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The essential role of DOCK8 in humoral immunity

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Abstract. The processes that normally generate and maintain adaptive immunity and immunological memory are poorly understood, and yet of fundamental importance when infectious diseases place such a major economic and social burden on the world's health and agriculture systems. Defects in these mechanisms also underlie the many forms of human primary immunodeficiency. Identifying these mechanisms in a systematic way is therefore important if we are to develop better strategies for treating and preventing infection, inherited disease, transplant rejection and autoimmunity. In this review we describe a genome-wide screen in mice for the genes important for generating these adaptive responses, and describe two independent DOCK8 mutant mice strains identified by this screen. DOCK 8 was found to play an essential role in humoral immune responses and to be important in the proper formation of the B cell immunological synapse.

Keywords: DOCK8, germinal center, immunodeficiency, DOCK family, guanine exchange factor

1. Introduction

The adaptive immune system of higher vertebrates is characterized by the ability to remember previous encounters with antigen and to respond more quickly with higher affinity antibodies, the second time an antigen is encountered. In part, this is due to long-lived bone marrow resident plasma cells and memory B cells which are formed during the primary response in a specialized structure within the secondary lymphoid tissue known as the germinal center [3]. The exact mechanisms responsible for the development and persistence of these adaptive responses are not known, and a systematic approach to gene discovery may allow new insights into these processes.

Genome wide screening in patients with particular primary immunodeficiencies has identified the genetic cause in many monogenic forms of immunodeficiency, while also discovering the role of many genes important in innate and adaptive immune responses [12]. Identifying mouse models of individual immune deficiencies can also be important to inform us about human disease, and provide insights into normal immune function. Characterization of the SAP−/− mice has allowed new insights into the extent of cellular abnormalities in X-linked lymphoproliferative disease [35, 45], while investigations of WASp −/− mice has provided insights into B cell abnormalities in patients with Wiskott-Aldrich syndrome [39]. Using mouse models to look at the cellular consequences of mutation can be useful in situations in primary immunodeficiency in which it is difficult to differentiate the cellular consequences of the mutation from the consequences of infection arising due to the mutation, or from the effects of antibiotic or antiviral medications used to treat the infections. Mouse models also provide a genetically homogenous environment to study the cellular consequences of the mutation and differentiate these from consequences that may arise due to genetic polymorphisms within a population.

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In this review we discuss the recent identification of murine DOCK8 mutations through the use of a genome-wide screen for mice with abnormal vaccination responses, and the consequences for humoral immunity of DOCK8 mutations. We also discuss the DOCK family of proteins in mice, and highlight particular DOCK proteins thought to play a role in the immune system and as well as discussing the parallels with the function that DOCK proteins are thought to play in the nervous system.

2. The use of ENU mutagenesis to identify novel causes of immunodeficiency

Long-lived plasma cells and memory B cells produced during adaptive immune responses form the basis of vaccination and protection from disease [3,59], yet the genes, mechanisms and processes behind the production of these important cell types are not fully understood. The genes important in the production of the adaptive immune responses are also likely to be defective in those human primary immunodeficiencies in which a genetic cause has not yet been found (such as in the majority of cases of common variable immunodeficiency (CVID)).

One way to search for these novel genes and mechanisms is to study animals with heritable defects in the immune response that are generated with the chemical mutagen ethylnitrosurea (ENU). Male C57BL/6 mice injected with ENU develop single nucleotide substitutions at a frequency of $\sim$1 per million base pairs in their spermatogonial stem cells [52,60], which are transmitted to progeny, thus creating libraries of mutant mice that can be screened for heritable phenotypes. Of those that lead to a detectable phenotypic effect, two thirds interfere with the function of a protein due to an amino acid substitution, while one-third lead to aberrant splicing [28]. We have already shown how the characterization of strains from screens for autoimmune disease and lymphocyte development – such as *sanroque*, *tiny* and most recently *themis* [27,46,61] – can reveal new and unexpected information relevant to human disease.

