carefully documented that in a simplified *in vitro* model, which used cytokine-activated human umbilical vascular endothelial cells (HUVECs), the combination of apically applied stromal cell derived factor 1α (or SDF-1α, which binds rapidly to the apical surface) and fluid shear stress promoted robust CD3$^+$ T cell transmigration[2] (**Fig. 1b**). Importantly, in parallel experiments with SLC (secondary lymphoid organ chemokine), which did not bind appreciably to the endothelial cells used, the chemokine triggered arrest and cell movement on the apical surface but did not promote transmigration[2]. These data reinforce the theory that, once immobilized, chemokines are more effective at promoting transmigration. The investigators found that >80% of adherent memory T cells (defined as CD3$^+$CD45RO^{hi} cells) migrated, whereas <30% of adherent naïve T cells actually transmigrated[2]. These data, however, contrast markedly with previous results with neutrophils and monocytes. *In vitro* and under either static or flow conditions, both neutrophils and monocytes can transmigrate efficiently across the lateral junctions of cytokine-activated endothelial cell monolayers and do so without exogenously applied chemokines[10]. In their discussion, Alon and colleagues state that chemokines adsorbed on cytokine-activated HUVECs also potentiate neutrophil transmigration under flow, as compared to static, conditions. Indeed, others have also reported that fluid shear, as compared to static conditions, can potentiate neutrophil transmigration, with the main observation that neutrophils migrated faster under flow but to the same extent[11].

What of the theory that a chemotactic gradient *per se* is not necessary for leukocyte transmigration? Alon and colleagues envision chemokine-triggered lymphocyte transmigration, or chemor-heotaxis, works because T cells acquire their promigratory potential only in response to adequate chemokine signals transmitted at the migratory zone and in the context of continuous application of shear flow. They discard the possibility that fluid shear stress redistributes the overlaid chemokine to subendothelial cell compartment in order to initiate a chemotactic gradient because T cell transmigration was not observed in a static analysis of chemokine-coated monolayers that were pre-exposed to shear flow. One significant issue remains: that of how to identify exactly what constitutes the promigratory zone alluded to by Alon and colleagues and whether fluid shear force can regulate its properties.

The physiological relevance of these *in vitro* observations remains to be determined in an appropriate *in vivo* experimental model. Endothelial cell propagated *in vitro* have their limitations. Similarly, *in vivo* endothelial cells and leukocytes are continuously exposed to fluid shear stresses, whereas these experiments used intermittent exposure to fluid shear conditions. Despite these shortcomings, this system gives valuable information on mechanisms of leukocyte transmigration and offers insight into how shear stress directly effects T cell transmigration. Finally, considering the numerous checks and balances that exist to regulate leukocyte emigration *in vivo*, appropriate caution should prevail when interpreting this study. However, the data do support the authors' conclusion that surface presentation of integrin-activating chemokines in a perfusion chamber can suffice for T cell transmigration.

1. Butcher, E. C. *Cell* **67**, 1033–1036 (1991).
2. Cinamon, G., Shinder, V. & Alon, R. *Nature Immunol.* **2**, 515–522 (2001).
3. Tozeren, A. & Ley, K. *Biophys. J.* **63**, 700–709 (1992).
4. Lawrence, M. B. & Springer, T. A. *Cell* **65**, 1–20 (1991).
5. Arnaout, M. A. *Immunol. Rev.* **114**, 147–180 (1990).
6. Etzioni, A. *et al. N. Engl. J. Med.* **327**, 1789–1792 (1992).
7. Goldsmith, H. L. & Spain, S. *Microvasc. Res.* **27**, 204–222 (1984).
8. Garcia-Cardena, G., Comander, J., Anderson, K. R., Blackman, B. R. & Gimbrone, M. A. *Proc. Natl Acad. Sci. USA* **98**, 4478–4485 (2001).
9. Moazzam, F., DeLano, F. A., Zweifach, B. W. & Schmid-Schonbein, G. W. *Proc. Natl Acad. Sci. USA* **94**, 5338–5343 (1997).
10. Shaw, S., Bamba, P. S., Perkins, B. N. & Luscinskas, F. W. *J. Immunol* (in the press, 2001).
11. Kitayama, J., Hidemura, A., Saito, H. & Nagawa, H. *Cell Immunol.* **203**, 39–46 (2001).

