CD38 and CD157: A long journey from activation markers to multifunctional molecules.

Published version:

DOI:10.1002/cyto.b.21092

(Article begins on next page)

CD38 and CD157: a long journey from activation markers to multifunctional molecules

Valeria Quarona,[1] Gianluca Zaccarello,[1] Antonella Chillemi, [1] Enrico Brunetti,[1] Kijay Kumar Singh,[3] Enza Ferrero[1,2], Ada Funaro[1,2], Alberto L. Horenstein [1,2] and Fabio Malavasi [1,2,4]

[1]Laboratory of Immunogenetics, Department of Medical Sciences and [2]Centro di Ricerca in Medicina Sperimentale (CeRMS), University of Torino Medical School, TORINO, Italy; [3]Cancer Genomics Laboratory, Fondazione Edo ed Elvo Tempia, BIELLA, Italy; [4] Transplantation Immunology Service, Città della Salute Hospital, TORINO, Italy.

Correspondence to:

Fabio Malavasi, M. D.

Lab of Immunogenetics

University of Torino Medical School

Via Santena 19, 10126, TORINO, Italy

Email: fabio.malavasi@unito.it,

Tel. (+39) 011-696-1734, Fax. (+39) 011-696-6155

Abbreviations

ADO= adenosine;

ADP= adenosine diphosphate;

ADPR= adenosine diphosphate ribose;

AMP= adenosine monophosphate;

ART= ADP-ribosyltransferase;

ATP= adenosine 5'-triphosphate;

ATRA= all-*trans* retinoic acid;

cADPR= cyclic adenosine diphosphate ribose;

CD= cluster designation;

CLL= chronic lymphocytic leukemia;

ECM= extracellular matrix;

HLA= human leukocyte antigens;

IP_3= inositol triphosphate;

mAb= monoclonal antibody;

NAD^+= nicotinamide adenine dinucleotide;

$NADP^+$= nicotinamide adenine dinucleotide phosphate;

OT= oxytocin;

OTR= oxytocin receptor;

PARP= poly (ADP-ribose) polimerase;

PBMC= peripheral blood mononuclear cells;

RA= retinoic acid;

RAR= retinoic acid receptor;

RARE= retinoic acid response element;

SNP= single nucleotide polymorphism;

Abstract

CD38 (also known as T10) was identified in the late 1970s in the course of pioneering work carried out at the Dana-Farber Cancer Center (Boston, MA) in the late 1970's that focused on the identification of surface molecules involved in antigen recognition. CD38 was initially found on thymocytes and T lymphocytes, but today we know the molecule is found throughout the immune system, although its expression levels vary. Because of this, CD38 was considered an "activation marker", a term still popular in routine cytofluorimetry. This review summarizes the findings obtained from different approaches, which led to CD38 being re-defined as a multifunctional molecule. Now CD38 and its homologue CD157 (BST-1), contiguous gene duplicates on human chromosome 4 (4p15), are part of a gene family encoding products that modulate the social life of cells by means of bidirectional signals. Both CD38 and CD157 play dual roles as receptors and ectoenzymes, endowed with complex activities related to signaling and cell homeostasis. The structure-function analysis presented here is intended to give clinical scientists and cytometrists a background knowledge of these molecules. The link between CD38/CD157 and human diseases will be explored here in the context of chronic lymphocytic leukemia, myeloma and ovarian carcinoma, although other disease associations are known. Thus CD38 and CD157 have evolved from simple leukocyte activation markers to multifunctional molecules involved in health and disease. Future tasks will be to explore their potential as targets for *in vivo* therapeutic interventions and as regulators of the immune response.

Preface

CD38, also known as T10, epitomizes a story shared by several other molecules, identified within a project aimed at probing the cell surface of human leukocytes using murine monoclonal antibodies (mAbs), reagents which became available in the late 1970s. Both probes and targets were unknown, prompting scientists to devise original strategies in order to identify the structure and functions of the target molecules. The Leukocyte Workshop adopted a strategy that proved successful in the early days of HLA studies: when two or more mAbs reacted with the same target, they were said to form a cluster centered on an unknown molecule. This approach (Cluster Designation, CD) made it possible to identify an amazing number of surface molecules in a limited number of years. However, it took many more years to identify the functions exerted *in vivo* by the majority of these molecules, an effort still far from being concluded.

CD38 was identified during the pioneering work of E.L. Reinherz (Dana-Farber Cancer Center, Boston, MA) (1), focused on the identification of membrane molecules involved in antigen recognition. The fact that those studies were performed in a medical institution influenced the first part of CD38's life history. The molecule was found in thymocytes and in activated normal lymphocytes, and also in selected leukemias and myelomas (2). Its initial deployment in clinical diagnosis gave CD38 the label of "activation marker", a term still quite popular in routine cytofluorimetry.

This review will attempt to summarize and update the findings obtained from different areas of research, which led to the re-definition of CD38 as a multifunctional molecule. We now know that CD38 is joined by CD157 (also referred to as bone marrow stromal cell antigen 1, BST-1) in being part of a gene family coding for products that modulate the social life of cells by means of bidirectional signals (from outside to inside and *vice versa*). Besides being receptors, the same molecules act also as ectoenzymes, endowed with complex activities.

A complete analysis of the structure and functions of CD38 and CD157 in humans is intended to give clinical scientists and cytometrists access to background knowledge which usually found only within the realm of basic science (3). The hope is that this set of information may improve the reading and comprehension of results obtained daily in clinics.

The *CD38* gene family

CD38 and *CD157* genes are located on human chromosome 4 (4p15) (4,5) (Fig.1). Over 98% of the 70 kb *CD38* gene is represented by intronic sequences. The gene encompasses 8 exons: the largest, exon 1, determines the intracytoplasmic and transmembrane regions and the 33 membrane-proximal amino acids of the extracellular region (6,7). *CD38* expression appears to be under a quite complex multilayered transcriptional regulation. The first layer of control lies in the promoter region characterized by the lack of a TATA box and the presence of a CpG island (6), a methylation-controlled region more frequently associated with housekeeping than tissue-specific genes. A second layer of control is likely to be upstream of the CpG island, where potential binding site for transcription factors (*e.g.* T cell transcription factor-1, nuclear factor for interleukin-6) lie (6), while the third gene control lies within the first intron of the *CD38* gene. The 5'-end of the intron 1 contains responsive elements for retinoic acid (8)and peroxisome proliferator-activated receptor Y (PPARY) (9)- Intron 1 is also the location of a single nucleotide polymorphism (SNP) (10) that binds the transcription factor E2A binding site (11) and whose variants are differentially expressed in pathology (*vide infra*).

