Brief Review

VAP-1 and CD73, Endothelial Cell Surface Enzymes in Leukocyte Extravasation

Sirpa Jalkanen, Marko Salmi

Abstract—Leukocyte extravasation from the blood into tissues is crucial for normal immune surveillance and in inflammation. Traditionally molecules belonging to selectin, chemokine, integrin, and immunoglobulin super families are thought to mediate the multiple adhesive and activation events needed for a successful emigration cascade. Recently, emerging evidence suggests that enzymes expressed on the surface of endothelial cells and leukocytes also contribute to the leukocyte extravasation cascade. Here we briefly review the role of vascular adhesion protein-1 (VAP-1) and CD73, 2 cell surface enzymes, in leukocyte migration form the blood into the tissues. Importantly, specific enzyme inhibitors, gene-deficient mice, and recombinant enzymes have recently unambiguously shown that the catalytic activity of these enzymes regulates the leukocyte traffic. The concept of enzymatic regulation of leukocyte extravasation provides new insight into the multi-step adhesion cascade and opens new possibilities for inhibiting inappropriate inflammatory reaction through the use of small molecule enzyme inhibitors. (Arterioscler Thromb Vasc Biol. 2008;28:000-000.)

Key Words: leukocyte traffic ■ migration ■ endothelium ■ inflammation ■ ectoenzymes

The immune defense is dependent on proper migration of leukocytes between the blood and tissues. Under normal conditions lymphocytes leave the blood in venules of secondary lymphoid tissues, such as peripheral lymph nodes and Peyer patches and patrol the tissue for the presence of their cognate antigens.1,2 On an inflammatory reaction, activated lymphocytes together with other subclasses of leukocytes recognize the inflamed endothelium at the site of insult and migrate into peripheral tissues. Both on leukocytes and endothelial cells, a myriad of molecules ensure proper execution of the complex extravasation cascade. Thus, selectins and their mucin-like ligands mediate the early tethering and rolling of the leukocyte on the endothelial wall. Subsequent triggering of chemokine receptors through chemokines then activates leukocyte integrins and allows firm adhesion of the cell.3–5 Thereafter, integrins with other molecules accomplish the final transmigration step, which may occur via endothelial cell junctions or through a transcytotic route.6,7

During the past few years, a number of additional molecules have been suggested to be involved in the extravasation cascade. Among these are several enzymes, which are expressed on the surface of leukocytes and endothelial cells and have their enzymatically active domains outside of the cell membrane (ecto-enzymes).8 Some ecto-enzymes can physically act as adhesion molecules but most contribute to the adhesion cascade by regulating cell recruitment through their catalytic activity.

Ecto-enzymes are a large and diverse class of molecules.9,10 Often a single catalytic activity can be conferred by several enzymes, and conversely, a given enzyme may display more than one enzymatic activity. Therefore the rapid advances in molecular identification of the genes responsible for a given catalytic activity and subsequent generation of gene-deficient mice have only recently allowed detailed analyses of these molecules. In terms of leukocyte extravasation most evidence is available for the role of nucleotidases and related enzymes (such as CD39 and CD73), ADP-ribosyl cyclases (CD38 and CD157), ADP-ribosyl transferases (ART-2), peptidases and proteases (CD10, CD13, CD26, CD156b), and oxidases (VAP-1, NADPH; Figure 1).8

In this review we will focus on the role of VAP-1 and CD73 in leukocyte extravasation. They serve as a good example of two totally different ecto-enzymes, which are, nevertheless, involved in the regulation of the same biological processes in the blood vasculature.

VAP-1: A Semicarbazide-Sensitive Amine Oxidase

VAP-1 was originally discovered using an antibody that inhibited lymphocyte binding to high endothelial venules in frozen section adhesion assays.11 Cloning of the antigen revealed that it belongs to semicarbazide-sensitive amine oxidases (SSAO).12 All SSAO contain copper as a cofactor and most of them topaquinone, a unique posttranslational modification of tyrosine, at their catalytically active site.13,14 In human there are altogether four copper-containing amine oxidase (AOC) genes, one of which is a pseudogene.15 VAP-1 gene is also known as HPAO (human placental amine oxidase) and, officially, as AOC3.12,16

