Cellular hypersensitivity to brain antigen in children of a family with hereditary ataxia

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SUMMARY Sensitisation to brain antigen was demonstrated in eight of 24 clinically normal first generation children in a family with hereditary ataxia. This ratio is consistent with that expected in a dominantly inherited condition. It suggests that immunological reactivity may precede the clinical expression of disease, with important implications for presymptomatic diagnosis and for pathogenesis of degenerative disease.

A new form of hereditary ataxia with defective vestibular function was reported recently by Philcox et al. (1975). It has several features which distinguish it from that previously described—notably, defective optokinetic nystagmus and abnormal or absent oculovestibular responses in all affected patients. The first symptom is usually unsteadiness of gait, intermittent in the early stages, but progressive over several years to total incapacity. This is associated with hypertonicity, hyperreflexia, and dysarthria which progresses to the extent that speech often becomes unintelligible. There is usually no sensory deficit. Most severely affected patients have intellectual impairment. First symptoms appear between the ages of 20 and 52 years.

The problem arose during management of this family of finding a method for identifying affected individuals before the disease expressed itself clinically. This has important implications for genetic counselling since the condition may not become apparent until after it has been transmitted to the next generation. We set out to detect sensitisation in affected individuals, and followed this by looking for it in clinically normal children, some of whom would be expected to develop the disease later in life. These findings may be the first demonstration of sensitisation to components of nervous tissue preceding the onset of clinically apparent neurological disease. They could have important implications for the pathogenesis of this and other chronic diseases such as Huntington’s chorea and multiple sclerosis.

Subjects and methods

Seven of the nine severely affected individuals in this family were studied, as well as 24 of their 29 children, all of whom were asymptomatic and normal on examination. Ages of the children ranged from 8 to 32 years. Of the five children not tested, one was known to be affected, another had died from unknown causes at a young age, and the remaining three could not be traced, or refused to undergo investigation. Nineteen healthy medical and laboratory staff between 20 and 45 years of age acted as control subjects, some of them on several occasions.

Antigen Preparation

Antigen was prepared by homogenisation of fresh whole rat brain in a ground glass tissue homogeniser. A 1% w/v suspension was made up in medium 199 (GIBCO) with 0.3 g/100 ml tris buffer (Fluka) and stored at −20°C in aliquots.

Assay for Leucocyte Migration Inhibition Factor

Leucocyte migration inhibition (LMI) was performed according to the method of Søborg and Bendixen (1967). Heparinised venous blood was sedimented with 5% dextran (mol wt 250 000) in saline at 30°C for 45 minutes. Leucocytes were washed three times and suspended in tris buffered medium 199 containing 10% horse serum (Burroughs Wellcome), penicillin 200 units and strepto-

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mycin 100 µg/ml. Cells were packed in capillary tubes and sealed with Cristaseal (Hawksley). After 20 hours incubation, areas of cell migration were projected with a photographic enlarger and measured by planimetry. Usually eight migrations (four chambers) were obtained for each antigen and 10 migrations for each control. Where less than six migrations were obtained the results were discarded.

Migration index = A/C where
A = area of migration with antigen
C = area of migration without antigen

Reproducibility of the method was established by repeating the study on seven control subjects and one patient up to four times on separate samples and on different days. Mean coefficient of interassay variation for each individual was 3.8%.

OTHER IMMUNOLOGICAL MEASUREMENTS
Sensitisation to rat brain antigen was also studied in affected individuals by passive haemagglutination (Ling, 1961) and by indirect immunofluorescence using frozen sections of rat brain as substrate (Edgington and Dalessio, 1970). Sheep antihuman IgG labelled with FITC (Burroughs Wellcome) was used in 1:60 dilution. Sera were diluted 1:10 in veronal buffered saline (pH8.6) with 0.3% triton X100.

The immunological status of patients was assayed by standard techniques. Delayed hypersensitivity was assessed by intradermal injections of common antigens (PPD 10 TU, Candida albicans, mumps, and streptokinase). Immunoglobulins G, A, and M, and complement components C3 and C4 were measured by radial immunodiffusion (Partigen plates, Behringwerke), total haemolytic complement by sheep red cell lysis (Mayer, 1961), rheumatoid factor by latex fixation (Singer and Plotz, 1956), and auto-antibodies by indirect immunofluorescence. Antibody responses to influenza vaccine were measured by haemagglutination inhibition.

Results

MEASUREMENTS IN CLINICALLY AFFECTED INDIVIDUALS
Using rat brain antigen preparation at varying concentrations, migration indices (MI) in six control subjects varied between 0.80 and 1.00 (Fig. 1). Of seven patients, four showed marked inhibition of migration at 0.1% w/v antigen which became less marked with increasing dilution. The remaining three showed stimulation of migration in at least two of three antigen concentrations. Responses of the patients showing inhibition and stimulation of migration both differed significantly from control subjects at 0.1% antigen concentration (P<0.005).

No evidence could be adduced for antibody production to neural antigens by passive haemagglutination. Indirect immunofluorescence of rat brain sections showed only nonspecific staining of ependymal cells and inconsistent streaky fluorescence which did not differ from that seen with serum from normal control subjects. There was also no evidence that affected individuals showed any consistent immunological abnormalities. All

![Figure 1](http://jnnp.bmj.com/)  
**Figure 1** Leucocyte migration inhibition (LMI) in affected individuals and control subjects at different concentrations of rat brain antigen. Horizontal lines at 0.8 and 1.0 migration indices (MI) represent mean ±2 standard deviations (SD) of normal values. Open circles represent normal control subjects, and closed circles affected individuals.
subjects produced delayed hypersensitivity reactions to at least one of four common antigens used in skin testing. Quantitative immunoglobulin levels were normal. Six of seven patients showed a rise in influenza antibodies after immunisation. One patient showed smooth muscle antibody in low titre of 1:40.