The *CD157* gene extends for over 35 kb, very close to its paralogue *CD38* and consists of nine exons (4,12). *CD38* and *CD157* are highly conserved, as demonstrated by the fact that exons 2-8 are similar in length and maintain the same phase of intron insertion (13). The 5'-flanking region of the human *CD157* gene shows consensus sequences for interferon responsive elements (Y-IREs and ISRE-like), for nuclear transcription factors (E2A, AP2, AP3, PEA3, C/EBP, CREB, Sp1), for cytokine-responsive factors (NF-IL-6, NF-kB), and for p53 (4). Also this gene lacks a TATA box, suggesting multiple transcription start sites (4,14). All these elements suggest that the *CD157* gene may be up regulated by events such as DNA damage, inflammation and infection, whereas NF-IL-6 and NF-kB binding sites may explain the increased expression of the CD157 molecule in patients affected by rheumatoid arthritis (12).

Tissue distribution of CD38 and CD157

CD38 expression was first observed on thymocytes and T lymphocytes (15). Today, CD38 is considered virtually ubiquitous, at least in the immune system, but with variable expression levels. Table 1 lists the main tissues and cells where CD38 and CD157 are detectable. Because CD157 joined the family relatively later, data on its distribution is still limited.

Cytofluorographic analyses indicate that human CD157 is constitutively expressed by myeloid cells in peripheral blood mononuclear cells (PBMCs). The molecule is also expressed by synovial, vascular endothelial and follicular dendritic cells (16). Moreover, CD157 is also present on other cell types and tissues, such as dermal fibroblasts, human mast cells from lung, uterus, foreskin and peritoneal mesothelial cells, among others (17-21). CD157 was recently reported to be expressed in Paneth cells, where it mediates the effects of calorie restriction and rapamycin on murine intestinal stem cell function (22).

CD38 as a receptor

An initial function attributed to CD38 was the regulation of activation and proliferation of human T lymphocytes. Early functional studies of unidentified molecules were pursued by monitoring the effects induced by the engagement of different domains of the target by a panel of specific mAbs. The rationale was that effects induced by ligation with a surrogate mAb would have been mimicking those induced by a ligand still unknown at the time. CD38 engagement was followed by the activation of selected PBMC populations (23). The identification of a first putative ligand was obtained by exploiting the observation that human T lymphocytes tended to adhere to endothelial cells (24). Experiments blocking this adhesion concluded that CD31 [also known as PECAM-1, a member of the immunoglobulin (Ig) superfamily crucial to leukocyte adhesion and transmigration (25)] was a non-substrate ligand for CD38. It was later demonstrated that CD38/CD31 interactions trigger the same signaling cascade and recapitulate the biological events observed using agonistic mAbs (26,27). The interplay between CD38 and CD31 is crucial for leukocyte migration through the endothelium (28). The CD38/CD31 cross-talk has been extensively analyzed in a number of different environments ranging from T to B, NK, and myeloid cells, in normal and pathological conditions (29). CD38-mediated signals are regulated at distinct levels: the first level concerns the ultrastructural organization of the molecule, which exists both in monomeric (2) and dimeric (30) (or multimeric) type I forms (31). A flip-flop mechanism of membrane positioning has been recently proposed, with a type III form of CD38 displaying its catalytic site in the cytoplasm (32). The second level is based on the dynamic localization of CD38 in lipid microdomains within the plasma membrane. Lateral associations with other proteins, which vary according to the cell lineage, determine a third level of control. Lipid raft localization and association with professional signaling complexes are pre-requisites for signals mediated through CD38 (33).

CD157 as a receptor

The receptor and signaling features of CD157 have also been investigated by using agonistic mAbs to mimic putative ligand(s), too. By doing so, it has been demonstrated that CD157 ligation induces tyrosine phosphorylation of a 130 kDa protein, identified as focal adhesion kinase (FAK), in myeloid cell lines (34,35), and that CD157 engagement regulates Ca^{2+} homeostasis and mediates superoxide (O^{2-}) production in the human myelomonocytic U937 line (36). Accumulating evidence indicates that CD157 is a key player in the control of leukocyte adhesion, migration and diapedesis (37,38). In this context, CD157 behaves as a receptor by establishing lateral interactions with other transmembrane molecules, thus overcoming its structural limitation (*i.e.*, of being a GPI-anchored molecule) and acquiring the ability to transduce signals (13). More in detail, CD157 interacts with β1 and β2 integrins and Ab-induced cross-linking of CD157 promotes relocation of these complexes into detergent-resistant membrane domains (21). Moreover, CD157 effectively contributes to the integrin-driven signaling network, which is critical during leukocyte transmigration, leading to optimal phosphorylation of tyrosine kinase receptors and activation of PI3K and MAPK signaling cascades (39).

CD38 and CD157 enzymatic functions

The enzymatic functions of the two proteins were investigated after the enzyme ADP-ribosyl cyclase (ADPRC, purified from the mollusk *Aplysia californica*) was observed to display a striking similarity in protein sequence with human CD38 (40). CD38 is a multifunctional enzyme that catalyzes the synthesis of cyclic ADP-ribose (cADPR) from nicotinamide adenine dinucleotide (NAD^+) and also mediates the hydrolysis of cADPR to ADPR (41-44). In acidic conditions, CD38 catalyzes the generation of nicotinic acid adenine dinucleotide phosphate (NAADP) from nicotinamide adenine dinucleotide phosphate ($NADP^+$) (45). cADPR, ADPR and NAADP bind different receptors and channels involved in the regulation of cytoplasmic Ca^{2+} fluxes, activating signaling pathways critical for several biological processes [*e.g.*, lymphocyte proliferation (46,47), cardiac (48) and intestinal longitudinal muscle contraction (49), glucose-induced insulin release in pancreas (50)]. The role of CD38 and of its products in regulating a wide range of physiological functions, is indicated by the multiple defects revealed in *CD38* knock-out (KO) mice. These include impairment of neutrophil chemotaxis, defective oxytocin (OT) release, and aberrant social behavior (51).

The recombinant soluble extracellular domain of CD38 mediates ADP-ribosylation of cysteine residues of several proteins, including CD38 itself (52). CD38 is also modified by ecto-ADP-ribosyltransferases (ARTs). Arginine ADP-ribosylation results in inactivation of both cyclase and hydrolase activities, whereas cysteine ADP-ribosylation leads only to inhibition of the hydrolase activity. Arginine ADP-ribosylation causes a decrease in intracellular cADPR and a subsequent decrease in Ca^{2+} influx, with consequent death of activated T lymphocytes (53). Moreover, CD38 is the major NAD glycohydrolase (NADase) in mammalian cells, regulating intracellular NAD^+ levels. CD38 thus modulates the activity of sirtuins, intracellular NAD^+-dependent deacetylases implicated in ageing, cell protection and energy metabolism (54,55).

