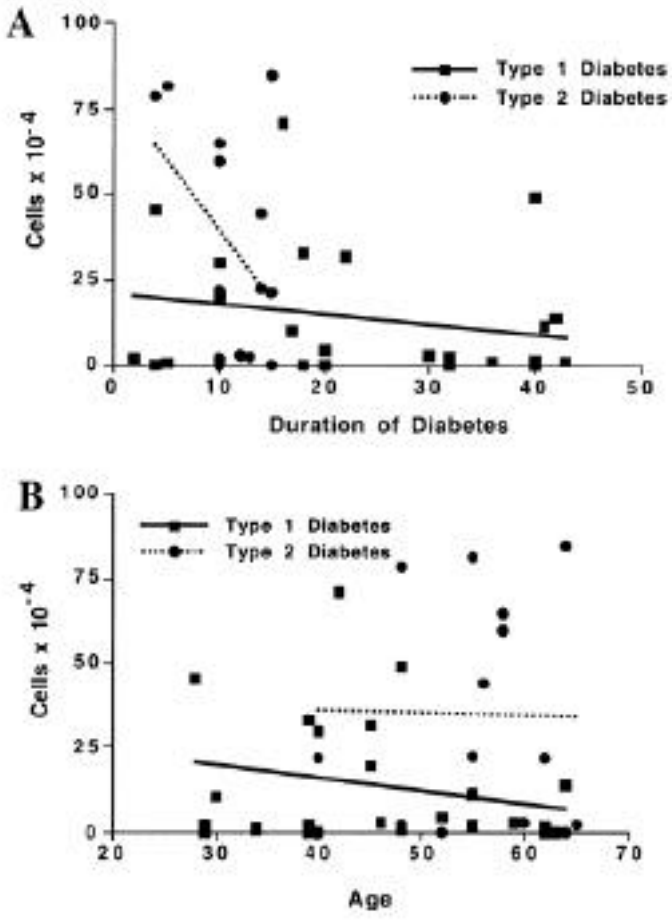

Figure 2—*Comparing (MANOVA) the response to sera from type 1 and type 2 patients across all 4 days of growth shows a significant effect of duration of diabetes (*P *< 0.02) and age (*P *< 0.02) on cell growth, an effect that is dependent on the type of diabetes. For illustration only, regressions of N1E-115 sensory/autonomic cell numbers on the 4th day of culture with serum from patients divided by type of diabetes are plotted against duration of diabetes (*A*) and age (*B*).*

RESULTS — In these studies, sera from patients with neuropathy and type 1 diabetes exhibited toxicity on the sensory/autonomic N1E-115 neuroblastoma cells more frequently (19 of 25) than sera from patients with type 2 diabetes (5 of 14). Sera from our population of nondiabetic neuropathy patients were intermediate to these two, with 50% (9 of 18) showing toxicity to N1E-115 cells, although none of the 3 patients with purely motor neuropathy exhibited toxicity. In the control population, toxicity was seen with only 1 of the 17 subjects' sera.

Sera from patients with type 1 diabetes and neuropathy were also significantly ($P < 0.05$) more toxic than sera from either patients with type 2 diabetes and neuropathy or healthy control subjects (Fig. 1*A*). N1E-115 cells exposed to sera from patients with nondiabetic peripheral, somatic neuropathies, in which autoimmunity has been implicated as a pathogenic mechanism (e.g., monoclonal gammopathy), were also significantly ($P < 0.05$) reduced in number compared with pooled human serum (Fig. 1*A*), indicating toxicity to sensory/autonomic neurons. In contrast, sera from healthy control patients were not toxic, and the cell growth was not significantly different from pooled human serum controls (data not shown).

