CD26/Dipeptidyl Peptidase IV in Context: the Different Roles of a Multifunctional Ectoenzyme in Malignant Transformation

By Satoshi Iwata* and Chikao Morimoto*‡

*From the *Division of Tumor Immunology, Dana-Farber Cancer Institute and Department of Medicine, Harvard Medical School, Boston, Massachusetts 02115; and the ‡Department of Clinical Immunology and AIDS Research Center, The Institute of Medical Science, The University of Tokyo, Minato-ku, Tokyo 108, Japan*

CD26, the T cell activation molecule, is a 110-kD glycoprotein that is also present on epithelial cells of various tissues, including the liver, kidney, and intestine. CD26 possesses a known dipeptidyl peptidase (DPP)IV activity in its extracellular domain (1). A paper by Wesley et al. in this issue indicates a possible role for CD26/DPPIV in suppressing malignant transformation of melanocytes to melanoma (2). The series of work by Houghton and colleagues, focusing on melanoma and melanocytes, has shown that loss of expression and its enzymatic activity of CD26/DPPIV occurs during malignant transformation of melanocytes into melanoma (3). The exact pathological role of its disappearance however, has been unclear. Wesley et al. now elegantly demonstrate that the inducible gene transduction of CD26 into melanoma cells reverses the malignant phenotype of the cells toward "benign" melanocyte-like phenotype, characterized by loss of tumorigenicity, reappearance of anchorage-dependent growth, restoration of a block in differentiation, and serum dependence. They further show that mutant DPPIV lacking serine protease activity is also able to restore the serum dependence in melanoma cells. Finally, the recruitment of another serine protease, fibroblast activation protein-α, may partly account for this reversal by mutant DPPIV.

What is the mechanism of such biological behavior of CD26/DPPIV in melanocytes and melanoma? The authors suggest the possibility that CD26/DPPIV might degrade autocrine growth factors, which are yet unidentified and hence regulate (suppress) the growth of benign melanocytes. These findings are quite impressive, as they present direct evidence that the loss of surface expression of CD26/DPPIV plays a pivotal role in malignant transformation of melanocytes toward melanoma.

Besides CD26/DPPIV, this serine protease family also consists of a group of membrane-associated enzymes (ectopeptidases) including CD10/NEP, CD13/APN, and BP-1/6C3/APA, which are zinc metallopeptidases (4). The expression of these ectopeptidases, including CD26/DPPIV, and their roles in various malignancies are under intense investigation.

Analogous to the loss of CD26/DPPIV in melanoma, the cell surface expression of CD10/NEP is lost during the development of androgen-independent prostate cancer from its androgen-dependent phenotype (5). Indeed, Shipp et al. (6) demonstrated that CD10/NEP inhibits the growth of small cell carcinoma (SCC) of the lung through the cleavage of bombesin-like peptides, which are autocrine growth factors for those cells. They further showed that expression levels of CD10/NEP in the SCC cells were reduced and that the growth of SCC was inhibited by CD10/NEP and potentiated by CD10/NEP inhibition.

In marked contrast to the inverse correlation between the expression of CD26 and malignant transformation of melanocytes, a direct correlation between disease aggressiveness and the expression of CD26 in T cell lymphomas has been reported by Carbone et al. (7). They also showed that the expression of CD26 and CD40L was mutually exclusive, with CD40L being expressed on slowly progressing diseases (7). In accordance with these findings, upregulation of CD26/DPPIV on the surfaces of tumor cells has been recently reported in cases of T cell acute lymphoblastic leukemia. The presence of CD13/APN on neoplastic lymphocytes showed a significant association with such unfavorable clinicoprognostic factors in B cell chronic lymphocytic leukemia as advanced clinical stage and the diffuse pattern of bone marrow infiltration. Besides hematologic malignancies, the upregulation of ectopeptidases has been reported in a variety of solid tumors. For example, it has been well established that follicular cell–derived thyroid carcinomas (papillary carcinoma, Hürthle cell carcinoma, and follicular carcinoma) express high levels of CD26/DPPIV, whereas thyroid glands with benign conditions are exclusively negative for CD26 expression. Therefore, the analysis of CD26/DPPIV expression and/or its enzymatic activity may be a powerful tool for the purpose of cytologic diagnosis of the specific types of thyroid carcinoma (8). In addition, DPPIV activity is higher in cases of basal cell carcinoma than in normal skin or benign lesions. Along with CD26/DPPIV, CD13/APN has been reported to be upregulated in breast carcinomas (9). Thus, the upregulation

or downregulation of these ectopeptidases appears to be tissue specific, and even cell type specific, in a variety of malignancies.

