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CD26: A novel treatment target for T-cell lymphoid malignancies? (Review)

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Abstract. CD26 is a surface glycoprotein with intrinsic dipeptidyl peptidase IV (DPPIV) enzyme activity with multiple biological roles, including being intricately involved in immunoregulation as a T-cell activation molecule and as a regulator of chemokine function. T-cell lymphoid malignancies represent a heterogeneous group of diseases that are generally aggressive and are for the most part resistant to current treatment modalities. Previous studies showed that CD26 is expressed on selected T-cell neoplasms, suggesting a potential role for CD26 in tumor development. We review herein recent classification schemes for T-cell lymphoid malignancies that take into account various facets of their clinical presentation. In addition, we discuss findings supporting the conclusion that CD26 has an essential role in human T-cell activation, as well as its ability to regulate the biological effects of selected chemokines through its DPPIV activity. Finally, we will present recent work from our laboratory that indicates a potential role for CD26 as a molecular target for novel treatment modalities for T-cell lymphoid malignancies.

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1. Introduction

CD26 is a 110 kDa cell surface glycoprotein with diverse functional properties, and is expressed on a variety of human tissues. An important facet of its biological property is its intrinsic dipeptidyl peptidase IV (DPPIV) activity, which is contained within its extracellular domain. Work since the late 1980s clearly demonstrated that CD26 has an essential role in immune regulation, particularly through its involvement in T lymphocyte activation and its ability to cleave selected biological factors to alter their receptor specificity and physiological effect. Meanwhile, although its exact role in cancer biology remains to be elucidated, CD26 also appears to play a part in the development or biological behavior of selected human neoplasms. In particular, preliminary results suggested that CD26 may contribute to the biology of selected T-cell hematological malignancies, being differentially expressed on certain subsets of T-cell tumors and influencing growth of tumor cell lines through its DPPIV enzyme activity.

T-cell lymphoid tumors represent a heterogeneous group of diseases that are generally aggressive and are relatively resistant to current treatment modalities. Recent classification schemes have attempted to take into consideration various aspects of disease presentation to better reflect disease biology and clinical behavior. In this paper, we will review the newer classification schemes for T-cell lymphoid tumors, issues relating to disease prognostic factors, and molecular defects. We will also discuss key aspects of CD26 biology, focusing particularly on its role in human T lymphocyte activation and chemokine processing. In addition, we will review data regarding the expression of CD26 and its potential role in T-cell hematological malignancies. We will specifically discuss our latest findings, which suggest that CD26 may be an appropriate target for novel treatment strategies for T-cell neoplasms.

2. T-cell lymphoid malignancies

The classification of lymphoid malignancies in the past has been controversial, and it has been difficult to establish an internationally accepted scheme. An important change in the lymphoma classification systems occurred recently with the publication of revised European-American classification of lymphoid neoplasms (REAL) (1). The REAL classification, relying heavily on original and updated Kiel classifications (2),

subdivides all lymphomas into T-cell and B-cell lymphomas. This modification is able to overcome the confusion involving the previously accepted morphology-based working formulation (3), in which T-cell lymphomas occurring less frequently and having a worse prognosis are included in different groups (diffuse-mixed, large-cell, or lymphoblastic) and are treated similarly to their B-cell counterparts.

The REAL classification system. The REAL classification emphasizes the fact that each disease is a distinct entity that is defined by a constellation of laboratory and clinical features, including morphologic features, immunophenotype, genetic features, clinical manifestations, and course. There is no 'gold standard'. While most of the REAL classification entities were recognized in the prior working formulation classification, new subtypes are also described (4).

The REAL classification divides T-cell lymphoid malignancies into two major groups based on the maturation stage of the malignant T-cells: a) precursor T-cell neoplasms and b) peripheral T-cell neoplasms (1). Additionally, the peripheral T- and NK-cell neoplasms are subdivided into 10 categories (1 provisional), with some containing provisional subtypes. Overall, the REAL classification represents a major advance in the characterization of lymphomas, especially the T-cell lymphoid malignancies. Furthermore, the REAL classification accentuates the fact that, for T-cell lymphomas, the site of disease presentation is an important facet of the disease, with the extranodal lymphomas being intrinsically different from their nodal counterparts.

Subsequent studies have demonstrated that the REAL classification is able to reliably define clinical entities that can be diagnosed by expert hematopathologists. Importantly, this classification scheme can be used to predict the patient's clinical course and prognosis. For example, the Non-Hodgkin's Lymphoma Classification Project (NHLCP) found the prevalence of T-cell lymphomas to be approximately 12%, the largest majority of which being the peripheral T-cell lymphoma group (7%) (5). However, in this analysis, the peripheral T-cell lymphoma group included 5 of 10 T-cell lymphoma subgroups defined by the REAL classification. Meanwhile, the anaplastic large T-/null-cell lymphoma and precursor T-lymphoblastic lymphoma subgroups each represented 2% of cases. In addition, immunophenotyping proved to be helpful in many cases in reaching the correct diagnosis and improved the diagnostic accuracy, especially for T-cell lymphomas. Overall, the new entities described by the REAL classification scheme comprised approximately 20% of all cases.

The WHO classification system. Since the publication of the REAL classification in 1994, new findings regarding some categories of lymphoma have been presented, hence clarifying the status of entities that were listed as provisional in the original REAL classification, particularly among T-cell lymphomas. The World Health Organization (WHO) classification for neoplastic diseases of the lymphoid tissues, developed under the joint auspices of the European Association for Hematopathology (EAHP) and the Society for Hematopathology (SH) during the last few years, uses the principles of the REAL classification and incorporates minor additional modifications (6). In additional to precursor T-cell neoplasm, the proposed WHO classification lists 14 subgroups among mature T- and NK-cell neoplasms. Of note is the fact that site of disease has an even more prominent role in subgroup delineation, resulting in the separation of subgroups as being predominantly leukemic, nodal, extranodal, or cutaneous (Table I).

Table I. Mature T- and NK-cell neoplasms in proposed WHO classification system.

Predominantly leukemic malignant neoplasms:
T-cell prolymphocytic leukemia
T-cell large granular lymphocytic leukemia
NK-cell leukemia
Sézary syndrome
Predominantly nodal malignant neoplasms:
Peripheral T-cell lymphoma
Angioimmunoblastic T-cell lymphoma
Adult T-cell leukemia/lymphoma
Anaplastic large-cell lymphoma
Predominantly nodal malignant neoplasms:
Nasal NK/T-cell lymphoma
Enteropathy-type intestinal T-cell lymphoma
Subcutaneous panniculitis-like T-cell lymphoma
Hepatosplenic γ/δ T-cell lymphoma
Predominantly cutaneous malignant neoplasms:
Mycosis fungoides
$CD30^+$ lymphoproliferative disease (primary cutaneous anaplastic large-cell lymphoma)

Adapted from Jaffe *et al* (6). NK, natural killer; WHO, World Health Organization.

Predominantly leukemic group. Predominantly leukemic group consists of four subtypes. T-cell prolymphocytic leukemia comprises up to 20% of all prolymphocytic leukemia cases, and is characterized by malignant cells expressing CD2, CD3, CD5, and CD7, with prominent nucleoli, some nuclear irregularity, and abundant cytoplasm. Cells that are smaller in some cases and that might be called T-cell chronic lymphocytic leukemia comprise 1% of all chronic leukemia cases. T-cell prolymphocytic/chronic lymphocytic leukemia is a more aggressive disease than its B-cell counterpart and is rarely curable with available therapy, with a median survival of approximately 2 years.

Large granular lymphocytic leukemia is divided in the WHO classification into two subgroups: *T- and NK-cell* leukemias. These entities are associated with lymphocytosis and neutropenia. The malignant cells in T-cell type LGL are often $CD8^+$ cells that express CD2, CD3, CD16, CD57.

T-cell type usually has more adverse futures, such as anemia, splenomegaly, and complications secondary to cytopenia, including life-threatening infections. While the disease course is usually indolent, an occasional case is associated with the Epstein-Barr virus (EBV), and has a more acute presentation and a more clinical aggressive course.

Sézary syndrome is the leukemic variant of mycosis fungoides (see below), although at times it can arise *de novo*. Tumor cells are predominantly small cells with cerebriform nuclei and express T-cell-associated antigens ($CD2^+3^+5^+$). While approximately one third are $CD7^+$, most cases are $CD4^+$, with erythroderma and lymphadenopathy being associated findings. It has an aggressive course, with expected survival being less than 1.5 years, particularly in the presence of extracutaneous disease.

Predominantly nodal group. The predominantly nodal group also consists of four entities. Peripheral T-cell lymphoma, unspecified, is the most common of all the T-cell subclasses, but it is a diagnosis of exclusion. It typically contains a mixture of small and large atypical cells that are difficult to subclassify. Tumor cells express variable T-cell-associated antigens and are lacking B-cell-associated antigens (may express CD45RA and lack CD45RO). Patients are usually adults with aggressive, generalized disease. Relapses in this disease are more common than in B-cell lymphomas of similar histological grades, with the prognosis being generally poor.

Angioimmunoblastic T-cell lymphoma is a relatively rare disorder with a generally poor outcome. The lymphoid cells are a mixture of small lymphocytes, immunoblasts, and a characteristic type of atypical 'clear' cells, which usually has abundant, pale or clear cytoplasm. Patients typically have systemic disease involvement, with generalized lymphadenopathy, B-symptoms, skin rash, and polyclonal hypergammaglobulinemia.

