

# Rac GTPases in human diseases

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**Abstract.** Rho GTPases are members of the Ras superfamily of GTPases that regulate a wide variety of cellular functions. While Rho GTPase pathways have been implicated in various pathological conditions in humans, to date coding mutations in only the hematopoietic specific GTPase, *RAC2*, have been found to cause a human disease, a severe phagocytic immunodeficiency characterized by life-threatening infections in infancy. Interestingly, the phenotype was predicted by a mouse knock-out of *Rac2* and resembles leukocyte adhesion deficiency (LAD). Here we review Rho GTPases with a specific focus on Rac GTPases. In particular, we discuss a new understanding of the unique and overlapping roles of Rac2 in blood cells that has developed since the generation of mice deficient in Rac1, Rac2 and Rac3 proteins. We propose that Rac2 mutations leading to disease be termed LAD type IV.

Keywords: Rho GTPases, Rac, neutrophils, leukocyte adhesion deficiency, RhoH, lymphocytes

## 1. Rho GTPases

The Rho family of GTPases are genes that encode small monomeric Ras-related proteins [9]. The Rho proteins can be divided into 6 families based on sequence homology, protein domains and function. These include Rac, RhoA, Cdc42, TC10 and TCL, Rnd, the Rho BTB subset and the Miro subfamily. There are at least 18 members of the Rho GTPase family of proteins. RhoA, Rac and Cdc42 are the best studied of these proteins. Studies in fibroblasts have provided the initial insights into the cellular function of Rho GTPases in actin cytoskeletal reorganization [42]. As confirmed by studies in many other mammalian cells, activation of RhoA, Rac1 and Cdc42 induces the assembly of three distinct filamentous(F)-actin structures respectively through separate signaling pathways [72]. In contrast to RhoA, which induces actin stress fiber formation, two other members of the Rho subfamily, RhoD

and RhoE, stimulate the disassembly of stress fibers and thereby inhibit cell motility [31,78]. Therefore, Rho GTPases appear to exert both positive and negative functional effects on regulating actin cytoskeleton reorganization and actin-based function in cells.

Rho GTPases have now been also recognized as intracellular switches to transduce signals from a wide variety of membrane receptors, including tyrosine kinase receptors and integrins, and more recently the T and B cell receptors [7,31,78,81]. They have been implicated in regulating a variety of cellular functions including cell proliferation, G1 cell cycle progression, adhesion, motility and apoptosis [31,78], including in hematopoietic cells (see below). Many studies have demonstrated that the c-Jun N-terminal kinase (JNK) and p38 MAP kinase pathways are activated by Rac and Cdc42 [80]. RhoA, Rac and Cdc42 have each been reported in different cell systems to regulate nuclear signaling through activation of serum response factor and nuclear factor  $\kappa$ B (NF- $\kappa$ B) [61]. In contrast to RhoA, Rac and Cdc42, RhoB appears to suppress the activation of NF- $\kappa$ B [32]. Therefore, RhoB, in combination with RhoD and RhoE, represents a distinct group of Rho GTPases that have functional effects antagonistic to those of other Rho GTPases, such as RhoA, Rac and Cdc42. Competition between these

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groups of Rho GTPases may provide another level for regulating particular Rho GTPase-activated signaling pathways related to disease development. For instance, Hansen et al. have demonstrated the competition between RhoA and RhoE in regulating Ras-induced transformation of epithelial cells via the Raf-Mek-ERK kinase pathways [43]. We and others have demonstrated that RhoH, which is a hematopoietic-specific, GTPase-deficient Rho GTPase, antagonizes Rac in lymphoblastic cell lines and primary myeloid progenitor cells [16, 54].

