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Homing-associated molecules CD73 and VAP-1 as targets to prevent harmful inflammations and cancer spread

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Abstract

Homing-associated molecules are of fundamental importance for proper functioning of our immune system as they direct the cells to sites of inflammation to create an immune response. However, they are also responsible for harmful cell trafficking, which takes place in acute and chronic inflammations as well as in tumor progression and metastatic spread of cancer. Therefore, these molecules are potential targets for developing drugs to prevent harmful inflammation and metastases of cancer. In this review, we will discuss about the most recent advances in studies elucidating the role of two homing-associated ecto-enzymes in physiological and pathological cell trafficking and their use as drug targets.

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1 Introduction

experimental autoimmune encephalomyelitis

HEV

high endothelial venules

MDSC

myeloid derived suppressor cells

Homing-associated molecules are important in targeting leukocytes and other cells into specific positions within the body. Molecules, which guide leukocyte trafficking, are of paramount importance in the function of normal innate and acquired immunity [1]. On the other hand, inappropriate migration of leukocytes to various target organs is a key pathogenetic factor in a wide spectrum of inflammatory diseases [2]. These include traditional autoimmune diseases such as multiple sclerosis or rheumatoid arthritis, as well as diseases like atherosclerosis, ischemia-reperfusion injury, obesity and Alzheimer's disease.

In addition to leukocytes, migratory nature is a hallmark of metastatic cancer cells. In fact, the malignant cells share with the leukocytes the routes of migration within the body (blood and lymphatic vasculature) and they also share many common molecular mechanisms by which the cellular targeting is accomplished [3]. Moreover, the active roles of different pro- and anti-tumorigenic leukocyte subclasses during the tumor progression have become increasingly evident during the past few years [4]. In particular, tumors seem to be able to evade the anti-tumor immunity by inducing and attracting various immunosuppressive leukocyte subtypes, such as regulatory T cells, type 2 macrophages and myeloid-derived suppressor cells, into the growing tumor.

The leukocyte trafficking molecules allow leukocytes to patrol throughout the whole body [1, 2]. Typically, the newly formed mononuclear and polymorphonuclear leukocytes circulate freely in the blood vasculature. At specific locations, such as in normal high endothelial venules (HEV) in lymphoid organs, or in inflamed venules in almost any organ they are able to leave the blood and extravasate into

the tissue. The extravasation cascade starts by tethering and rolling of the leukocyte on the endothelial cells. If followed by suitable activation stimuli, the leukocyte can then firmly adhere to the endothelial cell, and finally transmigrate through the inter-endothelial junctions, or through the endothelial cell, into the tissue to exert its effector functions.

The distinct steps of the extravasation cascade are typically mediated by different receptor–ligand pairs on the two opposing cell types. Thus, selectins and their oligosaccharide-based ligands presented on sialomucin-like proteins are key mediators of the transient first contacts, whereas chemokines and their receptors are heavily involved at the activation step [5, 6]. Thereafter, integrins on the leukocytes and their ligands belonging to the immunoglobulin super-family on the endothelium, together with certain homotypically interacting molecules such as CD31 and CD99, play essential roles in the firm adhesion and transmigration [7-9].

In addition to these classical homing-associated molecules several other proteins both on leukocytes and endothelial cells are known to be critical in controlling leukocyte trafficking. Among these are vascular adhesion molecule-1 (VAP-1) and CD73, two different cell-surface expressed enzymes. Here we focus on the potential role of these two molecules in controlling the movement of leukocytes in inflammatory and malignant conditions. We will give a brief overview on the earlier studies central to the concept of ecto-enzymatic control of leukocyte trafficking, but will focus on the new advances that have been published since our latest comprehensive review on this topic appeared in the beginning of 2008.

2 Discovery of the primary amine

oxidase VAP-1

VAP-1 was originally reported as an endothelial adhesion molecule able to support lymphocyte adhesion to HEV in man [10]. It is also inducible on the luminal surface of vessels with flat-walled endothelium under inflammatory conditions [11]. In addition to endothelial cells, VAP-1 is abundantly present in adipose cells, in which it is involved in glucose uptake, and in smooth muscle cells, in which its functions remain poorly understood [12-15].

