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Review

Immature thymocyte antigen, JL1, as a possible immunodiagnostic and immunotherapeutic target for leukemia

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= Abstract =

The identification of tumor-specific antigens has represented a critical milestone in cancer diagnosis and therapy. Clinical research in this area for leukemia has also been driven over the past few decades by the hope that surface antigens with restricted tissue expression would be identified. Disappointingly, only a small number of the leukemic antigens identified to date, meet sufficient criteria to be considered viable immunophenotypic markers. In this paper, we nominate anti-JL1 monoclonal antibody as an immunodiagnostic and immunotherapeutic candidate for leukemia. The JL1 molecule appears to be a novel cell surface antigen, which is strictly confined to a subpopulation of limited stages during the hematopoietic differentiation process. Despite the restricted distribution of the JL1 antigen in normal tissues and cells, anti-JL1 monoclonal antibody specifically recognizes various types of leukemia, irrespective of immunophenotypes. On the basis of these findings, we propose JL1 antigen as a tumor-specific marker, which shows promise as a candidate molecule for diagnosis and immunotherapy in leukemia, and one that spares normal bone marrow stem cells.

Key Words: JLI, leukemia, immunotherapy, immuodiagnosis, immunophentype, differentiation antigen

INTRODUCTION

Since the advent of the technology for producing monoclonal antibodies (mAbs) by Köhler and Milstein in 1975 (1), mAbs have been widely used for the cloning and functional characterization of many cell surface molecules, and have provided powerful diagnostic tools for malignant tumors. The initial work carried out on mouse tumors showed that distinct antigens can be produced in tumor cells by point mutations, abnormal gene fusions or the transcriptional activation of a gene not expressed in normal tissues (2). In addition, tumor cells can produce abnormally large amounts of proteins, which are also expressed in their normal counterparts. In particular, in the case of hematological malignancies, tumor antigens that belong to this category have long been sought among the many cell surface antigens. CD10 and CD34 are representative examples of this effort and have also been successfully used as markers of lineages, differentiation, and activation. Therefore, the mAbs that recognize such antigens have played an essential role in the diagnosis and classification of hematological malignancies, such as leukemia and lymphoma (3,4).

Major advances that have occurred over the past few years include the antibody engineering techniques, which have improved mAbs affinities for their antigens,

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minimized immunogenicity, and enhanced our ability to accurately deliver potent cytotoxic activity. These recent advances have led to a rapid increase in research and clinical trials of antibody-based tumor-targeted therapies (5,6). In particular, mAbs which target cell surface antigens, such as CD20, CD19, CD33, HER-2/neu, and Le^+, are under active investigation as potential therapeutic agents in combination with radioisotopes and toxins (3,7,8). However, their concomitant expression in normal cells poses a constraint on the use of these mAbs for clinical applications.

We previously developed and reported upon a mAb against a novel human thymocyte differentiation antigen, designated JL1 (9). JL1 is exclusively expressed on thymocytes and subpopulations of normal bone marrow cells. Despite this highly restricted distribution of the JL1 antigen, surprisingly, anti-JL1 mAb specifically recognizes various types of leukemias of myeloid and B-cell origin as well as T-lineage acute lymphoblastic leukemias (T-ALL) (10). These findings open up the possibility that JL1 antigen might be a candidate as an 'immunophenotypic signature' (11), that it creates an immunophenotypic distinction between tumor cells and normal cells, and in particular the possibility that anti-JL1 mAb may be of use as a therapeutic agent against leukemia.

JL1 antigen, unlike most of previously reported leukemic antigens, which are expressed in both normal and leukemic cells, appears to be sufficiently tumor-specific to allow the monitoring of individual responses to chemotherapeutic trials for the assessment of remission status prior to clinical relapse, and be of relevance to immunotherapy. In this article, we review the distribution patterns and the biochemical and functional features of JL1 molecules, and the potential use of anti-JL1 mAb in immunodiagnostic and immunotherapeutic trials.

THE RESTRICTED EXPRESSION OF THE JL1 MOLECULE, LEUKEMIA-ASSOCIATED ANTIGEN

Intrathymic T cell development is a continuous and orderly process, which is accompanied by coordinated changes in the expressions of cell surface antigens. Since the identification of new surface molecules on thymocytes is critical to the understanding of the mechanisms controlling T cell differentiation, we have developed several mAbs against human thymocytes, and one of these is anti-JL1 mAb. The JL1 molecule proved to be a novel glycoprotein of 120~130 kDa expressed on the surfaces of human thymocytes. The previous Immunohisto-chemical analysis showed that the JL1 antigen is strongly and specifically expressed on cortical thymocytes but absent from lymphoid tissues, such as tonsil, lymph node, and nonlymphoid tissues.

