Immunol Cell Biol. Author manuscript; available in PMC 2011 August 01.

Published in final edited form as:
Immunol Cell Biol. 2011 February ; 89(2): 197–206. doi:10.1038/icb.2010.147.

DARC and D6: silent partners in chemokine regulation?

Chris A H Hansell, **Catherine E Hurson**, and **Robert J B Nibbs***
Institute for Infection, Immunity & Inflammation, College of Medical, Veterinary & Life Sciences, Sir Graeme Davis Building, 120 University Place, Glasgow G12 8TA.

Abstract

Chemokine receptors adorn the surface of leukocytes and other cell types ready to translate the extracellular chemokine environment into functional cellular outcomes. However, there are several molecules that, in many respects, look like chemokine receptors, but which do not have the ability to confer chemotactic potential to cell lines. This apparent silence spurred the search for signalling-independent functions and led to the development of new paradigms of chemokine regulation. In this review, we summarise the experimental basis for these ideas focussing on DARC and D6, the most studied members of this group of molecules. We discuss data generated using *in vitro* systems and genetically deficient mice, include results from observational human studies, and summarise the key findings of recent research. We take a critical look at current models of *in vivo* function highlighting important gaps in our knowledge and demonstrating that there is still much to find out about these enigmatic molecules.

Keywords

chemokine; D6; DARC; leukocyte migration; scavenging; transcytosis

Introduction

The biological functions of chemokines are mediated by G-protein coupled chemokine receptors, which sense extracellular chemokines and transmit signals to change cell behaviour[1]. These receptors (CCR1-10, CXCR1-6, CX3CR1 and XCR1) are typically restricted to a particular chemokine subclass: some are highly promiscuous, such as CXCR2, CCR1 and CCR3, and play prominent roles during inflammation; others are more selective (e.g. CXCR4, CCR7 and CCR9) and serve critical homeostatic functions. Importantly, leukocyte function is intimately linked to migratory potential and this is defined, to a great extent, by the cell-specific profile of signalling-competent chemokine receptors. However, there are several chemokine receptor-like molecules that do not fit accepted paradigms of chemokine receptor function. These are referred to as Atypical Chemokine Receptors (ACRs), interceptors, or chemokine 'decoy' receptors, and include the Duffy Antigen Receptor for Chemokines (DARC), D6, CCX-CKR and CXCR7[2], [3]. They are structurally similar to other chemokine receptors, with seven predicted transmembrane helices and extracellular surfaces that bind chemokines with high affinity. However, when artificially expressed in immortalised cell lines *in vitro*, they are unable to couple to signal transduction pathways activated by typical chemokine receptors expressed in the same cellular system. Consequently, ACRs cannot direct the migration of transfected host cells. This inability to initiate conventional chemokine responses in cell lines is the principal defining feature of an ACR. It has led to the view that they are silent *in vivo* and driven the search for functions that do not rely on chemokine-induced signalling. These investigations have spawned

*corresponding author. Robert.Nibbs@glasgow.ac.uk; Tel: +44 141 330 3960; FAX: +44 141 330 4297. .

Europe PMC Funders Author Manuscripts

concepts of scavenging, sequestration, buffering, and transcellular transport that are now seen as critical for facilitating and regulating chemokine function. By summarising the data underpinning these ideas, we take a critical look at current models of DARC and D6 function and identify key remaining questions.

DARC

Red cell buffering of blood-borne chemokines

Human DARC can bind many, but not all, pro-inflammatory CC and CXC chemokines (Figure 1). The specificity of DARC in other species is less well understood, but assumed to be similar to human DARC. Cross-subfamily binding is unusual, but not unique to DARC[1], [4], [5]. DARC is the molecular determinant of the human Duffy blood group antigen, and an important entry factor for malarial species *Plasmodium vivax* into red blood cells (rbc)[6]. This is thought to have driven emergence of the Duffy null phenotype prominent in African populations, which is usually caused by a homozygous GATA-1 binding site mutation in the DARC promoter[7]-[9]. DARC-binding chemokines can block rbc infection by *P. vivax*[10], and the Duffy binding protein of this parasite is a potential vaccine target[11]. Interestingly, genetic variation in baboon DARC is thought to influence susceptibility to malaria-like pathogen *Hepatocystis*[12]. Human rbc DARC can also bind and present HIV. Some studies, though not others, report increased susceptibility of Duffy null individuals to HIV infection, but slower progression after infection[13]-[19]. Links between the Duffy null phenotype and IgE levels[20], complications of sickle cell anemia[21]-[24], transplant rejection[25], psoriasis[26], and prostate cancer[27] have also been investigated, but only weak associations, at best, have been found. However, exciting recent work, including genome wide association studies (GWAS), reveal strong association of the Duffy null phenotype with neutropenia[28]-[31], and perhaps monocytopenia[31]. Thus, DARC on human rbc influences infectious disease and leukocyte homeostasis.

Studies involving Duffy null humans have shown that DARC holds chemokines on the surface of rbc and regulates chemokine abundance in plasma. Recent GWAS have strongly linked DARC variant Asp42Gly with serum CCL2 levels in healthy people; revealed a weaker association with CXCL8 and CCL5; but found no association with some non-DARC binding chemokines or other inflammatory markers[32]. Compared to controls, Duffy null humans have lower resting levels of erythrocyte-bound CXCL1 and CCL2, but not CXCL8; less plasma CCL2 (according to some but not other studies); and more plasma CXCL8[31], [33]. After endotoxin challenge, they have lower peak levels of plasma CCL2 and CXCL1, but not CXCL8, and a substantial reduction in rbc-associated CCL2, CXCL1 and 8[31]. Resting and endotoxin-induced plasma levels of non-DARC ligand, CCL4, are unaffected[31]. Neither endotoxin-induced neutrophilia and monocytopenia, nor the pharmacokinetics of infused recombinant CCL2, are substantially affected by Duffy status, but Duffy null humans are reportedly more sensitive to CCL2-induced monocyte mobilisation[34]. In addition, DARC deficient mice have less plasma CCL2 and 11, and the plasma half-life of many DARC-binding chemokines delivered i.v. is significantly lower[35].

From these studies, and the nature of receptor/ligand interactions, rbc DARC may be seen as serving both the previously-proposed ‘sink’ and ‘reservoir’ functions, depending on context. When intact chemokines competent for DARC binding are released into blood during inflammation they will rapidly occupy available local DARC molecules (Figure 2B). With ~2000 chemokine binding sites per human rbc (i.e. ~15nmol of sites per litre of blood)[36], and ~10-fold more on mouse rbc[37], the DARC ‘sink’ clearly has a substantial capacity. Only excessive chemokine production will cause it to ‘overflow’ (Figure 2C). Chemokines in plasma are rapidly removed by DARC-independent mechanisms (e.g. clearance by the liver). As this occurs, the DARC ‘reservoir’ will release chemokines (Figure 2D) eventually

restoring the dynamic equilibrium of free versus bound chemokine (Figure 2A). This process of bind-and-release will buffer local vascular beds and the whole circulatory system against large rises and precipitous drops in chemokine abundance in the plasma. The precise role of DARC at a given time will therefore be influenced by a host of variables, including (i) chemokine release into the blood, (ii) the diversity of the blood chemokine repertoire, (iii) post-translational modifications of plasma chemokines[38], [39], (iv) the relative DARC on/off rates of these chemokines, and (v) the effectiveness of DARC-independent removal mechanisms. Importantly, DARC-bound chemokines are thought incapable of activating chemokine receptors on leukocytes[36], whilst those in plasma will be free to interact with blood-borne leukocytes (leading to receptor desensitisation) or become immobilised on endothelial surfaces (where they may encourage extravasation). Thus, buffering by DARC should ensure a relatively uniform level of chemokine responsiveness amongst blood-borne leukocytes, and limit inappropriate leukocyte adhesion to, and extravasation across, endothelium.

Whilst DARC buffering may be most significant during inflammation and its resolution, it is critical to highlight that many DARC ligands are constitutively produced in animals under normal living conditions. This is evident, for example, from defects in neutrophil and monocyte biology seen in resting mice deficient for CXCR2 and CCR2, respectively[40], [41]. Constitutive production will provide baseline rbc DARC occupancy that will influence both homeostasis and inflammation (Figure 2A). In this regard, exciting new work has shown that CXCL5, produced by platelets, is loaded onto DARC on rbc isolated from resting mice[38]. In a model of lethal pneumonia induced by high dose intratracheal *E. coli* challenge, this CXCL5 appears to limit DARC-mediated scavenging of other CXCR2 ligands (CXCL1 and 2), enhancing their ability to desensitise CXCR2 on neutrophils. As a consequence, CXCL5 deficient mice show increased neutrophil recruitment to the lung, enhanced bacterial clearance and reduced mortality[38]. Interestingly, other recent studies indicate that clotting causes a substantial release of CCL2 from DARC[32], and, remarkably, that DARC influences the formation of neutrophil/platelet aggregates and reduces bleeding time in mice[42]. These links between DARC, platelets and clot formation clearly merit further investigation.

DARC function on nucleated cells: transcytosis and more?

Duffy null humans usually retain DARC expression elsewhere[8], [9], [43]. Inflammation appears to substantially up-regulate this expression. DARC immunoreactivity has been reported in inflamed and resting human tissues on (i) blood vessel endothelial cells (BECs) of postcapillary venules, (ii) lymph node and tonsil high endothelial venules (HEV), (iii) littoral cells lining splenic sinusoids, (iv) glomerular and peri-bronchiolar capillaries, (v) lymphatic endothelial cells (LECs) of skin lymphatic pre-collectors, (vi) epithelium in lung and kidney collecting ducts, and (vii) Purkinje neurons in the cerebellum (from a distinct DARC transcript detected throughout the brain)[26], [43]-[59]. In contrast to humans, there are currently no detailed profiles of DARC expression by nucleated cells in experimental species, except for some evidence for DARC expression by mouse HEVs and lung ECs, and rabbit skin venules[60]-[62]. This is an important gap in our knowledge, particularly when it comes to the interpretation of phenotypes in DARC deficient mice.

