Regulation of integrin activation through the B-cell receptor

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Summary

Effective immune surveillance is absolutely dependent on the migration of lymphocytes throughout the body and on their successful recognition of specific antigens. Both of these functions rely on the capacity of integrins that are expressed on the surface of lymphocytes to respond in a highly regulated manner to a variety of chemokines and antigens. This Commentary is primarily concerned with the role of the B-cell integrins LFA-1 and VLA-4 in the antigen-recognition process, and summarises what is currently known about the molecular mechanisms of 'inside-out' integrin activation in response to B-cell-receptor stimulation. Recent investigations have identified Vav, PI3K and small GTPases as crucial regulators of the inside-out activation of B-cell integrins. These observations are of particular interest as they allude to an underlying mechanism by which B-cell-receptor-mediated signalling is linked to cytoskeleton reorganisation and subsequent integrin activation.

Key words: B cell receptor, B cells, Integrins

Introduction

Integrins are a widely expressed and diverse family of heterodimeric cell-surface receptors that comprise an α-subunit and a β-subunit, and are responsible for mediating cell-cell or cell-matrix adhesion (reviewed in Hynes, 1992). Two of the major integrins that are expressed on the surface of B cells are integrin αL (ITGAL; also known as leukocyte-function-associated antigen 1 and hereafter referred to as LFA-1) and very late antigen 4 (VLA-4; also known as integrin α4, ITA4) (reviewed by Springer, 1990). The principal ligands for these integrins – intercellular adhesion molecule 1 (ICAM1) for LFA-1, and vascular-cell adhesion molecule 1 (VCAM1) and fibronectin for VLA-4 – are expressed on a large variety of cell surfaces, including endothelial cells, leukocytes, follicular dendritic cells and dendritic cells.

When expressed on the surface of resting lymphocytes, integrins are found as monomers in a bent, inactive conformation (Takagi et al., 2002; Xiong et al., 2001). In response to a number of physiological stimuli, integrins become activated through the modulation of their conformation and/or distribution on the cell surface, leaving them competent for mediating high-affinity adhesion (Alon and Dustin, 2007; Bazzoni and Hemler, 1998; Carman and Springer, 2003; Dustin and Springer, 1989; Luo et al., 2007). This activation of integrins can occur either through the binding of ligand directly to the extracellular domains of the integrin ('outside-in' activation) or as a result of chemokine receptor or immunoreceptor engagement, leading to 'inside-out' activation (Dustin and Springer, 1989; Kim et al., 2003). It is this responsiveness of integrins to external stimuli that equips them to perform two predominant functions in B cells. The first is in directing B-cell migration in response to stimulation with chemokines during the development of the immune response. Indeed, it has been shown that LFA-1 has an important role in B-cell migration to peripheral lymph nodes (Berlin-Rufenach et al., 1999), and that both integrin activation and the CXC-chemokine receptor CXCR5 influence the migration of B cells between the marginal zones and follicles of splenic tissues (Cinamon et al., 2007; Lo et al., 2003; Lu and Cyster, 2002). The second key function of integrins in B cells is in mediating adhesion to antigen-presenting cells (APCs) during the antigen-recognition process and, thereby, lowering the threshold of antigen that is required for the activation of B cells (Carrasco and Batista, 2006b; Carrasco et al., 2004).

Although B cells can recognise antigens in a number of different forms, recent evidence points to a major role for the recognition of membrane-bound antigens during the development of an immune response (Carrasco and Batista, 2006a). Indeed, it has been demonstrated that membrane-bound antigen can be presented to B cells in vivo on the surface of follicular dendritic cells (Szakal et al., 1988; Wu et al., 1996), dendritic cells (Balázs et al., 2002; Wykes et al., 1998) and macrophages (Carrasco and Batista, 2007; Junt et al., 2007; Phan et al., 2007). We have observed that, upon initial contact with APCs, B cells rapidly undergo a spreading and contraction response along the antigen-containing surface (Fleire et al., 2006). In addition, following engagement of the B-cell receptor (BCR) with membrane-bound antigens, a dramatic reorganisation of the cell membrane occurs, resulting in the formation of an immunological synapse (IS) (Batista et al., 2001) (Fig. 1A). The IS allows the spatial segregation of antigen and BCR in a central supramolecular activation cluster (cSMAC) from a surrounding ring of LFA-1 in a peripheral SMAC (pSMAC) (Carrasco et al., 2004). The spreading and contraction process is absolutely dependent both on signalling through the BCR and rearrangements of the actin cytoskeleton. This process functions to increase the amount of antigen that is aggregated into the cSMAC and, thereby, accumulated by the B cell. Accumulated antigens can be extracted from the cSMAC and subsequently presented to helper T cells (Batista et al., 2001; Fleire et al., 2006), allowing recruitment of the helper $CD4^+$ T cells that are necessary to facilitate B-cell