In addition, the expression of JL1 antigen was not observed in peripheral blood mononuclear cells, regardless of their activation status, which is consistent with the finding that the expression of JL1 was completely absent in leukemic cell lines of peripheral T cell origin such as Hut78. In fact, $CD25^+$ or $CD69^+$ activated T cells obtained from hyperplastic lymph nodes did not also express JL1 antigen. Therefore, JL1 is a novel differentiation antigen expressed at certain developmental stages in the human thymus, which suggests that it might play a role in thymocyte differentiation and education. In fact, JL1 expression is detected in the earliest thymic precursors and disappears after CD1a downregulation in single positive thymocytes (12).

JL1 antigen was initially reported not to be expressed in the majority of normal unfractionated bone marrow cells (13). However, there remains a possibility that a small proportion of bone marrow cells do express JL1, since mononuclear cells in normal bone marrow include cells from various hematopoietic cell lineages and in various differentiation stages. By multiparameter flow cytometric analyses and fractionating mononuclear cells using lineage-specific markers, it is now evident that JL1 molecules are expressed on some of the precursor cells of the lymphoid and myelomonocytic lineages. Remarkably, $AC133^+CD34^+$ lineage non-committed stem cells did not undoubtedly express JL1 molecules (12).

As mentioned above, JL1 antigen is highly expressed on DP thymocytes. This suggests that JL1 might be

involved in the positive selection of thymocytes. In order to investigate the functional roles of the JL1 molecule in thymic development, anti-JL1 mAbs was added to a thymocyte suspension culture and tested with respect to its effect on thymocyte aggregation. Engagement of JL1 antigen was found to induce homotypic aggregation of cortical thymocytes, as did other surface molecules such as CD99, CD45, and CD46 (14,15). This aggregation was temperature-, energy-, and Mg^{++}-dependent, and required an intact cytoskeleton and actin polymerization (14,15). Adhesion properties are usually characteristics of integrin functions, and in fact treatment with anti-JL1 mAb mediated homotypic aggregation, generating pro-adhesive signals that activated the lymphocyte functions associated with antigen (LFA)-1 molecules at the cell surface. In addition, an immunoblotting study of tyrosine phosphorylation revealed that JL1 signaling generates autophosphorylation and induces the tyrosine phosphorylation of some cellular proteins (unpublished data). On the basis of these results, we propose that JL1 molecules mediate certain types of intercellular interaction, such as thymocytes-thymocytes (T-T) or thymocytes-thymic epithelium (T-TE) interactions during positive selection. This possibility was further emphasized using a human thymic re-aggregation culture system, because the differentiation of immature thymocytes into SP thymocytes was found to be significantly inhibited by anti-JL1 mAb treatment (unpublished data).

IMMUNODIAGNOSTIC POTENTIAL OF ANTI-JL1 MAB

The fact that the JL1 antigen is highly expressed on thymocytes prompted us to check its expression in T cell neoplasms from the corresponding stage of differentiation and evaluate its immunodiagnostic potential for lymphoma and leukemia. Surprisingly, the JL1 antigen was strongly expressed in 88.0% of lymphoblastic lymphomas and in 87.0% of T-ALL (12). In addition, investigations on the expression patterns of JL1 in various types of leukemic cells from different patients showed unexpectedly that the JL1 antigen was also positive in a considerable number of cases in other types of acute leukemia, and this was despite the application of strict criteria for JL1 positivity, namely, that the JL1 antigen should be expressed in more than 20% of total bone marrow cells. Nevertheless, JL1 positivity was found in 80.9% of non-T-ALL cases and in 90.1% of the acute myelogenous leukemia (AML) cases (12). These results posed the strong possibility that anti-JL1 mAb could be used for the routine diagnosis of acute leukemia and for imaging lymphoblastic lymphoma and leukemia. The expression of JL1 molecules in leukemias was further confirmed by immunoblotting, which showed slight variations in the size of JL1 at around 120 kDa.(13) These variations in the molecular weight of the JL1 antigen are considered to be due to differential glycosylation.

The JL1 antigen shares a common feature with the CD34 molecule in that both molecules are effective at detecting leukemic cells, regardless of leukemia type. In subsequent studies, we compared the clinical utility of JL1 with that of CD34. Preliminary result shows that JL1 positivity (87.0%) is slightly higher than CD34 positivity (76.4%) in all kinds of leukemia (208 cases) (12). Furthermore, either JL1 or CD34 were expressed in almost all most leukemic cases tested, indicating that flow cytometric analysis using both anti-JL1 and anti-CD34 mAbs might be able to detect almost all types of acute leukemia.