How to explain the distinct roles of CD26 in malignancies? How is this tissue and cell type specificity derived? First, it may be partly due to the multifunctional nature of CD26/DPPIV. CD26/DPPIV has several functions besides its DPPIV enzymatic activity, such as its association with adenosine deaminase (ADA) and CD45, its costimulatory effects on T cells, and its function as a receptor for extracellular matrix proteins such as fibronectin and collagens (10).

On human T cells, CD26 expression appears late in thymic differentiation and is preferentially restricted to the CD4^{+} helper/memory population. Remarkably, CD26 can deliver a potent co-stimulatory T cell activation signal to the CD3 and CD2 pathways or after PMA treatment in the absence of either exogenous IL-2 or accessory cells (1). The isolated cDNA for CD26/DPPIV predicted a type II membrane protein of 766 amino acids with G-W-S-Y-G at positions 628–632 in its putative extracellular domain, which fits the consensus sequence (G-X-S-X-G) of the catalytic site of the serine protease family (11). Studies performed with enzyme inhibitors have implicated DPPIV enzymatic activity in antigen-induced and/or mitogen-induced T cell proliferation and lymphokine production (12). These studies principally suggested that DPPIV activity is necessary for certain T cell activation pathways.

The human CD26 cDNA was mutated to encode a protein in which the putative catalytic serine residue at position 630 is replaced by alanine (1). We examined the comitogenic effect of stimulation through CD3 and CD26 Ag on IL-2 production using Jurkat T cells expressing wild-type CD26 (DPPIV[+]) or mutant CD26 (DPPIV[−]). Surprisingly, the DPPIV^{+} Jurkat cells produced substantially more IL-2 than the DPPIV^{−} cells on the coengagement of CD3, suggesting that the DPPIV enzymatic activity of CD26 by itself may contribute to IL-2 production in the costimulation of CD3 and CD26. These results directly support the notion that CD26 plays an important role in T cell costimulation

Because CD26 has only six amino acids in its cytoplasmic region, other signal-transducing molecules may be associated with CD26, which may account for the costimulatory activity of CD26. We have shown that CD26 was comodulated on the T cell surface with CD45RO, a known membrane-linked protein tyrosine phosphatase, and was associated with CD45 in T cells (1). Moreover, it was demonstrated that modulation of CD26 from T cell surface induced by anti-CD26 led to enhanced phosphorylation of CD3ζ tyrosine residues and increased CD4-associated p56[lck] tyrosine kinase activity (1). Thus, one possible mechanism for this costimulatory activity involves the association of CD26 with the membrane-linked protein tyrosine phosphatase CD45.

Another candidate for the signal transduction molecule is a 43-kD protein associated with CD26 in PHA-activated blast cells and CD26-transfected Jurkat cells (1) that was identified as the human ADA (13). ADA is present on the cell surface as well as in the cytoplasm of human fibroblasts, renal rabbit tubular cells, and human mononuclear blood cells (1) (highest expression in lymphocytes), and it catalyzes the conversion of adenosine and deoxyadenosine to inosine and deoxyinosine, respectively. Despite its wide distribution, ADA deficiency causes SCID in humans (14). The inhibition of ADA activity by deoxycoformycin results in reduction of TCR/CD3-mediated inositol phospholipid hydrolysis and calcium flux (15), implying possible involvement of ADA in early events of T cell activation. It was found that ADA coexpressed with CD26 on the cell surface can block the inhibitory effect of adenosine using CD26-transfected Jurkat cells. The CD26-transfected Jurkat cells that coexpressed ADA and CD26 on the cell surface were shown to be resistant to the inhibitory effect of ADA on cell proliferation and IL-2 production, showing a marked contrast with the case of the control cells without ADA and CD26 detected on their surfaces. These data strongly suggest that ADA expressed on the T cell surface can aid the cell in resisting an inhibitory effect of adenosine by reducing the effective extracellular concentration of adenosine.

This complex formation of CD26–ADA–adenosine may also be involved in the pathogenesis of some diseases. Indeed, hairy cell leukemia and chronic lymphocytic leukemia are lymphoid malignancies, which can express high levels of ADA and CD26. Perhaps their dramatic response to 2-deoxycoformycin and 2-chlorodeoxyadenosine, known inhibitors of ADA (16), relates to their surface expression of the ADA–CD26 complex.