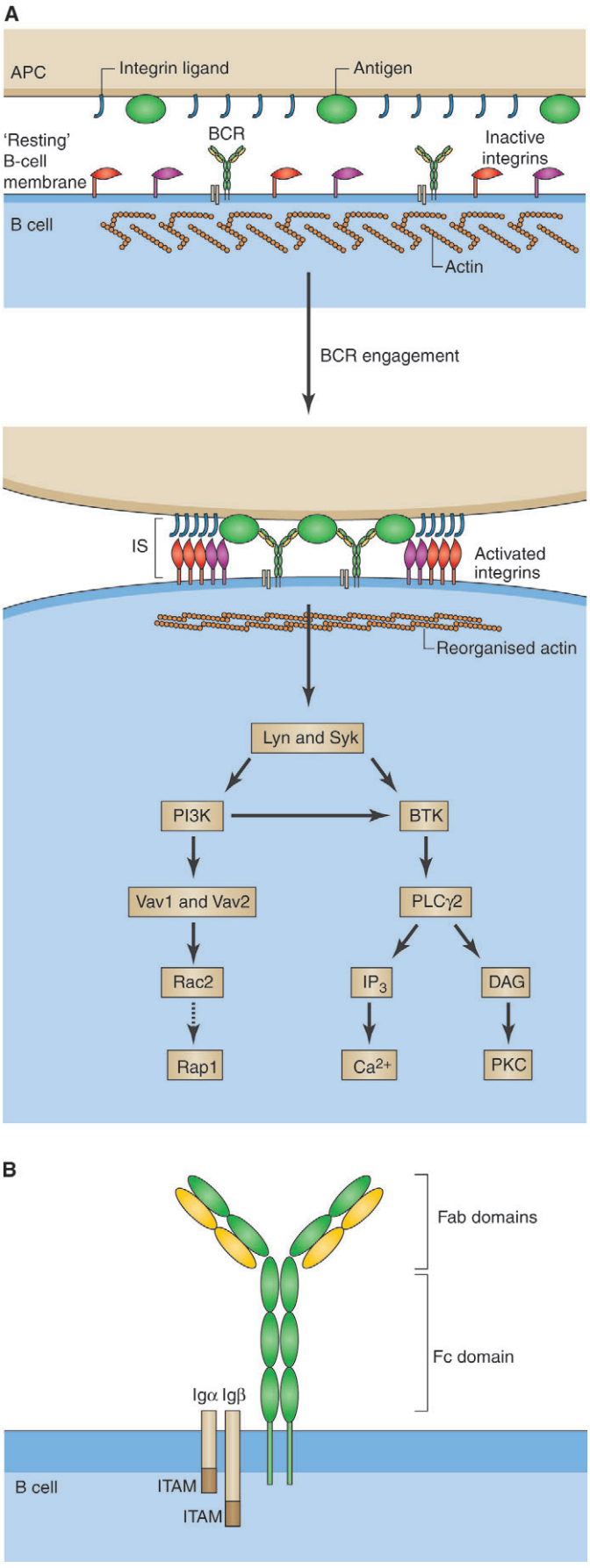

Fig. 1. Molecular mechanisms required for the activation of integrin-mediated B-cell adhesion in response to membrane-bound antigens. (A) Prior to antigen stimulation, the 'resting' B cell contains BCRs and inactive integrins that are distributed throughout the membrane. Following BCR engagement with antigen on the surface of an antigen-presenting cell (APC) that expresses integrin ligands, crosslinking of BCRs initiates intracellular signalling cascades that result in the inside-out activation of integrins. The molecular details of the intracellular pathways that are shown are based on those identified by Arana et al. and Spaargaren et al. (Arana et al., 2008; Spaargaren et al., 2003). These pathways operate by mechanisms that are dependent on the reorganisation of the cytoskeleton, and allow clustering and activation of integrins as well as the subsequent formation of the immunological synapse (IS). (B) Model of a BCR expressed on the surface of a naive B cell. This BCR comprises a membrane immunoglobulin M (mIgM) in complex with the Igα-Igβ sheath. The mIgM is a heterotetrameter, consisting of two light chains (yellow) and two heavy chains (green) that form distinct Fc and Fab domains. The Fab domains are responsible for binding to antigens, whereas the Fc domain mediates effector functions of antibodies by binding to Fc receptors. The Igα-Igβ sheath allows for transmission of signalling via the BCR through phosphorylation of their immunoreceptor tyrosine-based activation motifs (ITAMs) in their intracellular domains.

activation (Lanzavecchia, 1985; Rock et al., 1984). Subsequently activated B cells can develop into plasma cells that are competent for large-scale production of soluble antibodies, or memory cells that provide long-lasting immunological memory.

In this Commentary we highlight recent investigations of the mechanism underlying inside-out integrin activation in response to antigen stimulation through the BCR. Intriguingly, we observe a number of important differences in the mechanism that underlies inside-out activation according to the particular integrin activated and the cellular context of integrin expression. We discuss recent evidence that identifies key signalling molecules that govern the activity and distribution of LFA-1 and VLA-4 following engagement of the BCR. Finally, we propose a mechanism whereby these regulatory molecules mediate the cytoskeleton rearrangements that are required for the integrin activation and subsequent IS formation that occur during the antigen-recognition process.

Antigen-induced BCR signalling

The BCR can be described as a receptor tyrosine kinase, and its stimulation by a specific antigen results in the proliferation and differentiation of B cells. The BCR comprises a membrane immunoglobulin (Ig) in complex with the heterodimeric Igα-Igβ sheath (Fig. 1B), which allows its stable expression in the membrane (Reth, 1989; Venkitaraman et al., 1991). The constituents of the BCR act in concert to couple the recognition of extracellular antigens with the initiation of intracellular signalling through the immunoreceptor tyrosine-based activation motifs (ITAMs) of the Igα-Igβ sheath (Hombach et al., 1990; Reth, 1989). These activated ITAMs subsequently act as recruitment sites for the assembly of a complex array of intracellular adaptors and signalling molecules in a structure known as the signalosome (DeFranco, 2001; Fruman et al., 2000). The precise composition of a signalosome is dictated by the nature and context of the antigenic stimulant, and determines the outcome of signalling through the BCR (Depoil et al., 2008; Weber et al., 2008). In the specific case of inside-out activation of integrins it would be expected that this outcome involves the direct targeting of the cytoplasmic domains of the integrin itself, in addition to the extensive reorganisation of the cytoskeleton that is necessary for the recognition of antigens that are presented on a spatially constrained surface.

The mechanism of inside-out activation of integrins has been investigated extensively in T cells (reviewed in Kinashi, 2005). The

insight gained from this research has provided a foundation for similar studies in B cells. Although the number of these studies is currently very limited they have yielded useful information and have identified a number of important differences in the process of integrin activation in lymphocytes. In the following sections, we describe the current understanding of the molecular mechanism that underlies the inside-out activation of two of the major B-cell integrins, LFA-1 and VLA-4, and use these as a basis to propose a cellular mechanism by which the inside-out activation of integrins functions during the process of B-cell activation. In addition we discuss several differences that have been identified in the process of inside-out integrin activation in B and T cells.

Inside-out activation of LFA-1

A role for the interaction between LFA-1 and ICAM1 in mediating the adhesion of B cells and follicular dendritic cells was identified through the analysis of the binding of isolated follicular dendritic cells and B cells derived from human tonsils (Koopman et al., 1991). It has been proposed that the presentation of antigens on the surface of follicular dendritic cells within germinal centres has a key role during the affinity-maturation process in the selection of B cells that can produce high-affinity antibodies (MacLennan, 1994; Szakal et al., 1988). Interestingly, it has also been noted that the interaction between LFA-1 and ICAM1 prevents the apoptosis of follicular B cells (Koopman et al., 1994), thus suggesting a mechanism whereby the strength of adhesion provides a criterion for B-cell selection. B cells are required to respond to a far wider range of antigen affinities than other lymphocytes, and this is particularly important in the initial encounter of limited amounts of low-affinity antigens. It has been found that LFA-1 has an important role in promoting the adhesion of B cells under conditions of limited amounts of antigen, such that more antigen can be gathered into the cSMAC and thereby enhance B-cell activation (Carrasco et al., 2004). This study also demonstrated that, on formation of the IS, LFA-1 distribution is extensively coordinated and is segregated into the pSMAC.

We recently investigated the molecular mechanism that underlies the antigen-induced cytoskeleton reorganisation and subsequent activation of LFA-1-mediated B-cell adhesion, and found that the process of inside-out activation of LFA-1 required the activation of Src-family kinases and phosphoinositide 3-kinase (PI3K) (Arana et al., 2008) (Fig. 1A). In addition, LFA-1-mediated B-cell adhesion also requires the guanine-nucleotide-exchange factors (GEFs) Vav1 and Vav2, which have previously been observed to be essential for B-cell development (Doody et al., 2001; Tarakhovsky et al., 1995). Through their GEF activity, Vav1 and Vav2 activate RhoGTPases, such as Rho, Rac and Cdc42, molecules that are implicated in the regulation of cytoskeleton reorganisation (Jaffe and Hall, 2005). Surprisingly, we found that Rac2, but not the highly homologous Rac1, is necessary for the activation of LFA-1-mediated B-cell adhesion (Arana et al., 2008). Different roles for the Rac isoforms have not previously been observed in the regulation of integrin activation in lymphocytes, although distinct roles for Rac1 and Rac2 have been identified in macrophages (Pradip et al., 2003; Wheeler et al., 2006). This differential functioning of the Rac isoforms might be dependent on their distinct subcellular localisation and, in this regard, it has been shown that the C-terminal motifs of Rac2 are sufficient to determine its cellular location and associated function (Filippi et al., 2004). Indeed, we observed that the amount of activated Rac2 did not increase more than three times following BCR stimulation, suggesting that the spatial redistribution rather than a dramatic increase in the amount of activated protein is important in mediating its function (Arana et al., 2008). We found that, in the absence of Rac2 but not Rac1 the levels of Rap1-GTP were compromised, and there were severe actin-polymerisation defects, suggesting a mechanism by which Rac2 mediates its cellular effects (Arana et al., 2008).

