

# General Scientific Session on Neuroimmunology III/ Peripheral Nerve Morning Meeting Saturday, May 2, 1981

9:00 A.M.-12:00 Noon  
Ballroom Center

*Chairman:* Barry G.W. Arnason, Chicago, IL  
*Secretary:* David E. Pleasure, Philadelphia, PA

1-9:00

**Acute Canine Idiopathic Polyneuropathy: A Naturally Occurring Disorder**

JERRY W. NORTHINGTON and MARK J. BROWN, Philadelphia, PA

We reported a syndrome of acute flaccid quadriplegia and areflexia in previously healthy dogs not exposed to raccoons or neurotoxins. We now have identified 14 such dogs over the last 3 years from a large outpatient population. Their illness progressed quickly in all cases (1 to 20 days). This was followed by a short period of rapid recovery (2 to 4 weeks) in those that did not succumb to respiratory complications. Subsequent improvement required many months. Relapse occurred in two dogs; both responded to corticosteroid therapy. EMG examination consistently showed fibrillation potentials by 7 days. Motor conduction varied at different sites, even in the same individual. Velocities were normal (sometimes with slowed or absent F-wave responses), mildly slowed, severely slowed, or not measurable. Sensory conduction was normal or slowed. The morphology of biopsied mixed nerves ranged from normal to severe axonal degeneration. Segmental remyelination was found in two nerves. When examined, biopsied sensory nerves were normal. Present evidence suggests that this syndrome is a proximal predominantly motor demyelinating disorder with associated axonal degeneration. Its relationship to human Guillain-Barré syndrome is uncertain.

2-9:15

**Technique Assessment of Demyelinating Activity from Endoneurially Injected Test Solutions and Sera: Demyelinating Activity of HMSN, III Sera**

PETER JAMES DYCK, ALFRED C. LAIS, MARGARET F. SPARKS, and SEONA M. HANSEN, Rochester, MN

Injection of lysolecithin (Hall and Gregson, 1971) antigalactocerebrosides (Saida et al, 1977) and Guillain-Barré sera (Feasby, 1979) into rat nerve endoneurium is the method used to test for demyelinating activity. Does the methodology of injection, nonblinded conduct of experiments, and the lack of quantitation lead to false-positive or false-negative results? To test for the demyelinating effect of needle size, extraneous movement, rate of injection, and viscosity and constituents of serum, 10- $\mu$ l amounts of neutral buffered saline (NBS), syngeneic rat serum, human serum, NBS with 3.5% human albumin, NBS with 7% human albumin, and sera from patients with neuropathy, were injected into groups of 250-gm male syngeneic Lewis rat peroneal nerves. Solutions and sera were coded and randomly assigned for injection and histologic eval-

uation. Seven days later 100 fibers per nerve were teased and graded from six nerves per experimental condition. Hand-held injections with 30-gauge needles produce an unacceptable rate of segmental demyelination and of axonal degeneration. Micromanipulator and 33-gauge steel needle injections of NRS and serum produce a low and somewhat variable rate of abnormality. To recognize demyelinating activity, these optimal approaches in quite large groups of nerves should be used to detect demyelinating activity. Of human sera tested, two patients with HMSN, III sera showed significant levels of demyelinating activity.

3-9:30

**Cross-Reactive Antibodies to Influenza Vaccine and Peripheral Nerve in Guillain-Barré Syndrome (GBS)**

TERRY M. PHILLIPS, WILLIAM QUEEN, and JOSEPH BALLANTI, Washington, DC, and WILLIAM SHEREMATA, Miami, FL

The increased incidence of Guillain-Barré syndrome (GBS) following swine influenza vaccination has not been explained. Stored frozen sera from 35 normals and 44 CDC acute GBS sera have, for this reason, been studied for cross-reactive antibody binding to 50  $\mu$ l crude vaccine fixed to microwells. Indirect immunofluorescence (fluoresceinated goat antihuman IgG) was used to detect serum IgG binding to frozen human peripheral nerve sections. Normals gave 2-2.5  $\mu$ g IgG binding except for one (25  $\mu$ g). GBS sera were in two groups: (1) bindings of 0-15  $\mu$ g IgG and (2) bindings of 40-150  $\mu$ g IgG (2 of 21 unvaccinated and 15 of 23 vaccinated). Twelve of 21 unvaccinated and 17 of 23 vaccinated GBS had IgG binding to nerve. All ELISA-positive sera showed IgG binding to nerve. Affinity elution of nerve antibody gave Ouchterlony-type lines of identity between vaccine and nerve homogenate. No controls gave such results. These observations show presence of nerve antigens in swine influenza vaccine. Thus, immunologic tolerance in genetically or otherwise predisposed hosts may have been abrogated by the injection of minute quantities of viral antigens, and antigens resembling peripheral nerve constituents.