We have now used the same strategy to screen for ENU mutant strains with immunodeficiency detected by an abnormal antibody response to immunization. The immunization screen was devised to screen for mutations affecting the polarization of the immune response to either TH1 or TH2, to look for deficient responses to T – independent antigens, and lastly deficiencies in germinal center formation and affinity maturation in response to T dependent antigens [60].

The mice were first immunized with chicken gammaglobulin (CGG) coupled to arsonate hapten (ABA) and heat-killed *Bordetella pertussis* bacilli. The protein CGG normally elicits an IgG1 response, while the *B. pertussis* elicits an IgG2c response and these antibodies are detected in plasma 14 days after the initial immunization using an enzyme linked immunoassay (ELISA). Mice that produce a robust antibody reaction to CGG but do not respond to *B. pertussis* are inferred to have a low Th1 response, while those with normal responses to *B. pertussis* and low response to CGG are inferred to have a low Th2 response. Those in whom the response to both antigens is compromised, are classed as having deficiency of T-dependent immune responses (or mis-injection). To screen for mice with defects in secondary or memory responses, the mice were boosted with ABA-CGG 6 weeks after the first immunization, and antibodies detected by ELISA in plasma collected six days later. In C57BL/6 mice, antibodies to the arsonate hapten only emerge after mutation in germinal centers changes the specificity of the antibody so that it no longer cross-reacts with DNA [23], and antibodies to this particular compound also indicate the presence of germinal centers in the immune response. At the time of the booster injection, the mice were also immunized with NP-Ficoll to test the T-independent responses, with anti-NP antibodies also detected by ELISA six days later [60].

3. The discovery of DOCK8 immunodeficient mice

Two novel mutant mouse strains were identified due to abnormalities in the vaccination screen described above – captain morgan (*cpm*) and primurus (*pri*). Both were found to have recessive mutations in a poorly characterized 190 kDa guanine nucleotide exchange factor (GEF) called DOCK8.

The *cpm* mutation was in the exon 20 splice donor sequence and yielded frame shift mutations that eliminated the catalytic GEF domain due to truncating mutations. The GEF domain is also known as DOCK homology region 2 (DHR2) and is one of two conserved regions within all DOCK proteins (the other being DHR1). In *pri*, an exon 43 mutation caused substitution of a conserved Ser 1827 to Pro, which would be expected to break the predicted alpha helical structure of the same GEF domain. In DOCK9, where the GEF domain structure has recently been solved in complex

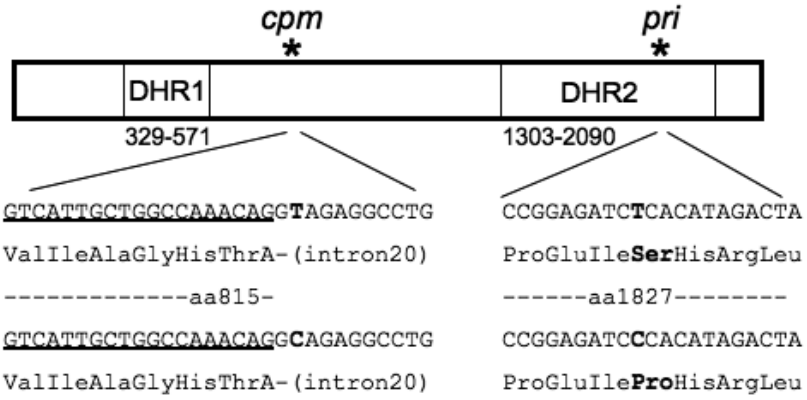

Fig. 1. Two independent mutations in mouse DOCK8. Schematic representation of the structure of murine DOCK8 showing the position of the two conserved protein domains – DHR1 and DHR2. The position of two independent mutations (*cpm* and *pri*) that arose through ENU mutagenesis are shown. Underlined text represents the sequence of Exon 20, while bold type indicates the mutated nucleotide. Figure modified from Fig. 3a original published in Nature Immunology; 10: 1283–1291 [53].

with Cdc42 [66], the serine is conserved and lies within alpha helix 6 that forms multiple contacts with Cdc42. The position of the two mutations and the conserved DHR1 and DHR2 domains within the DOCK8 protein are shown in Fig. 1.

