Histol Histopathol (2002) 17: 1213-1226

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Review

CD26: An expanding role in immune regulation and cancer

N.H. Dang and C. Morimoto

Department of Lymphoma/myeloma and Department of Clinical Immunology and AIDS Research Center, Institute of Medical Science, University of Tokyo, Minato-ku, Tokyo, Japan

Summary. In this review, we highlight major aspects of the biology of CD26, a dipeptidyl peptidase IV (DPPIV)-containing surface glycoprotein with multiple functions. In particular, we discuss findings demonstrating that CD26/DPPIV has an essential role in immune regulation as a T cell activation molecule and a regulator of chemokine function. We also review recent studies that identify key cellular molecules that physically associate with CD26 and the potential consequences of their interaction, including those with clinically-related implications. Furthermore, we present work suggesting a role for CD26 in the pathogenesis and behavior of selected human cancers, both solid tumors and hematological malignancies. We present recent studies that investigate the potential role of CD26 as a molecular target for novel treatment modalities for T cell lymphoid malignancies and possibly other hematological malignancies, with work involving the use of anti-CD26 monoclonal antibody, CD26-transfected cells as well as soluble CD26 molecules.

Key words: CD26/DPPIV, T cell activation, Chemokine, Hematological malignancies, Cell cycle

Introduction

CD26 is a 110 kD cell surface glycoprotein with diverse functional properties, and is expressed on a variety of tissues, including epithelial cells and selected leukocyte subsets (Morimoto and Schlossman, 1998). The isolated CD26 cDNA predicts a type II transmembrane protein comprising of 766 amino acids and belonging to the serine protease family. As members of this family share the consensus sequence Gly-Xaa-Ser-Xaa-Gly at the catalytic site (Brenner, 1988), the predicted CD26 amino acid sequence contains a putative catalytic site, Gly-Trp-Ser-Tyr-Gly at positions 628-632, which fits the consensus sequence, with Ser-630 being assumed to be the catalytic residue. Therefore, CD26 is a membrane-associated ectopeptidase with a short cytoplasmic domain comprising of 6 amino acids, a transmembrane region and an extracellular domain with dipeptidyl peptidase IV (DPPIV) activity, capable of cleaving NH2- terminal dipeptides from polypeptides with either L-proline or L-alanine at the penultimate position (Tanaka et al., 1992). The predicted amino acid sequence of human CD26 shows approximately 85% homology with the rat DPPIV enzyme (Hong and Doyle, 1987, 1988) and mouse thymocyte activating molecule (THAM) (Marguet et al., 1992). Work over the past decade has demonstrated that CD26 has a multitude of physiological roles, ranging from its role in immune regulation as a structure capable of transmitting T cell activation signals to its role as a regulator of biological processes through its cleavage of biological factors. Furthermore, recent work has also suggested that CD26 may be involved in the development of certain human cancers. In this review, we will examine data focusing on CD26 role in the regulation of various aspects of human immune system as well as its emerging role in cancer development, including its potential suitability as a novel target for therapy.

Offprint requests to: Dr. Nam H. Dang, Department of Lymphoma/Myeloma, MD Anderson Cancer Center, Box 429, 1515 Holcombe Boulevard, Houston, Texas 77030, USA. Fax: 713-794-5656. e-mail: nhdang@mail.mdanderson.org

CD26 and human immune regulation

Regarding the role of CD26/DPPIV in immune regulation, work done by our group and others have suggested that CD26 has a key role in T cell function. Although it is constitutively expressed in the liver, intestine and kidney, CD26 expression level is tightly regulated on T cells, with its density being markedly elevated following T cell activation (Fox et al., 1984; Morimoto et al., 1989; Dang et al., 1990a). In clinical settings involving activated immune responses, such as in autoimmune diseases, CD26 levels of circulating T lymphocytes are often elevated (Hafler et al., 1985; Eguchi et al., 1989). In the peripheral blood of normal donors, CD26 is expressed on a subset of CD4+ memory

T cells, and this CD4+CD26+ T cell population responds maximally to recall antigens (Morimoto et al., 1989). In addition, CD26+ peripheral blood T cells exhibit greater proliferation when stimulated with mitogenic signals than CD26- T cells, again consistent with the notion that CD26 is a marker of activated cells (Hafler et al., 1989). Besides its enhanced expression on activated T cells, various lines of evidence have converged to demonstrate that CD26 is functionally associated with T cell signal transduction processes, capable of transmitting signals relating to T cell activation. We have shown that CD26 is able to conduct co-mitogenic signals in conjunction with activation through the CD3/T cell receptor complex (TCR) or the CD2 pathway of mature human T lymphocytes. Binding of solid-phase immobilized anti-CD26 monoclonal antibody has a co-mitogenic effect in conjunction with submitogenic doses of anti-CD3 or anti-CD2 antibodies, associated with the IL-2 autocrine pathway. Likewise, crosslinking of CD26 enhances T cell activation induced by phorbol esters (Dang et al., 1990a). Similarly, CD26 conveys a co-mitogenic signal in CD3/TCR-mediated activation of human thymocytes. Interestingly, unlike the case with mature peripheral blood T cells, the ability of CD26 to mediate co-mitogenic signal in thymocytes is restricted to the CD3 pathway only and does not involve CD2-induced activation. Meanwhile, CD26 is mostly found on the surface of medullary thymocytes, the population of relatively mature thymocytes expressing high level of CD3. These findings hence suggested that CD26 may play a role in thymic differentiation and maturation via the differential engagement of the CD3 pathway (Dang et al., 1991). Furthermore, binding of anti-CD26 Ab leads to T cell activation in the presence of FcR+ antigen accessory cells, likely due to crosslinking of the antigen (Fleischer, 1987; Dang et al., 1990b). Meanwhile, antibody binding results in a decrease in CD26 surface expression due to its internalization. Importantly, this down-modulation of CD26 is associated with enhanced tyrosine phosphorylation of such key signaling molecules as CD3-zeta and $p56^{lck}$ (Hegen et al., 1997) as well as increased proliferative response to anti-CD3 and anti-CD2 stimulation (Dang et al., 1990c), additional evidence of CD26 ability to convey signals related to T cell activation.

Effect of DPPIV enzyme activity on CD26-mediated T cell function

Using the CD26 negative human T cell leukemia line Jurkat, we established stable CD26 transfectants to further investigate the role of CD26 in T cell activation. Consistent with findings relating to peripheral blood T lymphocytes, CD26-expressing Jurkat transfectants display enhanced intracellular calcium mobilization and IL-2 production when triggered with solid-phase immobilized anti-CD26 and anti-CD3 antibodies (Tanaka et al., 1993). By mutating human CD26 cDNA through oligonucleotide directed mutagenesis to encode a protein in which the putative catalytic serine residue at position 630 is replaced by alanine, we created Jurkat transfectants expressing a mutant CD26 antigen lacking DPPIV enzyme activity. The concurrent use of the wild type CD26+DPPIV+ and the corresponding mutant CD26+DPPIV- Jurkat transfectants allows for the characterization of the role of DPPIV in T cell activation mediated through CD26. Wild type CD26+DPPIV+ Jurkat transfectants consistently produce substantially higher level of IL-2 than the mutant CD26+DPPIV- or vector-only transfectants following stimulation with the combination of anti-CD3 and anti-CD26 antibodies or with stimuli not involving CD26, such as anti-CD3 and phorbol esters. These results hence suggested that the DPPIV enzyme activity of CD26 serves to enhance cellular responses of CD26 Jurkat transfectants to external stimuli through CD26 and/or the CD3/TCR complex, resulting in augmented IL-2 production and T cell activation.

Additional evidence of an essential role for the DPPIV enzymatic activity of CD26 in T cell proliferation is seen from studies involving soluble CD26 molecules. Experiments using either soluble recombinant CD26 that contains DPPIV (sCD26+DPPIV+) or soluble CD26 mutated at the DPPIV site (sCD26+DPPIV-) have demonstrated that the presence of DPPIV enzymatic activity enhances peripheral blood T cell proliferation induced by the recall antigen tetanus toxoid. However, sCD26+DPPIV+ itself does not provide a mitogenic signal and does not augment T cell responses to such mitogenic stimuli as phytohemagglutinin and anti-CD3, suggesting that sCD26 may exert its enhancing effect on T cell response to recall antigens via its effect on antigen-presenting cells (Tanaka et al., 1994). Our recent work supports this conclusion by demonstrating that the exogenously added sCD26 directly affects CD14+ peripheral blood monocytes in the early stages of immune response to the recall antigen tetanus toxoid. Furthermore, the M6P/IGFIIR appears to play a role in the transportation of sCD26 into monocytes, since M6P interferes with its uptake. In addition, the enhancing effect of sCD26 on tetanus toxoid-induced T cell proliferation does not result from trimming of the MHC-bound peptide on the monocyte surface, unlike the case described for another ectopeptidase, CD13 aminopeptidase N (Larsen et al., 1996). Rather, exogenously added sCD26 upregulates the expression of the costimulatory molecule CD86 on monocytes through its DPPIV activity, both at the protein and mRNA levels. Therefore, these recent data suggested that sCD26, particularly its DPPIV enzymatic activity, enhances T cell immune response to recall antigens through its direct effect on antigen-presenting cells (Ohnuma et al., 2001).

