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ORIGINAL ARTICLE

The CD38/CD157 mammalian gene family: An evolutionary paradigm for other leukocyte surface enzymes

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Abstract Human CD38 is the mammalian prototype of a family of phylogenetically conserved proteins which share structural similarities and enzymatic activities involved in the production of an intracellular second messenger with calcium mobilizing effects. Engagement of CD38 by agonistic monoclonal antibodies and the CD31 ligand initiates a cytoplasmic signaling cascade involving tyrosine phosphorylation of the proto-oncogene c-cbl and of the extracellular regulated kinase 1 of 2 complex. Further requirements for signal transduction include a privileged localization within the cholesterol-rich areas of the plasma membrane and physical association with specialized surface receptors. CD38-mediated signals are crucial in heterotypic cell adhesion and migration as well as in the activation of proliferation/survival programs by normal and neoplastic cells. Here we review the most recent literature on this complex topic and attempt to formulate a single model reconciling the enzymatic and receptorial activities of CD38.

Keywords calcium mobilization · cell surface enzymes · cell surface receptors · intracellular signaling

Abbreviations

BM	bone marrow
cADPR	cyclic ADP ribose
IP3	inositol triphosphate
ERK1/2	extracellular regulated kinase 1/2
mAbs	monoclonal antibodies
NAD	nicotinamide adenine dinucleotide

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Introduction

The concept of cell surface molecules with a catalytically active site in the extracellular environment was lastly brilliantly analyzed in a dedicated issue of *Immunological Reviews* edited by Goding and Howard [1]. At the time it was becoming clear that ectoenzymes could no longer be considered an oddity in leukocyte biology; on the contrary, it was seen that ~3.5% of the molecules expressed on the surface of human and murine cells shared such characteristics. This finding galvanized the attention and interest of basic scientists and, later, of clinical scientists as well.

Nucleotide-metabolizing ectoenzymes constitute a family within this larger family and are represented by a set of molecules involved in the catabolism and scavenging of extracellular nucleotides. This process results in the synthesis of compounds that play a critical role in cell homeostasis and metabolism, suggesting that the physiological role of this complex family goes beyond the simple recycling of nucleotides.

One of the characteristics initially thought to be shared by several, even though not all, nucleotide-metabolizing ectoenzymes was the ability to work in an environment where the substrate was generally present only in trace amounts and where the final product would be used prevalently inside the cell [2]. This initial view of enzymes working in an anti-economical environment was later refined thanks also

to the more sophisticated experimental approaches confirming that substrates and final products are not so topologically confined [3].

The majority of these molecules, identified using the grid of the CD International Workshop on Differentiation Antigens and considered to be orphan molecules, found initial clinical applications as disease markers. Indeed, CD38 is still considered an 'activation marker,' useful in the diagnosis of leukemias and myelomas [4], while CD39 was initially defined as a marker of EBV infection [5]. The same fate was shared by CD26 [6] and CD10/CALLA [7], probably the most commonly used clinical markers, as well as PC-1 [8], one of the first reliable markers of human plasma cells.

This review will focus on the CD38/CD157 family of ADP ribosyl cyclases and their relevance in physiology and human pathology.

CD38 and CD157: The early days

CD38 was identified during detailed analysis of the cell surface by means of monoclonal antibodies (mAbs) as carried out by EL Reinherz and SF Schlossman during their pioneer search for molecules acting as T-cell receptors and as transducers of the signals elicited by the encounter with the specific antigen [9]. The upshot of that approach was the definition of a vast array of molecules, including some involved in the economy of discrete subsets of cells (CD4 and CD8), some playing the more general role of metabolic regulators (CD71/TFR-1) and several others, such as CD38, that served as markers for the study of thymocytes, activated T cells and selected tissues [10, 11].

Our interest in investigating human CD38 was sparked by the distribution of the molecule, which ranged from discrete expression during thymic differentiation to an extremely limited presence during the normal physiological life of a T cell. The molecule was re-expressed at high density by T cells undergoing activation and in selected leukemias [12]. In apparent contrast to the simplistic view of CD38 an activation marker was the finding that terminally differentiated plasma cells and their pathological counter-parts expressed the highest surface amount among human cells.

