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LYMPHOCYTE SUBPOPULATIONS AND CELL-MEDIATED IMMUNITY IN MULTIPLE SCLEROSIS IN IRAN

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SUMMARY

Thirty seven randomly selected patients suffering from multiple sclerosis (MS)(17 males and 20 females)and 30 healthy control subjects were studied for the lymphocyte subpopulations and cell-Mediated immunity.We observed a decrease of the total T-cells and an increase of active T-Lymphocytes and B cells, In MS patients as compared to normal controls. The inability of MS lymphocytes to measles antigen by LMT or LTT in our experiments could be due to T-lymphocyte dysfunction.

INTRODUCTION

The aetiology and pathogenesis of multiple sclerosis (MS) is still unknown despite intensive research.How-

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ever it has recently become clear that genetic factors of the major histo-compatibility system (MHS),(Botchelor et al.,1977;Bernsohn et al.1967; Jersild et al.,1973;Levy et al.,1977;Naito et al., 1972) as well as environmental factors of nutritional,(Bernsohn et al.,1967)or toxic, (Campbell et al., 1950) origin as well as other agents especially viral,(Norrby et al.,1974) and immunological factors, (Acheson, 1977; Caspary,1977) are involved in the aetiology of this disease.

Research on MS is made difficult from the outset by three main problems, (McDonald and Halliday et al.,1977). Firstly, there is no established means of proving the diagnosis during life. Secondly, there is considerable variation in the course of the illness and its clinical manifestations from case to case, and thirdly, in some of the most progressive cases, it may be impossible to distinguish clinically between MS and certain pathologically distinct degenerative diseases of the central nervous system(CNS).

Virus infection plays a role in the pathegenesis of MS, (Bernsohn et al., 1967;Norrby et al., 1974).It has been claimed that measles virus and other paramixoviruses are involved in the development of MS, (Cunningham et al., 1973).

Recent studies have demonstrated a selective defect in the cell-mediated immune (CHi)response of MS patient leukocytes to paramixovirus antigens,(Cendrowski et al., 1970; Ciongoli et al.,1973; Dan et al.,1970;Fuccilo et al.,1975; Jensen, 1968) and it is interesting to investigate this function since studies in experimenial animals have shown that the major histocompatibility reaction(MHR) linked immune response (Ir)genes primarily co-

ntrol the T-cell dependent CM1 respones,(Levy et al., 1977).

In an attempt to evaluate the CM1 response, we have studies 37 MS patients randomly selected from those in a stable phase of the disease. The lymphocyte transformation and leukocyte migration tests, using phytohaemagglutinin (PHA) and measles antigen were employed for this investigation. Total and active T-cells and B lymphocytes were also enumerated in the peripheral blood.

MATERIALS AND METHODS:

Thirty seven randomly selected patients suffering from MS (17 males and 20 females with a mean age of 35 years)were studied.

The diagnosis was clinically confirmed in all cases by the criteria of Allison and Millar 1954;as modified by McDonald, Halliday (1977). None of the patients were in an acute phase of the disease at the time of investigation.

The control group was composed of 30 healthy blood donors, 19 males and 11 females, with a mean age of 42 years.

TESTS AND ANTIGENS:

The lymphocytes were obtained from peripheral blood using the Ficoll-Hypaque gradient technique, and lymphocyte transformation tests(LTT)were performed by the technique of Yamamura (Yamamura, 1973) using PHA and measles antigens.

Leukocyte migration test(LMT) was performed by the technique of Soborg and Bendixen, (1967)as described ea-

rlier (Massoud et al., 1976).

Detection of T-cell subpopulations or E-rosette forming cells (E-RFC)including total and active T-lymphocytes, were performed by the technique of Jondal et al., (Jondal et al., 1972) and Wybran, (Wybran et al.,1976).

The enumeration of B-lymphocytes or EAC-rosette forming cells(EAC-RFC)was carried out by the technique of (Mendes et al. 1973).

Final dilutions used were onehundred micro-liters of PHA-M (3.4 µg/ml)or 1/100 microliters of the measles antigen dilution per ml in culture tubes or migration chambers.

RESULTS:

Detection of lymphocyte subpopulations in MS patients (Table 1).

We found a slight reduction in the percentage of Total E-RFC in MS patients when we compared them to normal controls(58.5% $\pm$ 6.4 vs 73.9% $\pm$ 11.35). In addition we observed an increase of active E-RFC (46.1% $\pm$ 4.3 vs 30.4% $\pm$ 9.43) and EAC-RFC(32.3% $\pm$ 1.2 vs 20.2% $\pm$ 4.45), in patients as compared to normal controls. However this increase was not significant.