Adult T-cell lymphoma/leukemia (ATL) is a clinicopathologic entity caused by the retrovirus HTLV-1, which is transmitted either by infected cells via semen, blood products, needles or breast milk, or by transplacental migration, with an incubation period ranging from 20 to 30 years. HTLV-1 is endemic in the Caribbean basin and Southwestern Japan. Tumor cells express the T-cell-associated antigens CD2, CD3, CD5, CD4 and CD25 but usually lack CD7. Demonstration of monoclonal or oligoclonal integration of HTLV-1 provirus in the tumor cells by Southern blot analysis provides definitive evidence of viral infection. Four clinical subtypes are recognized, the majority being acute with a median survival duration of less than 1 year. While autologous stem-cell transplantation does not appear to offer substantial benefit in the treatment of adult T-cell leukemia, treatment with monoclonal antibodies to the CD25 antigen conjugated to toxins or radioactive moieties has shown activity in some patients (7).

Primary nodal anaplastic large T-/null-cell lymphoma is associated with the presence of large blastic cells that in most cases express CD30 and epithelial membrane antigen, and have a characteristic chromosomal abnormality, t(2;5) (see below). While patients often exhibit systemic disease with extranodal involvement (40-70%), treatment with aggressive chemotherapy can result in excellent response in many cases.

Predominantly extranodal group. The modern definition of primary extranodal lymphomas includes only cases with limited stage IE and IIE disease, with the majority of cases being of B-cell origin. Many cases of extranodal T- and NK-cell lymphomas are associated with EBV, with greater frequency of occurrence among the immunosuppressed patient population, particularly following organ transplantation. While they combine to account for approximately 25% of all T-cell lymphomas, each of four types comprise less than 1-2% of the total number of lymphoma cases.

Extranodal NK-/T-cell lymphoma represents a distinct clinicopathological entity associated with EBV, usually presenting in the clinical setting with destructive nasal or midline facial tumor. It is more common in Asia than in Europe and the United States. NK-/T-cell lymphoma is equivalent to angiocentric lymphoma in the REAL classification. The tumor cells usually express CD2, CD56 and cytoplasmic CD3 as well as cytotoxic molecules, such as TIA1, granzyme B, and perforin. Although the disease is often localized, chemotherapy is generally administered in conjunction with radiotherapy. However, the relapse rate is high, with overall prognosis being generally poor. The tumor cell appears to be primarily resistant to chemotherapy, which may partly be related to high expression of multidrug-resistant genes (8).

Approximately 10-25% of primary intestinal lymphomas have a T-cell phenotype. These lymphomas are often found in association with celiac disease and also are called enteropathy-type intestinal T-cell lymphomas. Many patients present with a short history of abdominal pain and weight loss, or are typically treated with emergency surgery due to small-bowel perforation prior to diagnosis. The tumor cells express CD3, CD7, and CD103. While a tumor mass is frequently absent, the clinical course is aggressive and death usually occurs from multifocal intestinal perforation caused by refractory malignant ulcers.

Subcutaneous panniculitis-like T-cell lymphoma usually presents with single and multiple subcutaneous nodules, most commonly affecting the extremities and trunk. While the malignant T-cells are typically $CD8^+$, they can express either the α-ß or γ-δ T-cell receptor. The clinical course may generally be indolent with spontaneously waxing and waning skin lesions or aggressive. A common complication is the hemophagocytic syndrome (HPS), which may be fatal if the disease does not respond to therapy. Clinically, patients often present with fever, pancytopenia, and hepatosplenomegaly. Of note is the fact that bone marrow aspiration may be instrumental in establishing the diagnosis of disease-associated HPS.

Hepatosplenic γ-δ T-cell lymphoma is characterized by primary extranodal disease with typical sinusoidal infiltration of the liver and sinusal infiltration of the spleen. Patients usually present with hepatosplenomegaly and bone marrow involvement but without lymphadenopathy. Tumor cells express CD2, CD3, CD7, and TCR-γδ. Furthermore, they frequently express such NK-related antigens as CD16 and CD56 (64). This lymphoma also is frequently associated with isochromosome 7q and trisomy 8 (9). The majority of patients are young males. Despite the use of a variety of treatment regimens including stem cell transplantation, this aggressive disease is generally refractory to treatment, having with a median survival of less than 3 years.

Predominantly cutaneous group. The definition of primary cutaneous lymphomas includes neoplasms manifesting exclusively in the skin without evidence of extracutaneous disease at the time of diagnosis or within the first 6 months after diagnosis. More than 80% of all primary cutaneous lymphomas are of T-cell origin.

There are two major types, with the prognosis for both being generally good. Mycosis fungoides is a low-grade lymphoma of mature T-cells. Tumor cells express T-cell-associated antigens ($CD2^+3^+5^+$); approximately one third are $CD7^+$; most case are $CD4^+$. This disease is characterized by three phases of evolution: patch, plaque, and tumor. Of note is that all three phases can present simultaneously in the same patient. In rare instances, mycosis fungoides can progress to the leukemic variant Sézary syndrome (see above).

The predominantly cutaneous T-cell lymphoma subgroup also comprises the rare primary anaplastic large-cell lymphoma (ALCL), which has an excellent prognosis. Primary cutaneous ALCL is part of the spectrum of $CD30^+$ T-cell lymphoma of the skin and should be distinguished from systemic ALCL. However, anaplastic lymphoma kinase $(ALK)^+$ ALCL can present as cutaneous disease. Therefore, immunophenotypic and molecular studies are advisable for this distinction. It usually occurs in adults as a solitary mass with an ulcerated surface, and satisfactory treatment generally consists of surgical excision with or without radiation. In approximately a quarter of patients, the skin lesions spontaneously regress, either partially or completely. Primary cutaneous ALCL and lymphomatoid papulosis have overlapping clinicopathological features, and are generally considered to represent a continuous spectrum of the disease.

Prognostic factors. Recent studies have presented data regarding clinical aggressiveness, prognostic factors, immunophenotype, and molecular genetics of various lymphoma subgroups. For example, the Non-Hodgkin's Lymphoma Classification Project (NHLCP) analysis of the relative clinical aggressiveness of different lymphoma groups (Table II) provided contrasting results with regard to the clinical behavior of different T-cell lymphoma subgroups (10). In this analysis, the anaplastic large-cell lymphoma group obviously exhibits the best prognosis (with an overall 5-year survival rate of 77%), while the T-cell lymphoblastic lymphoma group (26%) and peripheral T-cell lymphoma groups (25%) have the poorest prognosis.

In other studies, the T-cell phenotype was found to be an important prognostic factor in itself. Independent of the international prognostic index (IPI), the T-cell lymphomas exhibit significantly shorter disease-free survival and overall survival (except for $CD30^+$ anaplastic large-cell lymphoma) as compared with B-cell lymphomas (11,12). Furthermore, the T-cell phenotype appears to be associated with a greater probability of disseminated disease stage, older age, performance status, and elevated lactate dehydrogenase levels (12). Although the IPI project did not evaluate the influence of immunophenotype on overall survival, subsequent studies used the IPI to stratify T-cell lymphoma patients into prognostic groups. This effort was done mainly to compare outcomes between groups of different immunophenotypes (T-cell vs. B-cell) with the same IPI. Recently, Gisselbrecht *et al* (12) reported that the IPI could indeed stratify T-cell lymphoma patients into statistically significant prognostic groups. However, Armitage and Weisenburger (10) used the IPI to stratify patients in every major lymphoma subgroup and did not find any correlation between the T-cell lymphoma subgroups (PTCL, ALCL, and LBL). Recent analysis of the PTCL cases diagnosed within the NHLCP showed that while the frequency of PTCL (excluding ALCL) varied geographically, survival was relatively poor with the 5-year overall and failure-free survival for patients with PTCL treated with doxorubicin-containing regimens were only 26% and 20%, respectively. Furthermore, both failure-free and overall survivals were strongly correlated with the IPI and performance status, although there was no statistically significant difference in survival among the major histological types (13). Meanwhile, in a series consisting of 560 cases of aggressive NHL (68 T-cell, 492 B-cell lymphoma patients) at M.D. Anderson Cancer Center, the T-cell phenotype was found to be an independent and significant prognostic factor, with T-cell malignancies having worse outcome than B-cell lymphomas of comparable IPI or M.D. Anderson Prognostic Tumor Score (11).

Table II. REAL classification of lymphoma entities according to aggressiveness.

Subgroup	5-year overall survival rate (%)
Anaplastic large T-/null-cell	77
Marginal zone, MALT	74
Follicular	72
Lymphoplasmacytic	59
Marginal zone, nodal	57
Small lymphocytic	51
Primary mediastinal large B-cell	50
Burkitt's-like	47
Diffuse large B-cell	46
Mantle cell	27
Lymphoblastic, T-cell	26
Peripheral T-cell	25

Adapted from the Non-Hodgkin's Lymphoma Classification Project (5). MALT, mucosa-associated lymphoid tissue; REAL, revised European-American classification of lymphoid neoplasms.

Molecular genetics. The molecular genetics of T-cell lymphomas are less well understood than those of their B-cell counterparts (14), although it has been established that large number of T-cell lymphomas are characterized by chromosomal abnormalities involving the T-cell receptor genes. A notable exception is $CD30^+$ anaplastic large-cell lymphoma (ALCL), with respect to its chromosomal abnormality and our understanding of its molecular genetics.

Approximately half of ALCLs are associated with a typical t(2;5) translocation (15-17). As demonstrated by Morris *et al*

Table III. Expression of ALK fusion proteins in ALK+ anaplastic large-cell lymphoma.

Frequency (%)	Genetic alteration	Fusion proteins	Staining pattern
72.5	t(2;5)	NPM-ALK	Cytoplasmic and nuclear
17.5	t(1;2)	TPM3-ALK	Cytoplasmic
2.5	t(2;3)	TFG-ALK	Cytoplasmic
2.5	inv(2)	ATIC-ALK	Cytoplasmic
2.5	t(2;22)	CTLCL-ALK	Granular cytoplasmic

Adapted from Stein *et al* (16). NPM, nucleophosmin; ALK, anaplastic lymphoma kinase; TPM, non-muscle tropomyosin; TFG, tropomysin receptor kinase-fused gene; ATIC, 5-aminoimidazole-4-carboxamide-1-ß-D-ribonucleotide transformylase/inosine monophosphate cyclohydolase; CTLCL, clathrin heavy polypeptide-like gene.