Our analysis proceeded from the observation that CD38, a 45 kD molecule, was initially reported as being associated to a 12 kD single chain protein, reminiscent of a family of HLA Class I-like molecules known at that time as T6 (now CD1a) [13]. That view was disconfirmed once we generated an extensive panel of mAbs specific for the molecule, which enabled us to perform an initial structural analysis that ruled out the presence of β2-microglobulin. Moreover, using a somatic cell genetics approach, the gene coding for the structure was attributed to human chromosome 4 (4p15, and thus outside the canonical HLA supergene in chromosome 6) [14].

One of the mAbs in the panel used for the characterization of human CD38 was found to induce signals after binding to its target, a new and unforeseen characteristic of mAbs [15]. An agonistic mAb is usually a reagent with a very high affinity for the target, mimicking the interaction between ligand and receptor. Using that agonistic mAb, CD38 was initially reported as a surface molecule acting as a receptor engagable by an unknown ligand [16]. While we were busy analyzing the molecule from an immunological perspective, CD38 unexpectedly became the focus of a group of biochemists who were interested in the regulation of calcium signaling through a pathway independent from inositol triphosphate (IP3) [17]. H.C. Lee was able to identify cyclic ADP ribose (cAPDR) as an intracellular second messenger and to purify the enzyme responsible for its synthesis from *Aplysia californica*, a mollusk separated from humans by 80 million years. Surprisingly, the sequence of soluble ADP ribosyl cyclase purified from *Aplysia* proved strikingly similar to the leukocyte transmembrane antigen CD38.

The similarity in amino acid sequences between *Aplysia* ADP ribosyl cyclase and the human lymphocyte antigen CD38 was timidly reported as a letter which appeared in TIBS in December 1992 [18]. This led to an explosion in interest by basic and clinical scientists, astonished by the close similarity between two such phylogenetically distant molecules. Human and mouse CD38 were confirmed to be ectoenzymes [19, 20]. The underlying issue that had to be resolved concerned the relationship between the molecule's receptorial and enzymatic activities: In other words, are the signals implemented by CD38 ligation guided by the intermediate or final products synthesized by CD38, or are the two functions independent?

The identification of a second member of the CD38 family occurred during roughly the same years [21]. Bone marrow stromal antigen-1 (BST-1), later clustered as CD157, was defined as a stromal cell molecule facilitating pre-B cell growth [22]. Its deduced amino acid sequence exhibited 33% sequence homology with CD38. Originally, BST-1/CD157 gene was mapped to chromosome 14q32.3, where immunoglobulin heavy

chain genes are clustered [22]. Further studies did not confirm that initial finding and instead mapped CD157 to chromosome 4p15, in close proximity to the CD38 gene [23].

This review is an attempt to present a unified picture that encompasses both CD38 and CD157, providing answers to several issues that have remained unresolved after more than a decade of analysis.

CD38 as a signaling molecule

As mentioned, analysis using agonistic mAbs eventually led to characterization of the signaling pathway controlled by CD38. Because the molecule is widely expressed by lymphoid cells, the signaling events were analyzed in detail in distinct lineages and during discrete developmental stages. The emerging picture is complex and non-univocal, but some common threads seem to emerge.

a) CD38 engagement by agonistic mAbs is followed by a signaling cascade typical of the canonical receptors, including tyrosine phosphorylation of a sequential number of intracellular enzymes, nuclear events and more long-term effects dependent on active protein synthesis. The individual steps of the signaling pathway vary according to the model studied; however, a common player in the CD38 pathway is the proto-oncogene c-cbl, which is reported to be tyrosine phosphorylated in all the cell models tested, including T, B, NK and myeloid cells [24–28]. This observation suggests that CD38 may play a role in the fine tuning of antigen receptor signaling [29]. Moving further down, the signal appears to be funneled through the ERK1/2 cross-point, at least in T and NK cells [28, 30].

b) An increase of the cytoplasmic levels of calcium ions is also a common theme upon activation of CD38. The calcium wave is typically low and slow in rising as compared to the spikes obtained after signaling through the antigen receptors in T and B lymphocytes. Unlike the antigen receptors, a CD38-induced calcium wave may last several minutes before declining, indicating that the molecular mechanisms responsible for calcium mobilization might be different [27, 31, 32]. In several instances, it was possible to determine PLC-γ tyrosine phosphorylation upon CD38 cross-linking, suggesting that the conventional IP3 pathway may account for calcium mobilization [33, 34]; in other instances and mostly using murine models, no PLC-γ activation could be detected [35]. However, a direct contribution of cADPR extracellularly produced by CD38 to the signaling process cannot be convincingly ruled out, as the deletion of the catalytically active site by means of site-directed mutagenesis is followed by a lack of binding by the available agonistic mAbs [36, 37]. Moreover, activation of surface CD38 by preincubation of cells with nicotinamide adenine dinucleotide (NAD+) was found to lead to a dose-dependent increase in cADPR, with no recordable changes in the intracellular concentrations of calcium ions [38, 39].