Multiple studies using chemical inhibitors of DPPIV activity of CD26 have also demonstrated that DPPIV plays a key role in T cell activation. For example, the chemical inhibitor Pro-boroPro inhibits antigen-specific T cell proliferation and IL-2 production *in vitro* (Flentke

et al., 1991). In addition, the synthetic competitive DPPIV inhibitors Lys[Z(NO_2)])-thiazolidide, Lys[Z(NO_2)]-piperidide, and Lys[Z(NO_2)]-pyrrolidide significantly inhibit DNA synthesis and the production of IL-2, IL-10, IL-12 and IFN-gamma in pokeweed mitogen (PWM)-stimulated purified T lymphocytes. On the other hand, the presence of these inhibitors enhances secretion of the immunoinhibitory cytokine TGF-beta1 by the PWM-stimulated T cells, hence suggesting that TGF-beta1 helps regulate CD26/DPPIV effect on T cell function (Reinhold et al., 1997). Furthermore, these DPPIV inhibitors directly affect such early signaling events in T cells as tyrosine phosphorylation and Ca2+ flux, further support for the notion that CD26 affects T cell signaling pathways through its DPPIV activity. In particular, while capable of inducing tyrosine phosphorylation in resting human T cells, both Lys[Z(NO_2)])-thiazolidide, Lys[Z(NO_2)]-piperidide inhibit PMA-induced tyrosine phosphorylation of several molecules, including p56lck. While they do not affect protein kinase C activity or cAMP levels in human T cells, these inhibitors do suppress anti-CD3-mediated Ca2+ flux (Kahne et al., 1998). DPPIV inhibitors also inhibit lymphocyte proliferation induced by mitogenic lectins and immunoglobulin production by pokeweed mitogen-stimulated lymphocytes in vitro (Schon et al., 1987). DPPIV inhibitors have also been used in in vivo studies to evaluate their potential effectiveness in treating clinical conditions involving immune activation. Lys[Z(NO_2)]-pyrrolidide treatment blocks DPPIV activity in vivo and results in the clinical improvement of experimental autoimmune encephalomyelitis, while increasing the secretion of the immunosuppressive cytokine TGF-beta1 (Steinbrecher et al., 2001). Meanwhile, treatment with the low-molecular-weight inhibitor prodipine suppresses systemic DPPIV activity, impairs host cytotoxic T lymphocyte responses and abrogates acute rejection of cardiac allografts in rat recipients, leading to prolonged allograft survival (Korom et al., 1997). Besides providing additional evidence of a key role for CD26/DPPIV in immune activation in vivo, these findings therefore demonstrate that specific targeting of CD26/DPPIV to induce immunosuppression may have potential effectiveness in selected clinical conditions involving active immune system.

However, DPPIV enzyme activity does not appear to be necessary for CD26-mediated T cell function in certain experimental conditions. At short incubation time, inhibitors blocking up to 95% of DPPIV enzyme activity do not inhibit CD26-mediated cytotoxicity of T cell clones against Fc-receptor bearing target cells (Hegen et al., 1993; von Bonin et al., 1998). Meanwhile, studies using T cell receptor positive-mouse T cell hybridoma transfected with human CD26 showed that cells expressing mutant CD26 lacking DPPIV enzyme activity are able to be stimulated with anti-CD26 mAbs, as are cells expressing wild type CD26 (Steeg et al., 1995). Also, work using murine hybridomas transfected with human CD26 molecules demonstrated CD26 co-stimulatory effect is still present in mutants lacking most of the extracellular domain of the molecule, with the membrane proximal glycosylation rich domain of CD26 being sufficient for its co-stimulatory activity (Huhn et al., 2000). The reasons for the differing results regarding the role of DPPIV enzyme activity in CD26-mediated T cell function are not yet clearly elucidated. Relative specificity and non-specific toxicity of the chemical inhibitors of DPPIV used in various experiments are potential confounding factors. In addition, the biological effect of DPPIV activity may depend on the particular experimental model and to particular growth conditions, due to the potential association of the enzyme activity to differentially manifested cellular processes.

CD26-associated molecules

CD26-CD45

The fact that its cytoplasmic domain contains only 6 amino acids strongly suggests that CD26 is able to convey intracellular signals through its physical and functional association with structures involved in the signal transduction cascades. Recent work has indeed demonstrated that CD26 interacts with several molecules with key roles in T cell function. We showed that CD26 is co-modulated on the T cell surface with CD45RO, a membrane-linked tysosine phosphatase critical to T cell signal transduction, while anti-CD26 antibodies are capable of precipitating CD45 from T cell lysates (Torimoto et al., 1991). Moreover, antibody-induced modulation of CD26 results in augmented phosphorylation of CD3-zeta tyrosine residues and increased p56lck tyrosine kinase activity. Since CD45 regulates the phosphorylation status of these structures, these findings hence suggested that one possible mechanism for CD26 ability to convey activation signals involves its association with the membrane-linked protein-tyrosine-phosphatase CD45, perhaps by modulating CD45PTPase enzymatic activity or by affecting the accessibility of critical substrates. Recently, we demonstrated that CD26 localizes into lipid rafts, and targeting of CD26 to rafts is necessary for CD26-mediated signaling. We found that the presence of CD26 in rafts is necessary for the tyrosine phosphorylation of several cellular proteins in T cells, including Erk 1/2, TCR-zeta, and ZAP70, following anti-CD26 binding. Importantly, disruption of the structure of membrane rafts with such agents as cytochalasin D and nystatin markedly prevents the tyrosine phosphorylation of these molecules. Moreover, anti-CD26-induced aggregation of CD26 causes co-localization of CD45 molecules into lipid rafts, a process that involves CD26 binding to the cytoplasmic domain of CD45. Therefore, CD26-CD45 interaction may account for changes in the status of key signaling proteins, such as increased p56lck activity, enhanced CD3-zeta chain phosphorylation and intracellular calcium mobilization, leading to the

amplification of in vivo immune response (Ishii et al., 2001).

CD26-ADA

CD26 also physically associates with adenosine deaminase (ADA) as the ADA-binding protein (Kameoka et al., 1993; Morrison et al. 1993; De Meester et al., 1994). A key role for ADA itself in lymphocyte physiology is indicated by the fact that ADA deficiency is a cause of human severe combined immunodeficiency disease (SCID) (Morimoto and Schlossman, 1998). Anti-CD26 antibody co-immunoprecipitates a 43 kD band, which is identified as ADA by partial sequencing. ADA is associated with CD26 on T cell lines lacking either ADA or DPPIV enzyme activity, indicating that the physical association between these two molecules does not require their respective enzymatic activity. In addition, CD26 and ADA appear to co-localize on the cell surface but not inside the cells in in vitro studies, thereby suggesting that CD26 does not function to transport ADA to the cell surface, but rather binds to ADA present in the culture medium. Consistent with this notion are the findings that CD26 can associate with ADA secreted from other cells to form a CD26/ADA complex on the cell surface. In the absence of cell surface ADA, adenosine inhibits T cell proliferation and IL-2 production induced by various stimuli. However, cells expressing ADA and CD26 on the surface display greater resistance to adenosine-mediated inhibition. These data hence suggested an important role for the cell surface complex of ADA/CD26 in immunoregulation, potentially negating the suppressive effect of endogenous adenosine on T cell function (De Meester et al., 1994; Dong et al., 1996). Additionally, CD26-associated ADA has been suggested to have co-stimulatory function independent of its catalytic activity (Martin, 1995; Franco et al., 1998). Whereas many of the available anti-CD26 monoclonal antibodies recognize epitopes in the glycosylation-rich region of the antigen, the ADA binding site appears to be located in the cysteine-rich region of the extracellular domain of CD26 (Huhn et al., 1999). Meanwhile, studies using CD26 deletion, human-rat CD26 swap mutants and point mutations showed that residues L340, V341, A342, and R343 on the CD26 molecule are essential amino acids for ADA binding (Dong et al., 1997). Another study also reported that binding to CD26 by ADA and anti-CD26 antibodies that inhibit ADA binding involves discontinuous and overlapping sites on a predicted beta-propeller domain. Specifically, residues Leu294 and Val341 of CD26 appear to be essential for ADA binding, while the epitope of anti-CD26 antibodies that inhibit ADA binding contains amino acid region Leu340 to Arg343 as well as amino acids Thr440/Lys441 (Abbott et al., 1999).

A potential consequence of the interaction between CD26 and ADA in the clinical setting may be reflected in situations involving the use of ADA inhibitors to treat selected human diseases. For example, at MD Anderson Cancer Center, we conducted a phase II study to examine the effect of the ADA inhibitor pentostatin on relapsed T cell lymphomas (Dang et al., 2001). The objectives of the study are to determine the effectiveness of pentostatin in these diseases and to evaluate the lymphopenic effect of the drug on CD26+ T lymphocytes. Interim analysis of 11 evaluable cases showed an overall response rate of 45%. Intriguingly, pentostatin preferentially reduces the level of CD26+ T lymphocytes in the patients' peripheral blood and bone marrow in both responders and non-responders. While the mechanism behind the preferential reduction of CD26+ T lymphocytes induced by treatment with the ADA inhibitor pentostatin is unclear at the present time, a potential explanation may be due to the physical association between CD26 and ADA on the T lymphocyte surface. 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 level of CD26+ T lymphocytes induced by pentostatin has potentially significant implications for immune system function in treated patients.

CD26-M6P/IGFIIR

Another molecule that binds to cell surface CD26 is recently identified to be the mannose-6-phosphate/insulin-like growth factor II receptor (M6P/IGFIIR), with the M6P residues in the CD26 carbohydrate moiety being critical for this interaction (Ikushima et al., 2000). Activation of peripheral blood T lymphocytes results in the mannose-6-phosphorylation of CD26. In addition, antibody-mediated crosslinking of CD26 induces co-localization of CD26 with M6P/IGFIIR, along with capping and internalization of CD26. Importantly, both the process of antibody-mediated internalization of CD26 and the T cell proliferation induced by CD26-mediated costimulation are inhibited by the addition of M6P, but not by glucose-6-phosphate or mannose-1-phosphate. These data therefore indicated that the binding of CD26 with M6P/IGFIIR is important for antibody-mediated CD26 internalization and that the interaction between mannose-6-phosphorylated CD26 and M6P/IGFIIR has an essential role in CD26-mediated T cell activation.

CD26-CXCR4

Recent work has also shown that CD26 physically and functionally associate with the chemokine receptor CXCR4 on the surface of human peripheral blood lymphocytes (Herrera et al., 2001). CD26 colocalizes with CXCR4 on T and B cell lines, while the two molecules co-immunoprecipitate from cellular membranes. Down-regulation of CXCR4 induced by its physiological ligand stromal cell-derived factor 1 alpha (SDF-1 alpha) also results in co-internalization of CD26

on lymphocytes. Furthermore, treatment with SDF-1 alpha induces the formation of pseudopodia in which both CD26 and CXCR4 co-localize and in which ADA is absent. Since CD26 is capable of cleaving SDF-1 alpha and thereby affecting its biological function (Ohtsuki et al., 1998; Proost et al., 1998a; Shioda et al., 1998), the CD26/CXCR4 complex may be a functional unit allowing CD26 to directly modulate SDF-1 alpha activity.