ADA and CD26 expressed on T cells may play an important role in the catalytic removal of local adenosine, which in turn could exaggerate the inflammatory response or provide the growth advantage of lymphoid malignancies (1). Thus, the costimulatory nature and recruitment of ADA by CD26 may confer a proliferative advantage to tumor T cells that in turn allows cancer cells to escape from immunosurveillance through the downregulation of CD26/DPPIV on their cell surfaces. This reciprocal effect on both lymphoid lineage and epidermoid lineage may contribute to the tissue- and cell type–specific results. It should be noted that development of malignant tumors in vivo is regulated not only by their own cell growth but also by a variety of surrounding cells. Of particular interest is the report by Gruss et al. showing that T cells in the vicinity of Hodgkin's and Reed-Sternberg cells do not express the CD26 antigen, and that these CD26^{−} T cells remain CD26^{−} even after stimulation in vitro, indicating an ineffective activation of T cells in Hodgkin's disease–involved tumor lesions (17).

Another possibility, as proposed by Wesley et al. (2), is that chemokines might act as growth factors for malignant cells, which may be the substrate for CD26/DPPIV or other ectopeptidases. In fact, the existence of a transformed cancer cell line that produces and excretes CC chemokines into the culture supernatant has been reported previously (18), although it is not clear whether those chemokines act as autocrine growth factors.

Reported physiological substrates for CD26/DPPIV in-

clude substance P, β-casomorphin, kentsin, somatoliberin, growth hormone releasing factor, the neuropeptides, and the fibrin α chain. CD26/DPPIV and/or CD13/APN were unable to cleave rIL-1α, IL-2, rG-CSF, or natural IL-2 despite the presence of susceptible NH_2-terminal sequences. Therefore, physiological substrates related to the immunological aspects of CD26/DPPIV have not yet been identified.

Recently, Oravecz et al. (19) have demonstrated CD26-mediated cleavage of RANTES (regulated on activation, normal T cell expressed and secreted) on Ca^{2+} mobilization in T cells and monocytes. In accordance with their results, we have, and the differential effect of full length (AA 1-68) and truncated (AA 3-68) RANTES also found that RANTES was digested by soluble CD26 at the exact position of its specificity. Furthermore, we have demonstrated that soluble rCD26 enhances transendothelial migration of T cells induced by RANTES, whereas it reduces the migratory response of monocytes (20). This differential regulation of the transendothelial migration of T cells and monocytes seems to be partly explained by CD26-mediated cleavage of RANTES; however, synthetic RANTES (amino acid 3–68) lacking two NH_2-terminal amino acids showed equivalent chemotactic activity to its full length form on resting T cells, suggesting that other mechanisms may be involved in the enhancement of T cell migration by CD26. These findings have led us to propose that CD26/DPPIV may be involved in the switching of the innate and acquired immune responses. The receptors for

RANTES are CCR1, CCR3, CCR4, and CCR5 (21). According to the reports by Oravecz et al. (19) and Struyf et al. (21a), the soluble CD26/DPPIV-mediated loss of chemotactic activity of RANTES in monocytes may be due to its loss of signal transducing ability through CCR1 and/or CCR3. Furthermore, a growing body of evidence has shown that other CC chemokines, such as eotaxin, macrophage-derived chemokine, and stromal cell–derived factor 1 (22) are also cleaved and/or subsequently modulated in their receptor specificities. Another ectopeptidase, CD13/APN was also found to degrade IL-8 and inactivate its chemotactic activity. This cleavage and inactivation of IL-8 was significantly inhibited by the presence of bestatin or anti-CD13 mAb, indicating that CXC chemokines as well as CC chemokines are also processed and modulated by one of the ectopeptidases (23).