The vaccination responses in both *cpm* and *pri* mouse strains were further characterized with the antigens used in the initial vaccination screen. Both strains failed to develop a sustained IgG response after a primary immunization with CGG despite having a normal initial immune response and normal polarization to TH1 and TH2 [53]. Both mutations did not seem to compromise the B cell activation, selection, switching and plasma cell differentiation that occurs in extra-follicular reactions with Th1 and Th2 helper T cells and produces the initial wave of antibody [36]. Both strains of mice, however, failed to make antibodies to arsonate hapten indicating a possible failure of germinal center formation. This was confirmed by the failure of germinal center formation with sheep red blood cell (SRBC) immunization. Homozygous mutations had only small or non-significant effect on T-cell independent extra-follicular B cell responses to NP-Ficoll and compound heterozygote *cpm*+/−*pri*+/− mice failed to show complementation for the immunization defect, confirming that these phenotypes were due to the DOCK8 mutations [53].

The mutant strains showed no gross abnormalities in the differentiation or numbers of B cells, except for the absence of splenic marginal zone (MZ) B cells, and a reduction in B1 B cells in the peritoneum, both of which were shown to be B cell intrinsic defects in mixed bone marrow chimeras. In the T cell compartment there was a two-fold reduction in naïve CD8 and CD4 T cells in the blood, spleen and Peyer's patches of both strains, and preservation of cells with an activated/memory CD44hi phenotype [[53], unpublished data.]

Chemotaxis assays carried out using the three main chemotactic factors for germinal center, MZ, and B1 cells – S1P, CXCL12 and CXCL13 – found that mutant and wild-type spleen B cells had similar chemotactic responses. Likewise, *in vivo* accumulation of B cells in lymph nodes was indistinguishable between mutant and wildtype (WT) B cells in competitive mixed chimeras. The positioning of B cells within the germinal center depends upon CXCL12 and CXCR4 [2], and positioning of *cpm/cpm* MD4 (conventional IgM/IgD anti-HEL transgene) and SW_{HEL} anti-HEL (see below) B cells in the germinal center was not different from WT cells B cells in splenic cryosections [[53], unpublished data], confirming that these cells had no major defects in chemotaxis. Mutant B cells also showed no difference compared to wild-type cells in major assays of BCR signaling with normal intracellular calcium flux after stimulation by soluble antigen, and no differences in ERK phosphorylation, induction of the activation markers CD25, CD86 and CD69 on the B cell surface, or induction of DNA synthesis by varying concentrations of soluble HEL or anti-IgM [[53], unpublished data].

4. DOCK8 is required for germinal center B cell survival

As described in the previous section, DOCK8 mutant mice were found to have a failure of germinal center

formation in response to immunization with sheep red blood cells, which normally induce a robust immune response [57], and this failure was found to be B cell intrinsic in bone marrow chimeras [53].

To investigate whether the cell intrinsic abnormality in germinal center B cells in the DOCK8 mutant mice was due to a failure to produce or to sustain the germinal center response, antigen specific B cells were followed through a normal immune response using the SW_{HEL} adoptive transfer experimental model. SW_{HEL} mice carry two transgenes. The first is a rearranged VDJ exon of the HyHEL 10 hybridoma "knocked-in" to the Jh region of the endogenous Ig heavy chain, and the second transgene is a rearranged HyHEL10 kappa light chain at an independent locus [50]. In mice that carry both of the transgenes, between 10–20% of the B cells express the antigen specific BCR to hen egg lysozyme (HEL). The rest of the B cells express the non-targeted H chain and hence a polyclonal repertoire of B cell receptors [9].