CD26 and cleavage of cytokines

Recent studies have demonstrated that CD26/DPPIV cleaves a number of cytokines to alter their biological functions, by altering their chemotactic potency, impairing their signaling effects and modifying their receptor specificity. Initial studies by Oravecz et al. (Oravecz et al., 1997) demonstrated that the receptor specificity of CCL5 (regulated on activation, normal T cell expressed and secreted, RANTES) is altered by DPPIV-mediated cleavage, as signaling through CCR1 and CCR3 by RANTES is abolished by the 2 amino acid-truncation mediated by CD26/DPPIV while signaling through CCR5 is not affected. Further studies showed that CD26/DPPIV-mediated cleavage of RANTES influences its activity in monocyte chemotaxis and HIV-1 infection (Proost et al., 1998b). Work done subsequently by various investigators further showed that an emerging aspect of CD26 biology is the ability of its intrinsic DPPIV enzyme activity to cleave selected chemokines to alter their biological effects. For example, the chemotactic and anti-HIV activities of mature SDF 1-alpha are both abolished by cleavage of membrane-bound as well as soluble CD26/DPPIV (Ohtsuki et al., 1998; Proost et al., 1998a; Shioda et al., 1998). In addition, eotaxin, an important mediator of allergic reactions due to its ability to attract eosinophils, Th2 lymphocytes and basophils, is a substrate for CD26/DPPIV. DPPIV-cleaved eotaxin exhibits reduced chemotactic activity for eosinophils and impaired binding and signaling via its receptor CCR3, while differentially regulating the chemotactic and antiviral properties of intact eotaxin (Struyf et al., 1999). Other chemokines demonstrated to exhibit altered receptor specificity and biological activity following CD26/DPPIV-mediated cleavage include macrophage-derived chemokine (MDC) (Lambeir et al., 2001; Proost et al., 1999), LD78 beta isoform of macrophage inflammatory protein-1 alpha (Proost et al., 2000; Struyf et al., 2001), and the interferon-inducible chemokines (Proost et al., 2001).

CD26 and cancer

CD26 binding to the extracellular matrix is an aspect of its biology with potential implications in neoplastic diseases. CD26 binds to collagen and fibronectin in a variety of experimental conditions (Bauvois, 1988; Piazza et al., 1989; Dang et al., 1990d; Loster et al., 1995). Through its interaction with the extracellular matrix proteins, CD26 may further affect immune regulation by recruiting activated lymphoid effectors to

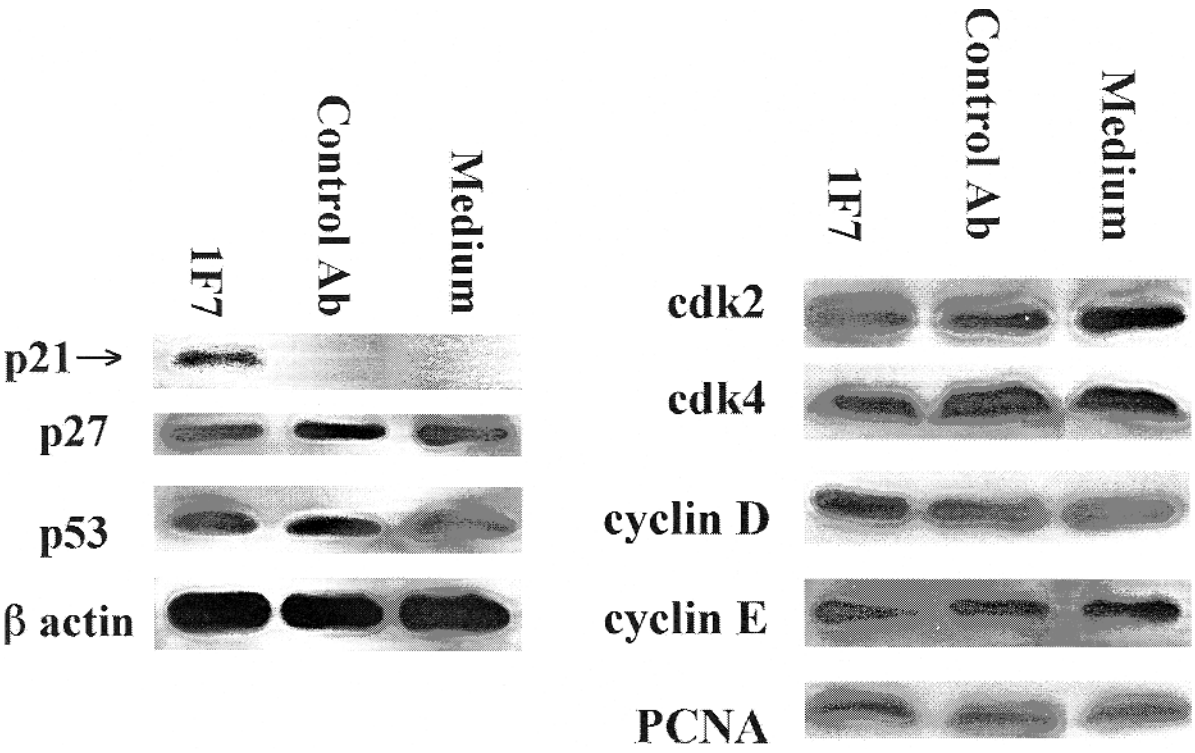

Fig. 1. Enhanced p21 expression following anti-CD26 treatment. T-cell CD30+ anaplastic large cell lymphoma Karpas 299 cells were incubated overnight at 37 °C with medium alone, medium containing isotype control mAb or the anti-CD26 monoclonal antibody 1F7 (2 μg/ml). Cells were then harvested, and SDS-PAGE and immunoblotting studies were performed. Adapted from Ho et al., 2001.

inflammatory sites (Masuyama et al., 1992). In addition, this interaction may play a role in the clinical behavior and progression of selected cancers. For example, CD26 molecules found on lung endothelial cells specifically interact with fibronectin assembled on breast cancer cell surface, potentially promoting tumor cell adhesion and metastasis (Cheng et al., 1998). Intriguingly, interference with this interaction inhibits tumor metastasis in animal models (Abdel-Ghany et al., 1998).

Besides influencing tumor cell progression through its interaction with the extracellular matrix, CD26 may have a role in the development of certain cancers, as suggested by several studies examining its expression in selected tumors. Most lung adenocarcinomas are DPPIV+, while other histological types of lung carcinoma are DPPIV- (Asada et al., 1993). In addition, CD26 is highly expressed on differentiated thyroid carcinomas but is absent in benign thyroid diseases (Tanaka et al., 1995). Aberrant CD26 expression is also seen in hepatocellular carcinomas, with its expression in most cases examined deviating distinctly from its expression in normal hepatocytes (Stecca et al., 1997). In other instances, CD26 expression is down-regulated during neoplastic transformation and tumor progression, and up-regulated during cellular differentiation. DPPIV expression is significantly decreased in metastatic prostate cancer specimens as compared to primary tumors (Bogenrieder et al., 1997), while colon cancer cell lines undergoing enterocytic differentiation show enhanced DPPIV expression (Yoshioka et al., 1991; Darmoul et al., 1992). Similarly, CD26 level is negatively correlated with melanoma growth phase, being expressed in cases involving the *in situ* or invasive radial growth phase to being progressively lost in late tumor progression stages, i.e. the vertical growth phase and metastatic phase (Van Den Oord, 1998). Meanwhile, in a series of elegant experiments, Houghton and colleagues demonstrated a potentially essential role for CD26 in melanoma development (Houghton et al., 1988; Morrison et al., 1993; Wesley et al., 1999). In vitro malignant transformation of melanocytes results in loss of expression, while CD26 reexpression in transfected melanoma cells leads to phenotypic changes characteristic of normal melanocytes, further supporting the view that regulation of CD26 expression is an important event in tumor pathogenesis. Meanwhile, serum levels of CD26/DPPIV are altered in cancer patients compared with normal controls (Uematsu et al., 1996; Cordero et al., 2000) and in certain instances,

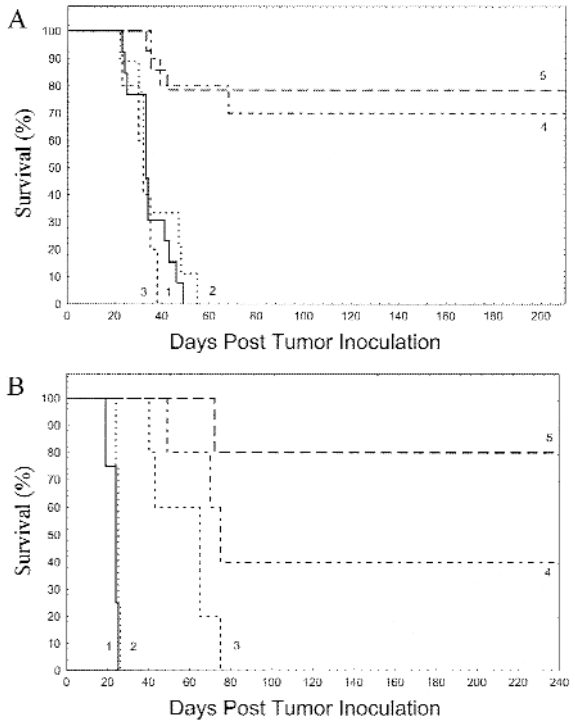

Fig. 2. Enhanced survival of Karpas 299-bearing SCID mice treated with 1F7. In **A**, 1 day after i.p. inoculation of SCID mice with 1 x 10^6 Karpas 299 cells per mouse, i.p. treatment with saline alone, isotype control Ab (5 μg/injection or 10 μg/injection), or anti-CD26 monoclonal antibody 1F7 (5 μg/injection or 10 μg/injection) was then administered 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, 1F7 (10 μg/injection, n=10); arm 5, 1F7 (5 μg/injection, n = 14). In **B**, 1 day after i.p. inoculation of SCID mice with 3 x 106 Karpas 299 cells per mouse, i.p. treatment with saline alone, isotype control Ab (20 μg/injection), or 1F7 (5 μg/injection, 10 μg/injection, 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., 2001.

