Hematopoietic Differentiation Antigens That Are Membrane-Associated Enzymes: Cutting Is the Key!

By Margaret A. Shipp and A. Thomas Look

MANY CELL SURFACE glycoproteins expressed during specific stages of lymphoid and myeloid cell development were initially characterized on the basis of their reactivity with monoclonal antibodies (MoAbs). Subsequent analysis of cloned cDNAs identified four of these hematopoietic differentiation antigens as membrane-associated enzymes with common structural and regulatory features: CD10/neutral endopeptidase 24.11 (CD10/NEP), CD13/aminopeptidase N (CD13/APN), BP-1/6C3/aminopeptidase A (BP-1/6C3/APA), and CD26/dipeptidyl peptidase IV (CD26/DPPIV). All four of these enzymes are type II integral membrane proteins with a single hydrophobic sequence that functions as both a signal peptide and a transmembrane region. Three of these enzymes (CD10/NEP, CD13/APN, and BP-1/6C3/APA) require zinc for biologic activity and share a pentapeptide consensus sequence that has been implicated in both zinc binding and catalysis. Although CD10/NEP, CD13/APN, BP-1/6C3/APA, and CD26/DPPIV are expressed by different hematopoietic cell types at unique stages of lymphoid or myeloid differentiation, they are coexpressed on the luminal surface of epithelial brush borders. All four enzymes participate in the final stages of peptide hydrolysis in the renal proximal tubules and the small intestine. In certain instances, these enzymes also work in concert to digest common substrates.

CD73/ecto 5'-nucleotidase is another hematopoietic differentiation antigen and cell surface enzyme that is also expressed on epithelial brush borders. A glycosyl phosphatidylinositol-linked protein, CD73/ESN, differs from the above-mentioned enzymes in its transmembrane orientation and its role in nucleoside dephosphorylation and transport.

The well-characterized activities of these enzymes in nonhematopoietic tissues have stimulated research into their potential regulatory roles in hematopoiesis. Recent studies implicate these proteins in a variety of processes including stromal cell-dependent B lymphopoiesis, peptide-mediated inflammatory responses, and T-cell activation. Although peptide or nucleotide cleavage remains the best-known activity of each enzyme, CD13/APN, CD26/DPPIV, and CD73/ESN have additional nonenzymatic roles, as illustrated by CD13/APN, which serves as a coronavirus receptor.

We review here recent progress in understanding the structural features, patterns of expression, and biologic functions of these hematopoietic differentiation antigens and ectoenzymes, emphasizing their roles in the regulation of hematopoiesis.

CD10/NEP (EC 3.4.24.11)

Pattern of Expression

The common acute lymphoblastic leukemia antigen (CALLA; CD10) was originally identified with heterosera and subsequently with MoAbs as a 100-Kd tumor-associated cell surface antigen expressed by the majority of acute lymphoblastic leukemias. Additional lymphoid malignancies, including lymphoblastic, Burkitt's, and nodular poorly differentiated lymphocytic lymphomas, as well as chronic myelogenous leukemias in lymphoid blast crisis, were CD10+, whereas acute myelogenous leukemias and lymphomas with a mature B- or T-cell phenotype lacked CD10 expression. For this reason, CD10 was a useful diagnostic marker long before its function was known. Although it was originally considered to be a tumor-specific antigen, CD10 was subsequently identified on normal early human lymphoid progenitors within the bone marrow, fetal liver, and thymus (Table 1). The phenotypic characteristics of these CD10+ cells indicated that they were either uncommitted to the B- or T-cell lineage or committed to only the earliest stages of B-cell differentiation. In fact, the expression of CD10 on certain lymphoid malignancies was thought to reflect the restricted expression of the antigen during the earliest stages of normal lymphoid differentiation. However, CD10 was also identified on multiple myelomas with an immature histologic appearance, suggesting that there may be discordant expression of the antigen in certain B-cell malignancies.

CD10 was also expressed on terminally differentiated granulocytes and a variety of nonhematopoietic cell types, including bronchial epithelial cells, cultured fibroblasts, bone marrow stromal cells, renal proximal tubular epithelial cells, breast myoepithelium, biliary canaliculi, fetal intestine, and certain solid tumor cell lines (Table 1), indicating that the biologic function of the antigen was not restricted to lymphoid development.
Table 1. Tissue-Specific Expression and Functions of Hematopoietic Differentiation Antigens Identified as Ectoenzymes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>General Functions</th>
<th>Hematopoietic Cells</th>
<th>Nonhematopoietic Cells</th>
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<tr>
<td></td>
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<td>Tissue Distribution</td>
<td>Regulatory Function</td>
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<tr>
<td>CD10/NEP</td>
<td>Reduces cellular responses to peptide hormones</td>
<td>Lymphoid progenitors</td>
<td>Regulates stromal cell-dependent B lymphopoiesis</td>
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<td>Granulocytes</td>
<td>Limits neutrophil inflammatory responses</td>
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<td>Acute lymphoblastic leukemias</td>
<td>Augments IL-2 production by certain T cells</td>
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<td>Certain nodular, lymphoblastic, and</td>
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<td>Burkitt’s lymphomas</td>
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<td>CML in lymphoid blast crisis</td>
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<td>Certain myelomas</td>
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<tr>
<td>CD13/APN</td>
<td>Reduces cellular responses to peptide hormones</td>
<td>Myeloid progenitors (CFU-GM)</td>
<td>Limits neutrophil inflammatory responses</td>
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<td></td>
<td>Reduces cellular responses to peptide hormones</td>
<td>Granulocytes</td>
<td>Promotes monocyte antigen processing</td>
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<td></td>
<td>Serves as coronavirus receptor</td>
<td>Myeloid leukemias</td>
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<td>Myeloid progenitors</td>
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<td>Monocytes</td>
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<td>Granulocytes</td>
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<td>Myeloid leukemias</td>
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<tr>
<td>BP-1/BC3/APA</td>
<td>Reduces cellular responses to peptide hormones</td>
<td>B-cell progenitors</td>
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<td>Abelson-transformed lymphoblastoid lines</td>
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<td>CD26/DPP IV</td>
<td>Reduces cellular responses to peptide hormones</td>
<td>Immature thymocytes</td>
<td>Mediates antigen-induced T-cell proliferation</td>
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<td>Adhesion molecule</td>
<td>Subpopulations of peripheral blood T cells</td>
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<td>Splenic B cells</td>
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<td>EBV-transformed</td>
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<td>lymphoblastoid lines</td>
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<td>B-cell lymphomas and surface Ig* acute</td>
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<td>lymphoblastic leukemias</td>
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<td>CD73/ESN</td>
<td>Regulates uptake of extracellular purines</td>
<td>Subpopulations of peripheral B and T cells</td>
<td>Maturation marker of B and T cells</td>
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<td>Processes ATP by dephosphorylating AMP to adenosine</td>
<td>EBV-transformed</td>
<td>Mediates generation of cytotoxic T cells</td>
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<td>lymphoblastoid lines</td>
<td>Regulates neutrophil inflammatory responses</td>
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<td>Many pre-B and progenitor</td>
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<td>B-cell leukemias</td>
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Abbreviations: CML, chronic myeloid leukemia; CNS, central nervous system.
Biochemical and Molecular Characterization

Biochemical analysis of CD10/NEP suggested that the glycoprotein contained ~20% to 25% carbohydrate and that there were tissue-specific differences in its posttranslational modification. In fibroblasts and kidney, the CD10/NEP molecular mass was ~90 Kd, whereas CD10/NEP in lymphoid progenitors and neutrophils was ~100 Kd and ~110 Kd, respectively.