The current view of DARC function on human BECs, and perhaps other polarised nucleated cells, is that it mediates transcellular chemokine transport, or transcytosis (Figure 3). This concept stemmed in part from the inability of DARC to couple to conventional chemokine-induced signalling pathways in transfectants[63], encouraging functions for DARC to be considered that were not reliant on this type of signalling. Chemokine transcytosis *in vivo* received direct support from elegant *in situ* studies with radiolabelled chemokines in human and rabbit skin explants, tracking chemokine transport from the interstitial space, through the caveolar network of ECs, to the luminal surface of venules[60], [64]. Notably, injected

chemokines co-localised with DARC immunoreactivity in venular ECs[65]. In *in vitro* model systems, DARC can transport chemokines, functionally intact, across polarised cell monolayers (including DARC-transfected immortalised ECs) and present it on apical surfaces to facilitate leukocyte transmigration[65], [66]. Moreover, mice transgenically over-expressing DARC on ECs show enhanced leukocyte recruitment in response to CXCL1 injection, and exaggerated contact hypersensitivity responses, compared with WT controls[65].

These observations do not exclude other roles for DARC on nucleated cells (Figure 3). First, DARC may contribute to chemokine destruction. There is little degradation of chemokines internalised via DARC in transfected polarised cells[65], [66], but it can deplete extracellular chemokines in other cell lines[67], [68]. Thus, cell-type or context-specific intracellular trafficking itineraries could lead to degradation of chemokines internalised by DARC-expressing primary cells enabling it to play a scavenging role akin to that proposed for D6 (see below). Second, DARC and CCR5 hetero-oligomerise when co-expressed in immortalised cell lines, and DARC can interfere with CCR5 function in this context[69], so DARC may modify the chemokine responsiveness of nucleated cells. DARC+ BECs are likely to express other chemokine receptors, such as CXCR2. Third, it remains possible that DARC is signalling-competent in some contexts. Lack of conventional signalling in transfectants is not surprising since DARC lacks the DRY motif essential for G-protein coupled signal transduction through typical chemokine receptors[37], [63], [70]. However, it has led to the assumption that DARC is never capable of initiating intracellular signals despite the fact that, to our knowledge, the behaviour of endogenous DARC on primary nucleated cells has not been explored in depth. Moreover, recent data showing that chemokines alter the subcellular localisation of DARC in transfected MDCK cells hint at chemokine-driven signals of some form being passed through DARC[65]. Thus, further investigations, ideally using DARC-expressing primary cells, would be helpful at this stage, although the rapid loss of DARC from cultured BECs[71] means that this will not be trivial.

The impact of DARC deficiency in mice: cancer and neutrophils

Unchallenged DARC deficient mice were originally reported to have no gross abnormalities, and lack the neutropenia and monocytopenia observed in Duffy null humans[72], although others have reported leukopenia, but associated neutrophilia, in these animals[42]. Interestingly, they do show high bone mineral density and reduced osteoclastogenesis[73], and in light of the critical osteoclastogenic role played by CCR2[74], it is possible that this phenotype results from dysregulation of CCL2 and other CCR2 ligands. Associations between human rbc DARC deficiency and bone pathophysiology have not been reported, although such studies would now be of interest.

Although there is no firm indication that Duffy null humans have altered tumour susceptibility, roles for DARC in cancer have been explored in mice. In the TRAMP (Transgenic adenocarcinoma of the mouse prostate) model of prostate cancer, tumour growth, intratumoral levels of CXCL1 and 2, and the density of vWF+ vessels were suppressed by DARC[75]. The role of stromal versus haemopoietic DARC was not defined. Melanoma cells expressing CXCL2 grew less well in mice transgenically expressing DARC in ECs than they did in wild-type (WT) recipients. Chemokine levels were not measured, but tumours in DARC transgenic mice contained more lymphocytes and fewer CD31+ vessels[76]. Over-expressing DARC in breast cancer cell lines suppressed their growth and metastasis *in vivo*[68], and lung cancer cell lines carrying ectopic DARC generated larger, but less metastatic, tumours containing more necrosis, fewer live cells and reduced CD31+ vessel density than DARC-negative controls[67]. In both these studies[67],[68], ectopic DARC scavenged chemokines *in vitro*. Remarkably, low DARC immunoreactivity in human breast cancer samples has been linked to high microvessel density, lymph node metastasis and poor

survival, although it was unclear in this study which cell types were DARC+[68]. This surprising finding awaits independent confirmation in larger cohorts. In all studies, effects of DARC were proposed to be due to suppression of angiogenesis through scavenging of CXCR2-binding chemokines, long thought to contribute to blood vessel growth during cancer progression[77]. However, direct evidence for this is limited, and changes in vessel density may be secondary to other effects of DARC deletion.

DARC on ECs has also been linked to metastasis suppression by binding tumour tetraspanin KAI1/CD82 to block proliferation and induce senescence[78]. DARC deficiency compromised the ability of KAI1/CD82 to suppress lung tumour formation from a mouse melanoma cell line, although a specific role for stromal DARC was not shown and chemokine-dependent effects on this phenotype cannot be excluded. It is not clear how these provocative results fit with other theories on CD82-mediated metastasis suppression, or whether DARC binds KAI1/CD82 on non-tumour cells, such as T cells[79].

In models of inflammation, studies have focussed on neutrophil recruitment. Early studies of DARC deficient mice reported reduced neutrophil recruitment into the peritoneal cavity in response to i.p. thioglycollate, but not zymosan, and into the peritoneal cavity, lungs and intestine 24 hours after lipopolysaccharide (LPS) delivery i.p. (200μg)[72]. Others reported increased neutrophil recruitment to liver and lung two hours after i.p. LPS (~600μg)[80]. Luo and colleagues published a correction stating that they too saw more neutrophil recruitment in DARC deficient mice, although it was unclear whether this related to all the models they used[72]. In addition, DARC enhanced neutrophil accumulation in bronchoalveolar lavage after intratracheal administration of CXCL8[66]. During lung injury induced by intratracheal LPS administration, Lee and colleagues reported that DARC deficiency causes a reduction in neutrophils in the airspaces, and in the lung as a whole[61], while others described an increase in airway neutrophils in DARC deficient mice in this model, although with fewer neutrophils in the lung interstitium and adhered to pulmonary vessels[81]. Notably, in both studies, bone marrow transplantation revealed that loss of DARC from the haemopoietic cell compartment was principally responsible for the phenotypes. In an acid-induced lung injury model, DARC was required for neutrophil recruitment to the lung within two hours of acid administration[42]. Reduced neutrophil recruitment, possibly linked to defective chemokine presentation on ECs, was proposed to underpin the reduced response of DARC deficient mice to acute kidney damage induced by ischemia or LPS[82]. In contrast, DARC deficiency provided no protection against renal inflammation after ureteral obstruction or during immune-mediated glomerulonephritis[83].

Although DARC clearly influences neutrophil biology, presumably by binding chemokines, it is difficult to develop an overarching explanation for the observed phenotypes. Moreover, infusion of neutrophils from naïve DARC deficient mice is reported to protect WT mice from acid-induced lung injury, indicative of an inherent resting neutrophil defect[42]. The neutropenia associated with the Duffy null phenotype in humans also points to a role for DARC in neutrophil homeostasis[28-31]. This may result from unsequestered homeostatic plasma chemokines (e.g. CXCL5) influencing neutrophil development, BM egress, or the basal phenotype of circulating neutrophils. Clearly, further work on DARC regulation of neutrophils is needed, and although DARC deficient mice show no change in susceptibility to *Staphylococcus aureus* infection[72] or high dose *E. coli*-induced pneumonia[38], detailed studies using others models of infectious disease could prove informative.

D6

Ligand specificity and expression profiles in mice and humans

In many respects, D6, also called chemokine-binding protein 2 (ccbp2), looks like a typical chemokine receptor. Its predicted amino acid sequence indicates a heptahelical structure with 30-37% identity and ~60% similarity to other chemokine receptors[84-86]. Its C-terminus however is markedly different from other chemokine receptors[84-87], and there are other notable differences, particularly in the second intracellular loop where the canonical DRYLAIV motif is present as DKYLEIV in all mammalian D6 proteins and as DTYLQIV in chickens. D6, like DARC, is a promiscuous high affinity receptor for inflammatory chemokines. It is thought to be restricted to CC chemokines, and there are currently 12 known ligands for human D6[84, 88-90], but a substantial number of chemokines have yet to be tested (Figure 1). The specificity of mouse D6 is less clear. CCL2, 3 and 4 appear be the only mouse chemokines that have been demonstrated to bind mouse D6[85], although human forms of other chemokines can also bind. Clearly, ligand specificity of D6 is a critical feature of its function, so it will be important to define the specificity of mouse D6 for mouse chemokines, ideally on primary cells, and generate a complete profile of ligands for human D6.