Rap has been shown to have a crucial role in the activation of integrin-mediated B-cell adhesion (McLeod et al., 2004) and in the response of B cells to particulate antigens (Lin et al., 2008). It has been suggested that Rap-GTP promotes the polymerisation of actin and, correspondingly, blocking the activation of Rap reduces the chemokine-induced increase in total F-actin (McLeod et al., 2004). A number of potential effectors for Rap have been identified, including the adaptor protein regulator for cell adhesion and polarization enriched in lymphoid tissue (RASF5; also known as RAPL, Nore1) (Katagiri et al., 2003), the Rap1-GTP-interacting adaptor molecule (RIAM) (Lafuente et al., 2004) and the tyrosine kinase Pyk2 (McLeod et al., 2004). One mechanism by which Rap1 might mediate reorganisation of the cytoskeleton is through the recruitment of Tiam1 and Vav2, which are activators of Cdc42 and Rac2, respectively (Arthur et al., 2004). Alternatively it has been demonstrated that Rap1 induces the formation of an 'integrin-activation complex' that functions to activate the cytoplasmic domains of integrin β-chains contains RIAM and the cytoskeleton protein talin (Han et al., 2006). However, the precise mechanism by which Rap regulates cytoskeleton reorganisation and LFA-1 activation remains unclear.

Inside-out activation of VLA-4

The interaction of VLA-4 and its ligands has a major role in B-cell development and function. It has been well-documented that VLA-4 functions to mediate B-cell migration and localisation to peripheral lymph nodes (Lo et al., 2003; Lu and Cyster, 2002); however, it has recently become apparent that VLA-4 is also involved in antigen-specific B-cell differentiation (Spaargaren et al., 2003; de Gorter et al., 2007). Indeed, VLA-4 facilitates B-cell activation following the recognition of an antigen that is presented in target membranes that contain VCAM1 (Carrasco and Batista, 2006b). VCAM1, the major ligand for VLA-4, is highly expressed on the surface of follicular dendritic cells and, as such, it has been proposed that VLA-4 acts not only as an initial tether for the B cell to allow antigen recognition by the BCR, but also in signalling synergistically with the BCR to promote tight adhesion and enhance B-cell activation.

Several intracellular effectors within the B cell have been implicated in mediating the inside-out activation of VLA-4 (which is expressed on the B-cell surface), resulting in increased VLA-4-mediated adhesion to VCAM1 and fibronectin (Spaargaren et al., 2003) (Fig. 1A). This study showed that the process of inside-out activation of VLA-4 requires the activity of the Src-family kinases and PI3K in a manner similar to that identified for LFA-1 (Arana et al., 2008). In addition, VLA-4 activation also requires the consecutive activation of Bruton tyrosine kinase (BTK), phospholipase Cγ2 (PLCγ2), IP_3-receptor-mediated Ca^{2+} release and protein kinase C (PKC) to mediate B-cell adhesion. Using a combination of FACS to determine the binding affinity of VLA-4 for soluble VCAM1, and confocal microscopy to visualise the distribution of molecules within the membrane, it was established that integrin activation occurs through clustering of VLA-4, as a result of cytoskeleton reorganisation (Spaargaren et al., 2003). It was postulated that this reorganisation is mediated by the Ca^{2+}-induced activation of the calpain proteases. In such a mechanism,

calpains cleave actin-associated cytoskeleton proteins, causing the release of VLA-4 from cytoskeleton constraints and allowing its movement to stabilised membrane rafts to permit integrin clustering. The involvement of BTK in the pathway of inside-out activation of VLA-4 was of particular significance, given that the B-cell immunodeficiency disease X-linked agammaglobulinaemia (XLA) results from loss-of-function germline mutations in the *BTK* gene (Nomura et al., 2000). Such a finding underlines the importance of the identified pathway during the development of B-cell-mediated immune responses in vivo.

Differential inside-out regulation of VLA-4 and LFA-1

From the molecular dissections detailed above, it is apparent that, whereas the activation pathways of LFA-1 and VLA-4 have a number of common signalling molecules downstream of the BCR (such as Src-family kinases and PI3K), distinct signalling pathways are employed in the inside-out activation of different integrins. The use of diverse pathways in the activation of various integrins would allow them to be subject to different spatiotemporal dynamic regulation and thereby support different functions during antigen recognition. Initial observations that compared the localisation of VLA-4 and LFA-1 within the IS following stimulation with membrane-bound antigen support this possibility. LFA-1 was found to be excluded from the antigen-BCR-containing cSMAC and to occupy the pSMAC, which corresponds to the classic description of the IS (Carrasco et al., 2004). A subsequent investigation of the role of VLA-4 within the B-cell IS showed that VLA-4, which was visualised using planar bilayers that contained fluorescently labelled GPI-linked VCAM1, is found within the cSMAC (Carrasco and Batista, 2006b). However, it is worth noting that, in the molecular context of an APC, VCAM1 was retained in the B-cell pSMAC in a mechanism that is dependent on its transmembrane domains. In agreement with these observations it has been demonstrated that, in the presence of antibodies that prevent its interaction with VCAM1, VLA-4 localises to the cSMAC in $CD4^+$ T cells (Mittelbrunn et al., 2004). The observed differential localisation within the IS following stimulation of the BCR suggests that the various integrins can mediate different interactions with the underlying cell cytoskeleton (Carrasco and Batista, 2006b).

It has been proposed that LFA-1 is retained within the pSMAC of the IS through its interaction with the cytoskeletal protein talin, which has been shown to be enriched in the pSMAC (Kim et al., 2003; Monks et al., 1998), whereas VLA-4 presumably exhibits an inverse pattern of interactions with components of the cytoskeleton (Carrasco and Batista, 2006b). In addition, it has been reported that the various α- and β-chains of integrins, indeed, recruit different intracellular adaptor and effector molecules, including some that are involved in the organisation of the cytoskeleton (Ginsberg et al., 2005). The adaptor protein paxillin, for example, has been shown to associate with α4 integrins, such as VLA-4, and can thereby function to regulate the spatial coordination of Rac activity (Barreiro et al., 2007; Rose et al., 2007). The observed differences in localisation of LFA-1 and VLA-4 would suggest that the levels of their activation are subject to regulation by different underlying molecular mechanisms. In line with this suggestion, we have observed that Rac2 is not required for VLA-4-mediated B-cell adhesion, although it is absolutely required for the inside-out activation of LFA-1 (Anne Vehlow and F.D.B., unpublished data). Differential control of integrin activation in the context of antigen recognition by B cells allows different integrins to perform distinct, possibly sequential, functions in response to BCR stimulation (Carrasco and Batista, 2006b). Thus, such mechanisms offer B cells greater functional diversity in terms of fine-tuning their responses to a wide range of conditions and antigens.