c) What ignites the signal? The lack of correlation between CD38-mediated signals and the production of cADPR, ADPR, and/or nicotinamide prompted the search for alternative ligands capable of initiating the signaling process. The finding that CD38 could modulate $CD4^+/CD45RA^+$ naïve T lymphocyte [40] adhesion to endothelial cells culminated with the identification of a 130 kD protein recognized by a soluble form of CD38 that was used as a probe in a Western blot system [41]. This molecule turned out to be CD31/PECAM-1, an Ig superfamily member mainly involved in the modulation of leukocyte adhesion to the vessels [42]. In subsequent years, most of the signals recorded using agonistic mAbs were reproduced by using the CD31 ligand. These included mobilization of calcium signaling as well as more structured events, such as proliferation and cytokine induction [31, 43]. More recently, a similar approach exploiting a soluble CD38-Ig recombinant protein was used to identify novel ligands in the murine system. As a result, a ligand of molecular weight 130 kD, apparently different from CD31, was detected on the surface of dendritic cells [44],

d) The structural requirements for signaling are as yet unclear. Of the various challenges facing defenders of the receptor hypothesis, determining how the signaling is initiated is perhaps the most compelling. The role of the cytoplasmic tail is controversial at this point. On the one hand, the Lund group convincingly showed that the presence of the intracellular residues of CD38 is irrelevant for signals and enzymatic activities [38]. Moreover it is generally accepted that the tail does not contain signaling motifs. However, other studies have determined – at least in T lymphocytes – a direct association between the tail of CD38 and the SH2 domain of the kinase lck [45].

We and others have shown that CD38 is located in critical areas of the plasma membrane in close physical proximity with professional signaling receptors [46], such as the T-cell receptor in T lymphocytes [34], the B-cell receptor complex in B cells [47], CD16 in NK cells [30], MHC Class II and CD9 in monocytes [27, 48] and the CCR7 chemokine receptor, CD83 and CD11b in mature dendritic cells [49]. Although these associations are clearly visible using confocal microscopy, they are lost when performing co-immunoprecipitation experiments, suggesting either that the interaction is weak or that there is an as yet unidentified third party protein which acts as a bridge between CD38 and the other receptors. In line with the latter hypothesis, recent papers have implied the existence of supramolecular complexes acting in conjunction with multiple partners [48, 50]. These complexes appear to reside within the cholesterol-rich areas of the plasma membrane [51] and to be dependent on their integrity [52]. The association between CD38 and other signaling receptors was deemed to have a functional nature, as implied by the results of experiments with T, B and NK cell line models genetically modified to lack functional signaling receptors [30, 34, 53]. This hypothesis was also confirmed by using normal cells that lack functional receptors [30].

e) The long-term events described as a consequence of the interaction of CD38 with the CD31 ligand and mimicked by agonistic mAbs vary according to cell lineage and differentiation status. A common theme in human T cells is the modulation of a pattern of cytokines, including the classical Th1 (IL-1, IL-6 and IFN-γ) as well as the Th2 cytokines (IL-4 and IL-10) [54, 55]. The result is implementation of a network of signals, which ultimately rule the processes of proliferation, survival and differentiation. This was recently shown in tests of the receptor hypothesis using chronic lymphocytic leukemia (CLL) as a model [47]. CD38 was found to be expressed by a subset of CLL cells characterized by shorter survival times and shorter intervals prior to therapy [56–58]. In these cells, CD38-mediated signals was shown to lead to cell proliferation and to increase the survival of the neoplastic clone by cooperating with other survival receptors, including semaphorin CD100 [43]. The signals induced by the agonistic mAb are fully mimicked after interaction with the CD31 ligand, suggesting that this receptor/ligand system may play a direct role in the pathogenesis of CLL.