The CD10 cDNA sequence predicts a 750 amino acid type II integral membrane protein with a single 24 amino acid hydrophobic segment that functions as both a transmembrane region and a signal peptide (Fig 1). The COOH-terminal 700 amino acids compose the extracellular region, whereas the 25 NH₂-terminal amino acids remaining after cleavage of the initiation methionine form the cytoplasmic tail (Fig 1). There are six potential N-linked glycosylation sites in the extracellular domain, suggesting that the tissue-specific differences in CD10 molecular mass may be related to glycosylation patterns. CD10 transcripts range in size from 2.7 to 5.7 kb, with major transcripts of 3.7 and 5.7 kb; the multiple mRNAs result, at least in part, from the use of multiple polyadenylation signals in the 3' untranslated region.

The CD10 cDNA sequence is virtually identical to the reported cDNA sequence for a cell surface zinc-dependent metalloprotease, neutral endopeptidase 24.11 (NEP, "enkephalinase," EC 3.4.24.11). The CD10 cDNA was formally shown to encode this enzyme by generating CD10 stable transfectants that had cell surface NEP enzymatic activity. In all cell types examined to date, the levels of the cell surface enzyme correspond to the levels of detectable CD10/NEP transcripts.

The enzyme was first described in 1974 as a neutral metalloendopeptidase from rabbit kidney brush border, and subsequently in 1978 as an "enkephalinase" from brain. Thereafter, the separately characterized neutral metalloendopeptidase and "enkephalinase" were shown to be the same enzyme in a variety of tissues. Although cell types including fibroblasts, reticular cells, bronchial epithelial cells, and peripheral blood granulocytes and tissues including placenta and intestine were known to express NEP, the presence of the enzyme on lymphoid cells was not confirmed until CD10 and NEP were found to be identical. CD10/NEP hydrolyzes a variety of physiologically active peptides, including chemotactic peptide (fMLP), substance P, atrial natriuretic factor, endothelin, neurotensin, oxy-

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**Fig 1.** Molecular structures of CD10/NEP, CD13/APN, BP-1/6C3/APA, CD26/DPPIV, and CD73/E5N. The size, monomeric or dimeric structure, and orientation of each protein with respect to the cytoplasm, transmembrane (TM) region, and extracellular domain are shown. Proven or potential active sites are indicated, as are zinc-binding (ZB), substrate-binding (SuB), and potential tyrosine sulfation (TS) sites. Glycosylation sites, cysteines, and known disulfide bonds are included.
<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Known Substrates</th>
<th>Enzymatic Activity</th>
<th>Cleavage Site</th>
<th>Inhibitors</th>
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<tbody>
<tr>
<td>CD10/NEP</td>
<td>Opioid peptides, fMLP, substance P, bombesin-like peptides, atrial natriuretic factor, endothelin, oxytocin, bradykinin, angiotensins I and II</td>
<td>Cleaves small peptides on the NH₂ terminal side of hydrophobic amino acids</td>
<td>NH₂ → Xaa Xaa Xaa   → Xaa Xaa Xaa Xaa COOH</td>
<td>Phosphoramidon, thiorphan, SCH32615</td>
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<tr>
<td>CD13/APN</td>
<td>Opioid peptides, enkephalins, tuftsin, fMLP</td>
<td>Catalyzes the removal of NH₂ terminal neutral amino acids from peptides</td>
<td>NH₂ → Xaa Xaa Xaa Xaa Xaa COOH</td>
<td>Bestatin, amastatin, actinonin</td>
</tr>
<tr>
<td>BP-1/6C3/APA</td>
<td>Angiotensins, 7IL-7</td>
<td>Catalyzes the removal of NH₂ terminal acidic amino acids from peptides</td>
<td>NH₂ → Xaa Xaa Xaa Xaa Xaa Xaa COOH</td>
<td>Bestatin, amastatin</td>
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<tr>
<td>CD26/DPPIV</td>
<td>Substance P, casomorphin, kenzin, α chain of fibrin, growth hormone releasing hormone</td>
<td>Cleaves Xaa-Pro and Xaa-Ala dipeptides from the NH₂ terminus of polypeptides and proteins</td>
<td>NH₂ → Xaa Pro Xaa Xaa Xaa Xaa COOH</td>
<td>Di-isopropyl fluorophosphate, diprotin A</td>
</tr>
<tr>
<td>CD73/5EN</td>
<td>Purine and pyrimidine ribonucleoside and deoxyribonucleoside monophosphates</td>
<td>Catalyzes the dephosphorylation of purine and pyrimidine ribonucleoside and deoxyribonucleoside monophosphates</td>
<td><img src="image" alt="Diagram" /></td>
<td>α, β methylene adenosine-5-diphosphate</td>
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tocin, bradykinin, angiotensins 1 and 2, the bombesin-like peptides, and the opioid peptides met- and leu-enkephalin (Table 2).59-66

Comparison of the CD10/NEP cDNA sequences from several human, murine, rat, and rabbit sources, including leukemic and bone marrow stromal cell lines, kidney, brain, and placenta, indicates that the gene has been tightly conserved throughout evolution. Additional studies also predict the existence of a CD10/NEP invertebrate homologue.17 There is approximately 90% identity of the predicted CD10/NEP amino acid sequences and 100% conservation of critical functional motifs.15,17,61,62-64 When these CD10/NEP inhibitors are added to in vitro or in vivo assays, the effect is that of increasing the local concentrations of the relevant CD10/NEP peptide substrate(s). In all of the cell types in which CD10/NEP function has been studied, the enzyme appears to reduce cellular responses to peptide hormones (Table 1). Target cells express both the cell surface enzyme and the receptor for the relevant CD10/NEP peptide substrate (Fig 2). By hydrolyzing the peptide substrate, CD10/NEP reduces the local concentrations of peptide available for receptor binding and signal transduction (Fig 2A).14,15,61,83-87 For example, CD10/NEP reduces atrial natriuretic factor-mediated hypotension and diuresis;61 enkephalin-mediated analgesia;62 and peptide-mediated inflammatory responses (Table 1).17,59,83,84 The concentrations of enkephalin needed to generate a neutrophil inflammatory response differ by several orders of magnitude in the presence or absence of the cell surface enzyme.17 CD10/NEP similarly affects the required concentrations of other inflammatory peptides such as substance P and fMLP,59,83,85-86. The cough, bronchial constriction, vasodilation, and neutrophil migration associated with substance P-mediated neurogenic inflammation of the lungs are significantly increased when cell surface CD10/NEP is inhibited (Table 1).83,86 In certain instances, inflammatory peptides such as fMLP are initially cleaved by CD10/NEP and further hydrolyzed by CD13/APN (Fig 2B). This pattern of cooperative cleavage of peptide substrates by CD10/NEP and CD13/APN occurs in a variety of tissues.