Wild-type C57BL/6 male mice (CD45.2) received an intravenous adoptive transfer of SW_{HEL} splenocytes containing 10^5 HEL-binding B cells (either *pri/pri* or wild-type). Included in this injection was 2×10^8 SRBC covalently coupled to HEL^{2X} – which binds to the HEL receptor with intermediate affinity [49]. A number of control mice received 10^5 wild-type HEL-binding B cells and 2×10^8 SRBC prepared as per the coupled cells but without the addition of HEL (and so the specific antigen for the transgenic B cells was not present). These control mice were designated "mock".

Two and a half days after this transfer, the number of SW_{HEL} B cells was determined by flow cytometry. Equal numbers of cells were found in primurus, wildtype and mock experimental groups. Analysis at day 3.5 and 4.5 showed expansion of the wild-type and primurus SW_{HEL} B cell populations, but this expansion did not occur in the "mock" experimental group.

An important marker of adequate T cell activation signals to B cells is class switching to IgG – which occurs in both the extrafollicular plasma cell response and the germinal center response. The same proportion of donor SW_{HEL} wild-type and primurus cells were noted to switch to IgG1 in the early phase of the response (day 3.5 and 4.5). This equal proportion of cells switched to IgG1 indicated that there were adequate T cell activation signals to the primurus SW_{HEL} B cells. Chan et al. [11] have shown that switching to IgG1 does not occur if SW_{HEL} B cells are CD40 deficient, and it is also known that the process of switching is linked to the number of divisions that the cells have undergone [25]. In mice that received SW_{HEL} B cells and SRBC, but where the SRBC had not been coated with HEL^{2X}, no class switching of the antigen specific cells occurred. Although the absolute number of plasma cells produced by *pri/pri* donor B cells was reduced – at a similar magnitude to the total number of SW_{HEL} B cells – the relative proportion of donor cells that had become plasma cells was comparable between mice that had received wild-type and *pri/pri* donor cells.

Adoptively transferred primurus SW_{HEL} B cells were able to differentiate to become germinal center B cells, in contrast to the situation in the primurus mouse in which no germinal centers were made. At day 5, as shown in Fig. 2a, robust germinal center reactions (B220+, GL7+, Fas+ cells) were seen in all mice. However, nine to ten days after adoptive transfer, despite ongoing germinal center reactions in all experimental groups, the antigen specific, donor derived *pri/pri* SW_{HEL} B cells had almost disappeared – dropping to 1/20 of the numbers seen in mice receiving wild-type SW_{HEL} B cells. This almost complete absence was confirmed by immunohistochemistry of splenic cryosections as shown in Fig. 2a. These experiments show that DOCK8 plays an important B cell intrinsic role in the persistence and survival of germinal center B cells.

5. A defect in the B cell synapse

Recognition of antigen is known to be important for germinal center B cell survival [3]. In the germinal center, antigen is displayed on the membranes of follicular dendritic cells (FDCs) [44] and when B cells interact with membrane-bound antigen, a B cell immunological synapse is formed [24].

The B cell immunological synapse has been found to consist of a central supramolecular activation complex (cSMAC) where antigen receptors and antigen are clustered on the B cell and antigen presenting cell respectively, and a peripheral SMAC (pSMAC) where the integrin LFA-1 and its ligand ICAM-1 are concentrated [10]. Integrins are a family of heterodimeric cell adhesion molecules composed of α and β subunits important for cell-cell and cell-matrix interactions [6]. Integrins are usually present on the cell surface in an inactive conformation, but can be rapidly activated by conformational changes brought about by external binding ("outside-in signaling") or by activation signals from within the cell brought about by antigen or chemokine receptor stimulation ("inside-out signaling") [1]. The