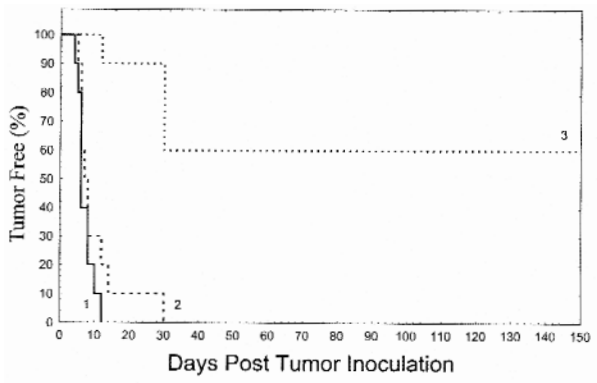

Fig. 3. Initial tumor appearance in SCID mice after s.c. tumor cell inoculation and s.c. treatment with antibodies. Karpas 299 tumor cells ($1x10^6$) incubated in saline alone were administered subcutaneously to SCID mice. Alternatively, $1x10^6$ Karpas 299 tumor cells were mixed on ice with 100 μg of 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 as treatment effects. Arm 1, saline alone (n = 10); arm 2, isotype control Ab (n=10); arm 3: 1F7 (n= 10). Adapted from Ho et al., 2001.

serum CD26 levels have been reported to correlate with tumor clinical behavior (Cordero et al., 2000). For example, in patients with colorectal cancer, pre-operative serum CD26 levels appear to have potential diagnostic and prognostic value (Cordero et al., 2000). Compared with healthy donors, patients with colorectal carcinoma have significantly lower levels of serum CD26. In addition, serum CD26 level is a distinct variable that has greater diagnostic efficiency than that demonstrated by other markers, especially in patients with early stage disease.

Besides its role in the pathophysiology of certain solid tumors, CD26 may also have a role in the development of selected hematological malignancies. B-chronic lymphocytic leukemia (B-CLL) cells express higher levels of CD26 protein and mRNA transcripts as compared to normal resting B cells (Bauvois et al., 1999). Moreover, treatment with interferons and retinoic acid enhances CD26 expression in B-CLL cells by engaging Stat1 alpha-mediated signaling (Bauvois et al., 2000). In addition, CD26 expression on T cell malignancies appears restricted to aggressive pathologic entities such as T cell lymphoblastic lymphomas/acute lymphoblastic leukemias (LBL/ALL) and T cells CD30+ anaplastic large cell lymphomas. Significantly, within the T cell LBL/ALL subset, CD26 expression is an independent marker of poor prognosis, although these findings were based on relatively small sample sizes and limited clinical data (Carbone et al., 1994, 1995). Recent work has also confirmed that the majority of patients with T cell ALL express CD26 on the tumor cell surface (Klobusicka and Babusikova, 1999). A strong correlation between CD26 antigen expression and DPPIV positivity in the majority of T-lymphoblasts is

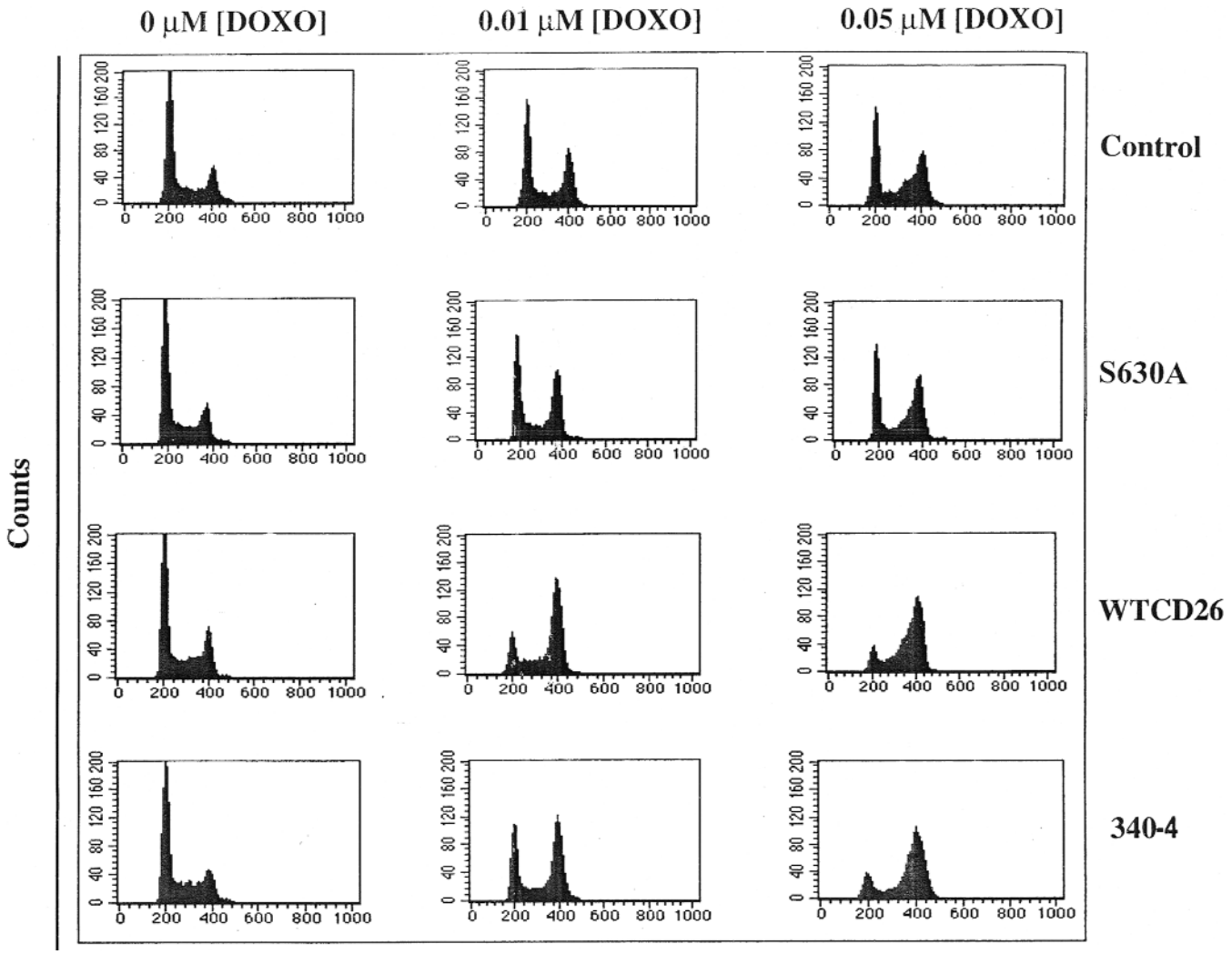

Fig. 4. Effect of CD26/DPPIV expression on doxorubicin-mediated cell cycle arrest at G2-M. In **A**, CD26 Jurkat transfectants were cultured at 37 °C in doxorubicin-containing media for 24 h. Cells were then harvested, and cell cycle analyses were done with propidium iodide staining. Data are representative of three separate experiments. Adapted from Aytac et al., 2001.

also noted, while CD26 expression is not detected on DPPIV-negative T lymphoblasts. CD26 and its associated DPPIV enzyme activity are also found on the surface of an aggressive hepatosplenic gamma-delta T-cell lymphoma (Ruiz et al., 1998). Conversely, in the case of the relatively indolent disease mycosis fungoides (MF), the neoplastic cells, which are typically CD4+, most often do not express surface CD26 (Bernengo et al., 2001; Jones et al., 2001). At MD Anderson Cancer Center, we performed flow cytometric characterization of CD26 surface expression in peripheral blood samples of patients with MF and its related disorder Sezary syndrome (SS). In 66 of 69 (96%) samples from 28 patients, we identified the tumor cell population in the peripheral blood as being CD26 negative/dim, distinct from the normal peripheral blood T lymphocytes. In contrast, expression of CD7, a commonly used marker to identify circulating MF/SS cells, is much more variable, allowing recognition of a distinct abnormal T cell population in only 34 of 69 (49%) of involved MF/SS samples (Jones et al., 2001). These findings suggested that CD26 can be a novel marker capable of being used in the clinical setting to identify MF/SS patients with circulating tumor cells. In fact, based on results mentioned above, CD26 is now included as part of the panel of flow cytometric markers used at MD Anderson Cancer Center to routinely screen clinical samples from patients with a confirmed or suspected diagnosis of T cell malignancy.

CD26 as a potential treatment target in hematological malignancies

T cell hematological malignancies represent a group of heterogeneous diseases that are generally noted for their aggressive clinical behavior and their relative refractoriness to current treatment modalities (Melnyk et al., 1997; Gisselbrecht et al., 1998). We recently conducted studies investigating the potential role of CD26 as a novel treatment target for selected T cell