There was no significant difference in the growth of the VSC4.1 motor cells exposed to control patients' sera or type 1 or type 2 sera. In contrast, sera from patients with nondiabetic peripheral, somatic neuropathies were toxic to the motor neuron–like VSC4.1 cells, significantly ($P < 0.05$) reducing cell growth (Fig. 1*B*). Because 15 of the 18 somatic neuropathies were characterized by motor nerve dysfunction, there appeared to be a relationship between motor neuropathy in patients and cytotoxicity to the motor neuron cell in culture. There were not enough non–motor neuropathy patients in this group to statistically evaluate this specificity.

The growth of N1E-115 cells exposed to the patients' sera showed significant effects related to duration of diabetes ($P < 0.01$) (Fig. 2*A*), age ($P < 0.02$) (Fig. 2*B*), and HbA_{1c} ($P < 0.02$) (Fig. 3) when crossed with effects of the type of diabetes and analyzed by MANOVA. Within the types of diabetes, the correlation of cell growth against HbA_{1c} showed opposite effects in response to type 1 (negative correlation, as expected) and type 2 (positive correlation) sera. There was also a significant effect related to the type of diabetes crossed with NDSs ($P < 0.05$) and vibration perception thresholds ($P < 0.03$) by MANOVA. These results suggest an association of in vitro toxicity with larger Aδ fibers and possibly even some motor fibers. This relationship was even more clear when the sera were divided according to severity into quartiles (mild, moderate,

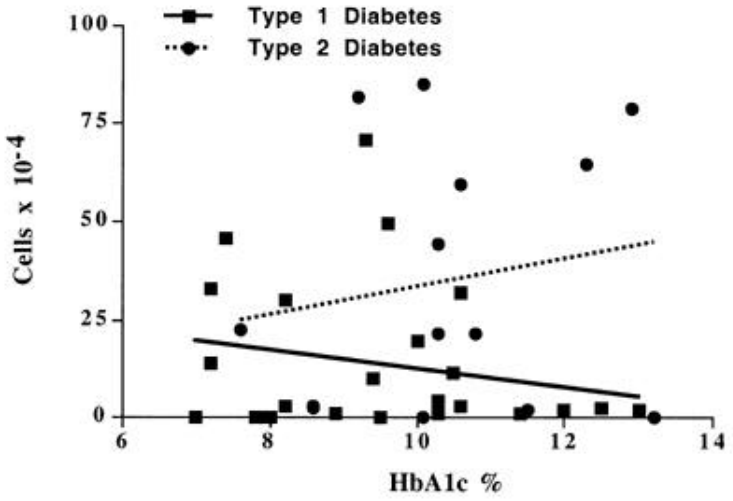

Figure 3—*Comparing (MANOVA) the response to sera from type 1 and type 2 patients across all 4 days of growth shows a significant effect ($P < 0.05$) of glycated hemoglobin (HbA_{1c}) on cell growth, an effect that is dependent on the type of diabetes. For illustration only, regression of N1E-115 sensory/autonomic cell numbers on the 4th day of culture with serum from patients divided by type of diabetes is plotted against HbA_{1c}.*

marked, and severe neuropathy), based on their total NSS and NDS. Analysis comparing the N1E-115 sensory/autonomic cell growth curves in response to treatment with sera from patients with diabetes grouped according to the severity of NDSs shows a significant trend ($P < 0.05$ by MANOVA) for more cytotoxicity with increasing severity of neuropathy.

There was no significant effect on cell growth for any of the other clinical measures taken. Quantitative autonomic function tests and nerve conduction velocities did not show a significant relationship with serum toxicity on either N1E-115 or VSC4.1 cells, although the cell reduction effects related to the lying/standing ratio almost achieved statistical significance ($P = 0.07$) in the tests on the N1E-115 sensory/autonomic cells.

CONCLUSIONS — These studies suggest that there are toxic serum factors, reflected by in vitro toxicity of serum to neuronal cells, that may be an important mechanism in the pathogenesis and persistence of diabetic neuropathy. The first important observation in these studies is the specificity of neurotoxicity for the type of neuronal cells (sensory/autonomic) that demonstrate the most marked clinical damage. Cytotoxicity to the motor cell line VSC4.1 occurred with sera from patients with predominantly motor neuropathies, suggesting a different toxic mechanism, possibly reflecting a different autoimmune antigen expressed by the target cell from that in diabetic neuropathy.