CD38 and CD157 as enzymes

As mentioned above, the extracellular domains of CD38 and CD157 contain an enzymatic site that can synthesize cADPR and nicotinic acid adenine dinucleotide phosphate (NAADP) from NAD^+ and $NADP^+$, respectively [19, 59]. Thus, CD38 and CD157 are ADP-ribosyl cyclases, a family of multifunctional enzymes apparently ubiquitous in eukaryotic cells [60]. CD38 and CD157 are the major enzymes with ADP-ribosyl cyclase activity, although a non-CD38/CD157 ADP-ribosyl cyclase has recently been detected in brain tissue from CD38 knock-out (KO) mice [61]. The most relevant product of the reaction is cADPR, a universal second messenger with the ability to control calcium levels in an IP3 independent way [62]. cADPR – and thus ADP ribosyl cyclases – play a key role in physiological processes, including cell proliferation [63], muscle contraction [64, 65], stem cell regeneration [66] and hormone secretion [67], among the most relevant. Under normal physiologic pH conditions, and unlike the *Aplysia* enzyme, CD38 is a very inefficient cyclase, with cADPR representing only 1%–3% of the final products [19]. ADPR and NAADP produced by CD38 [68] also cooperate in the regulation and modulation of intracellular calcium: Indeed, ADPR acts on plasma membrane LTRPC2 calcium channels, and NAADP mobilizes calcium from intracellular stores [69]. Basile et al. recently showed that CD38 and the other ADP ribosyl cyclases also synthesize three novel adenine dinucleotides from cADPR [70]. Pinpointing the role of these compounds in intracellular calcium homeostasis is in the early stages: Preliminary data indicate that they may play relevant roles in the network of positive and negative signals interchanged between cells expressing CD38 or CD157 and interacting partners.

The ADP ribosyl cyclase family of enzymes is present in a range of cells ranging from protists and unicellular algae to higher Metazoa and Metaphyta. Even if CD38 and CD157 share low sequence identities (~30%), genetic analyses have shown that the exon-intron structures of the two genes is remarkably similar, suggesting that they evolved through gene duplication phenomena from a common ancestor [71]. The similarities are maintained when comparing CD38/CD157 with the *Aplysia* cyclase [72]. The extracellular domain of CD38 was recently crystallized and compared to the structures of CD157 and of the *Aplysia* cyclase [73–76]. The results indicate that the overall topology of the three enzymes is similar: However, the large structural changes observed at the two termini of the extracellular domains of CD38

suggest that this gene underwent heavier evolutionary stress than the CD157 and *Aplysia* ADP ribosyl cyclase genes.

CD157

In contrast with CD38, CD157 is linked to the plasma membrane by means of a glycosylphosphatidylinositol (GPI) anchor. Another element distinguishing it from CD38 concerns cell and tissue distribution, with CD157 detected on human peripheral blood monocytes, eosinophils, basophils, neutrophils and on the majority of myeloperoxidase-positive bone marrow (BM) myeloid precursors. Erythrocytes, platelets and lymphoid cells from peripheral blood or from spleen and tonsils, as well as lymphoblastoid cell lines of T and B origin are negative [77]. Outside the immune system, CD157 is expressed by the endothelium, by synovial cells and derived lines and by follicular dendritic cells [78], suggesting that CD38 and CD157 are mutually exclusive in terms of expression, with the notable exception of the myeloid compartment.

The initial characterization of the structure and distribution of CD157 was performed by Todd et al., using the Mo-5 mAb [77]. Later on, a contribution to clustering as CD157 was independently provided by the cloning of BST-1, a molecule expressed by BM stromal cells and by similar lines derived from the stroma of rheumatoid arthritis patients [22]. The murine homologue of CD157 was cloned in the same period by Dong et al. [79].

CD157 shares the same enzymatic properties as CD38 and – as mentioned above – the two molecules have a similar genetic organization and a close chromosomal localization [23, 60, 80].

Again in analogy to CD38, CD157 acts as a receptor capable of generating signals which can be measured in terms of phosphorylation of a cascade of intracellular proteins. The signals mediated by CD157 were initially defined and characterized in the context of the interactions taking place between stromal and hemopoietic cells in closed environments, such as the BM or the joints [22, 81]. The pathway induced upon CD157 cross-linking by a polyclonal antibody involved tyrosine phosphorylation of selected substrates in different line models [82–84], amply reviewed in [85]. These findings imply the existence of a cell bound ligand for CD157, potentially alternative to NAD^+ and $NADP^+$, as was the case for CD38. The existence of a ligand is also supported by the finding that a soluble form of CD157 binds to human B-cell, lines [78].

However, the pathway controlled by CD157, the mechanisms of signal delivery, the molecular nature of the substrates involved and the identification of the ligand/counter-receptor await further investigation.