CD157 also cleaves extracellular NAD^+, generating cADPR and ADPR (13,36). However, the catalytic efficiency of CD157 in generating cADPR is much lower than that of CD38 (13).

The products derived from NAD^+ cleavage operated by CD38 or CD157 can also act as extracellular immunomodifiers (56). Emerging data indicate that these products may operate outside the cell as paracrine factors (57). Moreover, the catalytic reactions generate substrates for ARTs and poly-ADP-ribose polymerases (PARPs) involved in cell signaling, DNA repair and apoptosis (56).

CD38 is also found in soluble form in normal and pathological fluids (58) and in exosomes, which are membrane vesicles secreted by B cells and likely a component of an intercellular communication network (59).

CD38 and disease

Chronic lymphocytic leukemia (CLL). CLL is a common adult leukemia which results from the accumulation of small B ($CD19^+/CD5^+/CD23^+$) lymphocytes in blood, bone marrow (BM), lymph nodes (LN) and other lymphoid tissues (60). The latter districts represent permissive niches, where lymphocytes can proliferate in response to microenvironmental signals (61). Elevated expression of CD38 in CLL cells is generally associated with advanced disease stage, higher incidence of lymphadenopathy, high-risk cytogenetics, shorter lymphocyte doubling time, shorter time to first treatment and poorer response to therapy. Besides being a prognostic marker, CD38 is a component of a molecular network which delivers growth and survival signals to CLL cells (62). CD38 acts as a receptor in leukemic cells and its signals are mediated by ZAP70, another negative prognostic factor for the disease and a limiting factor for CD38-mediated activation (33,63). CD38 can work in association with chemokines and their receptors, mainly CXCL12/CXCR4, influencing the migratory responses and contributing to the recirculation of neoplastic cells from blood to lymphoid organs (64) and with specific adhesion molecules belonging to the integrin family (65,66).

Multiple Myeloma (MM). MM is a malignancy characterized by accumulation of monoclonal plasma cells in BM, a high concentration of monoclonal Igs in serum and urine and lytic bone lesions. The proliferation of neoplastic plasma cells in MM interferes with the normal production of blood cells, while the monoclonal Ig impairs humoral immunity.

There are several issues suggesting that CD38 plays significant role(s) in MM. First, CD38 is expressed by plasma cells (normal and tumoral) at top levels of cell surface density. Secondly, experiments in murine models showed that CD38 is a key regulator of OT levels in biological fluids while OT is also released by human osteoblasts (67). A receptor for OT (OTR) is detectable on myeloma cells and derived lines (unpublished results, 2012). CD38 is expressed by osteoblasts and osteoclasts, where it implements signals leading to IL-6 release and inhibition of bone resorption (68,69).

In light of these considerations, plasma cells (and their malignant counterpart) and bone niches are good testing grounds for assessing the presence of a connection between ectoenzymes and neuropeptides (70). The system is closed, and nucleosides represent additional signals to those led by cytokines/chemokines and other conventional regulators: ATP and NAD^+ operating *in loco* may complement the physiological regulatory systems of plasma cells.

Acute promyelocytic leukemia (APL). APL is a unique subtype of acute leukemia, which causes an arrest of leukocyte differentiation at the promyelocyte stage. Retinoic acid (RA) is included in therapeutical protocols for its ability to induce the differentiation of leukemic cells into mature granulocytes. This therapy may be associated with retinoic acid syndrome (RAS), a clinical manifestation characterized by fever, dyspnea, pulmonary edema and infiltrates (71). Normal granulocytes are $CD38^-$, while RA-treated APL cells express high amounts of the molecule (72,73). The aberrant expression of CD38 on leukemic cells enhances their propensity to interact with CD31, expressed by lung endothelial cells, resulting in a local production of inflammatory cytokines, apoptosis of endothelial cells and eventually contributing to the development of RAS (71).

CD157 and disease

Ovarian cancer. Leukocyte extravasation is a process which shares similarities with metastatic infiltration to secondary organs. CD157 is expressed by mesothelial cells, which share biological properties and embriological origin with ovarian surface epithelial cells (18). A working hypothesis was that CD157 might have also been expressed by epithelial ovarian cancer cells, guiding interactions between tumor cells and mesothelium. If confirmed, CD157 could have been involved in the control of ovarian cancer dissemination. This hypothesis was independently supported by a report that *BST-1/CD157* was among genes that were differentially expressed in primary cultures of epithelial ovarian cancer cells when compared to their normal counterparts (74). The results obtained have confirmed the hypothesis: indeed, CD157 is expressed by >90% of epithelial ovarian cancers and is involved in the interactions between epithelial ovarian cancer cells, extracellular matrix proteins and mesothelial cells. All these steps ultimately control migration of tumor cells and invasion of surrounding tissues. High expression of CD157 in human ovarian cancers is associated with clinical aggressiveness, confirming the role of CD157 as an independent prognostic factor of tumor relapse shortly after surgical debunking (75). The functional contribution of CD157 to the progression of epithelial ovarian cancer relies on its ability to switch on a differentiation program, which allows neoplastic cells to overcome the rules of epithelial tissue architecture, turning them toward a more mesenchymal state. The outcome in clinics is that these events boost the malignant progression of the disease (76).

Parkinson's disease. The *BST-1/CD157* gene has recently been associated with Parkinson's disease. Indeed, *BST-1* SNPs rs11931532, rs12645693, rs4698412 and rs4538475 were identified as risk factors in sporadic late-onset Parkinson's disease in a Japanese genome-wide association (GWA) study. rs4538475 showed the strongest association (77). The association between *BST-1* (rs4698412 SNP) and Parkinson's disease was confirmed in the European population (78), even if not present in a Northern Han Chinese population (79). A meta-analysis of GWA studies performed on a Northern American and European population indicates that *BST-1* did surpass the threshold for genome-wide significance (80). A conclusion is that ethnicity significantly influences the association between *BST-1/CD157* locus and Parkinson's disease.

Role of the CD38 family in other human diseases

Other human diseases showing correlation with CD38 or CD157 are reviewed in (13). The polymorphisms associated with genetic susceptibility to CLL have also been studied in other diseases, including systemic lupus erythematosus (SLE), where the CC genotype increases susceptibility to and the GC genotype confers protection from discoid rash development (81).

CD38 is also reported as target of autoantibodies in type 1 and type 2 diabetes mellitus (82,83), as well as in SLE (Pavòn E.J. *et al.*, 2013 in press).

