Lymphocyte sensitzation to nervous tissues and muscle in patients with the Guillain-Barré syndrome

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SUMMARY By means of an electrophoretic method lymphocytes from patients with the Guillain-Barré syndrome ('acute idiopathic polyneuritis') have been shown to be sensitized to both encephalitogenic factor (EF) and a similar basic protein prepared from human sciatic nerve (SNBP). Sensitization was more marked in the acute stage of the disorder during which there also appeared to be a degree of sensitization to muscle. The results are consistent with the view that lymphocytic infiltration of peripheral nerves in the condition is of pathogenetic significance.

Evidence has accumulated which suggests that lymphocyte-mediated hypersensitivity plays a part in the Guillain-Barré (G-B) syndrome (Asbury, Arna-son, and Adams, 1969). In an earlier communication we reported that lymphocytes from patients with this disorder were stimulated on culture with basic protein derived from human sciatic nerve (SNBP) and that the phenomenon persisted long after clinical resolution of the syndrome (Knowles, Saunders, Currie, Walton, and Field, 1969). This paralleled the persistence of lymphocytic infiltration in peripheral nerves for years after the acute syndrome (Asbury et al., 1969). No stimulation occurred on incubation with similar basic protein (encephalitogenic factor (EF) derived from central nervous tissue. The technique, which depends upon prolonged survival of lymphocytes in culture, presents several pitfalls which, despite the widespread use of the method, have only now been systematically studied (Hughes and Caspary, 1970). A more reliable and sensitive method has now been developed (Caspary and Field, 1970); it depends upon electrophoretic slowing of guinea-pig macrophages in the presence of lymphocytes which have been stimulated by incubation with a specific antigen. This method has now been applied to the study of lymphocytes from patients with G-B syndrome including some of those featured in the earlier communication (Knowles et al., 1969).

MATERIALS AND METHODS

There were sixteen patients with the G-B syndrome. Some clinical features of these cases are given in Table 1. The mean period over which the syndrome had evolved from onset to the point of maximum deficit was two weeks. Infection, usually respiratory, preceded the disorder in 10 patients. Other antecedent factors were surgery, the puerperium, and neoplasia, each in a single case. The amount of protein in the cerebrospinal fluid was increased in 12 out of the 15 patients in whom an estimation was made. A demyelinating neuropathy was suggested by a marked reduction in motor nerve conduction velocity in 10 of the 14 patients in whom neurophysiological studies were carried out. Sural nerve biopsy was performed in three patients and showed segmental demyelination. Steroid therapy (ACTH or prednisone) was followed by improvement in the eight patients so treated. Only one (no. 11) was receiving prednisone when blood was taken for testing. In five patients (nos. 1 to 5) the lymphocytes were studied during the acute stage of the syndrome. One of these (no. 2) was suffering from his third relapse in 25 years. Muscular atrophy was apparent in all five cases. In the remaining 11 patients the disorder had occurred between 0.6 and 4.5 years before the sampling of blood, with a mean interval of 1.9 years. In seven patients a deficit remained which was static in two and resolving in the other five. However, no neurogenic atrophy was noted in any of these 11 patients at the time of study.

BLOOD LYMPHOCYTES Lymphocytes were prepared from defibrinated venous blood by removal of polymorphs with saccharated iron and sedimentation with methyl cellulose (Hughes and Caspary, 1970). This gave a yield of about 10^6 lymphocytes/ml. blood.

GUINEA-PIG MACROPHAGES A macrophage rich exudate was obtained from guinea-pig peritoneal cavity by wash-
ing out with heparinized Hanks' solution (5 u/ml) eight
to 12 days after injection of 20 ml. sterile liquid paraffin.
The exudate was washed in heparinized Hanks' solution,
then in Hanks' solution without heparin (250 g for 10
minutes), and finally suspended in 10 ml. of medium 199.
The cells were counted and the volume adjusted to give
10⁷ macrophages/ml. This macrophage suspension (con-taining
10 to 20% of lymphocytes) was irradiated with
100 r (cobalt 60γ rays) in order to eliminate reactivity of
contaminating lymphocytes and so obviate a 'mixed
lymphocyte' reaction.

ANTILYMPHOCYTIC SERUM The rabbit anti-guinea-pig
lymphocyte serum which was used was that previously
shown to afford marked protection against the develop-ment of experimental allergic encephalomyelitis (Field,
1969), and to block antigen/lymphocyte interaction
(Field, Caspary, Hall, and Clark, 1970). Agglutination
titre of this serum was 1:1200.

