Abstract
B-cell development and function requires the products of the Vav family of genes. Vav proteins act as key components of the antigen receptor signal transduction machinery by integration of signals that control the cell cycle, differentiation and apoptosis. At the molecular level, Vav proteins regulate small-molecular-mass GTPases, phosphoinositide 3-kinase, TEC family tyrosine kinases and intracellular calcium flux.

Introduction
The development of B-cells proceeds through a well defined set of stages [1]. Signal transduction events elicited by the antigen receptor or its precursors are absolutely necessary for survival and selection of B-cells at multiple checkpoints. Thus the functional assembly of, and signal transduction through, a pre-B-cell receptor (BCR) that contains an immunoglobulin heavy chain is critical for the proliferation of pre-B cells. Furthermore, this receptor complex signals allelic exclusion, the process through which further heavy chain rearrangements are halted. Upon successful rearrangement of light chains, immature B-cells require signal transduction through newly expressed surface IgM molecules in order to undergo maturation into long-living mature recirculating B-cells. In mature B-lymphocytes, activation mediated through the antigen receptor orchestrates integration of the cell cycle, transcription and differentiation. Therefore, understanding the mechanism of BCR signalling will lead to a greater understanding of B-cell development, the balance between tolerance and autoimmunity, and the selection of high-affinity antibody-producing variants.

One of the hallmarks of BCR signal transduction is the mobilization of intracellular Ca\(^{2+}\). Ca\(^{2+}\) fluxes have been implicated in the selection events that regulate B-cell maturation and in antigen-receptor-triggered proliferation [2,3]. A number of mutations in signalling molecules have been identified in humans, chicken DT40 cells and in transgenic mice that compromise B-cell development and BCR-induced Ca\(^{2+}\) release [4,5]. Immunodeficient xid mice lack a functional Bruton’s tyrosine kinase (Btk) owing to a point mutation in the plekstrin homology domain that renders Btk unable to interact with phosphatidylinositol 3,4,5-trisphosphate [6]. As a consequence of this, Btk cannot be recruited to membrane-associated signalling complexes and thus cannot be activated. Mutations of the p85\(\alpha\) regulatory subunit of phosphoinositide 3-kinase (PI 3-kinase), phospholipase C\(\gamma\)2 (PLC-\(\gamma\)2) and B-cell linker protein [also known as SLP-65 (SH2-containing lymphocyte protein of 65 kDa) or BASH (B-cell adapter with SH2 domain)] also give rise to defects in peripheral B-cell maturation. All of these proteins may interact physically to form a signalling complex that is termed the ‘signalosome’ or ‘receptorsome’, the best characterized function of which is to regulate the PLC-\(\gamma\)2 after antigen-receptor ligation [4,5].

Vav proteins are guanine nucleotide exchange factors that activate a subgroup of Rho GTPases (Rac1, Rac2 and RhoG) by promoting the exchange of GDP for GTP. Vav proteins are phosphorylated and activated rapidly upon antigen-receptor engagement, and provide the major, and perhaps only, link between the antigen receptor and Rho family of GTPases [7]. By generating mice deficient in Vav-1 and Vav-2, we have identified Vav proteins as functional components of the BCR signalosome. Studies of signal transduction in Vav-deficient B-cells indicate that Vav proteins regulate PI 3-kinase activity. The ability of Vav proteins to regulate PI 3-kinase activity places them at a central position in the signalosome model, as both Btk and PLC-\(\gamma\)2 activation requires the function of the Vav proteins.

Results and discussion
Development of B-cells in Vav-1- and Vav-2-deficient mice
Initial reports suggested that B-cell development in Vav-1-deficient mice was normal, with the
exception of the Bl subset, which is Vav-1-dependent [8-11]. However, subsequent analysis has revealed there is a modest decrease in the most mature population of splenic B-cells, which are referred to as IgM⁺⁺IgD⁺⁺ cells [12,13]. The numbers of B-cells in the peripheral lymphoid organs of Vav-2-deficient mice were not different from littermate controls, which suggests that Vav-2 was not absolutely required for B-lymphocyte development. In the bone marrow of Vav-1⁺⁻/Vav-2⁺⁻ mice, immature B-cells are present in normal numbers, but there are far fewer recirculating B-cells [12,13]. In these mice, many splenic and lymph node B-cells remain immature, express high levels of IgM and do not upregulate expression of surface IgD. Thus the majority of B-cells in Vav-1⁺⁻/Vav-2⁺⁻ mice are phenotypically immature. It is thought that immature peripheral B-cells require BCR-mediated signals to progress to the more mature IgM⁺⁺IgD⁺⁺ stage [1,14]. A requirement for both Vav-1 and Vav-2 for optimal BCR signalling at this stage of B-cell development could explain the dramatic decrease in the numbers of mature peripheral B-cells in Vav-1- and Vav-2-deficient mice. However, our findings could reflect defective selection or migration of immature B-cells from the bone marrow, or that alternatively, the survival of mature B-cells, which is dependent upon low levels of 'tonic' BCR signalling [15], may require Vav function.

