

Cellular Hypersensitivity to Basic Myelin (P₂) Protein in the Guillain-Barré Syndrome

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The Guillain-Barre syndrome (GB) is an acute demyelinating disease of the peripheral nervous system (PNS). The etiology is not clearly established. An analogy between experimentally produced allergic neuritis (EAN) and the GB (Waksman and Adams, 1955) has suggested an immunological basis for this "most feared of human paralytic diseases" (Asbury, Arnason and Adams, 1969). This comparison has stimulated the search for a specific PNS antigen, sensitization to which results in EAN in animals. The recent discovery of a neuritogenic basic myelin protein by Brostoff and co-workers (1972) is a major step in this direction. Demonstration of hypersensitivity to

such an antigen in GB would greatly strengthen the theoretical concept of the pathogenesis of this disorder.

Intradermal injection of crude peripheral nerve and Freund's adjuvant produces EAN in many species of animals. Early attempts to characterize the neuritogenic component of peripheral nerve were largely unsuccessful. Using PNS basic protein extracts similar to preparations of encephalitogenic factor, Caspary et al (1965) failed to produce experimental disease. Crude pH₃ extracts prepared by Kies, although producing PNS lesions in guinea pigs, did not produce typical EAN (Sheremata and Behan, 1973). However, efforts by Kiyota and Egami (1972) were successful in producing disease in

rabbits with a purified fraction "MP component of P-IV". Inflammatory infiltrates in the CNS of these animals were found as well. Brostoff et al (1972) have recently produced a similar disease in monkeys, but not in rabbits, with a basic protein (P₂ protein) of rabbit sciatic nerve myelin. Isolation of this protein prompted us to determine if cellular hypersensitization to this myelin constituent was present in human disease.

MATERIALS AND METHODS

Macrophage Migration Inhibition Factor (MIF) Assay

The technique of macrophage migration inhibition (Rocklin et al, 1971) was utilized. Peripheral blood lymphocytes were obtained from heparinized venous blood by sedimentation with 5% dextran and incubation of the buffy coat on cotton for 30 minutes at 37°C. 3×10^6 lymphocytes/ml were incubated with and without antigen; crude peripheral nerve homogenate, or A₁ protein. Pooled supernatants collected daily for 3 days were lyophilized and then reconstituted to 1/5 of the original volume with TC¹⁹⁹ containing 15% guinea pig serum. Antigen controls were used in all experiments. Assay for macrophage migration inhibition factor (MIF) was carried out using normal guinea pig peritoneal macrophages in capillary tubes. Migration in MacKannes culture chambers was measured at 24 hours by projection and planimetry. Percent migration was calculated by the formula:

$$\% \text{ Migration} = X/Y \times 100$$

$$X = \frac{\text{Area of Migration in Supernatant with Antigen}}{\text{Area of Migration in Medium with Antigen}}$$

$$Y = \frac{\text{Area of Migration in Supernatant without Antigen}}{\text{Area of Migration in Medium without Antigen}}$$

SUMMARY: Lymphocytes of 29 subjects were assayed for MIF production in response to P₂ peripheral nerve protein, crude human peripheral nerve and human central nervous system A₁ basic myelin protein. Seven were performed in normal control subjects, 12 in Guillain-Barré patients (GB), 5 with other polyneuropathies and 5 in patients with multiple sclerosis (MS). Only GB patients with acute illness produced MIF in response to neuritogenic P₂ protein and crude human nerve. Two MS patients in the acute phase of an exacerbation and one GB patient produced MIF in Response to A₁ protein. The results of this study demonstrate cellular hypersensitivity to a neuritogenic constituent in peripheral nervous tissue and support the concept that this may be important in the pathogenesis of GB.

RÉSUMÉ: Les lymphocytes de 29 sujets ont été vérifiés pour la production de MIF en réponse à la protéine (P₂) du nerf périphérique, à la protéine A₁ du nerf périphérique humain et du système nerveux central humain. Il y avait sept sujets contrôles normaux, 12 patients atteints de Guillain-Barré (GB), 5 avec d'autres polyneuropathies et 5 patients atteints de sclérose en plaques (SEP). Seuls les patients (GB) avec maladie aiguë ont produit du MIF en réponse à la protéine neuritogénique P₂ et au nerf humain. Deux patients avec SEP en phase aiguë d'une exacerbation et un patient GB ont produit du MIF en réponse à la protéine A₁. Les résultats de cette étude démontrent une hypersensibilité cellulaire à un constituant neuritogénique dans le tissu nerveux périphérique et supportent le concept que ce phénomène peut être important dans la pathogénèse de GB.

