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Ectoenzymes and innate immunity: the role of human CD157 in leukocyte trafficking

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1. ABSTRACT

CD157 is a glycosylphosphatidylinositol-anchored molecule encoded by a member of the CD38/ADP-ribosyl cyclase gene family, involved in the metabolism of NAD. Expressed mainly by cells of the myeloid lineage and by vascular endothelial cells, CD157 has a dual nature behaving both as an ectoenzyme and as a receptor. Although it lacks a cytoplasmic domain, and cannot transduce signals on its own, the molecule compensates for this structural limit by interacting with conventional receptors. Recent experimental evidence suggests that CD157 orchestrates critical functions of human neutrophils. Indeed, CD157-mediated signals promote cell polarization, regulate chemotaxis induced through the high affinity fMLP receptor and control transendothelial migration.

2. INTRODUCTION

CD157/BST-1 was originally identified as a surface molecule highly expressed by human bone marrow (BM) stromal cell lines derived from patients with rheumatoid arthritis (RA) (1). However, the molecule had already been known for a long time as Mo-5 (2) but the identity between the BST-1 and Mo-5 antigens was clarified only ten years later, thanks to the effort of scientists involved in the VI Workshop on Differentiation Antigens that eventually led to the realization that the original Mo-5 and BST-1 molecules were actually one and the same molecule, henceforth known as CD157 (3).

Human CD157 is a glycosylphosphatidylinositol (GPI)-anchored glycoprotein encoded by a member of the NADase/adenosine diphosphate (ADP)-ribosyl cyclase

(ADPRC) gene family which also includes CD38 (4, 5). Both CD157 and CD38 are pleiotropic in function, acting both as ectoenzymes and receptors (6, 7). Expression of the two molecules coincides in a limited number of tissues and discrete lineage-specific differentiation stages; however, their patterns of expression are distinct in most tissues (including the hematopoietic system), which may indicate that they cooperate in the regulation of selected cell functions.

The ADPRC family of ectoenzymes is evolutionarily conserved (8) and controls complex processes, such as egg fertilization (9), cell activation and proliferation (10), muscle contraction (11), hormone secretion (12) and immune response (13). In human as well as in mouse, CD157 and CD38 play key roles in the regulation of both innate and adaptive immune responses (14-16).

Our interest in this family of molecules originally stemmed from the observation that CD38, or T10 as it was known at the time, was a receptor and not simply a marker of differentiation (17, 18). Our group has spent the last several years assessing the role of CD38 in the human immune response and, more recently, of CD157 in leukocyte transendothelial migration. This underlying theme has led us to evaluate different aspects of the immune response focusing alternately on innate and adaptive immunity.

This review describes the role of CD157 in the innate immune response with particular emphasis on neutrophil migration. Neutrophils are the most abundant leukocyte subpopulation in healthy individuals. These short-lived cells represent the first line of defense against pathogens, and are capable of accumulating within hours at sites of acute inflammation. In concert with monocytes and tissue macrophages, neutrophils carry out many of the major functional responses of the innate immune system. The regulation of neutrophil recruitment to the inflammatory site and neutrophil clearance are critical processes assuring effective host defence without tissue injury. Leukocyte migration and extravasation are coordinated by sequential steps mediated by specific molecular interactions between leukocytes circulating in the bloodstream and the endothelial cells within the vascular lumen (19). This multi-step paradigm initially involves tethering and rolling steps, prevalently mediated by selectin-based adhesive events (20), followed by integrin-mediated firm adhesion (21, 22). Leukocytes then polarize, migrate towards and through inter-endothelial cell junctions, thus crossing the endothelial barrier and penetrating the associated basement membrane (23). Following extravasation, neutrophils undergo chemotaxis towards the inflammatory site, where they carry out their immune functions (24) (Figure 1, top panel).

3. CD157 AND THE ADPRC GENE FAMILY

3.1. Phylogenetic analysis

The NADase/ADPRCs form a unique eukaryotic gene family derived from a common ancestor, with homologs in *Aplysia* (25), *Schistosoma mansoni* (8), the sea urchin *Strongylocentrotus purpuratus* (see NCBI Accession numbers ABQ09453, ABQ09454, ABQ09455), chicken (NCBI Acc. Nos. XP_42077, XP_420775), mouse (26-28), rat (29), macaque (30) and human (5, 25).

The oldest NADase/ADPRC gene isolated so far is from the sea slug *Aplysia*, whose origins date back to over 500 million years ago. The *Aplysia* cyclase is encoded by a compact 8-exon, 7 kb gene. CD38 and CD157 represent the two mammalian NADase/ADPRCs, and their genes have been molecularly characterized in human and mouse (4, 28, 31). Comparative analysis of their genes shows extensive conservation of their intron-exon structures with that of the *Aplysia ADPRC* gene, indicating their origin from a common ancestral gene.

3.2. CD157 and CD38 gene structure and regulation

CD157 and *CD38* are tandemly arranged gene duplicates (32) located in conserved syntenic blocks on human chromosome 4 and mouse chromosome 5. *CD157* spans ~35 kb and consists of nine exons (4) whereas *CD38* is ~76 kb long in humans, and consists of eight exons. Exons 1-8 of *CD157* and *CD38* are highly conserved as are their intron insertion phases. As described below, the ninth exon unique to *CD157* is necessary for GPI anchoring.

CD157 and *CD38* lack canonical TATA boxes in their promoter regions. Human *CD157* presents numerous potential binding sites for transcription factors involved in the immune response such as NF-*k*B, NF-IL6, CREB, PEA, E2A, C/EBP, AP3, AP2 and SP1 (4). More recently, *CD157* has been identified as a PAX5-responsive gene in mouse where it may be involved in B cell development (33). Transcriptional analysis revealed multiple start sites in human *CD38* (5, 25) which may also undergo epigenetic regulation via methylation of a CpG island at the 5' end of the gene.