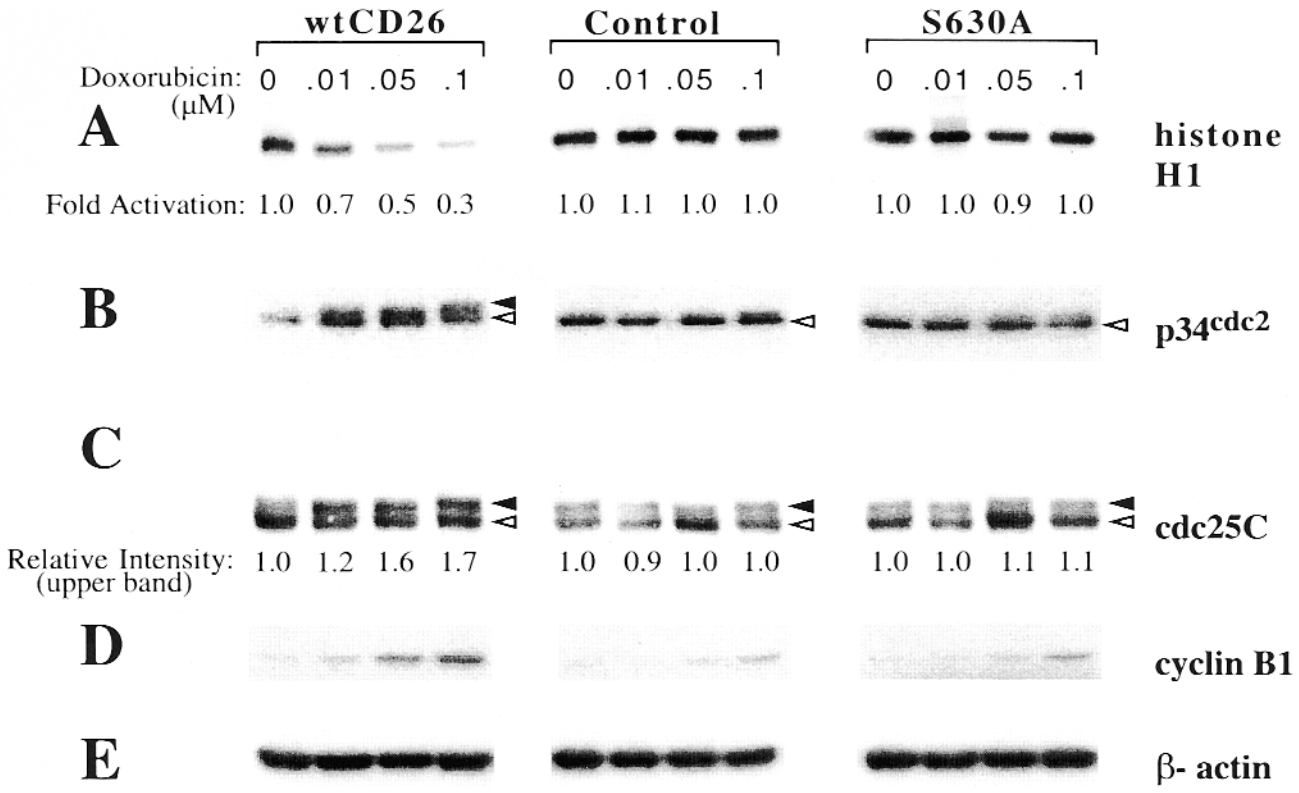

Fig. 5. Effect of CD26/DPPIV expression on $p34^{cdc2}$/cyclin B1 complex and cdc25C after doxorubicin treatment. Jurkat cells were incubated for 24 h at 37 °C with doxorubicin-containing media at the indicated doses. Cells were then harvested, and kinase assays and immunoblotting studies were performed. **A**, whole cell lysates were prepared and p34cdc2 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 p34cdc2 were evaluated by immunoblotting studies with anti-p34cdc2. Black triangles show the phosphorylated p34cdc2 kinase. **C**, protein levels of cdc^{25C} were evaluated by immunoblotting studies with anti-cdc^{25C}. The two major electrophoretic forms reflect the differences in serine-216 phosphorylation. Black triangle indicates the serine-216-phosphorylated form of cdc25C (cdc25C-P). After quantification with phosphoimager, intensity of the cdc^{25C}-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., 2001.

malignancies, using as models human T cell lines. We demonstrated that binding of the anti-CD26 monoclonal antibody 1F7 inhibits the growth of the human CD30+ anaplastic large cell T-cell lymphoma cell line Karpas 299 in both in vitro and in vivo experiments (Ho et al., 2001). In vitro studies showed that binding of 1F7 induces cell cycle arrest at the G1-S checkpoint, associated with enhanced expression of the key cyclin-dependent kinase inhibitor p21 that is dependent on de novo protein synthesis (Fig. 1). Our work also suggested that the ability of anti-CD26 antibody to inhibit cell growth is epitope-dependent, since the anti-CD26 monoclonal antibody 5F8, recognizing a distinct epitope than that recognized by 1F7, is much less potent in inducing growth arrest. This differential effect is concordant with previous work examining the role of various epitopes of CD26 in human T cell function, with the 1F7 containing the CD26 domain being consistently demonstrated to be capable of transmitting intracellular signals to modify cellular activity (Dang et al., 1990a; Dong et al., 1998; Torimoto et al., 1992). Expanding on the in vitro results, we subsequently conducted experiments with a severe combined immunodeficient mouse tumor model in which SCID mice were treated with 1F7 following injection with Karpas 299 cells. Our findings demonstrated that 1F7 treatment significantly enhances survival of tumor-bearing mice by inhibiting tumor formation, suggesting therefore that anti-CD26 antibody treatment may have potential clinical use for CD26+ T cell hematological malignancies (Figs. 2, 3).

As previously reported (Morrison et al., 1993; Carbone et al., 1995), CD26 expression is associated with changes in tumor cell line behavior in vitro, and the clinical behavior of certain T-cell tumors appears to correlate with differences in CD26 expression level. Furthermore, the DPPIV enzymatic activity of CD26 has

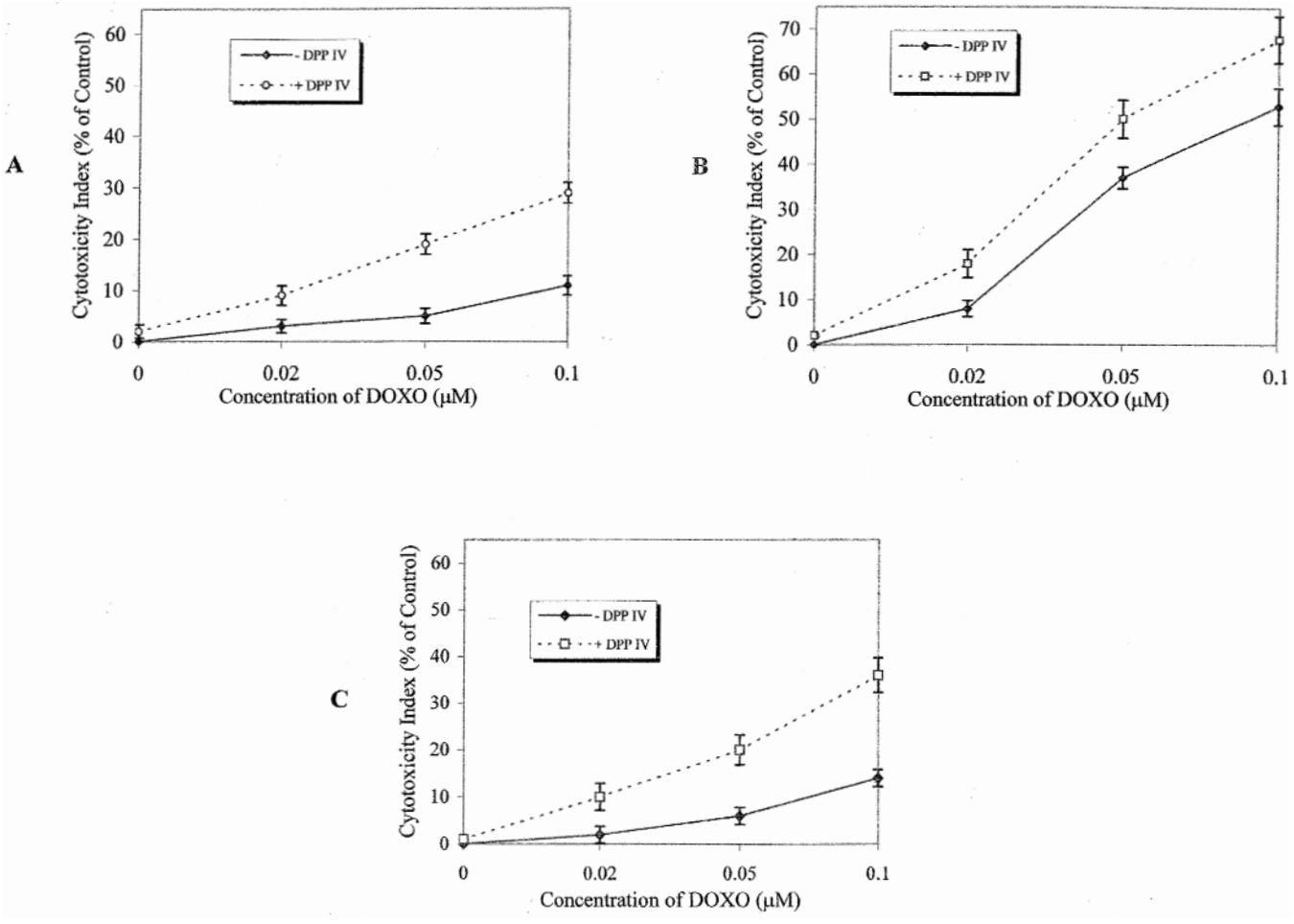

Fig. 6. Effect of exogenous sDPPIV on doxorubicin-mediated growth inhibition of Jurkat cells. Jurkat cells were cultured at 37° C in media alone, culture media with doxorubicin alone at the indicated concentrations, culture media with sDPPIV (50 μg/ml) alone, and culture media with doxorubicin at the indicated concentrations and sDPPIV (50 μg/ml). MTT uptake assays were performed. Data represent the means of three separate experiments. **A**, control; **B**, wtCD26; C, S630A. Cytotoxicity index was calculated as follows: Cytotoxicity index (% of control) = (1-absorbance of treated cells) / (absorbance of cells incubated in culture medium alone) x 100. Adapted from Aytac et al., 2001.