ENCEPHALITOGENIC FACTOR (EF) The encephalitogenic
factor which was used was prepared from fresh young
human brain (subject blood group O) (Caspary and Field,
1965).

BASIC PROTEIN FROM PERIPHERAL NERVE (BP) Basic
protein from human sciatic nerve was prepared in the
same way as EF. It was weakly neuritogenic in guinea-
pigs when used with the same proportion of Freund's
adjuvant as served to produce encephalitis when given
with EF (but it is possible that different proportions of
adjuvant and concentrations of BP might show a higher
pathogenicity).

MUSCLE Human erector spinae muscle (20% w/v)
was homogenized in saline and centrifuged at 250 g for
30 minutes. The supernatant fluid was used as muscle
antigen.

PROCEDURE The tests for sensitization of peripheral
lymphocytes were carried out as follows. 10 x 10⁶
irradiated guinea-pig macrophages in TC 199 were mixed
with 0-5 x 10⁴ human peripheral lymphocytes and the
total volume made up to 3 ml. with TC 199. Antigen in
0-1 ml. volume (EF 100 μg; BP 100 μg; Muscle supernatant
from 20% (w/v)) was added and the mixture incubated
at room temperature for 90 minutes before the electrophoretic mobility was measured.

ELECTROPHORETIC MEASUREMENTS Electrophoretic mobili-
ty was measured in a Zeiss cytopherometer. Each spec-
mimen was gently agitated and examined for cell aggre-gation before introduction into the cytopherometer.

Changes in migration time of 10 cells was measured to the
nearest 0-1 second, each in both directions of the potential
difference (180 V at 7-5 mA) and average values derived
from 20 readings were calculated. Changes in migration
time were expressed as a percentage of the control time-
that is, that of macrophages in the presence of human
blood cells under test without addition of antigen. All
measurements were made 'blind', the results being later
uncoded.
RESULTS

Table 1 shows sensitization of peripheral lymphocytes to antigens of both central and peripheral nervous origin (P < 0·001) in the G-B syndrome when compared with control subjects. There was a significant difference between cases in the acute stage and those after recovery in the response to EF (P < 0·001) and to SNBP (P < 0·001). Acute cases also showed a response to muscle not shown in those who had recovered (P < 0·001). However, the number of acute cases was small and these findings must be treated with caution. In the cases after recovery there was no correlation with the time which had elapsed since the acute syndrome.

The response to all three antigens was abolished by ALS (Table 2) confirming the immune nature of the reaction (Field, Hughes, and Caspary, 1969).

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Mobility reduction (%)</th>
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<tbody>
<tr>
<td>EF</td>
<td>20·5 (2) 10·0 (3)</td>
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<tr>
<td>ALS + EF</td>
<td>13·8 (8)</td>
</tr>
<tr>
<td>SNBP</td>
<td>27·6 (2) 13·4 (9)</td>
</tr>
<tr>
<td>ALS + SNBP</td>
<td>1·3 1·5</td>
</tr>
<tr>
<td>Muscle</td>
<td>12·9 (2) 17·7 (1)</td>
</tr>
<tr>
<td>ALS + Muscle</td>
<td>0·1 3·4</td>
</tr>
</tbody>
</table>

Figures in parentheses refer to patients in Table 1.

DISCUSSION

The results suggest that lymphocytes from patients with the G-B syndrome are sensitized to protein of both central and peripheral neural origin. The response to central protein is not surprising, since infiltrates of mononuclear cells and destructive changes are known to occur within the central as well as in the peripheral nervous system (Schmaus, 1904), though such changes are rarely prominent or of clinical significance (Haymaker and Kernohan, 1949). Failure to find stimulation by EF in our previous work (Knowles et al., 1969) probably resulted from the relative insensitivity of the method and the technical vagaries to which it is subject (Hughes and Caspary, 1970). However, Behan, Lamarche, Feldman, Behan, and Kies (1969) did record stimulation with central protein, though in their cases a response to either central or peripheral protein did not persist after resolution of the clinical syndrome. In the present cases sensitization persisted long after recovery, though reduced in degree. In the active phase, during which denervation atrophy was progressing, lymphocytes which were sensitized to muscle antigen were also present, though not subsequently. The apparent sensitization to muscle was probably a secondary phenomenon and illustrates once again the difficulty in deducing a pathogenetic role from findings of antibodies and cellular sensitization in ‘autoimmune’ disorders.

REFERENCES