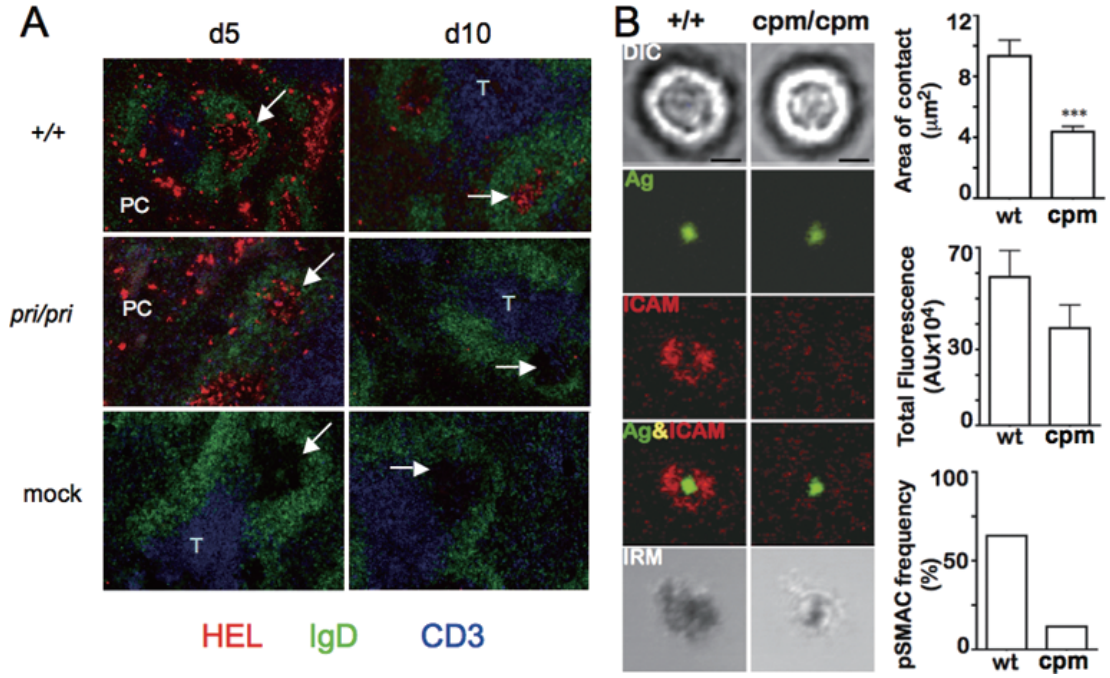

Fig. 2. DOCK8 is essential for germinal center B cell persistence and in the formation of the B cell immunological synapse. (A) Splenic cryosections taken 5 and 10 days after adoptive transfer of SW_{HEL} B cells (either mutant (*pri/pri*) or wildtype (+/+)) together with HEL^{2X} conjugated sheep red blood cells (SRBC). One group of mice received SW_{HEL} B cells and SRBC without HEL (mock). Arrows indicate germinal centers, T, the position of the T cell areas and PC, the extrafollicular plasma cell response. HEL positive B cells are shown in red, IgD positive cells in green and CD3 positive cells in blue. Originally published as Fig. 6a in Randall et al., Nature Immunology; 10: 1283–1291 [53]. (B) MD4 wild-type or DOCK8 mutant naïve B cells were settled into lipid bilayers containing mono-biotinylated HEL as the antigen (green) and Alexa-532 conjugated ICAM-1 (red) and imaged by confocal microscopy after 10 minutes of interaction. Differential interference contrast (DIC), fluorescence and interference reflection microscopy (IRM) images of representative cells are shown. Quantification of the area of B cell contact with the bilayer, the relative amounts of antigen accumulated (expressed as a sum of fluorescence) and the percentage of B cells forming a pSMAC. Columns are means and error bars standard error of the mean. Originally published as Fig. 7a in Randall et al., Nature Immunology; 10: 1283–1291 [53] (generated by Bebhinn Treanor, CRUK).

main integrins on B cells are LFA-1, which binds to ICAM−1, −2, −3 and −5, and VLA-4, which binds to VCAM-1 and fibronectin. The ligands for LFA-1 and VLA-4 are found on endothelial cells, leukocytes including B cells, dendritic cells and follicular dendritic cells [34] and are upregulated on follicular dendritic cells in the germinal center [5].