In humans, the principal cell types expressing D6 appear to be LECs, trophoblasts, leukocytes, and possibly hepatocytes. In situ radioligand binding, immunohistochemistry, and in situ hybridisation on healthy human skin provided compelling evidence of D6 expression by LECs, confirmed as such by co-expression of LEC marker podoplanin[91]. D6 was also detected on LECs lining most, but not all, lymphatics in lung and gut, and lymphatics within lymph nodes, tonsils and lymphoid aggregates in the gut[91-93]. LECs in many other organs lacked D6, as did BECs in all tissues analysed[91]. It was found on some cells lining vessels in the red pulp of human spleen[91]. The spleen has some limited lymphatic drainage, but the identity of these D6-expressing cells remains unclear. D6 mRNA has also been reported in isolated hepatocytes[94], is readily detectable by Northern blot in liver[84], and we have observed D6 immunoreactivity in human hepatocytes in liver sections (RJBN, unpublished). Notably, the only published study of D6 genetics reported that allelic variants of D6 are associated with liver inflammation in patients with chronic hepatitis C[94]. Trophoblasts in human placenta show particularly high D6 expression[84, 95, 96]. The precise pattern of immunoreactivity depends on trophoblast location, with syncytiotrophoblasts showing a marked polarisation of D6 towards their apical surface, which contacts maternal blood[95, 96]. Human trophoblasts can bind radiolabelled CCL2, but not CXCL8, *in situ*[95]. D6 mRNA, from foetal-derived cells, is detectable in mouse placenta by Northern blot, but at levels that are low compared to humans[84, 95]. It is much less abundant in placenta than lung, and at levels comparable to liver[95], while placenta is by far the richest source of D6 mRNA amongst human tissues[84]. Finally, despite immunohistochemistry of many tissues, including lymphoid tissues, only detecting D6 on a few tissue-resident leukocytes[91-93] (identified as mast cells[97] (RJBN, unpublished) and possibly macrophages[93]), D6 has surprisingly been detected by flow cytometry on B cells, monocytoid and plasmacytoid dendritic cells (mDC and pDC), and a subset of monocytes[97, 98]. Monocyte-derived DC generated *in vitro* also show high surface D6 immunoreactivity in flow cytometry[97]. Although these data point to broad expression of D6 by leukocytes in humans, and are supported by PCR detection of D6 mRNA in some leukocytes[97], the discordance between the immunohistochemistry and flow cytometry data is troublesome and requires resolution.

In contrast to humans, there are, in our experience, no reliable anti-mouse D6 antibodies currently available. This has seriously limited understanding of D6 expression in the mouse. D6 transcript is detectable by Northern blotting in liver and placenta, and particularly in

lung, and low levels can be detected by sensitive RT-PCR in many other tissues[85, 95, 97]. In the colon, D6 mRNA is predominantly found in non-leukocytes (CD45-negative), but not epithelial cells[99], and is up-regulated during DSS-induced colitis[92, 99]. Expression is unchanged in lung, liver and spleen during *Mycobacterium tuberculosis* infection[93], or in liver after carbon tetrachloride treatment[100]. The specific cell types responsible for mouse D6 expression have, in most cases, not been defined. Little, if any, direct evidence exists for D6 expression by LECs, trophoblasts, or hepatocytes *in situ* in mice, although sensitive Q-PCR approaches have provided some insight into D6 mRNA expression by leukocyte subsets[97]. However, the use of fluorescent human CCL2 to detect D6-expressing cells by FACS is now providing unprecedented, cell-by-cell analysis of D6 activity[97, 99]. This approach has revealed that, amongst mouse leukocytes, D6-mediated chemokine uptake is highly restricted to innate-like B cells, such as splenic marginal zone B cells and B1 B cells in the body cavities and elsewhere (CAHH, manuscript submitted). D6 mRNA is enriched in these cells (CAHH, manuscript submitted), in line with microarray data comparing follicular and marginal zone B cells[101]. This contrasts dramatically with the broad expression of D6 reported on human leukocytes[97]. This may be due to the different detection methods used, and their reliability, and it should be noted that the fluorescent chemokine assay measures D6-mediated chemokine uptake rather than D6 protein. However, D6 expression by all human blood-borne mDC and pDC was confirmed functionally (by CCL3 inhibition of fluorescent CCL2 uptake[97]), yet conventional DC in mice show no detectable D6-mediated CCL2 uptake (CAHH, unpublished).

Clearly, as with DARC, a collective look at existing data highlights our lack of understanding of D6 expression in mice, and potential differences between species. This knowledge gap, in addition to uncertainties about mouse D6 ligand specificity, are a significant cause for concern and have meant that the interpretation of phenotypes in D6 deficient mice have substantially relied on data from human studies.

Establishing the paradigm of D6 as a silent scavenger

D6 is currently viewed as a silent chemokine scavenger, an idea first developed in *in vitro* studies. Despite early evidence to the contrary[85], D6 over-expressed in immortalised cell lines, including trophoblast and LEC cell lines, is incapable of inducing Ca^{2+} flux or chemotaxis in response to ligand[89, 96, 102, 103]. Such responses are commonly initiated through typical chemokine receptors. In addition, D6 ligands are unable to induce these responses in the choriocarcinoma BeWo cell line that expresses endogenous D6[95, 96]. Thus, D6 is unable to couple to signal transduction pathways used by typical chemokine receptors. This has been attributed to the DRYLAIV to DKYLEIV change, and indeed, mutation of A to E in CCR5 prevents coupling to Ca^{2+} fluxes, while D6 carrying E in place of A can induce very small Ca^{2+} fluxes (RJBN, unpublished). However, the strict conservation of DKYLEIV in D6 across mammals is surprising if the sole purpose of this evolutionary change was to stop chemokine-induced signalling. Nonetheless, as with DARC, lack of conventional signalling spurred the search for alternative functions for D6 that were not reliant on signalling. A role in transcytosis was not favoured because D6 could not efficiently transport chemokines when artificially expressed in LEC or trophoblast cell lines[96, 103]. However, D6 over-expressed in immortalised cell lines can constitutively internalise from, and recycle to, the cell surface via the endosomal compartment[87, 104, 105]. The C-terminus of D6 plays a critical role, although the significance of its phosphorylation and interaction with β-arrestins is a matter of debate[87, 104]. Chemokine encountered by D6 transiting the cell surface can be internalised without the need for chemokine-induced signalling. In cell lines, internalised chemokine rapidly dissociates from D6 and is degraded, leaving the receptor free to recycle. Iterative rounds of chemokine internalisation result in progressive depletion of extracellular chemokine[87, 103-105]. In addition, transient RNAi

'knock-down' of endogenous D6 in BeWo cells prevents chemokine depletion by these cells[95]. In fact, chemokine may alter the subcellular distribution of D6 in transfected cell lines and BeWo cells to enhance scavenging[106]. The silent scavenger concept established by these *in vitro* observations remains a cornerstone of models of D6 function *in vivo*.

If correct, the context of this scavenging needs to be considered. Active D6 on circulating human leukocytes could act as a DARC-like 'sink' for plasma chemokines to prevent desensitisation of chemokine receptors on blood-borne leukocytes. Chemokine internalisation however would stop D6 serving a 'reservoir' function like rbc DARC. In contrast, removal of chemokines from the surface of ECs by D6 on blood-borne leukocytes could suppress leukocyte recruitment into inflamed tissue. Motile D6-expressing leukocytes in tissues could deplete bioavailable chemokines throughout interstitial spaces[107], while D6 on non-motile cells would cause local modification of chemokines. For example, D6 on LECs or trophoblasts might keep the surfaces of these cells chemokine-free, preventing adhesion of leukocytes expressing cognate receptors. In placenta, this could help limit maternal immune cell recognition of semi-allogeneic foetal cells[95], while on LECs it may prevent inflammatory leukocyte adhesion to the lymphatic vasculature to enable the free movement of tissue fluid and matured DCs. In pregnant humans, ~10m^2 of D6-expressing syncytiotrophoblasts are bathed in circulating maternal blood at term. D6-mediated scavenging would be expected to reduce plasma chemokine abundance, and interestingly plasma levels of CCL2, 3 and 11 are reduced during pregnancy[95]. Again, this would be predicted to enhance the chemokine responsiveness of circulating leukocytes because of less receptor desensitisation. Conversely, D6 on invasive cytotrophoblasts[95] might reduce chemokine abundance in the decidua to suppress leukocyte movement within the tissue. Thus, as with DARC, the temporospatial context of D6-mediated scavenging would be predicted to be of paramount importance. Of course, all these contextural considerations depend on how robustly the silent scavenger model reflects D6 function in vivo.

The impact of D6 deficiency on inflammation *in vivo*

The generation of D6 deficient mice allowed indispensable *in vivo* roles for D6 to be defined. First it was reported that these animals develop exaggerated skin inflammation after repeated cutaneous challenge with 12-O-tetradecanoyl-phorbol-13-acetate (TPA), characterised by enhanced leukocyte infiltration (primarily by T cells and mast cells), keratinocyte hyperproliferation and extensive neovascularisation[108]. They also show altered responses to subcutaneous Complete Freund's Adjuvant (CFA) injection, more rapidly developing granuloma-like lesions than WT controls, with some alterations in draining lymph node cellularity[102]. Chemokine levels were somewhat elevated in skin[108] and lymph node[102] in these studies. The impact of D6 deficiency on skin responses was further developed using models of cancer[109]. Topical application of mutagen 7,12-Dimethylbenz(a)antracene (DMBA), prior to repeated applications of TPA, leads to papilloma formation. D6 deficiency increased susceptibility to tumour development in this model, and was sufficient to make resistance strains of mice susceptible to tumour formation[109]. Conversely, transgenic over-expression of D6 in basal keratinocytes could reduce papilloma formation, and T cells and mast cells were less abundant in inflamed skin. D6 deficiency was associated with increased bioavailable CCL3 protein in chronically inflamed skin, and cultured transgenic keratinocytes scavenged CCL3. Another skin defect was unexpectedly revealed while examining susceptibility to autoimmune encephalitis[110]. The reduction in disease severity, spinal cord inflammation and demyelination in D6 deficient mice was attributed to a defect in T cell priming. $CD11c^+$ cell aggregates were found in the skin, which, it was argued, indicated that defective DC migration was responsible. However, for reasons we do not understand, we have not observed any defects

in T cell priming in D6 deficient mice in response to antigens delivered via the skin, and find no role for D6 in autoimmune encephalitis (unpublished).