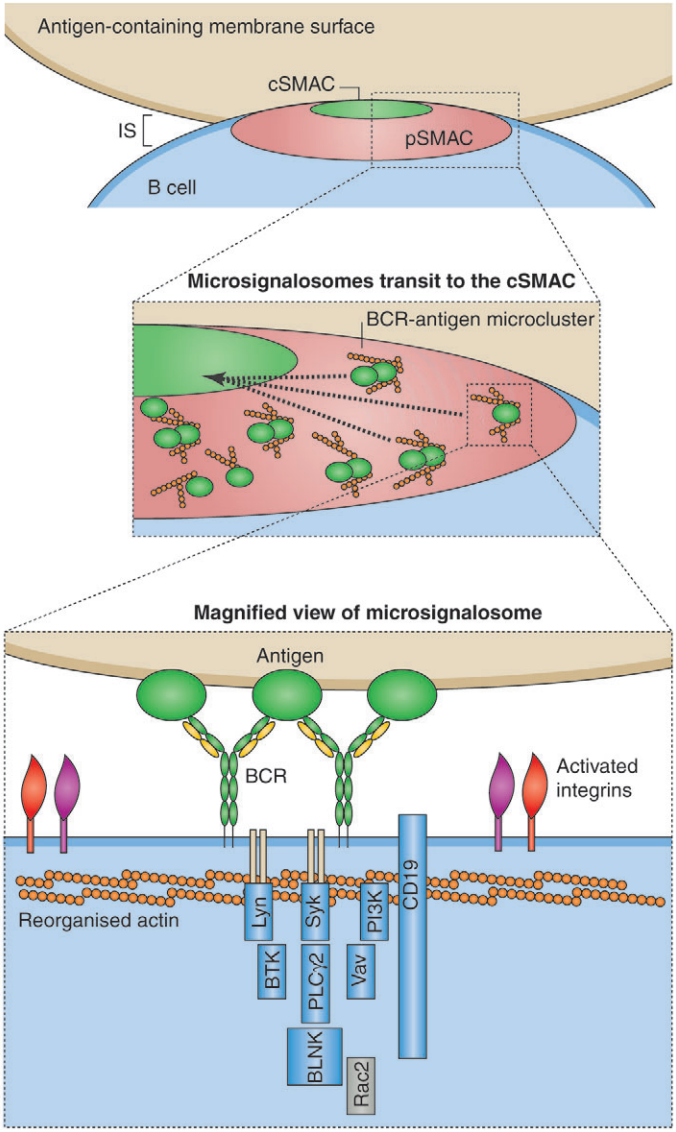

Fig. 2. Cellular mechanism for the inside-out activation of integrins. Following BCR stimulation with antigen on the surface of an APC, BCR-antigen microclusters form throughout the area of contact. These microclusters act as the sites for microsignalosome assembly and recruit molecules such as Syk, Vav, CD19, PLCγ2 (Weber et al., 2008) and PI3K (D. Depoil and F.D.B., unpublished data). We propose that these microsignalosomes provide a favourable environment for the activation of small GTPases such as Rac2 (shown in grey), which in turn mediate reorganisation of the cytoskeleton and activation of integrin-mediated B-cell adhesion. Enhanced adhesion and signalling through the BCR allow for propagation of the B-cell spreading response. We suggest that microsignalosomes are transported from the periphery to the cSMAC by centripetal retrograde actin flow in a manner similar to that recently observed in T cells (Kaizuka et al., 2007). The presence of activated integrins during spreading promotes B-cell adhesion, which stimulates the generation of further microsignalosomes and ultimately facilitates the activation of B cells.

Towards a cellular mechanism to describe inside-out integrin activation in B cells

In light of the recent experimental data that have been collected, we propose the following mechanism to describe the regulation of

integrin activity during the process of antigen recognition and activation of B cells. It has recently been established that, within the B-cell IS, multiple discrete antigen-BCR microclusters are generated throughout the pSMAC and these represent the sites of active signalling (Depoil et al., 2008). This observation is in contrast to the original assignment of the cSMAC as the site of signalling, but is in agreement with observations in T cells (Bunnell et al., 2002; Campi et al., 2005; Varma et al., 2006; Yokosuka et al., 2005). These antigen-BCR microclusters act as the sites of assembly of multiple BCR microsignalosomes in response to membrane-bound antigen recognition (Fig. 2). We have observed the recruitment of the GEF Vav to BCR microclusters within the pSMAC following stimulation with membrane-bound antigen (Weber et al., 2008). It would be expected that this recruitment of Vav mediates the subsequent localisation of Rac2, and we have demonstrated that Rac2 is, indeed, found in the pSMAC (E.A. and F.D.B., unpublished observations). We postulate that Rac2, but not Rac1, transiently associates with the microsignalosomes as they transit to the cSMAC (Fig. 2). Rac2 might then function to mediate the cytoskeleton rearrangements that are necessary to provide an organised, favourable environment for the coordinated activation of cellular effectors (Arthur et al., 2004; Bos, 2005). In support of this suggestion it has also been reported that the correct spatiotemporal coordination of Rap1 activity is important in integrin-mediated adhesion in T cells (Kliche et al., 2006). A more detailed investigation of the spatiotemporal regulation of the various components of the activation pathway, using high-resolution imaging techniques such as total internal reflection microscopy (TIRFM), will prove invaluable in the dissection of the precise mechanism of integrin activation.

The inside-out activation of integrins such as LFA-1 and VLA-4 allows for tighter B-cell adhesion and synergy with BCR signalling which, together with outside-in integrin activation, allow for the propagation of the B-cell spreading response. It has already been observed that the spreading response allows for the accumulation of greater amounts of antigen within the cSMAC of the IS (Fleire et al., 2006). Thus, it is our view that during B-cell spreading, integrins promote adhesion and the generation of a greater number of microsignalosomes, thereby facilitating B-cell activation.

One recent study has offered valuable insight into the dynamic coordination of the events prior to IS formation in the Jurkat T-cell line (Kaizuka et al., 2007). In this system, discrete microclusters of T-cell receptors (TCRs) and LFA-1 that are characterised by different associations with the underlying actin cytoskeleton were observed. Following their generation in the cell periphery, these microclusters were transported with different efficiencies by centripetal reterograde actin flow – the TCR microclusters to the cSMAC and the LFA-1 microclusters to the pSMAC. We expect a similar mechanism of microcluster transit, which is dependent on the interactions with components of the cytoskeleton, might operate during formation of the IS in B cells (Fig. 2).

Differences in inside-out activation of integrins in B and T cells

In view of the recent investigations of inside-out activation of VLA-4 and LFA-1 it is clear that, in spite of general similarities in the underlying mechanisms of these processes in lymphocytes, several important differences occur in B and T cells in terms of the composition of molecular adaptors and intracellular signalling

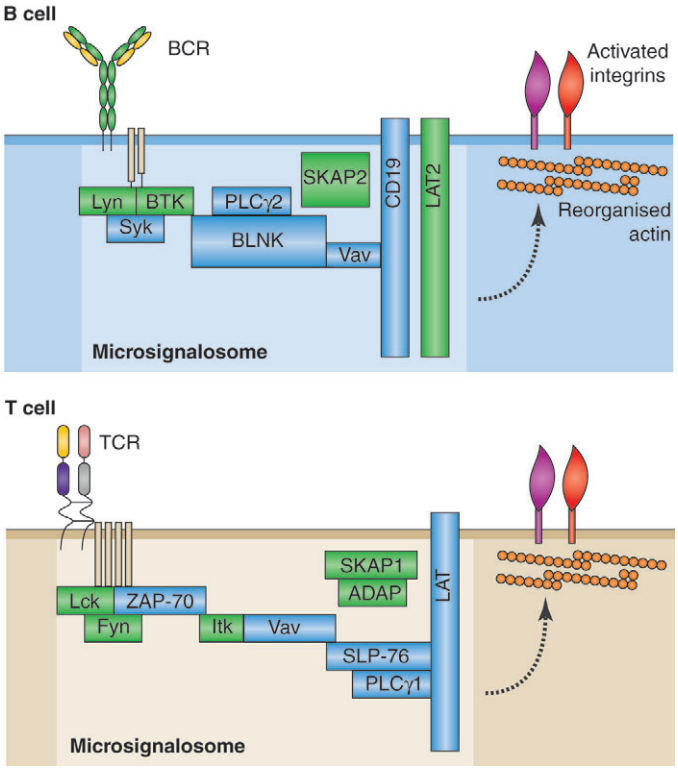

Fig. 3. Differences in inside-out activation of integrins in B and T cells. The current understanding of the early molecular events that underlie the inside-out activation of integrins in B and T cells following immunoreceptor stimulation is shown. Differences in the composition of the signalosomes that are assembled are illustrated, in terms of their differential use of proximal signalling and adaptor molecules. Molecules that have been demonstrated to be localised within the microsignalosome by high-resolution imaging are shown (blue), as well as those that have been implicated to be important by biochemical analysis (green). The Src-family kinases (Lyn and Lck) are not strictly considered to be part of the microsignalosome because they are localised to the membrane prior to antigenic stimulation; however, they are crucial for microsignalosome formation.

molecules that are recruited to the signalosome (Fig. 3). However, it is worth noting that, as integrins can be activated through alteration of their conformation and/or distribution on the cell surface, integrin activation can be measured using a variety of experimental approaches. For example, the alteration of integrin conformation can be examined by its capacity to bind soluble ligand or conformation-dependent antibodies, or alternatively, the location of integrin on the cell surface can be examined by high-resolution imaging. A number of discrepancies have arisen in the literature as to the importance of particular molecules in the activation of integrins in T cells as a consequence of the variety of experimental methods used to quantify integrin activation. Thus, the precise manner by which integrin activation is assessed must be considered during any comparison of these processes in lymphocytes.