3.3. Gene modifications underlie differences in NADase/ADPRC protein topology

The CD38, CD157 and *Aplysia* cyclase polypeptides share a central core of ~250 amino acids with 25-30% amino acid sequence similarity, which is sufficient for retention of their ancestral enzymatic function. The determinants of their diverse protein topology reside instead in the NH2- and COOH-terminal modifications. Being a soluble protein, the *Aplysia* cyclase has an N-terminal signal peptide. A longer form of this hydrophobic region is found in CD38, accounting for its being a type II membrane protein whereas CD157 is GPI-anchored, thanks to a gene modification which adds a ninth exon encoding the hydrophobic signal for GPI attachment. Thus changes in *CD157* exon 9 and *CD38* exon 1 are responsible for membrane attachment of the mammalian ADPRCs. There remains the teleological issue of *why* CD157 and CD38 are expressed on the cell surface, and why they are expressed there in leukocytes in a tightly regulated manner.

3.4. CD157 protein structure

CD157 consists of a variably glycosylated single polypeptide chain of 42-45 kDa (1) with a core protein of ~31 kDa (34). The COOH-terminal end of CD157 is

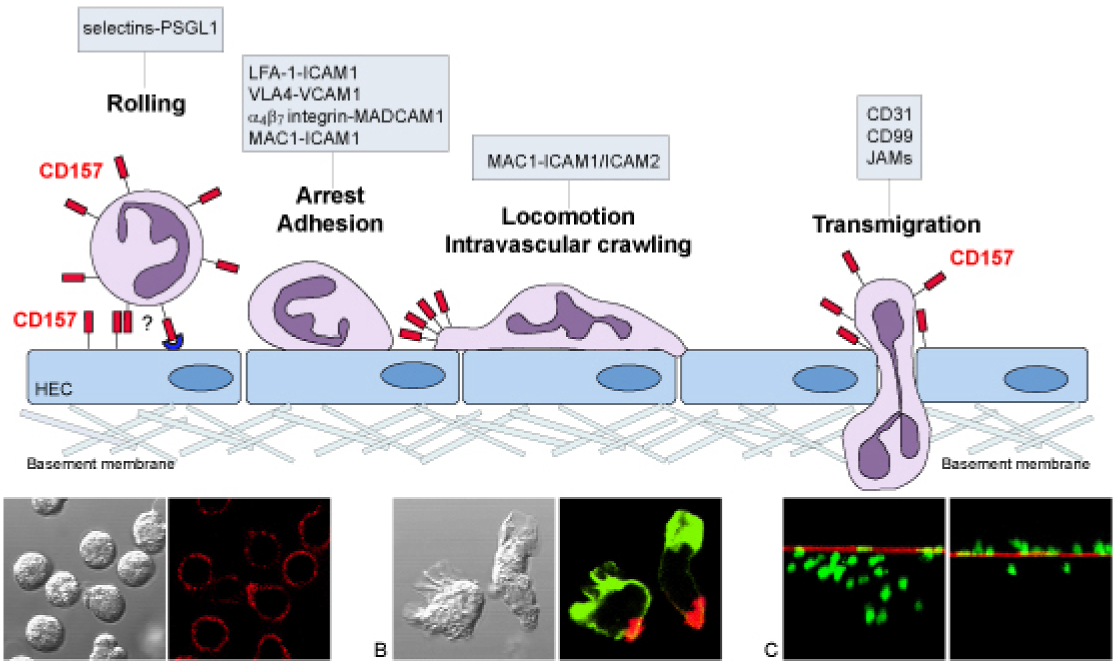

Figure 1. Key migratory steps of leukocytes at site of inflammation. Leukocytes expressing the appropriate set of trafficking molecules undergo a multi-step adhesion cascade, then polarize and move by diapedesis across the venular wall. Diapedesis involves transient disassembly of interendothelial junctions and penetration through the underlying basement membrane. The key molecules involved in each step are indicated in boxes. PSGL1, P-selectin glycoprotein ligand 1; LFA1, leukocyte function-associated antigen 1 ($\alpha_L\beta_2$-integrin); ICAM1, intercellular adhesion molecule 1; VLA4, very late antigen 1 ($\alpha_4\beta_1$-integrin); VCAM1, vascular cell-adhesion molecule 1; MADCAM1, mucosal vascular addressin cell-adhesion molecule 1; MAC1, macrophage antigen1 ($\alpha_m\beta_2$-integrin); JAM, junctional adhesion molecule. A) Expression of CD157 on membrane of resting neutrophils. Samples were observed by differential interference contrast (left panel) and fluorescence confocal microscopy (right panel); B) neutrophils treated with fMLP were fixed, permeabilised and stained with anti-CD157 labelled with Texas red (red) and phalloidin-FITC (green) to visualize F-actin polarization. C) Neutrophils from healthy donors were labelled with CSFE, treated with anti-CD157 (right panel) or with irrelevant IgG (left panel), and seeded on TNFα-activated HUVEC monolayers grown on collagen. After 30 min of migration, samples were fixed, washed, stained with anti-CD31 labelled with Texas red (to identify the HUVEC layers) and evaluated by laser-scanning confocal microscopy. The images show the position of neutrophils at the end of transmigration assay.

anchored to the plasma membrane by a GPI molecule, the distal NH2-terminal region includes the catalytic domain. CD157 is seen to occur in both monomeric and dimeric forms when the molecule is heterogeneously expressed at high epitope density in MCA102 and CHO fibroblasts (35). These dimers can dissociate into monomers upon exposure to reducing agents such as β-mercaptoethanol, indicating the presence of intermolecular disulfide bonds. When expressed and released into culture medium in a soluble form without the COOH-terminal GPI anchor, CD157 was found to further aggregate into oligomers, which can only be dissociated under reducing conditions (36).