VAP-1 is a primary amine oxidase (E.C. 1.4.3.21; also known as semicarbazide-sensitive amine oxidase (SSAO) or amine oxidase, copper dependent 3 (AOC3) [16]. The enzymatic activity of VAP-1 converts soluble primary amines into the corresponding aldehydes [17, 18]. At the first step of this two step reaction, the amine substrate forms a transient Schiff-base with the enzyme, the enzyme is reduced and then the corresponding aldehyde is released. In the second half-reaction the enzyme is oxidized back to its original state in a reaction, which also produces ammonium ions and hydrogen peroxide. The catalytic activity of VAP-1 is critically dependent on a tyrosine residue (at position 471 in human), which is spontaneously self-processed into a topaquinone (6-hydroxydopa) co-factor [19]. Despite the fact that the catalytic reaction driven by VAP-1 has been dissected in great detail, the identity of soluble endogenous VAP-1 substrates still remain enigmatic. It is known that both methylamine and aminoacetone, side products of intermediary metabolism in man, can serve as good VAP-1 substrates, but more abundant physiological substrates may well exist [17].

The VAP-1 molecule consists of an extracellular part, which harbors the catalytic site, a transmembrane segment, and a short intracellular N-terminal tail [20, 21]. On the plasma

membrane VAP-1 normally forms a homodimer of two 90 kDa glycoproteins. The extracellular part of each monomer consists of three domains (D2-D4). VAP-1 has a relatively narrow substrate channel formed by domains D4 and D3 and a key leusine (469 in human) guards the entry of substrates. The large D4 domains, from each subunit, form the dimer interface and each also contains a catalytic site, buried at the base of a deep cleft.

3 Anti-VAP-1 mAbs inhibit leukocyte migration in inflammation

VAP-1 has been shown to regulate leukocyte-endothelial interactions in a number of in vitro and in vivo models. Inhibition of VAP-1 by function-blocking monoclonal antibodies inhibits lymphocyte, monocyte and granulocyte binding to HEV and inflamed flat walled vessels in many different tissues such as peripheral lymph nodes, skin, heart, gut, synovium, kidney and liver in frozen section assays [10, 22-26]. The anti-VAP-1 mAbs also reduce rolling, firm adhesion and transmigration of granulocytes and/or lymphocytes under conditions of laminar flow on different types of VAP-1 positive endothelial cells [27, 28]. More recently, a unique subpopulation of CD16+ monocytes has also been shown to rely partially on VAP-1 in adhesion to and transmigration through hepatic endothelial cells under shear [29]. In intravital videomicroscopy studies, anti-VAP-1 mAbs reduce the number of rolling, firmly adherent and transmigratory granulocytes [30, 31]. In in vivo animal models function-blocking anti-VAP-1 mAbs diminish inflammatory cell infiltration in many experimental models including peritonitis, type 1 diabetes, hepatitis, skin inflammations and allograft rejection [30, 32-34].

4 VAP-1 enzyme inhibitors attenuate inflammation

The function of VAP-1 cannot only be blocked by mAbs, but also by specific inhibitors of its enzyme activity. Traditionally, small carbonyl reactive substances such as hydroxylamine, semicarbazide or 2-bromoethylamine, have been employed as VAP-1 inhibitors. Later on several new VAP-1 inhibitors belonging to hydrazine derivatives (such as BTT-2052/SZE5302 and LJP1207), haloalkylamines, arylalkylamines, propenyl- and propargylamines, and oxazolidines with improved specificity and biocompatibility have been developed [35, 36].