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Cellular Hypersensitivity to Crude PNS Antigen in PNS Disease and Multiple Sclerosis

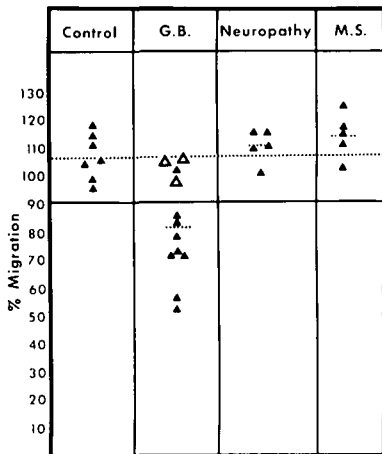


Figure 1—Cellular hypersensitivity to crude peripheral nervous system antigen. (GB = Guillain-Barré syndrome, MS = multiple sclerosis). The mean control migration is indicated by a dotted line across the figure. The line of significance — 2 standard deviations below — is indicated by a solid line. Mean migration for each disease group is indicated by a dotted line in each column. Open circles and/or triangles indicate patients receiving steroid therapy.

The formula used nullifies the effects of cytotoxic factors present in culture supernatants and nonspecific inhibition or stimulation by antigens themselves. All in vitro studies were carried out blindly. An average of 6 determinations was obtained for each culture, with and without antigen, and for each corresponding set of controls (medium with antigen, medium without antigen).

Antigens

1. A partially clarified homogenate of human crude peripheral nerve was prepared as previously described (Rocklin et al, 1971). A dilution of 1:3000 was used in cell cultures.
2. P₂ protein was obtained from defatted rabbit sciatic nerve by acid extraction, gel filtration and chromatography on Cellex B (Brostoff et al, 1972). Difficulty was consistently encountered in obtaining material completely free of A₁ protein antigen. A 10 mcg/ml concentration was used in cell cultures with this antigen.

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3. A₁ protein of human central nervous system was prepared as previously described. A 10 mcg/ml concentration was used in cultures.

Patient Material

Subjects studied were obtained from the Montreal Neurological Hospital and associated institutions*. Seven sets of normal control subjects, twelve sets in seven Guillain-Barré patients and five sets in other neuropathies were obtained. Five were performed in multiple sclerosis subjects.

Normal Control Subjects — Seven volunteers aged 18 to 40 years were selected for assay.

Subjects with Polyneuropathies — Seven Guillain-Barré patients varying in age from 13 to 40 and five subjects with other polyneuropathies aged 20 to 39 (two alcoholic and three diabetic neuropathies) were studied.

Eight assays in seven patients with Guillain-Barré syndrome were obtained within 3 weeks of onset, and five in the fourth to the eighth week of illness. Three acutely ill patients suffered respiratory paralysis; one died in the third week of his illness. The latter patient was on steroids at the time of the repeat assay. A second patient was on steroids in the second week of his illness, when assayed.

Multiple Sclerosis — Five patients with multiple sclerosis aged 21 to 35 were also assayed with each of the antigens. Two patients with multiple sclerosis were studied, one during the first, and the other during the second week of an exacerbation (the "acute" patients), and three had not had an exacerbation within 6 months of study.

RESULTS

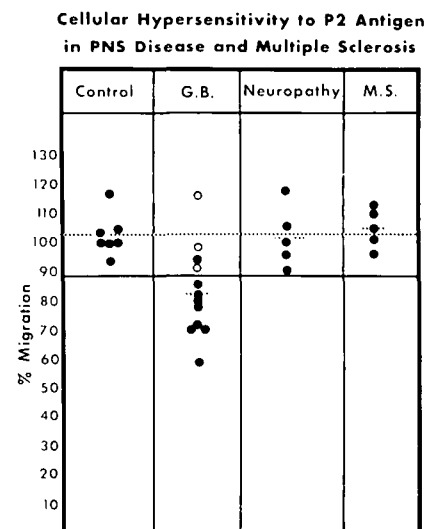
Results for peripheral nerve antigens are shown in figures 1 and 2. Mean migration index in normal subjects was 107 ± 7.8 for PN, and 103 ± 72. for P₂. The groups of

*St. Mary's Hospital, Montreal General Hospital and The Queen Mary Veterans' Hospital.

Guillain-Barré patients obtained results of 82 ± 18.9 and 83 ± 15.5 respectively. GB patients with acute illness and not on steroid therapy gave values of 72 ± 12 for PN and 74 ± 8.7 for P₂. Subjects with miscellaneous neuropathies gave values of 111 ± 6.1 for PN, and 102 ± 10.6 for P₂. Multiple sclerosis patients' results were 115 ± 8.1 and 105 ± 6.4 respectively. Of all subjects tested only those with Guillain-Barré showed evidence of sensitization to PN on P₂ antigen. No significant difference in sensitization to either peripheral nervous system antigen was apparent in any group of subjects.