(18), the translocation causes the nucleophosmin (NPM) gene located at 5q35 to fuse with a gene at 2p23 encoding the tyrosine protein kinase anaplastic lymphoma kinase (ALK). As a consequence, the ALK gene comes under the control of the NPM promoter, which induces a permanent and ubiquitous transcription of the hybrid gene, resulting in the production of an 80-kDa chimeric protein termed NPM-ALK (18) or p80 (19). This NPM-ALK protein contains the NPM oligomerization domain and the intracytoplasmic region of ALK, and oligomerization mediated by the NPM segment leads to activation of the catalytic ALK domain contained in the NPM-ALK fusion protein (16). The activated ALK domain binds to GRB2 and SH2 domains of phospholipase C-γ (15,16), and these proteins then activate several signaling pathways, such as those involving RAS, PLC-γ, PI3 kinase, and STAT3/5, resulting in induction of mitogenic activity, including cell proliferation and inhibition of apoptosis (15).

Several cytogenetic and molecular studies have recently suggested that translocations other than classic t(2;5) may also activate the ALK gene and participate in the pathogenesis of ALCL (20-22). Features of the various ALK fusion proteins occurring in ALK+ ALCL are summarized in Table III. Interestingly, the variant fusion proteins (other than NPM-ALK) appear to lack nuclear localization signals. These findings indicate that the oncogenic activity of rearranged ALK is independent of its nuclear localization.

Several authors considered ALK expression to be the most important favorable prognosis factor in establishing the correct prognosis (16,17,23). The overall survival rate for ALK+ patients is 71-80%, while being approximately 30-46% for ALK- patients (17). However, a recent analysis of the cases of ALCL-T/null identified in the NHLCP showed that patients with this diagnosis generally were found to be significantly younger, less likely to have advanced-stage disease of bone marrow involvement, more likely to have low IPI score and importantly, experienced significantly better survival as compared to patients diagnosed with other forms of PTCL. In addition, the absence or presence of ALK expression was not associated with significant differences in clinical features or survival (24).

Recently isochromosome 7q [i(7q)] have been reported to be associated with hepatosplenic γδT-cell lymphoma (9,25). Alonsozana *et al* (25) reported a consistent i(7q) abnormality in three cases of hepatosplenic γδT-cell lymphoma, with the first case having i(7q) as the sole abnormality and the others having i(7q) along with additional abnormalities. Weidmann (9) recently reviewed 45 cases of hepatosplenic T-cell lymphoma including 19 patients with the γδT-cell type. i(7q) has been detected in 13 of 19 hepatosplenic γδT-cell lymphoma patients. Interestingly, all of them were males, and there was association with trisomy 8 in 10 cases. However, the occurrence of trisomy 8 without i(7q) has never been reported in this disease. Although i(7q) and trisomy 8 exist as independent cytogenetic abnormalities in various types of hematological malignancies and solid tumors, the combination of both i(7q) and trisomy 8 appears to be a unique set of cytogenetic abnormalities in hepatosplenic γδT-cell lymphoma. The median survival for patients with i(7q) was 7.5 months as compared to 10 months for the total patient population. Taken together, these data suggest that i(7q) is an important marker for hepatosplenic γδT-cell lymphoma and indeed may have an essential role in the pathogenesis of this disease.

Topoisomerase IIα is an intracellular protein tightly linked to the cell cycle and proliferation (26,27). Essential for cellular proliferation as a key role for regulation for mitosis, DNA topoisomerase II enzyme catalyzes many types of interconversions between different DNA topological isomers (28,29). Two isoforms of topoisomerase IIα and ß, have been reported in eukaryotes (30,31). The 170 kDa topoisomerase IIα protein is regarded as the primary target of such antineoplastic agents as doxorubicin and etoposide that can stabilize the cleavable complex, which eventually leads to augmentation of double-stranded breaks and growth inhibition (32). Several studies demonstrated that increased enzyme level is associated with augmented sensitivity, and drug resistance is related with reduced topoisomerase IIα level (33-35). Preliminary studies with relatively small sample sizes suggested that aggressive T-cell lymphomas have relatively lower levels of topoisomerase IIα than the corresponding aggressive B-cell lymphomas (36,37), findings which may partly explain the relative resistance of subsets of T-cell lymphomas to chemotherapy (13).

3. CD26

In view of the heterogeneity of T-cell lymphomas in terms of clinical behavior and prognosis, there is a constant search for prognostic factors and molecular markers. One example involves research examining the potential role of CD26 in human T-cell lymphomas. CD26 has been extensively characterized as a molecule with an essential function in human T-cell physiology. It performs a number of diverse function, and has been described as a marker of T-cell activation, an alternative pathway of T-cell activation, an ectopeptidase with dipeptidyl peptidase IV (DPPIV) enzyme activity, a functional collagen receptor, a molecule involved in thymic ontogeny, and a peptidase involved in chemokine processing (38-45). For the rest of this review, we will focus

on two important physiological roles of CD26, its role in human T lymphocyte activation and chemokine processing. In addition, we will examine recent data that suggest an emerging role for CD26 in hematological neoplasms, including work from our laboratory indicating that CD26 is a potential novel treatment target for T lymphoid malignancies.

T-cell activation. The CD3/T-cell receptor (TCR) complex plays a central role in T-cell activation and function. In general, stimulation through the CD3/TCR complex alone cannot induce T-cell proliferation and lymphokine secretion. A co-stimulatory signal provided by accessory molecules expressed on the cell surface is generally required for CD3/TCR-mediated activation of T lymphocytes (46). CD26 is one such co-stimulatory molecule present on T-cell surface. It is a 110-kDa glycoprotein that is present on a number of tissues, including epithelial cells found in the liver, kidney and intestine, as well as certain leukocyte subsets (44,47). Although constitutively expressed on human T-cell surface, CD26 expression is markedly enhanced following T-cell activation (44,48). Within the human $CD4^+$ T lymphocyte subset, CD26 is preferentially expressed on the memory/helper ($CD45RO^+$ $CD29^+$) population. This unique subset of $CD4^+$ cells has the ability to respond to recall antigens, induce B-cell immunoglobulin production, and provide MHC-restricted help to cytotoxic T-cells. On the other hand, $CD4^+CD26^-$ cells cannot be triggered to elicit helper functions but are able to respond to mitogens and alloantigens (40,48,49). In addition, increased cell-surface expression of CD26 is associated with an increase in antigen sensitivity, allowing for the maintenance of T-cell memory despite decreasing antigen concentration (50). $CD26^+$ T lymphocytes also exhibit greater proliferation when stimulated with mitogenic signals than $CD26^-$ T-cells, again consistent with the notion that CD26 is a marker of T-cell activation (51).

CD26 also exhibits dipeptidyl peptidase IV (DPPIV; EC 3.4.14.5) activity in its extracellular domain. This ectoenzyme is capable of cleaving N-terminal dipeptides from polypeptides with either the Pro or Ala residue in the penultimate position (52,53). Previous studies using genetically modified CD26 or chemical inhibitors have shown that its intrinsic DPPIV activity is essential for CD26-mediated T-cell co-stimulation. To clarify the role of CD26 and its associated DPPIV activity with T-cell co-stimulation, the human T-cell leukemia cell line Jurkat, which was originally $CD26^-$, was transfected with the expression plasmid pSRα26, in which the CD26 cDNA was placed under the control of the SRα promoter (54). IL-2 production from the Jurkat transfectant expressing wild-type CD26 with DPPIV activity was significantly increased not only following CD3/CD26 co-stimulation but also stimulation not directly involving CD26, such as CD3/phorbol ester. In contrast, $CD26^-$ parental Jurkat cells and the transfectant expressing DPPIV activity-deficient mutant CD26 secreted significantly lower levels of IL-2 in response to the stimuli (55). In addition, DPPIV enzyme inhibitors inhibited T-cell proliferation and cytokine production (56-61). In experiments involving both *in vitro* mitogen and antigen induced T-cell activation, the addition of DPPIV inhibitors impaired DNA synthesis, Ig production and secretion, and production of IL-2 and IFN-γ (58). Similar results were observed in pokeweed

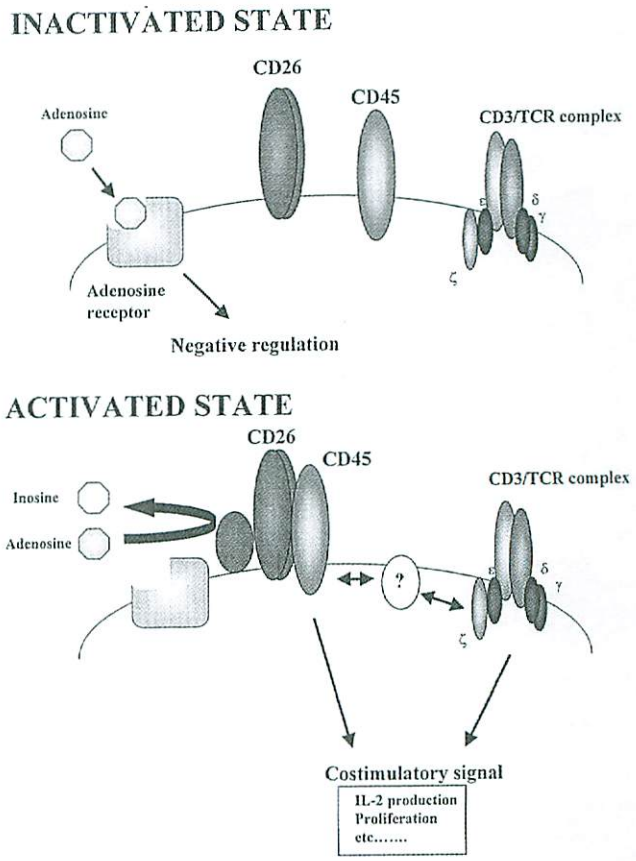

Figure 1. A model representing the possible roles of CD26 in T-cell activation. A stimulatory signal through only the CD3/TCR complex is insufficient for activation in inactivated T-cells. In addition, extracellular adenosine may possibly provide an inhibitory signal. In CD26-mediated T-cell activation, signaling through CD26 is communicated at least partly through CD45 besides the CD3/TCR complex signaling, with the DPPIV activity of CD26 being essential for CD26-associated signaling. Meanwhile, extracellular adenosine is converted to inosine by CD26-associated ADA. Additional molecules may be associated with CD26-mediated signaling since neither ADA nor CD45 interacts directly with the CD3 chain. Adapted from Ohtsuki *et al* (45).