The modulation of the NH_2 termini of chemokines is of great importance not only for binding to their receptors and the following reactions but also for altering the receptor specificity of the processed chemokine and producing endogenous inhibitor of intact chemokines. While interfering with the putative autocrine loop as suggested by Wesley et al. (2), cleavage of chemokines may have profound effects on the responder cells. Judging from the enormous complexity and redundancy of the various chemokines and their receptors, one could not easily predict the biological outcome of cleavage of specific chemokines by the ectopeptidases. Additionally, there may be interplays among different ectopeptidases, as there may be colocalization of
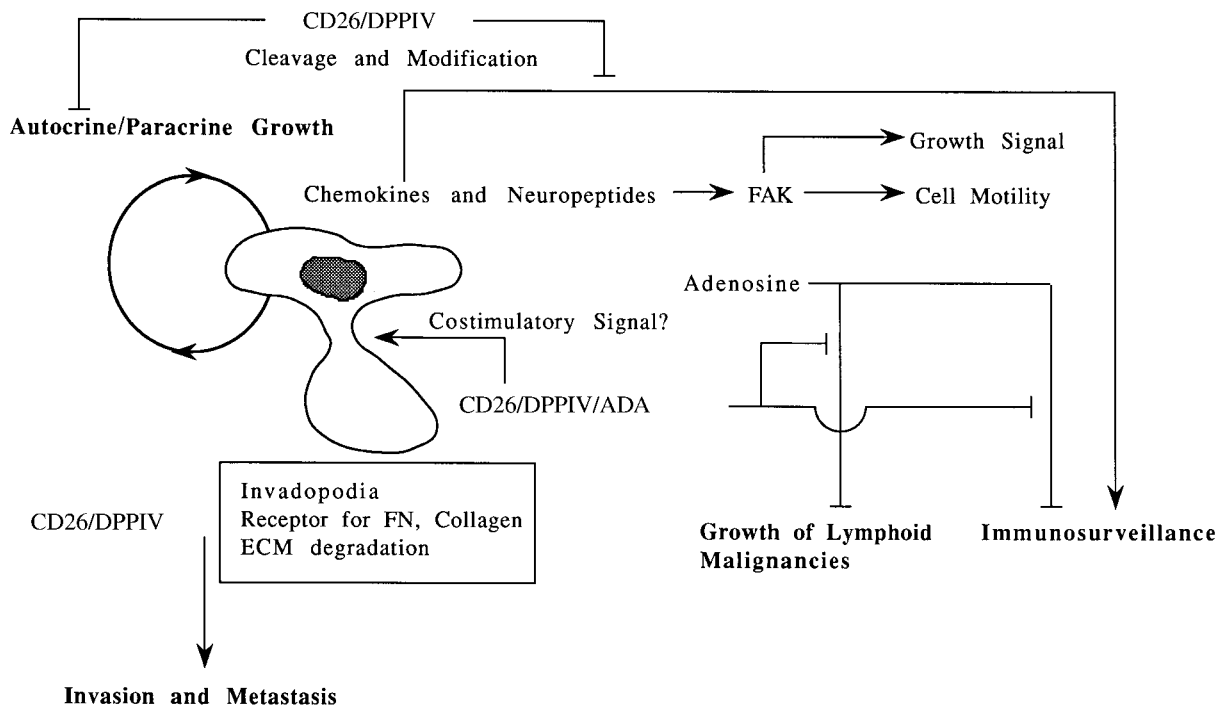
Figure 1. Schematic view: complexity of the site of action and clinical significance of CD26/DPPIV. CD26/DPPIV is a multifunctional ectoenzyme that may elicit biological effects on various aspects of development and progression of malignancy. The scheme summarizes some of these effects, including modulation of growth factors and adenosine, alteration of immunosurveillance, promotion of cellular invasion, and metastasis.

CD13, CD10, and CD26 in various cell types, with their expression differentially regulated by various stimuli (4). There may be cooperation among these ectopeptidases in processing the substrates and, thus, tissue specificity may depend on the interplays among these enzymes.