Migrations for cultures with A₁ protein are shown in figure 3. Control subjects gave a mean migration index of 101 ± 7.3. GB patients gave a mean of 98 ± 9.5, miscellaneous neuropathies 103 ± 12.4 and multiple sclerosis (MS) patients 89 ± 25.1. One GB patient and both of the MS patients in acute exacerbation yielded highly significant results.

Figure 2—Cellular hypersensitivity to P₂ antigen. (GB = Guillain-Barré syndrome, MS = multiple sclerosis). The mean control migration is indicated by a dotted line across the figure. The line of significance — 2 standard deviations below — is indicated by a solid line. Mean migration for each disease group is indicated by a dotted line in each column. Open circles and/or triangles indicate patients receiving steroid therapy.



Cellular Hypersensitivity to A₁ Antigen in PNS Disease and Multiple Sclerosis

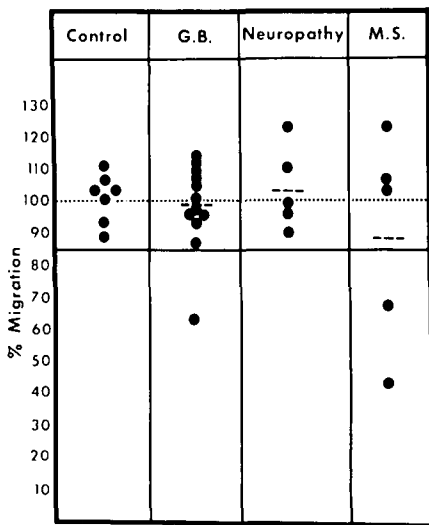


Figure 3—Cellular hypersensitivity to A₁ antigen. (GB = Guillain-Barré syndrome, MS = multiple sclerosis). The mean control migration is indicated by a dotted line across the figure. The line of significance — 2 standard deviations below — is indicated by a solid line. Mean migration for each disease group is indicated by a dotted line in each column.

Assay results of patients with the Guillain-Barré syndrome generally correlate with the acuteness of the illness. Lymphocytes of a patient with incipient respiratory paralysis, assayed on the second day of the neurological illness, gave a result of 54 in response to crude peripheral nerve, 60 with P₂ myelin protein, and 97 with myelin A₁ protein. Cells of another patient with respiratory paralysis, studied on his fourth day of illness, gave values of 57, 70, and 103 with crude nerve, P₂ protein, and A₁ protein respectively. A third patient with respiratory paralysis gave values of 70, 71, and 106. Lymphocytes of patients studied on their fourteenth, twentieth, and twenty-first day of illness responded with significant, but less striking, inhibition of migration. Results from three patients on steroids obtained on the seventh, fourteenth, and thirtieth days of their illness were not significant. A second set of assays on one of the patients was obtained on the fourteenth day of his illness. Repeat assays in a patient 4 weeks

after onset were again positive with the peripheral nervous system antigens. Results in another patient convalescing at the end of her eighth week of illness were negative for all antigens. Five patients recovered without significant deficit, one patient has been left with bilateral drop foot, and one died. Pathological findings in this case were typical of severe and extensive involvement with the Guillain-Barré syndrome.

DISCUSSION

Cellular hypersensitization to pure P₂ protein has been clearly documented in the Guillain-Barré syndrome. Particularly significant is the similar degree of sensitization seen using either crude human or purified rabbit P₂ antigen. Only lymphocytes from GB patients with active disease produced MIF. Of 3 subjects on steroids only 1 gave a significant result, on the third day of illness, but not a week later. A negative result was also seen in a boy with relapsing Guillain-Barré, who was asymptomatic at the time of assay. Subjects with other neuropathies and MS gave uniformly negative assays. Significant hypersensitization to a pure neuritogenic PNS antigen equivalent to that seen with crude preparations has been documented in the Guillain-Barré syndrome for the first time.

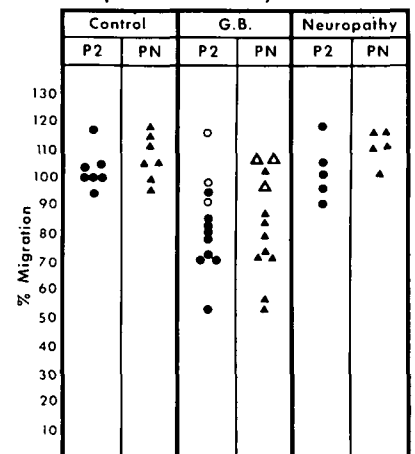
Asbury et al (1969) have emphasized the analogy between EAN and the Guillain-Barré syndrome. The pathology is similar in both, with perivenular lymphocytic infiltrates appearing in nerve early in the disease. Immunoblasts in peripheral blood are seen in EAN and GB. Sensitivity to peripheral nervous system antigen was seen in both, as determined by demyelination activity of lymphocytes in rat trigeminal ganglion cultures. Behan et al (1969, 1972), and Currie and Knowles (1971), using the technique of lymphoblastic transformation, demonstrated delayed (cellular) hypersensitivity to PN and an acid extract of sciatic nerve. However, the material used by Currie and Knowles does not produce disease in experimental animals, hence its relevance is not clear. We have established the an-