In models of allergic lung inflammation, D6 deficient mice developed a more substantial inflammatory infiltrate after challenge, primarily comprised of eosinophils and DCs[111]. Unexpectedly, they had less airway reactivity to aerosolised methacholine, even in the absence of challenge. Only subtle changes in chemokine abundance were found in bronchoalveolar lavage: after one antigen challenge, CCL17 but not CCL22 was elevated in D6 deficient mice compared with WT, while after repeated challenge the opposite was seen. Other likely D6 ligands (CCL3, 5 and CCL11) were unaffected. Responses to intra-nasal infection with *Mycobacterium tuberculosis* have also been explored[93]. D6 deficient mice became moribund 8-16 weeks after infection, while all WT animals survived beyond 16 weeks, providing compelling evidence for an indispensible role for D6 during infection. Bacterial load was unaffected, but there was a progressive accumulation of leukocytes in the lung, liver, kidney and mediastinal lymph node of D6 deficient mice that was not seen in WT animals. Unsurprisingly, the exaggerated inflammation seen twelve weeks after infection was associated with elevated levels of chemokines and cytokines in bronchoalveolar lavage and serum, and a cocktail of neutralising anti-chemokine antibodies (against CCL2, 3, 4, and 5) afforded some protection for D6 deficient mice.

D6 deficient mice are also more sensitive to carbon tetrachloride-induced acute liver damage[100]. This was associated with increased numbers of T and NK cells in the liver, and inflamed D6 deficient livers contained slightly more CCL2, 3 and 5 than the less inflamed livers of WT mice. In models of colitis, two studies report opposing outcomes of D6 deletion[92], [99]. Both used the dextran sodium sulphate (DSS) model of colitis, although differences in treatment regime, genetic background, or local microflora may explain disparities between the studies. Vetrano and colleagues observed that, compared with WT, D6 deficient mice had higher clinical disease scores and increased leukocyte recruitment to the colon (mainly T cells, DC and, surprisingly, B cells), and their more highly inflamed colons released more chemokines when cultured ex vivo[92]. They were also more susceptible to chronic colitis, and to colon tumour formation induced by azoxymethane mutagenesis then chronic DSS feeding. In contrast, we found that D6 deficient mice developed less severe colitis than WT. Chemokine release from explants, and the magnitude of the cellular infiltrate, were no different from WT[99]. Protection was associated with, and partially dependent on, an increase in IL-17A concomitant with the presence of more IL-17A-secreting γδ T cells in inflamed colons.

Finally, two papers have explored roles for D6 during mouse pregnancy motivated by D6 expression in placenta[95], [96]. The first study revealed that, compared with WT mice carrying WT foetuses, D6 deficient females carrying D6 deficient foetuses had increased susceptibility to foetal loss induced by endotoxin or anti-phospholipid antibodies[96]. Shortly after endotoxin challenge, male or pregnant female D6 deficient mice had higher levels of serum CCL2 and 11 (CCL3 was unaffected) compared to WT. There was also a small increase in CCL3 and 11 (but not CCL2) in placental lysates, accompanied by a large rise in macrophages. Endotoxin-induced foetal loss from WT and D6 deficient animals could be prevented with a cocktail of neutralising anti-chemokine antibodies (against CCL2, 3, 4, and 5). Although these exciting data revealed a role for D6 in fetal protection, they did not specifically implicate foetal/trophoblast D6. The phenotypes may have been caused by loss of maternal D6. However, in embryo transfer experiments into WT recipients, while foetal D6 was dispensable for the survival of syngeneic pups, it did provide some protection for allogeneic foetuses[95].

Model of D6 function in vivo: silence is golden

The phenotypes of D6 deficient mice clearly demonstrate the indispensability of D6 during inflammation. Incorporating expression data and the silent scavenger model, they have been presented as evidence that D6 on lymphatics, trophoblasts, leukocytes and possibly hepatocytes mediates chemokine scavenging *in vivo* without signalling. Since the first phenotypes of D6 deficient mice were reported[102, 108], this attractive theory has dominated studies of D6. In our view, however, a number of important issues still require resolution.

First, phenotypes in D6 deficient mice have been interpreted using cell-specific expression profiles from humans. We still await the identification of D6-expressing cells in most mouse tissues. In addition, it is important to clarify disparities in leukocyte expression between mice and humans, and define the relative contribution of D6 on blood versus stromal cells. This has only been attempted once using BM chimaeras. This led to the conclusion that D6 on stromal cells is required in mice for protection from weight loss during colitis[92]. Because of D6 expression by colonic LECs in humans[91], this stromal D6 was assumed to be on mouse lymphatics[92]. Confusingly, however, WT mice carrying D6 deficient blood appeared to have comparable weight loss to D6 deficient mice with WT blood in this study, with differences only apparent between control groups (i.e. WT mice reconstituted with WT BM[92]). Nonetheless, this approach, or the generation of lineage-specific D6 deficient mice, is important in helping to understand D6, particularly in light of the discussion above regarding the impact of context on scavenging outcome.

The second, more pressing, concern is that the model relies heavily on D6 behaviour in transfected cells. The principal evidence of D6 scavenging *in vivo* are elevated chemokine levels that are often, but not always, seen in challenged D6 deficient mice. However, it is very difficult to determine whether this is directly caused by D6 deletion, or is secondary to enhanced inflammation. Likewise, suppression of inflammation in D6 deficient mice by neutralising anti-chemokine antibody cocktails[93, 96] does not prove that loss of chemokine scavenging underpins the phenotype, but rather that they develop an aberrant response that is chemokine-dependent. Moreover, elevated chemokines are seen when conventional chemokine receptors are inhibited or deleted. For example, antibodies that block ligand binding to CCR2 lead to elevation in CCR2 ligands during inflammation[112]. More notably, mice lacking CCR2, CXCR2, CX3CR1 or CXCR3 show substantial increases in their cognate ligands, even in the absence of challenge, leading to desensitisation of other remaining receptors that bind these chemokines[113]. Such profound chemokine dysregulation is not seen in D6 deficient mice, even after challenge. Thus, chemokine scavenging appears to be an integral component of the biology of many, if not all, chemokine receptors, and dysregulated chemokines in D6 deficient mice do not provide unequivocal evidence that D6 is a specialised chemokine scavenger *in vivo*. In fact, we would argue that this definition relies exclusively on the silence of D6, a phenomenon that has only been reported in *in vitro* cell systems and, in our view, with a limited assessment of signalling competency. Notably, artificially over-expressed heptahelical receptors in in vitro cellular systems often show at least some degree of constitutive activation[114]. This can mimic receptor behaviour on primary cells, but this is not always the case. Thus, it is critical that the behaviour of native D6 on primary cells is examined in detail with an open mind to define if it has any signalling capacity or ability to change cell behaviour in its natural context. If nothing else, it offers the potential to make the silent scavenger model more robust. Until then, we believe it is premature to exclude other possible functions for D6 on primary cells, including transcytosis and signalling (Figure 3), and that some doubt should remain about the validity of the silent scavenger model of D6.

Europe PMC Funders Author Manuscripts

Future Prospects

ACRs, united by their unusual behaviour in transfected cells, are intriguing, enigmatic components of chemokine networks. We have attempted to provide an outline of existing theories about DARC and D6 function *in vivo*, some more robust than others, and present a comprehensive summary of the experimental bases for these theories. No doubt novel phenotypes will emerge from the study of DARC or D6 deficient mice in the future to reveal exciting new indispensable functions for these molecules. At the moment, there is no real option but to interpret these phenotypes using existing paradigms of chemokine sequestration, transcytosis and/or scavenging. However, there are critical gaps in our knowledge, outlined herein, that mean these paradigms are based on several important assumptions. New tools and experimental approaches are needed to allow these gaps to be filled, and it is imperative to determine if *in vitro* systems provide reliable insights into ACR function in vivo.

References

1. Rot A, von Andrian UH. Chemokines in innate and adaptive host defense: basic chemokinese grammar for immune cells. Annu Rev Immunol. 2004; 22:891–928. [PubMed: 15032599]
2. Mantovani A, Bonecchi R, Locati M. Tuning inflammation and immunity by chemokine sequestration: decoys and more. Nat Rev Immunol. 2006; 6:907–18. [PubMed: 17124512]
3. Hansell CA, Simpson CV, Nibbs RJ. Chemokine sequestration by atypical chemokine receptors. Biochem Soc Trans. 2006; 34:1009–13. [PubMed: 17073739]
4. Loetscher P, Pellegrino A, Gong JH, Mattioli I, Loetscher M, Bardi G, et al. The ligands of CXC chemokine receptor 3, I-TAC, Mig, and IP10, are natural antagonists for CCR3. J Biol Chem. 2001; 276:2986–91. [PubMed: 11110785]
5. Xanthou G, Duchesnes CE, Williams TJ, Pease JE. CCR3 functional responses are regulated by both CXCR3 and its ligands CXCL9, CXCL10 and CXCL11. Eur J Immunol. 2003; 33:2241–50. [PubMed: 12884299]
6. Allison AC. Genetic control of resistance to human malaria. Curr Opin Immunol. 2009; 21:499–505. [PubMed: 19442502]
7. Tournamille C, Colin Y, Cartron JP, Le Van Kim C. Disruption of a GATA motif in the Duffy gene promoter abolishes erythroid gene expression in Duffy-negative individuals. Nat Genet. 1995; 10:224–8. [PubMed: 7663520]
8. Mallinson G, Soo KS, Schall TJ, Pisacka M, Anstee DJ. Mutations in the erythrocyte chemokine receptor (Duffy) gene: the molecular basis of the Fya/Fyb antigens and identification of a deletion in the Duffy gene of an apparently healthy individual with the Fy(a-b-) phenotype. Br J Haematol. 1995; 90:823–9. [PubMed: 7669660]
9. Rios M, Chaudhuri A, Mallinson G, Sausais L, Gomensoro-Garcia AE, Hannon J, et al. New genotypes in Fy(a-b-) individuals: nonsense mutations (Trp to stop) in the coding sequence of either FY A or FY B. Br J Haematol. 2000; 108:448–54. [PubMed: 10691880]
10. Horuk R, Chitnis CE, Darbonne WC, Colby TJ, Rybicki A, Hadley TJ, et al. A receptor for the malarial parasite Plasmodium vivax: the erythrocyte chemokine receptor. Science. 1993; 261:1182–4. [PubMed: 7689250]
11. Grimberg BT, Udomsangpetch R, Xainli J, McHenry A, Panichakul T, Sattabongkot J, et al. Plasmodium vivax invasion of human erythrocytes inhibited by antibodies directed against the Duffy binding protein. PLoS Med. 2007; 4:e337. [PubMed: 18092885]
12. Tung J, Primus A, Bouley AJ, Severson TF, Alberts SC, Wray GA. Evolution of a malaria resistance gene in wild primates. Nature. 2009; 460:388–91. [PubMed: 19553936]
13. He W, Neil S, Kulkarni H, Wright E, Agan BK, Marconi VC, et al. Duffy antigen receptor for chemokines mediates trans-infection of HIV-1 from red blood cells to target cells and affects HIV-AIDS susceptibility. Cell Host Microbe. 2008; 4:52–62. [PubMed: 18621010]

