A SUPPRESSION OF CELLULAR IMMUNITY IN PATIENTS WITH MULTIPLE SCLEROSIS

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Many authors have entertained the possibility, mainly on epidemiological grounds, that multiple sclerosis is a consequence of a disease acquired in childhood (1). Among the number of common childhood infectious agents that have been impliacted in this disease, viral agents, in particular the paramyxoviruses, occupy a prominent place as possible etiological agents. First, a number of studies have demonstrated that both the sera and cerebrospinal fluid of multiple sclerosis patients contain unusually high titers of measles antibody (2–4). Secondly, at least two investigators have reported either isolation of paramyxoviruses or the presence of viral nucleocapsids in the brains of patients with multiple sclerosis (5, 6). Finally, pathological examination of the brains of experimental animals infected with viruses such as canine distemper virus, a virus related to human measles virus, may occasionally cause demyelination of the canine brain tissue (7).

In spite of these reports relatively little attention has been paid to the state of the cellular immunity to these viral agents in multiple sclerosis patients (8). Because of these findings we were encouraged to reexamine the question of cellular immunity to viral antigens in patients with multiple sclerosis. Particular attention was placed on the cellular reactivity to a group of paramyxoviruses known to cause childhood infections. Using the method of direct migration inhibition of peripheral blood leukocytes in the presence of a given sensitizing antigen, the present study demonstrates that patients with multiple sclerosis have a specific lack of cellular recognition for measles antigens yet maintain a normal cellular response to both parainfluenza and rubella viruses. These studies were presented as part of the Multiple Sclerosis Symposium at the Tenth International Congress of Neurology in Barcelona, Spain, on 11 September, 1973.

Materials and Methods

Subjects.—15 patients with multiple sclerosis in various stages of the disease were age and sex matched with an equal number of controls. The patients were also matched as nearly as possible as to the place of birth with the control subjects.

Laboratory Methods.—The method of direct migration inhibition of peripheral blood leukocytes as originally described by Bendixen (9) and modified by Zabriskie et al. (10) was used in all experiments. This method may be briefly described as follows: 30 cc of whole venous blood were drawn by syringe and placed in heparinized vacutainer tubes (Becton-

Dickinson and Co., Rutherford, N. J.). The blood was mixed in these tubes with a solution of 2% Knox gelatin in 0.85% saline, and sedimented at 37°C at 45° slant for 15 min and upright for 5 min. The leukocyte-rich plasma and gelatin supernatant is removed and spun down at 700 *g* for 10 min. The pellet is treated with ammonium chloride for 5–10 min to lyse the remaining erythrocytes; the leukocytes are then washed three times in saline, and concentrated in tissue culture medium (Eagle's-Earle's balanced salt solution with added vitamins and amino acids and 20% fetal calf serum [Flow Laboratories, Rockville, Md.]) to 20×10^7 lymphocytes per cc. Aliquots of this leukocyte suspension are loaded into 20-μl capillaries, the bottom of the capillaries is sealed with clay, and the capillaries are spun down at 300 *g* for 10 min. The capillaries are cut at the medium-cell interface, and the pieces containing the pellet are placed in planchettes (York Scientific, Ogdensburg, N. Y.) to which appropriate concentrations of antigens are added. The planchettes are covered with glass cover slips sealed on with stopcock grease and incubated at 37°C for 18 h. All tests are done in triplicate. The resultant fans are projected on tracing paper; these projections are cut out and weighed. The migration index is calculated as previously described (11).