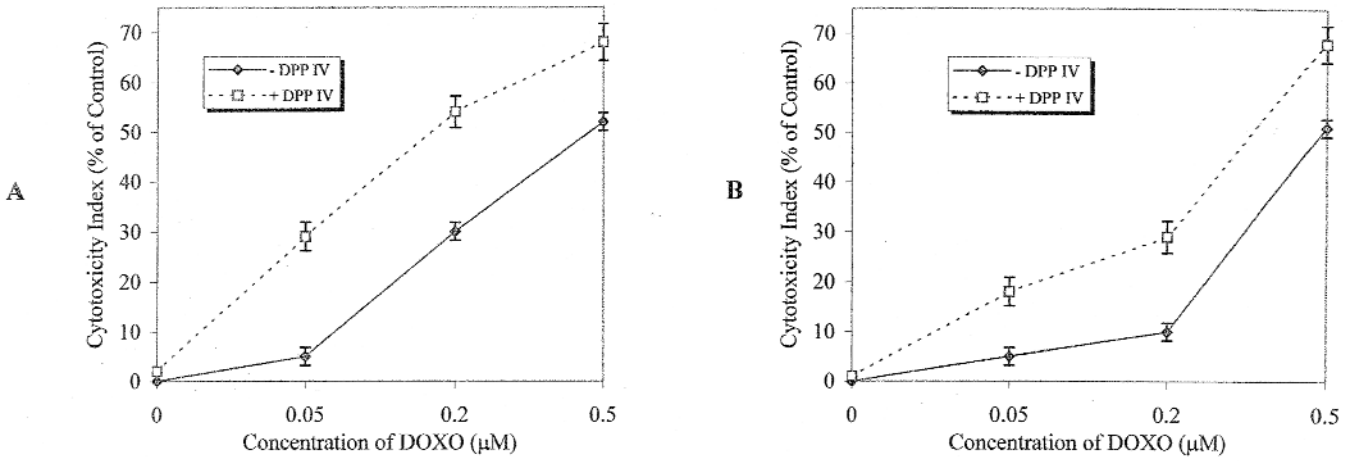

Fig. 7. Effect of exogenous sDPPIV on doxorubicin-mediated growth inhibition of B-lymphoid cell lines. Cells were incubated at 37 °C in media alone, culture media with doxorubicin alone at the indicated concentrations, culture media with sDPPIV (50 μg/ml) alone, and culture media with doxorubicin at the indicated concentrations and sDPPIV (50 μg/ml). MTT uptake assays were performed. Data represent the means of three separate experiments. **A**, Jiyoye; **B**, Namalwa. Cytotoxicity index was calculated as follows: Cytotoxicity index (% of control) = (1-absorbance of treated cells) / (absorbance of cells incubated in culture medium alone) x 100 Adapted from Aytac et al., 2001.

an essential role in the biology of selected lymphoid cell lines (Reinhold et al., 1994). These observations led to our hypothesis that CD26 expression or DPPIV activity affects tumor cell biology and potentially cellular sensitivity to antineoplastic agents. Doxorubicin is a widely used anthracycline antibiotic in cancer therapy and is a main component of chemotherapeutic regimens for the treatment of hematological malignancies and selected solid tumors. Using CD26-negative parental Jurkat and Jurkat cells stably transfected with wild type CD26 antigen (wtCD26); mutant CD26 molecule containing an alanine at the putative catalytic serine residue at position 630, resulting in a mutant CD26-positive/DPPIV-negative Jurkat transfectant (S630A); or mutant CD26 molecule containing point mutations at the ADA-binding 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-positive/DPPIV-positive Jurkat transfectant incapable of binding ADA (340-4), we investigated the effect of CD26/DPPIV on cellular sensitivity to doxorubicin (Aytac et al., 2001). Evaluating doxorubicin-induced cell cycle arrest at the G2-M checkpoint, we found that wtCD26 cells exhibit greater drug sensitivity than parental cells; moreover, S630A transfectants are less sensitive to doxorubicin, like parental cells. Conversely, 340-4 transfectants display greater sensitivity to doxorubicin, similar to the wtCD26 transfectants (Fig. 4). We also demonstrated that doxorubicin treatment has greater effect on the key regulators of G2-M in wtCD26 cells than in parental or S630A cells. Specifically, in wtCD26 as compared to parental and S630A cells, we observed greater phosphorylation of the inhibitory residues of Thr14Tyr15 of p34cdc2, associated with inhibition of p34cdc2 kinase activity, enhanced serine-216 phosphorylation of cdc25C, and elevated level of cyclin B1 (Fig. 5). Since our data indicated that the presence of DPPIV enzymatic activity is essential to enhanced doxorubicin sensitivity, we investigated the effect of exogenous soluble DPPIV (sDPPIV) on Jurkat cells. We found that the addition of exogenous sDPPIV also enhances Jurkat sensitivity to doxorubicin (Fig. 6). Importantly, we demonstrated that B cell lymphoid cell lines Jiyoye and Namalwa also exhibit CD26-associated enhancement in drug sensitivity, with potential implications for CD26 role in the clinical setting (Fig. 7). Taken together, our recent observations strongly suggest that CD26 is an appropriate molecular target for novel treatment approaches for T cell lymphoid malignancies as well as selected other neoplasms.

In this review, we focus on key aspects of CD26 function, presenting data that expand on its well-established role in immune regulation as well as findings that indicate an increasingly significant involvement in cancer biology. Meanwhile, our recent studies suggest that CD26 may be an appropriate target for novel treatment modalities for selected hematological malignancies. In view of its multifaceted roles in immune function and cancer, future therapeutic strategies targeting CD26 and its intrinsic DPPIV enzyme activity may be of potential value in the clinical setting.

Acknowledgements. NH Dang is supported by a grant from the V Foundation and the MD Anderson Physician-Scientist Program. C Morimoto is supported by a grant-in-aid from the Ministry of Education, Science, Sports and Culture and from the Ministry of Health, Labor and Welfare, as well as by National Institute of Health Grant AR33713. We also would like to thank Drs. Ugur Aytac, Linus Ho, Kei Ohnuma and Kazuya Sato for their contribution and helpful discussion.

References

Abbott C.A., McCaughan G.W., Levy M.T., Church W.B. and Gorrell M.D. (1999). Binding to human dipeptidyl peptidase IV by adenosine deaminase and antibodies that inhibit ligand binding involves overlapping, discontinuous sites on a predicted beta propeller domain. Eur. J. Biochem. 266, 798-810.

Abdel-Ghany M., Cheng H.-C., Levine R.A. and Pauli B.U. (1998). Truncated dipeptidyl peptidase IV is a potent anti-adhesion and anti-metastasis peptide for rat breast cancer cells. Invasion Metastasis 18, 35-43.

Asada Y., Aratake Y., Kotani T., Marutsuka K., Araki Y., Ohtaki S. and Sumiyoshi A. (1993). Expression of dipeptidyl aminopeptidase IV activity in human lung carcinoma. Histopathology 23, 265-270.

Aytac U., Claret F.-X., Ho L., Sato K., Ohnuma K., Mills G. B., Cabanillas F., Morimoto C. and Dang N. H. (2001). Expression of CD26 and its associated dipeptidyl peptidase IV enzyme activity enhances sensitivity to doxorubicin-induced cell cycle arrest at the G2/M checkpoint. Cancer Res. 61, 7204-7210.

Bauvois B. (1988). A collagen-binding glycoprotein on the surface of mouse fibroblasts is identified as dipeptidyl peptidase IV. Biochem. J. 252, 723-731.

Bauvois B., De Meester I., Dumont J., Rouillard D., Zhao H.X. and Bosmans E. (1999). Constitutive expression of CD26/dipeptidylpeptidase IV on peripheral blood B lymphocytes of patients with B chronic lymphocytic leukaemia. Br. J. Cancer 79, 1042-1048.

Bauvois B., Djavaheri-Mergny M., Rouillard D., Dumont J. and Wietzerbin J. (2000). Regulation of CD26/DPPIV gene expression by interferons and retinoic acid in tumor B cells. Oncogene 19, 265-272.

Bernengo M.G., Novelli M., Quaglino P., Lisa F., De Matteis A., Savoia P., Cappello N. and Fierro M.T. (2001). The relevance of the CD4+CD26- subset in the identification of circulating Sezary cells. Br. J. Dermatol. 144, 125-135.

Bogenrieder T., Finstad C.L., Freeman R.H., Papandreou C.N., Scher H.I., Albino A.P., Reuter V.E. and Nanus D.M. (1997). Expression and localization of aminopeptidase A, aminopeptidase N, and dipeptidyl peptidase IV in benign and malignant human prostate tissue. Prostate 33, 225-232.

Brenner S. (1988). The molecular evolution of genes and proteins: a tale of two serines. Nature 334, 528-530.

Carbone A., Cozzi M., Gloghini A. and Pinto A. (1994). CD26/dipeptidyl peptidase IV expression in human lymphomas is restricted to CD30-positive anaplastic large cell and a subset of T-cell non-Hodgkin's lymphomas. Human Pathol. 25, 1360-1365.

Carbone A., Gloghini A., Zagonel V., Aldinucci D., Gattei V., Degan M., Improta S., Sorio R., Monfardini S. and Pinto A. (1995). The expression of CD26 and CD40 ligand is mutually exclusive in human T-cell non-Hodgkin's lymphomas/ leukemias. Blood 86, 4617-4626.

Cheng H.-C., Abdel-Ghany M., Elble R. C. and Pauli B. U. (1998). Lung endothelial dipeptidyl peptidase IV promotes adhesion and metastasis of rat breast cancer cells via tumor cell surface-associated fibronectin. J. Biol. Chem. 273, 24207-24215.

Cordero O.J., Ayude D., Nogueira M., Rodriguez-Berrocal F.J. and de la Cadena M.P. (2000). Preoperative serum CD26 levels: diagnostic efficiency and predictive value for colorectal cancer. Br. J. Cancer 83, 1139-1146.

Dang N. H., Torimoto Y., Deusch K., Schlossman S. F. and Morimoto C. (1990a). Co-mitogenic effect of solid-phase immobilized anti-1F7 on human CD4 T cell activation via CD3 and CD2 pathways. J. Immunol. 144, 4092-4100.

Dang N.H., Hafler D.A., Schlossman S.F. and Breitmeyer J.B. (1990b). FcR-mediated crosslinking of Ta1 (CDw26) induces human T lymphocyte activation. Cell. Immunol. 125, 42-57.

Dang N.H., Torimoto Y., Sugita K., Daley J. F., Schow P., Prado C., Schlossman S. F. and Morimoto C. (1990c). Cell surface modulation of CD26 by anti-1F7 monoclonal antibody: analysis of surface expression and human T cell activation. J. Immunol. 145, 3963-3971.

Dang N.H., Torimoto Y., Schlossman S.F. and Morimoto C. (1990d). Human CD4 helper T cell activation: functional involvement of two distinct collagen receptors, 1F7 and VLA integrin family. J. Exp. Med. 172, 649-652.

Dang N.H., Torimoto Y., Shimamura K., Tanaka T., Daley J.F., Schlossman S. F. and Morimoto C. (1991). 1F7 (CD26): a marker of thymic maturation involved in the differential regulation of the CD3 and CD2 pathways of human thymocyte activation. J. Immunol. 147, 2825-2832.