Regulation of cellular proliferation Recent studies suggest that CD10/NEP also regulates peptide-mediated cellular proliferation.87 The enzyme hydrolyzes bombesin-like peptides (BLP), which are potent mitogens for fibroblasts and normal bronchial epithelial cells and essential autocrine growth factors for many small-cell carcinomas of the lung.87 The growth and proliferation of BLP-dependent small cell carcinomas is inhibited by CD10/NEP and potentiated by CD10/NEP inhibition.82 These results are of particular interest because (1) small-cell carcinomas of the lung occur almost exclusively in cigarette smokers;88 (2) smokers have increased levels of BLP in their bronchoalveolar lavage fluid;89 (3) cigarette smoke inactivates CD10/NEP; and (4) small-cell carcinomas have reduced levels of the cell surface enzyme.87 Thus, reduced CD10/NEP enzymatic activity may promote BLP-mediated proliferation of pulmonary neuroendocrine cells and facilitate the development of small-cell carcinomas of the lung.87 For this reason, the characterization of a lung cancer-derived CD10/NEP cDNA
that lacks an exon and encodes a cell surface protein with virtually no enzymatic activity is of particular interest.91

The temporal and cellular patterns of CD10/NEP expression in developing fetal lung also implicate the enzyme in the regulation of BLP-mediated normal lung growth.84 The enzyme is expressed at the highest levels in early fetal lung development and is primarily found on cells that are most sensitive to the effects of BLP, the undifferentiated airway epithelium in the distal developing airways.84 These observations led to in vitro and in vivo studies that directly linked the enzyme with peptide-mediated bronchial epithelial development (Table 1).84,92 In human fetal lung organ cultures, inhibition of cell surface CD10/NEP resulted in a significant increase in DNA synthesis84 and, in related murine studies, the in utero administration of a specific long-acting CD10/NEP inhibitor also increased the proliferation and maturation of developing murine fetal lungs.92

CD10/NEP function in lymphoid development. Although CD10/NEP has a defined function in many organ systems, until recently little was known about the enzyme’s role in lymphoid ontogeny. The absence of suitable human models for the study of lymphoid CD10/NEP prompted investigators to turn to murine systems in which discrete stages of B-cell development and models of in vitro and in vivo lymphoid differentiation were well-characterized. The majority of murine pre-B cells lacked CD10/NEP expression; however, a subpopulation of Thy-1lowB220+ murine lymphoid progenitors from bone marrow and modified Whitlock-Witte cultures had low levels of CD10/NEP enzymatic activity and CD10/NEP transcripts that were detectable using RNA-based polymerase chain reaction.14 These Thy-1lowB220+ lymphoid progenitors were of particular interest because they were large cycling cells that could effectively reconstitute the B-lymphoid compartment of lethally irradiated animals.93 Murine bone marrow stromal cells that supported the B-cell differentiation of hematopoietic progenitors including Thy-1lowB220+ cells also expressed high levels of CD10/NEP.14,51 For these reasons, CD10/NEP function was evaluated in modified Whitlock-Witte cultures in which uncommitted hematopoietic progenitors were plated on a stromal cell line that supports the earliest stages of B-cell differentiation.14 In such assays, in which uncommitted progenitors differentiate into Thy-1lowB220+ pro-B cells and Thy-1B220+ pre-B cells, inhibition of CD10/NEP significantly increased early lymphoid colony formation.14 The effects of CD10/NEP inhibition on early lymphoid development were further assessed using an in vivo congenic mouse model in which uncommitted hematopoietic progenitors from donor animals were used to rescue sublethally irradiated recipients treated with a specific long-acting CD10/NEP inhibitor (SCH32615).15 The lymphoid reconstitution of animals treated with the CD10/NEP inhibitor or vehicle alone was compared by analyzing the percentages of donor-derived hematopoietic cells, B-lymphocytes, and mature B cells in the lymphoid organs in both sets of animals.15 SCH32615-treated animals had significantly more donor-derived splenocytes, splenic B cells, and mature (sIg+) splenic B cells than animals treated with vehicle alone, suggesting that inhibition of CD10/NEP increased the proliferation and maturation of splenic B cells.15 Taken together, the in vitro and in vivo studies suggest that CD10/NEP functions to regulate B-cell development by inactivat-
ing a peptide that stimulates B-cell proliferation and differen-
tiation (Table 1).4,17 Alternatively, the enzyme may acti-
vate a propeptide that inhibits B-cell proliferation and differen-
tiation. These new insights into the role of the
enzyme in regulating early lymphoid proliferation are of partic-
ular interest because patients with CALLA+ acute lympho-
blastic leukemias were originally thought to have more
favorable prognoses.84 These studies also underscore the
similarities between the growth-regulatory effects of the
enzyme on lymphoid progenitors and developing epithelial
cells.14,15,84,92

Although the majority of T cells have undetectable
CD10/NEP transcripts and cell surface protein, a subclone of
the T-cell leukemia line Jurkat was recently reported to
express low levels of the enzyme.95 This CD10/NEP+ T-cell
subclone produced substantially lower levels of interleukin-
2 (IL-2) when it was activated in the presence of specific
CD10/NEP enzymatic inhibitors or an anti-CD10/NEP
MoAb, prompting speculation that CD10/NEP may also
function to regulate IL-2 production in certain T cells (Ta-
bles 1).95

Regulation of the enzyme in response to peptide stimu-
lization. In early studies, the treatment of CD10/NEP+ cells
with an anti-CD10 antibody resulted in the rapid cell sur-
face redistribution, internalization, and degradation of the
CD10/NEP-antibody complex.96,98 Subsequently, a variety of
inflammatory mediators were found to modulate the lev-
els of the cell surface enzyme. Treatment of CD10/NEP+ lymphoid, myeloid, fibroblastoid, or epithelial cells with
phorbol esters decreased cell surface CD10/NEP expres-
sion98-100; in several cell types, the cell surface protein
was rapidly internalized and the CD10/NEP transcripts were
also reduced.98-100 Epstein-Barr virus (EBV)-negative B-cell
lymphoma cell lines transfected with the EBV-associated
latent membrane protein also lost cell surface CD10/NEP
expression.101 In contrast, inflammatory mediators such as
tumor necrosis factor, IL-1, IL-6, granulocyte-macrophage col-
ony-stimulating factor (GM-CSF), CSa, and calcium iono-
phore increased cell surface CD10/NEP enzymatic activity
in human neutrophils.53,102 In additional studies, glucocto-
cid treatment of transformed human tracheal epithelial
cells increased CD10/NEP enzymatic activity and trans-
cripts.103 Investigators postulated that steroids might exert
their anti-inflammatory effects in part by upregulating cell
surface CD10/NEP,96 because the enzyme was known to
reduce substance P-mediated neurogenic inflammation of the
lung.81,86

Recent studies also associate the levels of CD10/NEP en-
zymatic activity with cell growth. Human tracheal bron-
chial epithelial cells and non-B, non-T lymphoblastic leukemias express increased levels of CD10/NEP at increased cell
density,102,104 suggesting that one mechanism for limiting peptide-mediated cell growth may be upregulation of
the cell surface enzyme.

