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■ REVIEW ■

Immunotherapeutic Potential of JL1, a Thymocyte Surface Protein, for Leukemia

Tumor-specific antigens for leukemia cells have been sought for the past decades, but none of cell surface markers met sufficient criteria as a 'phenotypic signature'. Here we suggest that JL1 antigen can be efficiently used for diagnosis and treatment. JL1 is a human thymocyte differentiation antigen strictly confined to a $CD4^+CD8^+$ double positive subpopulation of cortical thymocytes. Despite its restricted distribution in normal tissues and cells, the expression of JL1 is highly associated with hematopoietic malignancies, particularly various types of leukemia such as T-lineage acute lymphoblastic leukemia (T-ALL), non-T-ALL, and acute myelocytic leukemia (AML). The expression of JL1 antigen was observed in 75.6% of leukemic cases (117 out of 154 leukemic patients tested) with a high mean fluorescence intensity on flow cytometric analysis and confirmed by immunoblotting. Since JL1 antigen is selectively expressed on the surface of human leukemic cells, but not on mature human peripheral blood cells and normal bone marrow cells, anti-JL1 mAb can be used as a reagent of choice in the routine diagnosis of various types of leukemia, providing an excellent candidate for the treatment of these diseases.

Key Words : *JL1, Thymus, Leukemia, Differentiation antigen, Immunotherapy, Immunodiagnosis*

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INTRODUCTION

The cloning and functional characterization of many leukocyte surface molecules with the aid of monoclonal antibodies (mAbs) has had a profound effect on cancer immunology as well as basic immunology. The advent of mAbs provided a powerful new method to search for cancer antigens and made antibody-based therapies possible. The identification of tumor specific antigens, which currently implies that the molecules are expressed only at a certain developmental stage but are able to be reexpressed on malignant tissue later, would make it possible to distinguish cancer cells from normal cells, and to treat cancer with antibody-based therapies or vaccination. These tumor specific antigens fit more with a self/altered self paradigm than with a nonself paradigm for antigens recognized in infectious diseases. Such a distinction of tumor cells from normal cells is based on the premise that the tumor cell bears a distinct immunophenotype, referred to as a 'phenotypic signature' (1). Although these phenotypic signatures for cancer cells have been sought among many cell surface markers over the past decades, none of these trials have been successful so far.

Since they are also able to be used as markers for lineages, differentiation, and activation, the clinical application of some mAbs have played essential roles in the diagnosis and classification of a variety of malignancies such as leukemia and lymphoma (2). For instance, CD10 (CALLA) and CD34 have been used as immunodiagnostic and immunotherapeutic tools for leukemia (3). However, their concomitant expression in normal cells, outside tumors, has posed a constraint on the use of these mAbs in clinical applications.

We previously developed a mAb against a 120-130 kDa human thymocyte surface molecule, designated JL1. This antigen was exclusively expressed on double positive ($CD4^+CD8^+$) cortical thymocytes and was absent from all other types of normal tissues and cells (4). The fact that JL1 was also expressed in several leukemic cell lines with immature T cell phenotype led to the investigation of JL1 expression in various types of leukemias. Surprisingly, anti-JL1 mAb specifically recognized leukemias of myeloid and B cell origins as well as leukemias corresponding to the cortical stage of T cell differentiation. Indeed, the expression of JL1 antigen was observed in 75.6% of all of leukemic cases (5). The data opened up

the possibility that the molecular mechanism of these phenomena might have a relationship with an aberrant regulation of gene expression in leukemic cells which is a common feature in various types of leukemia.

In this review, two primary issues will be addressed. One is to introduce the biochemical and functional features of the JL1 molecule. The other is to discuss the potential usage of the anti-JL1 mAb in clinical aspects due to its ability to distinguish leukemic cells from normal cells.