The Vascular Research Division, Departments of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115, USA. (fluscinskas@rics.bwh.harvard.edu)

© 2001 Nature Publishing Group http://immunol.nature.com

The immunological relay race: B cells take antigen by synapse

T cells, move over. B cells are now reported, in a recent paper in *Nature*, to have their own immunological synapses with APCs. Only this time the antigen is whole and B cells don't keep it to themselves.

MICHAEL L. DUSTIN[1] AND LYNN B. DUSTIN[2]

The immune response depends upon coordinated recognition both of intact and fragmented forms of protein antigens. T lymphocytes use T cell antigen receptors (TCRs) to recognize peptides associated with major histocompatibility complex (MHC) molecules. The TCR and peptide-MHC complexes meet exclusively in an immunological synapse between the T cell and peptide-MHC–presenting cell (T synapse)[1]. In a report in *Nature*, Neuberger and colleagues focus our attention on a different form of antigen presentation, one which involves intact antigens on the surface of an antigen-presenting cell (APC) interacting with the B cell antigen receptor (BCR, also known as surface immunoglobulin)[2]. Presentation of intact antigen as a transmembrane protein triggers formation of a BCR-dependent immunological synapse (B synapse). The B cell synapse extracts intact antigen from the APC that is then processed to generate peptide-MHC complexes for T synapse formation. The report by Neuberger's lab emphasizes the multistage "relay race"—which involves at least two synapses that are specialized for recognition of intact and degraded antigens—that is mounted by the immune system in response to soluble toxins and viruses. The description of the B synapse calls our attention to emerging work on the unique aspects of molecular interactions in synapses, integration of innate and adaptive immunity, cellular trafficking of intact antigens and viral particles and the mechanism of B cell affinity maturation.

The BCR- and TCR-mediated immunological synapses are strikingly similar in terms of antigen receptor recruitment, exclusion of negative regulators such as CD45 and the stimulation of actin polymerization (**Fig. 1**). A key element of the T synapse is the specific relationship between the ring of integrin adhesion molecules and the central cluster of TCRs[3]. (The location of adhesion molecules in the B cell synapse was not explored by Neuberger and colleagues, however, and remains to be determined.)

The study[2] builds on decades of investigation into the effects of antigen-binding affinity on

© 2001 Nature Publishing Group http://immunol.nature.com

the humoral response. It was quickly appreciated that the association of multiple antibody-binding sites in a single particle (either by natural association or artificial linkage *via* covalent backbones such as high molecular weight dextrans) greatly enhances the antibody response both *in vitro* and *in vivo*. This type of multipoint binding of antigens to the BCR elicits microscopic clustering and capping through signaling and actin-myosin contractility[4]. More recently, it has been appreciated that tethering antigens to membranes also greatly increases their potency in inducing B cell activation *in vivo*[5]. Unlike the precise geometric organization of viral particles or the regular spacing of dextrans, the organization of proteins on cell membranes is less well understood. It is clear, however, that many membrane proteins are freely mobile and thus present an ever shifting array of independent binding sites. Nonetheless, it has been directly shown that very low affinity interactions can generate organized areas of synapsis in which interactions with low solution affinities are highly efficacious[6]. Therefore, recognition of intact antigens in a synapse allows more efficient recognition based on very low affinity interactions, but does not allow discrimination of interactions with affinities greater than 10^6 M^{-1}. How is this low-affinity ceiling reconciled with the selection of high affinity antibodies? In earlier work, Neuberger proposed that the physical nature of the antigen extraction process may itself provide discrimination of higher affinity interactions, as the resistance of a chemical interaction to applied force is related to its affinity[7]. Clathrin and dynamin, proteins involved in the formation of endocytic vesicles, apply significant forces to membrane receptors. This force may effectively test the affinity (in the range 10^6–10^{10} M^{-1}) of the antibody-antigen interaction in the B synapse and provide the necessary feedback for affinity maturation.