mitogen-stimulated peripheral blood mononuclear cells and U937-H cells, including a reduction in IL-6 and IL-1ß production (57,60). Besides, DPPIV inhibitors have been shown to suppress phosphorylation of $p56^{lck}$, Ca^{2+} flux and activation of phosphoinositol-3 and the MAP kinases ERK1 and ERK2 (61,62), all known to be involved in the T-cell activation cascades. These results therefore suggest that its intrinsic DPPIV activity is essential for CD26-mediated T-cell co-stimulation.

The CD26 cDNA sequence predicts a type II integral membrane protein with a large extracellular domain containing its DPPIV activity, a transmembrane segment, and a cytoplasmic tail. The fact that its cytoplasmic domain consists only of six amino acids suggests that CD26 may be physically and functionally associated with other molecules involved in signal transduction (54). Molecules that have been demonstrated to physically associate with CD26 include CD45, a membrane-linked protein-tyrosine phosphatase (43), and adenosine deaminase (ADA) (63-66). Fig. 1 shows the possible roles of CD26 in human T-cell activation. ADA is a soluble globular enzyme present in all mammalian tissues that

plays an important role in the development and function of lymphoid tissue (67,68). The main consequence of hereditary deficiency of ADA is severe combined immune deficiency, a profound lymphopenia causing impairment of cellular and humoral immunity. Although ADA is more than 90% intracellular, it is also located on the cell surface of T and B lymphocytes (44). Recent *in vitro* studies have demonstrated that ADA cell-surface expression was dependent on CD26 surface expression and that co-expression of ADA with CD26 on the cell surface could block the inhibitory effect of accumulated adenosine on T-cell proliferation (44,65). These findings hence identified a potentially novel role for cell surface ADA as a molecule acting in conjunction with CD26 on human T lymphocytes. Besides ADA, CD26 is physically associated with CD45, as antibody-induced modulation of CD26 on T-cells resulted in a concurrent decrease in CD45 expression, enhanced phosphorylation of the TCR-associated ζ chain and increased $p56^{lck}$ kinase activity (43). Meanwhile, recent work demonstrated that CD26 localizes into lipid rafts, and targeting of CD26 to rafts is necessary for signaling events through CD26. Furthermore, antibody-mediated aggregation of CD26 also caused CD45 co-aggregation into lipid rafts, through binding of CD26 to the cytoplasmic domain of CD45. This CD26-CD45 interaction then resulted in enhanced protein tyrosine phosphorylation of such key signaling molecules as $p56^{lck}$, ZAP-70 and TCR-associated ζ chain (69). It is therefore apparent that CD26 plays an important role in human T-cell physiology through numerous facets, including its intrinsic DPPIV activity and its association with such key molecules as CD45 and ADA.

Besides its function as a transmembrane molecule, CD26 also exerts its effect on T-cell biological programs as a soluble molecule, most likely through its DPPIV activity. For example, soluble CD26 (sCD26) molecules mediated enhanced transendothelial T-cell migration in a DPPIV-dependent manner. Furthermore, sCD26 appeared to directly target endothelial cells by interacting with the mannose 6-phosphate/insulin-like growth factor II receptor (M6P/IGFIIR) on the endothelial cell surface (70). Meanwhile, sCD26 enhanced proliferation of peripheral blood T lymphocytes induced by the recall antigen tetanus toxoid by interacting with the M6P/IGFIIR of the $CD14^+$ monocytes in the early stages of immune response. Importantly, sCD26 up-regulated the expression of the co-stimulatory molecule CD86 on monocytes through its DPPIV activity at both protein and mRNA levels, thereby affecting T-cell-monocyte interaction to enhance T-cell proliferative response to the recall antigen (71).

Chemokine processing. Originally named as a shortening of chemoattractant cytokines, chemokines are a superfamily of small (6-14 kDa) secreted proteins. To date, over 40 chemokines have been identified (72-75). While most chemokines cause migration of leukocytes, these molecules also affect angiogenesis, proliferation of hematopoietic precursors, and viral responses. The chemokine superfamily is divided into four subgroups, CC, CXC, C and CX3C families, based on a cysteine motif. The two most important subfamilies are CXC and CC chemokines, which differ in the spacing of the first two cysteine residues that form disulfide links with two other cysteines. In the CXC chemokine subset, these first cysteine

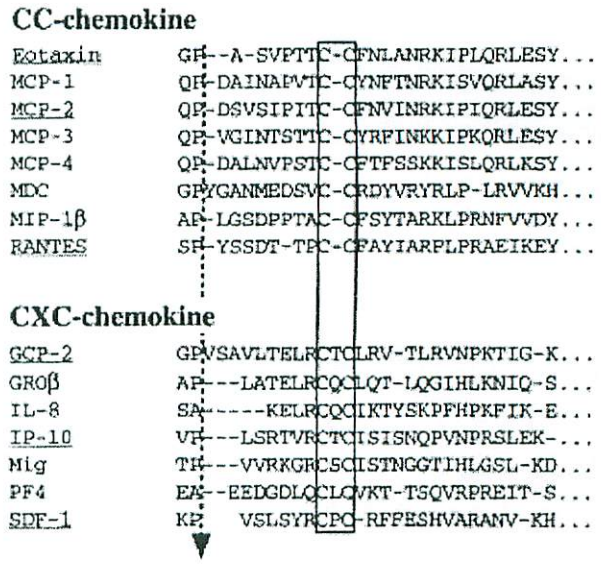

Figure 2. Alignment of N-terminal sequences of chemokines containing the conserved sequence for DPPIV-mediated cleavage. Underlined chemokines have been reported to be substrates of DPPIV (76,78,84,90-92). The conserved CC- and CXC-motifs are boxed, and dashed arrow indicates possible cleavage sites by DPPIV. Adapted from Ohtsuki *et al* (45).

residues are separated by one amino acid; whereas in the CC chemokine subgroup, they are directly neighbored. Some of the CC- and CXC-chemokines contain the conserved amino acid sequence for cleavage by DPPIV at the N-terminus, and hence they are potential substrates of CD26 (Fig. 2). CCL3 (macrophage inflammatory protein-1α, MIP-1α; isoform LD78ß), CCL4 (macrophage inflammatory protein-1ß, MIP-1ß), CCL5 (regulated on activation, normal T-cell expressed and secreted, RANTES), CCL11 (eotaxin), CCL22 (monocyte-derived chemokine, MDC), CXCL9 (monokine-induced by IFN-γ, Mig), CXCL10 (inflammatory protein-10, IP-10), CXCL11 (IFN-inducible T-cell α-chemoattractant, I-TAC) and CXCL12 (stromal cell-derived factor-1, SDF-1) are DPPIV substrates in their natural *in vivo* forms (76-84).

Since the initial studies by Oravecz and colleagues demonstrating that the receptor specificity of CCL5 is altered by DPPIV cleavage (76), an emerging aspect of CD26 biology is the ability of its intrinsic DPPIV activity to alter the receptor specificity and biological function of an increasing number of chemokines. CCL5 (RANTES), whose receptors are CCR1, CCR3 and CCR5, is known to inhibit HIV infection via its binding to CCR5 (85). This chemokine is cleaved by CD26/DPPIV, resulting in the removal of its N-terminal dipeptide (76,78,84). Of note is the fact that while signaling through CCR1 and CCR3 by RANTES is abolished by the 2 amino acid-truncation mediated by CD26/DPPIV, signaling through CCR5 is not affected (76,86,87), suggesting a differential effect of CD26/DPPIV cleavage on chemokine activity. It is therefore possible that CD26/DPPIV-mediated processing of RANTES and the receptor expression on target cells provide an integrated mechanism for differential cell recruitment and for the regulation of target cell specificity. In this regard, Iwata *et al* have shown that the 2 amino acid-truncated RANTES (-2) specifically attracts T-cells but fails to attract monocytes.

On the other hand, non-truncated RANTES attracts both T-cells and monocytes. These authors also demonstrated that the presence of soluble CD26 with DPPIV activity significantly enhances the chemotactic effect of RANTES (-2) as well as non-truncated RANTES against T-cells (84). These findings hence suggest that there may be additional interactions between CD26/DPPIV and RANTES besides N-terminal cleavage. It is of interest to note that RANTES (-2) has been reported to inhibit HIV infection more efficiently than non-truncated RANTES (78).

CXCL12 (SDF-1) is a ligand for CXCR4 and is capable of blocking HIV infection (88,89). The chemotactic and anti-HIV activities of mature SDF-1 are both abolished by cleavage with membrane-bound CD26/DPPIV as well as its soluble form (90-92). Since SDF-1 appears to bind principally to CXCR4, the disappearance of both the chemotactic and anti-HIV activities of 2 amino acid-truncated SDF-1 derived from CD26/DPPIV-mediated cleavage of SDF-1 may be due to its inability to bind to CXCR4. By the same token, truncated SDF-1 fails to induce signaling and inhibit the adaptation of HIV particles (93).