In contrast, neurotoxicity to N1E-115 cells showed significant effects, dependent on the type of diabetes, of neuropathy disability scores and vibration perception thresholds, indicating that these cells might serve as good models for studying toxic mechanisms attacking Aδ fibers in diabetes. Of interest, these fibers are normally thinly myelinated in vivo, while the N1E-115 cells are not. Thus, this model may also allow observation of neurotoxic mechanisms independent of demyelination and perhaps more indicative of pathogenic mechanisms resulting in axonal degeneration.

The failure to find effects dependent on quantitative autonomic function tests was surprising, particularly because anti-adrenal medullary antibodies have been found in patients with type 1 diabetes (13). These results might suggest that the N1E-115 cells are less sensitive to detecting serum components that are toxic to autonomic neurons than those that are toxic to sensory neurons. The number of patients with significant autonomic dysfunction was small, however, and studies with larger numbers of patients may be required to define further the role of neurotoxicity in diabetic autonomic neuropathy. Another complicating factor is the possibility that antibodies are highest midway in the course of disease during active destruction of the neurons, and lowest both when symptoms are the least, before the titer is high, and when the severity is highest, occurring with complete loss of neurons and antigens (14). Longitudinal studies will be required to confirm or deny this hypothesis.

Another observation in these studies is that duration of diabetes, age, and HbA_{1c}, dependent on type of diabetes, are important variables for predicting in vitro toxicity on N1E-115 cells. This further supports the possibility that glycation of either immune effector cells or antigen targets may play a role in the development of autoimmunity

Table 1—*Summary of clinical measures for patients with diabetes and neuropathy divided according to type of diabetes*

Clinical measure	Type 1 diabetes	Type 2 diabetes	Nondiabetic
n	25	14	18
Age (years)	45.9 ± 2.0	56.1 ± 2.7	64.9 ± 2.8
HbA_{1c} (%)	9.6 ± 0.4	10.4 ± 0.5	NA
Duration of diabetes (years)	24.2 ± 2.2	11.2 ± 3.0	NA
L/S ratio	1.05 ± 0.04	1.05 ± 0.05	—
E/I difference	12.8 ± 1.0	10.7 ± 1.3	—
NDS	4.44 ± 0.46	4.57 ± 0.61	1.7 ± 0.3
NSS	3.56 ± 0.45	4.00 ± 0.61	2.4 ± 0.3
VPT	24.0 ± 2.4	27.2 ± 3.2	15.8 ± 2.0
PMNCV (m/s)	38.6 ± 1.3	39.4 ± 1.7	39.7 ± 1.8

Data are means ± SEM. All patients with diabetes were seen and evaluated at the Manchester Royal Infirmary (Manchester, U.K.). The neuropathy patients without diabetes (nondiabetic) were seen and evaluated at the Diabetes Institutes (Norfolk, VA). Normal values for the ratio of the R-R intervals at the 15th and 30th beat after standing (lying/standing [L/S] ratio) were >1.04, and abnormal values were <1.04. Normal values for the mean maximal R-R interval during expiration subtracted by the mean shortest interval during inspiration (E/I difference) were ≥15. The NDS is out of 12 for diabetic patients and out of 288 for nondiabetic patients, and the NSS is out of 9 points for diabetic patients and out of 19 for nondiabetic patients. NA, not available; PMNCV, peroneal motor nerve conduction velocity; VPT, vibration perception threshold.

to neuronal tissues in patients with diabetes. This would be consistent with the results of the DCCT Study, demonstrating a 60% risk reduction over 10 years with improved blood glucose control (1). Because the relationship of toxicity with HbA_{1c} is different for type 1 and type 2 sera, these results suggest there might be different glycation targets causing neurotoxicity with N1E-115 neuronal cells in response to type 1 (e.g., immunoglobulins or antibodies) compared with type 2 (e.g., neurotrophins) sera. Whether this difference between type 1 and type 2 sera applies to the development of diabetic neuropathy in general remains to be determined.