Results from *CD38* KO mice have highlighted a role for CD38 in the release of OT from the neurohypophysis (51). The clinical diagnosis of a deficit in short-term social memory in these mice has drawn the attention to human conditions sharing this feature. One of these is autism spectrum disorder (ASD). Indeed, some polymorphisms of the *CD38* gene (rs6449197, rs3796863 and rs1800561) are associated with ASD (84,85). Beside this, it has recently been reported that a quantitative trait of CD38 expression correlates with social functions in ASD (86). The study was performed by analyzing CD38 expression in lymphoblastoid cell lines derived from PBMCs of ASD patients, which has been showed to be lower than CD38 expression in similar lines obtained from the parents. A possible explanation is that reduced cell surface expression negatively influences the enzymatic performances of CD38 in ASD, leading to a malfunction of the CD38/OT axis in this disorder (51,87). A further step of the study investigated the role of retinoids in up-regulating cell surface CD38, potentially suggesting a new therapeutic approach (88,89).

Emerging working hypothesis

From a phylogenetic perspective, the human *CD38* and *BST-1* genes share gene structure (6). A similar intron/exon organization is also shared by their respective orthologs in mouse, chicken and frog, and by the ADPRCs from *Aplysia*, *Schistosoma mansoni* and the purple sea urchin (*Strongylocentrotus purpuratus*) (NCBI and Ensembl genome databases). Thus the ADPRCs are believed to derive from a common ancestral gene. As the common taxonomic denominator of the ADPRC-bearing species is that they all belong to the bilateria (*i.e.*, animals with front/back and left/right symmetry), this suggests that the origin of the ADPRCs dates back to the last common bilaterian ancestor, about 555 million years ago (mya).

Read from an applied perspective, the evidence derived from a phylogenetic analysis of the CD38 family, prompts the hypothesis that early CD38/CD157 precursors were components of innate immunity, and that their passage to the surface of immune cells evolved in parallel with the transition to adaptive immunity (Fig.2). We hypothesized that CD38 (CD157 having not been considered in this context yet) might be part of a circuit generating activation or suppression of immune responses according to the environment. CD38 would also be a component of one of the multiple strategies adopted by tumoral cells to fool the immune system. The enzymes CD39 (ectonucleoside triphosphate diphosphohydrolase 1) and CD73 (ecto-5' nucleotidase) govern a metabolic pathway leading to the generation of adenosine (ADO) and thus likely to lead to immunosuppression when ADO is taken up by a specific receptor expressed on lymphocytes. This pathway is flanked by different mechanisms led by NAD^+, which is consumed by the CD38/CD157 ectoenzymes and - in some systems - by ART2. Endogenous signals released during physiological or pathological conditions may contribute to alarm the innate immune system, accompanied by the production of pro-inflammatory cytokines. In addition, extracellular NAD^+ may influence the immune system by altering the balance between activation and suppression led by specific lymphocyte subsets in different districts and organs.

CD38 has recently been associated with functions exerted by regulatory T cells (Tregs) in murine models. High CD38 espression in $Foxp3^+/CD4^+$ T cell populations correlates with extremely powerful modulatory properties of $CD4^+$ regulatory T lymphocytes (90). Furthermore, CD38 is part of the Treg transcriptional signature (91,92). The role of CD38 has been confirmed in *CD38* KO mice, where NAD^+ influences the survival, phenotype and function of Treg cells and provides proof of principle that acting on the ART2/P2X7 system may be a new strategy for manipulating these cells *in vivo* (93). A recent report states that $CD8^+/CD38^{high}$ T lymphocytes have strong immunosuppressive capabilities *in vitro* and *in vivo*. This subset may possess a regulatory potential that could work together with the innate immune response and control immune homeostasis during inflammation (94).

PC-1 (also known as CD203a) is a cell surface enzyme with nucleotidase pyrophosphatase phosphodiesterase (ENPP1) activity (95). We have recently verified the existence of a new pathway led by the CD38/PC-1 network, which provides substrates to CD73 and consequently feeds the production of ADO in different organs (Figure 3). Detailed knowledge about this pathway has been hindered by the fact that PC-1/CD203a had only been studied on human cells to answer questions related to diabetes and by technical difficulties in analyzing ADO and different substrates *in vitro* and in biological fluids (Horenstein A.L., 2013 submitted). However, a link between CD38 and CD73 was highlighted some years ago (96).

It is not known to what extent this unconventional ectoenzyme network will be able to provide some contribution to the generation of local tolerance in different disease models, such as the BM microenvironment in MM (Fig.4) and the mesenchymal stem cell (MSC) niche in recurrent pregnancy losses (Cecati M. *et al.*, 2013 submitted). Preliminary results show the existence of the CD38/PC-1/CD73 axis in different cells, where tolerance plays a part in maintaining homeostasis. Studies are currently being conducted on MM and melanoma patient samples to determine a possible strategy of immune escape operated by CD38 and CD157. In these samples, a chain of ectoenzymes capable of generating ADO independently from CD39 has already been confirmed (Morandi F *et al*., in preparation).

There are still several unanswered questions concerning CD38 and CD157. One deals with CD38 tissue distribution, which ranges from discrete expression during lymphocyte differentiation to a limited presence during the physiological resting state of both T and B cells. The molecule is strongly re-expressed by cells undergoing activation and in selected leukemias. In contrast to the notion of CD38 as an activation marker, terminally differentiated plasma cells (and derived tumors) express the highest surface density among human cells. This means that ontogeny has still a lot to teach and to unveil.

Furthermore, the fact that CD38 and CD157 are ectoenzymes should no longer be considered oddities in leukocyte biology; on the contrary, more than 4% of the molecules expressed on the surface of human cells show enzymatic features.

Nucleotide-metabolizing ectoenzymes constitute a subgroup of a larger family of ectoenzymes, involved in the catabolism and scavenging of extracellular nucleotides. This process results in the synthesis of compounds that play critical roles in cell homeostasis and metabolism, and not simply nucleotide recycling. Initially it was thought that nucleotide-metabolizing ectoenzymes would operate in environments containing only trace amounts of the substrate and that the final product were to be used prevalently inside the cell. This view has later been revised, and it is now known that substrates and final products are not topologically confined to one side or the other of the plasma membrane.

The author's Lab tackled these issues by 1) focusing exclusively on human model, 2) using the ontogeny and phylogeny of these molecules as a source of physiological clues and 3) trying to infer information from disease models, the best experiments performed by nature itself.

The aim of this review has been that of overviewing old and new facts enriching the field related to the CD38 family products and on the role of ectoenzymes in general. Even though interpretations are kept to a minimum, the perspective of this Laboratory has inevitably pervaded the vision of the gene family.