Similar to Ras proteins, Rho GTPases are subject to posttranslational modifications, in which the proteins are lipid modified by prenylation at a conserved cysteine in the carboxy(C)-terminal CaaL sequence (where C is cysteine, a is an aliphatic amino acid, and L is leucine). Immediately adjacent to this CaaL sequence, several members of the family also contain an important 'polybasic domain', which has been shown in Ras proteins to modify the strength of the membrane localization. Subsequent to prenylation, the C-terminal tripeptide (aaL) is removed by proteolysis, and the new C-terminal amino acid is methylated [27,33,34,73,89]. Lipid modifications of Rho GTPases are critical for membrane association and for protein-protein interactions [64]. As described below, both the 'polybasic' domain and the conserved CaaL sequence are particularly important in determining Rac protein localization and therefore function in blood cells.

In agreement with the wide variety of roles served by Rho GTPases [45], deregulation of Rho GTPase-related signaling pathways [for example the Cdc42 pathway and its effector Wiskott-Aldrich Syndrome protein (WASp)] have been implicated in human diseases. The abnormal activities of these proteins have been associated with cellular transformation and tumor progression, either by enhancing the metastasis of the transformed cells or by serving as ancillary factors that contribute to the transforming activity of oncoproteins such as Ras [79,92]. However, unlike Ras, the only GTPase found to have a mutated coding sequence in human disease is the *RAC2* gene. Additionally, mutations in the first intron of *RHOH*, a GTPase-deficient member of the Rnd family, have been implicated in B cell malignancies (reviewed in [70]). Interestingly, Rac2 and RhoH are the only Rho GTPases with expression restricted to the hematopoietic cells. This review will focus on Rho GTPases of the Rac family, and the role of *RAC2* specifically in human diseases.

## 2. Rac GTPases

Similar to Ras, Rac proteins act as molecular switches that cycle between inactive, guanosine diphosphate (GDP)-bound and active, guanosine triphosphate (GTP)-bound states. GTP-bound (active) Rac interacts with a variety of specific effectors or target molecules to trigger diverse cellular responses. Activation of Rac is typically induced by Dbl-family guanine nucleotide exchange factors (GEFs) that are activated by receptor-dependent kinases [91]. Acceleration of the intrinsic GTPase function of the proteins by GTPase activating proteins (GAPs) removes the  $\gamma$ -phosphate and returns the active GTPase to a GDP-bound (inactive) form of the protein. Additional regulation may involve sequestering or stabilizing the inactive, GDP-bound protein via GDP dissociation inhibitors (GDIs) within the cytoplasm. Almost all Rho family GTPases, including Rac, stimulate actin polymerization within the cell and regulate changes in cytoskeletal structure that affect cell shape, migration, and adhesion. These processes are clearly important for immune cells, including lymphocytes and neutrophils, but are also critical for more primitive hematopoietic stem and progenitor cells. The role of Rac GTPases in these more primitive hematopoietic cells has recently been reviewed [14,60] and will not be covered here.

## 3. Role of Rac GTPases in immune cells

There are three isoforms of Rac proteins in mammals, which are Rac1, Rac2, and Rac3. Rac1 and Rac2 share over 90% identity in primary sequence, and all three isoforms share a high degree of amino acid identity (~89% overall). As noted above, expression of Rac proteins differs substantially in tissue distribution and levels of expression. Rac1 and Rac3 proteins are widely expressed in different tissues, whereas as noted above Rac2 expression is restricted to cells of hematopoietic origin.

The cellular functions that are the result of activation of Rac GTPases are determined in part by a multitude of cellular effector proteins. These are likely both agonist- and cell lineage-specific, although much remains to be understood about Rho GTPases' effectors in primary cells. However, some downstream effectors of Rac have been implicated in primary hematopoietic cells. Depending on the specific lineage and agonist, Rac can activate p42/p44 and p38 MAPK, JNK, and Akt kinases [13,15,39,67,87,88]. These pathways reg-