With these inhibitors it has become evident that the enzymatic activity of VAP-1 is important in the leukocyte extravasation, in particular during the transmigration step. Thus, inhibition of exogenously or endogenously expressed VAP-1 by hydroxylamine or semicarbazide significantly reduces leukocyte transmigration through an endothelial monolayer in in vitro flow assays [28, 37]. The inhibitors are also effective in alleviating inflammation in in vivo models. This was initially demonstrated in air pouch inflammation and adjuvant and anti-collagen antibody induced arthritis models by BTT-2052 [27, 38]. Later, enzymatic inhibition of VAP-1 with different inhibitors has been successfully used to ameliorate colitis, ischemia-reperfusion injury of lung, stroke, experimental autoimmune encephalomyelitis (EAE), sepsis and transplant rejection [39-42]. In the EAE model, VAP-1 inhibitors showed notable efficacy in preventing an already established inflammatory response in the central nervous system even when initiated later during the relapsing-remitting course of the disease [42].

More recently, several publications have indicated that VAP-1 can inhibit leukocyte infiltration into the inflamed eye. In a

rat model of uveitis, the inflammation induced the expression of VAP-1 in the vasculature of the retina [43]. An SSAO inhibitor alleviated accumulation of leukocytes in the different anatomical compartments of the eye. Also in a rat model of diabetic retinopathy, inhibition of VAP-1 markedly reduced leukocyte transmigration into the retina [44]. Moreover, in another rat eye model relevant to age-related macular degeneration, SSAO inhibition significantly reduced the numbers of choroidal macrophages, and the amount of neovascularization and the leakiness of the newly formed vessels [45]. Very recently, VAP-1 was shown to be induced by IL-1 in a corneal neovascularization model in mice. VAP-1 inhibitors inhibited IL-1 induced type 2 macrophage infiltration and both angiogenesis and lymphangiogenesis in that model [46]. Although another member of the primary amine oxidase family, retina-specific amine oxidase AOC2, may contribute to the observed effects in mice (and humans), rats lack this protein [47]. Moreover, VAP-1 is expressed in the vasculature of the eye [48]. Nevertheless, all eye models were done using a single 1,3-thiazole based SSAO inhibitor, and it would be interesting to confirm the observations using VAP-1 deficient mice. In any case the elegant eye models show that the SSAO activity plays an important role in the generation of the intraocular inflammatory response.

Taken together, enzymatic inhibitors of VAP-1 have shown notable anti-inflammatory effects in multiple in vivo models. Nevertheless, the specificity of the inhibitors against other SSAO family members, including retinal amine oxidase and diamine oxidase, and possibly against some unrelated molecules, has not been tested very thoroughly under in vivo conditions.

5 VAP-1 deficient mice show

altered inflammatory reactions

Generation of VAP-1 deficient mice has confirmed the enzymatic and adhesive functions of this oxidase in vivo. Mice lacking VAP-1 are grossly normal but show almost complete absence of primary amine oxidase activity in the tissue [31]. These mice show faster rolling (less efficient leukocyte-endothelial cell contacts), reduced number of firmly adherent cells, and a clear inhibition of leukocyte transmigration into sites of inflammation. Moreover, inflammatory reactions such as peritonitis and arthritis, and responses to mucosal immunization are attenuated in the absence of VAP-1 [38, 49].

VAP-1 also seems to contribute to leukocyte homeostasis in the fat tissue. VAP-1 is induced in white adipose tissue in genetically obese mouse strains [50]. Moreover, VAP-1 deficient mice are slightly more obese with larger white adipose tissues than their wild-type controls [51]. Nevertheless, there were fewer T cells, macrophages, NK cells and NKT cells in the fat tissue in the mice lacking VAP-1. Thus, homing of different leukocyte subclasses into fat, which is now known to be important for the induction of the chronic low-state inflammation associated with obesity, is regulated by VAP-1. Moreover, the VAP-1 deficient mice provide a new model of mild obesity, in which the numbers of fat cells and infiltrating leukocytes are not directly associated to each other.

6 VAP-1 modulates leukocyte migration into tumors

VAP-1 may support tumor progression. In early studies we showed that anti-VAP-1 mAbs inhibit the adhesion of NK cells and tumor infiltrating lymphocytes to VAP-1 positive neovessels within the tumors [52]. More recently we have

found that in VAP-1 deficient mice melanoma and lymphoma tumors grew more slowly than in wild-type animals [53]. The tumors in VAP-1 –/– host had defective angiogenesis, and impaired recruitment of myeloid-derived suppressor cells (MDSC). Notably, if the MDSC were ablated from the mice, VAP-1 deficiency no longer protected the animals. Moreover, genetic experiments with a transgenic mice expressing an enzymatically inactive mutant of VAP-1 showed that the effects on MDSC accumulation were dependent on the oxidase activity of VAP-1.