What role does the APC play in the B synapse *in vivo*? Neuberger and colleagues have shown that immune complexes captured on FcR^+ cells can be presented to form a B synapse[2]. They did this by generating a chimeric protein composed of hen egg lysozyme, green fluorescent protein and the transmembrane and cytoplasmic domains of H-2K^d, which they expressed in a tumor cell, thereby generating a model APC with which to test the concept of generically membrane-anchored antigens participating in synapse formation. It is thought that, *in vivo*, through Fc and complement receptors, follicular dendritic cells (FDCs) capture the immune complexes on their surface and present them to germinal center B cells. Beadlike structures called iccosomes—which contain antibody, antigen and complement products—can be identified on the dendritic processes of FDCs. Centrocytes in the germinal centers capture these complexes from the FDCs and present peptides degraded from them in order to elicit T cell help for affinity maturation[8]. The presentation of intact antigens as immune complexes on FDCs is thought to be important for the affinity maturation of antibodies. How-ever, early in a primary response, secreted specific antibody may not be available. In the absence of antigen-antibody complexes, other mechanisms may serve to decorate an APC with intact native antigen.

Other recent evidence points to a role for dendritic cells (DCs) in antigen presentation to primary B cells. It has been reported that DCs pulsed with soluble antigen can prime naïve B cells in T cell–dependent responses *in vivo* and *in vitro*[9]. Direct contact between the DCs and the B cells was required for subsequent B cell antigen presentation to T cells. DCs that were antigen-pulsed and then lysed were ineffective, which suggests that DCs play an active role in antigen priming. In these studies it was found that a fraction of the native antigen internalized by DCs was protected from intracellular degradation for at least 48 h[9]. In addition, a subset of DCs that traffics to primary lymphoid follicles is effective at promoting primary B cell differentiation[10]. DCs are also implicated in carrying intact virus to the lymphoid organs for generation of an effective humoral immune response[11].

According to Neuberger's data[2] antigen-specific B cells can extract membrane-bound antigens from cells such as transfected fibroblasts, which clearly are not professional antigen presenters. The polymeric nature of the membrane-displayed antigen and the close cell-cell contact provided in this system[2] may permit amateur APCs to function under these circumstances. Not all antigens insert themselves into a cell membrane, however.

How might the immune system take advantage of the B cell synapse to optimize the effectiveness of encounters between B cells and soluble toxin or circulating viruses? The answer may lie, in part, in the innate mechanisms of antigen binding by professional APCs. DCs and macrophages express a variety of lectins and scavenger receptors that facilitate direct or indirect recognition of carbohydrate patterns, bacte-