JL1, A THYMOCYTE-SPECIFIC SURFACE ANTIGEN

JL1 was first described as a novel 120-130 kDa human T cell surface molecule in 1993 (4). The JL1 molecule is a single chain glycoprotein with intramolecular disulfide bridges and about a 5 kDa glycosylation. The size variation of JL1 antigen in different types of cells was considered to be due to a difference in glycosylation. Immunohistochemical and flow cytometric analysis revealed that it was strongly and specifically expressed on double positive ($CD4^+CD8^+$) cortical thymocytes (Fig. 1A). It is not likely that the JL1 antigen belongs to a category of activation molecules because peripheral blood mononuclear cells (PBMC), regardless of activation status, were JL1 negative. Furthermore, both $CD34^+$ and $CD34^-$ bone marrow cells as well as leukocytes of cord blood were clearly negative for JL1 (Fig. 1B). Whereas, many other cell surface proteins, such as CD1 which represents the immature cortical stage of thymocyte differentiation, are also expressed on extrathymic tissues or cells, the JL1 molecule is unique in that its expression was undetectable in normal tissues and cells other than cortical thymocytes. This unique distribution pattern of JL1 suggests that it may play a role in thymocyte differentiation and education. Recently, the functional role of JL1 antigen during positive selection has been investigated using a human reaggregate culture system. The differentiation of immature thymocytes into single positive mature T cells was inhibited with anti-JL1 mAb treatment (unpublished data). In addition, the engagement of the JL1 antigen increased tyrosine phosphorylation of cellular proteins and induced homotypic aggregation of cortical thymocyte through the LFA-1/ICAM-1 pathway, as was the case in other surface molecules such as CD99, CD45, and CD46 (6). JL1-mediated homotypic aggregation of thymocytes also requires functionally intact cytoskeletons, actin polymerization and protein tyrosine phosphatase (unpublished data). On the basis of these data, we propose that the engagement of JL1 surface molecules with a natural ligand might play an important role in positive selection of thymocytes.

EXPRESSION OF JL1 ON VARIOUS TYPES OF LEUKEMIA

We investigated the expression patterns of JL1 in various types of tumor cells from leukemic patients (5). Unexpectedly, we found that JL1 was widely expressed on many different types of leukemia. Most T-ALL cases showed the JL1 positivity (93%). The proportion of JL1-positive cases in acute non-T-lymphoblastic leukemia (non-T-ALL) (82%) was also very high although it was

