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Elevated Le^y antigen expression on T-lymphocytes in schizophrenic patients

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Summary. Le^y is a carbohydrate determinant of membrane glycoconjugates and is expressed in some tumor and embryonic cells. On T lymphocytes, it is known that human immunodeficiency virus (HIV)-infected human lymphocyte T-cell lines express elevated Le^y antigen and AIDS patients show the highest Le^y expression in T lymphocytes at lower CD4/CD8 ratio. Later, a comparative elevation of Le^y expression on T-cell subsets has been noticed to be mainly present in patients with viral diseases, such as acute and chronic hepatitits, implying an association of the highest Le^y expression with viral infection. We found that Le^y antigen was most expressed in both $CD4^+$ and $CD8^+$ subsets of peripheral T lymphocytes in hospitalized schizophrenic patients. On the other hand, atypical lymphocytes with stimulated morphology are known to appear in the blood circulation of schizophrenic patients. Similar atypical lymphocytes have also been described in viral and autoimmune diseases. Two possibilities have been discussed: viral association in the pathology in some schizophrenic patients; and immunological abnormalities including environmental effects under hospitalization on immune status, since normal controls (staff in psychiatric hospitals) showed higher Le^y expression than normal controls under non-psychiatric circumstances.

Key words: Le^y antigen – Stimulated lymphocyte – Schizophrenia – Viral agent – Environmental factors

Introduction

The pathogenesis of schizophrenia is still unknown, but autoimmune mechanism and viral infection in genetically predisposed individuals have been suggested as possible factors. The appearance of atypical lymphocytes was found in the peripheral lymphocytes of schizophrenia [10] and shown to be not always caused by neuroleptic medication [16, 24]. The morphology of atypical lymphocytes showed features similar to those which appeared in viral and autoimmune diseases. Recently, Ganguli et al. [12] reported an increase in $HLA\text{-}DR^+$ $CD4^+$ T lymphocytes. McAllister et al. [17] found an elevation in $CD5^+$ B cells in schizophrenia, suggesting a possible involvement of autoimmune mechanism in a subgroup of schizophrenic patients. To test viral etiology in schizophrenia, several studies have attempted to measure titers of antibodies to viruses in the serum and cerebrospinal fluid. During the last decade viruses characteristic of persistent infection and neurotropism, e.g., cytomegalovirus (CMV), herpes simplex type-1 (HSV-1), Epstein-Barr virus have been targetted [6, 19, 23]. But the data are often conflicting and possible involvement of these viral agents in schizophrenia has so far remained unclear.

Recently, human retroviruses have become of greater interest. Human T-cell leukemia virus (HTLV-I) and human immunodeficiency virus (HIV) are now well known as latent and neurotropic viruses, and have been associated with chronic neuropsychiatric syndromes, chronic progressive myelopathy [18], and AIDS encephalopathy presenting with various psychotic symptoms. Retroviruses integrate their genetic code randomly into the host somatic cell DNA or, in the case of endogenous type, germ cell DNA of the host. Crow [4] proposed a retrovirus/transposon theory to explain schizophrenia in the context of brain dysfunction and its genetic transmission. Although neither antibodies to HTLV and HIV antigens, and retrovirus-specific enzyme reverse transcriptase [5, 20], nor reverse transcriptase activity of a putative retrovirus were detected in lymphocyte cultures of schizophrenic patients [8], the retroviral etiology in the field of psychiatry still waits further investigations. Some authors, Adachi et al. [2] have reported that Le^y antigen was particularly highly expressed on the cell surface of an HIV-infected T-cell line and of peripheral lymphocyte from patients with AIDS. We preliminarily surveyed Le^y antigen levels in peripheral lymphocytes of

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psychiatric patients. To evaluate the influence of institutional environment on the Le^y expression of T cells, normal subjects unrelated to psychiatric circumstances were selected as the other control group in addition to medical staff in psychiatric hospitals as a further control group.