Dang N.H., Hagemeister F.B., Duvic M., Romaguera J.E., Younes A., Rodriguez M. A., Sarris A. H., Jones D., East K., Gomez R., Fayad L. E., Pro B., McLaughlin P., Samaniego F., Goy A. and Cabanillas F. (2001). Phase II study of pentostatin for relapsed T cell lymphomas: efficacy and effect on CD26+ T cells. Blood 98:130a.

Darmoul D., Lacasa M., Baricault L., Marguet D., Sapin C., Trotot P., Barbat A. and Trugnan G. (1992). Dipeptidyl peptidase IV (CD26) gene expression in enterocyte-like colon cancer cell lines HT-29 and Caco-2. J. Biol. Chem. 267, 4824-4833.

De Meester I., Vanham G., Kestens L., Vanhoof G., Bosmans E., Gigase P., and Scharpe S. (1994). Binding of adenosine deaminase to the lymphocyte surface via CD26. Eur. J. Immunol. 24, 566-570.

Dong R.-P., Kameoka J., Hegen M., Tanaka T., Xu Y., Schlossman S. F. and Morimoto C. (1996). Characterization of adenosine deaminase binding to human CD26 on T cells and its biologic role in immune response. J. Immunol. 156,1349-1355.

Dong R.-P., Tachibana K., Hegen M., Munakata Y., Cho D., Schlossman S. F. and Morimoto C. (1997). Determination of adenosine deaminase binding domain on CD26 and its immunoregulatory effect on T cell activation. J. Immunol. 159, 6070-6076.

Dong R.-P., Tachibana K., Hegen M., Scharpe S., Cho D., Schlossman S. F. and Morimoto C. (1998). Correlation of the epitopes defined by anti-CD26 mAbs and CD26 function. Mol. Immunol. 35, 13-21.

Eguchi K., Ueki Y., Shimomura C., Otsubo T., Nakao H., Migita K., Kawakami A., Matsunaga M., Tezuka H. and Ishikawa, N. (1989). Increment in the Ta1+ cells in the peripheral blood and thyroid tissue of patients with Graves' disease. J. Immunol. 142, 4233-4240.

Fleischer B. (1987). A novel pathway of human T cell activation via a 103 KD T cell activation antigen. J. Immunol. 138, 1346-1350.

Flentke G.R., Munoz E., Huber B.T., Plaut A.G., Kettner C.A. and Bachovchin W.W. (1991). Inhibition of dipeptidyl aminopeptidase IV (DP-IV) by Xaa-boro-Pro dipeptides and use of these inhibitors to examine the role of DP-IV in T cell function. Proc. Natl. Acad. Sci. USA 88, 1556-1559.

Fox D., Hussey R.E., Fitzgerald K.A., Acuto O., Poole C., Palley L, Daley J.F., Schlossman S F. and Reinherz E.L. (1984). Ta1, a novel 105 KD human T cell activation antigen defined by a monoclonal antibody. J. Immunol. 133, 1250-1256.

Franco R., Valenzuela A., Lluis C. and Blanco J. (1998). Enzymatic and extraenzymatic role of ecto-adenosine deaminase in lymphocytes. Immunol. Rev. 161, 27-42.

Gisselbrecht C., Gaulard P., Lepage E., Coiffier B., Briere J., Haioun C., Cazals-Hatem D., Bosly A., Xerri L., Tilly H., Berger F., Bouhabdallah R. and Diebold J. (1998). Prognostic significance of T-cell phenotype in aggressive non-Hodgkin's lymphomas. Groupe d'Etude des Lymphomes de l'Adulte (GELA). Blood 92, 76-82.

Hafler D. A., Fox D. A., Manning M. E., Schlossman S. F., Reinherz E. L. and Weiner H. L. (1985). In vivo activated T lymphocytes in the peripheral blood and cerebrospinal fluid of patients with multiple sclerosis. N.Engl. J. Med. 312, 1405-1411.

Hafler D. A., Chofflon M., Benjamin D., Dang N. H. and Breitmeyer, J. (1989). Mechanisms of immune memory- T cell activation and CD3 phosphorylation correlate with Ta1 (CDw26) expression. J. Immunol. 142, 2590-2596.

Hegen M., Mittrucker H. W., Hug R., Demuth H. U., Neubert K., Barth A. and Fleischer B. (1993). Enzymatic activity of CD26 (dipeptidyl peptidase IV) is not required for its signaling function in T cells. Immunobiol. 189:483-493.

Hegen M., Kameoka J., Dong R. P., Schlossman S. F. and Morimoto C. (1997). Cross-linking of CD26 by antibody induces tyrosine phosphorylation and activation of mitogen-activated protein kinase. Immunol. 90, 257-264.

Herrera C., Morimoto C., Blanco J., Mallol J., Arenzana F., Lluis C. and Franco R. (2001). Comodulation of CXCR4 and CD26 in human lymphocytes. J. Biol. Chem. 276, 19532-19539.

Ho L., Aytac U., Stephens L. C., Ohnuma K., Mills G. B., McKee K. S., Neumann C., LaPushin R., Cabanillas F., Abbruzzese J. L., Morimoto C. and Dang N. H. (2001). In vitro and in vivo antitumor effect of the anti-CD26 monoclonal antibody 1F7 on human CD30+ anaplastic large cell T-cell lymphoma Karpas 299. Clin. Cancer Res. 7, 2031-2040.

Hong W. and Doyle D. (1987). cDNA cloning for a bile canaliculus domain-specific membrane protein of rat hepatocytes. Proc. Natl. Acad. Sci. USA 84, 7962-7966.

Hong W. and Doyle D. (1988). Membrane orientation of rat gp110 as studied by in vitro translation. J. Biol. Chem. 263, 16892-16898.

Houghton A. N., Albino A. P., Cordon-Cardo C., Davis L. J. and Eisinger M. (1988). Cell surface antigens of human melanocytes and melanoma-expression of adenosine deaminase binding protein is extinguished with melanocyte transformation. J. Exp. Med. 167, 197-212.

Huhn J., Olek S., Fleischer B. and von Bonin A. (1999). The adenosine deaminase-binding region is distinct from major anti-CD26 mAb epitopes on the human dipeptidyl peptidase IV (CD26) molecule. Cell. Immunol. 192, 33-40.

Huhn J., Ehrlich S., Fleischer B. and von Bonin A. (2000). Molecular analysis of CD26-mediated signal transduction in T cells. Immunol. Lett. 72, 127-132.

Ikushima H., Munakata Y., Ishii T., Iwata S., Terashima M., Tanaka H., Schlossman S. F. and Morimoto C. (2000). Internalization of CD26 by mannose 6-phosphate/insulin-like growth factor II receptor contributes to T cell activation. Proc. Natl. Acad. Sci. USA 97, 8439-8444.

Ishii T., Ohnuma K., Murakami A., Takasawa N., Kobayashi S., Dang N. H., Schlossman S.F. and Morimoto C. (2001). CD26-mediated signaling for T cell activation occurs in lipid rafts through its association with CD45RO. Proc. Natl. Acad. Sci. USA. 98, 12138-12143.

Jones D., Dang N.H., Duvic M., Washington L.T. and Huh Y.O. (2001). Absence of CD26 expression is a useful marker for diagnosis of T-cell lymphoma in peripheral blood. Am. J. Clin. Pathol. 115, 885-892.

Kahne T., Neubert K., Faust J. and Ansorge S. (1998). Early phosphorylation events induced by DPPIV/CD26-specific inhibitors. Cell. Immunol. 189, 60-66.

Kameoka J., Tanaka T., Nojima Y., Schlossman S. F. and Morimoto C. (1993). Direct association of adenosine deaminase with a T cell activation antigen, CD26. Science 261, 466-469.

Klobusicka M. and Babusikova O. (1999). Expression of CD26 and DPPIV in T-acute lymphoblastic leukemia: comparison of immunocytochemistry with enzyme cytochemistry. Neoplasma 46, 299-303.

Korom S., De Meester I., Stadlbauer T. H. W., Chandraker A., Schaub M., Sayegh M. H., Belyaev A., Haemers A., Scharpe S. and Kupiec-Weglinski J. W. (1997). Inhibition of CD26/dipeptidyl peptidase IV activity *in vivo* prolongs cardiac allograft survival in rat recipients. Transplantation 63, 1495-1500.

Lambeir A.-M., Proost P., Durinx C., Bal G., Senten K., Augustyns K., Scharpe S., Van Damme J. and De Meester I. (2001). Kinetic investigation of chemokine truncation by CD26/dipeptidyl peptidase IV reveals a striking selectivity within the chemokine family. J. Biol. Chem. 276, 29839-29845.

Larsen S.L., Pedersen L.O., Buus S. and Stryhn A. (1996). T cell responses affected by aminopeptidase N (CD13)-mediated trimming of major histocompatibility complex class II-bound peptides. J. Exp. Med. 184, 183-189.

Loster K., Zeilinger K., Schuppan D. and Reutter W. (1995). The cysteine-rich region of dipeptidyl peptidase IV (CD26) is the collagen-binding site. Biochem. Biophys. Res. Comm. 217, 341-348.

Marguet D., Bernard A.M., Vivier I., Darmoul D., Naquet P. and Pierres M. (1992). CDNA cloning for mouse thymocyte-activating molecule. A multifunctional ecto-dipeptidyl peptidase IV (CD26) included in a subgroup of serine proteases. J. Biol. Chem. 267, 2200-2208.

Martin M., Huguet J., Centelles J.J. and Franco R. (1995). Expression of ecto-adenosine deaminase and CD26 in human T cells triggered by the TCR-CD3 complex. Possible role of adenosine deaminase as costimulatory molecule. J. Immunol. 155, 4630-4643.

Masuyama J.-I., Berman J. S., Cruikshank W. W., Morimoto C. and Center D. M. (1992). Evidence for recent as well as long term activation of T cells migrating through endothelial cell monolayers in vitro. J. Immunol. 148, 1367-1374.

Melnyk A., Rodriguez A., Pugh W. C. and Cabanillas F. (1997). Evaluation of the Revised European-American Lymphoma classification confirms the clinical relevance of immunophenotype in 560 cases of aggressive Non-Hodgkin's Lymphoma. Blood 89, 4514-4520.

Morimoto C., Torimoto Y., Levinson G., Rudd C. E., Schrieber M., Dang N. H., Letvin N. L. and Schlossman S. F. (1989). 1F7, a novel cell surface molecule, involved in helper function of CD4 cells. J. Immunol. 143, 3430-3439.

