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

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**ABSTRACT**

CD26 is a M₃ 110,000 surface glycoprotein with diverse functional properties, including having a potentially significant role in tumor development, and antibodies to CD26 mediate pleomorphic cellular functions. In this report, we show that binding of soluble anti-CD26 monoclonal Ab 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. In *vitro* experiments show that 1F7 induces cell cycle arrest at the G₁-S checkpoint, associated with enhanced p21 expression that is dependent on *de novo* protein synthesis. Furthermore, experiments with a severe combined immunodeficient mouse tumor model demonstrate that 1F7 treatment significantly enhances survival of tumor-bearing mice by inhibiting tumor formation. Our data therefore suggest that anti-CD26 treatment may have potential clinical use for CD26+ hematological malignancies.

**INTRODUCTION**

CD26 is a M₃ 110,000 surface glycoprotein with an array of diverse functional properties that is expressed on a number of tissues, including epithelial cells and leukocyte subsets (1, 2). Furthermore, it is a membrane-associated ectopeptidase that possesses DPPIV activity in its extracellular domain and is able to cleave NH₂-terminal dipeptides from polypeptides with either t-proline or t-alanine at the penultimate position. Although a significant physiological substrate related to the immunological aspects of CD26/DPPIV is yet to be identified conclusively, recent work has demonstrated that CD26 can cleave certain chemokines involved in T-cell and monocyte function (3, 4). Work over the past decade has shown CD26 to be a molecule with a plethora of functions in basic human T-cell physiology. Recently identified as the ADA binding protein, CD26 regulates ADA surface expression, with the CD26/ADA complex perhaps playing a key role in the catalytic removal of local adenosine to regulate immune system function (5–7). T-cell activation by various stimuli results in enhanced CD26 surface expression as a T-cell activation marker (8, 9). It is also a costimulatory surface molecule via the CD3 and CD2 pathways of activation as well as being capable of acting as an alternate pathway of T-cell activation when cross-linked by specific mAbs, possibly because of its physical association with the transmembrane protein tyrosine phosphatase CD45 (10–17). On the other hand, soluble anti-CD26 mAbs and DPPIV inhibitors suppress T-cell growth and function in certain instances (5, 18–20). Besides being a key immunoregulatory molecule, CD26 may have a potential role in the development of certain neoplasms, including aggressive T-cell hematological malignancies (21, 22).

In eukaryotic cells, cell cycle progression is controlled at the G₁-S checkpoint by a group of related enzymes known as the CDKs, which are positively regulated by their physical association with regulatory subunits called cyclins (23). However, enzymatic activities of the CDK-cyclin complexes are negatively regulated by a set of proteins termed CDK inhibitors. The p21 (WAF1, Cip1) CDK inhibitor blocks multiple cyclin-CDK complexes through its physical association with these structures (24, 25). In addition, through its direct interaction with PCNA, p21 can inhibit DNA replication (26). Various stimuli can induce p21 expression, including cellular damage, serum factors, and phorbol esters, with p21 induction having been shown to be both p53 dependent and p53 independent, depending on the stimuli (24, 27, 28). In this report, we demonstrate that binding of soluble anti-CD26 mAb 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. Anti-CD26 binding results in growth arrest at the G₁-S checkpoint, associated with increased p21 expression that is depend-

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3 The abbreviations used are: DPPIV, dipeptidyl peptidase IV; ADA, adenosine deaminase; CDK, cyclin-dependent kinase; PCNA, proliferating cell nuclear antigen; SCID, severe combined immunodeficiency; mAb, monoclonal antibody; MTT, 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide; CHX, cycloheximide.

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ent on de novo protein synthesis. Furthermore, studies using a SCID mouse tumor model show that 1F7 treatment leads to significantly enhanced survival of tumor-bearing mice. Besides showing a functional association between CD26 and regulators of the cell cycle, our study suggests that CD26-targeted therapy may have potential implications in the treatment of certain human hematological malignancies.