Among the other candidate chemokine substrates of CD26/DPPIV, CCL3 (MIP-1) and CCL22 (MDC) are also reported to inhibit HIV infection (85,94,95). Cocchi *et al* (85) identified MIP-1α and MIP-1ß, as well as RANTES, as the major HIV-SF (suppressive factors) released from immortalized and primary $CD8^+$ T-cells. Furthermore, these researchers demonstrated that recombinant human MIP-1α, MIP-1ß, and RANTES inhibited infection by different HIV strains in a dose-dependent fashion. Meanwhile, Pal *et al* (94) purified a protein produced by HIV-1 infected $CD8^+$ T-cells that is associated with HIV-SF and identified it to be MDC, which inhibits infection from both X4 and R5 HIV strains. In addition, Struyf *et al* (80) reported that cleavage of MDC at the NH2-terminus resulted in a reduction of the chemotactic potency and an increase in the inhibitory activity of HIV-1 infection, as compared to intact MDC. Since MDC truncated at the NH2-terminus showed reduced binding capacity to CCR4, and since CCR4 has not been shown to be an HIV-1 co-receptor, its increased antiviral effect is possibly caused by its interaction with another MDC receptor associated with HIV infection.

Other groups have recently suggested that chemokines are related with the balance between Th1 and Th2 cells as well as the polarized expression of chemokine receptors on Th1 and Th2 populations (95-97). Th1 cells particularly express high levels of CCR5 and CXCR3. On the other hand, Th2 cells express CCR4, CCR8, CXCR4 and lesser level of CCR3 (96,97). CD26 is preferentially expressed on Th1 cells (95) and is able to cleave such chemokines as RANTES, eotaxin, MDC, MIP-1ß, SDF-1, IP-10 [interferon (IFN)-γ-inducible protein-10; CXCL10], Mig (monokine induced by IFN-γ; CXCL9) and I-TAC (IFN inducible T-cell α-chemoattractant; CXCL11), which interact with these receptors. The cleaved products of these chemokines bind to Th1 but not Th2-specific chemokine receptors (96). Therefore, CD26/DPPIV potentially contributes to the down-regulation of Th2 responses by Th1 cells.

More recently, Proost *et al* (98) reported novel findings relating to the interaction between CXCR3, which is exclusively expressed on Th1 lymphocytes, and three chemokines induced by IFN-γ, IP-10, Mig and I-TAC. They reported that processing of IP-10 and I-TAC by CD26/DPPIV caused a reduction in CXCR3-binding potentials, loss of calcium-signaling capacity through CXCR3, and reduced chemotactic potency. In addition, they demonstrated that I-TAC and IP-10 processed by CD26/DPPIV played a role as antagonists of chemotaxis and that cleavage of Mig and IP-10 by CD26/DPPIV did not alter their ability to inhibit interleukin-8-mediated angiogenesis. These data suggested that CD26/DPPIV contributes to the negative feedback regulation of chemotaxis through CXCR3 as well as regulation of angiogenesis.

4. CD26 in hematological malignancies

The basic role of CD26 in T-cell function has been relatively well-understood (38-44). However, its potential contribution to the development of human hematological malignancies has only recently been investigated. Carbone *et al* (99) reported that CD26 was expressed mostly on $CD30^+$ anaplastic large-cell lymphoma (ALCL) (12 of 17 cases) regardless of antigenic phenotype, and on T-cell lymphomas that are not ALCL (7 of 15 cases). In contrast, CD26 was not detected among 26 B-cell lymphomas except for B-cell anaplastic large-cell lymphomas, and was rarely found in Hodgkin's disease (2 of 23 cases). In addition, while activated or reactive normal T-cells were found to co-express CD26 and CD40 ligand, the expression of these molecules was mutually exclusive in human T-cell lymphomas/leukemias (100). CD26 expression was restricted mainly to aggressive diseases, such as T-cell acute lymphoblastic leukemia (T-cell LBL/ALL) (12 of 23 cases) and T-cell $CD30^+$ anaplastic large-cell lymphoma (5 of 8 cases). Meanwhile, CD40 ligand was expressed on relatively indolent diseases, such as mycosis fungoides (11 of 21 cases). Intriguingly, within aggressive subgroups such as T-cell LBL/ALL or $CD30^+$ALCL, lymphomas expressing CD26 appeared to have poorer prognosis. On the other hand, Bauvois *et al* (101) showed that B-chronic lymphocytic leukemia (CLL) cells expressed high levels of CD26 protein and mRNA transcripts as compared to normal resting B-cells. Furthermore, treatment with interferons and retinoic acid enhanced CD26 gene and protein expression in B-CLL cells through the engagement of signaling pathways involving Stat1α and the GAS response element of the CD26 promoter (103). Meanwhile, at M.D. Anderson Cancer Center, we examined surface CD26 expression in peripheral blood samples from patients with morphologically identifiable tumor cells of a T-cell lymphoproliferative disorder, primarily mycosis fungoides/Sezary syndrome (MF/SS) (103). We demonstrated that an abnormal $CD26^-$ (23 of 28 cases)/dim (5 of 28 cases) T-cell population was distinguishable from the variable CD26 expression seen in normal peripheral blood T lymphocytes. Furthermore, based on the absent or dim expression of CD26 on tumor cells, it appeared to be a more sensitive marker than CD7 for the diagnosis of MF/SS. Interestingly, Kondo *et al* (104) reported that CD26 expression was significantly decreased in all 20 patients with adult T-cell leukemia/lymphoma as compared to healthy individuals. They concluded that HTLV-1 has possibly *in vivo* tropism to $CD26^-$ cells

because of a high copy number of viral DNA in the CD26$^-$ cells compared with CD26$^+$ cells.

Other researchers have recently reported additional evidence supporting a role for this molecule in the pathogenesis of T-cell malignancies. Extensive immunophenotypic characterization of T-acute lymphoblastic leukemia cells revealed a strong association between CD26 enzymatic activity, features of T-cell immaturity (i.e., lack of CD3 cell surface expression), and proliferative activity. It is interesting to note that CD26 expression increases in relatively immature human thymocytes, as indicated by membrane CD3 antigen absence (105). Moreover, CD26 enzymatic activity varied with the stage of differentiation, as well as activation of human alloreactive T-cell subsets, suggesting that CD26/DPPIV potentially plays an important role in the generation of specific alloimmunity (106). Similar observations were made with neoplastic T-cells, in which the presence (or intensity) of cell-surface CD26 expression was concordant with the level of DPPIV (107). Meanwhile, Reinhold *et al* (60) assessed the importance of CD26 expression and its DPPIV enzymatic function in CD26$^+$ U937 lymphoma cells. They reported that suppression of DPPIV enzymatic activity by specific inhibitors resulted in the inhibition of both DNA synthesis and cellular proliferation, as well as modulation of cytokine production. Their findings further support the notion that DPPIV enzymatic activity plays an important role in the biology of U937 lymphoma cells. Taken together, these data suggest that CD26 cell-surface expression and its DPPIV activity may be involved in the pathogenesis of T-cell malignancies.

5. CD26 as novel target in T-cell lymphoproliferative diseases

In vitro and in vivo antitumor effect of anti-CD26 monoclonal antibody 1F7 (108,109). In view of the multi-faceted role played by CD26 in T-cell physiology, it is our hypothesis that CD26 may be an appropriate target for novel treatment modalities for T-cell neoplasms. T-cell stimulation by various agents leads to enhanced CD26 surface expression as one of the T-cell activation markers (48,110). Furthermore, CD26 acts as a co-stimulatory surface molecule via the CD3 and CD2 pathways of activation as well as an alternate pathway of T-cell activation when cross-linked by its specific mAbs, possibly due to its interaction with signaling molecules such as the protein tyrosine phosphatase CD45 (38-43,111,112). Meanwhile, soluble anti-CD26 mAbs and DPPIV inhibitors suppress T-cell growth and function in certain experimental conditions (62,65,113,114). Since CD26 is able to transmit signals affecting cellular proliferation and is expressed on certain aggressive T-cell lymphomas (99,100), we recently investigated the potential use of soluble anti-CD26 mAb in CD26-expressing T-cell malignancies (108), using as our model the CD30$^+$ T-cell anaplastic large-cell lymphoma cell line Karpas 299 (115).

While surface expression of CD26 was down-modulated following incubation with anti-CD26 mAb (Fig. 3), consistent with previous reports demonstrating antibody-induced down-modulation of CD26 from normal T lymphocyte surface (41), binding of the anti-CD26 mAb recognizing the 1F7 epitope inhibited tumor growth *in vitro* as determined by the MTT

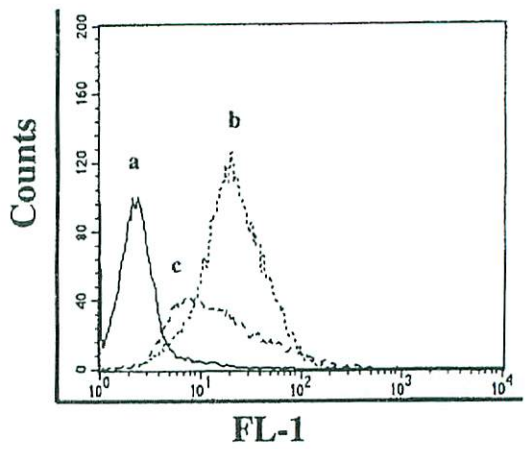

Figure 3. Phenotypic characterization of Karpas 299. After overnight incubation with the anti-CD26 mAb 1F7 (1 μg/ml) at 37°C, Karpas 299 cells were examined by flow cytometry for CD26 expression and compared with CD26 expression in non-treated cells. a, Negative control; b, CD26 expression in non-treated cells; c, CD26 expression following overnight Ab incubation. Adapted from Ho *et al* (108).