The CD157 protein sequence shares 36% identity with human CD38 and 33% with the soluble ADPRC from *Aplysia californica* (31, 37, 38). Murine and rat CD157 are highly homologous to the human molecule, sharing approximately 72% overall identity (28, 39). Sequence alignment of human CD157 and CD38 revealed the presence of 10 cysteine residues, conserved among the cyclase family, forming intrachain disulphide bonds (40, 41).

The crystal structures of the extracellular region of human CD157 have been resolved in the ligand-free state as well as in complexes with 5 substrate analogues (nicotinamide, nicotinamide mononucleotide, adenosine triphosphate (ATP), ethenoNADphosphate (NADP$^+$) and ethenoNAD revealing a substrate recognition mode and catalytic scheme common to the cyclase family (42) (Figure 2). Based on mutational analyses of CD38 and *Aplysia* cyclases the critical residues for CD157 cyclase activity are Trp77, His81, Ser98, Asp99, Asp107, Trp140, and Glu178 (43). In particular, Trp77, Trp140, and Glu178 - which are strictly conserved in the sequence alignment of CD157, CD38 and *Aplysia* cyclase (42) - play key roles in substrate recognition and cyclization.

3.5. CD157 tissue distribution

Human CD157 was originally identified as a bone marrow stromal cell molecule (BST-1) facilitating the growth of the DW34 murine pre-B cell line *in vitro* (34), and is prevalently expressed by cells belonging to the myelomonocytic lineage (2). CD157 expression parallels the differentiation pathway of the myeloid lineage: indeed,

Table 1. Tissue distribution of the CD157 molecule

TISSUE	CELL TYPE	SPECIES	REFERENCES	
		human	**mouse**	
Bone Marrow	B progenitors	-	●[1]	54-56, 70
	myeloid precursors	●	●	2, 54
	stromal cells	●	*nd*	1, 104, 105
	endothelial cells	●	*nd*	1
	nurse-like cells	●	*nd*	48
	macrophages	*nd*	●	106, 107
Blood	eosinophils	●	*nd*	3
	basophils	●	*nd*	3, 52
	neutrophils	●	●	3, 54, 60
	monocyte	●	●	2, 45, 54
	macrophages	●	●	7, 54, 107
	plasmacytoid dendritic cells	●	*nd*	44
Spleen	reticular cells of the white pulp area	*nd*	●	58
Lymph nodes	follicular reticular cells	*nd*	●	58
Thymus	T progenitors	-	●[1]	55-57, 73
Vessels	endothelial cells	●	*nd*	49, 108
Liver	fetal B progenitors	-	●	55
Lung	mast cells	●	*nd*	51, 52
Uterus	mast cells	●	*nd*	52
Kidney	collecting tubules	*nd*	●	58
Gut	brush border of epithelial cells	*nd*	●	58
	Peyer's patches	*nd*	●	58
	stromal cells in cryptopatches isolated lymphoid follicles in the small intestine	*nd*	●	109
Peritoneum	mesothelial cells	●	*nd*	53
	macrophages/peritoneal exudates	*nd*	●	58
Pancreas	α and β cells	*nd*	●	59
Skin	mast cells	●	*nd*	51
Heart		*nd*	●	38
Gingival fibroblasts		●	*nd*	50

[1] rat; nd = not determined; (**-**) negative

it is absent in early CD34-positive precursor cells, becoming clearly positive only at the CD36^{low} stage of maturation of the monocytic cell lineage and at the late pro-myelocyte stage in neutrophils (44, 45). CD157 is not detected in erythrocytes, platelets and lymphoid cells from peripheral blood or in spleen and tonsil. Expression of CD157 in hematological malignancies is restricted to the acute myelogenous leukemias (M4 and M5 express > than M1, M2 and M3) and mirrors its expression in the corresponding normal counterparts (46, 47). Flow cytofluorografic analysis indicates that human CD157 is expressed by synovial cells (48), vascular endothelial cells (49) and follicular dendritic cells (45). Moreover, CD157 is expressed by gingival fibroblasts (50), mast cells from lung, uterus and foreskin (51, 52), and on mesothelial cells from peritoneum (53).

Human and murine CD157 distribution differs in many tissues. Murine CD157 (BP-3) was first described on early progenitors of murine B and T lymphocytes, in B progenitors in fetal liver and in pre-T cells in fetal thymus (54-57): it is absent instead on the human lymphoid counterpart. The expression of murine CD157 during ontogenesis spans pre-B cells and circulating B cells with an immature phenotype (IgM^{high}/IgD^{low}) typical of cells recently emigrated from bone marrow (55). CD157 in the murine myeloid lineage overlaps the expression of the human counterpart, indeed, it is expressed at low levels by relatively mature myeloid cells in the bone marrow and at high levels by polymorphonuclear cells, adherent macrophages and stromal cells from the bone marrow and peritoneal exudate (54). Immunohistochemical staining of murine CD157 revealed the molecule in the collecting tubules of kidney, on the brush border of intestinal epithelial cells and on a subset of reticular cells in lymph nodes, Peyer's patches and splenic white pulp (58). Finally, CD157 has also been identified in mouse pancreatic islet cells, including α and β cells (59) (Table 1) .

3.6. CD157 expression under inflammatory conditions

The involvement of CD157 in leukocyte recruitment is supported by data showing that CD157 is upregulated and undergoes redistribution and membrane compartmentalization in the presence of proinflammatory cytokines, both in neutrophils and monocytes. *In vitro*, the expression of CD157 in neutrophils is upregulated by treatment with calcium ionophores and with the chemoattractant formyl-met-leu-phe (fMLP) (60) MCP-1 and GM-CSF upregulate CD157 expression in monocytes (A. F. and E. O. *unpublished data*).