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All SSAO oxidatively deaminate amines in a reaction that results in the generation of the corresponding aldehyde, ammonium, and hydrogen peroxide (Figure 2, center).\textsuperscript{13,14} The catalytic reaction proceeds in two steps. During the reductive step, a primary amine interacts with the topaquino of VAP-1 leading to the formation of a transient covalent interaction (a Schiff base) between the enzyme and the substrate before an aldehyde is formed. During the oxidative half-reaction, the enzyme is reoxidized and hydrogen peroxide and ammonium are released. SSAO reaction can be inhibited by carbonyl reactive compounds such as semicarbazide and hydroxylamine. Recently many more small molecule inhibitors with improved properties have been developed.\textsuperscript{17–19}

Solving of the crystal structure of VAP-1 dimer revealed that it is a heart-shaped dimer consisting of 4 domains per monomer.\textsuperscript{20,21} On the top of the molecule there are multiple O- and N-linked oligosaccharides, which may be important for its adhesive function.\textsuperscript{22} There is also a groove on the surface of VAP-1 which opens into narrow and deep substrate channel leading to the catalytic center of the molecule buried relatively deep inside each monomer. The amino acids guarding the entry into the channel differ between different SSAO and are likely to serve a key role in conferring the substrate specificity of these molecules.

The nature of physiological substrates for VAP-1 still remains largely unknown. However, VAP-1 can deaminate methylamine and aminoacetone, which are soluble amines produced during the intermediary metabolism in humans.\textsuperscript{23} There is also evidence that suitable free amino groups in proteins or aminosugars can interact with the catalytic center of VAP-1.\textsuperscript{24,25}

**VAP-1 Is Inflammation-Inducible in Endothelial Cells**

VAP-1 is expressed in endothelial cells in most organs and tissues, and adipocytes and smooth muscle cells are also uniformly VAP-1 positive.\textsuperscript{26} In endothelial cells VAP-1 is present in intracellular vesicles under resting conditions. Notably, the endothelial expression of VAP-1 is quite different between humans and mice in certain organs such as liver and kidney.\textsuperscript{27} On inflammation VAP-1 is rapidly translocated onto the luminal surface of endothelial cells both in animals and in humans.\textsuperscript{28,29} However, the individual inflammatory
mediators involved in the up-regulation and translocation of VAP-1 in endothelial cells remain unknown.30 VAP-1 is also present in a soluble form in plasma.31 This form is most likely formed by metalloprotease dependent shedding of the extracellular domains of VAP-1 in many tissues and cell types.32–34 In fat cells tumor necrosis factor (TNF)-α can induce the shedding, and in human plasma at least insulin can regulate the level of soluble VAP-1. Interestingly, soluble SSAO activity is increased only in a few inflammatory diseases such as type 1 and type 2 diabetes and certain liver disorders.35–38

VAP-1 and the Multistep Adhesion Cascade in Vitro
Anti–VAP-1 mAbs inhibit leukocyte binding to vessels in multiple in vitro assays. VAP-1–dependent adhesion of lymphocytes to high endothelial venules is seen in many lymphoid tissues.11,26 Because of the absence of selectins in liver VAP-1 appears to play a particularly prominent role in this organ.39 Anti–VAP-1 mAbs also reduce leukocyte binding to inflamed vessels of frozen sections in many nonlymphoid organs.40–42 Studies using defined laminar shear have shown that the anti–VAP-1 antibodies interfere with leukocyte rolling on and transmigration through VAP-1 positive endothelial monolayers.17,24,43 Small molecule SSAO enzyme inhibitors also block leukocyte-endothelial cell interactions in vitro. In real-time assays, they inhibit the rolling, firm adhesion, and transmigration of leukocytes.17,24,43 Moreover, catalytically inactive VAP-1 mutants are unable to support leukocyte extravasation.17 Importantly, the anti–VAP-1 antibodies do not inhibit SSAO activity.17,24,44 The anti–VAP-1 antibodies and SSAO inhibitors also do not show additive blocking effects in leukocyte binding. Therefore, current models propose that VAP-1 serves a dual adhesive function.8 Firstly, it can function as an adhesion molecule by itself through the anti–VAP-1 mAb defined surface epitopes. Secondly, the enzymatic activity of VAP-1 also contributes to leukocyte-endothelial binding. This may be mediated through the covalent binding of the enzyme (VAP-1 on endothelial surface) and its substrate (presumably expressed on the surface of a leukocyte) during the catalytic reaction. In addition, the biologically active products derived from the SSAO reaction may have signaling effects in the local microenvironment. In fact, very recent data indicate that VAP-1 activity can induce expression of E- and P-selectins.