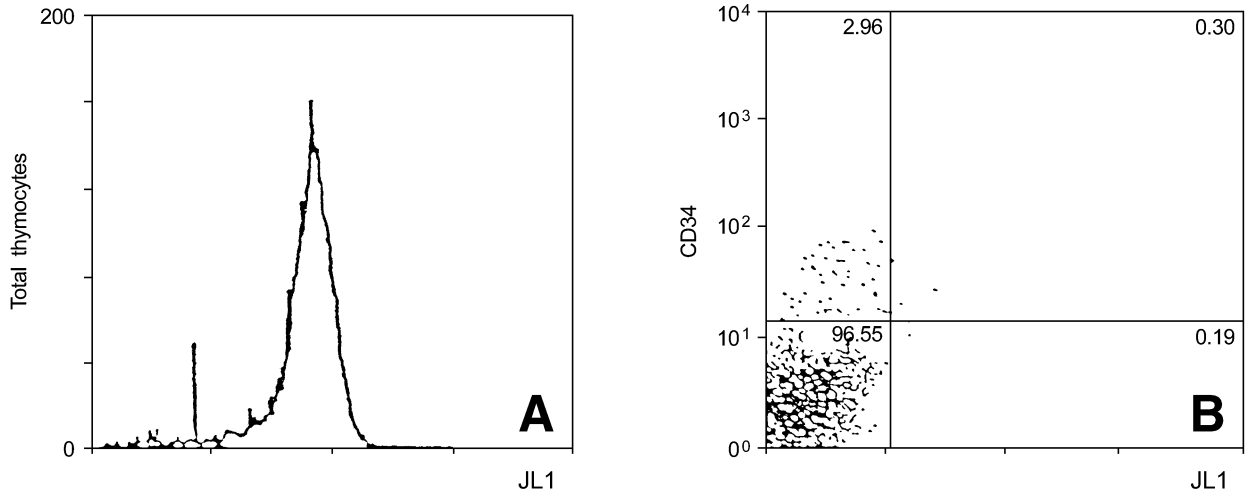

Fig. 1. Flow cytometric analysis of human thymocytes (A) and bone marrow cells (B). While most of human thymocytes express JL1 antigen, CD34-positive and -negative bone marrow cells do not express it.

Table 1. Flowcytometric profile of anti-JL1 mAb immunofluorescence in various leukemias

Type of leukemia	No. of cases tested	No. of JL1-positive cases	Percent of JL1-positive cases
T-ALL	27	25 (83.1±16.6)†	92.6
non-T-ALL*	50	41 (72.7±19.3)	82.0
AML	62	43 (61.5±29.7)	69.4
Acute biphenotypic leukemia	9	6 (63.3±18.2)	66.7
CML in blast crisis	4	1 (42.0)	25.0
CLL of B cell lineage	1	0 (0)	0
Adult T cell leukemia†	1	1 (58.0)	100
Total	154	117	75.6

* CALLA-positive or negative cases (one case was CALLA-negative and JL1-positive).
† HTLV-related case.
† average percent of positive cells ± standard deviation (adapted from W.S. Park et al., Leukemia, in press, 1998. Stockton Press).

lower than that in T-ALL. Even in AML, 70% of total cases were positive for JL1. As a whole, the expression of the JL1 antigen was observed in 76% of all types of leukemic cases (117 out of 154 leukemic patients tested) on flow cytometric analysis (Table 1). The data suggest that anti-JL1 mAb could be effectively used in the diagnosis of most types of leukemia.

There was a lineage-dependent difference in JL1 expression between types of leukemia in terms of fluorescence intensity. The average percentage of positive cells was 83.1±16.6% in $JL1^+$ T-ALL, 72.7±19.3% in $JL1^+$ non-T-ALL, and 61.5±29.7% in $JL1^+$ AML. The percentage of cases showing a mean fluorescence intensity of over 100 was 76% in T-ALL, 44% in non-T-ALL, and 30% in AML. These results suggest that JL1 expression could be efficiently used in discriminating between leukemic cells and normal stem cells.

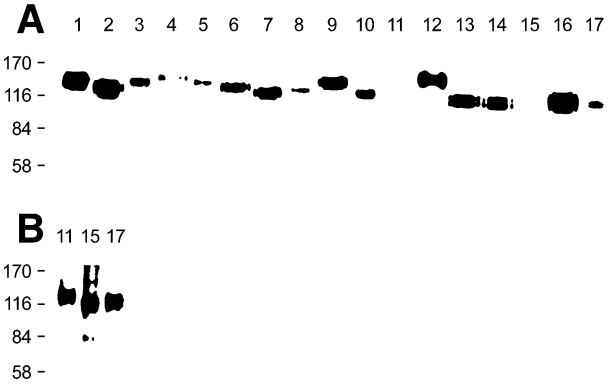

Fig. 2. Immunoblots of bone marrow cell suspensions from $CALLA^+$ non-T-ALL (lanes 3-7), AML (lanes 8-14), and T-ALL (lanes 15-17) stained by anti-JL1 mAb, show bands of varied molecular weights from 120 to 160 kDa (A). Molt-4 and thymocytes (lanes 1 and 2, 130 kDa and 120 kDa, respectively) also show different bands. The protein (JL1) bands are absent or faint in lanes 11, 15 and 17. Immunoprecipitation of ^{125}I-labeled cell lysates of those samples with low JL1 density on immunoblotting was performed (B), and prominent bands corresponding to JL1 antigen are demonstrated in all three samples (adapted from W.S. Park et al., Leukemia, in press, 1998. Stockton Press).