CD13/APN (EC 3.4.11.2)

Pattern of Expression

CD13 was originally identified as a 150-Kd cell surface
glycoprotein (gp150) expressed by committed granulo-
cyte-monocyte progenitors (CFU-GM) and by cells of the
granulocytic and monocyct lineage at all morphologically
distinct stages of differentiation (Table 1).105-109 The glyco-
protein is also expressed by leukemic blasts in a high percent-
age of acute myeloid leukemias106-114 and by a smaller sub-
est of acute lymphoid leukemias (Table 1).114,116 The
molecule appears to be highly immunogenic; 17 MoAbs
individually derived from mice immunized with human my-
eloid cells bound CD13 epitopes in studies reported at the
Third International Workshop.117 Antibodies specific for
CD13 do not bind to normal B or T lymphocytes, but do
react with nonhematopoietic cells including fibroblasts,
bone marrow stromal cells, osteoclasts, and cells that line
renal proximal tubules, small intestine, and bile duct cana-
luciae (Table 1).103 The significance of this pattern of CD13
expression became clear when the CD13 cDNA sequence
was found to be identical to that of aminopeptidase N
(APN; EC 3.4.11.2), a prominent membrane-anchored me-
tallopeptidase expressed by the brush borders of the small
intestine and renal tubules.118,119 Although CD13/APN
and CD10/NEP are expressed by many of the same nonhe-
matoopoietic cell types,120,121 the former is not found on early
lymphoid precursors,103,117 whereas the latter does not ap-
pear on monocytes or committed myeloid precursors (Ta-
ble 1).103,117

Biochemical and Molecular Characterization

Biochemical analysis. CD13-specific antibodies immu-
noprecipitate 130-Kd and 150-Kd glycoproteins (gp130
and gp150) from human myeloid leukemia cell lines.118,122
Metabolic labeling studies established that gp130 is an intra-
cellular precursor of gp150 that differs only in the composi-
tion of its carbohydrate chains. When cells were labeled in
the presence of tunicamycin, which blocks the addition of
asparagine-linked oligosaccharide chains, a single unglyco-
sylated polypeptide of 110 Kd was immunoprecipitated.118,122
Comparison of labeled tryptic cleavage products of
gp130 and gp150 molecules confirmed that these glyco-
proteins have an identical primary structure and are thus
posttranslationally modified products of a single gene.4

The biosynthesis of APN was independently character-
ed in epithelial cells from the small intestine and renal
proximal tubules.120,123 Pulse-chase experiments showed
that newly synthesized molecules of approximately 140 Kd
contained cotranslationally added asparagine-linked oligo-
saccharide chains rich in mannose. Within 30 to 60 minutes
of synthesis, the side chains were remodeled to form com-
plex oligosaccharides characteristic of the mature 160-Kd
molecule on the cell surface. Endoglycosidase H treatment
of the immature form of the molecule removed the high-
mannose oligosaccharide chains, producing a polypeptide
backbone of 115 Kd.

APN is expressed as a homodimer on the surface of pig,
rat, rabbit, and human intestinal epithelial cells.8,121,124
More recent studies by Danielscn125,128 show that APN
forms non–disulfide-linked homodimers intracellularly be-
fore Golgi-associated processing and suggest that dimeriza-
tion may be required for transport of the high-mannose in-
tracellular form of APN out of the endoplasmic reticulum.
Although myeloid cells display APN uniformly over the cell surface, epithelial cells and hepatocytes primarily express the enzyme on the apical surface. Cell type-specific pathways are responsible for this asymmetric expression, because APN is directly sorted to the apical membrane in canine kidney cells, but indirectly sorted via the basolateral membrane in hepatocytes. The targeting signal in the canine kidney cells is located in the extracellular catalytic domain of the glycoprotein, rather than the intracellular domain, transmembrane domain, or the serine- and threonine-rich stalk.

Predicted amino acid structure. Human APN and CD13 cDNA clones from intestinal epithelium and myeloid cells predict an identical type II integral membrane protein of 967 amino acids with 11 potential sites of asparagine-linked oligosaccharide addition (Fig 1). These findings account for the 110-kD molecular size of the unglycosylated polypeptide and the additional mass attributable to the carbohydrate moiety of the cotranslationally modified glycoprotein. Analysis of amino-terminal protein sequences indicated that CD13/APN molecules are synthesized with an uncleaved signal sequence and that proteolytic processing at the amino terminus is limited to removal of the initiator methionine. The retained signal sequence also functions as the membrane-spanning segment, orienting the CD13/APN amino terminus inside and the carboxyl terminus outside the cell in a configuration common to a subset of receptors and membrane-bound enzymes, including CD10/NPE (Fig 1). The large extracellular carboxy-terminal domain contains a pentapeptide signature sequence (His-Glu-Leu-Ala-His) characteristic of members of the zinc-binding metalloprotease family (Fig 1).

Regulation of expression. Little is known about the mechanisms that regulate the lineage-restricted expression of cell surface metalloproteases. Nevertheless, it is of interest that CD13/ APN and CD10/NPE are coexpressed by many of the same nonhematopoietic cells, although the two enzymes are expressed by different subsets of hematopoietic cells. Epithelial cells of the renal proximal tubule and small intestine, granulocytes, bone marrow stromal cells, and sympathetic membranes in the central nervous system express both enzymes, whereas monocytes and committed myeloid progenitors express only CD13/ APN, and early lymphoid progenitors express only CD10/NPE (Table 1). Because CD13/ APN and CD10/NPE are both type II metalloproteases with multiple alternatively spliced 5' untranslated regions, it is possible that distinctive regulatory elements control the expression of these enzymes in different tissues.

In the case of CD13/ APN, Shapiro et al identified separate promoters that control transcription in myeloid and intestinal epithelial cells and regulate independent transcripts that differ only in their 5' untranslated sequences. In small intestinal epithelial cells, transcription is controlled by a classical promoter containing a TATA box immediately upstream from the translation initiation codon. This promoter, which has been characterized by Olsen et al, appears to direct the expression of very high levels of APN on intestinal brush border membranes. By contrast, the longer transcripts found in myeloid cells and fibroblasts originate from several sites clustered in an upstream exon located 8 kb from the exon containing the initiator codon; these longer RNAs include sequences from a unique untranslated exon. Regulatory elements of the promoter used by epithelial cells, including the TATA box and the transcription origin, are included within the 5' untranslated region of the longer myeloid cell/fibroblast transcript. Both aminopeptidase N transcripts encode the same polypeptide, indicating that the physically distinct promoters must have evolved to regulate expression of this cell surface peptidase by cells of different tissues.

Enzymatic Activity and Functional Roles

CD13/APN catalyzes the removal of NH2-terminal amino acids from peptides; although the enzyme has a preference for neutral amino acids, it will also cleave basic and acidic residues (Table 2). Natural CD13/APN substrates appear to be small peptides rather than larger proteins, although the enzyme is more effective in removing residues from oligopeptides than dipeptides (Table 2). Inhibitors of CD13/APN activity that have been useful in characterizing the enzyme's function include bestatin, amastatin, and aminoin (Table 2). In the intestinal brush border, the CD13/APN carboxy-terminal enzymatic domain faces the lumen and most likely plays an important role in the final stages of the digestion of small peptides. In other tissues, the enzyme has been postulated to interact with CD10/NPE to modulate signal transduction by bioactive peptides (Fig 2). The two membrane-bound enzymes collaborate in the hydrolysis of oligopeptides in the small intestine and appear to act in concert to inactivate opioid peptides and enkephalins in the brain, as well as tuftsin and the chemotactic peptide Met-Leu-Phe during neutrophil-mediated inflammatory responses (Fig 2).