14. Horne KC, Li X, Jacobson LP, Palella F, Jamieson BD, Margolick JB, et al. Duffy antigen polymorphisms do not alter progression of HIV in African Americans in the MACS cohort. Cell Host Microbe. 2009; 5:415–7. author reply 418-9. [PubMed: 19454342]
15. Julg B, Reddy S, van der Stok M, Kulkarni S, Qi Y, Bass S, et al. Lack of Duffy antigen receptor for chemokines: no influence on HIV disease progression in an African treatment-naive population. Cell Host Microbe. 2009; 5:413–5. author reply 418-9. [PubMed: 19454341]
16. Kulkarni H, Marconi VC, He W, Landrum ML, Okulicz JF, Delmar J, et al. The Duffy-null state is associated with a survival advantage in leukopenic HIV-infected persons of African ancestry. Blood. 2009; 114:2783–92. [PubMed: 19620399]
17. Lachgar A, Jaureguiberry G, Le Buenac H, Bizzini B, Zagury JF, Rappaport J, et al. Binding of HIV-1 to RBCs involves the Duffy antigen receptors for chemokines (DARC). Biomed Pharmacother. 1998; 52:436–9. [PubMed: 9921412]
18. Walley NM, Julg B, Dickson SP, Fellay J, Ge D, Walker BD, et al. The Duffy antigen receptor for chemokines null promoter variant does not influence HIV-1 acquisition or disease progression. Cell Host Microbe. 2009; 5:408–10. author reply 418-9. [PubMed: 19454339]
19. Winkler CA, An P, Johnson R, Nelson GW, Kirk G. Expression of Duffy antigen receptor for chemokines (DARC) has no effect on HIV-1 acquisition or progression to AIDS in African Americans. Cell Host Microbe. 2009; 5:411–3. author reply 418-9. [PubMed: 19454340]
20. Vergara C, Tsai YJ, Grant AV, Rafaels N, Gao L, Hand T, et al. Gene encoding Duffy antigen/receptor for chemokines is associated with asthma and IgE in three populations. Am J Respir Crit Care Med. 2008; 178:1017–22. [PubMed: 18827265]
21. Nebor D, Durpes MC, Mougenel D, Mukisi-Mukaza M, Elion J, Hardy-Dessources MD, et al. Association between Duffy antigen receptor for chemokines expression and levels of inflammation markers in sickle cell anemia patients. Clin Immunol. 2010; 136:116–22. [PubMed: 20347396]
22. Mecabo G, Hayashida DY, Azevedo-Shimmoto MM, Vicari P, Arruda MM, Bordin JO, et al. Duffy-negative is associated with hemolytic phenotype of sickle cell anemia. Clin Immunol. 2010; 136:458–9. author reply 460-1. [PubMed: 20638342]
23. Afenyi-Annan A, Kail M, Combs MR, Orringer EP, Ashley-Koch A, Telen MJ. Lack of Duffy antigen expression is associated with organ damage in patients with sickle cell disease. Transfusion. 2008; 48:917–24. [PubMed: 18248572]
24. Schnog JB, Keli SO, Pieters RA, Rojer RA, Duits AJ. Duffy phenotype does not influence the clinical severity of sickle cell disease. Clin Immunol. 2000; 96:264–8. [PubMed: 10964545]
25. Akalin E, Neylan JF. The influence of Duffy blood group on renal allograft outcome in African Americans. Transplantation. 2003; 75:1496–500. [PubMed: 12792503]
26. Sticherling M, Baisch C, Bornscheuer E, Schroder JM, Christophers E. The role of the Duffy antigen-related chemokine receptor in psoriasis vulgaris. Cytokine. 2002; 18:329–33. [PubMed: 12160521]
27. Elson JK, Beebe-Dimmer JL, Morgenstern H, Chilkuri M, Blanchard J, Lentsch AB. The Duffy Antigen/Receptor for Chemokines (DARC) and Prostate-Cancer Risk among Jamaican Men. J Immigr Minor Health. 2011; 13:36–41. [PubMed: 20596779]
28. Nalls MA, Wilson JG, Patterson NJ, Tandon A, Zmuda JM, Huntsman S, et al. Admixture mapping of white cell count: genetic locus responsible for lower white blood cell count in the Health ABC and Jackson Heart studies. Am J Hum Genet. 2008; 82:81–7. [PubMed: 18179887]
29. Reich D, Nalls MA, Kao WH, Akylbekova EL, Tandon A, Patterson N, et al. Reduced neutrophil count in people of African descent is due to a regulatory variant in the Duffy antigen receptor for chemokines gene. PLoS Genet. 2009; 5:e1000360. [PubMed: 19180233]
30. Grann VR, Ziv E, Joseph CK, Neugut AI, Wei Y, Jacobson JS, et al. Duffy (Fy), DARC, and neutropenia among women from the United States, Europe and the Caribbean. Br J Haematol. 2008; 143:288–93. [PubMed: 18710383]
31. Mayr FB, Spiel AO, Leitner JM, Firbas C, Kliegel T, Jilma-Stohlawetz P, et al. Duffy antigen modifies the chemokine response in human endotoxemia. Crit Care Med. 2008; 36:159–65. [PubMed: 18007272]
32. Schnabel RB, Baumert J, Barbalic M, Dupuis J, Ellinor PT, Durda P, et al. Duffy antigen receptor for chemokines (Darc) polymorphism regulates circulating concentrations of monocyte

Europe PMC Funders Author Manuscripts

chemoattractant protein-1 and other inflammatory mediators. Blood. 2010; 115:5289–99. [PubMed: 20040767]

33. Jilma-Stohlawetz P, Homoncik M, Drucker C, Marsik C, Rot A, Mayr WR, et al. Fy phenotype and gender determine plasma levels of monocyte chemotactic protein. Transfusion. 2001; 41:378–81. [PubMed: 11274594]
34. Mayr FB, Spiel AO, Leitner JM, Firbas C, Schnee J, Hilbert J, et al. Influence of the Duffy antigen on pharmacokinetics and pharmacodynamics of recombinant monocyte chemoattractant protein (MCP-1, CCL-2) in vivo. Int J Immunopathol Pharmacol. 2009; 22:615–25. [PubMed: 19822078]
35. Fukuma N, Akimitsu N, Hamamoto H, Kusuhara H, Sugiyama Y, Sekimizu K. A role of the Duffy antigen for the maintenance of plasma chemokine concentrations. Biochem Biophys Res Commun. 2003; 303:137–9. [PubMed: 12646177]
36. Darbonne WC, Rice GC, Mohler MA, Apple T, Hebert CA, Valente AJ, et al. Red blood cells are a sink for interleukin 8, a leukocyte chemotaxin. J Clin Invest. 1991; 88:1362–9. [PubMed: 1918386]
37. Luo H, Chaudhuri A, Johnson KR, Neote K, Zbrzezna V, He Y, et al. Cloning, characterization, and mapping of a murine promiscuous chemokine receptor gene: homolog of the human Duffy gene. Genome Res. 1997; 7:932–41. [PubMed: 9314499]
38. Mei J, Liu Y, Dai N, Favara M, Greene T, Jeyaseelan S, et al. CXCL5 regulates chemokine scavenging and pulmonary host defense to bacterial infection. Immunity. 2010; 33:106–17. [PubMed: 20643340]
39. Loos T, Opdenakker G, Van Damme J, Proost P. Citrullination of CXCL8 increases this chemokine's ability to mobilize neutrophils into the blood circulation. Haematologica. 2009; 94:1346–53. [PubMed: 19608678]
40. Cacalano G, Lee J, Kikly K, Ryan AM, Pitts-Meek S, Hultgren B, et al. Neutrophil and B cell expansion in mice that lack the murine IL-8 receptor homolog. Science. 1994; 265:682–4. [PubMed: 8036519]
41. Serbina NV, Pamer EG. Monocyte emigration from bone marrow during bacterial infection requires signals mediated by chemokine receptor CCR2. Nat Immunol. 2006; 7:311–7. [PubMed: 16462739]
42. Zarbock A, Bishop J, Muller H, Schmolke M, Buschmann K, Van Aken H, et al. Chemokine homeostasis vs. chemokine presentation during severe acute lung injury: the other side of the Duffy antigen receptor for chemokines. Am J Physiol Lung Cell Mol Physiol. 2010; 298:L462–71. [PubMed: 20061440]
43. Peiper SC, Wang ZX, Neote K, Martin AW, Showell HJ, Conklyn MJ, et al. The Duffy antigen/receptor for chemokines (DARC) is expressed in endothelial cells of Duffy negative individuals who lack the erythrocyte receptor. J Exp Med. 1995; 181:1311–7. [PubMed: 7699323]
44. Middleton J, Americh L, Gayon R, Julien D, Mansat M, Mansat P, et al. A comparative study of endothelial cell markers expressed in chronically inflamed human tissues: MECA-79, Duffy antigen receptor for chemokines, von Willebrand factor, CD31, CD34, CD105 and CD146. J Pathol. 2005; 206:260–8. [PubMed: 15887283]
45. Hadley TJ, Lu ZH, Wasniowska K, Martin AW, Peiper SC, Hesselgesser J, et al. Postcapillary venule endothelial cells in kidney express a multispecific chemokine receptor that is structurally and functionally identical to the erythroid isoform, which is the Duffy blood group antigen. J Clin Invest. 1994; 94:985–91. [PubMed: 8083383]
46. Chaudhuri A, Nielsen S, Elkjaer ML, Zbrzezna V, Fang F, Pogo AO. Detection of Duffy antigen in the plasma membranes and caveolae of vascular endothelial and epithelial cells of nonerythroid organs. Blood. 1997; 89:701–12. [PubMed: 9002974]
47. Lee JS, Frevert CW, Thorning DR, Segerer S, Alpers CE, Cartron JP, et al. Enhanced expression of Duffy antigen in the lungs during suppurative pneumonia. J Histochem Cytochem. 2003; 51:159–66. [PubMed: 12533524]
48. Liu XH, Hadley TJ, Xu L, Peiper SC, Ray PE. Up-regulation of Duffy antigen receptor expression in children with renal disease. Kidney Int. 1999; 55:1491–500. [PubMed: 10201015]
49. Hadley TJ, Peiper SC. From malaria to chemokine receptor: the emerging physiologic role of the Duffy blood group antigen. Blood. 1997; 89:3077–91. [PubMed: 9129009]