Molecular adaptors

Differential requirements for adaptors that are present within the signalosome have been reported following the initial tyrosine kinase activation of the immunoreceptor (Fig. 3). As detailed earlier, PLCγ2 is required for the inside-out activation of VLA-4 in B cells

(Spaargaren et al., 2003). Interestingly, PLCγ1 has previously been implicated in the inside-out activation of integrins in T cells (Katagiri et al., 2004); however, this requires its recruitment to the signalosome, which is dependent on the activities of two adaptor proteins: linker for activation of T cells family member 1 (LAT) and lymphocyte cytosolic protein 2 (LCP2, also known and hereafter referred to as SLP-76) (reviewed by Bezman and Koretzky, 2007). As mature B cells do not express LAT or SLP-76 (Fu and Chan, 1997; Zhang et al., 1998), they must use an alternative adaptor(s) for the recruitment of PLCγ2 to the signalosome.

One such adaptor, the B-cell linker (BLNK), has been established as having an important role in the activation of PLCγ2 in B cells (Chiu et al., 2002; Fu et al., 1998; Hashimoto et al., 1999; Ishiai et al., 1999). BLNK might be recruited directly to the BCR complex, and from there mediate the recruitment of PLCγ2 (Engels et al., 2001; Kabak et al., 2002). It has proved difficult to assess the role of BLNK in the inside-out activation of integrins as a deficiency of BLNK severely impacts the development of the mature B-cell compartment (Xu et al., 2000). However, we have recently observed that BLNK is required for the recruitment of PLCγ2 during the initiation of the B-cell spreading response (Weber et al., 2008). In addition, it has been suggested that linker for activation of T cells family member 2 (LAT2; also known as NTAL or LAB) performs a similar function in B cells as LAT in T cells (Brdicka et al., 2002; Janssen et al., 2003). However, LAT2 does not associate with PLCγ2 and cannot, therefore, alone mediate its recruitment to the signalosome.

We have recently established an unexpected and essential role for the co-receptor CD19 in the activation of B cells by membrane-bound antigens (Depoil et al., 2008). As CD19 was found to immunoprecipitate with various intracellular signalling molecules, including PLCγ2 and Vav (Brooks et al., 2004), we postulate that CD19 functions as a 'LAT-like' adaptor that recruits signalling molecules to the B-cell signalosome following stimulation with membrane-bound antigen (Weber et al., 2008). However, the identification of the particular adaptor(s) that is used to recruit PLCγ2 during inside-out activation of VLA-4 remains a challenge to be addressed in the future, potentially through the generation of conditional knockouts of various candidate proteins.

An additional cytosolic adaptor protein that functions downstream of SLP-76, and is required for the inside-out activation of β1- and β2-integrin-mediated adhesion in T cells has been identified (Griffiths et al., 2001; Peterson et al., 2001). This protein was named adhesion- and degranulation-promoting adaptor protein (ADAP) and has been demonstrated to bind to and form a functional unit with the T-cell-specific adaptor SKAP1 (also known as SKAP55) following stimulation of the TCR (Liu et al., 1998; Marie-Cardine et al., 1997). Although the ADAP-SKAP1 module has been found to be crucial for optimal activation of integrin-mediated T-cell adhesion (Kliche et al., 2006), it has been shown that mature B cells do not express either component of this complex (da Silva et al., 1997; Dluzniewska et al., 2007; Marie-Cardine et al., 1997; Musci et al., 1997). Instead, B-cell adhesion is dependent on the adaptor protein SKAP2 (also known as SKAP-HOM), such that in the absence of SKAP2, adhesion to both fibronectin and ICAM1 is substantially reduced (Togni et al., 2005). However, the requirement for and identity of any additional B-cell adaptor molecule (which would be equivalent to ADAP in T cells) that is required for SKAP2 function remains to be determined.

Intracellular signalling

As well as differences in adaptors, differences in intracellular signalling molecules that are recruited to and activated at the signalosome have been observed. For example, although the p110δ subunit of PI3K is required for LFA-1-mediated B-cell adhesion (Arana et al., 2008), β2-integrin-mediated T-cell adhesion is not impaired in the absence of this subunit (Okkenhaug et al., 2002). Alongside the observed differences in the composition of the signalosome, which is assembled following antigen stimulation in lymphocytes, it has been demonstrated that distinct pathways can control immunoreceptor and integrin clustering in T cells. Notably, clustering of the TCR requires both Vav1 and Wiskott-Aldrich syndrome protein (WASP), whereas clustering of LFA-1 following TCR stimulation requires Vav1 but not WASP (Krawczyk et al., 2002). However, such distinct pathways have not yet been identified in B cells and represent a potentially significant difference in the mechanism of inside-out integrin activation that is used by lymphocytes. We anticipate that more detailed investigations of the activation of integrins in B cells in response to membrane-bound antigens will identify further differences in the mechanisms of these processes in lymphocytes.

Conclusions and Perspectives

It can be clearly seen that the inside-out activation of integrins in response to stimulation through the BCR requires extensive spatiotemporal coordination of numerous intracellular signalling molecules and adaptors. The importance of integrin activation can be appreciated when it is considered that membrane-bound antigens represent the major route for encountering B-cell antigen in vivo, and often these antigens are both scarce and low in affinity during the development of the B-cell response (Batista and Neuberger, 1998; Batista and Neuberger, 2000).

Recent investigations have concerned the characterisation of intracellular signalling pathways, and have identified a key role for small GTPases such as Rac2 and Rap (Arana et al., 2008; Lin et al., 2008). Such molecules have the capacity to act as the links between extracellular signals and cytoskeleton reorganisation, allowing for the activation of integrins and subsequent activation of B cells. Numerous challenges remain before a complete description of the mechanism of integrin activation and its role in the process of B-cell activation can be formulated. These challenges include: the identification of additional adaptor proteins that are involved in maintaining the integrity of the BCR signalosome, the investigation of the role of other RhoGTPases such as Cdc42 and Rho in the process of inside-out integrin activation, and the visualisation of the dynamic recruitment of constituents of the signalosome to signalling microclusters at high resolution using techniques such as TIRFM.

The combination of these and other investigations would be expected to yield insight into the role of integrins in the development of the immune response and, thus, offer pharmaceutically relevant information to treat and prevent autoimmune and infectious diseases as well as cancer.

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References

Alon, R. and Dustin, M. L. (2007). Force as a facilitator of integrin conformational changes during leukocyte arrest on blood vessels and antigen-presenting cells. *Immunity* **26**, 17-27.

Arana, E., Vehlow, A., Harwood, N. E., Vigorito, E., Henderson, R., Turner, M., Tybulewicz, V. L. and Batista, F. D. (2008). Activation of the Small GTPase Rac2 via the B Cell Receptor Regulates B Cell Adhesion and Immunological-Synapse Formation. *Immunity* **28**, 88-99.

Arthur, W. T., Quilliam, L. A. and Cooper, J. A. (2004). Rap1 promotes cell spreading by localizing Rac guanine nucleotide exchange factors. *J. Cell Biol.* **167**, 111-122.