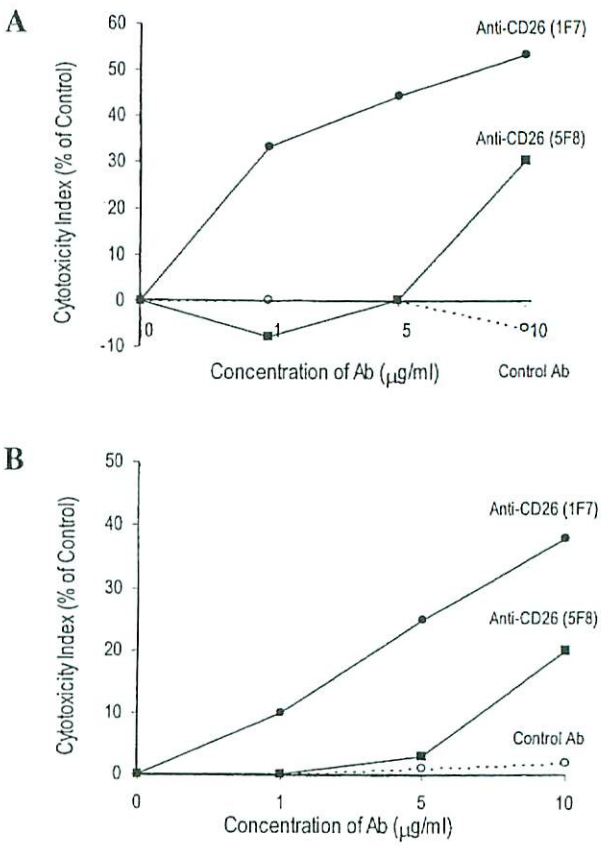

Figure 4. Inhibitory effect of soluble anti-CD26 mAb on cell growth. MTT uptake assays of Karpas 299 (A) or H9 (B) cells cultured in medium containing soluble anti-CD26 mAb 1F7, anti-CD26 mAb 5F8, or isotype control mAb. Adapted from Ho *et al* (108).

assay (Fig. 4). Moreover, 1F7 treatment enhanced cell cycle arrest at the G_1-S checkpoint, associated with increased p21 expression (Fig. 5). Extending our *in vitro* findings, we investigated the effect of 1F7 on Karpas 299 growth in a

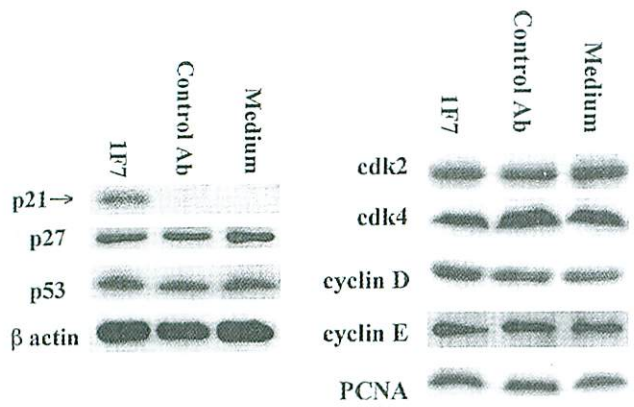

Figure 5. Enhanced p21 expression following anti-CD26 treatment. Karpas 299 cells were incubated overnight at 37°C in medium alone or medium containing isotype control mAb or 1F7 (2 μg/ml). After lysis, SDS-PAGE and immunoblotting studies were then performed with the appropriate Abs (108).

SCID mouse tumor model. SCID mice were inoculated with Karpas 299 cells by i.p. injection ($1x10^6$ cells/mouse) and starting at day 1 after tumor inoculation, the animals were treated with saline, isotype control Ab, or 1F7 at the indicated doses every other day for a total of 10 i.p. injections. As shown in Fig. 6A, mice treated with 1F7 at 5 or 10 μg/injection had statistically significant survival advantage as compared to those treated with saline ($P<0.0001$) or isotype control Ab ($P<0.001$). When mice were inoculated with higher i.p. doses of tumor cells ($3x10^6$ cells/mouse), we again observed statistically significant survival advantage for mice treated with 1F7 as compared with saline alone (for 1F7 at a dose of 5 μg/injection, $P=0.03$; for 1F7 at a dose of 10 μg/injection, $P=0.03$; for 1F7 at a dose of 20 μg/injection, $P<0.01$). Also, treatment with 20 μg-1F7 led to significant survival advantage as compared to treatment with 20 μg-isotype control ($P<0.01$). Comparing mice treated with different 1F7 doses, those treated with 20 μg-1F7 had statistically significant survival advantage over those treated with 5 μg-1F7 ($P<0.01$). Although the difference was not statistically significant ($P=0.2$), treatment with 20 μg-1F7 appeared to be associated with better survival than treatment with 10 μg-1F7 (Fig. 6B).

As another means of establishing the *in vivo* antitumor effect of 1F7, we evaluated its potential efficacy in *de novo* tumor growth inhibition when administered by another route. We investigated the time required for initial appearance of Karpas 299 tumors after subcutaneous injection of tumor cells and subcutaneous treatment with saline, 1F7, or isotype control Ab. Karpas 299 cells ($1x10^6$/mouse) were incubated in saline alone prior to being injected subcutaneously into the SCID mice; or the cells were mixed with 100 μg of 1F7 or isotype control Ab and immediately after mixing, they were injected subcutaneously into the animals. Starting 1 day after tumor cell inoculation, SCID mice received subcutaneous injections of saline, 20 μg-isotype control Ab, or 20 μg-1F7 every other day for 10 injections, placed at the original site of subcutaneous tumor injection. The day of initial appearance of a visible tumor was then documented to determine treatment efficacy. As shown in Fig. 7, there was a statistically significant difference in the rate of visible tumor development among

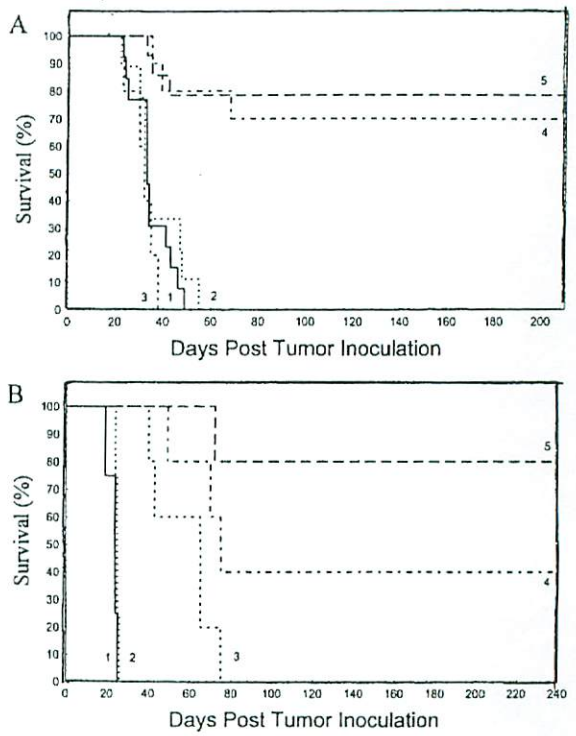

Figure 6. Enhanced survival of Karpas 299-bearing SCID mice treated with 1F7. A, 1 day after i.p. inoculation of SCID mice with $1x10^6$ Karpas 299 cells per mouse, saline alone, isotype control Ab (5 or 10 μg/injection), or 1F7 (5 or 10 μg/injection) was then administered by i.p. injections every other day for a total of 10 injections. Arm 1, saline alone (n=13); arm 2, isotype control Ab (5 μg/injection, n=10); arm 3, isotype control Ab (10 μg/injection, n=5); arm 4, anti-CD26 mAb 1F7 (10 μg/injection, n=10); arm 5, anti-CD26 mAb 1F7 (5 μg/injection, n=14). B, 1 day after i.p. inoculation of SCID mice with $3x10^6$ Karpas 299 cells per mouse, i.p. treatment with saline alone, isotype control Ab (20 μg/injection), or 1F7 (5, 10 or 20 μg/injection) was then administered every other day for a total of 10 injections. Arm 1, saline alone (n=5); arm 2, isotype control Ab (20 μg/injection, n=5); arm 3, 1F7 (5 μg/injection, n=5); arm 4, 1F7 (10 μg/injection, n=5); arm 5, 1F7 (20 μg/injection, n=5). Adapted from Ho *et al* (108).

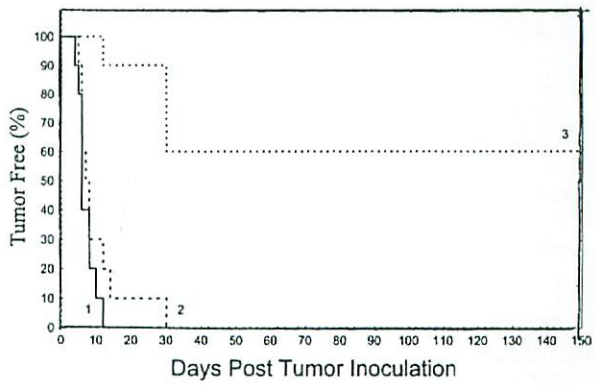

Figure 7. Initial tumor appearance in SCID mice after s.c. tumor cell inoculation and s.c. treatment with antibodies. Karpas 299 tumor cells ($1x10^6$) incubated in saline alone were administered s.c into SCID mice. Alternatively, $1x10^6$ Karpas 299 tumor cells were mixed on ice with 100 μg of 1F7 or isotype control Ab, and then the cells were immediately injected s.c. into SCID mice after mixing. Subsequently, starting 1 day after tumor cell inoculations, SCID mice then were subcutaneously treated with saline, isotype control Ab (20 μg/injection), or 1F7 (20 μg/injection) *in 0.1 ml of sterile saline every* other day for 10 injections, placed at the original site of s.c. tumor injection. The day of initial appearance of a visible tumor was documented to monitor treatment effects. Arm 1, saline alone (n=10); arm 2, isotype control Ab (n=10); arm 3, anti-CD26 mAb 1F7 (n=10). Adapted from Ho *et al* (108).

the mice treated with different conditions. The mice treated with 1F7 exhibited a lower rate of tumor development as compared to those treated with isotype control Ab or saline alone ($P<0.001$ and $P<0.001$, respectively), with most of 1F7-treated mice remaining free from tumor within the study duration.