Patients and methods

Patients

The psychiatric patients examined in the study did not have physical disease and were diagnosed by three psychiatrists. Thirty-five chronic schizophrenics and 23 patients with bipolar disorders were selected. They were being treated as inpatients at two Japanese mental hospitals and met the DSM-III-R criteria for the studied disorders (Table 1).

The schizophrenic patients were divided into two groups: 24 of active type (with prominent psychotic symptoms) and 11 of residual type. The patients with bipolar disorder included 12 manic and 11 depressive types. Therapeutic medication was administered uninterruptedly during the study period. The main drugs used were haloperidol, chlorpromazine, bromazepam and promethazine for schizophrenics, and lithium carbonate, carbamazepine and amitriptyline further for patients with bipolar disorders. The normal control subjects were healthy volunteers who were psychiatric medical staff (control group I) and people living in a local area screened by the adult diseases prevention program in a general hospital (control group II). Informed consent was obtained from all the patients and controls.

Flow cytometric analysis

Ten milliliters of peripheral blood were withdrawn using a heparinized tube from each subject under fasting conditions between 7:30 and 9:30 a.m. Blood samples from psychiatric patients and from at least three individuals in normal control group I were drawn and processed together at the same time. Individuals in the control group II were tested on different days in the same study period, but the other study conditions were the same as one in the above-mentioned groups. The heparinized blood was mixed with silica particles (KAC 2:JIMRO, Takasaki, Japan) at a ratio of 1:10 (v/v) and incubated at 37°C for 1 h with frequent shakings; then it was centrifuged over Ficoll-Hypaque at room temperature for 30 min at 400 *g* to separate phagocytic cells from mononuclear cells. Less than 1% of the collected nonphagocytic cells was peroxidase-positive. The enriched lymphocyte fraction was washed, and suspended in PBS containing 0.1% NaN_3. The suspension (100 µl, containing 1×10^6 cells) was mixed with 100 µl of anti-Le^y monoclonal antibody (BM-1:JIMRO, Takasaki, Japan), incubated for 1 h at 4°C, washed with PBS [1]. The mixture was added to 100 µl of FITC-labeled anti-mouse IgM ($F[ab]_2$ fragment, Tago Inc., Burlingame, Calif., USA), incubated for 30 min at 4°C, and washed with PBS. Purified mouse IgM was used instead of primary antibody for control. The lymphocyte suspension was mixed with one of two types of PE (phycoerythrin)-labeled monoclonal antibodies for helper/inducer T cell: anti-CD4 (Leu 3a) or for suppressor/killer T cell: anti-CD8 (Leu 2a), (Becton Dickinson & Co, Mountain View, Calif., USA). The mixture was allowed to stand at 4°C for 1 h. The lymphocytes were then washed with PBS, suspended in PBS, and subjected to flow cytometry using the two-color analysis in an EPICS-C (Coulter Electronics Inc., Hialeath, Fla., USA).

We also tested reactivities of the following four types of anti-carbohydrate monoclonal antibodies with peripheral blood lymphocytes from 9 schizophrenic patients and 5 normal controls (I); anti-Le^x, -trifucosyl Le^x (trimeric Le^x), -sialyl Le^x and -Le^y.

The results were expressed as mean SD for percentage of T-cell subsets and as median for percentage of Le^y expression in T-cell subsets. Statistical analysis of the results was performed using Mann-Whitney U test.

Table 1. Psychiatric and normal control groups tested for Le^y antigen expression

Groups	Number	Mean age (years)	Male	Female
Schizophrenia				
Active type	24	37.0	12	12
Residual type	11	39.6	6	5
Bipolar disorder	23	39.4	11	12
Normal control (I)	24	33.8	15	9
Normal control (II)	37	34.1	18	19

Table 2. Percentages of T-cell subsets in peripheral lymphocytes in psychiatric patients and normal control groups