**MIGRATION INHIBITION IN UNAFFECTED CHILDREN**

In the next experiment we compared LMI in the 24 unaffected first generation children with results in 19 healthy medical and laboratory staff aged between 20 and 45 years. We elected to use 0.1% rat brain extract as antigen since this preparation provided the clearest discrimination between patients and healthy control subjects. Migration indices outside two standard deviations of the mean of 28 control values (0.86 to 1.10) were taken to indicate hypersensitivity. Eight children showed abnormal LMI with stimulation in five and inhibition in three (Fig. 2), and there was no overlap of results with values obtained in normal control subjects (P<0.001 comparing normal control subjects with children showing inhibition of migration, and with those showing stimulation of migration). Ages of children showing hypersensitivity to brain antigen ranged from eight to 32 years and were of similar distribution to siblings without hypersensitivity.

**Discussion**

Immunological reactivity to components of nervous tissue has been demonstrated in a variety of neurological diseases including multiple sclerosis (Bartfeld and Atosyn, 1970; Rocklin et al., 1971; Alvord et al., 1974) and the Guillain-Barré syndrome (Sheremata et al., 1975), and the concept has arisen that autoimmune mechanisms are important in the pathogenesis of these diseases. It is difficult to deny the alternative explanation, however, that immunological reactivity may be secondary to pathological changes brought about by other mechanisms. For example, sensitisation to nervous tissue antigens can be detected after strokes (Rocklin et al., 1971; Alvord et al., 1974), and encephalitis (Behan et al., 1968; Nyerges et al., 1974). Such an issue is almost impossible to resolve in diseases like multiple sclerosis and Guillain-Barré syndrome because it cannot be predicted which individuals will develop the disease at some future time. Sheremata et al. (1975) attempted to answer this question in the case of the Guillain-Barré syndrome. They observed that maximal sensitisation to peripheral nerve antigens occurred soon after diagnosis and that it diminished with recovery. From this they extrapolated that hypersensitivity may precede the onset of the clinical picture.

The finding of sensitisation to nervous tissue antigen in affected members of this family is similar to findings in experimental allergic encephalitis and neuritis in animals (Waksman and Adams, 1955; Paterson, 1966) and in multiple sclerosis and the Guillain-Barré syndrome in man. However, we are not aware that hypersensitivity has been demonstrated in normal subjects who may be expected to develop disease in the future. Being dominantly inherited, about half of first generation children in this family might be expected to develop the illness. Our finding of abnormal results in eight out of 24 children is consistent with this ratio when allowance is made for the small size of the sample and the fact that five children, at least one of whom is known to be affected, could not be examined.

Leucocyte migration inhibition is regarded as a reliable in vitro index of sensitisation to a variety of antigens in man (Søborg and Bendixen, 1967; Ramsay et al., 1976). Leucocyte migration may be stimulated in conditions of low antigen concentra-
ation (Nelson, 1969) and lesser sensitivity of cells (Søborg, 1971). Søborg (1971) observed a biphasic migration pattern with inhibition at high antigen concentrations and stimulation with decreasing concentrations, and he considers that both inhibition and stimulation of migration indicate cellular hypersensitivity in vitro. A number of affected subjects and their children showed significantly increased migration at some concentrations of antigen, and these results were taken to indicate hypersensitivity in this study. This pattern was not observed in any normal control subjects. Purification of the tissue extract would be expected to increase antigen concentration and to lead to more consistent inhibition.

Clearly, long-term follow-up will be required to determine whether children in whom sensitisation to brain has been demonstrated, will develop clinical disease. If this proves to be the case the implications would be of considerable interest.

It could prove to be a marker for identifying affected individuals before the disease becomes clinically apparent. Allowance needs to be made for variability in expression of the disease and in its rate of progression, but these studies could be of great value for genetic counselling. Abnormal oculovestibular responses were demonstrated in one young asymptomatic member of the family, and it was hoped that they would be of value as a genetic marker. However, investigation of other normal family members failed to reveal any more cases with this abnormality.

It could also be the first demonstration that hypersensitivity to neural antigens precedes the onset of the clinical syndrome. This raises the possibility that cell-mediated immune mechanisms may be implicated in development of the disease. For example, some genetically determined biochemical or structural abnormality of nervous tissue may provoke an immunological reaction which is responsible to a variable degree for progression of the pathological process. We have not had an opportunity for studying pathological changes in this condition. The amount of information yielded by such studies might be disappointing unless death occurred early in the course of the disease. With progression, widespread pathological changes would be expected which could obscure the primary process.

Another approach to identifying a primary defect would be to use different fractions of rat brain extract in order to show which antigens are responsible for sensitisation. This information has not been looked for in these preliminary studies.

It remains to be seen whether similar observations pertain to other hereditary neurological diseases such as Huntington's chorea. In vitro sensitisation to antigen derived from brains of patients dying with Huntington's chorea was recently reported by Barkley et al. (1977) in patients with this disease. However, children and unaffected family members were not studied. If the implications of our findings can be substantiated, a new tool may become available for genetic counselling, and a new approach to these hopeless diseases may be offered by methods aimed at suppressing or deviating the immune response.

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References


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