CD13/APN also serves as a receptor for coronaviruses, which are RNA viruses that cause respiratory disease in humans and several species of animals (Table 1). In humans, coronaviruses are responsible for 15% to 20% of common upper respiratory tract infections, and probably also cause gastroenteritis. Recent studies show that two coronavirus strains use CD13/APN to enter respiratory and intestinal epithelial cells. One strain, 229E (HCV-229E), which is an important cause of upper respiratory tract infections in humans, uses CD13/APN as its receptor. Similarly, transmissible gastroenteritis virus (TGEV), a major source of fatal gastroenteritis in newborn pigs, uses porcine APN as its portal of entry. In both instances, the receptor role of CD13/APN was suggested by the finding that MoAbs able to block infection of susceptible cells specifically recognized extracellular epitopes of the enzyme. Because the host range of these viruses was restricted, it was also possible to render resistant cells susceptible to infection by introducing recombinant cDNAs that encoded either human or porcine APN. These studies provide the means to define key CD13/APN residues that are required for virus binding and infectivity. For example, CD13/APN encoded by a mutant human cDNA clone and lacking key residues near the active site did not bind HCV-229E virus or have enzymatic activity; these
results suggest that the enzyme active site and the virus attachment site are closely linked.\textsuperscript{21,148}

\textbf{BP-1/6C3/APA (EC 3.4.11.7)}

\textit{Pattern of Expression}

BP-1/6C3 is expressed in a developmentally restricted pattern by immature murine B lymphocytes (Table 1). This finding can be traced to studies, begun nearly a decade ago, on lineage-restricted molecules recognized by the MoAbs BP-1 and 6C3. These antibodies were independently derived in the laboratories of Max Cooper and Irving Weissman\textsuperscript{149,150} and subsequently found to recognize epitopes on the same murine cell surface molecule (BP-1/6C3).\textsuperscript{151} The BP-1/6C3 antigen is expressed by cytoplasmic Ig\textsuperscript{+} pre-B-lymphoid cells in bone marrow and fetal liver, but not by mature B-lineage cells in blood, spleen, Peyer’s patches, or lymph nodes (Table 1).\textsuperscript{149} The antigen is also expressed at high levels by early B-lineage cells grown in Whittlock-Witte cultures and by Abelson virus-transformed pre-B cells (Table 1). BP-1/6C3 expression can be abolished by fusogen pre-B cells with plasmacytoma cells.\textsuperscript{146,152} The developmentally restricted binding pattern of the BP-1 and 6C3 antibodies suggested that their cell surface target was a murine early B-lineage differentiation antigen, a finding supported by marked upregulation of the antigen in pre-B cells stimulated to proliferate with IL-7.\textsuperscript{153,154} The pattern of BP-1/6C3 expression in human lymphocytes remains to be determined.

It soon became apparent that the murine BP-1/6C3 antigen was also expressed by nonhematopoietic cell types, including bone marrow-derived stromal cell lines and epithelial cells along the brush borders of the renal proximal tubule and small intestine, and a subset of epithelial cells in the thymic cortex (Table 1).\textsuperscript{16,155} These results suggested a role for BP-1/6C3 beyond B-lymphocyte development and drew attention to the similarities between the patterns of expression of this antigen and the two human metalloproteases, CD10/NEP and CD13/APN.

\textit{Biochemical and Molecular Characterization}

\textit{Biochemical analysis.} Both the BP-1 and 6C3 MoAbs precipitate a disulfide-linked homodimer from the surface of B cells and bone marrow stromal cells.\textsuperscript{151} The protein backbone observed in cells treated with tunicamycin is consistently 110 Kd in size, and the intracellular high-mannose form of the glycoprotein is 125 Kd, although various glycosylated forms of the molecule are expressed at the cell surface by different cell types. For instance, the cell surface form of the molecule expressed by pre-B cells has the apparent electrophoretic mobility of a 140-Kd homodimer, whereas a more rapidly migrating 135-Kd homodimer is observed on bone marrow-derived stromal cell lines.\textsuperscript{151}

\textit{Predicted amino acid structure.} As was the case for CD10/NEP and CD13/APN, BP-1/6C3 was identified as a cell surface metalloprotease by virtue of its predicted amino acid sequence.\textsuperscript{3} cDNA clones for BP-1/6C3, isolated by Wu et al.,\textsuperscript{7} predicted a 945 amino acid integral membrane protein with a type II configuration and a large extracellular carboxyl terminal domain (Fig 1).\textsuperscript{3} Within this extracellular region, the BP-1/6C3 protein contains the pentapeptide consensus sequence (His-Glu-Leu-Val-His) that is characteristic of zinc-binding metalloproteases (Fig 1). Among previously characterized members of the metalloprotease family, the BP-1/6C3 protein is most similar, but not identical, to aminopeptidase N, with murine BP-1/6C3 and human APN showing 36% overall sequence identity.\textsuperscript{3}

\textit{Enzymatic Activity and Functional Roles}

Although the predicted amino acid sequence of the BP-1/6C3 protein suggested that it might be an aminopeptidase, the sequence was not identical to that of a previously characterized enzyme. In recent studies, this murine enzyme was found to have the substrate specificity and sensitivity to inhibitors characteristic of aminopeptidase A (APA; EC 3.4.11.7),\textsuperscript{155} BP-1/6C3 specifically catalyzed the hydrolysis of N-terminal glutamic or aspartic acid residues and was inhibited by chelating agents and competitive inhibitors such as amastatin and bestatin (Table 2).\textsuperscript{156,159} In addition, BP-1/6C3 enzymatic activity was augmented by Ca\textsuperscript{2+},\textsuperscript{158} a property characteristic of APA.\textsuperscript{160,161} Taken together, these data indicated that the recently cloned BP-1/6C3 antigen was APA, which had not previously been cloned or sequenced.

In contrast to other members of the zinc metalloprotease family, the enzymatic activity of BP-1/6C3/APA is inhibited rather than potentiated by increasing concentrations of zinc.\textsuperscript{155} This observation was initially confusing, because the amino acid sequence BP-1/6C3/APA\textsuperscript{3} contains the pentapeptide motif required for zinc coordination and enzymatic activity by other metalloproteases (Fig 1). This discrepancy was resolved when both wild-type and mutant BP-1/6C3/APA cDNA clones were examined in heterologous cells, and the enzyme was found to require low levels of zinc for activity and to be inhibited by higher concentrations of the metal.\textsuperscript{162} Moreover, a missense mutation that converted one of the histidine residues of the signature pentapeptide to a phenylalanine residue completely abolished enzymatic activity, indicating that the integrity of the zinc-binding motif is required for aminopeptidase activity.\textsuperscript{162}

The wide tissue distribution of BP-1/6C3/APA suggests that it may function differently in different cell types. As with other members of the cell surface metalloprotease family, BP-1/6C3/APA is thought to participate in the final stages of the hydrolysis of peptides in the small intestine (Table 1). It may also be involved in the termination of peptide signals, particularly those mediated by angiotensin II (Table 2).\textsuperscript{159,160} APA catalyzes the removal of the N-terminal amino acid aspartic acid from angiotensin II, markedly reducing its biologic activity and rendering it susceptible to further degradation by APN.