4-9:45

**Polyinosinic-Polycytidylic Acid Poly-L-lysine (Poly-ICLC): A New Anti-Dysimmune Effect Remarkably Beneficial in Neuropathies**

W. KING ENGEL, PETER G. BERNAD, and HILTON B. LEVY, Bethesda, MD

We introduced PolyICLC (stabilized with carboxymethyl cellulose) as a new treatment for dysimmune dyschwannian neuropathy (DSN) in one patient (*Lancet* 1:503, 1978). Off all other drugs for more than 1.5 years, he continues after 3 years to benefit and be dependent upon PolyICLC. Now age 31, he has had motor DSN from age 15, and eventually was refractory to prednisone, azathioprine, and plasmapheresis. Confined to an electric wheelchair and hardly able to use his limbs before PolyICLC, now, at the peaks of PolyICLC action, he is normal proximally and much improved distally, and can walk 8 miles a day. Four of seven other motor-sensory DSN and two of two dysimmune dysneuronal motor-sensory neuropathy patients refractory to other drugs now have beneficial responses extending over 1-2 years, allowing elimination or major reduction of any other medications. Each IV dose (20-100  $\mu$ g/kg) of PolyICLC causes benefit lasting 2-6 weeks. Acutely, there is 4-18 hours flu-like syndrome, 4-5 days selective lymphocytopenia to 1-20 percent of baseline, 24-48 hours granulocytosis 2-3  $\times$  baseline, 48 hours hypercorticoidemia, and minimal <

24 hours hyperinterferonemia. The beneficial effect of Poly-ICLC is unlikely to be simply glucocorticoid because responsive patients had severe prednisone side effects at the beginning of treatment, which dissipated as prednisone was withdrawn.

5-10:00

**The Prevalence of Monoclonal Gammopathy in Peripheral Neuropathy: A Prospective Survey**

JOHN J. KELLY, Jr, R.A. KYLE, P.C. O'BRIEN, and P.J. DYCK, Rochester, MN

To assess the prevalence of monoclonal gammopathy in peripheral neuropathy, we attempted to perform serum protein electrophoresis in all 692 patients diagnosed as having a peripheral neuropathy over a 1-year period. About 80% of the entire group had a serum protein electrophoresis performed. Three hundred thirty four of the 692 patients (48%) proved to have a neuropathy not due to a systemic disease and not inherited. In this "idiopathic" group, there was a 10% prevalence of monoclonal gammopathy. Among the patients with a monoclonal gammopathy, further investigation showed that 8 patients had 1° systemic amyloidosis, 3 patients had multiple myeloma, 1 had Waldenstrom macroglobulinemia, 1 had gamma-heavy chain disease, and 15 had a monoclonal gammopathy of undetermined significance. This 10% prevalence rate was significantly higher than corresponding rates reported for the general population. The increase was statistically significant at the  $p < 0.025$  level in each decade from 50-80 years of age, but not for patients 80+. We conjecture that the lack of statistical significance in older patients may be due at least in part to smaller sample size in the 80+ group.

The result suggests that: (1) Serum protein electrophoresis should be obtained in all patients with peripheral neuropathy of unknown cause, and (2) a disturbance of the immune system probably plays a role in some cases of peripheral neuropathy associated with a monoclonal protein.