The work of Poduslo and Curran (15) relating nonenzymatic glycation to autoimmune nerve destruction in diabetes suggests that increased permeability of the blood-nerve barrier to glycated immunoglobulins may be one mechanism involved in neurotoxicity. Alternatively, glycation of the antigens on neuronal cells might render them more antigenic, resulting in a heightened autoimmune response. This type of mechanism occurs in LDL oxidation, where glycation makes the LDL more susceptible to oxidation, rendering it more antigenic and promoting macrophage ingestion (16–19).

Slow neuronal cell death may be mediated by glucose-disrupting cellular metabolic homeostasis, with subsequent neuronal death, spilling antigens into the circulation (20). The resulting secondary autoimmune response might then result in accelerated neuronal destruction, accounting for the limited success of tight glucose control or aldose reductase inhibitors in significantly reversing the progression of established neuropathy. In contrast, there is mounting evidence that immunotherapy can cause significant, relatively rapid improvement in neurologic symptoms in diabetic neuropathy (21) and with even more marked improvements in diabetic proximal motor neuropathy and classic distal symmetric polyneuropathy with predominantly large fiber dysfunction (22).

The lack of neurotoxicity displayed by sera from diabetic patients on the motor cell line may relate to the difference of N1E-115 cells with other neuronal cultures, in that these cells require a 25-mmol/l glucose culture medium. This raises the potential for glycation of putative antigens over time and with increasing passage of the cell line, similar to that which may develop in vivo with neurons exposed to high plasma glucose loads. This might explain difficulties finding similar toxicity in other cell types, including VSC4.1 cells, for sera from patients with diabetes. Furthermore, other investigators with other cell types have shown that high glucose itself can be toxic to neuronal cells in culture (23). However, high glucose in N1E-115 cell cultures cannot be the whole story, because of the difference in the pattern of toxicity of serum between type 1 and type 2 patients related to HbA_{1c}.

Another possible mechanism of cell loss might be failure of cell adhesion due to components of the sera. One report from Jude et al. (24) suggests that elevated serum P-selectin and E-selectin, cell attachment factors, are predictive markers for neuropathy, although their role in neurodegeneration in diabetic neuropathy is as yet unknown. Further, serum P-selectin and intercellular adhesion molecule-1 were found to be inversely related to nerve conduction velocity in patients with diabetic neuropathy (24), suggesting that these serum factors might play a role in the continuing neuronal destruction in diabetic neuropathy. It would be interesting to test whether neuroblastoma cells might also produce P- and E-selectins, perhaps as a compensatory attempt to promote neuronal attachment.

Since the early 1980s, investigators suggested that autoimmunity might contribute to the pathogenesis of diabetic neuropathy. Guy et al. (25) found an association between diabetic autonomic neuropathy and iritis, suggesting an immunologic background. Since iritis itself is an immunologically mediated disorder with circulating immune complexes, they speculated that the associated small fiber damage, which results in autonomic neuropathy, might have been due to autoimmunity. Organ-specific, complement-fixing autoantibodies against unknown antigens from adrenal medulla (CF-ADM) and sympathetic ganglia (CF-SG) have been demonstrated in type 1 patients (26–30). With duration of diabetes >5 years, CF-ADM occurs in both islet cell antibody–positive and –negative patients, suggesting that the antigenic targets in adrenal medulla and pancreatic islets are different. Because these experiments were done as cross-sectional studies, it is not known whether the generation of antibody preceded neuronal destruction. Observations by Zanone et al. (30) on the prevalence of at least one of the autoantibodies directed to autonomic nervous system structures (CF-SG, CF-ADM, or complement-fixing anti-vagal autoantibody) in patients with diabetic autonomic neuropathy support the relationship between autoantibodies to autonomic structures and autonomic neuropathy in type 1 diabetes. A number of studies, including our own, have shown that neurotoxic antibodies play a role in in vitro cytotoxicity (6–10,31). However, the connection between circulating antibodies and the development of neuropathy has not been satisfactorily established.

