Direct Leukocyte Migration Inhibition In Multiple Sclerosis — A Possible Assessment of Activity

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SUMMARY: *Twenty-four patients with multiple sclerosis were evaluated and classified according to their clinical state. Specific migration inhibition studies were carried out on blood samples from each using myelin basic protein, an acid soluble protein fraction isolated from normal human CNS white matter, and multiple sclerosis myelin basic protein isolated from patients who had the disease, as the antigenic material. This test system employed selected media. Results were compared with those of normal controls and patients with other neurological disease states. Antigen concentration of 500 μ g/ml in serum free medium combined to produce the greatest inhibiting effect on leukocytes in patients with apparent multiple sclerosis and differentiated these patients from those in the other groups tested.*

Leukocytes in patients who had probable multiple sclerosis with partial impairment, signifying possible current activity of the disease, were especially inhibited as compared to the leukocytes from other groups tested against myelin antigen. Cerebrospinal fluid from affected patients when used as a media enhanced the test.

This study suggests that migration inhibition of peripheral leukocytes using myelin protein may be useful in the diagnosis of patients with multiple sclerosis. There is additional evidence that the degree of leukocyte migration inhibition may reflect activity of the disease with its consequent implications on treatment and prognosis.

RÉSUMÉ: *Vingt-cinq patients atteints de sclérose en plaques furent évalués et classifiés selon leur état clinique. Des études de l'inhibition spécifique de la migration ont été faites sur des échantillons de sang de chacun en utilisant comme matériel antigénique la protéine myélinique de base, une fraction d'une protéine acide soluble isolée de la matière blanche du SNC d'un humain normal, et la protéine myélinique de base de patients ayant la sclérose en plaques. Des milieux pré-sélectionnés furent utilisés pour ces tests. Les résultats furent comparés avec cèux de contrôles normaux et de patients avec autres maladies neurologiques. Une concentration d'antigène de 500 μ g/ml dans le milieu libre de sérum produisit le plus grand effet inhibiteur sur les leukocytes de patients avec des signes apparents de sclérose en plaques et différenciait ces patients de ceux des autres groupes analysés.*

Les leukocytes des patients ayant probablement la sclérose en plaques avec atteinte partielle, signifiant une activité probable de la maladie, étaient spécialement inhibés en comparaison avec les leukocytes des autres groupes analysés contre l'antigène myélinique. Le liquide céphalo-rachidien des patients atteints, quand il était utilisé comme milieu, augmentait la positivité du test.

Cette étude suggère que l'inhibition de migration des leukocytes périphériques par la protéine myélinique peut être utile dans la diagnostic de patients avec sclérose en plaques. Le degré d'inhibition de la migration des leukocytes peut refléter l'activité de la maladie.

INTRODUCTION

Multiple sclerosis is a chronic and usually relapsing disease of the central nervous system commonly beginning in adult life and characterized by signs and symptoms pointing to multiple episodes of randomly located lesions of the central nervous system. It is pleomorphic in nature, but usually the diagnosis becomes apparent after a variable clinical course. Specific diagnostic tests have yet to be developed.

The resemblance to other hypersensitivity diseases such as allergic encephalomyelitis (Behan, 1973), the occurrence of elevated CSF globulins, some of which are oligoclonal (Olsson, 1973), increased incidence of the disease in persons with specific HLA haplotypes (Paty, 1974) and responses to massive immunosuppression (Rose, 1974) is strong evidence that an immunological event is critical in this disease.

A recent symposium (Bergman, 1974) emphasized the difficulties of diagnosis and classification of this disease. The problems are the insidious and relapsing nature of the disease plus the difficulty in assessing the activity of the process at any clinical stage.

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Clinical classifications, although useful in management, cannot equate activity which must be a basis of treatment. A technique, first described by Bendixen and Søborg, 1969, using leukocyte migration inhibition was used with myelin basic protein and multiple sclerosis myelin protein as the antigens and tested on patients in separate clinical categories. Results were compared with a variety of control sera.