6-10:15

**Peripheral Neuropathy and Plasma-Cell Dyscrasia: The Range of Pathologic Findings in Sural Nerve**

R. NEMNI, Boston, MA, and G. GALASSI, N. LATOV, W.H. SHERMAN, M.R. OLARTE, and A.P. HAYS, New York, NY

We reported marked demyelination in sural nerve of a patient with neuropathy and monoclonal antibodies directed against peripheral nerve (PN) myelin (*N Engl J Med* 303:618, 1980). We have now performed a similar morphologic study of five more cases of plasma-cell dyscrasia to determine whether the pathogenesis of the associated neuropathy is similar. Immunologic studies showed antibodies directed against PN myelin in cases 1 and 2 and directed against PN axonal pellet in case 3. No antibodies directed against PN components were detected in cases 4-6. On light microscopy no inflammatory cells were seen in any biopsy. Axonal degeneration (AD) was marked in the small bridging interfascicular bundles in cases 4 and 5. In cases 3 and 4 small calcium deposits were observed in the endoneurium. Histometric studies showed an almost complete loss of myelinated fibers (62/mm<sup>2</sup>) in case 3 while the other five cases had a variable loss (3292-6024/mm<sup>2</sup>); the large-diameter fibers were particularly lost. Teased single-fiber studies were performed in four patients and showed segmental demyelination (SD) in case 1, SD and AD in case 2, and AD with secondary SD in cases 5 and 6. Ultrastructural studies showed thinly myelinated fibers and onion bulb formations in case 1, axonal abnormalities and SD in case 2, and

different stages of AD in cases 3-6. These studies suggest that multiple pathogenetic factors are responsible for PN disease in plasma cell dyscrasia.

7-10:30

**Immunocytochemical Staining of Peripheral Nerve with Serum from Patients with Polyneuropathy and Paraproteinemia**

GARY M. ABRAMS, NORMAN LATOV, ARTHUR P. HAYS, WILLIAM SHERMAN, and EARL A. ZIMMERMAN, New York, NY

Immunohistochemistry was used to demonstrate antibodies directed against peripheral nerve in patients with peripheral neuropathy with associated monoclonal gammopathy. Serum was obtained from two patients with peripheral neuropathy and monoclonal IgMk paraproteinemia. Previous work has shown that these immunoglobulins cross-reacted with components of myelin prepared from human peripheral nerve. Normal postmortem peripheral nerve was fixed in formalin and embedded in paraffin. Six  $\mu$ m sections were prepared and processed by a modification of the Sternberger immunoperoxidase technique. The tissue was incubated with sera from patients for 4-24 hours at 4° C. Subsequent incubations were (1) rabbit antiserum to human IgM (2) goat antirabbit serum, and (3) rabbit peroxidase-antiperoxidase complexes. Immunoreaction products were developed with peroxide-activated 3, 3'-diaminobenzide and sections were examined by light microscopy. Characteristic immunoprecipitate was seen within the myelin sheath. Substitution of normal human serum eliminated specific staining. Patient serum that was preabsorbed with a preparation of human myelin so as to eliminate the IgMk spike by electrophoresis showed markedly reduced immunostaining. Immunohistochemistry should be valuable in the investigation of patients with monoclonal gammopathy and various neurologic disorders.

8-10:45

**Monoclonal Antibodies to Peripheral Nerve in Patients with Polyneuritis and Plasma Cell Dyscrasia**

NORMAN LATOV, WILLIAM A. SHERMAN, ROBIN GROSS, JOANNA S. SHYONG, MARCELO R. OLARTE, AUDREY S. PENN, ELLIOTT F. OSSERMAN, and LEONARD CHESSE, New York, NY

Sera from 10 patients with polyneuritis and nonmalignant plasma cell dyscrasia were studied for evidence of monoclonal antibody activity to peripheral nerve. In eight patients the paraprotein was IgMk and in two, IgGk. All presented with slowly progressive neuropathy beginning in the arms or legs and without autonomic or cranial nerve involvement. Sera were tested for antibody activity to human peripheral nerve myelin or axons using complement fixation and immunoadsorption. In two patients with IgMk, there was complement-fixing antimyelin activity and the paraproteins were selectively absorbed by incubating with myelin. Antibody activity resided in the IgM but not in the IgG serum fractions. In another patient with IgMk the paraprotein was selectively absorbed by the axonal pellet but no complement-fixing activity was detected. No antibody activity to peripheral nerve was found in the other seven patients.

The data indicate that in some patients with plasma cell dyscrasia and polyneuropathy, paraproteins are directed at antigens in the myelin or axonal pellets and may cause the neuropathy.