These results further indicate that toxicity assays using N1E-115 cells may detect toxic factors resulting in sensory dysfunction dependent on the type of diabetes within the patient group, while VSC4.1 cell cytotoxicity reflects toxic factors resulting in strictly motor neuropathies. Further studies will be needed to establish whether this approach might prove useful in pointing toward specific diagnosis, and therefore treatment, of these groups of patients.

Acknowledgments— The authors would like to acknowledge the support of the Diabetes Institutes Foundation for this work.

References

1. Diabetes Control and Complications Trial Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 329:977–986, 1993
2. U.K. Prospective Diabetes Study (UKPDS) Group: Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet* 352:837–853, 1998
3. U.K. Prospective Diabetes Study (UKPDS) Group: Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). *Lancet* 352:854–865, 1998
4. U.K. Prospective Diabetes Study (UKPDS) Group: Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 38. *BMJ* 317:703–713, 1998
5. Diabetes Control and Complications Trial Research Group: The effect of intensive diabetes therapy on measures of autonomic nervous system function in the Diabetes Control and Complications Trial (DCCT). *Diabetologia* 41:416–423, 1998
6. Juntti-Berggren L, Larsson O, Rorsman P, Ämmälä C, Bokvist K, Wåhlander K,

Nicotera P, Dypbukt J, Orrenius S, Hallberg A, Berggren P-O: Increased activity of L-type Ca^{2+} channels exposed to serum from patients with type I diabetes. *Science* 261: 86–90, 1993
7. Kimura F, Smith RG, Delbono O, Nyormoi O, Schneider T, Nastainczyk W, Hofmann F, Stefani E, Appel SH: Amyotrophic lateral sclerosis patient antibodies label Ca^{2+} channel alpha 1 subunit. *Ann Neurol* 35:164–171, 1994
8. Smith RG, Alexianu ME, Crawford G, Nyormoi O, Stefani E, Appel SH: Cytotoxicity of immunoglobulins from amyotrophic lateral sclerosis patients on a hybrid motoneuron cell line. *Proc Natl Acad Sci U S A* 91:3393–3397, 1994
9. Pittenger GL, Liu D, Vinik AI: The toxic effects of serum from patients with type I diabetes mellitus on mouse neuroblastoma cells: a new mechanism for development of autonomic neuropathy. *Diabet Med* 10: 925–932, 1993
10. Pittenger GL, Liu D, Vinik AI: The neuronal toxic factor in serum of type 1 diabetic patients is a complement-fixing autoantibody. *Diabet Med* 12:380–386, 1995
11. Pittenger GL, Liu D, Vinik AI: The apoptotic death of neuroblastoma cells caused by serum from patients with insulin-dependent diabetes and neuropathy may be Fas-mediated. *J Neuroimmunol* 76:153–160, 1997
12. Young MJ, Boulton AJM, Macleod AF, Williams DRR, Sonksen PH: A multicentre study of the prevalence of diabetic peripheral neuropathy in the United Kingdom hospital clinic population. *Diabetologia* 36:150–154, 1993
13. Brown FM, Kamalesh M, Adri MNS, Rabinowe SL: Anti-adrenal medullary antibodies in IDDM subjects and subjects at high risk for developing IDDM. *Diabetes Care* 11:30–33, 1988
14. Pittenger GL, Milicevic Z, Vinik AI: Autoimmune mechanisms of diabetic neuropathy. In: *Diabetes Mellitus: A Fundamental and Clinical Text*. LeRoith D, Olefsky JM, Taylor S, Eds. Philadelphia, Lippincott-Raven, 1996, p. 751–758