MATERIALS AND METHODS

Twenty-four adult patients with diagnosed multiple sclerosis (of whom seventeen were females) were assessed, using the criteria previously outlined (McAlpine, 1972; Gilland, 1965; Offner, 1974) as an index of disability. These patients were divided into three categories; probable multiple sclerosis (20 patients), possible multiple sclerosis (3 patients) and latent probable multiple sclerosis (1 patient). (Table 1)

The probable group which contained the largest number of patients (20/24) showed multiple lesions, remitting in character, with partial or severe impairment. Of these 13/20 patients had severe disability requiring considerable help and in many instances were bedridden. The time of onset was always in excess of five years. The remaining seven patients had partial disability impairment, either managing by themselves with difficulty or needing a small degree of help; the time of onset being in excess of two years.

TABLE 1 — *Clinical Classification of Multiple Sclerosis Related to Percent Migration.*

Classification		Number of Patients	Percent Migration (Average)
I	PROBABLE Multiplicity of lesions, remitting character, partial or severe impairment.	13 severe / 20 \ 7 partial	78 / 43
II	LATENT PROBABLE More than one bout, transient character, slight or no impairment.	1	81
III	POSSIBLE Monofocal lesion, remission not mandatory, at least some impairment.	3	74

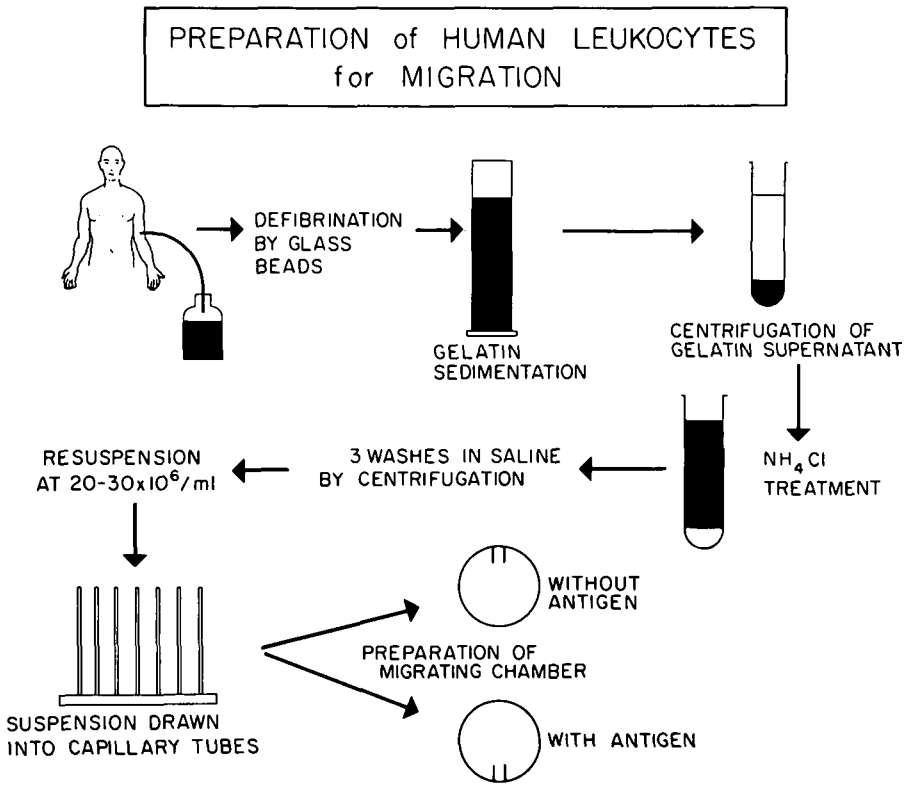

Figure 1— Preparation of human leukocytes for migration.

Possible and latent probable categories (4/24) were grouped together as they had slight or no disability at the time of testing. Symptoms were typical but mild. All managed themselves without difficulty and had symptoms in excess of four years.

In order to control this study blood samples from six patients with recent cerebrovascular events and eight healthy individuals were also assessed. Some patients were on medications which did not include corticosteroids or immunosuppressive agents.

LABORATORY METHODS

The method used for leukocyte migration inhibition studies was a modification of the original capillary tube migration system described by Bendixen and Søborg in 1969. (Falk, 1973)

Peripheral blood leukocytes from multiple sclerosis patients and normal controls, as well as patients with other neurological diseases were tested for their response to various concentrations of appropriate antigens in three systems (serum free, serum free supplemented with autologous serum and serum free supplemented with cerebrospinal fluid from multiple sclerosis patients). (Figure 1)

White blood cells were separated from defibrinated blood by sedimentation with 3% gelatin. (Coulson, 1964) The white blood cells were washed three times with isotonic saline after lysing the red cells with 0.85% ammonium chloride. Leukocyte concentration was adjusted to 20 - 30 x 10^6 cells per ml with medium consisting of NCTC 109 (DIFCO) buffered with 7.5% sodium bicarbonate with added Penicillin 10^5 u/litre (basic NCTC 109).