### Table 1. Efficacy of Targeting VAP-1 and CD73 by Antibodies, Inhibitors, and Recombinant Proteins in In Vivo Models

<table>
<thead>
<tr>
<th>Disease</th>
<th>Effect</th>
<th>Comment</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peritonitis</td>
<td>50–70% reduction in emigrated leukocytes</td>
<td>IL-1 + PP-induced</td>
<td>Tohka et al49, Merinen et al47</td>
</tr>
<tr>
<td>Hepatitis</td>
<td>ALT level decreased by 90%</td>
<td>ConA-induced</td>
<td>Bonder et al44</td>
</tr>
<tr>
<td>Insulitis</td>
<td>Incidence of diabetes decreased by 50%</td>
<td>NOD-mice</td>
<td>Merinen et al47</td>
</tr>
<tr>
<td>Skin inflammation</td>
<td>Monocyte infiltration decreased by 60%</td>
<td>CCL21 in air pouch</td>
<td>Merinen et al47</td>
</tr>
<tr>
<td>Rejection</td>
<td>60% reduction in infiltrating cells</td>
<td>Liver allograft</td>
<td>Martelius et al48</td>
</tr>
<tr>
<td>Arthritis</td>
<td>Clinical score decreased by 40–80%</td>
<td>Adjuvant or mAb-induced</td>
<td>Marttila-Ichihara et al53</td>
</tr>
<tr>
<td>Skin inflammation</td>
<td>Leukocyte infiltration decreased by 50%</td>
<td>Carrageenan-induced</td>
<td>Koskinen et al17</td>
</tr>
<tr>
<td>Lung inflammation</td>
<td>BAL leukocyte counts decreased by 40%</td>
<td>LPS-induced</td>
<td>Kinemuchi et al43</td>
</tr>
<tr>
<td>Collitis</td>
<td>Survival increased by 50%</td>
<td>Oxazolone-induced</td>
<td>Saltar-Cid et al18</td>
</tr>
<tr>
<td>EAE</td>
<td>Clinical score decreases by &gt;50%</td>
<td>PLP-induction, treatment started at peak of disease</td>
<td>O’Rourke et al50</td>
</tr>
<tr>
<td>Endotoxemia</td>
<td>Mortality reduced by 50%</td>
<td>LPS-induced</td>
<td>Kinemuchi et al43</td>
</tr>
<tr>
<td>IR-injury</td>
<td>Neurological ability increased by 80%</td>
<td>Stroke</td>
<td>Xu et al51</td>
</tr>
<tr>
<td>CD73 enzyme inhibitor treatment</td>
<td>Inhibition of the positive effect of IP</td>
<td>Heart, kidney</td>
<td>Eckle et al79, Grenz et al80</td>
</tr>
<tr>
<td>Ischemic preconditioning (IP)</td>
<td>Inhibition of positive effects of statins</td>
<td>Heart</td>
<td>Sanada et al82</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>Increase in epithelial permeability</td>
<td>Intestine</td>
<td>Synnestvedt et al84</td>
</tr>
<tr>
<td>Ischemic preconditioning</td>
<td>Restoration of the positive effect in CD73 mice</td>
<td>Heart, kidney</td>
<td>Eckle et al79, Grenz et al80</td>
</tr>
<tr>
<td>Soluble CD73 treatment</td>
<td>Acute lung injury</td>
<td>Reversion of the symptoms in CD73</td>
<td>Eckle et al73, Thompson et al85</td>
</tr>
<tr>
<td>Vascular leakage</td>
<td>Reversed in CD73</td>
<td>Ventilator-induced</td>
<td>Eckle et al73, Thompson et al85</td>
</tr>
</tbody>
</table>