In conclusion, CD38 and CD157 have shifted from being considered simple activation markers of leukocyte populations to being recognized as molecules exerting multiple functions in health and disease. A last daunting task is now to demonstrate that these surface structures may become appropriate targets for *in vivo* therapeutic interventions (Chillemi A. *et al*., 2013 submitted) and regulators of the immune response (Horenstein A.L. *et al.*, 2013 submitted).

Acknowledgements

This work was supported by grants from PRIN (Ministry of Education, University, and Innovation), from FIRB (Fondo per gli Investimenti della Ricerca di Base), from ex-60% Project (University of Torino) and from Associazione Italiana Ricerca Cancro (AIRC grant 11602 and partly AIRC 5x1000).

Antonella Chillemi and Valeria Quarona are students of the PhD Program "Biomedical Sciences and Oncology", University of Torino. Andrea Zito provided much-appreciated technical assistance.

The Fondazione Ricerca Medicina Sperimentale (FIRMS) provided a valuable assistance and support to this research project.

The authors report no biomedical financial interests or potential conflicts of interest.

References

1. Reinherz EL, Kung PC, Goldstein G, Levey RH, Schlossman SF. Discrete stages of human intrathymic differentiation: analysis of normal thymocytes and leukemic lymphoblasts of T-cell lineage. Proc Natl Acad Sci U S A 1980;77:1588-92.
2. Terhorst C, van Agthoven A, LeClair K, Snow P, Reinherz E, Schlossman S. Biochemical studies of the human thymocyte cell-surface antigens T6, T9 and T10. Cell 1981;23:771-80.
3. Maecker HT, McCoy JP, Nussenblatt R. Standardizing immunophenotyping for the Human Immunology Project. Nat Rev Immunol 2012;12:191-200.
4. Muraoka O, Tanaka H, Itoh M, Ishihara K, Hirano T. Genomic structure of human BST-1. Immunol Lett 1996;54:1-4.
5. Katz F, Povey S, Parkar M, Schneider C, Sutherland R, Stanley K, Solomon E, Greaves M. Chromosome assignment of monoclonal antibody-defined determinants on human leukemic cells. Eur J Immunol 1983;13:1008-13.
6. Ferrero E, Malavasi F. Human CD38, a leukocyte receptor and ectoenzyme, is a member of a novel eukaryotic gene family of nicotinamide adenine dinucleotide+-converting enzymes: extensive structural homology with the genes for murine bone marrow stromal cell antigen 1 and aplysian ADP-ribosyl cyclase. J Immunol 1997;159:3858-65.
7. Ferrero E, Saccucci F, Malavasi F. The making of a leukocyte receptor: origin, genes and regulation of human CD38 and related molecules. Chem Immunol 2000;75:1-19.
8. Kishimoto H, Hoshino S, Ohori M, Kontani K, Nishina H, Suzawa M, Kato S, Katada T. Molecular mechanism of human CD38 gene expression by retinoic acid. Identification of retinoic acid response element in the first intron. J Biol Chem 1998;273:15429-34.
9. Song EK, Lee YR, Kim YR, Yeom JH, Yoo CH, Kim HK, Park HM, Kang HS, Kim JS, Kim UH and others. NAADP Mediates Insulin-Stimulated Glucose Uptake and Insulin Sensitization by PPARgamma in Adipocytes. Cell Rep 2012;2:1607-19.

10. Ferrero E, Saccucci F, Malavasi F. The human CD38 gene: polymorphism, CpG island, and linkage to the CD157 (BST-1) gene. Immunogenetics 1999;49:597-604.

11. Saborit-Villarroya I, Vaisitti T, Rossi D, D'Arena G, Gaidano G, Malavasi F, Deaglio S. E2A is a transcriptional regulator of CD38 expression in chronic lymphocytic leukemia. Leukemia 2011;25:479-88.

12. Ortolan E, Vacca P, Capobianco A, Armando E, Crivellin F, Horenstein A, Malavasi F. CD157, the Janus of CD38 but with a unique personality. Cell Biochem Funct 2002;20:309-22.

13. Malavasi F, Deaglio S, Funaro A, Ferrero E, Horenstein AL, Ortolan E, Vaisitti T, Aydin S. Evolution and function of the ADP ribosyl cyclase/CD38 gene family in physiology and pathology. Physiol Rev 2008;88:841-86.

14. Dong C, Willerford D, Alt FW, Cooper MD. Genomic organization and chromosomal localization of the mouse Bp3 gene, a member of the CD38/ADP-ribosyl cyclase family. Immunogenetics 1996;45:35-43.

15. Bhan AK, Reinherz EL, Poppema S, McCluskey RT, Schlossman SF. Location of T cell and major histocompatibility complex antigens in the human thymus. J Exp Med 1980;152:771-82.

16. Hernandez-Campo PM, Almeida J, Sanchez ML, Malvezzi M, Orfao A. Normal patterns of expression of glycosylphosphatidylinositol-anchored proteins on different subsets of peripheral blood cells: a frame of reference for the diagnosis of paroxysmal nocturnal hemoglobinuria. Cytometry B Clin Cytom 2006;70:71-81.

17. Ghannadan M, Baghestanian M, Wimazal F, Eisenmenger M, Latal D, Kargul G, Walchshofer S, Sillaber C, Lechner K, Valent P. Phenotypic characterization of human skin mast cells by combined staining with toluidine blue and CD antibodies. J Invest Dermatol 1998;111:689-95.

18. Ross JA, Ansell I, Hjelle JT, Anderson JD, Miller-Hjelle MA, Dobbie JW. Phenotypic mapping of human mesothelial cells. Adv Perit Dial 1998;14:25-30.

19. Shimaoka Y, Attrep JF, Hirano T, Ishihara K, Suzuki R, Toyosaki T, Ochi T, Lipsky PE. Nurse-like cells from bone marrow and synovium of patients with rheumatoid arthritis promote survival and enhance function of human B cells. J Clin Invest 1998;102:606-18.

20. Wimazal F, Ghannadan M, Muller MR, End A, Willheim M, Meidlinger P, Schernthaner GH, Jordan JH, Hagen W, Agis H and others. Expression of homing receptors and related molecules on human mast cells and basophils: a comparative analysis using multi-color flow cytometry and toluidine blue/immunofluorescence staining techniques. Tissue Antigens 1999;54:499-507.

21. Lavagno L, Ferrero E, Ortolan E, Malavasi F, Funaro A. CD157 is part of a supramolecular complex with CD11b/CD18 on the human neutrophil cell surface. J Biol Regul Homeost Agents 2007;21:5-11.

22. Yilmaz OH, Katajisto P, Lamming DW, Gultekin Y, Bauer-Rowe KE, Sengupta S, Birsoy K, Dursun A, Yilmaz VO, Selig M and others. mTORC1 in the Paneth cell niche couples intestinal stem-cell function to calorie intake. Nature 2012;486:490-5.