Conversely, the constitutive expression and surface distribution of CD157 in HUVEC (particularly in the interendothelial contacts) are not affected by cell activation induced by a panel of cytokines (such as TNF-α, IL1-β, IFN-α, IFN-γ and IL-4) or by selected chemical mediators (such as, fMLP, LPS, PMA, and dibutyryl-cAMP) at any of the concentrations or incubation times

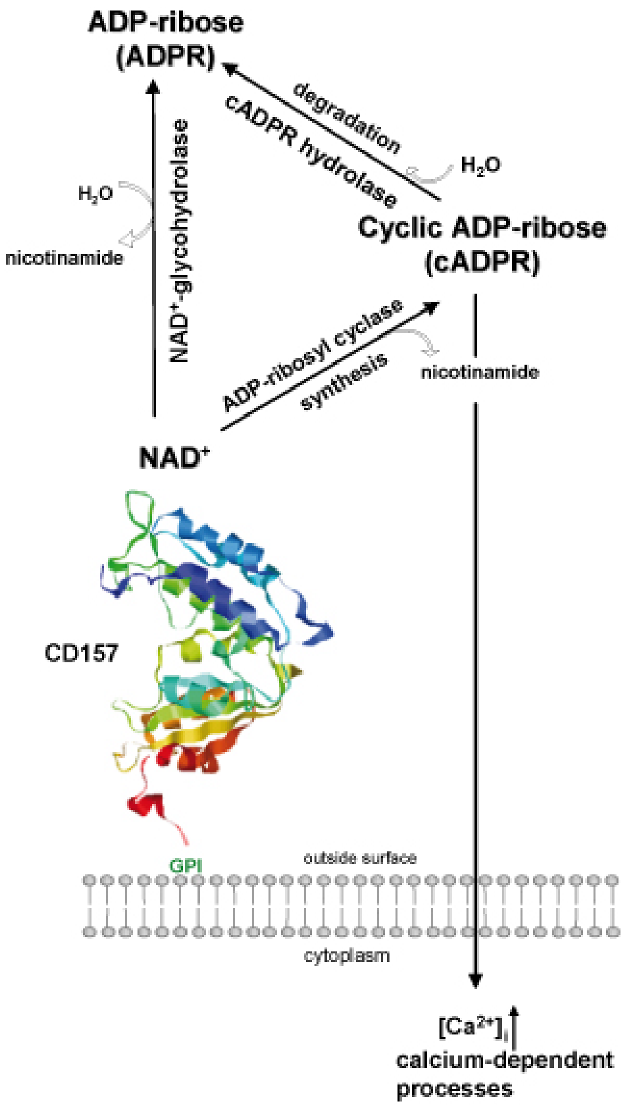

Figure 2. Catalytic reactions of CD157 and predicted structure. The ectoenzyme CD157 catalyzes the production of cyclic ADP-ribose (cADPR) and ADP-ribose (ADPR) from its substrate NAD^+. The CD157 molecule includes two domains: the N domain (residues 2–68, 98–150) and the C domain (residues 69–97, 151–251), which are connected by a hinge region of three peptide chains. The internal architecture of each domain is essentially identical with that of *Aplysia* cyclase.

considered (49). *In vivo*, CD157 is overexpressed in the superficial lining cells of the synovium of patients with RA (48). Nurse-like cell lines established from synovium or bone marrow of RA patients express CD157, where the molecule's expression is upregulated by IFN-γ (48). Furthermore, elevated levels of soluble CD157, at concentrations 30-50 fold higher than those of healthy donors, have been detected in the sera of a subset of patient with severe RA, suggesting a correlation between CD157 and severity or progression of the disease (61). Recently, analysis of differential gene expression between pristane-induced arthritis (PIA)-susceptible DA rats and PIA-resistant E3 rats, identified bone marrow stromal cell antigen 1 (*Bst1*) as being one of the arthritis susceptibility genes (62).

4. CD157 FUNCTIONS

4.1. Enzymatic functions

The identification of sequence similarities between human CD38 and *Aplysia* ADPR cyclase triggered a synergistic interaction between biochemists, biologists and immunologists in their attempt to elucidate the implications of the enzymatic properties in physiology and pathology (37). The importance of these enzymatic pathways has been demonstrated not only in the immune system, but also in different tissues and organs (63). However, in spite of the efforts of scientists, several issue remain unclear. Perhaps the most intriguing concerns the relationship between the enzymatic and receptor functions of both CD38 and CD157.

The homology between *Aplysia* ADPRC, CD38 and CD157 implies that they share the same enzymatic activity, cyclising NAD to produce cADPR a potent Ca^{2+}-mobilizing second messenger (63, 64). Furthermore, CD157 and CD38 (but not *Aplysia* ADPRC) have very similar catalytic properties, possessing both cADPR synthesizing and hydrolyzing activities (65) (Figure 2). The predominant enzymatic product of CD38 and CD157 from NAD thus is ADP-ribose, with cADPR being only a minor component (66).

Recombinant soluble CD157 shows both cyclase and hydrolase activities (34). However, its catalytic efficiency is one hundred-fold lower than that of CD38 (67). The weak cyclase activity of CD157 in an acidic pH suggests that it is unlikely that CD157 is enzymatically active in physiological conditions. Furthermore, the ADPRC activity of CD157 requires Zn^{2+} and Mn^{2+} ions; by contrast, Cu^{2+} has an inhibitory effect on both the cyclase and hydrolase activities of CD157, while it enhances the cyclase activity of CD38 (68). These discrepancies suggest that the enzymatic activities of each molecule can play distinct roles in different environments. In myeloid cells, CD157 has proven to be a very inefficient cyclase, as inferred from the inability of both neutrophils and differentiated HL-60 cells to produce cGDPR from NGD (60).

4.2. Immunoregulatory functions

Like CD38, CD157 may act as a receptor that generates transmembrane signals. Early evidence of its receptorial activity derives from the analysis of the bone marrow microenvironment, where the molecule is expressed by stromal cells and supports the growth of a murine pre-B-cell line (1). The signal-transduction ability of CD157 has been analyzed using specific antibodies mimicking a natural ligand which has yet to be identified. Cross-linking of CD157 by a polyclonal serum induced tyrosine phosphorylation of a 130-kDa protein in the human myeloid cell lines U937 and THP-1. Cross-linking in a transfectant overexpressing CD157 instead induced tyrosine phosphorylation of p130, dephosphorylation of a 100-kDa protein, and growth inhibition (7). The identification of p130 as the focal adhesion kinase (FAK) is controversial, indeed the correspondence has been demonstrated in some cell lines but not in others (35, 69).