ALT indicates alanine aminotransferase; BAL, bronchoalveolar lavage; ConA, concanavalin A; EAE, experimental autoimmune encephalomyelitis; NOD, nonobese diabetic; IP, ischemic preconditioning; IR, ischemia reperfusion; LPS, lipopolysaccharide; PLP, proteolipid protein; PP, proteose peptone.
intercellular adhesion molecule-1 (ICAM-1), and CXCL8 in human endothelial cells and that this activity leads to increased lymphocyte binding to endothelial cells in vitro (Figure 2).45,46 Induction of E- and P-selectin is most likely mediated by hydrogen peroxide produced during the VAP-1 catalyzed enzyme reaction.45

**VAP-1 Is Needed for Leukocyte Migration In Vivo**

Anti–VAP-1 antibodies impair leukocyte extravasation in many animal models (Table 1). They have been reported to partially inhibit migration of lymphocytes, granulocytes, or macrophages into inflamed peritoneum, liver, and pancreas.44,47–50 Moreover, SSAO inhibitors also alleviate inflammation in multiple models including colitis, skin inflammation, ischemia-reperfusion injury, sepsis, experimental allergic encephalomyelitis, arthritis, and lung inflammation (Table 1).17,18,51,52 It is intriguing that an SSAO inhibitor has been very effective in ameliorating clinical disease activity in EAE not only in a pretreatment model but also when the treatment is first started at the peak of disease or at remission.50 Both the anti–VAP-1 antibodies and SSAO inhibitors increase leukocyte rolling velocity and decrease the number of firmly adherent and transmigrated leukocytes in inflamed vascular beds in vivo.44,49,53

The fact that both anti–VAP-1 antibodies and SSAO enzyme inhibitors diminish leukocyte traffic into sites of inflammation in vivo is in line with the proposed dualistic function of VAP-1.4 Thus, the antibody binding masks surface epitopes of VAP-1 that are needed for leukocyte binding. The enzyme inhibitors, on the other hand, can prevent the formation of the covalent bond that is formed during the catalytic reaction between the VAP-1 enzyme and its substrate(s) on leukocytes. Moreover, inhibition of the enzymatic activity of VAP-1 also prevents the formation of bioactive signaling molecules and thus alters the inflammatory cascades in the local microenvironment. The reduced production of VAP-1 derived hydrogen peroxide, for instance, reduces the expression of E- and P-selectins and thereby attenuates the inflammatory reaction.46,54 Although dissection of the relative importance of adhesive and enzymatic functions of VAP-1 in vivo remains to be determined, both functional modalities apparently contribute to leukocyte influx into sites of inflammation.

Leukocyte traffic is also compromised in VAP-1–deficient mice (Table 2). These animals recapitulate the effects seen when VAP-1 is blocked by antibodies or inhibitors, ie, in VAP-1–deficient mice leukocytes roll faster, and there are less firmly adhered and transmigrated leukocytes than in wild-type control mice.53 Moreover, granulocyte recruitment to inflamed peritoneum and joints is diminished in the absence of VAP-1.52,53 Under normal conditions VAP-1–deficient mice are apparently healthy and only show minor defects in constitutive lymphocyte homing. However, in young VAP-1–deficient mice the lymphocyte number in Peyer patches is diminished by about 50%.54 Moreover, after oral immunization the immune responses of both T- and B-cells are attenuated in the absence of VAP-1. Here again the modulation of the local microenvironment at the site of inflammation by the catalytic activity of VAP-1 may contribute to the observed phenotype in addition to direct VAP-1–dependent effects on lymphocyte homing.

**SSAO Activity Can Contribute to the Pathogenesis of Vasculopathies**

VAP-1 transgenic mice overexpressing human VAP-1 on endothelium show increased formation of advanced glycation end products when they ingest additional VAP-1 substrates.55 These mice also suffer from impaired glucose tolerance and show glomerulosclerosis (an atherosclerotic manifestation) later in their life. It therefore appears that although VAP-1 activity has short-term insulin-like effects in fat cells,56 exaggerated, long-lasting SSAO activity can lead to vascular complications. According to the prevailing hypothesis, increased glucose levels in conjunction with VAP-1–derived aldehydes can lead to accelerated nonenzymatic glycation of proteins.57 The oxidation of lipids through VAP-1–generated hydrogen peroxide can also contribute to the vascular damage. In addition, it has been suggested that SSAO activity may contribute to the pathogenesis of amyloid formation and hypertension.34,58