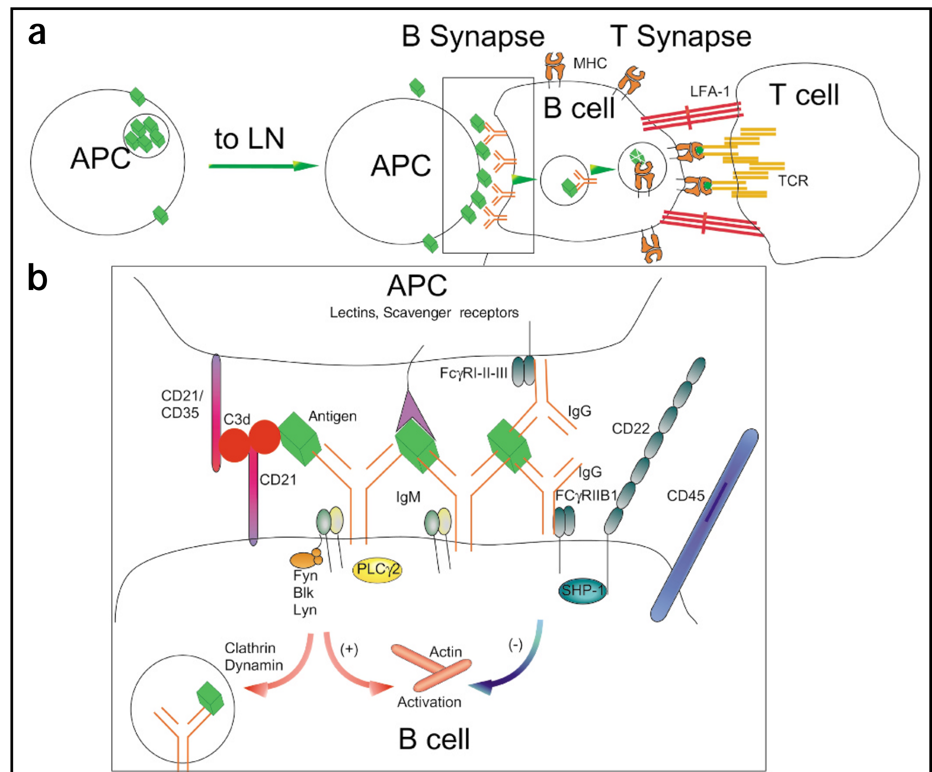

Figure 1. Passing the antigenic "baton" in the immunological relay race. (**a**) Antigens or immune complexes captured by DCs are presented to B lymphocytes (B synapse), which extract antigen for processing and presentation to helper T lymphocytes (T synapse). (**b**) In the B synapse, antigen and the BCR are clustered along with many effectors of signal transduction. Molecules that may negatively regulate BCR signal transduction are excluded from the synapsis area. (LN, lymph node; LFA-1, lymphocyte function–associated antigen 1.)

© 2001 Nature Publishing Group http://immunol.nature.com

rial structures, apoptotic cells and debris associated with cell damage[12]. These receptors may represent a branch of the innate immune system that is specialized to capture specific classes of intact antigens, which are evolutionarily connected to pathogens. Maturing DCs have a remarkable capacity to internalize and protect intact antigens in lysosome-like compartments. With maturation of the DC, many of these antigens are degraded, but many may also be moved to the surface as intact antigens.

A number of pathogens can initiate the mannan-binding lectin and alternative pathways of complement activation. The classical and alternative pathways of complement activation all tag antigens with C3 cleavage products, which are ligands for the CD21 and CD35 complement receptors. Thus, antigens can be bound to complement receptors even in the absence of complement-fixing antibody. In addition, even antigen-naïve animals often express "natural antibodies". These are immunoglobulin M (IgM) and, less commonly, IgG or IgA; they bind many toxins, carbohydrates and other common repetitive structures that are associated with various pathogens. The immunoglobulins may fix complement or bind to Fc receptors, thus permitting the construction of immune complexes for presentation before the development of acquired immunity.

Thus, a division of labor may emerge in intact antigen presentation. DC subsets may present antigen to naïve B cells, whereas the distinct lineage of FDCs may present antigens to activated B cells and evolving memory cells in the germinal centers. B cells may take the relayed antigen and present pro-cessed forms to T cells. Further study is required to establish the physiological importance of this chain of events now that the synaptic basis of a new link has been highlighted by Neuberger and colleagues[2]. Appre-ciating the immunological relay race may provide clues to enhancing the collaboration between DCs, B and T cells in vaccine development and provide approaches for strategically fumbling the antigenic baton, to help prevent or treat autoimmune diseases.

1. Grakoui, A. *et al. Science* **285**, 221–227 (1999).
2. Batista, F. D., Iber, D. & Neuberger, M. S. *Nature* **411** (in the press, 2001).
3. Monks, C. R., Freiberg, B. A., Kupfer, H., Sciaky, N. & Kupfer, A. *Nature* **395**, 82–86 (1998).
4. Braun, J., Fujiwara, K., Pollard, T. D. & Unanue, E. R. *J. Cell Biol.* **79**, 409–418 (1978).
5. Brink, R. *et al. J. Exp. Med.* **176**, 991–1005 (1992).
6. Dustin, M. L. *et al. J. Biol. Chem.* **272**, 30889–30898 (1997).
7. Batista, F. D. & Neuberger, M. S. *EMBO J.* **19**, 513–520 (2000).
8. Tew, J. G. *et al. Immunol. Rev.* **156**, 39–52 (1997).
9. Wykes, M., Pombo, A., Jenkins, C. & MacPherson, G. G. *J. Immunol.* **161**, 1313–1319 (1998).
10. Berney, C. *et al. J. Exp. Med.* **190**, 851–60 (1999).
11. Ludewig, B. *et al. Eur. J. Immunol.* **30**, 185–196 (1999).
12. Banchereau, J. *et al. Annu. Rev. Immunol.* **18**, 767–811 (2000).