ulate cell survival and proliferation via cell cycle progression. Wnt/ $\beta$ -catenin signaling has also been proposed as a key signal in maintenance of hematopoietic stem cell (HSC) quiescence [30,65,66]. For instance, Wu et al. found that Rac1 complexes with JNK2 and  $\beta$ -catenin [84]. In this complex, Rac1 acts through JNK2 kinase to promote phosphorylation of  $\beta$ -catenin at Ser191 and Ser605 residues, which in turn induces nuclear translocation via the canonical Wnt-dependent  $\beta$ -catenin activation pathway. Interestingly, Rac and McgRacGAP also are required for nuclear localization of Stat5a [49], suggesting that Rac proteins directly or indirectly regulate nuclear localization of factors involved in gene regulation. PAK, POR1, and STAT5 have been implicated in Rac functions in primary hematopoietic cells, particularly in cytoskeletal functions and transcription regulation. Many of these studies in primary cells have been the result of the availability of mouse knock-out lines leading to a much more precise understanding of how Rac GTPases regulate cell behavior.

Because the sequences of all three mammalian Rac proteins are quite similar, it has been difficult to understand how these related proteins regulate different cell functions. Importantly Rac2 differs from Rac1 and Rac3 in the primary sequence located near the C-terminal CaaL sequence, in a domain analogous the Ras "hypervariable region". This region in Rac1 (and in K-Ras) is highly poly-basic in nature. We and others have utilized gene-targeted mice to understand the unique functions of each Rac protein in mammalian cells. For instance, we have shown that in spite of the overall sequence similarity between Rac1 and Rac2, Rac2 serves unique functions in hematopoietic cells related to intracellular localization, which is controlled in large part by the divergent sequences in the 'polybasic domain' between Rac1 and Rac2 [28,76]. These differences in Rac1 and Rac2 functions will be described in more detail below.

#### 4. In vitro studies

Much of the early knowledge of the roles of Rac GTPases in hematopoietic cells were derived from *in vitro* studies utilizing either constitutive active (CA) or dominant negative (DN) mutants. Many of these studies were carried out in cell lines and focused on roles in immune cells, specifically phagocytic cells and lymphocytes. In phagocytic cells, two critical and linked functions have been studied: cell shape change asso-

ciated with adhesion and migration and the generation of phagocytic oxidants required for killing bacteria. In these studies, transient expression of mutant Rac cDNAs supported a role for Rac in membrane ruffling, lamellipodia formation, and migration of a macrophage cell line [4,5,21]. Both Rac1 and Rac2 have been known for some time to be required in cell-free systems to generate NADPH-dependent phagocytic oxidase [2,52]. Interestingly, the difference in the C-terminal tail of Rac has been shown to affect the efficiency of interaction with p67<sup>phox</sup>, one of the key proteins in the oxidase complex. Previous studies also have suggested a role for Rac in lymphocyte development and function. For instance, Rac2 mRNA increases 30- to 50-fold in human T cells stimulated with hemagglutinin A [63], and transgenic expression of constitutively active Rac2 in the thymus leads to increased apoptosis and thymic atrophy [55]. When the activities of both Rac1 and Rac2 are inhibited by overexpression of a DN form of Rac in T-cell lines, T-cell activation downstream of the T-cell receptor, as well as the costimulatory molecules CD28 and CD5, are substantially reduced [8,37,44]. In Jurkat cells, antisense inhibition of Rac2 results in diminished actin polymerization triggered by ligation of L-selectin [11]. Thus, prior to the development of knock-out mice of each Rac GTPase, there was already considerable evidence that Rac proteins played a critical role in various immune cells.

