Adenosine Deaminase and 5′Nucleotidase Activities in Peripheral Blood T Cells of Multiple Sclerosis Patients

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Multiple sclerosis (MS) is one of the most common causes of neurological disability in young and middle-aged adults and is thought to be mediated by autoreactive T cells. Activities of adenosine deaminase (ADA) and 5′(nucleotidase (5′NT), which are involved in the differentiation and maturation of the lymphoid system, were measured in peripheral blood T cells from 21 MS patients and in 23 age and sex matched healthy controls to determine whether an association existed between these enzyme abnormalities and cellular immune functions. ADA and 5′NT activities were found significantly decreased in MS patients (P < .001 and P < .01 respectively) when compared with controls. Low levels of ADA and 5′NT activities were found irrespective of whether patients had relapsing–remitting or chronic progressive MS. These findings suggest that low levels of these enzyme activities in T cells may be related to the persistent abnormalities in T cell function in the clinical course of MS.

INTRODUCTION

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS) of unclear etiology and pathogenesis. This enigmatic, relapsing and often eventually progressive disorder of the central nervous system continues to challenge investigators trying to understand the pathogenesis and prevent its progression (1). The pathological hallmark of chronic multiple sclerosis includes focal demyelination, gliosis, inflammation and axonal injury. Diagnosis rests upon identifying typical clinical symptoms and interpreting supportive laboratory and radiological investigations.

Hallmarks of inflammation in the CNS, clonal expansion of B cells, their antibody products, and T cells are found in MS (2). Many MS patients display oligoclonal bands (OCBs) and an abnormal predominance of IgG in cerebrospinal fluid (CSF) (3). Enhanced antibody levels of CSF anti myelin oligodendrocyte glycoprotein (MOG), a minor myelin protein that is exclusively expressed in the CNS, and anti myelin basic protein (MBP) were also reported in MS patients (4). The pathogenic potential of anti-MOG antibodies may be critically dependent on synergy with a T cell mediated inflammatory response in the CNS (4). Studies on T-lymphocyte subset function and numbers suggest that an abnormality of T suppressor/cytotoxic cells may exist and is correlated with disease activity (5). The importance of these findings to disease pathogenesis and the mechanisms involved remains undefined.
Adenosine deaminase (ADA, EC 3.5.4.4) irreversibly converts adenosine to inosine and deoxyadenosine to deoxyinosine (6) and is widely distributed in human tissues. The activity of ADA is 10 times greater in lymphocytic cells than in erythrocytes and in relation to the former, it is greater in T-lymphocytes than in B-lymphocytes (6). It has been demonstrated that this enzyme play a putative role in lymphocyte differentiation and is reported to be essential for the normal growth, differentiation and proliferation of T lymphoid cells (7). ADA deficiency is one of the most severe of the immunodeficiencies in humans and the metabolic basis for this immunodeficiency is likely related to the sensitivity of lymphocytes to the accumulation of the ADA substrates adenosine and 2'-deoxyadenosine (8). High accumulation of deoxyribonucleotides is found to be toxic to lymphocytes and induces cell death (9). The accumulation of adenosine and the activation of adenosine receptors in T cells may lead to impaired T cell development or function (10). Lower values of ADA activity in severe combined immunodeficiency disease (SCID) (11), in T-lymphocytes of Rheumatoid arthritis patients and in lymphocytes of other autoimmune diseases (12).

5’Nucleotidase (5’NT, EC 3.1.3.5), a rate limiting catabolic enzyme of the purine catabolic pathway, hydrolyses mononucleotides and deoxynucleotides to their respective nucleosides or deoxynucleosides, both of which play an important role in immunoregulation. 5’NT also represents the major enzyme responsible for the formation of extracellular adenosine from AMP (13) and has a critical role in the functional activation of alloreactive cytotoxic T-lymphocytes (14). Decreased 5’Nucleotidase activity in human peripheral blood has been reported in a number of immunodeficiency diseases, lymphoproliferative disorders (15), acquired immune deficiency syndrome (16) and in systemic lupus erythematosus (17).

Abnormal levels of one or both of these enzymes may be associated with the occurrence of autoimmune disorders. To determine whether abnormal ADA and 5’NT levels are present in lymphocytes from patients with MS and whether such abnormalities correlate with the diseases status, we examined ADA and 5’NT activity in T-lymphocytes from MS patients and compared with healthy controls.

EXPERIMENTAL PROCEDURE

Samples. Twenty-one MS patients (16 men and 5 women) undergoing treatment in Department of Neurology, All India Institute of Medical Sciences, New Delhi, India were enrolled after obtaining informed consent following the institutional ethical committee approval. For comparison, 23 normal healthy persons of similar sex and matched for age (± 5 years) were included in the study. All patients had a definite diagnosis of Multiple sclerosis as per McDonald’s criteria (18). Twelve patients were in relapsing–remitting and 9 were in chronic progressive disease course. Disease duration ranged from 2 to 12 years. Lumbar puncture disclosed normal biochemical content and OCBs were present in 55% of patients (whereas in isolated studies CSF OCBs have been seen in only 30% of the Indian MS patients, (19)). MRI studies showed typical white mater lesions compatible with the diagnosis of MS in all patients.

Methods. Lymphocytes were separated from the whole blood using lymphoprep (Sigma) and monocytes were removed by the plastic adherent method (20). The lymphocytes were layered onto a pre-washed nylon-wool column and the non-adherent T cells were eluted using warm medium (21). Purity of T cells was tested by AET-SRBC rosetting. Lymphocytes were disrupted by homogenization, centrifuged and the supernatant fraction was assayed for ADA activity by the method of Giusti (22) and 5’NT by the method of Arkestijn using the kit and quality controls purchased from Sigma diagnostics (23). The statistical correlation was done using unpaired Student’s t-test.

RESULTS

ADA and 5’NT activities were expressed in Units/10⁶ T lymphocytes and given in the Table I as mean ± standard error of the mean (SEM). ADA activity was found to be decreased significantly in both relapsing–remitting and chronic progressive MS (P < .001) when compared with controls. 5’NT activity was also found to be significantly decreased in relapsing–remitting as well as chronic progressive MS (P < .01) when compared with controls. There was no significant difference when the 5’NT and ADA levels were compared within the MS group either in exacerbation state or in progressive state.

DISCUSSION

Strong evidences suggest that MS is an autoimmune disease, directed against CNS myelin or oligodendrocytes and both cell-mediated and humoral immune mechanisms contribute to pathological injury. The ubiquitous enzyme, ADA, has been found to play a role in the differentiation and maturation of the lymphoid system in association with 5’NT. These enzyme levels are significantly decreased in T-lymphocytes in patients with MS irrespective of chronic progressive or relapsing–remission state, arguing for the existence of a metabolic T cell defect irrespective