Commentary

Of ITAMs and ITIMs: Turning On and Off the B Cell Antigen Receptor

By Matthew L. Thomas

From the Howard Hughes Medical Institute and the Department of Pathology, Washington University School of Medicine, St. Louis, Missouri 63110

For any given receptor, there are mechanisms that regulate the activation of signal transduction pathways, as well as mechanisms that extinguish those signaling pathways. Responding to environmental signals requires that both positive and negative regulation proceed in an ordered manner. Much research has focused on lymphocyte antigen receptor–induced signal transduction pathways, and an understanding of some of the initial events has been gained. The mechanisms that terminate signaling are equally important but less well understood. The inability to properly extinguish activation cascades may result in inappropriate responses and perhaps even cell death. Concerning lymphocyte antigen receptors, antigen recognition results in the activation of intracellular protein tyrosine kinases, particularly members of the Src family kinases, as well as the ZAP-70/syk kinases. The key to antigen-induced signal transduction is the phosphorylation of critical tyrosines within the cytoplasmic domains of the TCR CD3/ζ chain complex or the α and β chains of the B cell antigen receptor (BCR) (1–3). The critical tyrosines are located within a characteristic sequence motif termed immunoreceptor tyrosine-based activation motif (ITAM). Inactivating antigen receptors requires dephosphorylation, yet the involved enzyme(s) or process(es) have not previously been identified. Several recent reports, including one in this issue, provide important information about the mechanisms of BCR inactivation (3a–6).

Potential insight into the negative regulation of immunoreceptors was gained by the discovery that the *motheaten* mice (*me*) harbor a mutation in the SHP gene (also known as PTP1C, HCP, SH-PTP1) (7, 8). The mutation renders *me* mice deficient in SHP protein. SHP is an SH2 domain containing protein tyrosine phosphatase (PTPase) and belongs to a subfamily that contains another mammalian member, syp (also known as PTP2C, PTP1D and SH-PTP2), and a *Drosophila* member, *corkscrew* (*csw*) (9–18). syp is thought to function in the positive regulation of receptor tyrosine kinases and to be the mammalian orthologue of *csw*. In contrast, the phenotype of the *me* mice suggests that SHP functions in the negative regulation of leukocyte activation. *me* mice have numerous immunological disorders, including chronic macrophage and neutrophil activation, lymphopenia, and circulating autoantibodies (19). The B lymphocyte populations are markedly disproportionate; there is an absence of $CD5^-$ B lymphocytes (B2 cells), while the $CD5^+$ B lymphocytes (B1 cells) are present and increased in relative numbers in the spleen. The B1 lymphocytes are activated and produce autoantibodies. Indeed, in both the spleen and lymph nodes there are atypical plasmacytoid cells that accumulate immunoglobulin as insoluble inclusion bodies. The phenotype of *me* mice suggests that SHP regulates B lymphocyte differentiation and may regulate BCR signal transduction, albeit there may be fundamental differences between B1 and B2 lymphocytes.

In this issue, Pani et al. provide support for SHP-negative regulation of the BCR (6). Using a $CD5^+$ B cell line, CH12, Pani and co-workers convincingly demonstrate an association between SHP (PTP1C) and the BCR. Significantly, cross-linking of the BCR results in an immediate disassociation of the phosphatase. These data imply that SHP may function to keep the BCR inactivated before stimulation. Furthermore, these investigators demonstrate that *me* B cells are increased in responsiveness to suboptimal concentrations of BCR cross-linking (6). Therefore, SHP may also regulate the threshold with which the BCR responds to antigen.

Further support for this model comes from the recent work of Cyster and Goodnow (4). Using B cells from a transgenic mouse expressing anti–hen egg lysozyme crossed onto the *me* background, these investigators have demonstrated accentuated signaling induced by antigen. Thus, SHP functions in the negative regulation of the BCR. By using bone marrow chimeras to examine the antigen threshold required for clonal deletion, it was demonstrated that a soluble form of lysozyme autoantigen was able to arrest and delete immature B cells in the bone marrow, whereas B cells with normal SHP were only arrested and deleted if the same autoantigen was displayed on the surface of neighboring cells in a highly multivalent form. Therefore, the absence of SHP results in a reduced signaling threshold and increased sensitivity to self-antigen. The function of SHP in B cell development is critical in determining the threshold by which cells respond to self-antigens.