50. Horuk R, Martin A, Hesselgesser J, Hadley T, Lu ZH, Wang ZX, et al. The Duffy antigen receptor for chemokines: structural analysis and expression in the brain. J Leukoc Biol. 1996; 59:29–38. [PubMed: 8558064]
51. Gardner L, Wilson C, Patterson AM, Bresnihan B, FitzGerald O, Stone MA, et al. Temporal expression pattern of Duffy antigen in rheumatoid arthritis: up-regulation in early disease. Arthritis Rheum. 2006; 54:2022–6. [PubMed: 16732566]
52. Patterson AM, Siddall H, Chamberlain G, Gardner L, Middleton J. Expression of the duffy antigen/receptor for chemokines (DARC) by the inflamed synovial endothelium. J Pathol. 2002; 197:108–16. [PubMed: 12081195]
53. Segerer S, Regele H, Mac KM, Kain R, Cartron JP, Colin Y, et al. The Duffy antigen receptor for chemokines is up-regulated during acute renal transplant rejection and crescentic glomerulonephritis. Kidney Int. 2000; 58:1546–56. [PubMed: 11012889]
54. Girard JP, Baekkevold ES, Yamanaka T, Haraldsen G, Brandtzaeg P, Amalric F. Heterogeneity of endothelial cells: the specialized phenotype of human high endothelial venules characterized by suppression subtractive hybridization. Am J Pathol. 1999; 155:2043–55. [PubMed: 10595934]
55. Horuk R, Martin AW, Wang Z, Schweitzer L, Gerassimides A, Guo H, et al. Expression of chemokine receptors by subsets of neurons in the central nervous system. J Immunol. 1997; 158:2882–90. [PubMed: 9058825]
56. Wick N, Haluza D, Gurnhofer E, Raab I, Kasimir MT, Prinz M, et al. Lymphatic precollectors contain a novel, specialized subpopulation of podoplanin low, CCL27-expressing lymphatic endothelial cells. Am J Pathol. 2008; 173:1202–9. [PubMed: 18772332]
57. Geleff S, Draganovici D, Jaksch P, Segerer S. The role of chemokine receptors in acute lung allograft rejection. Eur Respir J. 35:167–75. [PubMed: 19608592]
58. Desbaillets I, Diserens AC, Tribolet N, Hamou MF, Van Meir EG. Upregulation of interleukin 8 by oxygen-deprived cells in glioblastoma suggests a role in leukocyte activation, chemotaxis, and angiogenesis. J Exp Med. 1997; 186:1201–12. [PubMed: 9334359]
59. Le Van Kim C, Tournamille C, Kroviarski Y, Cartron JP, Colin Y. The 1.35-kb and 7.5-kb Duffy mRNA isoforms are differently regulated in various regions of brain, differ by the length of their 5′ untranslated sequence, but encode the same polypeptide. Blood. 1997; 90:2851–3. [PubMed: 9326260]
60. Middleton J, Neil S, Wintle J, Clark-Lewis I, Moore H, Lam C, et al. Transcytosis and surface presentation of IL-8 by venular endothelial cells. Cell. 1997; 91:385–95. [PubMed: 9363947]
61. Lee JS, Wurfel MM, Matute-Bello G, Frevert CW, Rosengart MR, Ranganathan M, et al. The Duffy antigen modifies systemic and local tissue chemokine responses following lipopolysaccharide stimulation. J Immunol. 2006; 177:8086–94. [PubMed: 17114483]
62. Kashiwazaki M, Tanaka T, Kanda H, Ebisuno Y, Izawa D, Fukuma N, et al. A high endothelial venule-expressing promiscuous chemokine receptor DARC can bind inflammatory, but not lymphoid, chemokines and is dispensable for lymphocyte homing under physiological conditions. Int Immunol. 2003; 15:1219–27. [PubMed: 13679391]
63. Neote K, Mak JY, Kolakowski LF Jr. Schall TJ. Functional and biochemical analysis of the cloned Duffy antigen: identity with the red blood cell chemokine receptor. Blood. 1994; 84:44–52. [PubMed: 7517217]
64. Hub E, Rot A. Binding of RANTES, MCP-1, MCP-3, and MIP-1alpha to cells in human skin. Am J Pathol. 1998; 152:749–57. [PubMed: 9502417]
65. Pruenster M, Mudde L, Bombosi P, Dimitrova S, Zsak M, Middleton J, et al. The Duffy antigen receptor for chemokines transports chemokines and supports their promigratory activity. Nat Immunol. 2009; 10:101–8. [PubMed: 19060902]
66. Lee JS, Frevert CW, Wurfel MM, Peiper SC, Wong VA, Ballman KK, et al. Duffy antigen facilitates movement of chemokine across the endothelium in vitro and promotes neutrophil transmigration in vitro and in vivo. J Immunol. 2003; 170:5244–51. [PubMed: 12734373]
67. Addison CL, Belperio JA, Burdick MD, Strieter RM. Overexpression of the duffy antigen receptor for chemokines (DARC) by NSCLC tumor cells results in increased tumor necrosis. BMC Cancer. 2004; 4:28. [PubMed: 15214968]

68. Wang J, Ou ZL, Hou YF, Luo JM, Shen ZZ, Ding J, et al. Enhanced expression of Duffy antigen receptor for chemokines by breast cancer cells attenuates growth and metastasis potential. Oncogene. 2006; 25:7201–11. [PubMed: 16785997]

69. Chakera A, Seeber RM, John AE, Eidne KA, Greaves DR. The duffy antigen/receptor for chemokines exists in an oligomeric form in living cells and functionally antagonizes CCR5 signaling through hetero-oligomerization. Mol Pharmacol. 2008; 73:1362–70. [PubMed: 18230715]

70. Chaudhuri A, Polyakova J, Zbrzezna V, Williams K, Gulati S, Pogo AO. Cloning of glycoprotein D cDNA, which encodes the major subunit of the Duffy blood group system and the receptor for the Plasmodium vivax malaria parasite. Proc Natl Acad Sci U S A. 1993; 90:10793–7. [PubMed: 8248172]

71. Lacorre DA, Baekkevold ES, Garrido I, Brandtzaeg P, Haraldsen G, Amalric F, et al. Plasticity of endothelial cells: rapid dedifferentiation of freshly isolated high endothelial venule endothelial cells outside the lymphoid tissue microenvironment. Blood. 2004; 103:4164–72. [PubMed: 14976058]

72. Luo H, Chaudhuri A, Zbrzezna V, He Y, Pogo AO. Deletion of the murine Duffy gene (Dfy) reveals that the Duffy receptor is functionally redundant. Mol Cell Biol. 2000; 20:3097–101. [PubMed: 10757794]

73. Edderkaoui B, Baylink DJ, Beamer WG, Wergedal JE, Porte R, Chaudhuri A, et al. Identification of mouse Duffy antigen receptor for chemokines (Darc) as a BMD QTL gene. Genome Res. 2007; 17:577–85. [PubMed: 17416748]

74. Binder NB, Niederreiter B, Hoffmann O, Stange R, Pap T, Stulnig TM, et al. Estrogen-dependent and C-C chemokine receptor-2-dependent pathways determine osteoclast behavior in osteoporosis. Nat Med. 2009; 15:417–24. [PubMed: 19330010]

75. Shen H, Schuster R, Stringer KF, Waltz SE, Lentsch AB. The Duffy antigen/receptor for chemokines (DARC) regulates prostate tumor growth. FASEB J. 2006; 20:59–64. [PubMed: 16394268]