In humans diminished insulin secretion appears to increase the concentration of soluble VAP-1 in plasma. Moreover, in diabetic patients higher levels of soluble VAP-1 correlate positively with micro- and macroangiopathic complications.35–38 Hence, VAP-1 may be involved in the pathogenesis of vasculopathies via two distinct processes. Firstly, it may increase leukocyte recruitment into vascular wall, and secondly it may induce vascular dysfunction by enhancing the generation of AGE, causing direct oxidative damage and possibly also by increasing amyloid aggregation and blood pressure (Figure 2).

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**Table 2. VAP-1 and CD73-Deficient Mice and Leukocyte Extravasation**

<table>
<thead>
<tr>
<th></th>
<th>VAP-1&lt;sup&gt;−/−&lt;/sup&gt;</th>
<th>CD73&lt;sup&gt;−/−&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gross morphology and breeding</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Leukocyte counts</td>
<td>Reduced PP cells in young mice</td>
<td>Normal</td>
</tr>
<tr>
<td>Vascular barrier function</td>
<td>Normal</td>
<td>Leaky</td>
</tr>
<tr>
<td>Adhesion cascade</td>
<td>Impaired</td>
<td>Abnormal</td>
</tr>
<tr>
<td></td>
<td>-faster rolling, decreased</td>
<td>firm adhesion and</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-transmigration</td>
</tr>
<tr>
<td>Lymphocyte homing</td>
<td>Slightly impaired in PP and spleen</td>
<td>Not determined</td>
</tr>
<tr>
<td>Inflammatory response</td>
<td>Decreased</td>
<td>Increased</td>
</tr>
<tr>
<td></td>
<td>-TNF-α-induced peritonitis</td>
<td>-ischemia reperfusion</td>
</tr>
<tr>
<td></td>
<td>-reduced CAIA</td>
<td>-acute lung injury</td>
</tr>
<tr>
<td></td>
<td>-oral OVA immunization</td>
<td></td>
</tr>
</tbody>
</table>

PP indicates Peyer patch; CAIA, anti-collagen antibody induced arthritis; OVA, ovalbumin.
CD73 is a key player in extracellular ATP metabolism and regulates leukocyte trafficking. CD73 dephosphorylates AMP to adenosine (Ado). Lymphocyte binding to endothelium inhibits enzymatic activity of CD73. Adenosine level drops and the remaining adenosine is degraded to inosine (Ino) by adenosine deaminase (Ada) leading to increased transmigration of lymphocytes.

Figure 3. CD73 is a key player in extracellular ATP metabolism and regulates leukocyte trafficking. CD73 dephosphorylates AMP to adenosine (Ado). Lymphocyte binding to endothelium inhibits enzymatic activity of CD73. Adenosine level drops and the remaining adenosine is degraded to inosine (Ino) by adenosine deaminase (Ada) leading to increased transmigration of lymphocytes.

**CD73 Is A Nucleotidase that Produces Antiinflammatory Adenosine**

CD73 (also known as 5’-ecto-nucleotidase) is a key regulator of the proinflammatory and antiinflammatory balance of purines in the extracellular milieu. ATP is physiologically released into the extracellular space through regulated transport, and at the sites of injury additional ATP becomes available from the lytic cells. On resting vascular endothelium extracellular ATP is dephosphorylated to ADP and further to AMP by CD39. CD73 dephosphorylates AMP to adenosine. ATP that binds to purino-receptors of the P2X and P2Y families, is proinflammatory, whereas adenosine that binds to P1 type of purino-receptors, is antiinflammatory and regulates several molecules and mechanisms involved in leukocyte extravasation. The extracellular purinergic catabolism with the other enzymes involved is depicted in Figure 3.