Antigens.—All the antigens were obtained from Flow Laboratories. The measles virus (lot no. M944034) is the Edmonston 84F strain, final passage being in a primary African Green monkey kidney culture with BME (Earle's salts) and 2% fetal bovine serum. The activated antigen preparation is prepared by freeze-thawing of virus infected cells, followed by sonication of the disrupted cells and finally mild centrifugation to remove large cellular debris. The supernatant fluid containing measles antigens is used in the test system. Type 1 parainfluenza antigen (lot no. E921036) is from the Sendai strain and is grown in embryonated hens' eggs (allantoic fluid). The rubella antigen was prepared as an alkaline extract of rubella (Gilchrist)-infected BHK-21 (C/13) cell cultures and reconstituted in the medium described above (Flow Laboratories). Lots nos. L960133 and C961207 were used. None of the antigens contain preservatives and are stored at −60°C until use. They are diluted to the appropriate concentrations immediately before use. Control antigens (i.e., supernatants from the appropriate tissue culture lines but without virus, were also provided by Flow Laboratories) are treated in the same way as the viral antigens. These antigens were added to the medium in the appropriate concentrations to create the control fans.

RESULTS

The results of the migrations are shown on the accompanying graph (see Fig. 1). Cellular sensitivity is presumed to exist if the migration inhibition index is greater than 15%. The mean direct migration inhibition to measles antigen in the patients with multiple sclerosis was 21.3%(SE = ±6.7%) at a 1:10 dilution and 1.9% (SE = ±4.2%) at 1:100 dilution of the antigen in medium. These values are significantly different ($P < 0.001$) from the means obtained to the same antigen dilutions in the normal age- and sex-matched controls (48.7%, SE = ±4.8% and 26.3%, SE = ±6.2, respectively).

On the other hand, no significant differences were found between patients and controls with either rubella antigens or with parainfluenza type 1 antigen. The mean direct inhibition to rubella antigen diluted 1:40 for patients with multiple sclerosis is 22% (SE = ±6.8%) and for controls 18% (SE = ±5.8%) ($P > 0.7$). The mean direct inhibition to parainfluenza type 1 antigen was 33% (SE = ±5.6%) for patients and 26% (SE = ±7.8%) for controls at 1:10 dilution ($P > 0.5$) and 30% (SE = ±6.8%) for patients and 30% (SE = ±5.4%) for controls at a 1:100 dilution ($P \simeq 1$).

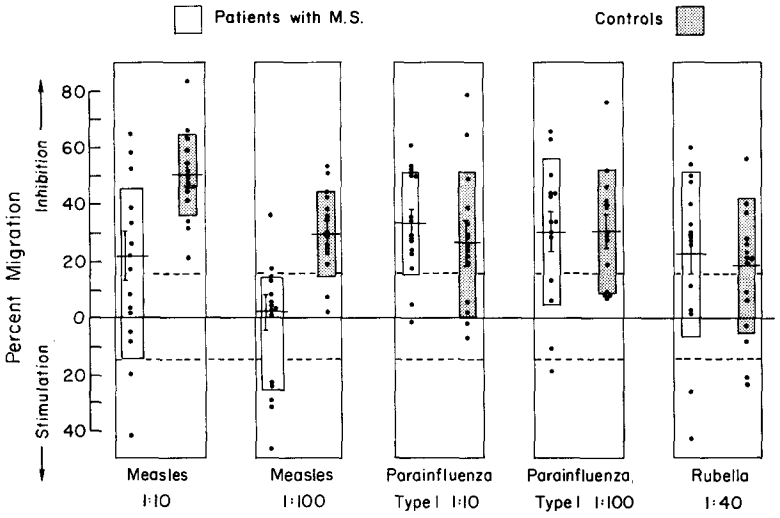

FIG. 1. A comparison of the migration inhibition values to viral antigens in patients with multiple sclerosis and normal subjects. Note the marked absence of cellular reactivity to measles antigens in multiple sclerosis patients as compared with the controls.

Studies with a human brain homogenate in a number of these patients further support our observation that patients with multiple sclerosis have a loss of cellular reactivity that is specific for measles antigens: at several concentrations of brain antigens (100, 10, and 1 μl) there was no significant difference ($P > 0.3$ and <0.5) in the degree of cellular reactivity to these antigens in multiple sclerosis patients and normal control subjects (31.0% $\pm$ 8.1 for multiple sclerosis and 20.0% $\pm$ 7.2 for normal subjects).