15. Poduslo JF, Curran GL: Glycation increases the permeability of proteins across the blood-nerve and blood brain barriers. *Brain Res Mol Brain Res* 23:157–162, 1994
16. Lopes-Virella MF, Virella G: Cytokines, modified lipoproteins, and arteriosclerosis in diabetes. *Diabetes* 45 (Suppl. 3):S40–S44, 1996
17. Mironova M, Virella G, Lopes-Virella MF: Isolation and characterization of human antioxidized LDL autoantibodies. *Arterioscler Thromb Vasc Biol* 16:222–229, 1996
18. Klein RL, Laimins M, Lopes-Virella MF: Isolation, characterization, and metabolism of the glycated and nonglycated subfractions of low-density lipoproteins isolated from type I diabetic patients and nondiabetic subjects. *Diabetes* 44:1093–1098, 1995
19. Virella G, Munoz JF, Galbraith GM, Gissinger C, Chassereau C, Lopes-Virella MF: Activation of human monocyte-derived macrophages by immune complexes containing low-density lipoprotein. *Clin Immunol Immunopathol* 75:179–189, 1995
20. Greene DA, Lattimer-Greene S, Sima AA: Pathogenesis of diabetic neuropathy: role of altered phosphoinositide metabolism. *Crit Rev Neurobiol* 5:143–219, 1989
21. Krendel DA, Costigan DA, Hopkins LC: Successful treatment of neuropathies in patients with diabetes mellitus. *Arch Neurol* 52:1053–1061, 1995
22. Vinik AI, Milicevic Z: New agents in the management of diabetic neuropathy. *Curr Opin Endocrinol Diab* 4:63–76, 1997
23. Matthews CC, Feldman EL: Insulin-like growth factor I rescues SH-SY5Y human neuroblastoma cells from hyperosmotic induced programmed cell death. *J Cell Physiol* 166:323–331, 1996
24. Jude EB, Abbott CA, Young MJ, Anderson SG, Douglas JT, Boulton AJ: The potential role of cell adhesion molecules in the pathogenesis of diabetic neuropathy. *Diabetologia* 41:330–336, 1998
25. Guy RJC, Richards F, Edmonds ME, Watkins PJ: Diabetic autonomic neuropathy and iritis: an association suggesting an immunological cause. *Br Med J* 289:343–345, 1984
26. Rabinowe SL, Brown FM, Watts M, Kadrofske MM, Vinik AI: Anti-sympathetic ganglia antibodies and postural blood pressure in IDDM subjects of varying duration and patients at high risk of developing IDDM. *Diabetes Care* 12:1–6, 1989
27. Sundkvist G, Lind P, Bergstrom B, Lilja B, Rabinowe SL: Autonomic nerve antibodies and autonomic nerve function in type 1 and type 2 diabetic patients. *J Intern Med* 229:505–510, 1991
28. Brown FM, Vinik AI, Ganda OP, Adri MN, Rabinowe SL: Different effects of duration on prevalence of anti-adrenal medullary and pancreatic islet cell antibodies in type I diabetes mellitus. *Horm Metab Res* 21:434–437, 1988
29. Brown FM, Brink SJ, Freeman R, Rabinowe SL: Anti-sympathetic nervous system autoantibodies: diminished catecholamines with orthostasis. *Diabetes* 38:938–941, 1989
30. Zanone MM, Peakman M, Purewal T, Watkins PJ, Vergani D: Autoantibodies to nervous tissue structures are associated with autonomic neuropathy in type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 36:564–569, 1993
31. Vinik AI, Pittenger GL, Stansberry KB, Powers A: Phospholipid and glutamic acid decarboxylase autoantibodies in diabetic neuropathy. *Diabetes Care* 18:1225–1232, 1995