While JL1 is not expressed on normal $CD33^{+}CD34^{+}$ myeloid progenitors, JL1 and CD34 antigens are co-expressed in 87.9% of AML. This interesting correlation suggests that this peculiar immunophenotype ($JL1^{+}CD34^{+}$) of AML might be useful for monitoring minimal residual disease after treatment. At present, when fewer than 5% of the cells in bone marrow are morphologically identifiable blasts, patients with acute leukemia are considered to be in complete remission. During the period between this morphological complete remission and overt relapse, the level of residual leukemia is largely unknown and, therefore, patients are treated using routine chemotherapeutic regimens re-

gardless of the total burden of leukemic cells in the body. Consequently, the concept of minimal residual disease was adopted to estimate more accurately the true number of leukemic cells. Although the sensitivity of morphologic studies has improved, the detection of leukemic cells, which comprise less than 1% of a cell population, remains beyond the resolution of present morphological methods. It is, therefore, a reasonable challenge to use anti-JL1 mAb for monitoring individual responses to chemotherapy, for the detection of impending relapses prior to clinical recurrence.

JL1 ANTIGEN AS POSSIBLE IMMUNOTHERAPEUTIC CANDIDATE FOR LEUKEMIA

Recent innovative antibody engineering techniques and a better understanding of a growing list of tumor antigens, imply a new round of clinical trials for antibody-based cancer therapies (16). However, the effective therapeutic use of mAbs for acute leukemias is extremely limited, mainly due to the absence of suitable tumor-specific targets. Unlike many other leukemia antigens, the JL1 molecule is unique in that its expression is almost undetectable in all types of normal tissues and cells, other than the thymus and a subpopulation of bone marrow mononuclear cells. In addition, anti-JL1 mAb has a high affinity constant of 1.4-1.9$\times 10^9$ L/mol, which thought to be enough to label tumor cells with high specificity, as it shows no evidence of shedding and has the ability to target tumor sites (17).

This unique distribution of JL1 antigen and its high affinity of its antibody further suggests that anti-JL1 mAb may be one of the most promising candidates for therapeutic trials in acute leukemias. In particular, JL1 antigen on the surface of leukemic cells can be internalized into an intracellular compartment with anti-JL1 mAb. In fact, the endocytosis of the JL1 antigen-antibody complex was clearly visualized by confocal microscopic examination (unpublished data). These characteristics of JL1 antigen further facilitate antibody-based tumor-targeted therapies for leukemias using anti-JL1 mAb. Concrete applications involving the targeting of cytotoxic agents toward only tumor sites without killing normal bystander cells, that is the so-called “magic bullets”, are to be found amongst the immunotoxins (ITs) (18,19,20,21). Using IT, the wide-ranging cytotoxicity caused by conjugated radioisotopes (approximately 50 cells diameters) can be avoided, in case of JL1 targeting. This IT may be useful for killing any remaining leukemic cells in a patient's bone marrow after high-dose chemotherapy and for removing any leukemic cells contaminating transplanted bone marrow cells by an immunochemopurging protocol before autologous bone marrow transplantation. Recently, in order to evaluate the possibility of IT-mediated immunotherapy targeting $JL1^+$ leukemic cells, we conjugated anti-JL1 mAb with gelonin, a ribosome inhibitor protein which is known to be toxic only when it is internalized, and tested its effect on JL1-expressing cell lines, including Molt-4. This resulted in the selective cell death of tumor cells. Whether this IT has also an effect on leukemia *in vivo* should be under active investigation, using leukemia xenograft in (SCID) severe cobined immunodeficierxy mouse.

Furthermore, in order to develop anti-JL1 mAbs as an anti-leukemic drug, the mouse anti-JL1 mAb should be converted into mouse-human chimerized Ab, or humanized reagents, in which only the Ab complementary-determining regions are of murine origin. These genetically-engineered Abs, must maintain their affinity to JL1 antigen without new cross-reactivity and have reduced immunogenicity with longer plasma half-life.

Besides antibody-based therapies, cancer-related antigens have long been studied as a clue for vaccine-based therapies (22). Cancer vaccines are designated to activate immune systems through T cells or other components, thereby, allowing them to recognize and vigorously attack malignant cells. Although JL1 antigen is expressed on thymocytes and subpopulations of bone marrow mononuclear cells, there is possibility that the administration of JL1 antigen or mimotopes, after high-dose chemotherapy, can effectively inhibit the proliferation of remaining leukemic cells. Further studies

on these issues are warranted.

In summary, we conclude that JL1 antigen presents us with one of the best 'immunophenotypic signatures' of acute leukemias. Since the JL1 antigen is expressed on the majority of leukemic blasts, we propose that it could be used to define remission status prior to clinical recurrence and be of relevance to immunotherapy. With the help of recent advances in protein chemistry, we are currently in the process of cloning the human cDNA of the JL1 antigen. This JL1 gene will permit us to elucidate function of JL1 in hematopoiesis and the role of JL1 in the pathogenesis of leukemia.

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FOOTNOTES

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