Adenosine produced by CD73 exerts its function via 4 receptors: A1, A2A, A2B, and A3. Activation of the adenosine receptors A2A and A2B on neutrophil surface leads to an antiadhesive signal that diminishes binding of neutrophils to microvascular endothelial cells. Treatment of neutrophils by A2A agonists inhibits shedding of L-selectin and reduces upregulation of beta 2 integrins. Both changes are known to impede leukocyte trafficking into inflammatory sites. Moreover, adenosine decreases cytokine release from the vasculature and leukocytes and thus inhibits leukocyte extravasation and immune reactions. It also increases endothelial cell barrier function by phosphorylation of tight junction-associated proteins such as vasodilator-stimulated phosphoprotein.

**Inducible CD73 Is Present Both on Leukocytes and Endothelial Cells**

CD73 is a glycosylphosphatidylinositol (GPI) linked 70-kDa glycoprotein that is abundantly expressed on vascular endothelial cells. 5% to 15% of peripheral blood lymphocytes also express CD73, whereas granulocytes and monocytes are completely negative. Among lymphocytes regulatory T cells and primed uncommitted CD4-positive T cells are CD73 positive. Catalytic activity of CD73 accounts for the immunosuppressive functions of these cells because the adenosine produced suppresses proliferation and cytokine secretion of T helper 1 and 2 effector cells.63,64

Although lymphocyte and endothelial cells express structurally similar CD73 molecules, the expression of this ectoenzyme is differently regulated in these two types of cells. Engagement of lymphocyte CD73 triggers a rapid shedding of surface CD73 and leads to clustering of CD11a/CD18 integrin and thereby enhances the integrin-mediated binding of lymphocytes to endothelium.66,67 This phenomenon is most likely independent of the enzymatic activity of CD73. In contrast, engagement of endothelial CD73 does not result in shedding of the molecule. Moreover, interferon (INF) alpha upregulates CD73 expression on endothelial cells. The induction takes place both in vitro and in human patients in vivo and correlates positively with increased enzymatic activity and adenosine production.68 On lymphocytes, in contrast, INF alpha has no effect on the levels of CD73. Finally, hypoxic environment may also induce expression of CD73. This is mediated by hypoxia-inducible factor-1 (HIF-1) because CD73 has a HIF-1 alpha responsive element in its promoter region.69

**Lymphocyte-Endothelial Interaction Regulates CD73 Activity**

When lymphocytes bind to the endothelium the enzymatic activity of CD73 is inhibited. The mechanism of this enzymatic inactivation remains open. However lymphocyte binding to endothelium masks endothelial CD73 without any covalent modification of this ectoenzyme or without its sequestration within plasma membrane subdomains.70 Binding results in the reversal of the enzymatic cascade regulating extracellular purine levels toward an ATP generating pathway.71 The residual adenosine left in the microenvironment is rapidly degraded by adenosine deaminase, which is a soluble enzyme bound to the lymphocyte surface via CD26 (a simplified scheme is presented in Figure 3). Decreased adenosine levels on lymphocyte contact with endothelial cells is thought to increase endothelial cell permeability and enhance leukocyte transmigration.

**CD73-Deficient Mice Display Exacerbated Inflammatory Reactions**

The importance of CD73-dependent adenosine production has been demonstrated by gene-deficient mice, CD73 inhibitor, and administration of soluble enzyme (Tables 1 and 2). CD73-deficient mice show increased neutrophil binding to endothelium in an in vivo ischemia-reperfusion model.72 Mechanical damage to the lungs of CD73-deficient mice increases the number of neutrophils in the lung tissues.73 CD73 deficiency also leads to increased constitutive activation of nuclear factor kappa B, upregulation of vascular cell adhesion molecule (VCAM)-1 expression, and enhanced monocyte adhesion to carotid arteries that is VLA-4-VCAM-1-mediated.72,74
Moreover, mice lacking CD73 are prone to more severe vascular leakage than wild-type animals in hypoxic conditions, and this can be reversed by administration of soluble CD73. Prominent leakage can especially be seen in the lungs simultaneously with increased neutrophil infiltration around larger pulmonary vessels.