Groups	T-cell subset (%)			
	CD4		CD8	
Schizophrenia				
Active type	45.9	(8.2)[a]	28.9	(7.3)
Residual type	49.6	(6.6)	26.4	(7.0)
Bipolar disorder	46.7	(10.4)	26.1	(7.9)
Normal control (I)	47.1	(11.3)	28.4	(7.8)
Normal control (II)	45.4	(8.5)	27.5	(6.1)

[a] Mean (SD)

Results

There was no significant difference in $CD4^+$ and $CD8^+$ T-cell rates among these five groups (Table 2). Table 3 shows the median percentages of Le^y antigen expression of both CD4 and CD8 T-cell subpopulations in active- and residual-type schizophrenia, bipolar disorder, normal control I and II. Significant group differences obtained are also indicated in Table 3. The highest Le^y expression rate in $CD4^+$ and $CD8^+$ T-cells was found in active-type schizophrenia as compared with others except residual-type schizophrenia ($P < 0.01$–0.001). Significantly higher Le^y expression was also found in the $CD4^+$ T-cell subset of bipolar disorder and in the $CD8^+$ T-cell subset of residual-type schizophrenia compared with normal control (I) ($P < 0.01$). It was noted that Le^y expression in $CD4^+$ and $CD8^+$ T-cells in normal control (I) was significantly elevated, compared with those of normal control (II) ($P < 0.01$ and $P < 0.001$). The data on individual Le^y expression rates in $CD4^+$ and $CD8^+$ T-cell subsets are shown in Fig. 1 (i) and (ii), respectively.

On the expression of Le^x-antigen derivatives, i.e., Le^x, sialyl dimeric Le^x, trimeric Le^x, no significant difference was observed between schizophrenic patient and normal control (I). Statistically significant difference in Le^x antigen expression was observed between them, but even less than 3% positive at maximum level.

Table 3. Le^y expression rate in T-cell subsets in peripheral lymphocytes in psychiatric patients and normal control groups

Group	Number	Le^y antigen expression (%)					
		CD4		P^a	CD8		P
Schizophrenia							
Active type	24	17.3[b]			22.5		
			vs N(I)	<0.001		vs N(I)	<0.001
			vs N(II)	<0.001		vs N(II)	<0.001
			vs BD	<0.01		vs BD	<0.01
			vs S(r)	NS[c]		vs S(r)	NS
Residual type	11	15.5			18.3		
			vs N(I)	NS		vs N(I)	<0.01
			vs N(II)	<0.001		vs N(II)	<0.001
			vs BD	NS		vs BD	NS
Bipolar disorder	23	12.4			12.4		
			vs N(I)	<0.01		vs N(I)	NS
			vs N(II)	<0.01		vs N(II)	<0.001
Normal control (I)	24	9.6			12.1		
			vs N(II)	<0.01		vs N(II)	<0.001
Normal control (II)	37	6.3			3.7		

[a] P value by Mann-Whitney U test; [b] median; [c] NS = $P > 0.05$

vs (versus): compared with N(I); normal control (I), N(II); normal control (II), BD; bipolar disorder and S(r); residual type of schizophrenia