To further understand the mechanism involved in antibody-mediated growth inhibition, particularly the role of the DPPIV enzyme activity in this process, we examined 1F7 effect on the human T-leukemia cell line Jurkat transfected with wild-type CD26 molecules (J.CD26/DP$^+$) or CD26 molecules mutated at the DPPIV site (J.CD26/DP$^-$) (109). We demonstrated that the presence of DPPIV enzyme activity was important for antibody-mediated growth inhibition, since 1F7 inhibited growth of J.CD26/DP$^+$ but not J.CD26/DP$^-$ cells. Similar to the case with Karpas 299 cells, 1F7 binding to J.CD26/DP$^+$ led to G_1/S arrest associated with enhanced $p21^{cip1}$ expression. In addition, expression of $p21^{cip1}$ after 1F7 treatment appeared to be induced through activation of Cb1 and ERK pathway.

Taken together, our findings demonstrated that anti-CD26 Ab can inhibit growth of human T-cell lines, suggesting that antibody therapy targeting CD26 surface expression may be of potential significance in the treatment of selected human hematological malignancies. Furthermore, its intrinsic DPPIV enzyme activity may play an important role in this process, with potential implications in the clinical setting.

Effect of CD26/DPPIV expression on Jurkat sensitivity to doxorubicin-induced cell cycle arrest (116). As reported previously (64,100), CD26 expression is associated with changes in tumor cell line behavior *in vitro*, while the clinical behavior of certain T-cell tumors possibly correlates with differences in the level of CD26 expression. In addition, DPPIV enzyme activity appears to have a significant role in the biology of certain lymphoma cell lines (60). Based our hypothesis that the presence of CD26/DPPIV affects tumor cell biology, we investigated the role of CD26 in cellular response to cytotoxic agents. The anthracyclines are widely used antineoplastic agents with broad-spectrum anticancer activity. As shown previously (117,118), doxorubicin-mediated-DNA damage leads to cell cycle arrest at the G_2-M checkpoint by inhibiting $p34^{cdc2}$ kinase dephosphorylation and inducing cyclin B1 accumulation. In view of the integral role of the anthracyclines in the treatment of hematological malignancies, we investigated the effect of CD26/DPPIV expression on cellular sensitivity to doxorubicin against the human T leukemia cell line Jurkat and its various CD26 transfectants.

Besides the CD26$^-$ parental Jurkat cells, we have created the following stable CD26 transfectants, as described previously (55,65,66): a) wild-type CD26-transfected Jurkat (wtCD26); b) Jurkat transfected with mutant CD26 containing an alanine at the putative catalytic serine residue at position 630, resulting in a mutant CD26$^+$/DPPIV$^-$ Jurkat transfectant (S630A); c) Jurkat transfected with mutant CD26 containing point mutations at ADA-binding site residues 340-343, with amino acids L340, V341, A342, and R343 being replaced by amino acids P340, S341, E342, and Q343, resulting in a mutant CD26$^+$/DPPIV$^+$ Jurkat transfectant incapable of binding ADA [340-4]; d) vector-only Jurkat transfectant (neo). Of note is the fact that the wtCD26 transfectants still retained DPPIV activity following doxorubicin treatment (data not shown).

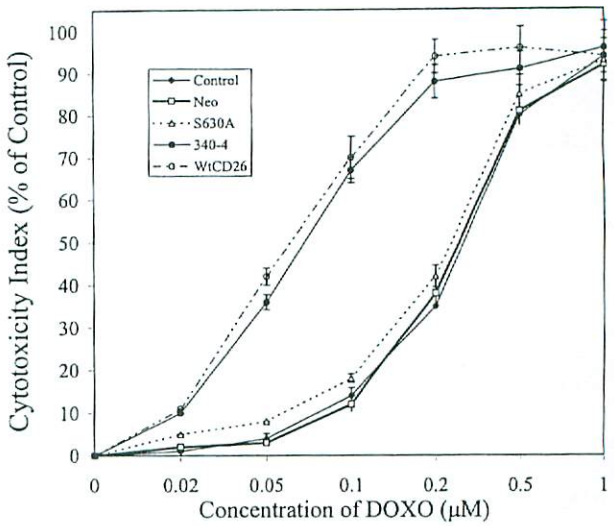

Figure 8. Effect of CD26/DPPIV expression on doxorubicin-mediated growth inhibition. CD26 Jurkat transfectants were treated at 37°C with culture media alone or culture media containing doxorubicin at the indicated concentrations, and MTT uptake assays were performed. wtCD26, wild-type CD26 Jurkat transfectant; S630A, CD26$^+$/DPPIV$^-$ mutant CD26 Jurkat transfectant; control, non-transfected Jurkat; 340-4, CD26$^+$/DPPIV$^+$ mutant CD26 Jurkat transfectant; neo, plasmid-only Jurkat transfectant. Data represent the means of three separate experiments. Adapted from Aytac *et al* (116).

MTT uptake assays demonstrated that wtCD26 exhibited greater sensitivity to doxorubicin as compared with parental Jurkat or vector-only transfectants (Fig. 8). Importantly, CD26 transfectants mutated at the DPPIV catalytic site (S630A) were also less sensitive to doxorubicin. Similar to the wtCD26 cells, Jurkat cells transfected with CD26 mutated at the ADA-binding site while still retaining DPPIV activity [340-4] showed enhanced sensitivity to doxorubicin. Consistent with these results were cell cycle analyses showing greater doxorubicin-induced G_2/M arrest in wtCD26 and 340-4 transfectants (Table IV). Evaluating the status of key regulators of the G_2-M checkpoint (118-123) (Fig. 9), we found that doxorubicin had a greater effect on $p34^{cdc2}$ Thr14Tyr15 phosphorylation and kinase activity, cdc25C phosphorylation of serine-216, and cyclin B1 expression in wtCD26 cells as compared to parental Jurkat and S630A transfectants. These findings hence indicated that the presence of CD26, in particular its intrinsic DPPIV enzymatic activity, enhanced cellular sensitivity to DNA damage by doxorubicin.

We also investigated the effect of exogenous soluble CD26 (sCD26) molecules on parental Jurkat and CD26 Jurkat transfectants treated with low doses of doxorubicin. Although sCD26 by itself did not affect MTT uptake in cells incubated in medium alone, significant enhancement in sensitivity to doxorubicin was observed in the presence of exogenous sCD26 (Fig. 10). Besides the Jurkat cells, the addition of sCD26 also enhanced the growth inhibitory effect of doxorubicin in the B-cell lines Namalwa and Jiyoye (Fig. 11), implying potential role in the clinical setting for CD26/

Table IV. Enhanced doxorubicin-induced G_2/M arrest in association with CD26/DPPIV expression.[a]

	% G_0/G_1	% S	% G_2/M
Media alone			
Control	53	33	14
S630A	50	40	10
wtCD26	46	42	12
340-4	49	41	10
Doxorubicin (0.01 μM)			
Control	40	26	34
S630A	33	32	35
wtCD26	11	27	62
340-4	25	25	50
Doxorubicin (0.05 μM)			
Control	32	31	37
S630A	27	35	38
wtCD26	6	25	69
340-4	11	11	78

Adapted from Aytac *et al* (116). [a]CD26 Jurkat transfectants were incubated at 37°C in media containing doxorubicin for 24 h. Cells were then harvested, and cell cycle analyses were performed. Data are representative of three separate experiments.

DPPIV in the treatment of hematological malignancies of both B and T-cell lineages.

6. Clinical trial for T-non-Hodgkin's lymphomas with the ADA inhibitor pentostatin

Adenosine deaminase (ADA) is essential for purine metabolism, and lack of ADA activity is linked to immunosuppression. Previous work has shown that CD26 is physically associated with surface expressed ADA on human T lymphocytes (63-66). Based on our knowledge of CD26 biology, we initiated a phase II study at M.D. Anderson Cancer Center to examine the effect of the ADA inhibitor pentostatin on relapsed T-cell hematological malignancies (Dang NH, *et al*, Blood 98: abs. 130, 2001). The objectives of the study are to correlate response to tumor cell CD26 status and to evaluate the lymphopenic effect of pentostatin on $CD26^+$ T lymphocytes. Interim analysis of 11 evaluable cases showed an overall response rate of 45%. One of 2 $CD26^+$ cases responded; 4 of 7 $CD26^-$ cases responded; while tumor CD26 status of 2 non-responders was not examined. Pentostatin also preferentially reduces the level of $CD26^+$ T lymphocytes in the patients' peripheral blood or bone marrows in both responders and non-responders. Since the $CD26^+$ T-cell population represents activated T lymphocytes, with the $CD4^+CD26^+$ subset belonging to the helper/memory population, a decrease in the levels of $CD26^+$ T lymphocytes induced by pentostatin has potentially significant implications for immune system function in treated patients.