**Ectoenzymes as Dynamic Regulators of Leukocyte Extravasation**

Although the involvement of ectoenzymes in leukocyte extravasation has been appreciated relatively recently, they have proven to be essential for dynamic regulation of leukocyte endothelial cell interactions. Ectoenzymes can control the inflammatory status of the microenvironment in different ways. For example, VAP-1 can function as an adhesion that directly mediates leukocyte binding through the antibody defined surface epitopes. On the other hand, the catalytic activity of VAP-1 is involved in leukocyte emigration in two ways. The formation of a transient Schiff base between VAP-1 on endothelial cells and its ligand(s) on leukocytes can physically bring the two cell types together. Secondly, the products of catalytic activity of VAP-1 function like signaling molecules and up-regulate other molecules involved in the extravasation cascade. In the case of CD73, engagement of this ecto-enzyme on the lymphocyte surface increases integrin-mediated adhesiveness to endothelium. Again there is also another mode of action which is dependent on the catalytic activity of the molecule inasmuch the adenosine produced by CD73 increases endothelial cell barrier function.

Enzymatic reactions are extremely fast and well suited for signal amplification. Moreover, they can be regulated at multiple levels, because substrate availability, natural inhibitors, and further metabolism of the end-products are key control mechanisms. In the case of CD73, for instance, availability of the substrate (AMP) partially controls the amount of adenosine produced, and other purines like ATP and ADP can inhibit the function of CD73. Therefore, ecto-enzymes are ideally suited for rapid tuning of the leukocyte extravasation cascade.

**Ectoenzymes as Drug Development Targets**

Elucidation of the role of ectoenzymes in leukocyte extravasation has opened new possibilities to design antiinflammatory drugs. As the catalytic centers of enzymes are often easily accessible to analogues of known substrates and inhibitors and the crystal structures of many enzymes are resolved, ecto-enzymes are optimal targets for rational drug design. Furthermore, small molecule enzyme inhibitors can often be administered orally. This is a clear benefit in comparison to antibodies against other adhesion molecules (eg, natalizumab and efalizumab), which need to be injected intravenously or subcutaneously.

Silencing of VAP-1 appears to be desirable for antiinflammatory purposes. Several animal models with antibodies and inhibitors have demonstrated the potential of VAP-1 as a drug target (see above). Importantly, the first clinical Phase I/IIa trials with a prototype murine monoclonal antibody against human VAP-1 have shown that VAP-1 can be targeted safely. Further clinical trials with a humanized anti-VAP-1 antibody will allow evaluation of the efficacy and possible indications for this potential antiinflammatory agent.

In contrast to VAP-1, the catalytic activity of CD73 appears to be beneficial in fight against inflammation. Therefore, the primary therapeutic option with CD73 is to increase its expression and enzymatic activity. In fact, adenosine has already been shown to have beneficial effects in controlling inappropriate inflammation, but its administration is not feasible because of its short half-life in the body (>10 seconds). However, animal models have demonstrated that soluble, exogenously administered CD73 has protective effects for example in acute lung injury and renal and myocardial ischemia (Table 1). On the other hand, the expression and activity of endogenous CD73 can be upregulated by interferons alpha and beta. In theory, this provides an attractive way to maintain and enhance adenosine production and consequently to improve endothelial barrier function. In fact, INF beta apparently protects mice from acute lung injury in a CD73-dependent manner in vivo (our unpublished results). Also statins increase expression and activity of CD73 on endothelial cells by inhibiting its endocytosis in a Rho-GTPase-dependent manner. Statin-mediated CD73 induction leads to limited infarct size after coronary occlusion in a canine model of myocardial infarction. Thus, statins may also provide an applicable therapeutic option to target CD73. As interferons and statins are approved drugs and in wide clinical use for other diseases, they may also be potential drug candidates without serious side effects to treat diseases manifested with acute vascular leakage and leukocyte extravasation.

**Concluding Remarks**

Although we have concentrated in this review only on two ectoenzymes, VAP-1 and CD73, they serve as good examples about the multifunctional nature of ectoenzymes. Moreover, they provide examples as to how ectoenzymes can be targeted, when leukocyte trafficking needs to be manipulated. The number of ectoenzymes participating in leukocyte trafficking is increasingly expanding and their mechanistic role in this process is being better understood. It has become clear that they act in concert with the traditional adhesion molecules to ensure proper execution of the leukocyte extravasation cascade. Hence, future research on ecto-enzymes is likely to further alter the classic paradigm of multistep adhesion cascade and offer conceptually new possibilities in the development of antiadhesive therapies.

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**References**


suppresses effector CD4 T cells by converting 5


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