The SSAO inhibitors also retard tumor progression. The SSAO inhibitor treated mice show diminished numbers of Gr-1+CD11b+ MDSC in the tumor as well as impaired neoangiogenesis [54]. These experiments not only provide two independent lines of evidence for the role of VAP-1 in leukocyte migration into the tumors, but also suggest that targeting of VAP-1 might have potential as an adjunctive treatment for the inhibition of tumor progression.

Currently we hypothesize that the VAP-1 expressing neoangiogenic vessels in the tumor bind MSDC. As a consequence, the intratumoral numbers of this particular pro-tumorigenic leukocyte subtype are selectively increased, with a concomitant stimulation of the neoangiogenesis and enhancement of the immune-suppressing gene signature of the tumor microenvironment.

7 VAP-1 as a signaling molecule

Hydrogen peroxide has emerged to be a powerful signaling molecule at low concentrations [55]. VAP-1 derived hydrogen peroxide, together with low dose vanadate, was initially shown to modulate glucose transport and GLUT4 recruitment by phosphorylating insulin receptor substrate-1 and -3, and PI-3-kinase in rat fat cells [56]. Later on, VAP-1

derived H_2O_2 (without vanadate) has been found to activate several key transcription factors including NF-kB and p53 [57, 58]. Hydrogen peroxide emerging from the catalytic activity of VAP-1 increases the expression of classical adhesion molecules such as E-selectin, P-selectin and MadCAM-1, and chemokines such as CXCL8 in different in vitro and in vivo models [57, 59, 60]. Use of mutant mice expressing an enzymatically inactive point mutation in VAP-1 has verified in vivo that VAP-1 dependent up-regulation of P-selectin and MadCAM-1 are dependent on the oxidase activity of VAP-1 [59, 60]. Thus, the enzyme-activity dependent adhesive functions of VAP-1 may be at least partially mediated through the H_2O_2-dependent signaling cascades, which alter the expression and/or function of other homing-associated molecules.

8 Novel counter-receptors of VAP-1 on the leukocyte

The leukocyte molecules(s) binding to VAP-1 have escaped characterization for almost 20 years. Very recently, panning of phage display libraries with recombinant VAP-1 has led to the identification of the first cellular counter-receptors of VAP-1. These experiments showed that VAP-1 binds to Siglec-9 and Siglec-10 proteins both in cell free protein-protein interaction assays and in different cell based models [61] (and our unpublished results).

Siglecs belong to a family of lectin molecules, which bind to sialic acids and mediate various adhesive and signaling events both within the immune system and elsewhere in the body [62]. The cellular distributions of Siglec-9 and -10 are very different: Siglec-9 is expressed on all granulocytes, whereas Siglec-10 is present mainly on B-cells. Based on molecular modeling it is plausible that both Siglecs can

present specific arginine residues into the enzymatic cavity of VAP-1. Although the side chain of arginine terminates in a complex guanidinium structure rather than in a normal primary amine, the arginine 293 of Siglec-10 has been experimentally demonstrated to function as a substrate of VAP-1 [61]. Thus, these molecules can apparently serve as surface-bound substrates of VAP-1. Currently it is not clear, whether the sialic acid modifications of VAP-1 are necessary for the binding to Siglecs.

Siglec-VAP-1 interaction can be utilized for imaging of inflammation in vivo (our unpublished results). Short synthetic Siglec-9 peptides (containing the VAP-1 interacting core sequence) localize selectively to sites of inflammation in vivo in VAP-1 expressing transgenic mice, but not in VAP-1 deficient mice. This finding is compatible with the fact that in normal non-inflamed vessels in the periphery VAP-1 resides in cytoplasmic vesicles, from which it is translocated onto the luminal surface only in inflammation [63].