MATERIALS AND METHODS

**Animals, Cells, and Reagents.** Female C.B-17 SCID mice were obtained from Taconic Farms, Inc. at 3–4 weeks of age and were housed in microisolator cages, and all food, water, and bedding were autoclaved before use. The human CD30+ anaplastic large cell T-cell lymphoma cell line Karpas 299 was established from the peripheral blood blast cells of a 25-year-old white man with the diagnosis of CD30+ anaplastic large cell T-cell lymphoma, bearing surface markers CD4, CD5, HLADR, and CD30, with the t(2;5) translocation and with rearranged T-cell receptor β-chain gene (29, 30). Cells were incubated at 37°C in culture medium, consisting of RPMI 1640 supplemented with 10% FCS, 100 units/ml penicillin, and 100 μg/ml streptomycin (Life Technologies, Inc.). Anti-CD26 mAbs used in this study are 1F7 and 5F8, both of which are murine IgG1 recognizing a CD45 RA epitope not expressed on mouse IgG1 recognizing a CD45 RA epitope not expressed on Karpas 299 cell line and was developed as described previously (9). Control mAb used is an isotype control mouse IgG1 recognizing a CD45 RA epitope not expressed on Karpas 299 cell line and was developed as described previously (9). Anti-CD3 and anti-CD2 mAbs were purchased from Coulter. For Western blotting studies, anti-p21 and anti-p27 were from Transduction Laboratories; anti-cdk2, anti-cdk4, and anti-cyclin D were from Upstate Biotechnology; anti-cyclin E and anti-PCNA were from biochem; anti-cdk2, anti-cdk4, and anti-cyclin D were from Transduction Laboratories; anti-p53 was from Calbiochem; anti-CD3 and anti-CD2 were purchased from Upstate Biotechnology; anti-cyclin E and anti-PCNA were from Santa Cruz Biotechnology; and anti-actin was from Sigma Chemical Co. Tetrazolium salt MTT (Sigma Chemical Co.) was dissolved at a concentration of 5 mg/ml in sterile PBS at room temperature, with the solution being further sterilized by filtration and stored at 4°C in the dark. Extraction buffer was prepared as follows. Twenty % w/v of SDS was dissolved at 37°C in a solution of 50% each of N,N-dimethyl formamide (Sigma Chemical Co.) and distilled water; pH was adjusted to 4.7 by the addition of 1 M HCl. CHX (Sigma Chemical Co.) was used at a concentration of 20 μg/ml.

**In Vivo Experiments.** All mice were pretreated i.p. with 0.2 ml of anti-asialo-GM1 monoclonal antiserum 25% (v/v; Wako, Richmond, VA) 1 day before tumor transplant to eliminate host natural killer cell activity and facilitate tumor engraftment (30). For survival studies, tumor cells were then inoculated by i.p. injection. One day after tumor cell inoculation, SCID mice then received saline, isotype control Ab, or anti-CD26 mAb 1F7 i.p. injections in 0.1 ml of sterile saline at the indicated doses and schedules. Tumor-bearing mice were then monitored for tumor development and progression, and moribund mice were euthanized and necropsied for evidence of tumors. In addition, mice with visible or palpable tumors measuring 2 cm at its greatest dimension were also euthanized and necropsied to minimize suffering to the animals. For some animals, organs were also harvested for histopathological analyses. In some experiments, SCID mice were injected with tumor cells by s.c. injection, and after tumor size had reached 0.5 cm at its greatest dimension, saline, isotype control mAb, or 1F7 (5 μg/injection) was injected intratumorally every other day for seven injections. Mice were then euthanized, and tumor mass at the site of injection was harvested for histopathological analyses. In other experiments, 1 × 10^6 Karpas 299 tumor cells were mixed on ice initially with saline alone, 100 μg of 1F7 or isotype control Ab, and immediately after mixing, tumor cells/antibody mixture was injected s.c. into SCID mice. Subsequently, starting 1 day after tumor cell inoculations, SCID mice then received saline, isotype control Ab (20 μg/injection), or 1F7 (20 μg/injection) s.c. injections 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 evaluate treatment effects.

**MTT Assay.** Cell growth assay was performed as described previously (32). Cells were incubated in microplates in the presence of culture medium alone or culture medium plus antibodies as described to a total volume of 100 μl (50,000 cells/well). After 48 h of incubation at 37°C, 25 μl of MTT were added to the wells at a final concentration of 1 mg/ml. The microplates are then incubated for 2 h at 37°C, followed by the addition of 100 μl of extraction buffer. After overnight incubation at 37°C, absorbance measurements at 570 nm were performed. Values reported represent the means of triplicate wells, and the standard errors of the mean were <15%.