# General Scientific Session on Neuroimmunology III/ Peripheral Nerve Morning Meeting Saturday, May 2, 1981

9:00 A.M.-12:00 Noon  
Ballroom Center

Chairman: Barry G.W. Arnason, Chicago, IL  
Secretary: David E. Pleasure, Philadelphia, PA

1-9:00

**Acute Canine Idiopathic Polyneuropathy: A Naturally Occurring Disorder**

JERRY W. NORTHINGTON and MARK J. BROWN, Philadelphia, PA

We reported a syndrome of acute flaccid quadriplegia and areflexia in previously healthy dogs not exposed to raccoons or neurotoxins. We now have identified 14 such dogs over the last 3 years from a large outpatient population. Their illness progressed quickly in all cases (1 to 20 days). This was followed by a short period of rapid recovery (2 to 4 weeks) in those that did not succumb to respiratory complications. Subsequent improvement required many months. Relapse occurred in two dogs; both responded to corticosteroid therapy. EMG examination consistently showed fibrillation potentials by 7 days. Motor conduction varied at different sites, even in the same individual. Velocities were normal (sometimes with slowed or absent F-wave responses), mildly slowed, severely slowed, or not measurable. Sensory conduction was normal or slowed. The morphology of biopsied mixed nerves ranged from normal to severe axonal degeneration. Segmental remyelination was found in two nerves. When examined, biopsied sensory nerves were normal. Present evidence suggests that this syndrome is a proximal predominantly motor demyelinating disorder with associated axonal degeneration. Its relationship to human Guillain-Barré syndrome is uncertain.

2-9:15

**Technique Assessment of Demyelinating Activity from Endoneurially Injected Test Solutions and Sera: Demyelinating Activity of HMSN, III Sera**

PETER JAMES DYCK, ALFRED C. LAIS, MARGARET F. SPARKS, and SEONA M. HANSEN, Rochester, MN

Injection of lysolecithin (Hall and Gregson, 1971) antigalactocerebrosides (Saida et al, 1977) and Guillain-Barré sera (Feasby, 1979) into rat nerve endoneurium is the method used to test for demyelinating activity. Does the methodology of injection, nonblinded conduct of experiments, and the lack of quantitation lead to false-positive or false-negative results? To test for the demyelinating effect of needle size, extraneous movement, rate of injection, and viscosity and constituents of serum, 10- $\mu$ l amounts of neutral buffered saline (NBS), syngeneic rat serum, human serum, NBS with 3.5% human albumin, NBS with 7% human albumin, and sera from patients with neuropathy, were injected into groups of 250-gm male syngeneic Lewis rat peroneal nerves. Solutions and sera were coded and randomly assigned for injection and histologic eval-

uation. Seven days later 100 fibers per nerve were teased and graded from six nerves per experimental condition. Hand-held injections with 30-gauge needles produce an unacceptable rate of segmental demyelination and of axonal degeneration. Micromanipulator and 33-gauge steel needle injections of NBS and serum produce a low and somewhat variable rate of abnormality. To recognize demyelinating activity, these optimal approaches in quite large groups of nerves should be used to detect demyelinating activity. Of human sera tested, two patients with HMSN, III sera showed significant levels of demyelinating activity.

3-9:30

**Cross-Reactive Antibodies to Influenza Vaccine and Peripheral Nerve in Guillain-Barré Syndrome (GBS)**

TERRY M. PHILLIPS, WILLIAM QUEEN, and JOSEPH BALLANTI, Washington, DC, and WILLIAM SHEREMATA, Miami, FL

The increased incidence of Guillain-Barré syndrome (GBS) following swine influenza vaccination has not been explained. Stored frozen sera from 35 normals and 44 CDC acute GBS sera have, for this reason, been studied for cross-reactive antibody binding to 50  $\mu$ l crude vaccine fixed to microwells. Indirect immunofluorescence (fluoresceinated goat antihuman IgG) was used to detect serum IgG binding to frozen human peripheral nerve sections. Normals gave 2-2.5  $\mu$ g IgG binding except for one (25  $\mu$ g). GBS sera were in two groups: (1) bindings of 0-15  $\mu$ g IgG and (2) bindings of 40-150  $\mu$ g IgG (2 of 21 unvaccinated and 15 of 23 vaccinated). Twelve of 21 unvaccinated and 17 of 23 vaccinated GBS had IgG binding to nerve. All ELISA-positive sera showed IgG binding to nerve. Affinity elution of nerve antibody gave Ouchterlony-type lines of identity between vaccine and nerve homogenate. No controls gave such results. These observations show presence of nerve antigens in swine influenza vaccine. Thus, immunologic tolerance in genetically or otherwise predisposed hosts may have been abrogated by the injection of minute quantities of viral antigens, and antigens resembling peripheral nerve constituents.