23. Alessio M, Roggero S, Funaro A, De Monte LB, Peruzzi L, Geuna M, Malavasi F. CD38 molecule: structural and biochemical analysis on human T lymphocytes, thymocytes, and plasma cells. J Immunol 1990;145:878-84.

24. Dianzani U, Malavasi F. Lymphocyte adhesion to endothelium. Crit Rev Immunol 1995;15:167-200.

25. Newman PJ. Switched at birth: a new family for PECAM-1. J Clin Invest 1999;103:5-9.

26. Deaglio S, Dianzani U, Horenstein AL, Fernandez JE, van Kooten C, Bragardo M, Funaro A, Garbarino G, Di Virgilio F, Banchereau J and others. Human CD38 ligand. A 120-KDA protein predominantly expressed on endothelial cells. J. Immunol. 1996;156:727-34.

27. Horenstein AL, Stockinger H, Imhof BA, Malavasi F. CD38 binding to human myeloid cells is mediated by mouse and human CD31. Biochem. J. 1998;330 (Pt 3):1129-35.

28. Deaglio S, Morra M, Mallone R, Ausiello CM, Prager E, Garbarino G, Dianzani U, Stockinger H, Malavasi F. Human CD38 (ADP-ribosyl cyclase) is a counter-receptor of CD31, an Ig superfamily member. J Immunol 1998;160:395-402.

29. Deaglio S, Mallone R, Baj G, Arnulfo A, Surico N, Dianzani U, Mehta K, Malavasi F. CD38/CD31, a receptor/ligand system ruling adhesion and signaling in human leukocytes. Chem Immunol 2000;75:99-120.

30. Mallone R, Ferrua S, Morra M, Zocchi E, Mehta K, Notarangelo LD, Malavasi F. Characterization of a CD38-like 78-kilodalton soluble protein released from B cell lines derived from patients with X-linked agammaglobulinemia. J Clin Invest 1998;101:2821-30.

31. Hara-Yokoyama M, Kukimoto-Niino M, Terasawa K, Harumiya S, Podyma-Inoue KA, Hino N, Sakamoto K, Itoh S, Hashii N, Hiruta Y and others. Tetrameric interaction of the ectoenzyme CD38 on the cell surface enables its catalytic and raft-association activities. Structure 2012;20:1585-95.

32. Zhao YJ, Lam CM, Lee HC. The membrane-bound enzyme CD38 exists in two opposing orientations. Sci Signal 2012;5:ra67.

33. Deaglio S, Vaisitti T, Billington R, Bergui L, Omede P, Genazzani AA, Malavasi F. CD38/CD19: a lipid raft-dependent signaling complex in human B cells. Blood 2007;109:5390-8.

34. Hussain AM, Lee HC, Chang CF. Functional expression of secreted mouse BST-1 in yeast. Protein Expr Purif 1998;12:133-7.

35. Okuyama Y, Ishihara K, Kimura N, Hirata Y, Sato K, Itoh M, Ok LB, Hirano T. Human BST-1 expressed on myeloid cells functions as a receptor molecule. Biochem Biophys Res Commun 1996;228:838-45.

36. Ishihara K, Hirano T. BST-1/CD157 regulates the humoral immune responses in vivo. Chem Immunol 2000;75:235-55.

37. Funaro A, Ortolan E, Ferranti B, Gargiulo L, Notaro R, Luzzatto L, Malavasi F. CD157 is an important mediator of neutrophil adhesion and migration. Blood 2004;104:4269-78.

38. Ortolan E, Tibaldi EV, Ferranti B, Lavagno L, Garbarino G, Notaro R, Luzzatto L, Malavasi F, Funaro A. CD157 plays a pivotal role in neutrophil transendothelial migration. Blood 2006;108:4214-22.

39. Lo Buono N, Parrotta R, Morone S, Bovino P, Nacci G, Ortolan E, Horenstein AL, Inzhutova A, Ferrero E, Funaro A. The CD157-integrin partnership controls transendothelial migration and adhesion of human monocytes. J Biol Chem 2011;286:18681-91.

40. States DJ, Walseth TF, Lee HC. Similarities in amino acid sequences of Aplysia ADP-ribosyl cyclase and human lymphocyte antigen CD38. Trends Biochem Sci 1992;17:495.

41. Howard M, Grimaldi JC, Bazan JF, Lund FE, Santos-Argumedo L, Parkhouse RM, Walseth TF, Lee HC. Formation and hydrolysis of cyclic ADP-ribose catalyzed by lymphocyte antigen CD38. Science 1993;262:1056-9.

42. Kim H, Jacobson EL, Jacobson MK. Synthesis and degradation of cyclic ADP-ribose by NAD glycohydrolases. Science 1993;261:1330-3.

43. Zocchi E, Franco L, Guida L, Calder L, De Flora A. Self-aggregation of purified and membrane-bound erythrocyte CD38 induces extensive decrease of its ADP-ribosyl cyclase activity. FEBS Lett 1995;359:35-40.

44. Takasawa S, Tohgo A, Noguchi N, Koguma T, Nata K, Sugimoto T, Yonekura H, Okamoto H. Synthesis and hydrolysis of cyclic ADP-ribose by human leukocyte antigen CD38 and inhibition of the hydrolysis by ATP. The Journal of biological chemistry 1993;268:26052-4.

45. Aarhus R, Graeff RM, Dickey DM, Walseth TF, Lee HC. ADP-ribosyl cyclase and CD38 catalyze the synthesis of a calcium-mobilizing metabolite from NADP. J Biol Chem 1995;270:30327-33.

46. Guse AH, da Silva CP, Berg I, Skapenko AL, Weber K, Heyer P, Hohenegger M, Ashamu GA, Schulze-Koops H, Potter BV and others. Regulation of calcium signalling in T lymphocytes by the second messenger cyclic ADP-ribose. Nature 1999;398:70-3.

47. Morra M, Zubiaur M, Terhorst C, Sancho J, Malavasi F. CD38 is functionally dependent on the TCR/CD3 complex in human T cells. Faseb J 1998;12:581-92.

48. Meszaros LG, Bak J, Chu A. Cyclic ADP-ribose as an endogenous regulator of the non-skeletal type ryanodine receptor Ca2+ channel. Nature 1993;364:76-9.

49. Kuemmerle JF, Makhlouf GM. Agonist-stimulated cyclic ADP ribose. Endogenous modulator of Ca(2+)-induced Ca2+ release in intestinal longitudinal muscle. J Biol Chem 1995;270:25488-94.

50. Takasawa S, Nata K, Yonekura H, Okamoto H. Cyclic ADP-ribose in insulin secretion from pancreatic beta cells. Science 1993;259:370-3.