The recruitment and activation of the integrin components of the immunological synapse is a consequence of antigen signaling through the BCR, but the exact pathway is still not known. Recent work has shown that the proper formation of the B cell immune synapse after antigen stimulation is dependent on signaling by src family tyrosine kinases, and is disrupted in mice treated with inhibitors of these kinases, and is also disrupted in mice carrying mutations of PI3K, Vav and Rac2 [7]. Disruptions in any of these signaling components affect the BCR dependent activation of Rap1. Rap1 is an important regulator of cell polarity and actin [32].

DOCK8 mutant B cells were assessed for their ability to form a B cell immune synapse using the *in vitro* assay developed by Facundo Batista and his laboratory [7]. As shown in the left panel of Fig. 2b, addition of wild-type HEL specific B cells led to rearrangement of the antigen and ICAM to reflect the formation of the immunological synapse, with antigen clustered centrally in a cSMAC surrounded by a ring of ICAM. The right hand panel of Fig. 2b shows the parallel analysis of *Dock8* deficient (*cpm/cpm*) HEL specific cells. Antigen clustering into a cSMAC occurred but there was no surrounding ring of ICAM indicating that the pSMAC had not formed.

Integrins have been found to provide important costimulatory signals for germinal center cell survival *in vitro* notably via the integrin ligand ICAM1 [30] Therefore, the inability to fully engage with FDCs through the failure to form a proper immune synapse might explain the failure of germinal center B cell survival in the DOCK8 mutants.

6. The DOCK gene family

The identification of the role of DOCK8 in germinal center B cell survival and in the formation of the B cell synapse highlights the importance of the DOCK family of RhoGTPase GEFs in cellular function. RhoGTPases exert their effect only when bound with GTP and rapidly lose their activity as they hydrolyze GTP to GDP. Two principal groups of proteins act as GEFs for Rho GTPases and activate them by exchanging GDP for GTP [15], the Dbl-homology domain containing proteins such as Vav1 (classical GEFs) and DOCK family proteins (non-classical GEFs).

There are 11 mammalian members of the DOCK family divided into four groups based on the degree of homology of two conserved DHR domains [13,38]:

- DOCK-A – containing DOCK180 (also known as DOCK1), DOCK2 and DOCK5
- DOCK-B – containing DOCK3 (also known as MOA) and DOCK4
- DOCK-C – containing DOCK6 (also known as Zir1), DOCK7 (also known as Zir2) and DOCK8 (also known as Zir3)
- DOCK-D – containing DOCK9 (also known as zizimin1), DOCK10 (also known as zizimin3) and DOCK11 (also known as zizimin 2).

A number of these proteins have been found to be critical activators of Rho/Rac/Cdc42 small GTPase proteins in worms, flies and mammals [15,63].

The first family member, DOCK180, was cloned in 1996 during a search for proteins interacting with the proto-oncogene Crk28 [26]. DOCK180's *C. elegans* orthologue *Ced-5* controls cell migration and phagocytosis [64], whilst the *Drosophila* orthologue, *Myoblast city*, is essential for myoblast fusion and dorsal closure [18]. DOCK8 was originally isolated in a yeast two-hybrid screen for binding partners of Cdc42 (though this was not confirmed in a pull-down assay) and found to localize at lamellipodia of fibroblasts [55].