Role of CD38 and CD157 in adhesion and migration

A significant number of cell surface molecules involved in the processes of cell adhesion and migration have unexpectedly turned out to be enzymes [86]. A role for CD38 in cell adhesion was initially hypothesized more than a decade ago during analysis of the dynamic adhesion phenomena taking place between T-cell, if adjectival subsets and endothelial cells [87]. That work demonstrated involvement of CD38 in the early phases of lymphocyte adhesion to the endothelium [40] through direct interaction with CD31 [42]. CD38/CD31 interactions up-modulate integrin expression, thus promoting the ensuing phases in the adhesion cascade, and induce the synthesis and secretion of a panel of cytokines on T cells [88] and of prostaglandin on endothelial cells [89], suggesting the existence of a regulatory loop.

Another possibility is that these interactions depend on modulation of the enzymatic activities of CD38. This scenario seems likely in mice, where cADPR catalyzed by CD38 is essential for eliciting a sustained calcium response in murine neutrophils, triggering chemotactic movements to infected tissues [90]. The initial observation that CD38 KO mice are considerably more sensitive to intratracheal infections with culminated in the finding that the CD38 expressed by murine neutrophils is a modulator of innate immune responses to bacteria [91]. The effects mediated by CD38 are directly dependent on calcium mobilization regulated through cADPR production [91].

Transfer of these observations from mice to men has only been partial. For one thing, CD38 is expressed at minimal levels by normal human neutrophils, where, at least in resting conditions, the dominant enzyme is CD157. CD157 is a key molecule in the regulation of polarization and migration of neutrophils in humans, whereas the same has not been observed in mice. Further, the mechanisms differ significantly in that they are apparently independent of the enzymatic activity and rely mostly on localization in membrane rafts and on physical interaction with β2 integrins [92].

A recent expansion of these findings to dendritic cells adds to the list of differences between men and mice. In both instances, CD38 is expressed by human and murine mature dendritic cells and in both instances it controls migration and polarization of T cells

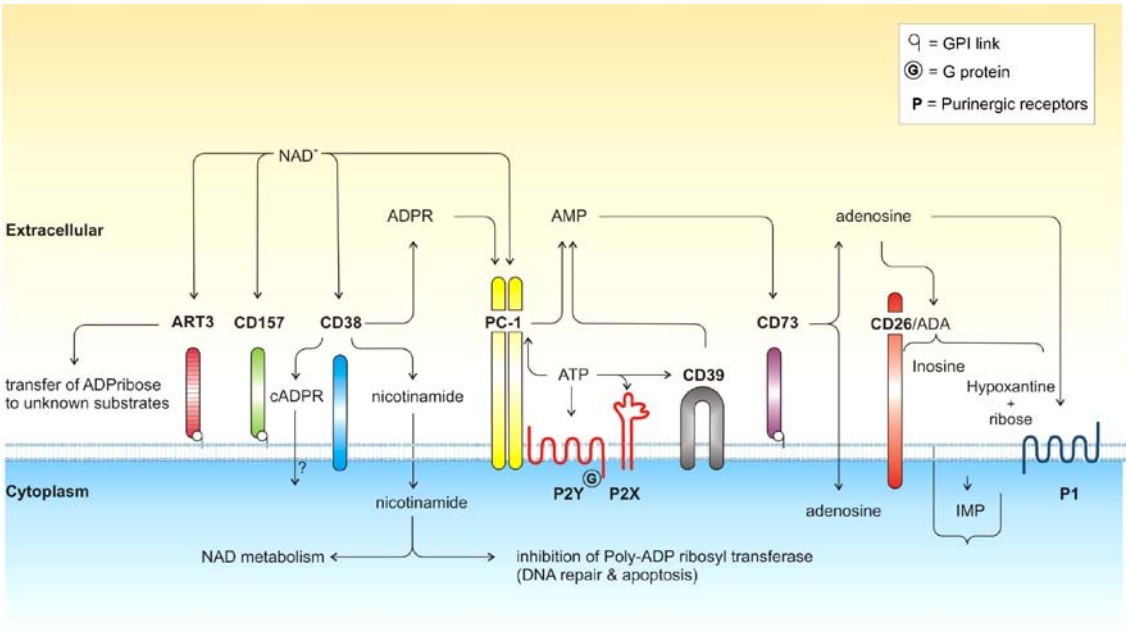

Fig. 1 Schematic representation of a virtual cell expressing a set of functionally related nucleotide-metabolizing cell surface enzymes. These enzymes are involved in (i) scavenging of nucleotides freed in the extracellular environment during inflammatory processes; (ii) modulation of purinergic receptor signaling and ART activity, and (iii) generation of immunomodulatory compounds, with extra- and intra-cellular effects.

towards a Th1 phenotype. However, in murine dendritic cells cADPR is the crucial second messenger regulating dendritic cell functions [93], whereas these events appear independent of cADPR production in humans [49, 94].