4-9:45

**Polyinosinic-Polycytidylic Acid Poly-L-lysine (Poly-ICLC): A New Anti-Dysimmune Effect Remarkably Beneficial in Neuropathies**

W. KING ENGEL, PETER G. BERNAD, and HILTON B. LEVY, Bethesda, MD

We introduced PolyICLC (stabilized with carboxymethyl cellulose) as a new treatment for dysimmune dyschwannian neuropathy (DSN) in one patient (*Lancet* 1:503, 1978). Off all other drugs for more than 1.5 years, he continues after 3 years to benefit and be dependent upon PolyICLC. Now age 31, he has had motor DSN from age 15, and eventually was refractory to prednisone, azathioprine, and plasmapheresis. Confined to an electric wheelchair and hardly able to use his limbs before PolyICLC, now, at the peaks of PolyICLC action, he is normal proximally and much improved distally, and can walk 8 miles a day. Four of seven other motor-sensory DSN and two of two dysimmune dysneuronal motor-sensory neuropathy patients refractory to other drugs now have beneficial responses extending over 1-2 years, allowing elimination or major reduction of any other medications. Each IV dose (20-100  $\mu$ g/kg) of PolyICLC causes benefit lasting 2-6 weeks. Acutely, there is 4-18 hours flu-like syndrome, 4-5 days selective lymphocytopenia to 1-20 percent of baseline, 24-48 hours granulocytosis 2-3  $\times$  baseline, 48 hours hypercorticoidemia, and minimal <

24 hours hyperinterferonemia. The beneficial effect of Poly-ICLC is unlikely to be simply glucocorticoid because responsive patients had severe prednisone side effects at the beginning of treatment, which dissipated as prednisone was withdrawn.

5-10:00

**The Prevalence of Monoclonal Gammopathy in Peripheral Neuropathy: A Prospective Survey**

JOHN J. KELLY, Jr, R.A. KYLE, P.C. O'BRIEN, and P.J. DYCK, Rochester, MN

To assess the prevalence of monoclonal gammopathy in peripheral neuropathy, we attempted to perform serum protein electrophoresis in all 692 patients diagnosed as having a peripheral neuropathy over a 1-year period. About 80% of the entire group had a serum protein electrophoresis performed. Three hundred thirty four of the 692 patients (48%) proved to have a neuropathy not due to a systemic disease and not inherited. In this "idiopathic" group, there was a 10% prevalence of monoclonal gammopathy. Among the patients with a monoclonal gammopathy, further investigation showed that 8 patients had 1° systemic amyloidosis, 3 patients had multiple myeloma, 1 had Waldenstrom macroglobulinemia, 1 had gamma-heavy chain disease, and 15 had a monoclonal gammopathy of undetermined significance. This 10% prevalence rate was significantly higher than corresponding rates reported for the general population. The increase was statistically significant at the  $p < 0.025$  level in each decade from 50-80 years of age, but not for patients 80+. We conjecture that the lack of statistical significance in older patients may be due at least in part to smaller sample size in the 80+ group.

The result suggests that: (1) Serum protein electrophoresis should be obtained in all patients with peripheral neuropathy of unknown cause, and (2) a disturbance of the immune system probably plays a role in some cases of peripheral neuropathy associated with a monoclonal protein.