9 Dual mode of function of VAP-1: the current hypothesis

VAP-1 is believed to have both enzyme activity dependent and enzyme activity independent adhesive functions [20]. The latter ones are those blocked by anti-VAP-1 mAbs, which do not inhibit the oxidase activity of VAP-1. On the other hand, a single point mutation at the critical Tyr 471 renders VAP-1 unable to support leukocyte transmigration under flow conditions. This mutant still harbors the epitopes for anti-VAP-1 mAbs, but its adhesion supporting properties cannot any more be inhibited by anti-VAP-1 antibodies. Moreover, in vitro flow assays have shown that the anti-VAP-1 and SSAO inhibitor treatments do not display additive effects. Therefore, we favor a model, in which a

leukocyte first makes a contact with VAP-1 expressed on endothelial cells by using its counter-receptor (Siglecs or other cell-surface expressed ligands) to interact with the surface of VAP-1 (anti-VAP-1 antibody epitopes). Then, the leukocyte can present a cell surface amine into the substrate channel of VAP-1. This leads to the oxidative reaction involving a covalent, but transient interaction of the counter-receptor (a substrate) with the endothelial VAP-1 (an enzyme), modification of the leukocyte cell-surface substrate into an aldehyde and release of biologically active hydrogen peroxide and ammonium.

10 VAP-1 based clinical trials: a prognostic biomarker and a potential drug target

A soluble form of VAP-1 (sVAP-1) is found in the blood of normal persons [64]. It is most likely formed by a proteinase-mediated shedding of the endothelial and other surface expressed forms of VAP-1 [65, 66]. The concentration of sVAP-1 is increased in a limited set of inflammatory diseases. The most notable elevations are seen in type 1 and type 2 diabetes and in certain liver diseases such as alcholic and primary biliary chirrosis. [66-69]. In a striking contrast to the majority of classical endothelial adhesion molecules, sVAP-1 is not induced in many other inflammatory diseases such as rheumatoid arthritis or inflammatory bowel diseases [66]. In type 2 diabetes, sVAP-1 even serves as an independent prognostic marker for the diabetic complications and it predicts the risk for cardiovascular and cancer mortality in these patients [26].

Increased sVAP-1 levels are also seen in cancer patients. Recent studies indicate that patients with low sVAP-1 levels

have significantly worse prognosis of colorectal cancer, and that sVAP-1 is an independent marker of hepatic and lymph node metastasis in these patients [70]. A similar correlation with low sVAP-1 and poor prognosis was reported in gastric cancer [71].

The ample evidence from in vitro and preclinical in vivo models demonstrates that VAP-1 contributes to the inflammatory reaction in several different settings, and therefore it is a potential target for anti-inflammatory therapeutics. It should be noted, however, that there are certain important differences in the expression and function of VAP-1 between different species. One of the biggest differences is in liver. Sinusoidal endothelial cells in human liver express high levels of VAP-1, whereas the corresponding vessels in mouse express VAP-1 only very faintly or not at all under normal conditions [22, 72]. Moreover, mouse VAP-1 oxidates a wider repertoire of substrates than human VAP-1 [72], which suggests the existence of subtle differences in the active sites of the enzymes between the two species. These differences should be taken into account when designing inhibitors for human use and testing them in preclinical mouse models.

To date only anti-VAP-1 antibodies have been administered to patients. Initially, the original anti-VAP-1 mouse mAb 1B2 was administered to contact dermatitis patients, and found to be safe, but cleared from the circulation very rapidly [73]. This has led to the development of chimeric and humanized, and lately, fully human anti-VAP-1 mAbs for clinical trials. The first phase I/IIa studies show that function-blocking fully human anti-VAP-1 mAbs are safe and pharmacokinetically feasible reagents, when administered into rheumatoid arthritis or psoriasis patients. Importantly, they also suggested preliminary therapeutic efficacy (**http://www.biotie.com/en/recearch_and_development/in**

flammation/vap1_antibody). Analyses of the efficacy of anti-VAP-1 blockade in clinical settings await for further studies. The development of multiple novel small molecular VAP-1 enzyme inhibitors by different pharmaceutical companies also indicates that blocking of this modality of VAP-1 function seems to be therapeutically attractive.