-Lymphocyte transformation test in the presence of PHA and measles antigen (Table 2).

The measles antigen was unable to stimulate normal and MS lymphocytes, so that no significant difference between these two groups was demonstrated. In contrast, PHA stimulated both normal and MS patients lymphocytes.

-Leukocyte Migration Test in the presence of PHA

and measled antigen(Table 3).

We have not been able to demonstrat any significant difference in migration index(Ml)with PHA and measled antigens between MS patients and normal controls. On the other hand, the leukocyte migration of 40% of normal groups and 44.44% of MS patients could be inhibited by measled antigen, this difference is not significant.

PHA produced inhibition of both MS and normal leukocytes. (74% vs 79%).

Table 1: Relative distribution of peripheral blood lymphocytes(Tt, Ta, B) among normal controls and MS patients.

Tests	Cells	Normal Controls	MS Patients
E-RFC and EAC-RFC	Tt	73.9% + 11.35	58.5% + 6.4
	Ta	30.4% + 9.43	46.1% + 4.3
	B	20.2% + 4.45	32.2% + 1.2

E-RFC = Erythrocyte Rosette Formlng Cells.

EAC- RFC = Erythrocyte Antibody Complement Rosette Forming Cells.

Table 2: Lymphocyte Transformation Test in the presence of PHA and Measles Ag among normal controls and MS patients.

	NC	MS
PHA 1/100/ml	24253 + 13887	17990 + 7344
Measles Ag 1/100/ml	2718 + 1132	3707 + 1559

Table 3: PHA and Measles Ag induced leukocyte inhibition among normal controls and MS patients.

Test	Ag	Normal Controls	Case	MS Patients	Case
LMT	PHA	79 %	15	74 %	10
	Measles Ag	40 %	10	44.44%	10

Ml $\leq$ 0.75

PHA = 0.01/ml

Measles Ag = 0.01/ml

DISCUSSION:

Multiple selerosis (MS) is an uncommon neurological disorder in Iran, and this may be due to certain regional, immunological, or genetic factors. The clinical and loboratory profile of the immunological system of MS patients suggests that many specific immune deficiencies exist in this group of patients,(Bartfeld et al.,1976; Ciongoli et al.,1973; Field et al.,1971;Knight,1977). It is possible that the CM1 deficiency seen in MS is not the primary aetiological factor, although it may still play a part in the pathogenesis,(Knawles et al.,1970; Saunders et al., 1969; Ultermohlen et al., 1973;Zweiman, 1971).Our results showing a decrease of total E-RFC in the peripheral blood of MS patients are similar to those of Lisak and collaborators 1975. In contrast, the number of active E-RFC in our MS patients showed an increase,as compared to normal controls, and this is in agreement with the findings of Platz et al. 1976,who found an increase of E-RFC in the peripheral blood of MS patients. It may be that, the increase of E-RFC in Platz work is related to increase of active E-RFC in our patients.

The number of EAC-RFC showed a slight increase in MS patients as demonstrated by Nowak et al. 1975, and Oger et al.1975, This is in agreement with our finidings.

The inability of MS lymphocytes to respond to measles antigen by LMT or LTT in our experiments could be due to T-lymphocyte dysfunction.

Ultermohlen et al. 1973, using the LMT with different concentrations of measles antigen, showed that the average index of inhibition to this antigen is signifi-

cantly lower in MS patients that in normal controls,and concluded that these patients exhibit a depression of CM1, specific for measles antigen. It was noted that in his group there were occasional patients who reacted relatively nomally to the measles antigen.

In a recent report, Lisak et al. 1977, using LMT and measles nuclear core antigen in MS,have not obtained any correlation between the index of migration and time of exacerbation. They concluded that patients with MS were more likely to show a CM1 response to basic protein and saline extracts of CNS.

Other factors may influence dysfunction of the immune system in MS patients such as: fatty acids, (Mertin et al. 1977,)and humoral products,(Caspary,1977; Thomson, 1977).

Lotfi et al.(1978)have observed a signifint increase of HLA, A3 and B7 in Iranian MS patients, and recently Levy et al. 1977, have suggested that an immune response (Ir)gene for the lymphocyte response to PHA is linked to the combination of HLA-B7 and DW2 in MS patients.

It may be that the identification of immune associated (Ia)antigens on thman cells(Kurdi et al.,1977)(McDonald et al.,1977) and demonstration of their stronger association with MS that HAL-A3, B7 or DW2,could explain the altered immunological reactivity in MS patients.

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