Microhematocrit capillary tubes (1.1 - 1.2 x 70 mm. - John's Scientific) were filled with this cell suspension, one end sealed with Sealease (Clay Adams) and spun at 400 r.p.m. for 5 minutes. The tubes were

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cut at the cell fluid interface and the cell containing portion placed in a culture chamber (Planchette, 20 mm. in diameter, Universalex, AB Sweden) and fixed to the side of the planchette by means of a silicone lubricant grease.

Leukocyte reactivity was measured using the following culture conditions:

1. Serum free - i.e. basic NCTC 109
2. 10% autologous serum (heat inactivated) with basic NCTC 109
3. 10% CSF (from 3 multiple sclerosis patients, clinically established and on no medication) with basic NCTC 109.

Myelin basic protein at 5 mg/ml saline was diluted to final concentrations of 500 μ g., 100 μ g., 50 μ g., and 10 μg/ml. media in planchettes. It had been prepared by mercaptoethanol extraction of myelin followed by 0.2 N sulphuric acid and the supernatant precipitated with 30% ethanol to form an acid soluble protein. (Gagnon, 1971) This myelin basic protein was obtained through the generosity of Dr. M. A. Moscarello, Hospital for Sick Children, Toronto, Ontario. Multiple sclerosis myelin protein prepared in the same manner was derived from patients who had multiple sclerosis.

BCG (Connaught) was reconstituted in 1 ml. of diluent and incorporated in planchettes at final concentrations of 10 μ g/ml. medium. Antigen was added using separate culture conditions listed above and the planchette was covered with a round cover glass (Corning No 2), done in triplicate and incubated at 37°C. for 18 hours in a 5% CO_2/95% air humidified incubator. The outer border of the cell fan was traced onto paper by projection microscopy, cut out and weighed. (Figure 2)

RESULTS

The data showed that the major leukocyte migration inhibition effect was at the 500 μ g/ml in myelin basic protein in the serum free media.

Figure 3 is a comparison of migration inhibition using myelin basic protein antigen in patients with multiple sclerosis on the left, other forms of CNS injury (mostly CVA) in the center and controls on the right. This is analysed using 500 μ g/ml myelin basic protein in serum free media and bars descending from the 100% level indicate the average percent of migration.

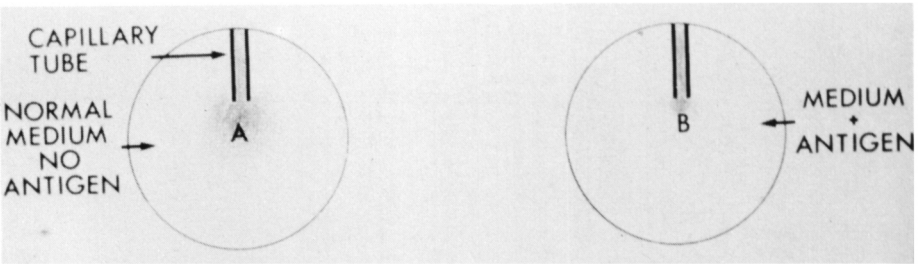

Figure 2—A pictorial representation of the leukocyte migration test. On the left is a planchette showing normal non inhibited migration of leukocytes from the capillary tube, while on the right is a planchette showing inhibited migration produced by the influence of specific antigen. The results are expressed as the percent migration, where

$$\text{percent migration} = \frac{\text{mean weight of area of migration with antigen (area B)}}{\text{mean weight of area of migration without antigen (area A)}} \times 100$$

Figure 4 compares the average leukocyte migration inhibition effect in patients with multiple sclerosis and controls in three media — serum free, 10% autologous and 10% cerebrospinal fluid. With autologous media the average effect was similar to the control. There was enhancement of the test using the CSF and there was a significant difference between tests of multiple sclerosis patients with CSF in media and serum free controls ($p < 0.05$).