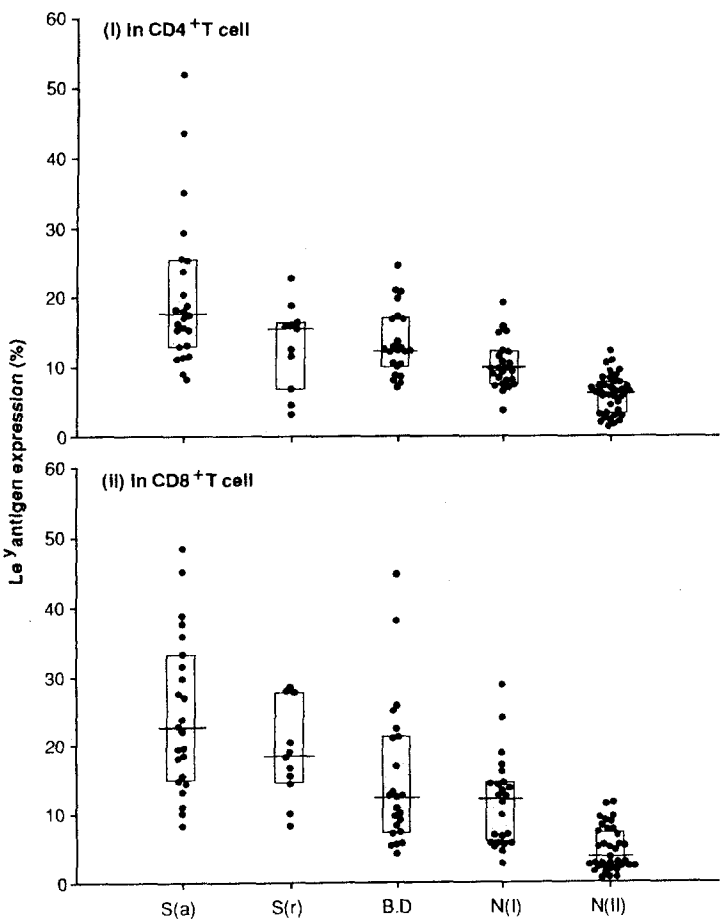

Fig. 1. Le^y antigen expression in (i) $CD4^+$ T cells (helper/inducer T cell) and (ii) $CD8^+$ T cells (suppressor/killer T cell). S(a), schizophrenia (active type); S(r), schizophrenia (residual type); B.D., bipolar disorder; N(I), normal control (psychiatric hospital staff); N(II), normal control (under non-psychiatric circumstances). (—) median, and (□) 75 and 25% limit

Discussion

In this pilot study, Le^y antigen expression on the cell surface of both $CD4^+$ and $CD8^+$ subsets of peripheral T lymphocytes was observed to increase remarkably in patients with active-type schizophrenia, compared with bipolar disorder and both the normal control groups in and out of psychiatric hospitals. Two out of 12 patients in a manic state and two out of 11 patients in a depressive state, for $CD4^+$ T cells, and the two manics, for $CD8^+$ T cells showed higher Le^y expression than the maximum Le^y level of the psychiatric hospital staff. Eight out of 24 patients with active-type schizophrenia showed highly elevated Le^y expression in $CD4^+$ and $CD8^+$ T cells, but one and none of patients with residual-type schizophrenia in $CD4^+$ and $CD8^+$ T cells respectively. Four months later we retested some patients who showed a high percentage of Le^y-positive T cells at the first test and found constant Le^y elevation.

The cell surface Le^y determinant is one of the specific carbohydrate structures arising from aberrant glycosylation in proliferating tumor cells [14] and have been observed at various stages of embryogenesis [9]. Lectin-stimulated lymphoblasts are also found to express the Le^y determinant together with other Le^x derivatives and activation antigens: HLA-DR as well as CD25, Tac antigen/interleukin-2 receptor (unpublished data). These results may suggest the possibility that the expression of the Le^y determinant appears to associate with cell activation/proliferation and some population of lymphocytes in certain patients mainly with schizophrenia are in an activated state.

Morphological findings support this idea, because atypical blastoid lymphocytes have been reported in the peripheral blood of medicated and non-medicated schizophrenic patients [10, 11, 16, 24]. Such blastoid lympho-

cytes as atypical lymphocytes observed in schizophrenics have also been seen in the peripheral blood of viral diseases [26] and autoimmune and infectious diseases [3], in which conditions the increase in peripheral lymphocytes are often seen. However, the increase, if any, in the number of T-cell subsets is not prominent in schizophrenia.