76. Horton LW, Yu Y, Zaja-Milatovic S, Strieter RM, Richmond A. Opposing roles of murine duffy antigen receptor for chemokine and murine CXC chemokine receptor-2 receptors in murine melanoma tumor growth. Cancer Res. 2007; 67:9791–9. [PubMed: 17942909]

77. Keeley EC, Mehrad B, Strieter RM. CXC chemokines in cancer angiogenesis and metastases. Adv Cancer Res. 2010; 106:91–111. [PubMed: 20399957]

78. Bandyopadhyay S, Zhan R, Chaudhuri A, Watabe M, Pai SK, Hirota S, et al. Interaction of KAI1 on tumor cells with DARC on vascular endothelium leads to metastasis suppression. Nat Med. 2006; 12:933–8. [PubMed: 16862154]

79. Miranti CK. Controlling cell surface dynamics and signaling: how CD82/KAI1 suppresses metastasis. Cell Signal. 2009; 21:196–211. [PubMed: 18822372]

80. Dawson TC, Lentsch AB, Wang Z, Cowhig JE, Rot A, Maeda N, et al. Exaggerated response to endotoxin in mice lacking the Duffy antigen/receptor for chemokines (DARC). Blood. 2000; 96:1681–4. [PubMed: 10961863]

81. Reutershan J, Harry B, Chang D, Bagby GJ, Ley K. DARC on RBC limits lung injury by balancing compartmental distribution of CXC chemokines. Eur J Immunol. 2009; 39:1597–607. [PubMed: 19499525]

82. Zarbock A, Schmolke M, Bockhorn SG, Scharte M, Buschmann K, Ley K, et al. The Duffy antigen receptor for chemokines in acute renal failure: A facilitator of renal chemokine presentation. Crit Care Med. 2007; 35:2156–63. [PubMed: 17855830]

83. Vielhauer V, Allam R, Lindenmeyer MT, Cohen CD, Draganovici D, Mandelbaum J, et al. Efficient renal recruitment of macrophages and T cells in mice lacking the duffy antigen/receptor for chemokines. Am J Pathol. 2009; 175:119–31. [PubMed: 19498001]

84. Nibbs RJ, Wylie SM, Yang J, Landau NR, Graham GJ. Cloning and characterization of a novel promiscuous human beta-chemokine receptor D6. J Biol Chem. 1997; 272:32078–83. [PubMed: 9405404]

85. Nibbs RJ, Wylie SM, Pragnell IB, Graham GJ. Cloning and characterization of a novel murine beta chemokine receptor, D6. Comparison to three other related macrophage inflammatory

protein-1alpha receptors, CCR-1, CCR-3, and CCR-5. J Biol Chem. 1997; 272:12495–504. [PubMed: 9139699]

86. Bonini JA, Steiner DF. Molecular cloning and expression of a novel rat CC-chemokine receptor (rCCR10rR) that binds MCP-1 and MIP-1beta with high affinity. DNA Cell Biol. 1997; 16:1023–30. [PubMed: 9324304]
87. McCulloch CV, Morrow V, Milasta S, Comerford I, Milligan G, Graham GJ, et al. Multiple roles for the C-terminal tail of the chemokine scavenger D6. J Biol Chem. 2008; 283:7972–82. [PubMed: 18201974]
88. Savino B, Borroni EM, Torres NM, Proost P, Struyf S, Mortier A, et al. Recognition versus adaptive up-regulation and degradation of CC chemokines by the chemokine decoy receptor D6 are determined by their N-terminal sequence. J Biol Chem. 2009; 284:26207–15. [PubMed: 19632987]
89. Bonecchi R, Locati M, Galliera E, Vulcano M, Sironi M, Fra AM, et al. Differential recognition and scavenging of native and truncated macrophage-derived chemokine (macrophage-derived chemokine/CC chemokine ligand 22) by the D6 decoy receptor. J Immunol. 2004; 172:4972–6. [PubMed: 15067078]
90. Nibbs RJ, Yang J, Landau NR, Mao JH, Graham GJ. LD78beta, a non-allelic variant of human MIP-1alpha (LD78alpha), has enhanced receptor interactions and potent HIV suppressive activity. J Biol Chem. 1999; 274:17478–83. [PubMed: 10364178]
91. Nibbs RJ, Kriehuber E, Ponath PD, Parent D, Qin S, Campbell JD, et al. The beta-chemokine receptor D6 is expressed by lymphatic endothelium and a subset of vascular tumors. Am J Pathol. 2001; 158:867–77. [PubMed: 11238036]
92. Vetrano S, Borroni EM, Sarukhan A, Savino B, Bonecchi R, Correale C, et al. The lymphatic system controls intestinal inflammation and inflammation-associated Colon Cancer through the chemokine decoy receptor D6. Gut. 2010; 59:197–206. [PubMed: 19846409]
93. Di Liberto D, Locati M, Caccamo N, Vecchi A, Meraviglia S, Salerno A, et al. Role of the chemokine decoy receptor D6 in balancing inflammation, immune activation, and antimicrobial resistance in Mycobacterium tuberculosis infection. J Exp Med. 2008; 205:2075–84. [PubMed: 18695004]
94. Wiederholt T, von Westernhagen M, Zaldivar MM, Berres ML, Schmitz P, Hellerbrand C, et al. Genetic variations of the chemokine scavenger receptor D6 are associated with liver inflammation in chronic hepatitis C. Hum Immunol. 2008; 69:861–6. [PubMed: 18822328]
95. Madigan J, Freeman DJ, Menzies F, Forrow S, Nelson SM, Young A, et al. Chemokine scavenger D6 is expressed by trophoblasts and aids the survival of mouse embryos transferred into allogeneic recipients. J Immunol. 2010; 184:3202–12. [PubMed: 20147628]
96. Martinez de la Torre Y, Buracchi C, Borroni EM, Dupor J, Bonecchi R, Nebuloni M, et al. Protection against inflammation- and autoantibody-caused fetal loss by the chemokine decoy receptor D6. Proc Natl Acad Sci U S A. 2007; 104:2319–24. [PubMed: 17283337]
97. McKimmie CS, Fraser AR, Hansell C, Gutierrez L, Philipsen S, Connell L, et al. Hemopoietic cell expression of the chemokine decoy receptor D6 is dynamic and regulated by GATA1. J Immunol. 2008; 181:3353–63. [PubMed: 18714007]
98. McKimmie CS, Fraser AR, Hansell C, Gutierrez L, Philipsen S, Connell L, et al. Hemopoietic cell expression of the chemokine decoy receptor D6 is dynamic and regulated by GATA1. J Immunol. 2008; 181:8171–81. [PubMed: 19039854]
99. Bordon Y, Hansell CA, Sester DP, Clarke M, Mowat AM, Nibbs RJ. The atypical chemokine receptor D6 contributes to the development of experimental colitis. J Immunol. 2009; 182:5032–40. [PubMed: 19342683]
100. Berres ML, Trautwein C, Zaldivar MM, Schmitz P, Pauels K, Lira SA, et al. The chemokine scavenging receptor D6 limits acute toxic liver injury in vivo. Biol Chem. 2009; 390:1039–45. [PubMed: 19642876]
101. Kin NW, Crawford DM, Liu J, Behrens TW, Kearney JF. DNA microarray gene expression profile of marginal zone versus follicular B cells and idiotype positive marginal zone B cells before and after immunization with Streptococcus pneumoniae. J Immunol. 2008; 180:6663–74. [PubMed: 18453586]

102. Martinez de la Torre Y, Locati M, Buracchi C, Dupor J, Cook DN, Bonecchi R, et al. Increased inflammation in mice deficient for the chemokine decoy receptor D6. Eur J Immunol. 2005; 35:1342–6. [PubMed: 15789340]
103. Fra AM, Locati M, Otero K, Sironi M, Signorelli P, Massardi ML, et al. Cutting edge: scavenging of inflammatory CC chemokines by the promiscuous putatively silent chemokine receptor D6. J Immunol. 2003; 170:2279–82. [PubMed: 12594248]
104. Galliera E, Jala VR, Trent JO, Bonecchi R, Signorelli P, Lefkowitz RJ, et al. beta-Arrestin-dependent constitutive internalization of the human chemokine decoy receptor D6. J Biol Chem. 2004; 279:25590–7. [PubMed: 15084596]
105. Weber M, Blair E, Simpson CV, O'Hara M, Blackburn PE, Rot A, et al. The chemokine receptor D6 constitutively traffics to and from the cell surface to internalize and degrade chemokines. Mol Biol Cell. 2004; 15:2492–508. [PubMed: 15004236]
106. Bonecchi R, Borroni EM, Anselmo A, Doni A, Savino B, Mirolo M, et al. Regulation of D6 chemokine scavenging activity by ligand- and Rab11-dependent surface up-regulation. Blood. 2008; 112:493–503. [PubMed: 18480427]
107. Graham GJ, McKimmie CS. Chemokine scavenging by D6: a movable feast? Trends Immunol. 2006; 27:381–6. [PubMed: 16814608]
108. Jamieson T, Cook DN, Nibbs RJ, Rot A, Nixon C, McLean P, et al. The chemokine receptor D6 limits the inflammatory response in vivo. Nat Immunol. 2005; 6:403–11. [PubMed: 15750596]
109. Nibbs RJ, Gilchrist DS, King V, Ferra A, Forrow S, Hunter KD, et al. The atypical chemokine receptor D6 suppresses the development of chemically induced skin tumors. J Clin Invest. 2007; 117:1884–92. [PubMed: 17607362]
110. Liu L, Graham GJ, Damodaran A, Hu T, Lira SA, Sasse M, et al. Cutting edge: the silent chemokine receptor D6 is required for generating T cell responses that mediate experimental autoimmune encephalomyelitis. J Immunol. 2006; 177:17–21. [PubMed: 16785491]
111. Whitehead GS, Wang T, DeGraff LM, Card JW, Lira SA, Graham GJ, et al. The chemokine receptor D6 has opposing effects on allergic inflammation and airway reactivity. Am J Respir Crit Care Med. 2007; 175:243–9. [PubMed: 17095748]
112. Maus UA, Wellmann S, Hampl C, Kuziel WA, Srivastava M, Mack M, et al. CCR2-positive monocytes recruited to inflamed lungs downregulate local CCL2 chemokine levels. Am J Physiol Lung Cell Mol Physiol. 2005; 288:L350–8. [PubMed: 15516494]
113. Cardona AE, Sasse ME, Liu L, Cardona SM, Mizutani M, Savarin C, et al. Scavenging roles of chemokine receptors: chemokine receptor deficiency is associated with increased levels of ligand in circulation and tissues. Blood. 2008; 112:256–63. [PubMed: 18347198]
114. Milligan G. Constitutive activity and inverse agonists of G protein-coupled receptors: a current perspective. Mol Pharmacol. 2003; 64:1271–6. [PubMed: 14645655]
115. Gardner L, Patterson AM, Ashton BA, Stone MA, Middleton J. The human Duffy antigen binds selected inflammatory but not homeostatic chemokines. Biochem Biophys Res Commun. 2004; 321:306–12. [PubMed: 15358176]
116. Chaudhuri A, Zbrzezna V, Polyakova J, Pogo AO, Hesselgesser J, Horuk R. Expression of the Duffy antigen in K562 cells. Evidence that it is the human erythrocyte chemokine receptor. J Biol Chem. 1994; 269:7835–8. [PubMed: 8132497]