The function of BP-1/6C3/APA in murine early B-cell development is still not completely understood; however, several provocative associations suggest a regulatory role. The IL-7 growth factor induces proliferation of early B cells and simultaneously upregulates cell surface BP-1/6C3/APA expression.\textsuperscript{153,154} Intriguingly, both IL-7 and the IL-7 receptor contain acidic N-terminal peptides, although neither has
yet been directly shown to be an APA substrate. Preliminary studies suggest that the BP-1 antibody may inhibit the proliferative effect of IL-7 on early B cells.\textsuperscript{153} In addition, the ability of cloned bone marrow stromal cell lines to support B lymphopoiesis is correlated with the levels of stromal cell surface expression of BP-1/6C3.\textsuperscript{16} The identification of BP-1/6C3 as APA and the availability of defined cDNA clones, MoAbs, and enzymatic inhibitors should allow the role of the enzyme in early B-cell development to be defined at a mechanistic level. The human BP-1/6C3/APA gene has recently been cloned (L. Li, J. Wang, and M. Cooper, personal communication, March 1993), so that studies of the enzyme in normal and malignant human B lymphopoiesis will also be possible.

**CD26/DPPIV (EC 3.4.14.15)**

**Pattern of Expression**

CD26 is a cell surface glycoprotein that was originally characterized as a T-cell differentiation antigen. The murine CD26 homolog, known as the thymocyte-activating molecule (THIAM), is expressed at high levels by immature CD4\textsuperscript{−}CD8\textsuperscript{−} thymocytes and at progressively lower levels by double-positive thymocytes and mature single-positive cells (Table 1).\textsuperscript{6,145} In humans, CD26 is primarily expressed by CD3\textsuperscript{+} medullary thymocytes, variable numbers and double-positive thymocytes and mature single-positive cells CD4\textsuperscript{−}CD8\textsuperscript{−} thymocytes and at progressively lower levels by peripheral blood T cells with mitogen, antigen, or IL-2 results in increased numbers of CD26\textsuperscript{+} cells and increased levels of the cell surface antigen.\textsuperscript{169,170}

Like CD10/NEP, CD13/APN, and BP-1/6C3/APA, CD26/DPPIV participates in the final stages of hydrolysis of certain peptides in both the small intestine and the renal proximal tubules.

CD26 is also expressed by certain splenic B cells, EBV-transformed lymphoblastoid cell lines, B-cell lymphomas, and surface Ig\textsuperscript{+} B-cell ALLs. In many respects, the nonhematopoietic tissue distribution of CD26 resembles that of CD10/NEP, CD13/APN, and BP-1/6C3/APA; CD26 is found on renal proximal tubules, intestinal epithelium, biliary canaliculi, and alveolar pneumocytes (Table 1).\textsuperscript{19,164,171,172} with the highest levels of protein on epithelial cell brush borders.\textsuperscript{164}

**Biochemical and Molecular Characterization**

The human CD26 protein is identified by multiple MoAbs\textsuperscript{19,168,175,177} that precipitate a 110- to 120-Kd protein under reducing and nonreducing conditions. However, the murine and rat CD26 proteins have been reported to form a 200- to 220-Kd dimer on the cell surface, raising the possibility that the protein may be a nondisulfide-linked dimer. The size of the CD26 protein and its wide tissue distribution on nonhematopoietic cells prompted further studies that showed that CD26 is the cell surface ectoenzyme dipeptidyl peptidase IV (DPPIV).\textsuperscript{19,164,167,177} After an incorrect identification of murine CD26 as an aminopeptidase,\textsuperscript{179} its identity with DPPIV was confirmed.\textsuperscript{7}

The recently characterized murine and human CD26/ DPPIV cDNAs predict a highly conserved approximately 766 amino acid polypeptide with type II membrane topology and a cytoplasmic domain of 6 amino acids (Fig 1).\textsuperscript{5,179,180} In contrast to CD10/NEP, CD13/APN, and BP-1/6C3/APA, the predicted CD26/DPPIV protein sequence contains no zinc-binding motif.\textsuperscript{180} However, it does possess structural homology with a previously described 110-Kd bile canaliculus domain-specific membrane protein, differing in only the 25 carboxy-terminal residues.\textsuperscript{6,181} The predicted CD26/DPPIV protein also contains regions of localized homology with a type II membrane dipeptidyl aminopeptidase from yeast (DPAPB).\textsuperscript{180,182}

**Enzymatic Activity and Functional Roles**

CD26/DPPIV is a serine-type protease that preferentially cleaves Xaa-Pro and Xaa-Ala dipeptides from the NH\textsubscript{2} terminus of peptides and proteins (Table 2).\textsuperscript{164,173,183,184} The optimal size and additional features of substrates for this enzyme have not been fully characterized; however, CD26/DPPIV hydrolyzes natural peptides including substance P, casomorphin, growth hormone releasing hormone (GRH), kenzin, and \( \alpha \) chain of fibrin (Table 2).\textsuperscript{164,183,186} The enzyme also cleaves a family of antibacterial peptides from insects,\textsuperscript{187} peptides from frog skin that resembled mammalian hormones and neurotransmitters,\textsuperscript{188} and a potent neurotoxin from bee venom.\textsuperscript{189} Many other human brain and gut peptides contain NH\textsubscript{2}-terminal sequences that predict susceptibility to CD26/DPPIV hydrolysis.\textsuperscript{186}

**CD26/DPPIV in nonhematopoietic cells.** Like CD10/NEP, CD13/APN, and BP-1/6C3/APA, CD26/DPPIV participates in the final stages of hydrolysis of certain peptides in both the small intestine and the renal proximal tubules. This was shown by investigators who took advantage of the fact that a specific strain of Fischer rats lack brush border CD26/DPPIV enzymatic activity.\textsuperscript{190,191} In these animals, the hydrolysis and absorption of Xaa-Pro-peptides in both the kidney and small intestine was markedly reduced.\textsuperscript{190,191}

Additional studies suggest that CD26/DPPIV is also the major enzyme responsible for the inactivation of circulating GRH in plasma.\textsuperscript{186}

**CD26/DPPIV in T cells.** CD26/DPPIV was initially associated with T-cell activation and proliferation because DPPIV inhibitors suppressed mitogen- and antigen-induced T-cell proliferation and impaired T-cell-directed B-cell differentiation and Ig production.\textsuperscript{164,192,194} Recent studies using more potent and specific DPPIV inhibitors suggested that these compounds primarily inhibit antigen-induced T-cell proliferation (Table 1).\textsuperscript{195} In contrast, MoAbs directed against the cell surface enzyme stimulated T-cell proliferation; immature and mature thymocytes proliferated in response to anti-CD26/DPPIV and PMA, IL-1, or IL-2 and mature peripheral blood T cells proliferated after treatment with anti-CD26/DPPIV and submitogenic doses of either anti-CD3 or anti-CD2.\textsuperscript{165,196,197} Furthermore, transfection of a CD26 T-cell hybridoma with either of the murine or human CD26 cDNAs results in the expression of a 10- to 128-Kd protein that functions as both an ectopeptidase and an activation signal-transducing structure.\textsuperscript{179,180} CD26/DPPIV may also function as an auxiliary adhe-
sion factor as well as an ectoenzyme. Purified CD26/DPPIV protein was found to bind fibronectin,171 which was of particular interest because CD26/DPPIV and fibronectin colocalized in many tissues.171 In additional studies, CD26/DPPIV+ cell types including hepatocytes and fibroblasts also bound collagen; this adhesive interaction was inhibited by either a tripeptide enzyme substrate or anti-CD26/DPPIV antibodies.198,199 The interaction between CD26/DPPIV and fibronectin or collagen in nonhematopoietic cells prompted analyses of CD26/DPPIV-mediated adhesion in T lymphocytes.200 Human CD4+ T cells proliferated in response to a combination of anti-CD3 MoAbs and type I, III, or IV collagen; an anti-CD26/DPPIV antibody inhibited this effect.206