**Immunofluorescence.** All procedures were carried out at 4°C, and flow cytometry analyses were performed (FACScan, Becton Dickinson) as described previously (16). Cells were stained with the appropriate antibodies and washed two times with PBS and then with goat antimouse IgG FITC (Coulter). Cytokine producing cells were then washed two times with PBS prior to flow cytometry analyses. Negative controls were stained with second antibody alone. For some experiments, SCID mice were inoculated with tumor cells i.p. (1 × 10^6 cells/mouse) as described above. When tumors were palpable, animals were euthanized, and tumor mass was harvested. Single cell suspensions were then isolated from tumor mass, and flow cytometry was then performed.

**Cell Cycle Analysis.** Cells were cultured in medium alone or in the presence of antibodies at a concentration of 5 μg/ml at 37°C. At the appropriate time intervals, cells were then collected, washed twice with PBS, and resuspended in PBS containing 10 μg/ml propidium iodide, 0.5% Tween 20, and 0.1% RNase at room temperature for 30 min. Samples were then analyzed (FACScan; Becton Dickinson) as described above. When tumors were palpable, animals were euthanized, and tumor mass was harvested. Single cell suspensions were then isolated from tumor mass, and flow cytometry was then performed.

**SDS-PAGE and Immunoblotting.** After incubation at 37°C, cells were harvested from wells, washed with PBS, and lysed in lysis buffer consisting of 1% Brij 97, 5 mM EDTA, 0.02 mM HEPES (pH 7.3), 0.15 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, 0.5 mM NaF, 10 μg/ml aprotinin, and 0.2 mM sodium orthovanadate. After incubating on ice for 15 min, nuclei were removed by centrifugation, and supernatants were collected. 2× sample buffer consisting of 20% glycerol, 4.6% SDS, 0.125 M Tris (pH 6.8), and 0.1% bromphenol blue was added to the appropriate aliquots of supernatants. Protein samples were sub-
mitted to SDS-PAGE analysis on a 20% gel under standard conditions using a mini-Protean II system (Bio-Rad). For immunoblotting, the proteins were transferred onto nitrocellulose (Immobilon-P; Millipore). After overnight blocking at 4°C in blocking solution consisting of 0.1% Tween 20 and 5% BSA in TBS, membranes were blotted with the appropriate primary antibodies diluted in blocking solution for 1 h at room temperature. Membranes were then washed with blocking solution, and appropriate secondary antibodies diluted in blocking solution were then applied for 1 h at room temperature. Secondary antibodies were goat antimouse or goat antirabbit horseradish peroxidase conjugate (Dako). Membranes were then washed with blocking solution, and proteins were subsequently detected by chemiluminescence (Amersham Pharmacia Biotech).

RESULTS

CD26 Expression on the Karpas 299 Lymphoma Cell Line. Expression of CD26 on the CD30+ anaplastic large cell T-cell lymphoma cell line Karpas 299 was evaluated by flow cytometry prior to tumor implantation into the SCID mouse. As shown in Fig. 1A, Karpas 299 cells have high surface expression of CD26, whereas CD3 and CD2 surface expression is not detectable. In addition, overnight incubation with 1F7 led to a decrease in expression of CD26 surface expression (Fig. 1B), consistent with findings reported previously of anti-CD26-mediated modulation of CD26 surface expression on normal T cells (16).

CD26-mediated Inhibition of Cell Growth at the G1-S Checkpoint. We next examined the effect of soluble anti-CD26 antibody binding on growth of Karpas 299 cells in in vitro experiments. As shown in Fig. 2A, the addition of 1F7 resulted in decreased cell growth as measured by MTT reduction. Of note is the fact that 5F8 exerted a significant inhibitory effect on cell growth only at relatively high concentrations. The anti-CD26 mAbs did not exert any growth-inhibitory effect on.
CD26-negative cell lines at the concentrations tested (data not shown). Besides its growth-inhibitory effect on Karpas 299 cells, Fig. 2B showed that 1F7 binding led to decreased MTT uptake in the T-cell line H9, which is also CD26+ (Ref. 9; data not shown). Additional evidence of the inhibitory effect of 1F7 on cell growth was obtained through cell cycle analysis. As shown in Table 1, binding of 1F7 resulted in enhanced blockade of cell cycle progression at the G1-S checkpoint, leading eventually to decreased cell metabolism and cell growth as detected by reduction in MTT uptake.