Europe PMC Funders Author Manuscripts

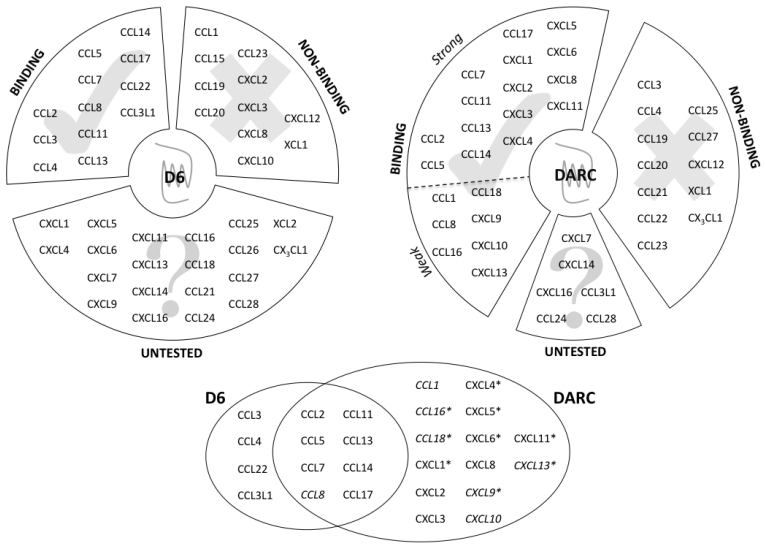

Figure 1. Chemokine binding profiles of human DARC and human D6

Chemokines are separated into those that show binding; those that do not bind; and those that, to our knowledge, have not been reported to have been tested[10, 38, 63, 84, 88-90, 115, 116]. The size of a segment is proportional to the number of chemokines in that segment. DARC-binding chemokines are divided into strong (K_i < 100 nM) and weak binders (K_i = 100 nM − 1 μM), while non-binding chemokines have K_i > 1 μM[38, 115, 116]. Compared to CCL3L1, its closely-related non-allelic variant CCL3 is a relatively low affinity ligand for D6[90]. All designations are based on radioligand displacement experiments using a limited repertoire of radiolabelled chemokines, and do not take into account the impact that N-terminal processing or other post-translational chemokine modifications may have on affinity[38, 39]. The bottom panel shows known specific and common ligands for human D6 and DARC. Chemokines that have not been tested on human D6 are denoted by an asterisk, and those with weak binding to DARC are italicised.

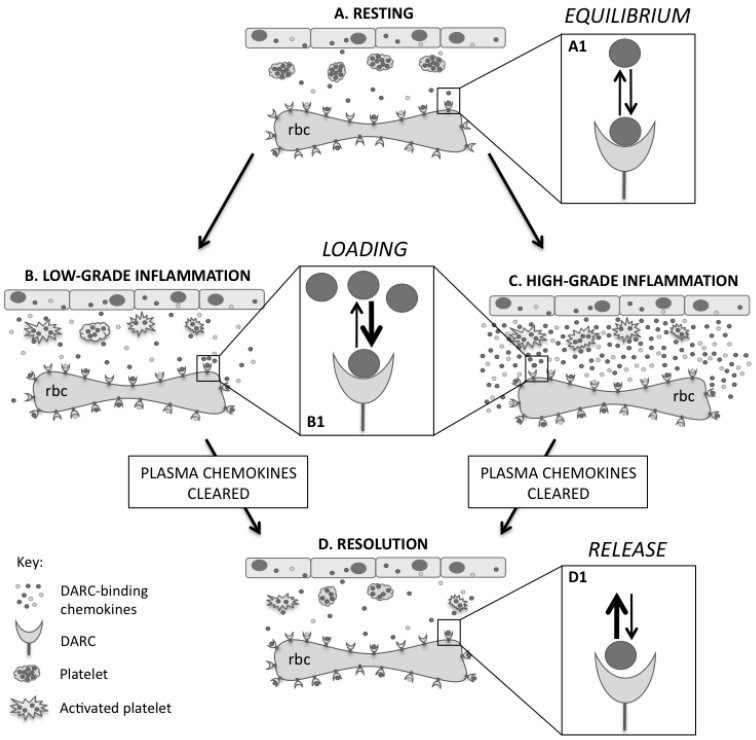

Figure 2. Erythrocyte DARC as a chemokine buffering system

During homeostasis (A), constitutively produced chemokines (CXCL5, CCL2 and others) occupy some of the available DARC molecules on rbc. These molecules will be in dynamic equilibrium with plasma-borne chemokines (A1), which will be constantly 'topped up' by chemokine release; subject to post-translational modification; and removed by DARC-independent mechanisms. Platelets are a rich source of CXCL5. Bioactive plasma chemokines will provide tonic stimulation of cognate leukocytic chemokine receptors. Upon induction of low-grade local inflammation (B), DARC-binding chemokines from platelets and other sources (e.g. ECs and circulating leukocytes) will transiently elevate plasma chemokine levels favouring rapid local rbc DARC loading (B1). This will buffer against large rises in chemokines. During high-grade inflammation (C), excessive levels of inflammation-induced chemokines, along with pre-existing chemokines produced at rest, may overwhelm the rbc DARC 'sink'. In both scenarios (B, C), there are downstream implications for leukocytic chemokine receptors and leukocyte trafficking, discussed in more detail in the text. Since plasma-borne chemokines appear to be more rapidly cleared than those on rbc, there will be a rapid switch to a state where the kinetics of receptor/ligand interactions will favour net chemokine release from the rbc 'reservoir' back into plasma (D and D1). This will dampen the rate of chemokine removal from the plasma as inflammation resolves. The kinetics of this DARC loading and release cycle, the nature of the equilibrium resting state, and the impact on leukocytic chemokine receptors will be dictated by the diversity of the chemokine repertoire, and by the rate of input, modification, and removal of plasma chemokines.

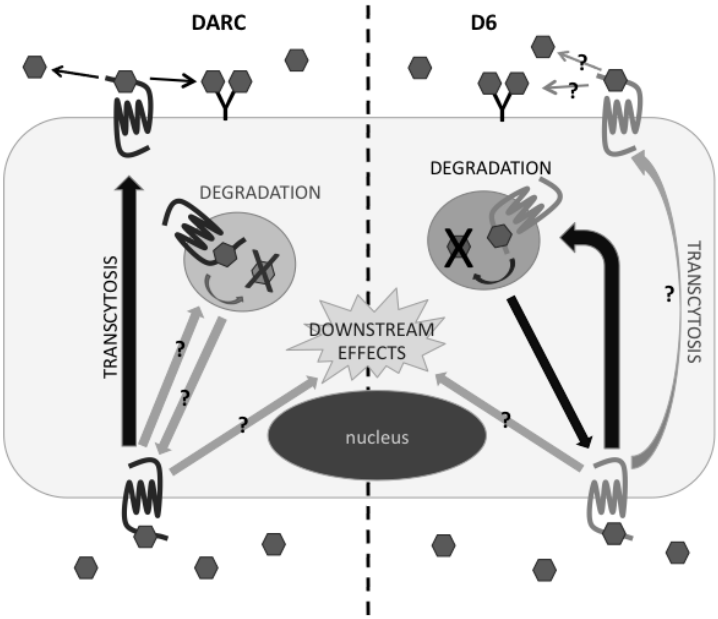

Figure 3. Putative functions of DARC and D6 on nucleated cells

Large black arrows denote roles for DARC (left) and D6 (right) that are thought to occur on nucleated cells i.e. transcytosis by DARC and scavenging by D6. Chemokines are shown as hexagons. Grey arrows with question marks, denote hypothesized functions i.e. transcytosis by D6 and scavenging by DARC. Also included are possible 'downstream effects' that both molecules may have on the behaviour of cells, such as the regulation of chemokine responsiveness or the induction of intracellular signals. These signals may facilitate transcytosis or scavenging, or perhaps induce other, currently unknown, biological responses (see text for further discussion). Note that transcytosed chemokine at the top of the figure may be transferred to cell surface glycosaminoglycans[60, 65], or released into the extracellular space.

Europe PMC Funders Author Manuscripts