While previous work using mutants of Rac (and other Rho GTPases) have contributed much to the understanding of the role of Rho GTPases in various cells, these methods have limitations. For instance, the levels of expressed proteins are non-physiologic and work in cell lines may not accurately reflect primary cells. In addition, constitutively active and dominantly negative mutants often have non-specific effects on multiple pathways due to abnormal binding of GEFs and effectors. A significant advance in the field was the use of gene-targeting in mice, which both allows analysis of effects in primary cells and eliminates some of the non-specific effects of expressing mutant proteins. The first reported use of mouse genetics to study Rho GTPases was the knock-out of *Rac2* [67], which showed a phagocytic immunodeficiency that facilitated identification of the first patient with a GTPase mutation. This study also demonstrated conclusively that Rac2 has functions unique from Rac1 (Table 1). Subsequent reports of conditional knock-outs of Rac1 and knock-out of Rac3, allowed a better understanding of both the unique and overlapping roles of these GTPases in blood and other tissues [14]. For instance, genetic ablation of

Table 1  
Summary of laboratory abnormalities described in patients and mice with *Rac2* deficiency

Function tested	Assay used	Case 1	Case 2	<i>Rac2</i> <sup>-/-</sup> mouse
Hematologic	Neutrophil count	Increased	Increased	Increased
	Hemoglobin	Normal	Reduced	Normal*
	T cell count	Low-normal	Reduced	Normal*
	TREC	?	Reduced	-
	Exudate formation	Reduced	Reduced	Reduced
	Susceptibility to bacterial infection	Increased	Increased	Normal
	Susceptibility to fungal infection	-	-	Increased
NADPH oxidase	Response to PMA	Normal	Normal	Reduced
	Response to fMLP	Absent	Absent	Reduced
	Response to PMA and TNF $\alpha$	-	-	Normal
	Response to serum-opsonized zymosan	-	-	Normal
	Response to IgG-SRBC	-	-	Reduced
	Response to Zymosan activated serum	Reduced	-	-
	Expression of L-selectin	-	-	Normal
Adhesion	Tethering to GlyCAM-1	Reduced	-	Reduced
	Tethering to P-selectin	-	-	Normal
	Spreading on anti-integrin antibodies	-	-	Reduced
	Chemotaxis	Response to fMLP	Reduced	-
Migration by videomicroscopy	Response to IL-8	-	-	Reduced
	Response to LTB4	-	-	Reduced
	Response to fMLP	Reduced	-	Reduced
	F-actin formation	Response to zymosan activated serum	Reduced	Reduced
Response to fMLP		Reduced	Reduced	Reduced
Response to IL-8		Reduced	-	Reduced
Response to TNF- $\alpha$		-	-	Normal
Response to GM-CSF		-	-	Normal
Response to fMLP + TNF- $\alpha$		-	-	Normal
Response to fMLP + GM-CSF		-	-	Normal
Phagocytosis	FcR-mediated	Reduced	-	Reduced
Degranulation	Azurophilic granule release	Absent	-	Reduced
	Specific granule release	Normal	-	Normal

References: [6, 7, 27, 53, 61, 79, 84, 87, unpublished data, C. Kim and M. Dinauer, manuscript submitted, J. Verbsky and J. Routes].

\*These parameters are reduced in mice with deficiency of both *Rac1* and *Rac2*.

*Rac1* in the mouse embryonic limb bud ectoderm disrupts canonical Wnt signaling and phenocopies deletion of  $\beta$ -catenin in causing severe truncations of the limb development [75,84].

### 5. Studies of *Rac* GTPases in neutrophils and macrophages using gene targeted mice

*Rac2*<sup>-/-</sup> mice have a phenotype similar to the human diseases of leukocyte adhesion deficiency and chronic granulomatous disease, including increased susceptibility to *Aspergillus* infection [67]. The mice show a prominent leukocytosis likely due to reduced shear-dependent endothelial capture via L-selectin and defective neutrophil chemotaxis in response to multiple agonists. Neutrophils have reduced F-actin assembly, reduced phagocytosis and reduced superoxide production in response to fMLP. In activated neutrophils, *Rac2*

has a subcellular microlocalization distinct from that of *Rac1*, and absence of *Rac2* is associated with mislocalization of *Rac1* [28]. As mentioned above, the localization of *Rac2* is regulated by specific sequences in the 'polybasic domain' of the C-terminus and an additional unique residue outside this region that had not previously been associated with GTPase function. These two distinct structural determinants determine the specificity of *Rac2* function in neutrophil chemotaxis and superoxide generation, via Pak1, Por and p67<sup>phox</sup>, implicating multiple, distinct and potentially parallel effector pathways [15]. Furthermore, *Rac2* has distinct functions in neutrophils [35,51,54], and the C-terminus differences are critical to *in vivo* function of neutrophils [86]. Additionally, *Rac2*<sup>-/-</sup> macrophages also demonstrate selective defects in phagocytosis and superoxide generation [85].