CD157 regulates calcium homeostasis in human myeloid cells and mediates superoxide production in the U937 cell line (70). Recent experimental evidence suggests that CD157 orchestrates critical functions of human neutrophils. Indeed, CD157-mediated signals promote cell polarization, and control neutrophil migration (60).

The relationship between the enzymatic activities and the receptorial functions continues to elude researchers. CD157-catalyzed generation of extracellular cADPR, followed by the concentrative uptake of the cyclic nucleotide by hemopoietic progenitors, may be a potentially relevant step in normal hematopoiesis (71). However, the implication of CD157 cyclase activity remains controversial since independent groups have demonstrated that CD157 may not be enzymatically active in physiological situations (67, 68). However, both CD157 and CD38 catalyze the production of other metabolites, including nicotinic acid adenine dinucleotide phosphate and adenine homodinucleotides whose functional roles remain largely unknown (72).

There is ample evidence that CD157 is an immunoregulatory molecule in the murine system: CD157 cross-linking enhanced the proliferative response of purified pre-T cells to CD3-mediated stimulation and accelerated the development of fetal thymic organ cultures (57). Moreover, the expression of CD157 by murine B cell progenitors parallels DJ rearrangement of the immunoglobulin heavy chain genes (55). This finding suggests that CD157 plays a role at critical stages of lymphopoiesis, and is involved in early T and B lymphocyte growth and development. However, the hypothesized involvement of CD157 in the development of the lymphoid compartment was not confirmed by the murine knock-out (KO) model, which demonstrated normal hematopoiesis and highlighted a central role of CD157 in the regulation of the humoral T-independent immune responses and mucosal thymus-dependent response (70). Indeed, KO mice showed impairment of thymus-independent antigen-induced IgG_3 secretion; furthermore, oral immunization with thymus-dependent antigens led to low production of specific IgA and IgG in the fecal extract, due to a reduced number of antigen-specific antibody-producing cells in the intestinal *lamina propria* (73).

Recently, we demonstrated that CD157 orchestrates a signal transduction pathway crucial to the function of human neutrophils. CD157 cross-linking by means of agonistic antibodies regulates calcium homeostasis. The Ca^{2+} current elicited by CD157 is both of extracellular and intracellular origin and is not controlled by the products of the enzymatic functions of the molecule (*i.e.*, cADPR and ADPR), since it is unaffected by 8Br-cADPR, a specific antagonist of cADPR (60). The amplitude of the signal is dependent on the extent of cross-linking, suggesting that the redistribution of the molecule on the membrane is crucial for the generation of signalling-competent microdomains. The observation that wortmannin interferes with the increase in intracellular calcium induced by CD157 ligation suggests that phosphoinositide-3-kinase (PI3K) is part of the downstream signal transduction pathway. This is in line with the knowledge that PI3K controls polarity and motility of neutrophils (74).

5. ROLE OF CD157 IN LEUKOCYTE TRAFFICKING

5.1. Role of CD157 on neutrophil polarization.

Cell migration requires the compartmentalization of specific membrane receptors and signalling molecules in particular cell locations, a process defined as polarization. Like other cells, in order to move, leukocytes must acquire and maintain morphological and functional asymmetry characterized by two poles: the leading edge, which protrudes at the cell front, and the rear edge, which retracts (75). Although there are differences among cell types, the leading edge usually contains the machinery driving actin polymerization and sensing the chemotactic gradient; in leukocytes the rear edge (uropod) contains receptors and signalling molecules mainly involved in cell adhesion (76). Localization to the uropod of the polarized neutrophils is a characteristic shared by many GPI-anchored proteins (77, 78), including CD157 (60) (Figure 1B). Moreover, cross-linking of CD157 by means of agonistic antibodies on neutrophils is associated with very rapid clustering of the molecule and subsequent profound modifications in cytoskeletal organization, culminating in cell polarization. These effects are prevented by pre-treatment of cells with a blocking anti-β_2 integrin mAb (60) suggesting that there is crosstalk between CD157 and CD11b-CD18 complex (see below).

When activated neutrophils transform from resting to migratory cells, the actin-based cytoskeleton dissolves, actin polymers reassemble as stress fibers near the leading edge of the polarized neutrophil and CD157 localizes into ganglioside GM1-enriched rafts (78) into the trailing uropod (Figure 1B). Treatment with methyl-β-cyclodextrin, a cyclic heptasaccharide that selectively extracts cholesterol from the plasma membrane, thereby destabilizing the ordered packing of glycolipids (79), prevents CD157 translocation into rafts and inhibits CD157-mediated Ca^{2+} influx. This finding suggests that the CD157-mediated signaling pathway requires organized rafts. Flow cytometry demonstrated that methyl-β-cyclodextrin does not affect plasma membrane expression of CD157, confirming that the effects of methyl-β-cyclodextrin are attributable to raft disruption and not to modulation of the molecule on the cell surface. Similar results were obtained with filipin, a cholesterol-sequestering antibiotic (E.O. and A.F., *unpublished data*).

5.2. Functional and molecular interactions between CD157 and the CD11b/CD18 complex

CD157 lacks a cytoplasmic domain, therefore, it must associate with membrane-spanning receptors to transduce signals. A reasonable hypothesis is that CD157 exploits its lateral mobility to establish functional interactions with conventional receptors, much in the same way as CD38 does (18, 80, 81).