11 CD73 – regulator of adenosine production

CD73 is an ectoenzyme (ecto-5′-nucleotidase, EC 3.1.3.5) mainly expressed on endothelial and epithelial cells and on a subset of lymphocytes, especially on regulatory T cells. It is a part of extracellular ATP metabolism and catalyzes conversion of AMP to adenosine. Adenosine is highly anti-inflammatory, since it increases endothelial barrier function and has suppressive effects on leukocytes. It exerts its effects via adenosine receptors A1, A2A, A2B and A3. Depending on the triggering pathway and adenosine concentration the effects can be either activating or inhibiting. In inflammatory conditions, increased adenosine levels lead to diminished production of activating cytokines and decreased expression of certain adhesion molecules such as E-selectin and ICAM-1 on endothelium and beta 2 integrins on leukocytes [74-77].

12 Distinct roles of CD73 on lymphatic and vascular endothelium

CD73 participates in leukocyte extravasation from the blood into the inflamed tissues. Experimental evidence suggests that when a leukocyte binds to endothelial CD73, its enzymatic activity becomes inhibited leading to decreased adenosine production. The remaining adenosine is rapidly

degraded by adenosine deaminase. Thus, this process facilitates leukocyte transmigration through the vascular endothelium [78].

Besides vascular endothelium, the lymphatic endothelium is also CD73 positive. Interestingly, CD73 seems to be so far the only molecule discriminating the afferent and efferent arms of the lymphatics. It is expressed on afferent but not on efferent lymphatics. In general, lymphocyte recirculation routes between blood and lymphoid tissues are well known. Also the molecular mechanisms mediating lymphocyte entry from the blood via the postcapillary venules into the tissues have been thoroughly analyzed [1, 2, 5-9]. In contrast, much less is known about the trafficking mechanisms of leukocytes within lymphatics. All leukocytes can enter the lymph node via the afferent lymphatics, but only lymphocytes can leave the node via the efferent ones. Thus, the trafficking mechanisms via the afferent lymphatics into the draining lymph nodes and those responsible for the continuation of the lymphocyte journey via the efferent lymphatics are presumably different. Therefore, we recently analyzed the role of CD73 in lymphatics and, against our expectations, found that lymphatic CD73 did not contribute to the migration. Instead, lymphocyte CD73 was important in mediating lymphocyte migration via the afferent lymphatics. Thus, we can currently conclude that CD73 on blood vessel endothelium and on lymphatic endothelium have distinct adhesive roles [79].

13 CD73 both on cancer cells and tumor infiltrating leukocytes contribute to the cancer behavior

CD73 is also expressed on malignancies such as leukemia and certain cancers of epithelial cell origin [80, 81]. It is

thought to provide survival advantage to the cancer cells by providing a salvage pathway in situations, where antimetabolites are used as cancer treatments to block the de novo synthesis of purines [82]. In fact, expression and activity of CD73 on cancer cells is associated with poor prognosis and may promote metastasis [83, 84].

Experimental cancer models have provided excellent tools to dissect the mechanisms behind this phenomenon more thoroughly. Gene expression profiling of tumor xenografts has shown CD73 to associate to lymph node metastases of breast cancer [85] and expression arrays have revealed that CD73 can protect leukemic cells from TRAIL-induced apoptosis [86]. Involvement of cancer cell CD73 in metastasis is well in line with the role of lymphocyte CD73 in normal migration of lymphocytes via afferent lymphatics into the draining lymph nodes [79]. Moreover, CD73-derived adenosine acting via A2A receptor has been shown to increase tumor cell chemotaxis. This may contribute to metastasis of tumor cells as well [87]. An additional mode of action of tumor CD73 is down-regulation of anti-tumor immunity, since blockade of its adenosinergic effects improves adoptive T cell therapy [88]. Although many of the CD73 effects are consequences of its adenosine production, also more direct interactions have been reported. The protection from TRAIL-induced apoptosis does not involve enzymatic activity of CD73. Instead, it is thought to be mediated by direct binding of CD73 to the death receptor 5 [86].