51. Jin D, Liu HX, Hirai H, Torashima T, Nagai T, Lopatina O, Shnayder NA, Yamada K, Noda M, Seike T and others. CD38 is critical for social behaviour by regulating oxytocin secretion. Nature 2007;446:41-5.

52. Grimaldi JC, Balasubramanian S, Kabra NH, Shanafelt A, Bazan JF, Zurawski G, Howard MC. CD38-mediated ribosylation of proteins. J Immunol 1995;155:811-7.

53. Han MK, Cho YS, Kim YS, Yim CY, Kim UH. Interaction of two classes of ADP-ribose transfer reactions in immune signaling. J Biol Chem 2000;275:20799-805.

54. Malavasi F, Deaglio S, Zaccarello G, Horenstein AL, Chillemi A, Audrito V, Serra S, Gandione M, Zitella A, Tizzani A. The hidden life of NAD+-consuming ectoenzymes in the endocrine system. J Mol Endocrinol 2010;45:183-91.

55. Audrito V, Vaisitti T, Rossi D, Gottardi D, D'Arena G, Laurenti L, Gaidano G, Malavasi F, Deaglio S. Nicotinamide blocks proliferation and induces apoptosis of chronic lymphocytic leukemia cells through activation of the p53/miR-34a/SIRT1 tumor suppressor network. Cancer Res 2011;71:4473-83.

56. Haag F, Adriouch S, Brass A, Jung C, Moller S, Scheuplein F, Bannas P, Seman M, Koch-Nolte F. Extracellular NAD and ATP: Partners in immune cell modulation. Purinergic Signal 2007;3:71-81.

57. Moreschi I, Bruzzone S, Bodrato N, Usai C, Guida L, Nicholas RA, Kassack MU, Zocchi E, De Flora A. NAADP+ is an agonist of the human P2Y11 purinergic receptor. Cell Calcium 2008;43:344-55.

58. Funaro A, Horenstein AL, Calosso L, Morra M, Tarocco RP, Franco L, De Flora A, Malavasi F. Identification and characterization of an active soluble form of human CD38 in normal and pathological fluids. Int Immunol 1996;8:1643-50.

59. Zumaquero E, Munoz P, Cobo M, Lucena G, Pavon EJ, Martin A, Navarro P, Garcia-Perez A, Ariza-Veguillas A, Malavasi F and others. Exosomes from human lymphoblastoid B cells express enzymatically active CD38 that is associated with signaling complexes containing CD81, Hsc-70 and Lyn. Exp Cell Res 2010;316:2692-706.

60. Chiorazzi N, Ferrarini M. Cellular origin(s) of chronic lymphocytic leukemia: cautionary notes and additional considerations and possibilities. Blood 2011;117:1781-91.

61. Chiorazzi N. Implications of new prognostic markers in chronic lymphocytic leukemia. Hematology Am Soc Hematol Educ Program 2012;2012:76-87.

62. Malavasi F, Deaglio S, Damle R, Cutrona G, Ferrarini M, Chiorazzi N. CD38 and chronic lymphocytic leukemia: a decade later. Blood 2011;118:3470-8.

63. Deaglio S, Capobianco A, Bergui L, Durig J, Morabito F, Duhrsen U, Malavasi F. CD38 is a signaling molecule in B-cell chronic lymphocytic leukemia cells. Blood 2003;102:2146-55.

64. Vaisitti T, Aydin S, Rossi D, Cottino F, Bergui L, D'Arena G, Bonello L, Horenstein AL, Brennan P, Pepper C and others. CD38 increases CXCL12-mediated signals and homing of chronic lymphocytic leukemia cells. Leukemia 2010;24:958-69.

65. Zucchetto A, Benedetti D, Tripodo C, Bomben R, Dal Bo M, Marconi D, Bossi F, Lorenzon D, Degan M, Rossi FM and others. CD38/CD31, the CCL3 and CCL4 chemokines, and CD49d/vascular cell adhesion molecule-1 are interchained by sequential events sustaining chronic lymphocytic leukemia cell survival. Cancer Res 2009;69:4001-9.

66. Zucchetto A, Vaisitti T, Benedetti D, Tissino E, Bertagnolo V, Rossi D, Bomben R, Dal Bo M, Del Principe MI, Gorgone A and others. The CD49d/CD29 complex is physically and functionally associated with CD38 in B-cell chronic lymphocytic leukemia cells. Leukemia 2012;26:1301-12.

67. Colaianni G, Di Benedetto A, Zhu LL, Tamma R, Li J, Greco G, Peng Y, Dell'Endice S, Zhu G, Cuscito C and others. Regulated production of the pituitary hormone oxytocin from murine and human osteoblasts. Biochem Biophys Res Commun 2011;411:512-5.

68. Iqbal J, Zaidi M. Extracellular NAD+ metabolism modulates osteoclastogenesis. Biochem Biophys Res Commun 2006;349:533-9.

69. Colaianni G, Sun L, Di Benedetto A, Tamma R, Zhu LL, Cao J, Grano M, Yuen T, Colucci S, Cuscito C and others. Bone marrow oxytocin mediates the anabolic action of estrogen on the skeleton. J Biol Chem 2012;287:29159-67.

70. Krause DS, Scadden DT, Preffer FI. The hematopoietic stem cell niche-home for friend and foe? Cytometry B Clin Cytom 2012.

71. Gao Y, Camacho LH, Mehta K. Retinoic acid-induced CD38 antigen promotes leukemia cells attachment and interferon-gamma/interleukin-1beta-dependent apoptosis of endothelial cells: implications in the etiology of retinoic acid syndrome. Leuk Res 2007;31:455-63.

72. Drach J, McQueen T, Engel H, Andreeff M, Robertson KA, Collins SJ, Malavasi F, Mehta K. Retinoic acid-induced expression of CD38 antigen in myeloid cells is mediated through retinoic acid receptor-alpha. Cancer Res 1994;54:1746-52.

73. Mehta K, Shahid U, Malavasi F. Human CD38, a cell-surface protein with multiple functions. Faseb J 1996;10:1408-17.

74. Le Page C, Sanceau J, Drapier JC, Wietzerbin J. Inhibitors of ADP-ribosylation impair inducible nitric oxide synthase gene transcription through inhibition of NF kappa B activation. Biochem Biophys Res Commun 1998;243:451-7.

75. Ortolan E, Arisio R, Morone S, Bovino P, Lo-Buono N, Nacci G, Parrotta R, Katsaros D, Rapa I, Migliaretti G and others. Functional role and prognostic significance of CD157 in ovarian carcinoma. J Natl Cancer Inst 2010;102:1160-77.