Our group demonstrated that CD157 associates with the CD11b/CD18 complex for signal transduction in human neutrophils. Immuno-localization and co-capping experiments showed that CD157, CD11b and CD18 appear to closely associate spatially, as ligand-induced clustering of β2 integrin (CD18) causes co-localization with CD157. Furthermore, observed changes in cell shape and cytoskeleton reorganization following CD157 ligation are prevented by antibodies that block CD18 (60).

Co-immunoprecipitation experiments demonstrated that CD157 and CD11b/CD18 are spatially juxtaposed and are actually physically bound to one another (82). The experimental model adopted was centred on human neutrophils, where membrane perturbation induced by the isolation procedure leads CD11b/CD18 to translocate from intracellular pools to the plasma membrane, where the dimer is joined by CD157. The CD157 domain involved in the interaction with CD11b/CD18 described here is not yet known. A number of studies demonstrated that other GPI-anchored molecules such as CD14, CD16, CD87 and GPI-80 also signal through membrane-spanning integrins (83-85). Several of these GPI-anchored receptors appear to form *cis* interactions with a lectin site on the integrin contributing to its acquisition of an active conformation which is absolutely necessary for firm adhesion of migrating leukocytes on endothelial cells (85-87). Indeed, the acquisition of the active conformation of the CD11b/CD18 complex is significantly reduced by treatment with phosphatidylinositol-specific phospholipase C, which removes approximately 70% of GPI-anchored molecules (87).

The association between CD157 and the CD11b/CD18 complex is not unique. It was recently reported that CD38 also associates with the complex in human dendritic cells (88).

5.3. Role of CD157 on vascular endothelial cells

The potential involvement of CD157 in endothelial cell signaling was evaluated by measuring calcium influx in physiological conditions using single-cell calcium measurements. Perfusion of anti-CD157 mAb induces a rapid and sustained cytosolic calcium rise in the majority of human umbilical vascular endothelial cells (HUVEC) and the amplitude of Ca^{2+} currents is unaffected by surface clustering of CD157 (Figure 3A-B). Previous studies have shown that cross-linking of selected surface molecules (such as CD54 and CD62E/E-selectin, among others), which increases endothelial intracellular Ca^{2+} concentration, also induces rearrangements of endothelial actin, which is indicative of increased contractility (89). Having established that engagement of CD157 is followed by increased $[Ca^{2+}]_i$ in HUVEC, the relationship between signals mediated by mAb ligation and rearrangement of cytoskeletal actin filaments was analyzed. Cell priming with PMA, which induces over-expression of adhesion molecules, shows rare and randomly oriented actin fibers in some cells (Figure 3C, left panel). Under these conditions, ligation of CD157 is followed by a dramatic reorganization of the cytoskeleton, with formation of a large amount of stress fibers in virtually all the cells (Figure 3C, middle panel).

CD157-mediated signal transduction is instrumental in actin reorganization; indeed, pre-treatment of HUVEC with BAPTA-AM (a cell-permeable calcium chelator) totally abolished CD157-induced formation of stress fibers (Figure 3C, right panel). Neutrophil transendothelial migration requires a transient increase of intracellular free calcium within the endothelial cells surrounding the transmigrating cell. The underlying molecular mechanism responsible for this increase in intracellular calcium is unknown. However, compelling evidence indicates that neutrophils migrating across endothelial cells elicit local transient elevation of $[Ca^{2+}]$ and retraction signals that are thought to enhance inter-endothelial gap formation near the migrating cell (90).