Enhancement of p21 Expression after CD26-mediated Cell Cycle Arrest. In view of the integral role played by CDK inhibitors at the G1-S checkpoint, we next examined the expression of p21, p27, and p16 after anti-CD26 antibody binding. Fig. 3A showed that p27 expression was not affected by anti-CD26 mAb binding, whereas p16 expression was not detectable in cells incubated in medium alone or in the presence of control antibodies as well as anti-CD26 mAbs (data not shown). On the other hand, p21 expression was enhanced after CD26 ligation. Compared with incubation under control conditions, treatment with anti-1F7 resulted in increased expression of p21, because Western blotting with specific anti-p21 mAb showed the appearance of the expected band migrating at the M₆ 21,000 position. It is known that under certain conditions, induction of p21 expression is dependent on p53 (24, 27). In our experimental system with the Karpas 299 cell line, we showed that there was no change in p53 expression in anti-CD26-treated cells as compared with controls, although the functional status of p53 has not been determined. In addition, previous work has demonstrated that p21 forms complexes with cyclins and CDKs to inhibit cell cycle progression at G1-S. Although anti-CD26 binding resulted in enhanced p21 expression, protein levels of cyclin D, cyclin E, cdk2, and cdk4, which are all present within the cyclin/CDK/p21 complex, remained unchanged. In addition, PCNA protein levels were unaffected by treatment with anti-CD26 (Fig. 3A). Meanwhile, Fig. 3B showed that enhanced p21 expression was detected within 3 h of treatment with 1F7, with its level rising during continuing antibody treatment.

As noted above, the 1F7 mAb was more effective at inducing cell growth arrest as compared with the 5F8 mAb. Consistent with this conclusion were our findings that treatment of Karpas 299 cells with 1F7 mAb resulted in elevated p21 expression at both doses of antibody tested, whereas only the higher dose of 5F8 mAb led to a modest enhancement in p21 expression (Fig. 3C).

Enhanced p21 Expression Is Dependent on de Novo Protein Synthesis. To determine whether the enhancement in p21 expression after anti-CD26 binding is dependent on increased protein synthesis, p21 expression in Karpas 299 cells treated with anti-CD26 in the presence of CHX was examined and compared with p21 expression in cells treated with anti-CD26 alone. As demonstrated in Fig. 4, p21 was seen in the cells treated with 1F7 but not those treated with 1F7 and CHX.
hence indicating that expression of p21 after CD26 ligation was dependent on de novo protein synthesis.

**Antitumor Effects of 1F7 in SCID Mice Bearing Karpas 299 in an in Vivo Tumor Model.** Extending our in vitro findings, we investigated the effect of the anti-CD26 mAb 1F7 on Karpas 299 growth in a SCID mouse tumor model. We showed that the process of tumor formation in the in vivo model did not have an effect on CD26 surface expression. Karpas 299 cells ($1 \times 10^6$) were implanted by i.p. injection into the SCID mouse, and tumor was allowed to develop. Subsequently, tumor mass was then removed, and single-cell suspensions were established. Fig. 5 showed that CD26 expression on Karpas 299 cells after tumor implantation into the SCID mouse was similar to its level prior to tumor implantation. Meanwhile, postmortem histopathological analyses of tissue sections of the i.p. mass were performed as described in “Materials and Methods.”

SCID mice were then inoculated with Karpas 299 cells by i.p. injection ($1 \times 10^6$ cells/mouse) and starting at day 1 after tumor inoculation, treatment with saline, isotype control Ab, or 1F7 was started at the indicated doses every other day for a total of 10 i.p. injections. As shown in Fig. 7A, mice treated with 1F7 at 5 µg/injection had statistically significant survival advantage over those treated with saline ($P < 0.0001$) or isotype control Ab at 5 µg/injection ($P < 0.001$). Similarly, the survival advantage of mice treated with 1F7 at 10 µg/injection was statistically significant as compared with saline-treated mice ($P < 0.0001$) or mice treated with isotype control Ab at 10 µg/injection ($P < 0.001$). Our data also indicated that there was no statistically significant difference in survival between 1F7 doses of 5 µg/injection and 10 µg/injection ($P = 0.7$).

When the mice were injected with higher i.p. doses of tumor cells ($3 \times 10^6$ cells/mouse) and then subsequently treated by i.p. injections of saline alone, isotype control Ab (20 µg/injection) or 1F7 at doses of 5, 10, or 20 µg/injection given every other day for a total of 10 injections, again there was 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, mice treated at 1F7 dose of 20 µg/injection had statistically significant survival advantage over those treated with isotype control Ab at a dose of 20 µg/injection ($P < 0.01$). Comparing mice treated with different 1F7 doses, those treated with 20 µg/injection had statistically significant survival advantage over those treated with the 1F7 dose of 5 µg/injection ($P < 0.01$). Although the mice treated with 1F7 at a dose of 20 µg/injection appeared to have better survival than those treated at a dose of 10 µg/injection, the difference was not statistically significant ($P = 0.2$). Likewise, there was no statistically significant difference in survival for those treated with the 1F7 dose of 10 µg/injection as compared with 5 µg/injection ($P = 0.09$), although those treated at the higher dose again appeared to have better survival than those treated at the lower dose (Fig. 7B).