Furthermore, CD73 of the host origin contributes to tumor behavior. Tumor infiltrating lymphocyte population contains variable number of regulatory T cells. They are considered to be harmful as they dampen the anti-tumor immune response. CD73 on regulatory T cells and adenosine A2A receptor on activated T cells form immunosuppressive

loops [89] and this may be envisaged to take place between the lymphocyte pools in cancer. The existing literature clearly demonstrates that CD73 exerts its functions in several ways during cancer development and acts both on cancer cells and non-malignant host cells. Overall, the mechanisms seem to work for the benefit of the cancer cells by helping them to escape the immuno-surveillance.

Knockout mice have been valuable in searching for the role of the host CD73 in cancer development [90, 91]. In colon cancer, lymphoma, mammary tumor and melanoma models lack of host CD73 leads to retarded tumor growth. This is due to decreased numbers of suppressive type 2 macrophages within the tumors and increased activity of CD8 positive T cells. The studies have further revealed that pro-tumorigenic effects of regulatory T cells are dependent on their CD73 expression and endothelial CD73 may be critical in promoting lung metastases [91].

14 Lack of CD73 leads to different consequences in mouse and man

The central role of CD73 at sites of inflammation and hypoxic conditions can be realized in knockout mice lacking CD73 [92-94]. These animals show enhanced leukocyte adhesion to vascular endothelium in an ischemia-reperfusion model [95] and increased vascular leakiness and leukocyte migration to inflammatory sites in acute lung injury [96]. All this is in perfect agreement with the known role of adenosine in inflammation. However, the findings regarding brain inflammation in EAE model have been surprising, because mice lacking CD73 are protected from the disease [97]. The results obtained in EAE suggest that both lymphocyte and endothelial CD73 is needed for proper entrance of lymphocytes into the brain in EAE. This suggests

an organ specific role for CD73 in inflammation and less significant contribution of CD73-derived adenosine to endothelial leakiness in murine blood-brain-barrier than in vasculature elsewhere in the body. In this context it is important to note, that expression of CD73 in the murine brain is different from that in human brain [97, 98]. Only occasional CD73 positive vessels can be found in murine brain, whereas CD73 is expressed abundantly in the vasculature of human brain.

Regarding CD73 another difference can be detected on afferent lymphatics, most of which are CD73 positive in humans. In contrast, only a minority of these lymphatics expresses CD73 in mouse [76].

Recently mutations in human CD73 gene have been identified leading to decreased CD73 concentrations and enzymatic activity. These patients suffer from symptomatic calcifications in lower-extremity arteries and in hand and foot joint capsules. Therefore, it is believed that CD73 is involved in the pathways preventing ectopic calcification [99]. This has not been observed in CD73 deficient mice.

15 Clinical implications of CD73

When evaluating the potential of a particular target for drug development it should be remembered that the data obtained with mouse disease models may not be directly transferable to humans, because the expression and/or function of the murine homolog may differ from that of humans. Thus, caution is also warranted when evaluating the results obtained from CD73 studies performed in mouse.

Due to its anti-inflammatory effects adenosine is a potent substance to alleviate inflammation. However, due to its

extremely short half-life its use is not very feasible in clinics. Theoretically, mediators up-regulating endogenous CD73 could serve as alternatives providing a more constant increase in CD73-derived adenosine. Such factors are interferon-beta and alpha. Both these interferons up-regulate human CD73 on vasculature without having any effects on CD73 on lymphocytes and cancer cells [81, 98]. Similarly, interferon-beta up-regulates murine endothelial CD73 and gives protection in acute lung injury in mice [96]. A clinical phase II study with interferon-beta is ongoing in patients suffering from severe lung inflammation (**http://www.faronpharmaceuticals.com/**).