Researchers are still grappling with the challenge of devising a unifying view. One appealing possibility is that CD38 and CD157 provide a connection between calcium modulation via cADPR and classical signaling cascades mediated by the tyrosine phosphorylation of activating and inhibitory enzymes. The crystal structure of the molecules as well as biochemical evidence indicate that they may work as dimers or dimers of dimers [73, 95], creating a central cavity which could operate as a channel for the internalization of the enzymatic products. Following this hypothesis, the NAD^+ and $NADP^+$ present in the extracellular space would be trapped by CD38 and/or CD157 and the cADPR produced immediately 'microinjected' in the cytoplasm, eventually binding ryanodine receptors and inducing a calcium wave. This perturbation of calcium homeostasis might be responsible for the activation of calcium-dependent kinases, thus initiating a signaling cascade closely resembling a canonical receptorial pathway. CD31 and other putative ligands could act as modulators of the three-dimensional structure of CD38, modifying its propensity to bind the substrates and start a signaling event. The localization of CD38 and CD157 in critical areas of the plasma membrane in close association with signaling receptors would create the necessary coupling of enzymatic and receptorial machinery and would thus be a requisite for the signal to begin.

CD38 and CD157 are part of a network of cell surface enzymes

It seems that there is a natural blueprint linking CD38 and CD157 to other leukocyte ectoenzymes, which share signaling abilities, enzymatic functions and localization in specialized areas of the membrane. Clustering enzyme/receptor molecules may serve as a strategy for collecting precious elements from dead cells, pooling together signaling receptors and adhesion molecules, as well as generating products acting as recruiters of different cells. A schematic representation of a virtual cell shows the mutual advantages that encourage aggregation of the ectoenzymes (Fig. 1). For instance, NAD^+ and ATP, intracellular metabolites of energy metabolism, are candidates as signaling molecules in extracellular environments [96, 97]. It is hypothesized that high concentrations of NAD^+ and ATP would be present at sites of cell necrosis and tissue damage: When the compounds reach the extracellular compartment, their levels are controlled by a

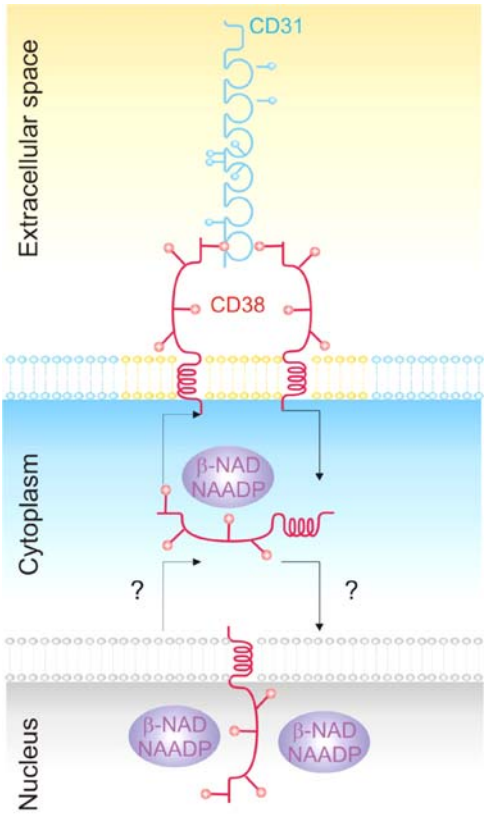

Fig. 2 Schematic model representing a possible dual function for CD38. Following this model, CD38 would perform as an adhesion/homing receptor when surface expressed and as an enzyme in the substrate-rich intracellular environments (cytoplasm and nucleus). The mechanisms exploited by CD38 to translocate to and from the three compartments are currently being analyzed.

number of ectoenzymes. This pathway has clearly been demonstrated for CD39 and related ectonucleotidases [98], which hydrolyze ATP to ADP and/or AMP. Similarly, Koch-Nolte et al. recently reported that CD38 enzymatically controls the availability of extracellular NAD^+ as a substrate for ADP-ribosyltransferase (ART)-catalyzed ADP-ribosylation of cell surface proteins [99].