These data hence indicated that the efficacy of antibody treatment was dependent on the relative amount of tumor present. Meanwhile, postmortem histopathological analyses of tissue sections showed that tumor-bearing mice treated under control conditions developed tumor infiltrates at local sites as well as distant organs. On the other hand, 1F7-treated mice had no evidence of tumor involvement at these sites (data not shown).

Another indication of the antitumor effect of 1F7 in the SCID mouse model is demonstrated in Fig. 8. In these sets of studies, SCID mice were inoculated with $1 \times 10^6$ Karpas 299 cells by s.c. administration. After the development of a visible tumor mass, mice were then treated by intratumoral injections of either saline alone or 1F7 (5 µg/injection) given every other day.
for seven injections. Histopathological analyses showed that 1F7 treatment resulted in tumor necrosis, because most of the tumor had undergone coagulative necrosis. In contrast, saline treatment resulted in tumor cells being viable in the vast majority of the tumor mass. Similarly, treatment with isotype control Ab at 5 μg/injection led to similar results as those obtained with saline treatment, because most of the tumor cells were found to be viable in the tumor mass.

Having established that 1F7 was effective in inhibiting de novo tumor growth when administered i.p., we evaluated its potential efficacy in de novo tumor growth inhibition when given by another route. Therefore, we examined the time required for initial appearance of Karpas 299 tumors after s.c. injection of tumor cells and s.c. treatment with saline, 1F7, or isotype control Ab, as another means of evaluating the efficacy of 1F7 treatment. For these studies, 1 × 10⁶ Karpas 299 cells incubated in saline alone were injected s.c. into the SCID mice; or the cells were mixed on ice with 100 μg of 1F7 or 100 μg of isotype control Ab and immediately after mixing, they were injected s.c. into the animals. Subsequently, starting 1 day after tumor cell inoculations, SCID mice then received saline, isotype control Ab (20 μg/injection), or 1F7 (20 μg/injection) s.c. injections 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 evaluate treatment effects.

As can be seen in Fig. 9, there was a statistically significant difference in the rate of visible tumor development among the mice treated with different conditions. The group treated with 1F7 had a lower rate of tumor development than those treated with isotype control Ab or saline alone (P < 0.001 and P < 0.001, respectively), with the majority of 1F7-treated mice remaining free of tumor during the length of the study. These data thus demonstrated that the ability of 1F7 to inhibit de novo Karpas 299 tumor growth in SCID mice was not limited by a particular method of antibody delivery. Rather, 1F7 was effective in vivo when given through different routes of administration.

**DISCUSSION**

In this study, we have demonstrated the antitumor effect of the anti-CD26 mAb 1F7 in vitro and in vivo studies. By showing that anti-CD26 mAb binding led to growth arrest of the CD30+ T-cell anaplastic large cell lymphoma cell line Karpas 299, these data demonstrated that CD26 mediates a growth-inhibitory effect through its functional association with the cell cycle, independent from its previously reported association with the established pathways of T-cell activation (11, 13, 15). In addition, although previous reports showed that CD26 ability to mediate activation signals is dependent on a functional CD3/T-cell receptor complex (2, 16), our data provided evidence that
CD26 can transmit signals resulting in alterations of T-cell biological responses in the absence of a functional CD3/T-cell receptor complex. In normal T cells, engagement of CD26 results in increase phosphorylation of proteins involved in T-cell signal transduction, mediated in part through the physical association of CD26 and CD45 (15, 17). On the other hand, the particular mechanism involved in CD26 engagement leading to cell cycle arrest is the subject of active research in our laboratory at the present time. Also, although previously published data showing G1 arrest after enforced CD26 expression in