One large patient group getting interferon-beta medication is patients suffering from multiple sclerosis (MS). CD73 in these patients' sera and endothelial CD73 increase subsequent to interferon-beta therapy and this correlates with clinical improvement of the patients [98]. Thus, part of the efficacy of interferon-beta therapy in MS disease may be mediated by CD73. It can be envisioned that interferon-beta up-regulates CD73 on brain vasculature leading to increased adenosine production. Adenosine improves endothelial barrier function that results in diminished infiltration of the inflammatory cells into the brain.

Statins have also been shown to up-regulate CD73 and adenosine production. They may be beneficial as a short-term therapy, when transient CD73 increase is needed for example in ischemia-reperfusion injury or as a pre-conditioning treatment [100, 101]. Their long-term effects, however, may be questionable as in vitro studies have demonstrated that statins increase CD73 expression and activity by preventing its endocytosis without increasing its synthesis [102]. Clinical trials with statins meant to target CD73 independently of their cholesterol lowering effects are ongoing or recently completed (**http://clinicaltrials.gov/**).

In oncology, CD73 seems to be harmful as it enhances tumor growth and metastasis both in various pre-clinical models and in human tumors [84, 87, 88, 90]. In this disease group, inhibition of CD73 would hypothetically be beneficial. In preclinical models anti-CD73 antibody therapy, small molecular inhibitors of CD73 as well as targeting of adenosine receptor A2A have shown their potential [87, 88, 90]. Whether they will also show efficacy in the clinical settings remains to be seen in future. Potentially also other adenosine receptors can be targeted to prevent tumor growth. A3 adenosine receptor agonists are able to suppress survival and proliferation of colon carcinoma cells in murine tumor models [103]. They can also decrease proliferation and metastasis of rat prostate cancer cells [104]. Moreover, an A3 receptor agonist induced apoptosis and inhibited tumor growth of hepatocellular carcinoma in rats [105]. Whether A3 receptor agonists are also beneficial for patients will be evaluated in a currently ongoing clinical trial targeting hepatocellular carcinoma (**http://www.canfite.com/CF102.htm**).

16 Conclusions

Aberrant leukocyte trafficking to sites of inflammation is often harmful leading to tissue damage and migrating malignant cells form the metastases in cancer. Therefore, molecules responsible for the harmful traffic are theoretically excellent targets to cure inflammations and prevent cancer spread. VAP-1 and CD73 are both ectoenzymes, which act via direct interactions with their counter-receptors and more importantly, exert their effects via the end-products of their enzymatic activity. The end-products are potent mediators that have several effects modulating the cellular microenvironments. In the case of VAP-1 the end-products are pro-inflammatory, whereas

adenosine produced by CD73 is highly anti-inflammatory. Thus it would be beneficial to suppress VAP-1 and activate CD73 to alleviate inflammatory reactions. However, in cancer both CD73 and VAP-1 have serious unwanted properties leading to accelerated cancer growth, metastasis formation and suppression of the anti-tumor immune response. Therefore, in this diverse entity inhibition of both VAP-1 and CD73 appears desirable. In the context of CD73 it is important to note that up-regulation of CD73 by interferon-beta therapy is unlikely to cause a threat of cancer progression, since in cancer the contribution of lymphocyte and cancer cell CD73 rather than endothelial CD73 appears to be critical [81]. A schematic presentation of the role of CD73 and VAP-1 in cancer and inflammations is shown in Fig. 1 .

Figure 1 Open in figure viewer | PowerPoint

The role of CD73 and VAP-1 in cancer and inflammation. Expression of CD73 and VAP-1 on different cell populations is indicated (symbols or negative) and their key functional consequences are summarized. Also the enzymatic reactions catalyzed by these enzymes are shown (top). Big yellow arrows indicate the overall contribution of these molecules in cancer and inflammation. Immunosuppressive leukocytes include regulatory T cells, type 2 macrophages and MDSC. See the text for the details.

In comparison to other trafficking-associated molecules, ectoenzymes provide pharmaceutical industry with unique targets, because their enzymatic activity offers additional approaches for modulation with small molecular drugs. Based on their properties and results obtained so far from

pre-clinical and clinical studies VAP-1 and CD73 are potential candidates to treat inflammation and cancer. The current and future clinical trials will show their true value in clinical medicine.

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