Balázs, M., Martin, F., Zhou, T. and Kearney, J. (2002). Blood dendritic cells interact with splenic marginal zone B cells to initiate T-independent immune responses. *Immunity* **17**, 341-352.

Barreiro, O., de la Fuente, H., Mittelbrunn, M. and Sánchez-Madrid, F. (2007). Functional insights on the polarized redistribution of leukocyte integrins and their ligands during leukocyte migration and immune interactions. *Immunol. Rev.* **218**, 147-164.

Batista, F. and Neuberger, M. (1998). Affinity dependence of the B cell response to antigen: a threshold, a ceiling, and the importance of off-rate. *Immunity* **8**, 751-759.

Bazzoni, G. and Hemler, M. (1998). Are changes in integrin affinity and conformation overemphasized? *Trends Biochem. Sci.* **23**, 30-34.

Batista, F. and Neuberger, M. (2000). B cells extract and present immobilized antigen: implications for affinity discrimination. *EMBO J.* **19**, 513-520.

Batista, F. D., Iber, D. and Neuberger, M. S. (2001). B cells acquire antigen from target cells after synapse formation. *Nature* **411**, 489-494.

Berlin-Rufenach, C., Otto, F., Mathies, M., Westermann, J., Owen, M., Hamann, A. and Hogg, N. (1999). Lymphocyte migration in lymphocyte function-associated antigen (LFA)-1-deficient mice. *J. Exp. Med.* **189**, 1467-1478.

Bezman, N. and Koretzky, G. (2007). Compartmentalization of ITAM and integrin signaling by adapter molecules. *Immunol. Rev.* **218**, 9-28.

Bos, J. L. (2005). Linking Rap to cell adhesion. *Curr. Opin. Cell Biol.* **17**, 123-128.

Brdicka, T., Imrich, M., Angelisová, P., Brdicková, N., Horváth, O., Spicka, J., Hilgert, I., Lusková, P., Dráber, P., Novák, P. et al. (2002). Non-T cell activation linker (NTAL): a transmembrane adaptor protein involved in immunoreceptor signaling. *J. Exp. Med.* **196**, 1617-1626.

Brooks, S., Kirkham, P., Freeberg, L. and Carter, R. (2004). Binding of cytoplasmic proteins to the CD19 intracellular domain is high affinity, competitive, and multimeric. *J. Immunol.* **172**, 7556-7564.

Bunnell, S. C., Hong, D. I., Kardon, J. R., Yamazaki, T., McGlade, C. J., Barr, V. A. and Samelson, L. E. (2002). T cell receptor ligation induces the formation of dynamically regulated signaling assemblies. *J. Cell Biol.* **158**, 1263-1275.

Campi, G., Varma, R. and Dustin, M. (2005). Actin and agonist MHC-peptide complex-dependent T cell receptor microclusters as scaffolds for signaling. *J. Exp. Med.* **202**, 1031-1036.

Carman, C. and Springer, T. (2003). Integrin avidity regulation: are changes in affinity and conformation underemphasized? *Curr. Opin. Cell Biol.* **15**, 547-556.

Carrasco, Y. R. and Batista, F. D. (2006a). B cell recognition of membrane-bound antigen: an exquisite way of sensing ligands. *Curr. Opin. Immunol.* **18**, 286-291.

Carrasco, Y. R. and Batista, F. D. (2006b). B-cell activation by membrane-bound antigens is facilitated by the interaction of VLA-4 with VCAM-1. *EMBO J.* **25**, 889-899.

Carrasco, Y. R. and Batista, F. D. (2007). B cells acquire particulate antigen in a macrophage-rich area at the boundary between the follicle and the subcapsular sinus of the lymph node. *Immunity* **27**, 160-171.

Carrasco, Y. R., Fleire, S. J., Cameron, T., Dustin, M. L. and Batista, F. D. (2004). LFA-1/ICAM-1 interaction lowers the threshold of B cell activation by facilitating B cell adhesion and synapse formation. *Immunity* **20**, 589-599.

Chiu, C., Dalton, M., Ishiai, M., Kurosaki, T. and Chan, A. (2002). BLNK: molecular scaffolding through 'cis'-mediated organization of signaling proteins. *EMBO J.* **21**, 6461-6472.

Cinamon, G., Zachariah, M., Lam, O., Foss, F. and Cyster, J. (2007). Follicular shuttling of marginal zone B cells facilitates antigen transport. *Nat. Immunol.* **9**, 54-62.

da Silva, A., Li, Z., de Vera, C., Canto, E., Findell, P. and Rudd, C. (1997). Cloning of a novel T-cell protein FYB that binds FYN and SH2-domain-containing leukocyte protein 76 and modulates interleukin 2 production. *Proc. Natl. Acad. Sci. USA* **94**, 7493-7498.

de Gorter, D., Beuling, E., Kersseboom, R., Middendorp, S., van Gils, J., Hendriks, R., Pals, S. and Spaargaren, M. (2007). Bruton's tyrosine kinase and phospholipase Cgamma2 mediate chemokine-controlled B cell migration and homing. *Immunity* **26**, 93-104.

DeFranco, A. (2001). Vav and the B cell signalosome. *Nat. Immunol.* **2**, 482-484.

Depoil, D., Fleire, S., Treanor, B. L., Weber, M., Harwood, N. E., Marchbank, K. L., Tybulewicz, V. L. and Batista, F. D. (2008). CD19 is essential for B cell activation by promoting B cell receptor-antigen microcluster formation in response to membrane-bound ligand. *Nat. Immunol.* **9**, 63-72.

Dluzniewska, J., Zou, L., Harmon, I., Ellingson, M. and Peterson, E. (2007). Immature hematopoietic cells display selective requirements for adhesion- and degranulation-promoting adaptor protein in development and homeostatsis. *Eur. J. Immunol.* **37**, 3208-3219.

Doody, G., Bell, S., Vigorito, E., Clayton, E., McAdam, S., Tooze, R., Fernandez, C., Lee, I. and Turner, M. (2001). Signal transduction through Vav-2 participates in humoral immune responses and B cell maturation. *Nat. Immunol.* **2**, 542-547.

Dustin, M. and Springer, T. (1989). T-cell receptor cross-linking transiently stimulates adhesiveness through LFA-1. *Nature* **341**, 619-624.

Engels, N., Wollscheid, B. and Wienands, J. (2001). Association of SLP-65/BLNK with the B cell antigen receptor through a non-ITAM tyrosine of Ig-alpha. *Eur. J. Immunol.* **31**, 2126-2134.

Filippi, M. D., Harris, C. E., Meller, J., Gu, Y., Zheng, Y. and Williams, D. A. (2004). Localization of Rac2 via the C terminus and aspartic acid 150 specifies superoxide generation, actin polarity and chemotaxis in neutrophils. *Nat. Immunol.* **5**, 744-751.

Fleire, S. J., Goldman, J. P., Carrasco, Y. R., Weber, M., Bray, D. and Batista, F. D. (2006). B cell ligand discrimination through a spreading and contraction response. *Science* **312**, 738-741.

Fruman, D., Satterthwaite, A. and Witte, O. (2000). Xid-like phenotypes: a B cell signalosome takes shape. *Immunity* **13**, 1-3.

Fu, C. and Chan, A. (1997). Identification of two tyrosine phosphoproteins, pp70 and pp68, which interact with phospholipase Cgamma, Grb2, and Vav after B cell antigen receptor activation. *J. Biol. Chem.* **272**, 27362-27368.

Fu, C., Turck, C., Kurosaki, T. and Chan, A. (1998). BLNK: a central linker protein in B cell activation. *Immunity* **9**, 93-103.