Another explanation may be that malignant transformation or tumor development is the end result of a complex set of processes with multiple steps. Particularly, the development of metastasis and invasive ability of malignancies should be taken into account, as well as tumor growth, which might cause variation in the biological and clinical effects of these ectopeptidases. In this context, the binding ability of CD26/DPPIV with fibronectin (FN) and collagen may confer an aggressive or metastatic phenotype to tumor cells. Indeed, we reported that peripheral T cells migrating through a monolayer of endothelial cells on collagen gels expressed the highest CD26, whereas CD26 of adherent (nonmigrating) cells was divided into negative and high expression (24). Along this line, Cheng et al. (25) have demonstrated the existence of DPPIV and FN-mediated adhesion and metastasis in lung endothelial cells and breast cancer cells, in which case DPPIV is expressed on the endothelial cells and FN is expressed on the cell surfaces of the malignant cells. Furthermore, the peptidase activity of the ectoenzymes has been implicated in the process of invasion and metastasis through the degradation of the extracellular matrix, as has been suggested in the case of the metalloprotease superfamily (26). The possible role of ectopeptidases in these processes has been supported by a pharmacological study showing the inhibition of tumor cell invasion and matrix degradation by aminopeptidase inhibitors. Furthermore, there may be considerable importance in the series of work by Chen et al. (27), suggesting the existence of CD26/DPPIV and fibroblast activation protein-α, another cell surface serine protease, at the "invadopodia," or the site of invasion of tumor cells. Interestingly, in their recent review on CD13/APN (28), Riemann et al. propose that focal adhesion kinase (FAK) might be a point of convergence between adhesion and peptidase substrates, as FAK is activated and auto-phosphorylated by ligation of integrins as well as peptidase substrates such as selected neuropeptides and chemokines (29). FAK may transduce growth-promoting and/or antiapoptotic signals through complex formation with various kinases such as Src, phosphatidylinositol 3 kinase, and adaptor proteins such as Grb-2, p130/Cas, and pp105/Cas-L (30). It is particularly interesting that peptidase-mediated regulation of FAK may have some effects on cell adhesion and motility as well as contact inhibition or anchorage-dependent growth of normal and malignant cells. Fig. 1 schematically summarizes the complex functions of CD26/PPIV.

As discussed above, CD26/DPPIV demonstrates a plethora of biological functions in a variety of tissue types, both benign and malignant. These diverse functions may also be responsible in part for the different roles of CD26 in various clinical settings. At this time, it is not possible to fully discern the molecular mechanisms that explain its various functional effects. However, as can be seen in the elegant work by Houghton and colleagues, the precise role of this intriguing molecule in malignant transformation is beginning to be elucidated, at least at the cellular level. Future work specifically targeting the expression of CD26 and its DPPIV enzymatic activity will undoubtedly contribute to a further understanding of its role in various physiological states in a variety of tissue contexts, hopefully leading to therapeutic approaches that are based on our precise understanding of CD26 biology.

Address correspondence to Chikao Morimoto, Division of Tumor Immunology, Dana-Farber Cancer Institute and Dept. of Medicine, Harvard Medical School, Boston, MA 02115. Phone: 617-632-3357; Fax: 617-632-4569; E-mail: asaharan@mbcrr.harvard.edu

References

1. Morimoto, C., and S.F. Schlossman. 1998. The structure and function of CD26 in the T-cell immune response. *Immunol. Rev.* 161:55–70.

2. Wesley, U.V., A.P. Albino, S. Tiwari, and A.N. Houghton. 1999. A role for dipeptidyl peptidase IV in suppressing the malignant phenotype of melanoma cells. *J. Exp. Med.* 190: 311–322.

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

4. Shipp, M.A., and A.T. Look. 1993. Hematopoietic differentiation antigens that are membrane-associated enzymes: cutting is the key! *Blood.* 82:1052–1070.

5. Papandreou, C.N., B. Usmani, Y. Geng, T. Bogenrieder, R. Freeman, S. Wilk, C.L. Finstad, V.E. Reuter, C.T. Powell, D. Scheinberg, et al. 1998. Neutral endopeptidase 24.11 loss in metastatic human prostate cancer contributes to androgen-independent progression. *Nat. Med.* 1:50–57.

6. Shipp, M.A., G.E. Tarr, C.Y. Chen, S.N. Switzer, L.B. Hersh, H. Stein, M.E. Sunday, and E.L. Reinherz. 1991. CD10/neutral endopeptidase 24.11 hydrolyzes bombesin-like peptides and regulates the growth of small cell carcinomas of the lung. *Proc. Natl. Acad. Sci. USA.* 88:10662–10666.

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

8. Aratake, Y., T. Kotani, K. Tamura, Y. Araki, T. Kuribayashi, K. Konoe, and S. Ohtaki. 1991. Dipeptidyl aminopeptidase IV staining of cytologic preparations to distinguish benign from malignant thyroid diseases. *Am. J. Clin. Pathol.* 96: 306–310.

9. Dixon, J., L. Kaklamanis, H. Turley, I.D. Hickson, R.D. Leek, A.L. Harris, and K.C. Gatter. 1994. Expression of aminopeptidase-N (CD 13) in normal tissues and malignant neoplasms of epithelial and lymphoid origin. *J. Clin. Pathol.* 47: 43–47.

10. Hanski, C., T. Huhle, R. Gossrau, and W. Reutter. 1988. Direct evidence for the binding of rat liver DPP IV to collagen in vitro. *Exp. Cell. Res.* 178:64–72.