While it is admitted that a number of patients in the study were on steroid therapy, these patients did not exhibit a general depression of cellular immunity since their response to other viral antigens, as well as brain antigens, was similar to that observed with normal control subjects.

In view of lack of cellular response to measles antigen, it was of interest to determine whether this cellular energy could be reversed in vitro by the use of transfer factor obtained from subjects with normal cellular response to measles antigen. Indeed, previous studies in this laboratory have demonstrated that transfer from purified protein derivative (PPD) skin test-positive individuals will markedly enhance the in vitro degree of inhibition of cells obtained from individuals who are PPD skin test negative (11). Accordingly, transfer factor (12) prepared from subjects with an unusually good cellular response to measles antigen (>60% inhibition) was incorporated into the medium in which leukocytes from multiple sclerosis patients migrated. In a total of five patients tested to date, there was an increase of migration inhibition index of 10–20%, the average increase being 14%, upon addition of this transfer factor. Transfer

factor alone had no effect on the migrating cells and, as expected, the incorporation of measles antigen in the medium without transfer factor gave results similar to that observed on the initial testing of these patients.

DISCUSSION

Suppression of cellular reactivity to measles antigen demonstrated in this test system by patients with multiple sclerosis contrasts sharply with these patients' normal cellular reactivity to the other viral antigens used, notably parainfluenza type 1 and rubella virus. Of particular interest was the fact that these patients react normally to the parainfluenza type 1 virus (Sendai) since this virus has been isolated from the brains of two patients with multiple sclerosis (5). However, it must be emphasized that Ter Meulen and Koprowski maintain that this virus differs in a number of biological characteristics from the parainfluenza virus used in our studies. This suppression of cellular reactivity to measles antigen is particularly remarkable in light of the increased measles antibody titers said to be present in this disease, and would suggest that measles virus, or an agent antigenically very similar to it, may be important in the pathogenesis of multiple sclerosis. There are at least two possible explanations for our findings:

(*a*) During the initial measles infection the clone of cells reacting to measles antigen has been wiped out, resulting in suppressed cellular response to measles virus. This anergy could permit persistence of the measles virus in the central nervous system and ongoing latent infection affecting the astrocyte population. As the astrocytes are essential to the integrity of the myelin sheath (7), such persistent viral infection would result in patchy demyelinization and the clinical picture of multiple sclerosis.

(*b*) Due to a genetically determined abnormal immune response, patients with multiple sclerosis are unable to make an effective antibody against the measles virus, with resultant persistence of the infection in the central nervous system. As the virus remains, the patient is stimulated to make ineffective antibody continuously. These high antibody titers of measles antibody (humoral immunity) may, in a manner not yet completely understood, suppresses cellular reactivity (cellular immunity) to this viral antigen. In support of this hypothesis are numerous observations that suggest that high humoral immunity results in poor cellular response to a given antigen (13, Paterson, P. Y., personal communication). In addition, patients with multiple sclerosis have an increased incidence of HL-A 3,7 histocompatibility markers, thereby providing evidence for a possible genetic predisposition to disease (14).

While we find cellular suppression only to measles antigen at present, it must be admitted that only three viral antigens were tested. A larger number of viral antigens will have to be tested before we can be absolutely sure this cellular suppression is specific for measles antigen alone.

If indeed this cellular anergy to measles is a phenomenon of basic importance in multiple sclerosis, it was of major interest to see whether this phenomenon could be reversed in vitro. Our preliminary experiments using transfer factor from measles-immunocompetent individuals to transfer immunocompetence to measles-anergic individuals (multiple sclerosis patients) have been successful and suggest that anergy to measles antigen may also be reversed in vivo. The findings described in this paper may provide both a rationale for this kind of therapy and a means of following—by direct migration inhibition—in vitro parameters of the effect of such therapy. These studies are now in progress.

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