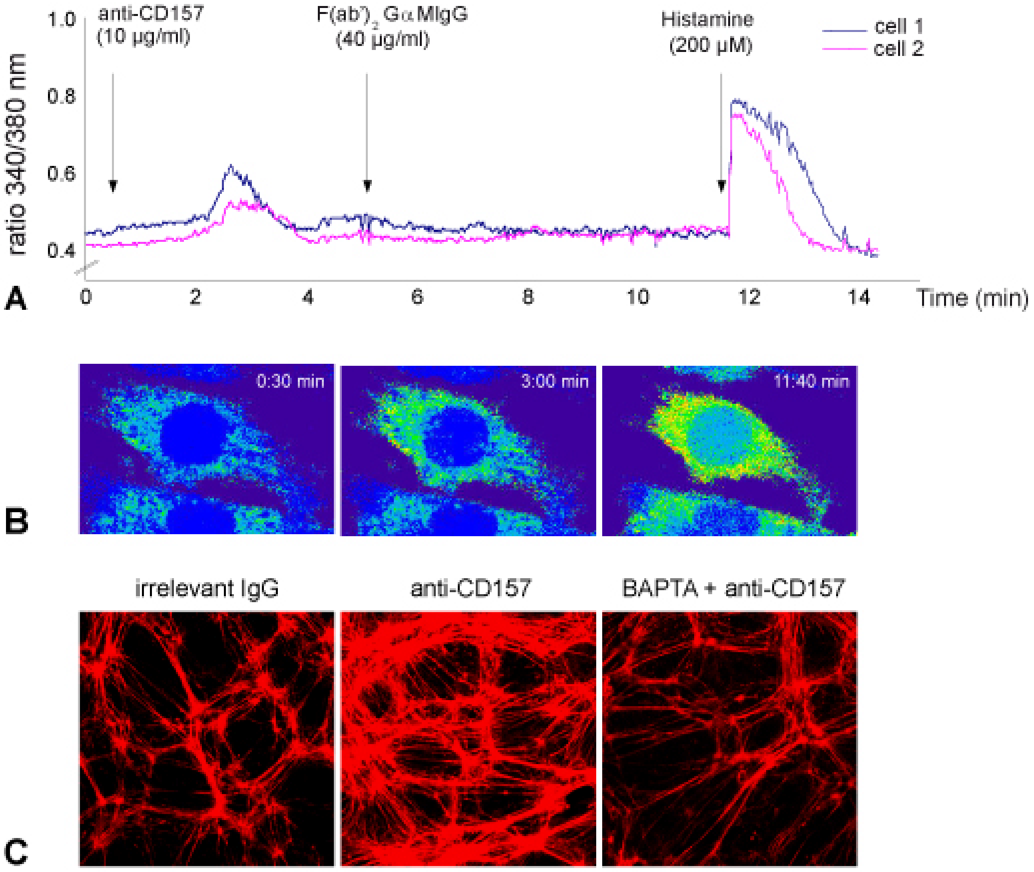

Figure 3. Receptorial activities of CD157 on vascular endothelial cells. Sub-confluent HUVEC grown on gelatin-coated glass coverslips were loaded with Fluo-3AM for 30 min at 37°C. Samples were assembled on a flow chamber at 37° C, placed on an inverted IX70 Olympus microscope and continuously perfused. Perfusion of anti-CD157 mAb induces a rapid (2-3 min) and sustained cytosolic calcium rise in the majority of HUVEC and the amplitude of Ca^{2+} currents is not affected by CD157 cross-linking (A). The increase of [Ca2^{+}]$_i$ peaks 3 min after CD157 ligation (A and B, middle panel). C) HUVEC monolayers stimulated with PMA were fixed, permeabilised and stained with phalloidin-TRITC. Samples were analyzed by confocal laser scanning microscopy. PMA-activated HUVEC show rare and randomly oriented actin fibers in some cells (left panel). Under these conditions, ligation of CD157 is followed by the formation of a large amount of stress fibers in virtually all cells (middle panel). Pre-treatment of HUVEC with BAPTA-AM completely abrogates the cytoskeleton reorganization induced by CD157 ligation (right panel).

5.4. Role of CD157 on neutrophil transendothelial migration

Leukocyte recruitment to sites of inflammation is a highly controlled process governed by the coordinated interplay of distinct adhesion and signaling molecules (91, 92). The multiple molecular interactions driven by adhesion molecules belonging to different families (21) are backed by the contribution of other molecules. Upon identification, several of these molecules, unexpectedly, turned out to be ectoenzymes (93). The findings that CD157 is constitutively expressed both by neutrophils and vascular endothelial cells mainly at interendothelial junctions and is implicated in the control of neutrophil behavior, were highly suggestive of potential involvement in transendothelial migration, and eventually, in neutrophil recruitment to the inflammatory site. Our studies demonstrated that CD157 plays a role in neutrophil extravasation: neutrophils treated with anti-CD157 mAb show an impaired transendothelial migration: they adhere to the apical surface of the endothelium looking for a junction, but appear to lose their path, meandering with prolonged and disoriented motility over the endothelial cell surface.

Real-time microscopy revealed that neutrophils move by locomotion an average distance of 20 µm before reaching a junction and beginning diapedesis. Neutrophils ligated by anti-CD157 mAb are able to move by locomotion towards interendothelial junctions, but it seems that they do not perceive the signal for subsequent diapedesis. Therefore, they move along the junction without squeezing through. Only a small percentage of cells, after traveling a long distance, escape the block and transmigrate (14, 49) (Figure 1C). These findings indicate that CD157 is crucial for neutrophil migration through endothelial junctions. However, the molecular details governing the CD157-mediated effects and the non-substrate ligand(s) of the molecule are still unknown and deserve further investigation.

5.5. Role of CD157 on neutrophil adhesion and chemotaxis

Cell polarity is an absolute requirement for cell movement and a prelude to chemotaxis, so molecules promoting polarization are potential coordinators of chemotaxis itself. CD157 regulates chemotaxis stimulated through the high affinity fMLP receptor. Indeed, ligation of CD157 is followed by significantly reduced neutrophil chemotactic response *in vitro*. The same inhibitory effect was obtained in HL-60 that underwent granulocytic differentiation with both dimethyl sulfoxide (DMSO) and all *trans*-retinoic acid (ATRA) (60).

An important indication that members of the NADase/ADPRC gene family are involved in the regulation of chemotaxis stemmed from the observation that CD38$^{-/-}$ mice show greater susceptibility to *S. pneumoniae* infection than controls, owing to a defect in neutrophil chemotaxis (94). Receptors critical for immune function may differ in human and mouse: the results obtained *in vitro* by chemotaxis experiments showed that human neutrophil migration is not influenced by CD38 but by CD157. These findings could also be read as the functional replacement of one gene family member by another in different species, helping to explain the lack of a marked phenotype in CD38$^{-/-}$ mice. Another discrepancy between human and mouse is that while cADPR seems to be a crucial element in the regulation of Ca^{2+} homeostasis in fMLP-induced neutrophil chemotaxis in the mouse model (95), cADPR is not required at all for chemotaxis of human neutrophils induced through the high affinity fMLP receptor, as demonstrated by the efficient chemotaxis of DMSO-differentiated HL-60 cells, whose cyclase activity is undetectable (60). It is conceivable that mouse and human neutrophils behave differently: moreover, requirements for calcium mobilization will vary according to many factors, including cell type, cell differentiation stage and the chemokine receptor being examined.

Following extravasation, neutrophils enter the tissues where they interact with the extracellular matrix components. The functional and structural interaction between CD157 and β_2 integrin prompted us to investigate the role of CD157 in mediating neutrophil adhesion to fibrinogen, which is one of the ligands of β_2 integrin. The results demonstrated that ligation of CD157 inhibits the adhesion of both resting and activated neutrophil to fibrinogen, supporting the idea of a functional interaction between the two molecules (60). Taken together the results suggest that CD157 relies on β_2 integrin for signal transduction. Indeed, β_2 integrin and CD157 appear to have a close structural and functional association.

5.6. The Paroxysmal Nocturnal Hemoglobinuria (PNH) disease model

PNH is an acquired blood disease characterized clinically by chronic hemolysis and hemoglobinuria, bone marrow failure, and a tendency for thrombosis (96). The unique feature of PNH is clonal proliferation of a hematopoietic stem cell with a somatic mutation in the X-linked *PIG-A* gene, as a result of which all GPI-anchored proteins are deficient on the cell surface (97, 98). Although the most striking manifestation of the *PIG-A* mutation is in erythrocytes, other leukocyte populations, including granulocytes, also show altered expression of GPI-anchored molecules.