Neutrophils from mice simultaneously deficient in both *Rac1* and *Rac2* have a more pronounced migra-

tion and adhesion defect compared with mice deficient in Rac2 [39], while neutrophils from mice deficient in only Rac1 have no migration or oxidase production defect to fMLP, emphasizing both unique and overlapping roles for Rac1 and Rac2. The more pronounced phenotype of murine neutrophils in the absence of both Rac1 and Rac2 may be relevant to the human disease discussed later in this review, since over-expression of a patient-derived DN mutant (D57N) suppresses both Rac1 and Rac2 activities [83]. Rac1 and Rac2 may also contribute to a differential response to ligand concentrations, and also show different kinetics of activation after stimulation with fMLP leading to distinct kinetics of F-actin assembly and cell shape changes [90]. Degranulation of primary granules in murine neutrophils is Rac2-dependent [1] and primary human neutrophil granule exocytosis is Rac-dependent [57]. Rac has been demonstrated to mediate neutrophil and macrophage responses that are critical to acute and chronic lung injury in two murine models [24,29]. Rac2 appears to have a modest effect on neutrophil development [36], and in one study expression of a DN mutant also led to myeloid cell dysplasia *in vitro* [76]. Overall, these data generated from gene-targeted mice define critical roles for both Rac1 and Rac2 in several aspects of neutrophil biology. Unanswered questions focus on which upstream GEFs and downstream effectors are important in neutrophil behavior related to Rac and to what degree these other proteins regulate different functions of Rac1 and Rac2 in these cells.

## 6. Rac2 in lymphocytes studied in gene targeted mice

Crocker et al. [22] has shown that Rac2 deficiency results in an abnormal T lymphocyte distribution *in vivo*, which is associated with defects in both T-lymphocyte migration and F-actin generation in response to chemoattractants *in vitro*. The defects in T cell distribution may be of relevance to the abnormalities in T cells reported in the second patient with the D57N Rac2 mutation (see below). However, despite the abnormalities in cytokine production and migration by T cells, Rac2-deficient mice were not overly sensitive to *L. major* infection, and cytotoxic T-lymphocyte responses to cytomegalovirus and ovalbumin were also normal. Thus, Rac2 is required for normal T-lymphocyte migration, but its role in the generation of TH1 responses to infection *in vivo* appears to be redundant.

Two groups generated double deficient Rac1/Rac2 mice using a floxed allele of *Rac1* crossed into transgenic Cre and *Rac2*<sup>-/-</sup> mice (*Rac1*<sup>fllox</sup>/*CD2-Cre/Rac2*<sup>-/-</sup> and *Rac1*<sup>fllox</sup>/*lck-Cre/Rac2*<sup>-/-</sup>). These mice show defects in thymocyte survival, beta selection and positive selection, that are moderate to severe, depending on the strength of the deleting Cre transgene [26,41]. Both models exhibit profound loss of T cells in the periphery, which implies a post-thymic survival or activation defect. Notably, deficiency of Rac1 alone does not perturb T cell development. Guo et al. [41] utilized mice conditionally deleted of Rac1 in either the stem cell compartment or the T cell compartment, in the setting of Rac2 deficiency. They noted that loss of both Rac1 and Rac2 in the stem cell compartment leads to significant deficiency of common lymphoid progenitors, whereas loss of Rac1 only modestly affected the number of CLP and moderately reduced T cell development. T cell-specific deletion of Rac1 did not affect T-cell development, whereas deletion of both Rac1 and Rac2 reduced immature CD4<sup>+</sup>CD8<sup>+</sup> and mature CD4<sup>+</sup> populations in thymus as well as CD4<sup>+</sup> and CD8<sup>+</sup> populations in spleen.