Ginsberg, M., Partridge, A. and Shattil, S. (2005). Integrin regulation. *Curr. Opin. Cell Biol.* **17**, 509-516.

Griffiths, E. K., Krawczyk, C., Kong, Y. Y., Raab, M., Hyduk, S. J., Bouchard, D., Chan, V. S., Kozieradzki, I., Oliveira-Dos-Santos, A. J., Wakeham, A. et al. (2001). Positive regulation of T cell activation and integrin adhesion by the adapter Fyb/Slap. *Science* **293**, 2260-2263.

Han, J., Lim, C., Watanabe, N., Soriani, A., Ratnikov, B., Calderwood, D., Puzon-McLaughlin, W., Lafuente, E., Boussiotis, V., Shattil, S. et al. (2006). Reconstructing and deconstructing agonist-induced activation of integrin alphaIIbbeta3. *Curr. Biol.* **16**, 1796-1806.

Hashimoto, S., Iwamatsu, A., Ishiai, M., Okawa, K., Yamadori, T., Matsushita, M., Baba, Y., Kishimoto, T., Kurosaki, T. and Tsukada, S. (1999). Identification of the SH2 domain binding protein of Bruton's tyrosine kinase as BLNK-functional significance of Btk-SH2 domain in B-cell antigen receptor-coupled calcium signaling. *Blood* **94**, 2357-2364.

Hombach, J., Tsubata, T., Leclercq, L., Stappert, H. and Reth, M. (1990). Molecular components of the B-cell antigen receptor complex of the IgM class. *Nature* **343**, 760-762.

Hynes, R. (1992). Integrins: versatility, modulation, and signaling in cell adhesion. *Cell* **69**, 11-25.

Ishiai, M., Kurosaki, M., Pappu, R., Okawa, K., Ronko, I., Fu, C., Shibata, M., Iwamatsu, A., Chan, A. and Kurosaki, T. (1999). BLNK required for coupling Syk to PLC gamma 2 and Rac1-JNK in B cells. *Immunity* **10**, 117-125.

Jaffe, A. B. and Hall, A. (2005). Rho GTPases: biochemistry and biology. *Annu. Rev. Cell Dev. Biol.* **21**, 247-269.

Janssen, E., Zhu, M., Zhang, W., Koonpaew, S. and Zhang, W. (2003). LAB: a new membrane-associated adaptor molecule in B cell activation. *Nat. Immunol.* **4**, 117-123.

Junt, T., Moseman, E., Iannacone, M., Massberg, S., Lang, P., Boes, M., Fink, K., Henrickson, S., Shayakhmetov, D., Di Paolo, N. et al. (2007). Subcapsular sinus macrophages in lymph nodes clear lymph-borne viruses and present them to antiviral B cells. *Nature* **450**, 110-114.

Kabak, S., Skaggs, B., Gold, M., Affolter, M., West, K., Foster, M., Siemasko, K., Chan, A., Aebersold, R. and Clark, M. (2002). The direct recruitment of BLNK to immunoglobulin alpha couples the B-cell antigen receptor to distal signaling pathways. *Mol. Cell. Biol.* **22**, 2524-2535.

Kaizuka, Y., Douglass, A., Varma, R., Dustin, M. and Vale, R. (2007). Mechanisms for segregating T cell receptor and adhesion molecules during immunological synapse formation in Jurkat T cells. *Proc. Natl. Acad. Sci. USA* **104**, 20296-20301.

Katagiri, K., Maeda, A., Shimonaka, M. and Kinashi, T. (2003). RAPL, a Rap1-binding molecule that mediates Rap1-induced adhesion through spatial regulation of LFA-1. *Nat. Immunol.* **4**, 741-748.

Katagiri, K., Shimonaka, M. and Kinashi, T. (2004). Rap1-mediated lymphocyte function-associated antigen-1 activation by the T cell antigen receptor is dependent on phospholipase C-gamma1. *J. Biol. Chem.* **279**, 11875-11881.

Kim, M., Carman, C. and Springer, T. (2003). Bidirectional transmembrane signaling by cytoplasmic domain separation in integrins. *Science* **301**, 1720-1725.

Kinashi, T. (2005). Intracellular signalling controlling integrin activation in lymphocytes. *Nat. Rev. Immunol.* **5**, 546-559.

Kliche, S., Breitling, D., Togni, M., Pusch, R., Heuer, K., Wang, X., Freund, C., Kasirer-Friede, A., Menasche, G., Koretzky, G. A. et al. (2006). The ADAP/SKAP55 signaling module regulates T-cell receptor-mediated integrin activation through plasma membrane targeting of Rap1. *Mol. Cell. Biol.* **26**, 7130-7144.

Koopman, G., Parmentier, H. K., Schuurman, H. J., Newman, W., Meijer, C. J. and Pals, S. T. (1991). Adhesion of human B cells to follicular dendritic cells involves both the lymphocyte function-associated antigen 1/intercellular adhesion molecule 1 and very late antigen 4/vascular cell adhesion molecule 1 pathways. *J. Exp. Med.* **173**, 1297-1304.

Koopman, G., Keehnen, R. M., Lindhout, E., Newman, W., Shimizu, Y., van Seventer, G. A., de Groot, C. and Pals, S. T. (1994). Adhesion through the LFA-1 (CD11a/CD18)-ICAM-1 (CD54) and the VLA-4 (CD49d)-VCAM-1 (CD106) pathways prevents apoptosis of germinal center B cells. *J. Immunol.* **152**, 3760-3767.

Krawczyk, C., Oliveira-dos-Santos, A., Sasaki, T., Griffiths, E., Ohashi, P. S., Snapper, S., Alt, F. and Penninger, J. M. (2002). Vav1 controls integrin clustering and MHC/peptide-specific cell adhesion to antigen-presenting cells. *Immunity* **16**, 331-343.

Lafuente, E., van Puijenbroek, A., Krause, M., Carman, C., Freeman, G., Berezovskaya, A., Constantine, E., Springer, T., Gertler, F. and Boussiotis, V. (2004). RIAM, an Ena/VASP and Profilin ligand, interacts with Rap1-GTP and mediates Rap1-induced adhesion. *Dev. Cell* **7**, 585-595.

Lanzavecchia, A. (1985). Antigen-specific interaction between T and B cells. *Nature* **314**, 537-539.

Lin, K. B., Freeman, S. A., Zabetian, S., Brugger, H., Weber, M., Lei, V., Dang-Lawson, M., Tse, K. W., Santamaria, R., Batista, F. D. et al. (2008). The rap GTPases regulate

B cell morphology, immune-synapse formation, and signaling by particulate B cell receptor ligands. *Immunity* **28**, 75-87.

Liu, J., Kang, H., Raab, M., da, Silva, A., Kraeft, S. and Rudd, C. (1998). FYB (FYN binding protein) serves as a binding partner for lymphoid protein and FYN kinase substrate SKAP55 and a SKAP55-related protein in T cells. *Proc. Natl. Acad. Sci. USA* **95**, 8779-8784.

Lo, C., Lu, T. and Cyster, J. (2003). Integrin-dependence of lymphocyte entry into the splenic white pulp. *J. Exp. Med.* **197**, 353-361.

Lu, T. and Cyster, J. (2002). Integrin-mediated long-term B cell retention in the splenic marginal zone. *Science* **297**, 409-412.

Luo, B., Carman, C. and Springer, T. (2007). Structural basis of integrin regulation and signaling. *Annu. Rev. Immunol.* **25**, 619-647.

MacLennan, I. (1994). Germinal centers. *Annu. Rev. Immunol.* **12**, 117-139.