An increasing number of GPI-anchored proteins have been attributed a role in the regulation of neutrophil behavior and, eventually, in the inflammatory response. Recently, some of the functional consequences of the deficient expression of GPI-anchored proteins in PNH patients have been investigated (99). We focused our attention on CD157 which has been shown to be a crucial mediator of neutrophil adhesion and migration. In 12 patients with PNH expressing CD157 on a variable percentage of cells (from 0 to 27%) we observed i) consistently impaired transendothelial migration, ii) impaired adhesion to extracellular matrix proteins, and iii) reduced chemotactic response in the presence of fMLP, producing a variable degree of impaired neutrophil migration as compared to neutrophils from healthy donors. In two patients, with ~70 % of GPI$^-$ neutrophils, neutrophil migration was further reduced by increasing the amount of the GPI$^-$ neutrophils, by negative selection with immune-magnetic beads coated with anti-CD157 mAb. This confirmed that the GPI$^-$ population is indeed the one which is defective in this function. Those PNH patients who suffer recurrent infectious diseases display more severe defects in cell migration. These observations are in line with previous evidence indicating that diapedesis of neutrophils from PNH patients is impaired (100). The scenario observed with neutrophils obtained from patients with PNH mirrors the experimental findings mediated by CD157 mAb ligation in normal neutrophils. Indeed, CD157-blocked neutrophils and neutrophils from patients with PNH display multiple functional similarities, including reduced chemotaxis, impaired migration through endothelial cells and collagen and efficient adhesion to vascular endothelium (49, 60). These findings show that the engagement of CD157 *in vitro* by appropriate mAb in normal neutrophils leads to the same functional consequences as its absence *in vivo* secondary to a somatic mutation in neutrophils from patients with PNH, thus highlighting the role of CD157 in diapedesis. However, a variety of GPI-anchored proteins expressed by normal neutrophils and playing a role in neutrophil functions (such as CD14, CD16, CD24, CD87, GPI-80) (83, 84, 101-103) are missing on PNH neutrophils; therefore, the possibility that GPI-anchored molecules other than CD157 may contribute to the functional defects cannot be ruled out.

These observations controvert the current view concerning the origin of recurrent infections in PNH patients which are not merely attributable to the leucopenia which frequently accompanies the disease. Nonetheless, PNH patients do not necessarily show enhanced susceptibility to infections, whereas *in vitro* PNH neutrophils are constantly functionally impaired. Possible explanations might be i) that a variable proportion of normal cells circulating in the blood of PNH patients (98) may be sufficient to establish a host defense; or ii) that circulating soluble CD157 may compensate for the lack of membrane-anchored molecules. CD157, like many other GPI-anchored molecules, is present in soluble biologically active form in the blood (61). This implies that soluble CD157 may behave as an hormone or a cytokine in endocrine- or paracrine-like systems.

6. SUMMARY AND PERSPECTIVES

Over the last twenty years, a large volume of information has emerged from different perspectives, contributing to an overall picture of the human ADPRC family. Nonetheless, several problems remain. Among these, the most intriguing is that of determining the relationship between enzymatic activities and receptorial functions. Substantial evidence from human models has consistently indicated that many immunoregulatory functions mediated by CD157 and CD38 occur independently from their enzymatic activities. One example of this dichotomy is human leukocyte migration. However, this is not an absolute assumption: indeed, leukocyte migration and enzymatic activities are strictly interdependent in mouse, at least in selected contexts. At best, we can say that the human and murine counterpart show marked differences, both in terms of tissue distribution and of functions, so results cannot necessarily be extrapolated across species. CD157 and CD38 are not unique in having a dual personality; it is a common trait of many ectoenzymes.

We may speculate that ectoenzymes exert a dual control of leukocyte trafficking by acting as enzymes and/or signaling molecules, with the biological outcome determined by the specific environment. For example, in the mouse model cADPR and ADPR play a role in regulating leukocyte migration in response to certain chemokines, but not to others.

Experimental data suggest that human CD157 controls two sequential steps in neutrophil trafficking. First, polarization and cytoskeletal remodeling mediated by CD157 engagement allow neutrophils to sense the presence of the inflammatory environment. Then, CD157 expressed at the interendothelial junctions behave as a gatekeeper, regulating an early step in diapedesis. These effects, mimicked *in vitro* by antibody ligation, are mediated *in vivo* by homotypic and/or heterotypic interactions with a cell-bound ligand(s) which is still unknown. CD157 exerts receptorial function in different cells, hence major challenges for the future will be to understand the molecular signals implicated in the CD157-mediated pathway, and to identify a non-substrate ligand.

It is now apparent that inhibiting leukocyte trafficking is an effective strategy for controlling chronic inflammation. Therefore, the finding that CD157 coordinates neutrophil migration and extravasation offers new perspectives for the design of treatment strategies in inflammatory conditions in which aberrant recruitment of neutrophils is deleterious to host tissues.

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Abbreviations: BM: bone marrow; RA: rheumatoid arthritis; GPI: glycosylphosphatidylinositol; ADP: adenosine diphosphate; ADPRC: ADP-ribosyl cyclase; cADPR: cyclic ADP-ribose; ATP: adenosine triphosphate; NADP: ethenoNADphosphate; fMLP: formyl-met-leu-phe; PIA: pristane-induced arthritis; cGDPR: cyclic guanosine diphosphoribose; FAK: focal adhesion kinase; PI3K: phosphoinositide-3-kinase; DMSO: dimethyl sulfoxide; ATRA: all *trans*-retinoic acid; HUVEC: human umbilical vascular endothelial cells; PNH: Paroxysmal Nocturnal Hemoglobinuria

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