Rac-deficient mice also show abnormalities of the B lineage, particularly in tissues requiring robust B cell migration. In spite of a peripheral blood B cell lymphocytosis, *Rac2*<sup>-/-</sup> mice show reduced numbers of B1a and marginal zone B cells and reduced IgM-secreting plasma cells. *Rac2*<sup>-/-</sup> B cells demonstrate a defect in responses to combined stimulation of the B cell receptor and CD19 [23]. Walmsley et al. [81] also reported a severe block in B cell development in *Rac1*<sup>Δ/Δ</sup>; *Rac2*<sup>-/-</sup> mice (where *Rac1*<sup>Δ/Δ</sup> denotes the Rac1 gene deletion from the flox allele). This block was particularly pronounced in the spleen and peritoneal cavity. The B cells from the double knockout mice show defective proliferation and survival associated with alterations in Akt and cyclin D activation. Furthermore, these B cells fail to transduce B cell receptor signals for upregulation of BAFF-R. This receptor for BAFF is required for B cell development and maintenance.

## 7. Rac2 in mast cells studied in gene targeted mice

Mast cells can be derived in large numbers from the bone marrow of mice after culture *in vitro* with interleukin-3. Using this procedure, *Rac2*<sup>-/-</sup> mice yield mast cells that have significantly defective cell growth and reduced survival [88]. These cells show a pro-apoptotic profile characterized by reduced Akt

activation, increased Bad expression, and reduced Bcl- $X_L$  expression. Reduced activation of Akt is also consistently found in multiple blood lineages of *Rac2*<sup>-/-</sup> mice at least as assayed *in vitro*. *Rac2*-deficient mast cells also demonstrate reduced migration to stem cell factor (SCF) and reduced mast cell degranulation to IgE receptor cross-linking. Use of gene targeted mice also demonstrated that *Rac2* plays a unique role in regulation of gene expression in mast cells [38]. In this study cytokine-stimulated activation of MMCP-7 gene transcription, a major granule protein, was selectively regulated in mast cells by *Rac2*-dependent activation of JNK. Overall these studies show *Rac2* plays an important role in mast cell biology.

### 8. *Rac2* in other blood cells studied in gene targeted mice

There is much less known and published on the role of Rac GTPases in other blood cells. Rac GTPases are important in maintaining red blood cell membrane integrity [47] and appear to be critical for enucleation [46]; *Rac1*<sup>Δ/Δ</sup>; *Rac2*<sup>-/-</sup> mice develop microcytic anemia with a hemoglobin drop of about 20% and significant anisocytosis and poikilocytosis associated with reticulocytosis suggestive of a hemolytic process. These changes are associated with abnormal actin and spectrin cytoskeleton and reduced deformability. Furthermore, deficiency of both *Rac1* and *Rac2* inhibits the expansion of very early erythroid cells in mice [48]. *Rac2* is also important in platelet secretion and aggregation [3]. Finally, abnormal activation of Rac has been shown in a number of acute and chronic leukemias, including chronic myelogenous leukemia, chronic lymphocytic leukemias and acute myeloid leukemias [59, 74, 77, 82]. Genetic or pharmacologic inhibition of Rac leads to reduced proliferation and increased apoptosis in some of these leukemias.