Fig. 8  Histopathological examinations of antitumor effect of 1F7. SCID mice were inoculated by s.c. administration with $1 \times 10^6$ Karpas 299 cells, and a visible tumor mass was allowed to develop. Mice were then treated by intratumoral injections of either saline, isotype control mAb (5 μg/injection), or 1F7 (5 μg/injection) given every other day for seven injections. Histopathological analyses showed that saline (A and D) or isotype control mAb (B and E) treatment resulted in tumor cells being viable in the vast majority of the tumor mass, whereas 1F7 treatment resulted in tumor necrosis (C and F). In A, the homogeneous staining of this mass treated with saline is attributable to the uniform viability of the neoplastic cells. In B, the homogeneous staining of this mass treated with isotype control mAb is attributable to the uniform viability of the neoplastic cells. In C, this mass shows areas of irregular staining that is the result of necrosis (the cleft is an artifact of tissue preparation). In D, the viable neoplastic cells from saline-treated tumor resemble those shown in Fig. 6. In E, similar to D, the viable neoplastic cells from isotype control mAb-treated tumor resemble those shown in Fig. 6. In F, this is representative of coagulative necrosis in a 1F7-treated tumor.
Although CD26 function in immune regulation has been well studied, its role in the clinical setting is not yet clearly defined, although available data suggest that it may be involved in the development of certain human diseases. Consistent with the findings that CD26 is a marker of T-cell activation and has a functional role in this process, CD26 may have a role in certain autoimmune diseases as a regulator of T-cell activation and lymphokine synthesis, including rheumatoid arthritis, Graves’ disease, and multiple sclerosis (35–38). Circulating T lymphocytes from patients with these autoimmune diseases express a high level of CD26 surface expression, and in several instances, the level of expressed CD26 correlates with disease activity (35–38). Furthermore, in patients with rheumatoid arthritis, antibody-induced modulation of CD26 expression results in enhanced interleukin 2 and IFN-γ synthesis in peripheral blood T cells, but it leads to decreased IFN-γ production while having no effect on interleukin 2 production in synovial fluid T cells (38). In addition, inhibition of CD26/DPPIV enzymatic activity in vivo prolongs cardiac allograft survival in rat recipients, suggesting a role for CD26 in alloantigen-mediated immune regulation in vivo and in the mechanism of allograft rejection (39). Besides its involvement in immunoregulation, CD26 may have a role in the development of certain human tumors. Most lung adenocarcinomas are DPPIV positive, whereas other histological types of lung carcinoma are DPPIV negative (40). In addition, CD26 expression is high in differentiated thyroid carcinomas but is absent in benign thyroid diseases (41). It also appears to have a role in melanoma development because its expression is lost with malignant transformation of melanocytes (7, 33). High levels of CD26 protein expression and mRNA transcripts are found in B-chronic lymphocytic leukemia cells and activated B cells, as compared with normal resting B cells (42). Meanwhile, CD26 expression on T-cell malignancies appears to be restricted to aggressive pathological entities such as T-cell lymphoblastic lymphomas/acute lymphoblastic leukemias and T-cell CD30+ anaplastic large cell lymphomas, being detected only on a small percentage of indolent diseases such as mycosis fungoides. Significantly, within the T-cell subset of T-cell lymphoblastic lymphoma/acute lymphoblastic leukemia, CD26 expression may be an independent marker of patients with poor prognosis (21, 22).

It is of significance to note that previous work has shown treatment with anti-CD26 mAb at effective doses is well tolerated in patients without inducing severe toxicity. In a pilot study, Bacigalupo et al. (43) treated eight patients with severe refractory graft versus host disease with a murine anti-CD26 mAb recognizing human CD26 (44). Along with a decrease in the number of circulating CD26+ T cells, there was a significant improvement in the severity of the disease. There were two complete responders and two partial responders, with five of eight patients surviving at least 1 year after treatment. Importantly, treatment with anti-CD26 monoclonal antibody was well tolerated with acceptable immediate adverse reactions, suggesting that future therapies involving anti-CD26 mAb can be administered with tolerable side effects. In view of CD26 expression in certain human cancers, including aggressive T-cell
malignancies, our findings that treatment with anti-CD26 mAb can inhibit tumor growth in vitro and in vivo, using a SCID mouse tumor model, may thus have potential clinical implications. The ability to inhibit tumor growth through the use of anti-CD26 mAbs may lead to the eventual development of new reagents targeting CD26+ human tumors, including aggressive hematological malignancies resistant to current treatment modalities. Furthermore, given its enhanced expression and the potential role in the pathophysiology of diseases involving activated immune status, including graft versus host disease and autoimmune diseases, CD26-targeted treatment may similarly prove to be effective in these settings.

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