tigenicity of our extracts of human nerve (Sheremata and Behan, 1973; Rocklin et al, 1971), and rabbit P₂ protein (Brostoff et al, 1972). In a previous study, using the present assay system, we have demonstrated hypersensitivity to PN in this disease (Rocklin et al, 1971). Despite the apparent potential for producing CNS lesions in monkeys, in our present study we did not observe any positive results in MS, as we have with CNS basic A₁ myelin protein (Rocklin et al, 1971; Levine and Wenk, 1963).

In control subjects and patients, lymphocytes cultured with A₁ protein gave results similar to data obtained in earlier studies. A GB patient who exhibited a significant result displayed psychotic behaviour during her attack, but other evidence of central nervous system involvement was not documented. Psychotic behaviour is occasionally seen and 7 fatal cases have been studied by Haymaker and Kernohan (1949). Currie and Knowles have correlated clinical central nervous system involvement and in vitro evidence of hypersensitivity to "encephalitogenic factor" (A₁ protein) in GB patients. The sensitivity to A₁

Figure 4—Comparison of MIF production in response to crude PN and P₂ antigens in peripheral nervous system disease. (GB = Guillain-Barré syndrome, MS = multiple sclerosis). Open circles and/or triangles indicate patients receiving steroid therapy.

Comparison of MIF Production in Response to Crude PNS and P₂ Antigens in Peripheral Nervous System Disease



protein in our patient may suggest such involvement, although Haymaker and Kernohan did not observe this. The highly significant results obtained in two multiple sclerosis patients studied within a week of onset are consistent with our earlier studies (Rocklin et al, 1971; Sheremata, Cosgrove and Eylar, 1974).

The P₂ protein is one of the major components of PNS myelin, along with the A₁ protein and the P₀ protein. Studies indicate that it comprises from 5-15% of the total protein of PNS myelin, depending on animal species and perhaps anatomical localization. The P₂ protein has a molecular weight near 12,000 and contains 19% basic residues (moles %). Amino acid composition differs markedly from the A₁ protein. The P₂ protein contains more hydrophobic and less histidine and proline residues. Certain features suggest secondary and tertiary molecular structure. There are, however, similarities to certain sequences of A₁ protein, including the encephalitogenic tryptophan region.

In the experimental situation, sensitization with appropriate amounts of crude nerve produces EAN. Freund's adjuvant is not essential in all species (Levine and Wenk, 1963) and the use of large amounts of nerve may result in the production of CNS lesions. A₁ protein normally present in peripheral nerve could be liberated by processing crude nerve antigen, and EAN might be expected if sufficiently large amounts of such material were to be used. Contamination of P₂ preparations with A₁ also could account for CNS lesions in sensitized animals. Alternatively, this phenomenon may be related to the presence of encephalitogenic sites on the P₂ molecule, normally masked by other constituents of PNS myelin. Whatever the explanation for central nervous system lesions in experimental animals, the P₂ preparation used did not stimulate MIF production in any MS lympho-

cyte cultures despite the fact that two acutely ill patients produced highly significant amounts with A₁ protein.

The reason for hypersensitization to P₂ protein in GB is not apparent. Onset of symptoms may follow viral infections, but no one virus or group of viruses has been specifically implicated. Other factors including drugs and trauma may precipitate this disorder. Genetic factors have not been reported as being important. However, GB is unusually common in the South African Black population. Sensitization was not seen in other neuropathies where it might have been anticipated if the observation in GB was purely a secondary phenomenon. Negative results in MS patients were of interest since we have shown sensitization to A₁ in this group of patients. This observation makes it less likely that our preparation contained significant amounts of the A₁ protein.

Our report supports the clinical separation of a distinct group of polyneuropathies in which immunological parameters are operative. The demonstration of cell mediated hypersensitization to a specific protein constituent of peripheral nerve, capable of producing experimental disease (experimental allergic neuritis), similar to the Guillain-Barré syndrome, is strong evidence for hypersensitization in the pathogenesis of the Guillain-Barré syndrome. Sensitization might result from similarities of protein sequences in myelin and viral nucleocapsid basic proteins or from the inclusion of myelin protein in the envelope of infecting viruses. The resulting immune response may thus be directed against that myelin. More observations will be necessary to support the present data. Studies of human peripheral basic myelin and viral nucleocapsid as well as other basic proteins by biochemical and immunological techniques will be necessary to support or refute such an hypothesis.

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