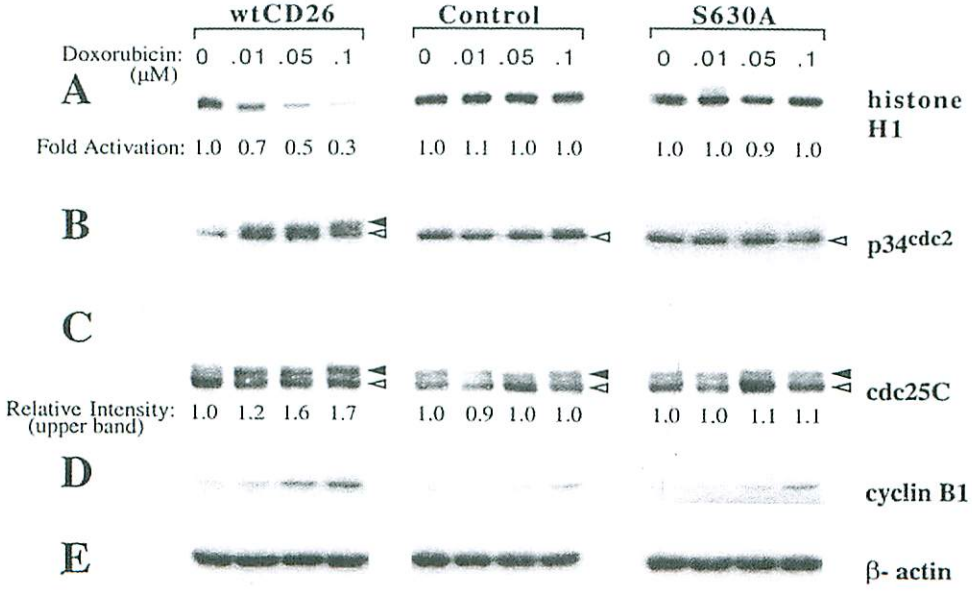

Figure 9. Effect of CD26/DPPIV expression on $p34^{cdc2}$/cyclin B1 complex and cdc25C after doxorubicin treatment. Jurkat cells were incubated for 24 h at 37°C with doxorubicin-containing media at the indicated doses. Cells were then harvested, and kinase assays and immunoblotting studies were performed. A, Whole cell lysates were prepared and $p34^{cdc2}$ kinase activity was measured by immunocomplex kinase assay with histone H1 as a substrate. After quantification with phosphoimager, $p34^{cdc2}$ kinase activity of untreated cells was given an arbitrary value of 1, and others were measured relative to this value. B, Protein levels of $p34^{cdc2}$ were evaluated by immunoblotting studies with anti-$p34^{cdc2}$. Black triangles show the phosphorylated $p34^{cdc2}$ kinase. C, Protein levels of cdc25C were evaluated by immunoblotting studies with anti-cdc25C. The two major electrophoretic forms reflect differences in serine-216 phosphorylation. Black triangle indicates the serine-216-phosphorylated form of cdc25C (cdc25C-P). After quantification with phosphoimager, intensity of the cdc25C-P band of untreated cells was given an arbitrary value of 1, and other activities were measured as comparison to this value. D, Protein levels of cyclin B1 were evaluated by immunoblotting studies with anti-cyclin B1. E, Protein levels of actin were evaluated by immunoblotting studies with anti-actin. Adapted from Aytac *et al* (116).

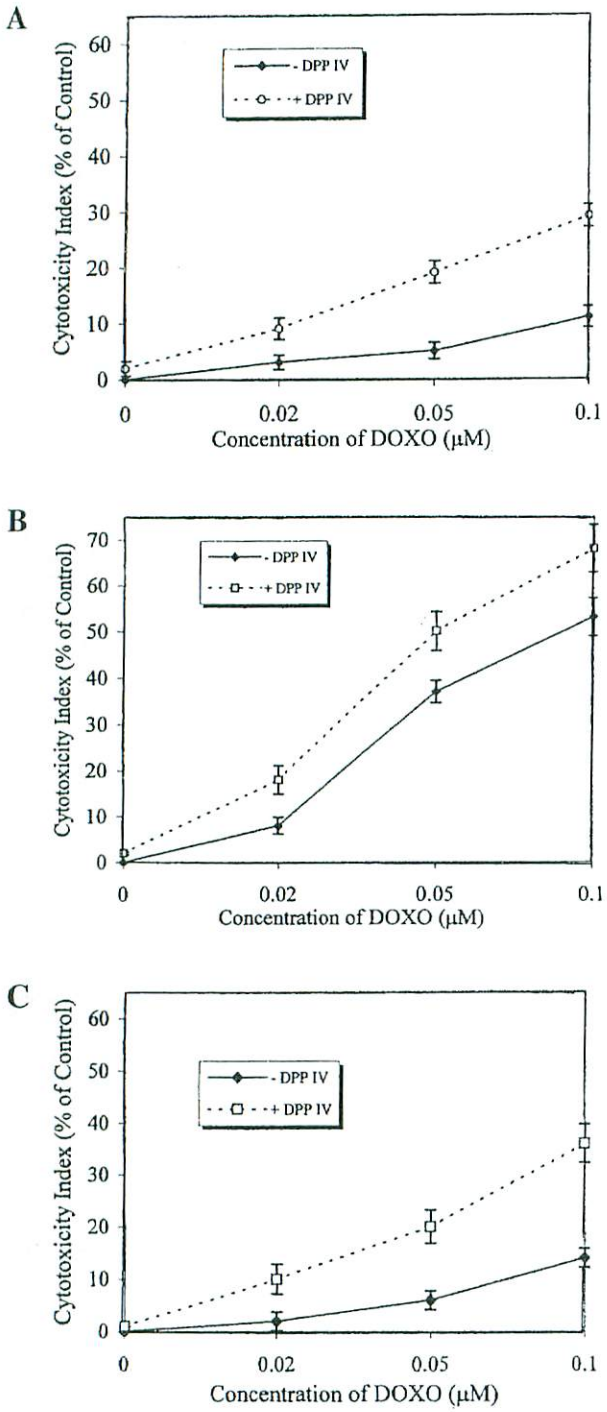

Figure 10. Effect of exogenous sCD26 on doxorubicin-mediated growth inhibition of Jurkat cells. Jurkat cells were cultured at 37°C in media alone, media with doxorubicin alone at the indicated concentrations, media with sCD26/DPPIV (50 μg/ml) alone, and media with doxorubicin at the indicated concentrations and sCD26/DPPIV (50 μg/ml). MTT assays were performed. Data represent the means of three separate experiments. A, Control; B, wtCD26; C, S630A. Adapted from Aytac *et al* (116).

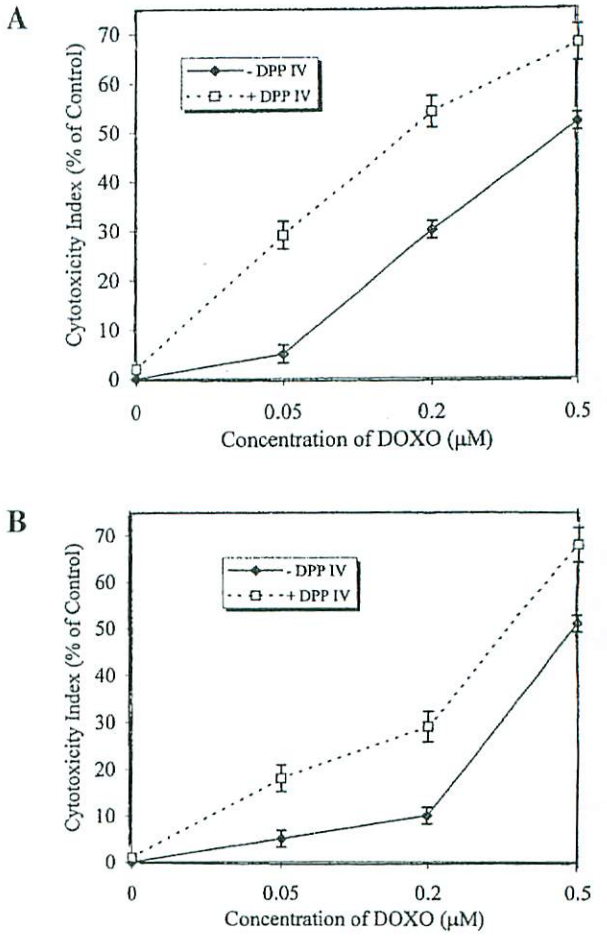

Figure 11. Effect of exogenous sCD26 on doxorubicin-mediated growth inhibition of B-lymphoid cell lines. Cells were incubated at 37°C in media alone, media with doxorubicin alone at the indicated concentrations, media with sCD26/DPPIV (50 μg/ml) alone, and media with doxorubicin at the indicated concentrations and sCD26/DPPIV (50 μg/ml). MTT assays were performed. Data represent the means of three separate experiments. A, Jiyoye; B, Namalwa. Adapted from Aytac *et al* (116).

7. Conclusion

In this review, we have highlighted some of the major aspects of CD26 biology, particularly its integral role in human T-cell activation and chemokine processing. It is evident from the wealth of published studies that CD26 has an essential role in the biology of normal T lymphocytes as a key immunoregulator. Meanwhile, emerging data suggest that it may be a factor in the development and behavior of selected T-cell lymphoid malignancies. Its expression on selected subsets of T-cell tumors may reflect its potential role in disease pathogenesis and clinical behavior. Based on the fact that T-cell lymphoid malignancies are generally aggressive and resistant to current treatments, the development of new treatment approaches based on a detailed understanding of the molecular processes involved in disease development would have potential benefit in the clinical setting. Our recent findings suggest that CD26 indeed may be an appropriate target for novel treatment modalities in T-cell lymphoid tumors. Future work will focus on further developing treatment strategies based on CD26 biology, and to understand the molecular mechanisms of CD26 involvement in tumor behavior.

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