Marie-Cardine, A., Bruyns, E., Eckerskorn, C., Kirchgessner, H., Meuer, S. and Schraven, B. (1997). Molecular cloning of SKAP55, a novel protein that associates with the protein tyrosine kinase p59fyn in human T-lymphocytes. *J. Biol. Chem.* **272**, 16077-16080.

McLeod, S. J., Shum, A. J., Lee, R. L., Takei, F. and Gold, M. R. (2004). The Rap GTPases regulate integrin-mediated adhesion, cell spreading, actin polymerization, and Pyk2 tyrosine phosphorylation in B lymphocytes. *J. Biol. Chem.* **279**, 12009-12019.

Mittelbrunn, M., Molina, A., Escribese, M., Yáñez-Mó, M., Escudero, E., Ursa, A., Tejedor, R., Mampaso, F. and Sánchez-Madrid, F. (2004). VLA-4 integrin concentrates at the peripheral supramolecular activation complex of the immune synapse and drives T helper 1 responses. *Proc. Natl. Acad. Sci. USA* **101**, 11058-11063.

Monks, C., Freiberg, B., Kupfer, H., Sciaky, N. and Kupfer, A. (1998). Three-dimensional segregation of supramolecular activation clusters in T cells. *Nature* **395**, 82-86.

Musci, M., Hendricks-Taylor, L., Motto, D., Paskind, M., Kamens, J., Turck, C. and Koretzky, G. (1997). Molecular cloning of SLAP-130, an SLP-76-associated substrate of the T cell antigen receptor-stimulated protein tyrosine kinases. *J. Biol. Chem.* **272**, 11674-11677.

Nomura, K., Kanegane, H., Karasuyama, H., Tsukada, S., Agematsu, K., Murakami, G., Sakazume, S., Sako, M., Tanaka, R., Kuniya, Y. et al. (2000). Genetic defect in human X-linked agammaglobulinemia impedes a maturational evolution of pro-B cells into a later stage of pre-B cells in the B-cell differentiation pathway. *Blood* **96**, 610-617.

Okkenhaug, K., Bilancio, A., Farjot, G., Priddle, H., Sancho, S., Peskett, E., Pearce, W., Meek, S. E., Salpekar, A., Waterfield, M. D. et al. (2002). Impaired B and T cell antigen receptor signaling in p110delta PI 3-kinase mutant mice. *Science* **297**, 1031-1034.

Peterson, E. J., Woods, M. L., Dmowski, S. A., Derimanov, G., Jordan, M. S., Wu, J. N., Myung, P. S., Liu, Q. H., Pribila, J. T., Freedman, B. D. et al. (2001). Coupling of the TCR to integrin activation by Slap-130/Fyb. *Science* **293**, 2263-2265.

Phan, T. G., Grigorova, I., Okada, T. and Cyster, J. G. (2007). Subcapsular encounter and complement-dependent transport of immune complexes by lymph node B cells. *Nat. Immunol.* **8**, 992-1000.

Pradip, D., Peng, X. and Durden, D. (2003). Rac2 specificity in macrophage integrin signaling: potential role for Syk kinase. *J. Biol. Chem.* **278**, 41661-41669.

Reth, M. (1989). Antigen receptor tail clue. *Nature* **338**, 383-384.

Rock, K., Benacerraf, B. and Abbas, A. (1984). Antigen presentation by hapten-specific B lymphocytes. I. Role of surface immunoglobulin receptors. *J. Exp. Med.* **160**, 1102-1113.

Rose, D., Alon, R. and Ginsberg, M. (2007). Integrin modulation and signaling in leukocyte adhesion and migration. *Immunol. Rev.* **218**, 126-134.

Spaargaren, M., Beuling, E. A., Rurup, M. L., Meijer, H. P., Klok, M. D., Middendorp, S., Hendriks, R. W. and Pals, S. T. (2003). The B cell antigen receptor controls integrin activity through Btk and PLCgamma2. *J. Exp. Med.* **198**, 1539-1550.

Springer, T. (1990). Adhesion receptors of the immune system. *Nature* **346**, 425-434.

Szakal, A., Kosco, M. and Tew, J. (1988). A novel in vivo follicular dendritic cell-dependent iccosome-mediated mechanism for delivery of antigen to antigen-processing cells. *J. Immunol.* **140**, 341-353.

Takagi, J., Petre, B., Walz, T. and Springer, T. (2002). Global conformational rearrangements in integrin extracellular domains in outside-in and inside-out signaling. *Cell* **110**, 599-511.

Tarakhovsky, A., Turner, M., Schaal, S., Mee, P. J., Duddy, L. P., Rajewsky, K. and Tybulewicz, V. L. J. (1995). Defective antigen receptor-mediated proliferation of B and T cells in the absence of Vav. *Nature* **374**, 467-470.

Togni, M., Swanson, K. D., Reimann, S., Kliche, S., Pearce, A. C., Simeoni, L., Reinhold, D., Wienands, J., Neel, B. G., Schraven, B. et al. (2005). Regulation of in vitro and in vivo immune functions by the cytosolic adaptor protein SKAP-HOM. *Mol. Cell. Biol.* **25**, 8052-8063.

Varma, R., Campi, G., Yokosuka, T., Saito, T. and Dustin, M. L. (2006). T cell receptor-proximal signals are sustained in peripheral microclusters and terminated in the central supramolecular activation cluster. *Immunity* **25**, 117-127.

Venkitaraman, A., Williams, G., Dariavach, P. and Neuberger, M. (1991). The B-cell antigen receptor of the five immunoglobulin classes. *Nature* **352**, 777-781.

Weber, M., Treanor, B., Depoil, D., Shinohara, H., Harwood, N. E., Hikida, M., Kurosaki, T. and Batista, F. D. (2008). Phospholipase C-gamma2 and Vav cooperate within signaling microclusters to propagate B cell spreading in response to membrane-bound antigen. *J. Exp. Med.* **205**, 853-868.

Wheeler, A., Wells, C., Smith, S., Vega, F., Henderson, R., Tybulewicz, V. and Ridley, A. (2006). Rac1 and Rac2 regulate macrophage morphology but are not essential for migration. *J. Cell. Sci.* **119**, 2749-2757.

Wu, J., Qin, D., Burton, G., Szakal, A. and Tew, J. (1996). Follicular dendritic cell-derived antigen and accessory activity in initiation of memory IgG responses in vitro. *J. Immunol.* **157**, 3404-3411.

Wykes, M., Pombo, A., Jenkins, C. and MacPherson, G. (1998). Dendritic cells interact directly with naive B lymphocytes to transfer antigen and initiate class switching in a primary T-dependent response. *J. Immunol.* **161**, 1313-1319.

Xiong, J., Stehle, T., Diefenbach, B., Zhang, R., Dunker, R., Scott, D., Joachimiak, A., Goodman, S. and Arnaout, M. (2001). Crystal structure of the extracellular segment of integrin alpha Vbeta3. *Science* **294**, 339-345.

Xu, S., Tan, J., Wong, E., Manickam, A., Ponniah, S. and Lam, K. (2000). B cell development and activation defects resulting in xid-like immunodeficiency in BLNK/SLP-65-deficient mice. *Int. Immunol.* **12**, 397-404.

Yokosuka, T., Sakata-Sogawa, K., Kobayashi, W., Hiroshima, M., Hashimoto-Tane, A., Tokunaga, M., Dustin, M. L. and Saito, T. (2005). Newly generated T cell receptor microclusters initiate and sustain T cell activation by recruitment of Zap70 and SLP-76. *Nat. Immunol.* **6**, 1253-1262.

Zhang, W., Sloan-Lancaster, J., Kitchen, J., Trible, R. and Samelson, L. (1998). LAT: the ZAP-70 tyrosine kinase substrate that links T cell receptor to cellular activation. *Cell* **92**, 83-92.