As described above, the proteins of the DOCK family are classified according to their two DOCK homology domains. The DHR1 domain of DOCK1 has been shown to bind to the lipid PtdIns(3,4,5)P3 (PIP3) in the cell membrane and it is thought that this binding mediates Rac-dependent actin re-organization of the leading edge of the cell during migration [14]. The DHR1 domains of other DOCK proteins have also been found to bind to PIP3 in *in vitro* studies – including DOCK2, DOCK7 [15] and DOCK4 and its splice variants [29]. The DHR2 domain binds to Rho-family GTPase-proteins (Rac, Cdc42) and provides catalytic GEF activity to stimulate these proteins and promote integrin reorganization and adhesion, lamellipodia formation, cell polarization, phagocytosis or cell fusion [15].

Dock8 mRNA is expressed at ten times greater quantity in B and T lymphocytes than in other tissues (BioGPS, SymAtlas) and our studies of DOCK8 immunodeficient mice, which appear normal in growth and behaviour, indicate that it plays a relatively specialized role within the immune system.

7. Other murine DOCK family members with roles in the immune system

The specificity of *Dock8* functions within the immune system contrast with those of *Dock2*, which is the other DOCK family member characterized in detail with respect to its function in the immune system. DOCK2 mutations in mice result in lymphopenia and disrupted lymphoid architecture with crippled B cell migration to lymph nodes and a general defect in chemotaxis [19].

Despite other differences, *Dock8* and *Dock2* deficient mice share the absence of MZ B cells, and DOCK2 and other guanine exchange factor proteins have also been shown to play a role in integrin signaling.

The DOCK2$^{-/-}$ strain has not been assessed for B cell synapse formation, but DOCK2 has been shown to be important for integrin activation in response to chemokine signaling in B cells [48]. DOCK2 has however been shown to be important for proper T cell synapse formation after activation by antigen. It is essential for translocation of TCR and lipid rafts into the synapse, but is not essential for proper placement of PKC-θ and LFA-1 in the synapse [56].

The localization of MZ B cells in the marginal zone depends upon both expression of LFA-1 and $\alpha 4\beta 1$ integrins [33], and the high expression of S1P1 and S1P3 receptors, whose activation is thought to counteract CXCL13 chemoattractant signals from the follicles [37]. MZ development is also tightly regulated by interactions between Notch2 and delta-like 1 receptors [58] and by the strength of BCR signaling, which may involve positive and/or negative selection by self-antigen [51]. In this context, DOCK8 might be involved in BCR signaling, or forms of signaling and adhesion critical to MZ B cell development or survival, perhaps in a manner similar to that in germinal center B cells. It is also possible that cytoskeletal changes due to

DOCK8 mutation affect cell-cell contacts or the morphology of the MZ cells. Relative and absolute defects in MZ B cells are shared by several mice lacking signaling molecules involved in cytoskeletal changes and cell localization including Rac2 [16], and Lsc (orthologue of human p115RhoGEF). Lsc is part of the signaling cascade downstream of $G\alpha_{12}$ and $G\alpha_{13}$ (part of the S1P receptor) and important for lymphocyte chemotaxis [20] and a decrease in marginal zone B cells is also seen with conditional deletions of $G\alpha_{12}$ and $G\alpha_{13}$ in B cells [54] and Pyk-$2^{-/-}$ mice [22], another component of this signaling pathway.

Other members of the DOCK family have also been shown to have possible functions in the immune system. DOCK11 (also known as zizimin2) was cloned in a screen for genes enriched in germinal center B cells using a cDNA library derived from C57BL/6 mice. DOCK11 was found to be expressed at 2.6 fold higher levels in germinal center cells as opposed to non-germinal center B cells [47], but its role in these cells has not been further elucidated and the consequences of mutating this gene in mice is not known. DOCK10 was identified as an IL-4 inducible gene in human chronic lymphocytic leukemia (CLL) and has been found to be expressed predominantly in peripheral blood leukocytes [67], but again the consequences of its absence have not been studied.