76. Morone S, Lo-Buono N, Parrotta R, Giacomino A, Nacci G, Brusco A, Larionov A, Ostano P, Mello-Grand M, Chiorino G and others. Overexpression of CD157 contributes to epithelial ovarian cancer progression by promoting mesenchymal differentiation. PLoS One 2012;7:e43649.

77. Satake W, Nakabayashi Y, Mizuta I, Hirota Y, Ito C, Kubo M, Kawaguchi T, Tsunoda T, Watanabe M, Takeda A and others. Genome-wide association study identifies common variants at four loci as genetic risk factors for Parkinson's disease. Nat Genet 2009;41:1303-7.

78. Saad M, Lesage S, Saint-Pierre A, Corvol JC, Zelenika D, Lambert JC, Vidailhet M, Mellick GD, Lohmann E, Durif F and others. Genome-wide association study confirms BST1 and suggests a locus on 12q24 as the risk loci for Parkinson's disease in the European population. Hum Mol Genet 2011;20:615-27.

79. Zhu LH, Luo XG, Zhou YS, Li FR, Yang YC, Ren Y, Pang H. Lack of association between three single nucleotide polymorphisms in the PARK9, PARK15, and BST1 genes and Parkinson's disease in the northern Han Chinese population. Chin Med J (Engl) 2012;125:588-92.

80. Nalls MA, Plagnol V, Hernandez DG, Sharma M, Sheerin UM, Saad M, Simon-Sanchez J, Schulte C, Lesage S, Sveinbjornsdottir S and others. Imputation of sequence variants for identification of

genetic risks for Parkinson's disease: a meta-analysis of genome-wide association studies. Lancet 2011;377:641-9.

81. Gonzalez-Escribano MF, Aguilar F, Torres B, Sanchez-Roman J, Nunez-Roldan A. CD38 polymorphisms in Spanish patients with systemic lupus erythematosus. Hum Immunol 2004;65:660-4.

82. Mallone R, Ortolan E, Pinach S, Volante M, Zanone MM, Bruno G, Baj G, Lohmann T, Cavallo-Perin P, Malavasi F. Anti-CD38 autoantibodies: characterisation in new-onset type I diabetes and latent autoimmune diabetes of the adult (LADA) and comparison with other islet autoantibodies. Diabetologia 2002;45:1667-77.

83. Mallone R, Ortolan E, Baj G, Funaro A, Giunti S, Lillaz E, Saccucci F, Cassader M, Cavallo-Perin P, Malavasi F. Autoantibody response to CD38 in Caucasian patients with type 1 and type 2 diabetes: immunological and genetic characterization. Diabetes 2001;50:752-62.

84. Lerer E, Levi S, Israel S, Yaari M, Nemanov L, Mankuta D, Nurit Y, Ebstein RP. Low CD38 expression in lymphoblastoid cells and haplotypes are both associated with autism in a family-based study. Autism Res 2010;3:293-302.

85. Munesue T, Yokoyama S, Nakamura K, Anitha A, Yamada K, Hayashi K, Asaka T, Liu HX, Jin D, Koizumi K and others. Two genetic variants of CD38 in subjects with autism spectrum disorder and controls. Neurosci Res 2010;67:181-91.

86. Riebold M, Mankuta D, Lerer E, Israel S, Zhong S, Nemanov L, Monakhov MV, Levi S, Yirmiya N, Yaari M and others. All-trans retinoic acid upregulates reduced CD38 transcription in lymphoblastoid cell lines from Autism spectrum disorder. Mol Med 2011;17:799-806.

87. Salmina AB, Lopatina O, Ekimova MV, Mikhutkina SV, Higashida H. CD38/cyclic ADP-ribose system: a new player for oxytocin secretion and regulation of social behaviour. J Neuroendocrinol 2010;22:380-92.

88. Malavasi F. Editorial: CD38 and retinoids: a step toward a cure. J Leukoc Biol 2011;90:217-9.

89. Ebstein RP, Mankuta D, Yirmiya N, Malavasi F. Are retinoids potential therapeutic agents in disorders of social cognition including autism? FEBS Lett 2011;585:1529-36.

90. Patton DT, Wilson MD, Rowan WC, Soond DR, Okkenhaug K. The PI3K p110delta regulates expression of CD38 on regulatory T cells. PLoS One 2011;6:e17359.

91. Blair PA, Norena LY, Flores-Borja F, Rawlings DJ, Isenberg DA, Ehrenstein MR, Mauri C. CD19(+)CD24(hi)CD38(hi) B cells exhibit regulatory capacity in healthy individuals but are functionally impaired in systemic Lupus Erythematosus patients. Immunity 2010;32:129-40.

92. Yu A, Zhu L, Altman NH, Malek TR. A low interleukin-2 receptor signaling threshold supports the development and homeostasis of T regulatory cells. Immunity 2009;30:204-17.

93. Hubert S, Rissiek B, Klages K, Huehn J, Sparwasser T, Haag F, Koch-Nolte F, Boyer O, Seman M, Adriouch S. Extracellular NAD+ shapes the Foxp3+ regulatory T cell compartment through the ART2-P2X7 pathway. J Exp Med 2010;207:2561-8.

94. Bahri R, Bollinger A, Bollinger T, Orinska Z, Bulfone-Paus S. Ectonucleotidase CD38 Demarcates Regulatory, Memory-Like CD8(+) T Cells with IFN-gamma-Mediated Suppressor Activities. PLoS One 2012;7:e45234.

95. Goding JW, Terkeltaub R, Maurice M, Deterre P, Sali A, Belli SI. Ecto-phosphodiesterase/pyrophosphatase of lymphocytes and non-lymphoid cells: structure and function of the PC-1 family. Immunol Rev 1998;161:11-26.

96. Massaia M, Perrin L, Bianchi A, Ruedi J, Attisano C, Altieri D, Rijkers GT, Thompson LF. Human T cell activation. Synergy between CD73 (ecto-5'-nucleotidase) and signals delivered through CD3 and CD2 molecules. J Immunol 1990;145:1664-74.

Figure Legends

Table 1 Distribution of CD38 and CD157 molecules in normal human tissues.

Fig. 1 Schematic representation of human chromosome 4, with attention to *CD38* (and its regulatory elements) and *CD157*. The right side shows the schematic features of membrane CD38 and CD157 proteins.

Fig.2 Phylogeny of the *CD38* family.

Fig. 3 Distribution of CD38, PC-1, CD39 and CD73 in human thymus, lymph node and spleen assessed by immunohistochemistry.

Fig. 4 Schematic model of human bone marrow highlighting cross-talks taking place among plasma cells, osteoblasts, osteoclasts, endothelium and stroma.