The expression of JL1 in leukemia was further confirmed by immunoblotting (Fig. 2). Leukemic cells from 15 cases of high, intermediate, and low relative antigen index with flow cytometric analysis showed clear bands with a slight variation in their sizes around 120 kDa. This data support that the flow cytometric results even in leukemic cells of the lowest intensity were not due to nonspecific binding of anti-JL1 mAb.

We have shown that JL1 was specifically expressed on the surface of leukemic cells, but not on those of peripheral blood lymphocytes and bone marrow cells from healthy donors. It is also worthwhile to note that the affinity of anti-JL1 mAb to leukemic cells (affinity constant = 1.69×10^9 L/mol) is high enough to label tumor cells with high specificity (7).

DIAGNOSTIC AND THERAPEUTIC POTENTIAL OF ANTI-JL1 MAb

The identification of cell surface molecules, which are normally restricted in distribution but predominantly expressed on leukemic cells, would be very helpful in the diagnosis and immunophenotypic classification of leukemia (8). Our data showed that the presence of $JL1^+$ PBMC is highly indicative of an attack by leukemia. Especially, the clinical usefulness of JL1 antigen shares a common feature with CD34 molecule in that both molecules are the most effective in the detection of leukemic cells among the currently available mAbs. On the basis of molecular characteristics of JL1, we suggest that when leukemic cells are identified as JL1 positive, we are able to determine whether patients came to a remission during chemotherapy by monitoring them in terms of

JL1 expression.

Besides their diagnostic utility, tumor specific antigens have been recognized as a clue to target the cancer cells by two distinct approaches; antibody-based therapies and vaccine-based therapies (9, 10). Although the clinical usefulness of currently available mAbs has been controversial, with a debate centering around the crossreactivity between normal and tumor cells, JL1 molecule is the most plausible candidate for this purpose due to its extremely high specificity. Anti-JL1 mAb is immediately applicable to antibody-based therapy only if it proves that it does not cause toxicity in human. The criterion for deciding whether a given mAb is to be tested as a therapeutic tool, is the likelihood that the mAb will be taken up by tumors in significantly greater amounts than by normal tissues (9, 11). Since our group showed specific localization of JL-1 in Molt-4 tumors induced in SCID mice without antigenic modulation or shedding, anti-JL1 mAb is thought to meet this requirement (7). With the specific localization and satisfactory affinity of anti-JL1 mAb, we suggest that JL1 would be the best candidate for therapeutic trials of leukemia.

Cancer vaccines are intended to induce T cells or other components of the immune system to recognize and vigorously attack malignant cells. Although it has yet to be studied whether immunizing leukemia patients against JL1 antigen can induce immunity against leukemia with minimal side effects, it appears unlikely that it will cause harmful immunological side effects by JL1 expression in the cortical thymocytes. In fact, thymectomized patients show few immunological deficits, since the thymus undergoes an involution and loss of function once the peripheral T cell repertoire has been established (12). Recently, it has been suggested that tumor specific antigens, MAGE, BAGE and GAGE, which are expressed in 60% of Caucasian melanoma, might possibly induce protection against parental tumor cells without severe side effects (13, 14). Likewise, JL1 antigen may be the best candidate for the development of a cancer vaccine for leukemia. In addition, there is still another possibility that anti-JL1 mAb is applicable to autologous bone marrow transplantation through a immunochemopurging protocol (unpublished data).

In summary, we conclude that JL1 would be the best 'phenotypic signature' of leukemia among "tumor-specific" molecules reported so far due to its two unique characteristics. The first is the narrow distribution pattern of its expression in normal tissues and cells despite its expression in wide range of leukemia. The second is the high affinity of anti-JL1 mAb to tumor cells. On the basis of these features of JL1 molecule, we suggest that JL1 antigen would be most useful in immunodiagnosis and immunotherapy of all kinds of acute leukemia.

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