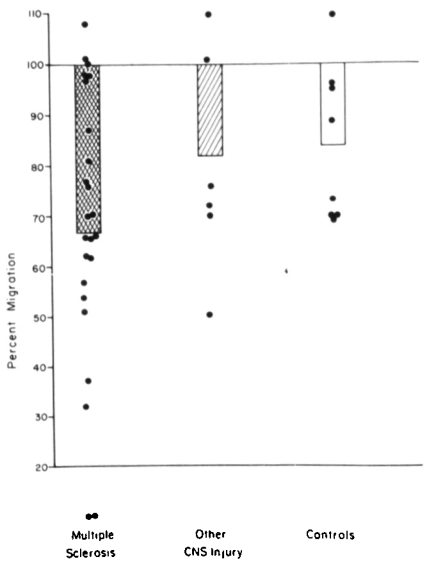

Figure 3—A comparison of leukocyte migration inhibition test in patients with multiple sclerosis, other CNS injury and controls.

Table II shows the effect of antigen specificity on migration inhibition in patients with multiple sclerosis using separate media (serum free, 10% autologous, and 10% CSF). This reveals that BCG at 10 μ g/ml did not cause significant migration inhibition of leukocytes of affected patients, nor did multiple sclerosis myelin protein at 500 μ g/ml. However, myelin basic protein at 500 μ g/ml showed a uniform trend to increased migration inhibition using the three media.

Figure 5 details graphically the clinical classification of multiple sclerosis with leukocyte migration inhibition, along with the controls. Each bar is the average result of

TABLE II — *The average percent migration of the leukocyte migration inhibition test using various media, and adding different antigens in multiple sclerosis patients.*

ANTIGEN	MEDIA		
	SERUM FREE (18-24 patients)	10% AUTOLOGOUS (12 patients)	10% CSF (14 patients)
1) BCG 10 μ g/ml	82%(24)	81%	73%
2) Multiple Sclerosis Myelin Protein 500 μ g/ml	80%(18)	84%	79%
3) Myelin Basic Protein 500 μ g/ml	67%(24)	75%	62%

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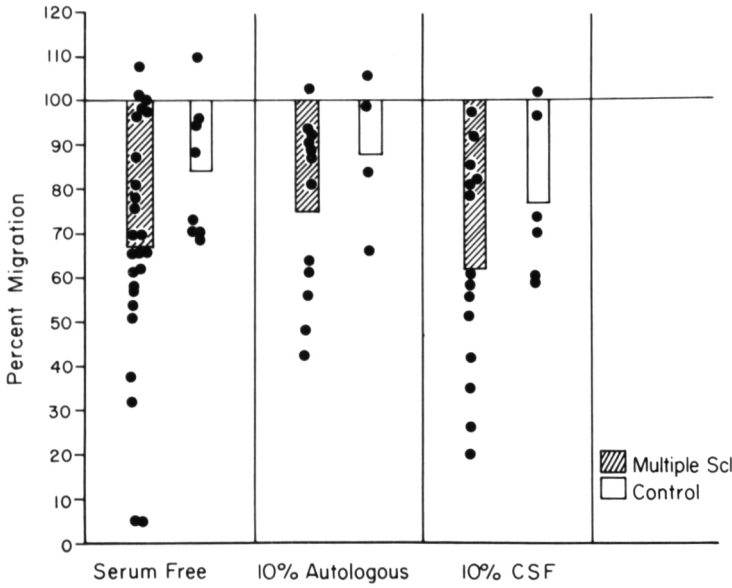

Figure 4—A comparison of the Leukocyte migration inhibition test in patients with multiple sclerosis and controls in different media.