Recent studies also suggest an association between CD26/DPPIV and the T200 tyrosine phosphatase, CD45. CD26/DPPIV comodulates with CD45, and a CD26/DPPIV MoAb also precipitates CD45 from the T-cell surface.201 Because anti-CD26/DPPIV treatment also leads to enhanced phosphorylation of CD3 ε on tyrosine residues and increased CD4-associated P56 lck tyrosine kinase activity, it is possible that the antibody stimulates T-cell proliferation in part by decreasing CD45-mediated dephosphorylation of key substrates.201

CD73/ECTO-5’-NUCLEOTIDASE

Pattern of Expression: Enzymatic, Immunologic, and Biochemical Characterization

Enzymatic activity. Ecto-5'-nucleotidase (E5N) is a glycosyl phosphatidylinositol-linked cell surface enzyme that catalyzes the dephosphorylation of purine and pyrimidine ribonucleoside and deoxyribonucleoside monophosphates to the corresponding ribonucleosides and deoxyribonucleosides (Table 2).202,203 The 70- to 74-Kd dimer converts nontransportable 5' nucleotides into a transportable form and contributes to the extracellular hydrolysis of ATP by dephosphorylating AMP to adenosine (Table 1).202,203 The previously shown association between inherited enzyme deficiencies in purine nucleotide degradation and human immunodeficiency syndromes204,205 prompted further evaluation of E5N activity in various immunodeficiency states. Patients with adult-onset “variable” primary hypogammaglobulinemia, congenital X-linked agammaglobulinemia, and IgA deficiency were found to have reduced levels of E5N enzymatic activity in their peripheral blood lymphocytes,206-208 raising the possibility that decreased E5N levels in patients with immunodeficiencies resulted from another inborn error of purine metabolism.

Cell surface E5N expression was initially evaluated by enzymatic assays of lymphoid subpopulations.209 Early studies suggested that E5N activity was a maturation marker on both B and T cells because peripheral T cells had 10-fold higher levels of enzymatic activity than thymocytes210 and adult peripheral B cells had fivefold to sixfold higher levels of enzymatic activity than fetal spleen and cord blood B cells (Table 1).211,212 Further analyses of lymphoid subpopulations from patients with congenital agammaglobulinemia, common variable immunodeficiency, or acquired immunodeficiency syndrome (AIDS) showed that these patients had decreased numbers of E5N+ T cells.213-215 In AIDS patients, circulating CD8+ T cells with reduced E5N activity had additional phenotypic characteristics suggesting that the reduced enzymatic activity resulted from a maturation block in these cells.215 These data supported the hypothesis that E5N is a differentiation antigen on peripheral blood lymphocytes and that enzyme levels are reduced in patients with certain immunodeficiency syndromes because these patients have more immature circulating cells rather than a genetic abnormality in E5N expression.210,215

Immunologic and biochemical characterization. In studies to assess the role of E5N in lymphoid maturation, the enzyme was purified from human placenta and used to generate a highly specific goat antihuman E5N antiserum and murine MoAbs; an additional MoAb was made by immunization with a human pre-B-cell line.202,216-219 Thereafter, the enzyme was given a CD designation, CD73.202 Immunophenotypic analysis showed that CD73/E5N was expressed on subpopulations of peripheral blood T cells (eg, 20% to 30% of CD3+ cells, 10% to 20% of CD4+ cells, 50% of CD8+ cells, and 25% of CD28+ cells) and B cells (eg, 70% to 80% of CD19+ cells) (Table 1).218,219 In frozen sections of lymphoid tissues, CD73/E5N antibodies stained mantle zone B cells and follicular dendritic reticulum cells in the basal part of the light zone and the dark zone of the germinal center.202,219

Immunophenotyping studies on peripheral blood lymphocytes from patients with immunodeficiency syndromes confirmed the data derived from enzymatic assays. Specifically, patients with congenital agammaglobulinemia had reduced numbers of CD73/E5N+ peripheral T cells.214,218 Furthermore, most hypogammaglobulinemic patients also had reduced levels of CD73/E5N+ B cells, suggesting that their B cells were blocked in maturation before expression of the cell surface enzyme.218 However, some hypogammaglobulinemia patients had normal levels of B-cell CD73,220 suggesting that there may be multiple sites at which B-cell development can be blocked.

CD73/E5N was also expressed on EBV-transformed lymphoblastoid cell lines, certain myeloma cell lines, and many pre-B- and progenitor B-cell leukemias (Table 1).202 The B-cell leukemias that had the highest levels of CD73/E5N expression were CD10/NEP+ /μμ+/slg- or CD10/NEP+/ /μμ+/slg-.211 High levels of CD73 expression correlated with a poor prognosis in patients with CD10/NEP+ leukemia.222,223 Nonhematopoietic cell types, including mesenchymal cells, follicular dendritic cells, hepatocytes, and small intestinal epithelium, also expressed the enzyme (Table 1).202

CD73/E5N is an ~70- to 74-Kd dimer with interchain disulfide bridges (Fig 1).202 MoAbs to CD73/E5N precipitate a single ~70- to 74-Kd band from myeloma and pre-B cells and placenta under reducing conditions. Treatment of CD73/E5N+ cells with the phosphatidylinositol-specific phospholipase C releases most of the enzyme activity, indicating that the cell surface protein is anchored in the membrane by a glycosylphosphatidylinositol linkage. This mode of membrane attachment was confirmed by detection of inositol in highly purified preparations of the enzyme.224-226
Biologic Activity of CD73/E5N

CD73/E5N and lymphocyte function. The functional significance of CD73/E5N expression in human lymphocyte subpopulations was further evaluated by separating enzyme-positive and -negative cells and performing standard immunologic assays in the presence or absence of a specific biochemical inhibitor of CD73/E5N activity (α, β methylene adenosine 5'-diphosphate [AOPCP]; Table 2).20,227,228 or an inhibitory antisera or MoAbs.