8. Parallels between GEF functions in the central nervous system and immune system

RNA microarray studies of genes expressed by germinal center B cells have found that a large proportion of genes expressed by these cells are also involved in axon growth and guidance [68], and we know that germinal center cells have been shown to take on a dendritic-like appearance after *in vitro* activation [68] and in two photon studies of the germinal center [4]. It is therefore interesting to note that a number of DOCK family proteins are thought to play an important signaling role within the nervous system, particularly in the formation of axons and dendrites [41].

Does DOCK8 also play a role within the nervous system? Heterozygous deletions in *DOCK8* have been reported in two people with mental retardation [21], although this effect might be explained by defects in the adjacent gene with a well established role in mental retardation, *ANKRD15* [31], as no neurological or cognitive impairment has been described in people with homozygous mutations and immunodeficiency [17,69].

The DOCK-C family, to which DOCK8 belongs, also contains DOCK6 and DOCK7, both of which have been found to have possible roles in the nervous system. DOCK6 has been shown in GTPase binding studies to have dual specificity for both Rac1 and Cdc42, and when the DHR2 domain of DOCK6 was transfected into COS cells it led to lamellipodia and filopodia formation. Neurite outgrowth in the N1E-115.5 cell line was shown to be associated with higher expression of *Dock6* and addition of siRNA specific for *Dock6* was shown to prevent neurite outgrowth in this cell line [40]. The exact role of *Dock6 in vivo* has not yet been determined.

DOCK7 was isolated in a yeast two-hybrid screen for Rac1 binding partners in brain tissue and this binding has been confirmed by GTPase assays. It does not have dual GTPase specificity and does not bind to Cdc42. DOCK7 has been found in a polarized distribution in hippocampal neurons and has been found to be essential for normal axonal growth – with decreased DOCK7 due to siRNA knock-down causing loss of axons, while over-expression gives rise to multiple axons [62]. DOCK7 has also been shown to be important for control of Schwann cell migration through binding of ErbB2 [65] and has been found to associate with the protein product of the tumor suppressor genes TSC1 and TSC2. Mutations in the TSC1 and TSC2 genes give rise to the human disease tuberous sclerosis characterized by the formation of multiple benign tumors [43]. However, more recently, two strains of mice with mutations in *Dock7* have been described – in one strain, misty, the mutation arose spontaneously and in the other a splicing error arose by ENU mutagenesis. Both strains of mice show no overt neurological abnormalities but have a general hypopigmentation and show white spotting of the coat [8]. The differences between the *in vivo* and *in vitro* phenotype may be due to the nature of the mutation that has arisen in the two mice strains, which may leave the guanine exchange function intact, or due to redundancy in the function of this protein within the nervous system. Interestingly, the proteins in the DOCK-C family that have been associated with nerve growth abnormalities and neural development rely on many of the same molecules as those found in lymphocyte signaling pathways such as Rac, Vav2 and TIAM [41].

DOCK3 has also recently been found to play a role in the nervous system affecting axonal length both *in vitro* and *in vivo*. Primary hippocampal neurons transfected with a plasmid containing *Dock3* showed increased axonal growth, while transgenic mice expressing increased Dock3 had structurally normal neural tissues, but increased axon length [42].

9. Conclusion

Mutation of the DOCK8 protein in mice has profound effects on humoral immunity with a failure to sustain the antibody response and failure of germinal center B cell persistence. Mice with *Dock8* mutations also have absent marginal zone B cells, and decreased peritoneal B1 cell and naïve T cell numbers. While B cell signaling is not affected in measures such as proliferation, calcium flux and expression of activation markers, *Dock8* deficiency prevents formation of the normal B cell immune synapse, affecting the pSMAC. This is likely to limit B cell survival during the germinal center response when survival is determined by competitive interaction of B cells with small amounts of antigen and other survival factors on FDCs. Further study of DOCK8 mutant mice will help to elucidate the mechanisms underlying this selection point, Other studies of *Dock8* mutant mice, including assessments of T cell proliferation, signaling and cell survival may help to further elucidate the mechanisms of immunodeficiency arising in patients with combined immunodeficiency due to homozygous mutations of DOCK8.

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