Conclusions

The dual nature of CD38 can be formulated in terms of its independent functions as receptor and as enzyme, reflecting two separate strands in its long evolutionary history. A reasonable alternative, which finds itself the focus of intense but still unsuccessful investigation, is that CD38 is a receptor that exploits its enzymatic characteristics for signaling. These views reflect – or, more appropriately, are biased by – the points of view of the groups analyzing the molecules. Immunologists tend to privilege the receptorial activities and the existence of a counter-receptor, fulfilling the needs for being a genuine adhesion/homing marker. Biochemists are attracted by the complexity of the functions exerted by these multifunctional and unique enzymes and their ability to regulate cytoplasmic calcium via an IP3-independent pathway.

Other controversies derive from the use of different models, mainly man and mouse. Most of the experiments on human samples have been performed on leukocytes derived from blood, while the evidences in murine and rat models are predominantly inferred from cells derived from lymph nodes and spleen. Nonetheless, we have also seen how the mouse system benefited from the existence of a CD38 KO model, enabling an accurate and causative comparison with the normal counterpart. The search for naturally occurring CD38- individuals was attempted in humans by analyzing more than 5,000 blood samples from newborns, reproducing the experience gathered with individuals lacking complement C2 and C4 [100]. In the Italian sample analyzed, none of the newborns was CD38−, suggesting that the absence of CD38 is incompatible with life (F. Malavasi, unpublished results). These results stand in apparent contradiction to the mouse model, where the KO animal is characterized by selected deficiencies in the immune effectors and innate responses, but it is still able to live and reproduce [90, 101]. The search for $CD157^-$ individuals (i.e., human KO) was pursued in a sample of patients affected by paroxysmal nocturnal hemoglobinuria, a clonal disease characterized by the inability to synthesize the GPI linkage. Indeed, these patients are $CD157^-$ in the pathological clone: However, this approach is not straightforward, for the inability to form GPI anchors is shared not only by CD157, but by all the molecules with this linkage to the membrane [92].

What does seem evident, however, is that we are faced with a family of molecules which descended from the same distant ancestors, but which diverged during evolution because of the pressures of natural selection and the different needs of the organisms. The cost of maintaining such systems must be counterbalanced by a definite profit for living systems. Another hint favoring a common view comes from analysis of the limited polymorphisms observed for the genes of human CD38 [23]. Indeed, evaluation of the allele distribution in a Caucasoid population reveals no differences between individuals from continental Italy and Sardinians, the latter considered the ancient core of the genetic reservoir of Europeans and whose unique characteristics have been conserved as a consequence of geographic restraints [102]. These

findings suggest that the selective pressure behind the two alleles reaches far into the distant past. Future research would benefit from detailed study of the differences between homozygous and heterozygous individuals in terms of enzymatic functions and signal transduction ability. The relationship between CD38 and selected diseases may also indicate that the members of this ectoenzyme family played a role in the defense against (or, better, resistance to) the diseases that in more recent centuries modeled the genome that we see today. Perhaps the greatest challenge to resolving these questions and controversies rests in defining the proper framework within which to interpret the results achieved by the limited number of groups working on ectoenzymes.

Another possibility awaiting confirmation is that CD38 may play a double function, according to its localization (Fig. 2). Indeed, it may act as a receptor when surface expressed and as an enzyme when internalized upon request or during its cytoplasmic transit [103]. There is no current evidence that this model may also hold true for CD157, mainly because of its GPI anchor, nor has the role of soluble CD38 [104, 105] and CD157 [106] been analyzed in any great detail.

The CD38 paradigm is also representative of a more general pattern involving several nucleotide-metabolizing ectoenzymes, such as CD39 [98] and CD26 [107]. Indeed, it appears that all these enzymes evolved from very well conserved ancestors, usually acquiring a membrane anchorage (either transmembrane or GPI) and developing parallel and apparently independent functions. Relevant in an immunological context is the acquisition of functions classically associated with receptor activity, such as signal transduction. These include calcium mobilization and protein tyrosine phosphorylation [108]. Another common trait is localization in membrane lipid microdomains and physical and functional association with partners specialized in signal transduction [109, 110].

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