B lymphocytes. CD73/E5N-positive and -negative human B-cell subpopulations synthesized equivalent quantities of IgM in response to a T-cell-dependent or T-cell-independent stimulus; however, CD73/E5N+ lymphocytes synthesized 8- to 26-fold more IgG per cell than B cells lacking this activity.227 These data provided further evidence that the enzyme is a marker for the functional maturation of human B cells (Table 1) and supported the hypothesis that CD73/E5N deficiency in hypogammaglobulinemic patients results from a block in B-cell development.227

T lymphocytes. In peripheral T cells, the inhibition of CD73/E5N with a specific inhibitor (AOPCP) selectively suppressed the cytotoxic responses of alloreactive T cells.20 In additional studies, CD73/E5N+ peripheral T cells proliferated when they were cocultured with submimetic doses of PMA and either goat antisera or murine MoAbs to CD73/E5N.229 Pretreatment of the T cells with phospholipase C removed the majority of CD73/E5N from the cell surface and inhibited the ability of the cells to proliferate in response to the CD73/E5N antibodies and PMA.229 Immunized CD73/E5N MoAbs also increased T-cell activation via the CD3 or CD2 pathway,230 although it is not clear whether the CD73/E5N antibodies enhanced T-cell proliferation by modulating enzymatic activity or by affecting the enzyme's glycosphingolipid anchor. In recent studies, T cells from transgenic animals expressing a glycosphingolipid-anchored form of a nonmitogenic protein proliferated in response to antibodies directed against that protein, prompting speculation that glycosphingolipid membrane anchors of phosphoinositols-linked cell surface proteins like CD73/E5N may function directly in transmembrane signaling.231

CD73/E5N in nonhematopoietic cells. Like CD10/NEP and CD13/APN, E5N has also been implicated in the regulation of neutrophil inflammatory responses (Table 1).18 In the intestine, inflammation results in the development of crypt abscesses in which neutrophils are in physical contact with the apical intestinal epithelial membrane.18 Neutrophils release 5'AMP that is converted by intestinal brush border CD73/E5N to adenosine; the newly liberated adenosine triggers adenosine receptors on intestinal epithelial cells and results in a secretory diarrhea.18

Recent studies suggest that CD73/E5N may also participate in cell-cell or cell-matrix interactions.203

Molecular Structure

The molecular structures of the human and rat CD73/E5N cDNAs have recently been elucidated and the predicted amino acid sequences of the respective CD73/E5N cDNAs show 89.4% identity.13,222 The human CD73/E5N cDNA encodes a 574 residue polypeptide with a calculated size of 63.4 Kd (Fig 1).13 The NH2-terminal 26 residues comprise a signal peptide that is followed by the NH2-terminal sequence of the purified protein (Fig 1).13 The mature CD73/E5N protein lacks the predicted COOH-terminal extension (aa524 through aa548), which has been replaced by glycosphingolipid functioning as the membrane anchor (Fig 1).13 The cDNA contains four potential N-linked glycosylation sites, suggesting that glycosylation may account for the larger size of the cell surface protein (71 Kd).13 CD73/E5N has been reported to bind zinc and to contain three cysteine and histidine residues that may function as potential Zn2+-binding sites (Fig 1).233,244 In contrast to CD10/NEP, CD13/APN, and BP-1/6C3/APA, CD73/E5N does not contain a characteristic zinc-binding pentapeptide sequence.

SUMMARY AND FUTURE DIRECTIONS

The identification of five membrane-associated enzymes as hematopoietic differentiation antigens underscores the potential importance of cell surface enzymes in the biology of normal and malignant hematopoietic cells. Studies to date suggest that certain membrane-associated enzymes (CD10/NEP, CD13/APN, BP-1/6C3/APA, and CD26/DPPIV) may function as part of a negative regulatory loop, limiting cellular responses to a given peptide or protein substrate. These enzymes, which are coexpressed in a variety of epithelial cells and expressed by overlapping subsets of hematopoietic cells, may also cooperate to limit some of the same physiologic processes and hydrolyze many common peptide substrates. For example, CD10/NEP and CD13/APN cooperate to limit neutrophil-mediated inflammatory responses triggered by peptides that are substrates for both enzymes (eg, fMLP and met-enkephalin). The overlapping patterns of expression of CD10/NEP and BP1/6C3/APA prompt speculation that these enzymes may have complimentary roles in the regulation of stromal cell-dependent B lymphopoiesis. That additional peptides such as substance P are substrates for both CD10/NEP and CD26/DPPIV suggests that these enzymes may also cooperate in still-undefined processes. The fact that "neuropeptides" such as substance P and met-enkephalin are substrates for enzymes expressed by a restricted population of hematopoietic cells has stimulated further investigation into the potential roles for these peptides in immune function and inflammation.

Preliminary findings on the biologic functions of these ectoenzymes have already begun to have therapeutic applications. In certain settings, enzyme inhibitors are being used to potentiate the biologic activity of endogenous peptide hormones. For example, oral inhibitors of CD10/NEP, which are being promoted as antidiarrheal medications,235,236 are also under investigation as antihypertensive agents. In other settings, the recombinant enzymes are being exploited to reduce local peptide concentrations, as illustrated by trials of aerosolized and systemic versions of recombinant CD10/NEP in reactive airways disease. The growth-regulatory effects of CD10/NEP on normal and malignant bronchial epithelial cells and normal lymphoid pro-
genitors suggest additional uses for this recombinant enzyme.

Characterization of the relevant peptide substrates for these enzymes may also have important clinical implications. For example, the identification of potential enzyme cleavage sites in endogenous enkephalins and to the development of more effective synthetic analgesics and GRH analogs. The characterization of bioactive substrates for membrane-associated enzymes expressed by hematopoietic cells is likely to be of similar benefit.

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REFERENCES


52. Tran-Paterson R, Boileau G, Gueguere V, Letarte M: Comparative levels of CALLA neutral endopeptidase on normal granulocytes, leukemic cells, and transfected COS-1 cells. Blood 76:775, 1990


85. Shipp MA, Stefano GB, Switzer SN, Griffin JD, Reinherz EL: CD10 (CALLA)/neutral endopeptidase 24.11 modulates inflammatory peptide-induced changes in neutrophil morphology, migration and adhesion proteins and is itself regulated by neutrophil activation. Blood 78:1834, 1991


131. Holland EC, Leung JO, Drickeamer K: Rat liver asialoglycoprotein receptor lacks a cleavable NH2-terminal signal sequence. Proc Natl Acad Sci USA 81:7338, 1984


138. Shapiro LH, Ashmun RA, Roberts WM, Look AT: Separate promoters control transcription of the human aminopeptidase...
152. Ramakrishnan L, Wu Q, Yue A, Cooper MD, Rosenberg N: BP-1/6C3 expression defines a differentiation stage of transformed pre-B cells and is not related to malignant potential. J Immunol 145:1603, 1990
162. Wang J, Cooper MD: A histidine residue in the zinc-binding motif of aminopeptidase A is critical for enzymatic activity. Proc Natl Acad Sci USA (in press)


195. Flinttke GR, Munoz E, Huber BT, Plaut AG, Kettner CA, Buchovcin WW: Inhibition of dipeptidyl aminopeptidase IV (DP-IV) by Xaa-boroPro dipeptides and use of these inhibitors to examine the role of DP-IV in T-cell function. Proc Natl Acad Sci USA 88:1536, 1991


198. Bovais B: A collagen-binding glycoprotein on the surface of mouse fibroblasts is identified as dipeptidyl peptidase IV. Biochem J 252:723, 1988


211. Rowe M, DeGast GC, Platts-Mills TAE, Asherson GL,


Hematopoietic differentiation antigens that are membrane-associated enzymes: cutting is the key!

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