Response of Vav-deficient mice to immune challenge
Vav-1-deficient mice are immunodeficient and respond poorly to immunization with thymus-dependent (T-D) antigens. These antigens are typically proteins that lead to the activation of helper T-cells. Vav-1-deficient B-cells respond normally to T-D antigens if T-cell help is provided [10]. Thus Vav-1⁺⁻ B-cells can undergo class-switching and the failure of T-cells to produce cytokines, such as interleukin-4, may account for the deficient helper function and T-D response. The response of Vav-2-deficient mice to T-D antigens is also defective; however, the cellular basis has not been established [12]. The response to type-2 thymus-independent (TI-2) antigens is often considered to be T-cell-independent because the antigenic stimuli are often polymeric, repetitive structures, such as those found in bacterial capsular antigens. These repeating units drive B-cell responses by extensively cross-linking the BCRs. To determine the roles of Vav proteins in these responses, we immunized Vav-deficient mice intraperitoneally with the TI-2 antigen, dinitrophenol-ficoll. B-cells from Vav-1⁺⁻ mice respond relatively well to such antigens, while the response of Vav-2⁺⁻ mice is impaired [10,12,13]. Vav-1⁺⁻/Vav-2⁺⁻ mice are further impaired, which highlights a redundancy between these two proteins [13]. Recently, marginal zone B-cells have been identified as the population that is principally responsible for the T-1 response [16]. In Vav-2⁺⁻ mice, CD21⁺⁺CD23⁺⁺B220⁺ marginal zone B-cells were not reduced in number. Our results favour the suggestion that there is a defect in the ability of Vav-2-deficient B-cells to respond to TI-2 antigens.

Signal transduction in Vav-deficient B-cells
In both Vav-1⁺⁻ and Vav-1⁺⁻/Vav-2⁺⁻ mice, the ability of mature B-cells to proliferate following BCR stimulation is impaired [12,13]. The level of impairment is particularly evident when surface IgM is cross-linked under conditions of low avidity. In Vav-1⁺⁻ B-cells, the BCR-elicited induction of cyclin D2 expression is blocked and this may account for the defective replicative capacity of the cells [17]. To examine the role of Vav-1 and Vav-2 in BCR-elicited calcium flux, we measured changes in intracellular Ca²⁺ concentrations after cross-linking of the BCR complex. These studies showed that Ca²⁺ fluxes were reduced in the Vav-1- or Vav-2-deficient B-cells. This was particularly evident when a low dose of anti-IgM was used. At higher doses, Vav-1- or Vav-2-deficient B-cells responded at almost normal levels; however, B-cells that lacked both Vav-1 and Vav-2 remained unresponsive. These studies indicate that both Vav-1 and Vav-2 are required for the full coupling of BCR activation to Ca²⁺ mobilization.