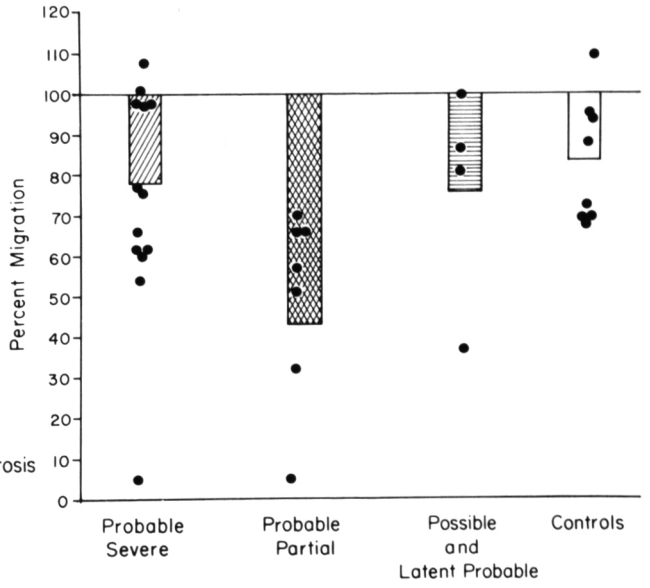

Figure 5—The clinical classification of multiple sclerosis using the leukocyte migration inhibition test and controls.

leukocyte migration inhibition using serum free media with added myelin basic protein at 500 μ g/ml media. This shows that the probable partial group of patients with multiple sclerosis had significantly increased leukocyte migration inhibition, when compared with controls ($p < 0.01$ or the probable severe group ($p < 0.05$).

Taking these results together it would seem that the degree of clinical manifestations may not reflect activity, since the leukocytes from severely affected patients showed less inhibitory activity, than those from the patients who had lesser clinical evidence of the disease.

DISCUSSION

This study uses a modification of a direct migration inhibition test described by Bendixen and Søborg, in which the leukocytes are allowed to migrate from capillary tubes and where inhibition of migration is observed using various antigens.

The majority of the reports related to in vitro studies of leukocyte inhibition in patients with multiple sclerosis have used the indirect migration test utilizing guinea pig macrophages or human monocytes as indicator cells. Bartfeld and Atoynatan (1970), using human macrophages to test 15 patients with multiple sclerosis, indicated a correlation of clinical activity with degree of inhibition using this test on cerebrospinal fluid or brain extract and later myelin basic protein (Bartfeld et al., 1972). Rocklin and Sheremata (1971) demonstrated leukocyte inhibition in 5/15 patients with multiple sclerosis and 6/9 patients with cerebrovascular accidents and suggested that a positive test might indicate non specific nervous tissue damage. Later studies by Sheremata (1974) noted an apparent temporal relation between production of migration inhibition factor and clinical attacks of illness in multiple sclerosis. This relationship was not a consistent feature of CVA and hence brain damage as such.

Recently Rocklin (1974) reported that the leukocyte migration system has been shown to be mediated by a separate factor (L.I.F.) and different indicator cells (PMN leukocytes) than the MIF — mediated system of migration inhibition - which utilizes guinea pig macrophages or human monocytes as indicator cells.

We have used the direct migration inhibition method employing autologous cells for several reasons. It is simple to perform, offers more versatility and possibilities for testing various types and concentrations of antigens. Standgaard and Jorgensen (1972) found only equivocal results in patients with chronic progressive multiple sclerosis using the direct method. Myers (1972), on the other hand, reviewed the history of this method and noted a possible correlation with the activity and later reported, in abstract, that 17/27 patients with active MS and 11/59 with inactive disease had positive migration indices. The loss of activity with autologous serum suggested that there was possible interaction of cellular and humoral factors (Myers, 1973).

Our results using the direct migration inhibition method have shown that myelin basic protein at 500 μ g/ml used as antigen in serum-free media is probably an effective method of determining the presence of active disease state. It is seen in a significant number of patients with partial disability as opposed to those with complete disability. When autologous serum was used in the media there was a tendency for the percent migration to return to more normal limits. On the other hand, when cerebrospinal fluid was added to the media there was enhancement of inhibition suggesting a possible enhancement factor.

The antigen effect of human myelin basic protein was most noticeable at 500 μ g/ml final concentration. With decreasing concentrations the percent migration returned to normal levels. It is be-

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lieved that an assay such as that described by Day and Wexler (1974) would be necessary to assay the different batches of myelin basic protein in future studies.

Patients who had clinical probable multiple sclerosis of partial type signifying possible current activity of the disease had the greatest migration inhibition when tested against the myelin basic protein antigen. This study suggests that migration inhibition of peripheral leukocytes using myelin basic protein is a useful test in the diagnosis of patients with multiple sclerosis. There is additional evidence that the degree of migration inhibition may reflect activity of the disease with its consequent implications on treatment and prognosis.

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