11. Brenner, S. 1988. The molecular evolution of genes and proteins: a tale of two series. *Nature.* 334:528–530.

12. Flentke, G.R., E. Munoz, B.T. Huber, A.G. Plaut, C.A. Kettner, and W.W. Bachovchin. 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.

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

14. Hirshhorn, R. 1990. Adenosine deaminase deficiency. *Immunodefic. Rev.* 2:175–198.

15. Buc, H.A., A. Moncion, M. Hamet, A.-M. Houllier, L. Thuilier, and J.-L. Perignon. 1990. Influence of adenosine deaminase inhibition on the phosphoinositide turnover in the initial stages of human T cell activation. *Eur. J. Immunol.* 20: 611–615.

16. Kraut, E.H., M.R. Grever, and B.A. Bouroncle. 1994. Long-term follow-up of patients with hairy cell leukemia after treatment with 2′-deoxycoformycin. *Blood.* 84:4061–4063.

17. Gruss, H.J., A. Pinto, J. Duyster, S. Poppema, and F. Herrmann. 1997. Hodgkin's disease: a tumor with disturbed immunological pathways. *Immunol. Today.* 18:156–163.

18. Wang, J.M., O. Chertov, P. Proost, J.J. Li, P. Menton, L. Xu, S. Sozzani, A. Mantovani, W. Gong, V. Schirrmacher, et al. 1988. Purification and identification of chemokines potentially involved in kidney-specific metastasis by a murine

lymphoma variant: induction of migration and NFκB activation. *Int. J. Cancer.* 75:900–907.

19. Oravecz, T., M. Pall, G. Roderiquez, M.D. Gorrel, M. Ditto, N.Y. Nguyen, R. Boykins, E. Unsworth, and M.A. Norcross. 1997. Regulation of the receptor specificity and formation of the chemokine RANTES (regulated on activation, normal T cell expressed and secreted) by dipeptydyl peptidase IV (CD26)-mediated cleavage. *J. Exp. Med.* 186: 1865–1872.

20. Iwata, S., N. Yamaguchi, Y. Munakata, H. Ikushima, J.F. Lee, O. Hosono, S.F. Schlossman, and C. Morimoto. 1999. CD26/dipeptidyl peptidase IV differentially regulates the chemotaxis of T cells and monocytes toward RANTES: possible mechanism for the switch from innate to acquired immune response. *Int. Immunol.* 11:417–426.

21. Premack, B.A., and T.J. Schall. 1996. Chemokine receptors: gateways to inflammation and infection. *Nat. Med.* 11:1174–1178.

21a. Struyf, S., I. De Meester, S. Scharpe, J.P. Lenaerts, P. Menten, J.M. Wang, P. Proost, and J. Van Damme. 1998. Natural truncation of RANTES abolishes signaling through the CC chemokine receptors CCR1 and CCR3, impairs its chemotactic potency and generates a CC chemokine inhibitor. *Eur. J. Immunol.* 28:1262–1271.

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

23. Kanayama, N., Y. Kajiwara, J. Goto, E. el Maradny, K. Maehara, K. Andou, and T. Terao. 1995. Inactivation of interleukin-8 by aminopeptidase N (CD13). *J. Leukoc. Biol.* 57: 129–134.

24. Masuyama, J., J.S. Berman, W.W. Cruikshank, C. Morimoto, and D.M. Center. 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.

25. Cheng, H.C., M. Abdel-Ghany, R.C. Elble, and B.U. Pauli. 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.

26. Werb, Z. 1997. ECM and cell surface proteolysis: regulating cellular ecology. *Cell.* 91:439–442.

27. Chen, W.T. 1996. Proteases associated with invadopodia, and their role in degradation of extracellular matrix. *Enzyme Protein.* 49:59–71.

28. Riemann, D., A. Kehlen, and J. Langner. 1999. CD13—not just a marker in leukemia typing. *Immunol. Today.* 20:83–88.

29. Morimoto, C., S. Iwata, and K. Tachibana. 1998. VLA-4-mediated signaling. *Curr. Top. Microbiol. Immunol.* 231:1–22.

30. Minegishi, M., K. Tachibana, T. Sato, S. Iwata, Y. Nojima, and C. Morimoto. 1996. Structure and function of Cas-L, a 105-kD Crk-associated substrate-related protein that is involved in β1 integrin–mediated signaling in lymphocytes. *J. Exp. Med.* 184:1365–1375.