Morimoto C. and Schlossman S. (1998). The structure and function of CD26 in the T-cell immune response. Immunol. Rev. 161, 55-70.

Morrison M.E., Vijayasaradhi S., Engelstein D., Albino A. P. and Houghton A. N. (1993). A marker for neoplastic progression of human melanocytes is a cell surface ectopeptidase. J. Exp. Med. 177, 1135-1143.

Ohnuma K., Munakata Y., Ishii T., Iwata S., Kobayashi S., Hosono O., Kawasaki H., Dang N. H. and Morimoto C. (2001). Soluble CD26/dipeptidyl peptidase IV induces T cell proliferation through CD86 up-regulation on APCs. J. Immunol. 167, 6745-6755.

Ohtsuki T., Hosono O., Kobayashi H., Munakata Y., Souta A., Shioda T. and Morimoto C. (1998). Negative regulation of the anti-human immunodeficiency virus and chemotactic activity of human stromal cell-derived factor 1-alpha by CD26/dipeptidyl peptidase IV. FEBS Lett. 431, 236-240.

Oravecz T., Pall M., Roderiquez G., Gorrell M. D., Ditto M., Nguyen N. Y., Boykins R., Unsworth E. and Norcross M.A. (1997). Regulation of the receptor specificity and function of the chemokine RANTES (Regulated on Activation, Normal T cells Expressed and Secreted) by dipeptidyl peptidase IV (CD26)-mediated cleavage. J. Exp. Med. 186, 1865-1872.

Piazza G.A., Callanan H.M., Mowery J. and Hixson D.C. (1989). Evidence for a role of dipeptidyl peptidase IV in fibronectin-mediated interactions of hepatocytes with extracellular matrix. Biochem. J. 262, 327-334.

Proost P., Struyf S., Schols D., Durinx C., Wuyts A., Lenaerts J.-P., De Clercq E., De Meester I. and Van Damme J. (1998a). Processing by CD26/dipeptidyl-peptidase IV reduces the chemotactic and anti-HIV-1-activity of stromal-cell-derived factor-1 alpha. FEBS Lett. 432, 73-76.

Proost P., De Meester I., Schols D., Struyf S., Lambeir A.-M., Wuyts A., Opdenakker G., De Clercq E., Scharpe S. and Van Damme J. (1998b). Amino-terminal truncation of chemokines by CD26/dipeptidyl-peptidase IV: conversion of RANTES into a potent inhibitor of monocyte chemotaxis and HIV-1-infection. J. Biol. Chem. 273, 7222-7227.

Proost P., Struyf S., Schols D., Opdenakker G., Sozzani S., Allavena P., Mantovani A., Augustyns K., Bal G., Haemers A., Lambeir A.-M., Scharpe S., Van Damme J. and De Meester I. (1999). Truncation of macrophage-derived chemokine by CD26/dipeptidyl-peptidase IV beyond its predicted cleavage site affects chemotactic activity and CC chemokine receptor 4 interaction. J. Biol. Chem. 274, 3988-3993.

Proost P., Menten P., Struyf S., Schutyser E., De Meester I. and Van Damme J. (2000). Cleavage by CD26/dipeptidyl peptidase IV converts the chemokine LD78beta into a most efficient monocyte attractant and CCR1 agonist. Blood 96, 1674-1680.

Proost P., Schutyser E., Menten P., Struyf S., Wuyts A., Opdenakker G., Detheux M., Parmentier M., Durinx C., Lambeir A.-M., Neyts J., Liekens S., Maudgal P. C., Billiau A. and Van Damme J. (2001). Amino-terminal truncation of CXCR3 agonists impairs receptor signaling and lymphocyte chemotaxis, while preserving antiangiogenic properties. Blood 98, 3554-3561.

Reinhold D., Bank U., Buhling F., Kahne T., Kunt D., Faust J., Neubert K. and Ansorge S. (1994). Inhibitors of dipeptidyl peptidase IV (DP IV, CD26) specifically suppress proliferation and modulate cytokine production of strongly CD26 expressing U937 cells. Immunobiol. 192, 121-136.

Reinhold D., Bank U., Buhling F., Lendeckel U., Faust J., Neubert K. and Ansorge S. (1997). Inhibitors of dipeptidyl peptidase IV induce secretion of transforming growth factor-B1 in PWM-stimulated PBMC and T cells. Immunology 91, 354-360.

Ruiz P., Mailhot S., Delgado P., Amador A., Viciana A.L., Ferrer L. and Zacharievich N. (1998). CD26 expression and dipeptidyl peptidase IV activity in an aggressive hepatosplenic T-cell lymphoma. Cytometry 34, 30-35.

Schon E., Jahn S., Kiessig S. T., Demuth H. U., Neubert K., Barth A., Von Baehr R. and Ansorge S. (1987). The role of dipeptidyl peptidase IV in human T lymphocyte activation: inhibitors and antibodies against dipeptidyl peptidase IV suppress lymphocyte proliferation and immunoglobulin synthesis in vitro. Eur. J. Immunol. 17, 1821-1826.

Shioda T., Kato H., Ohnishi Y., Tashiro K., Ikegawa M., Nakayama E.E., Hu H., Kato A., Sakai Y., Liu H., Honjo T., Nomoto A., Iwamoto A., Morimoto C. and Nagai Y. (1998). Anti-HIV-1 and chemotactic activities of human stromal cell-derived factor 1 alpha (SDF-1 alpha) and SDF-1 beta are abolished by CD26/dipeptidyl peptidase IV-mediated cleavage. Proc. Natl. Acad. Sci. USA 95, 6331-6336.

Stecca B.A., Nardo B., Chieco P., Mazziotti A., Bolondi L. and Cavallari A. (1997). Aberrant dipeptidyl peptidase IV (DPPIV/CD26) expression in human hepatocellular carcinoma. J. Hepatol. 27, 337-345.

Steeg C., Hartwig U. and Fleischer B. (1995). Unchanged signaling capacity of mutant CD26/dipeptidyl peptidase IV molecules devoid of enzymatic activity. Cell. Immunol. 164, 311-315.

Steinbrecher A., Reinhold D., Quigley L., Gado A., Tresser N., Izikson L., Born I., Faust J., Neubert K., Martin R., Ansorge S. and Brocke S. (2001). Targeting dipeptidyl peptidase IV (CD26) suppresses autoimmune encephalomyelitis and up-regulates TGF-beta1 secretion in vivo. J. Immunol. 166, 2041-2048.

Struyf S., Proost P., Schols D., De Clercq E., Opdenakker G., Lenaerts J.-P., Detheux M., Parmentier M., De Meester I., Scharpe S. and Van Damme J. (1999). CD26/dipeptidyl-peptidase IV down-regulates the eosinophil chemotactic potency, but not the anti-HIV activity of human eotaxin by affecting its interaction with the CC chemokine receptor 3. J. Immunol. 162, 4903-4909.

Struyf S., Menten P., Lenaerts J.-P., Put W., D'Haese A., De Clercq E., Schols D., Proost P. and Van Damme J. (2001). Diverging binding capacities of natural LD78beta isoforms of macrophage inflammatory protein-1alpha to the CC chemokine receptors 1,3 and 5 affect their anti-HIV-1 activity and chemotactic potencies for neutrophils and eosinophils. Eur. J. Immunol. 31, 2170-2178.

Tanaka T., Camerini D., Seed B., Torimoto Y., Dang N. H., Kameoka J., Dahlberg H.N., Schlossman S F. and Morimoto C. (1992). Cloning and functional expression of the T cell activation antigen CD26. J. Immunol. 149, 481-486.

Tanaka T., Kameoka J., Yaron A., Schlossman S.F. and Morimoto C. (1993). The costimulatory activity of the CD26 antigen requires dipeptidyl peptidase IV enzymatic activity. Proc. Natl. Acad. Sci. USA 90, 4586-4590.

Tanaka T., Duke-Cohan J.S., Kameoka J., Yaron A., Lee I., Schlossman S.F. and Morimoto C. (1994). Enhancement of antigen-induced T-cell proliferation by soluble CD26/dipeptidyl peptidase IV. Proc. Natl. Acad. Sci. USA 91, 3082-3086.

Tanaka T., Umeki K., Yamamoto I., Sakamoto F., Noguchi S. and Ohtaki S. (1995). CD26 (dipeptidyl peptidase IV/DPPIV) as a novel molecular marker for differentiated thyroid carcinoma. Int. J. Cancer 64, 326-331.

Torimoto Y., Dang N.H., Vivier E., Tanaka T., Schlossman S.F. and Morimoto C. (1991). Coassociation of CD26 (dipeptidyl peptidase IV) with CD45 on the surface of human T lymphocytes. J. Immunol. 147, 2514-2517.

Torimoto Y., Dang N.H., Tanaka T., Prado C., Schlossman S.F. and Morimoto C. (1992). Biochemical characterization of CD26

(dipeptidyl peptidase IV): functional comparison of distinct epitopes recognized by various anti-CD26 monoclonal antibodies. Mol. Immunol. 29, 183-192.

Uematsu T., Urade M., Yamaoka M. and Yoshioka W. (1996) Reduced expression of dipeptidyl peptidase (DPP) IV in peripheral blood T lymphocytes of oral cancer patients. J. Oral Pathol. Med. 25, 507-512.

Van Den Oord J.J. (1998). Expression of CD26/dipeptidyl-peptidase IV in benign and malignant pigment-cell lesions of the skin. Br. J. Dermatol. 138, 615-621.

Von Bonin A., Huhn J. and Fleischer B. (1998). Dipeptidyl-peptidase IV/CD26 on T cells: analysis of an alternative T-cell activation pathway. Immunol. Rev. 161, 43-53.

Wesley U.V., Albino A.P., Tiwari S. and Houghton A.N. (1999). A role for dipeptidyl peptidase IV in suppressing the malignant phenotype of melanocytic cells. J. Exp. Med. 190, 311-322.

Yoshioka M., Erickson R.H., Matsumoto H., Gum E. and Kim Y.S. (1991). Expression of dipeptidyl aminopeptidase IV during enterocytic differentiation of human colon cancer (Caco-2) cells. Int. J. Cancer 47, 916-921.

Accepted May 31, 2002