6-10:15

**Peripheral Neuropathy and Plasma-Cell Dyscrasia: The Range of Pathologic Findings in Sural Nerve**

R. NEMNI, Boston, MA, and G. GALASSI, N. LATOV, W.H. SHERMAN, M.R. OLARTE, and A.P. HAYS, New York, NY

We reported marked demyelination in sural nerve of a patient with neuropathy and monoclonal antibodies directed against peripheral nerve (PN) myelin (*N Engl J Med* 303:618, 1980). We have now performed a similar morphologic study of five more cases of plasma-cell dyscrasia to determine whether the pathogenesis of the associated neuropathy is similar. Immunologic studies showed antibodies directed against PN myelin in cases 1 and 2 and directed against PN axonal pellet in case 3. No antibodies directed against PN components were detected in cases 4-6. On light microscopy no inflammatory cells were seen in any biopsy. Axonal degeneration (AD) was marked in the small bridging interfascicular bundles in cases 4 and 5. In cases 3 and 4 small calcium deposits were observed in the endoneurium. Histometric studies showed an almost complete loss of myelinated fibers (62/mm<sup>2</sup>) in case 3 while the other five cases had a variable loss (3292-6024/mm<sup>2</sup>); the large-diameter fibers were particularly lost. Teased single-fiber studies were performed in four patients and showed segmental demyelination (SD) in case 1, SD and AD in case 2, and AD with secondary SD in cases 5 and 6. Ultrastructural studies showed thinly myelinated fibers and onion bulb formations in case 1, axonal abnormalities and SD in case 2, and

different stages of AD in cases 3-6. These studies suggest that multiple pathogenetic factors are responsible for PN disease in plasma cell dyscrasia.

7-10:30

**Immunocytochemical Staining of Peripheral Nerve with Serum from Patients with Polyneuropathy and Paraproteinemia**

GARY M. ABRAMS, NORMAN LATOV, ARTHUR P. HAYS, WILLIAM SHERMAN, and EARL A. ZIMMERMAN, New York, NY

Immunohistochemistry was used to demonstrate antibodies directed against peripheral nerve in patients with peripheral neuropathy with associated monoclonal gammopathy. Serum was obtained from two patients with peripheral neuropathy and monoclonal IgMk paraproteinemia. Previous work has shown that these immunoglobulins cross-reacted with components of myelin prepared from human peripheral nerve. Normal postmortem peripheral nerve was fixed in formalin and embedded in paraffin. Six  $\mu$ m sections were prepared and processed by a modification of the Sternberger immunoperoxidase technique. The tissue was incubated with sera from patients for 4-24 hours at 4° C. Subsequent incubations were (1) rabbit antiserum to human IgM (2) goat antirabbit serum, and (3) rabbit peroxidase-antiperoxidase complexes. Immunoreaction products were developed with peroxide-activated 3, 3' diaminobenzide and sections were examined by light microscopy. Characteristic immunoprecipitate was seen within the myelin sheath. Substitution of normal human serum eliminated specific staining. Patient serum that was preabsorbed with a preparation of human myelin so as to eliminate the IgMk spike by electrophoresis showed markedly reduced immunostaining. Immunohistochemistry should be valuable in the investigation of patients with monoclonal gammopathy and various neurologic disorders.

8-10:45

**Monoclonal Antibodies to Peripheral Nerve in Patients with Polyneuritis and Plasma Cell Dyscrasia**

NORMAN LATOV, WILLIAM A. SHERMAN, ROBIN GROSS, JOANNA S. SHYONG, MARCELO R. OLARTE, AUDREY S. PENN, ELLIOTT F. OSSERMAN, and LEONARD CHESSE, New York, NY

Sera from 10 patients with polyneuritis and nonmalignant plasma cell dyscrasia were studied for evidence of monoclonal antibody activity to peripheral nerve. In eight patients the paraprotein was IgMk and in two, IgGk. All presented with slowly progressive neuropathy beginning in the arms or legs and without autonomic or cranial nerve involvement. Sera were tested for antibody activity to human peripheral nerve myelin or axons using complement fixation and immunoadsorption. In two patients with IgMk, there was complement-fixing antimyelin activity and the paraproteins were selectively absorbed by incubating with myelin. Antibody activity resided in the IgM but not in the IgG serum fractions. In another patient with IgMk the paraprotein was selectively absorbed by the axonal pellet but no complement-fixing activity was detected. No antibody activity to peripheral nerve was found in the other seven patients.

The data indicate that in some patients with plasma cell dyscrasia and polyneuropathy, paraproteins are directed at antigens in the myelin or axonal pellets and may cause the neuropathy.