[1]Department of Pathology, Skirball Institute of Molecular Medicine, New York University School of Medicine, New York, NY 10016, USA. [2]Center for the Study of Hepatitis C, Rockefeller University, New York, NY 10021, USA. (dustin@saturn.med.nyu.edu and dustinl@rockeller.edu)

Vav and the B cell signalosome

ANTHONY L. DEFRANCO

Genetic ablation experiments have shown that Vav is critical for TCR signaling. Evidence is now emerging that the Vav family of signaling molecules play a critical role in antigen receptor signaling in B cells as well as in T cells.

Although the signaling mechanisms of B and T cell antigen receptors (BCRs and TCRs) are for the most part analogous, one way in which they were thought to differ was in the relative importance of the Vav signaling component. Vav-1$^{-/-}$ mice show profound defects in thymocyte cellularity, thymocyte positive and negative selection, peripheral T cell numbers and peripheral T cell responsiveness[1,2]. Thus, TCR and pre-TCR signaling appear to be substantially compromised in the absence of Vav-1. In contrast, alterations in the B cell compartment of these mice are modest: a loss of B1 cells and a partial decrease in B cell proliferation upon BCR cross-linking. In this issue of *Nature Immunology*, Tedford *et al.* and Doody *et al.* report data obtained *via* genetic ablation of the highly related *Vav2* gene[3,4]. Their studies have shown that Vav-2 is an important participant in antigen-receptor signaling in B cells and that its functions partially overlap with those of Vav-1, so that genetic ablation of both Vav-1 and Vav-2 results in greatly compromised BCR signaling capability and B cell functional responses.

Table 1. Lymphocyte defects in Vav-deficient mice

Process	Vav1$^{-/-}$	Vav2$^{-/-}$	Vav1$^{-/-}$Vav2$^{-/-}$
B cell development	Normal	Normal	↓
B cell maturation in periphery[a]	↓	Normal	↓
B1 cell development	↓↓	Normal	↓↓
TI-2 antibody response	Normal or ↓	↓	↓↓
BCR-induced proliferation	↓[b]	↓[b]	↓↓
BCR-induced Ca^{2+} elevation	↓	↓[b]	↓↓
T cell development	↓ or ↓↓	Normal	↓↓
TCR-induced Ca^{2+} elevation of thymocytes	↓	Normal	—
T cell proliferation	↓	Normal	—
T cell–dependent antibody response	↓ Normal[c]	↓	—

Data are from references 1–4 and 9. [a]Effect could have been due to a deficit in peripheral maturation of transitional B cells or a survival deficit of mature B cells. [b]Defect was seen primarily at moderate doses of stimulation. [c]Defective response is due to a T cell defect, Vav-1$^{-/-}$ B cells respond if given wild-type helper T cells. (↓, response or process decreased compared to wild-type; ↓↓, response or process greatly decreased or absent.)

Although the functions of the Vav family of signaling components (which, in addition to Vav-1 and Vav-2, includes Vav-3) are not completely understood, it is clear that they are members of the large family of guanine nucleotide exchange factors (GEFs) for the Rho family GTPases. These Ras-like proteins are molecular switches that are active in the GTP-bound state and can promote site-specific actin polymerization to create alterations in plasma membrane structures such as filapodia and lamellapodia. They can also stimulate signaling reactions *via* the c-Jun NH_2-terminal kinase (Jnk) and p38 mitogen-activated protein kinase cascades and *via* the p21-activated kinases (PAKs)[5]. Another function of Rho family GTPases is to promote sustained phosphatidylinositol(4,5)bisphosphate (PIP_2) hydrolysis by stimulating the PIP 5-kinase that synthesizes PIP_2 in order to replenish this molecule as it is being hydrolyzed by the phospho-

© 2001 Nature Publishing Group http://immunol.nature.com