### 9. *Rac3* knock-out mice

*Rac3* is a third member of the Rac subfamily which was originally identified from a chronic myelogenous leukemia cell line and has been implicated in human breast cancer [10, 56], ovarian cancer [58], cellular transformation [50] and tumor invasion [17]. *Rac3* interacts with the integrin binding protein, CIB, and promotes integrin-mediated adhesion and spreading in immortalized cell lines. In addition, *Rac3* is differential-

ly expressed during myeloid differentiation. *Rac3* null mutant mice [18, 20] are viable, fertile and without obvious physical anomalies, although one group has reported a mild neurological phenotype [12]. In addition, in a p190 Bcr-abl transgenic mouse model of acute lymphoblastic leukemia, *Rac3*, but not *Rac1* or *Rac2*, is activated and *Rac3* deficiency attenuates the development of leukemia in mice, although for an unexplained reason this attenuation was only seen in female mice [18]. In the absence of *Rac1* and *Rac2*, *Rac3* can mediate an attenuated myelodysplastic phenotype in mice transplanted with p210 Bcr-abl transduced HSC [77]. However, no systematic analysis of hematopoiesis has been reported in *Rac3*<sup>-/-</sup> mice. Using RT-PCR and Northern blot analysis, Knaus and co-workers have reported that the expression of *Rac3* is dramatically down-regulated during terminal myeloid differentiation, but the functional significance of this observation is unknown [12]. We have demonstrated normal neutrophil differentiation in *Rac1*<sup>-/-</sup>; *Rac2*<sup>-/-</sup> cells [28, 39], but observed abnormal myeloid development *in vitro* after transduction of HSC/P with the DN D57NRac2 mutant, which most likely inhibits *Rac3* in addition to *Rac1* and *Rac2* [76]. Interestingly, HSC/P expressing D57NRac2 fail to reconstitute hematopoiesis when transplanted into lethally irradiated recipients [38]. These data suggest that *Rac3* may be important in myelopoiesis, but suffer from the weaknesses of the use of DN mutants: the effects of can be both non-specific and may be different depending on the level of expression of the mutant in the cell.

### 10. *Rac2* dysfunction in humans, a complex phenotype showing features of leukocyte adhesion deficiency and chronic granulomatous disease

As discussed above, cellular and whole animal studies have demonstrated that *Rac2* has critical functions in neutrophil adhesion, migration and killing. *Rac2* has additional roles in neutrophil function and in T cell development that are redundant with *Rac1* and more clearly defined when *Rac1* is absent. To date only one case of *RAC2* mutation in humans has been described in detail [6, 53, 83]. The findings from this case have now been corroborated by a second case, which came to attention in the course of universal newborn screening in Wisconsin for severe combined immunodeficiency (SCID) [69] and unpublished data (courtesy of J. Routes, J. Verbsky et. al.). Below we describe how

these two cases in many ways recapitulate the mouse data, and in other ways demonstrate features of Rac2 function unique to humans.

The clinical presentation of both patients was characterized by defects in neutrophil adhesion and migration. The patients came to medical attention as newborns due to infection around the umbilical stump. They were noted to have neutrophilia and leukocytosis, and underwent surgical debridement of umbilical area wounds. The first child had an infected urachal cyst and also developed severe and recurrent perirectal abscesses that grew *Escherichia coli* and *Enterococcus*. The second child had omphalitis and a paratracheal abscess that grew *Stenotrophomonas* and *Prevotella*. Both patients had a dramatic absence of pus; however, biopsies revealed detectable neutrophils, with acute inflammatory infiltrate in the first case and but minimal infiltrate in the second. Leukocyte adhesion deficiency type I (LAD type I, due to defects in *ITGB2*) (J. Routes and J. Verbsky manuscript in preparation) was initially suspected but ruled out on the basis of normal expression of the CD11b/CD18 complex by flow cytometry [6, 53,83]. Treatment consisted of aggressive antibiotics, granulocyte transfusions, and ultimately hematopoietic stem cell transplantation.