Ganguli et al. and Villemain et al. [13, 25] reported that the increase in number of $CD25^+$ lymphocytes was not observed in schizophrenic patients. As to HLA-DR, the former found the increment but the latter did not. We also failed to detect the increase in CD25 and HLA-DR activation antigens in T-cell populations (data not shown). The reason for the lack of increased CD25 antigen expression in schizophrenic patients is unclear since their lymphocytes could be activated. But the phenotypic expression of lymphocyte following the activation seems not to be an invariable pattern. In a representative case of the diseases with atypical lymphocytes, infectious mononucleosis, CD25 and CD71 (transferrin receptor), which is also known as an activation antigen expressed in proliferating lymphocytes and in various tumor cells, are not detected on lymphocytes even with the presence of marked lymphocytosis [7, 22].

A series of studies on the elevated Le^y expression of both $CD4^+$ and $CD8^+$ circulating T lymphocytes in several viral and autoimmune diseases has revealed that the highest increase of Le^y-positive T-cell subsets was not always seen in the above diseases, in which atypical blastoid lymphocytes appeared in the circulation, but was strikingly seen in the viral diseases so far tested. Adachi et al. [2] have reported an increased Le^y expression in the peripheral lymphocytes of AIDS patients and in an HIV-infected T-cell line. Sata et al. [21] reported extremely high Le^y expression in $CD8^+$ lymphocytes in the acute phase of acute viral hepatitis and in $CD4^+$ lymphocytes in chronic viral hepatitis. The highest Le^y expression on peripheral T lymphocytes was also found in $CD4^+$ and $CD8^+$ subsets of patients with HTLV-1-associated myelopathy (HAM), while patients with rheumatoid arthritis showed elevated but not so high Le^y expression on both subsets of peripheral T lymphocytes (unpublished data).

Thus, Le^y antigen on T lymphocytes seems to be associated with lymphocyte activation, but not to be expressed just like other phenotypes, such as CD25 and HLA-DR, in T-cell activation. Recently, positive Le^y expression in human cancerous tissues has been demonstrated by immunostaining to be restricted in the apoptotic area which is characterized by nuclear condensation, PCNA (proliferating cell nuclear antigen)-negative and DNA nick-end labeling positive staining, indicating the presence of DNA fragmentation, but not in the PCNA-positive area showing the proliferating tumor cells [15]. If it is the case when there is lymphocyte stimulation, the higher rate of Le^y-positive T cells may imply that the signal for apoptosis operates after the stimulation/activation of lymphocytes in viral infections as well as active-type schizophrenia. However, the relationship between atypical lymphocytes and Le^y-positive lymphocytes has not yet been determined. The stimuli and mechanisms inducing high Le^y expression in schizophrenia also remain obscure.

The blastoid atypical lymphocytes observed in schizophrenics have been considered to be caused by stimulation by unknown factors, i.e., genetic influence, viral involvement, immunologic response to certain antigen and endogenous stimulating agents including neuropeptides. Close association of heightened Le^y expression of T cells with viral infection will indicate a possible involvement of virus in schizophrenia. While laboratory data exclude the possibility of HIV infection in our schizophrenic patients, endogenous retroviruses integrated in the human genome is not excluded as one of the etiologic factors of psychoses [4]. The elevation of Le^y is not exclusively found in HIV infection, but the present results may encourage the hypothesis that some virus might be involved in the pathology of putative group of schizophrenia.

The following issues require clarification before definite conlcusions can be drawn: (a) the institutional environment could have contributed to the increased in Le^y-positive lymphocytes, since the normal control (I) showed a higher Le^y expression than the control (II). (b) All the tested patients were treated with psychotropics, which are known to affect immunological reactivities of the host. In a preliminary test, we observed that no Le^y expression was induced by psychotropics on T lymphocytes in the lymphocyte culture in vitro at a drug concentration below the cytotoxic level. (c) Another possibility that unusual immunological reactions with some particular antigens, e.g., food or non-specific infectious agents could develop in medicated patients is not ruled out. Further investigations on Le^y expression in non-medicated outpatients with psychoses and patients with various autoimmune or viral diseases are needed to know whether Le^y antigen expression on lymphocytes could be useful in evaluating any immunological abnormality or viral association in psychiatric patients.

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