We have explored the mechanisms responsible for regulating Ca²⁺ release following BCR stimulation. Studies of Vav-1-deficient mice provided support for the suggestion that Vav-1-Rac-mediated activation of phosphatidylinositol-4-phosphate 5-kinase may control production of phosphatidylinositol 4,5-bisphosphate, the substrate for PLC-γ2 [18,19]. In this model, depletion of phosphatidylinositol 4,5-bisphosphate leads to reduced inositol 3,4,5-trisphosphate production and Ca²⁺ release would cease before the opening of plasma membrane Ca²⁺ channels. We have now obtained evidence to suggest that the mechanism
by which Vav proteins control Ca\(^{2+}\) flux in B-cells is, in part, through the regulation of PLC-\(\gamma\) activity. In B-cells from Vav-1\(^{-/-}\)/Vav-2\(^{-/-}\) mice, BCR-stimulated PLC-\(\gamma\) phosphorylation and lipase activity are reduced (E. Clayton, E. Vigorito, C. O’Malley, T. Kurosaki, D. J. Rawlings, and M. Turner, unpublished work). Importantly, this experiment analysed PLC-\(\gamma\) activity under conditions with an excess of substrate, thus revealing an intrinsic defect in enzyme activation. As Btk is required for proper activation of the lipase [20], we explored the possibility that the failure to activate PLC-\(\gamma\) is caused by the defective activation of Btk in B-cells. BCR-stimulated phosphorylation of Btk was found to be reduced in the absence of Vav-1 or Vav-2 and undetectable in Vav-1\(^{-/-}\)/Vav-2\(^{-/-}\) B-cells (E. Clayton, E. Vigorito, C. O’Malley, T. Kurosaki, D. J. Rawlings, and M. Turner, unpublished work). Furthermore, it was demonstrated, using monoclonal antibodies specific for individual sites of phosphorylation on Btk, that the activation of the kinase activity of Btk required the function of Vav-1 and Vav-2. In addition, Btk phosphorylation in trans by upstream kinases required Vav-1 and Vav-2. Thus deficiency in Vav proteins does not lead to a global defect in tyrosine phosphorylation, but appears to affect a pathway that leads to the activation of Tec family protein tyrosine kinases and their downstream substrates. Btk like other Tec family protein tyrosine kinases contains a plektrin homology domain that is required for the activation of the enzyme [21]. The above results point to a Vav-regulated pathway that leads to the activation of Btk. The finding that Vav proteins become inducibly associated with PI 3-kinase immune complexes suggests that Vav and PI 3-kinase activity may be associated (E. Clayton, E. Vigorito, C. O’Malley, T. Kurosaki, D. J. Rawlings, and M. Turner, unpublished work and [22-25]). We measured BCR-stimulated PI 3-kinase activity by immunoprecipitation of p85, followed by an in vitro kinase assay. We found BCR-stimulated activation of PI 3-kinase was defective in Vav-1\(^{-/-}\)/Vav-2\(^{-/-}\) murine B-cells. Taken together with the observation that Rac GTPases can enhance the activity of Class Ia PI 3-kinases by direct binding to the regulatory p85 subunit of the p85/p110 heterodimer [18,26,27], these studies suggest a guanine-nucleotide-exchange-factor-dependent role for Vav proteins in the activation of PI 3-kinase. In addition, a deficiency in the generation of phosphatidylinositol 3,4,5-trisphosphate may not only adversely affect the activation of Tec family kinases and the subsequent tyrosine phosphorylation and activation of PLC-\(\gamma\) isoforms, but may also affect the proper localization and activation of other lipid-binding-domain-containing molecules, such as PLC-\(\gamma\) isoforms and perhaps Vav proteins.

In conclusion, there now exists strong evidence of a role for Vav proteins in the signal transduction by the BCR. Studies using genetically manipulated mice have proved fruitful in defining both the physiological possesses and the molecular mechanism of Vav function. Vav proteins act to control the activity of multiple tyrosine and lipid kinases and serve as a point of integration of signalling pathways.

I thank my colleagues for our discussions and Dr L. Webb for reading the manuscript. This work was supported by Biotechnology and Biological Sciences Research Council, the Leukaemia Research Fund, the Association for International Cancer Research, and the Cancer Research Campaign.

References

Hyper-IgM syndromes: a model for studying the regulation of class switch recombination and somatic hypermutation generation

A. Durandy

Inserm U429, Hôpital Necker-Enfants Malades, 149 Rue de Sèvres, Paris, France

Abstract
Several genetic defects in class switch recombination, which lead to a hyper-IgM syndrome, have been described recently in humans. In addition to the well known role of CD40-ligand-CD40 interaction, these pathologies demonstrate definitively the requirement of CD40-mediated nuclear factor κB activation and the essential role of a recently described molecule, the activation-induced cytidine deaminase in an efficient humoral response, which includes class switch recombination and the production of high-affinity antibodies.

Introduction
The generation of the antibody repertoire requires two successive steps. The first step is antigen- and T-cell-independent, and takes place in the foetal liver and the bone marrow. Immature B-lymphocytes rearrange their immunoglobulin V (variable), D (diversity) and J (joining) gene segments, which produces a functionally integrated VDJ segment that is linked to the μ constant region (Cμ). A primary antibody repertoire, composed of IgM antibodies, is thus generated. The second step is antigen- and T-cell-dependent and takes place in secondary lymphoid organs. After encountering antigen, which they recognize via their membrane IgM, B-cells proliferate and form germinal centres. In this unique anatomic formation, two genetic events lead to the generation of the secondary antibody repertoire. The first event, immunoglobulin class switch recombination (CSR), occurs by a recombination process between two different switch (S) regions that are located upstream of each C region. Replacement of the C region Cμ by a C region of another class of immunoglobulin (Cγ, Cα or Cε) leads to the production of IgG, IgA and IgE molecules which harbour the same V specificity [1]. The second genetic event involves somatic hypermutations (SHMs), i.e. mutations located in the genes that encode the V regions of the immunoglobulin; this step precedes the positive selection of B-cells that harbour a B-cell receptor with high affinity for antigen and the negative selection of B-cells carrying a B-cell receptor with a low affinity for antigen or specific for an auto-antigen. These selection processes occur within the germinal centres in close interaction with follicular dendritic cells [2].