Investigators noted the striking similarity between the first child and what was at that time the recently published *Rac2*<sup>-/-</sup> mouse [68]. Indeed, examination of patient cells recapitulated the major findings seen in the *Rac2*<sup>-/-</sup> mouse, including neutrophilia, defects in chemotaxis, migration, F-actin formation, and selective defects in NADPH oxidase activity, which is a feature of chronic granulomatous disease (CGD) (Table 1). Many of these findings were also seen in the second patient. As noted above, the defect in NADPH oxidase activity confirms a long known requirement for Rac GTPase activity in the generation of the oxidase in cell free systems [2,52], although no patient had been described in the CGD literature with a disease attributable at the molecular level to mutation of any Rac gene.

Both children were found to have a c.169G>A mutation that results in a predicted protein change of p.Asp57Asn (D57N). This mutation corresponds to mutations in the GTP binding pocket of other Rho GTPases and Ras superfamily members, such as p21Ras D57A, that result in dominant negative activity. Thus some of the clinical effects of this mutation are due not only to lack of Rac2, but also due to inhibition of Rac1 activity [40], which in the mouse can compensate for deletion of the *Rac2* gene.

The second case points out additional interesting functions of Rac GTPases in human hematopoiesis. This patient had a mild defect in T cell development, which was identified by universal newborn screening for SCID [19,69]. SCID is a heterogeneous group of congenital disorders of lymphocyte development caused by at least 15 different genetic mutations. The aim of this screening is to detect extreme T cell lymphopenia, the hallmark of SCID, by measuring T cell receptor excision circles (TREC), an episomal circle of DNA generated after T cell receptor alpha gene rearrangement in the late stages of thymocyte development [25,62]. This child had complete absence of TREC (0-4/microliter, mean value reported for normal newborns 225/microliter, cutoff for screening < 25/microliter). In contrast to patients with a classical SCID phenotype who lack T cells, the child had a milder T cell lymphopenia (1434/microliter) and a mild increase in the proportion of previously activated or memory phenotype cells, as indicated by expression of CD45RO [69]. This latter finding may reflect the tendency of T cells under lymphopenic conditions to undergo so-called homeostatic proliferation, and thereby gain expression of CD45RO. Low TREC typically results from the additive effects of decreased thymic output, resulting in low numbers of newly generated TREC-containing naïve T cells, compounded by homeostatic proliferation, which dilutes out the TREC as T cells divide. Why the TREC are disproportionately low in this case of mild lymphopenia remains unclear. Overall the lymphocyte phenotype of the human mutation is less severe than that seen in the doubly deficient mouse, which may reflect the differences between a null and a dominant negative mutant. In fact transgenic expression of a different Rac dominant negative mutant, Rac1N17 under control of the CD2 promoter, is only mildly T cell lymphopenic [71].

In summary, two separate cases of Rac deficiency due to the presence of dominant negative mutations of the *Rac2* gene have now been described. The predominant phenotype of the disease is leukocytosis with a failure of neutrophil migration into infected tissues. Both infants suffered from repeated life-threatening bacterial infections, whose severity necessitated allogeneic stem cell transplantation. In both cases, the affected infants were suspected of suffering from LAD. While there are more subtle aspects to the phenotypes, we propose the term LAD type IV for this disease, as it most clearly resembles other forms of LAD.

## 11. Concluding remarks

Rho GTPases regulate a large number of cellular functions, including cytoskeletal rearrangements that control cell shape, adhesion and migration. Rac GTPases have been implicated in cell migration, lamellipodia formation and membrane ruffling in a variety of mammalian cells, including some blood cells. The development of gene-targeted mice deficient in Rac2, a hematopoietic-specific GTPase, identified a phagocytic immunodeficiency in these mice that confirmed the important and non-redundant role of this GTPase in several blood cell lineages, particularly neutrophils. The description of the murine *Rac2*<sup>-/-</sup> phenotype aided in the identification of a mutation in a patient with recurrent serious infections in infancy and a similar blood phenotype. Subsequently, a second patient has been identified with a similar phenotype and identical mutation. The phenotype of these patients, which includes leukocytosis, reduced neutrophil chemotaxis and L-selectin mediated endothelial rolling, most closely resembles human LAD and we propose the syndrome be named LAD type IV.

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