Insight into the molecular mechanisms by which SHP inactivates the B cell receptor has come from two further studies (3a, 5). It was previously demonstrated that BCR signal transduction can be abolished by cross-linking with FcγRIIB (20, 21), indicating that FcγRIIB functions to block BCR signaling. A 13–amino acid sequence within the cytoplasmic domain of FcγRIIB is responsible for the inhibitory signal. Furthermore, a specific tyrosine residue within the 13 amino acids is critical to the process (21). This sequence motif is termed an immunoreceptor tyrosine-based inhibitory motif (ITIM). D'Ambrosio and co-workers demonstrate that SHP

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/95/06/1953/04 $2.00
Volume 181 June 1995 1953–1956

mouse FcγRIIB	303	EAENTIT(p)YSLLKH
mouse CD22	780	TVS(p)YAILRFP
mouse CD22	840	SIH(p)YSELVQF
mouse CD22	859	EDVD(p)YVTLKH
mouse EPOR	425	Ac-PHLK(p)YLYLVVSDK
human IL-2βR	351	ASQV(p)YFTYDPYSE
human IL-3βR	624	GSLE(p)YLCLPAGDK
consensus		(T/S)XX(p)YXXL

Figure 1. Phosphotyrosyl peptide sequences that bind SHP SH2 domains and serve to increase catalytic activity (3a, 6, 30). The numbers indicate the position of the first amino acid in the sequence.

binds to the ITIM within the cytoplasmic domain of FcγRIIB (5). Importantly, BCR cross-linking and tyrosine phosphorylation of the ITIM sequence is required for the association with SHP. Evidence that this association is important in the negative regulation of BCR signaling comes from the observation that FcγRIIB does not block signaling in B cells from *me* mice (5, 6).

Given the specificity of FcγRIIB, it is unlikely that the SHP/ FcγRIIB interaction is the only mechanism of negative BCR regulation. It is satisfying, therefore, that a second potential mechanism has been reported by Doody and colleagues (3a). These researchers report that BCR cross-linking results in the rapid association of SHP with the transmembrane lectin, CD22. CD22 will associate with the BCR and becomes tyrosine phosphorylated in response to BCR ligation (22–24). The association of SHP with CD22 is mediated through three distinct phosphotyrosine residues within the cytoplasmic domain of CD22 and the amino-terminal SH2 domain of SHP. Importantly, the three phosphotyrosine residues that interact with SHP are located within regions that are similar to the ITIM sequence of FcγRIIB (Fig. 1). The identification of three distinct sites within the cytoplasmic domain of CD22 suggests that these sites may be redundant. Antibody-induced clustering of CD22 before BCR ligation increases the proliferative capacity, suggesting that CD22 may deliver a negative regulatory signal (3a). This observation is supported by earlier work that demonstrated that monoclonal antibody-induced cross-linking of CD22 can potentiate BCR signaling (25, 26).

To terminate signaling, SHP must only dephosphorylate tyrosine residues at the appropriate time. Since SHP is a cytosolic enzyme, preventing SHP from dephosphorylating substrates prematurely implies that the catalytic activity must be regulated. Indeed, this is the case. Full-length SHP has poor phosphatase activity. However, truncation of the SH2 domains results in a marked increase in enzymatic activity (3a, 27, 28). This suggests that the catalytic activity of SHP may be regulated by an allosteric mechanism involving the SH2 domains. It is of much interest, therefore, that peptides that bind the SH2 domains of either syp or SHP result in a dramatic increase in phosphatase activity (3a, 5, 29, 30). Thus, SHP SH2 domains serve to both recruit the enzyme to appropriate sites of tyrosine phosphorylation and to regulate the catalytic activity. Importantly, synthetic phosphopeptides representing the potential sites of tyrosine phosphorylation within FcγRIIB and CD22 serve to increase SHP catalytic activity (3a, 5). Therefore, the ITIM sequences represent SHP binding sites, and they function to increase phosphatase activity. A comparison of peptides that stimulate SHP activity indicates sequence similarities (Fig. 1).

A potential model to account for the termination of BCR signal transduction is that tyrosine phosphorylation of either CD22 or FcγRIIB recruits SHP to the membrane and, upon association with the BCR, allows for dephosphorylation of appropriate substrates (Fig. 2). It is unknown whether these interactions account for the association with the BCR in unstimulated cells, as observed by Pani et al. (6). It will be important, therefore, to determine whether SHP can directly bind the BCR, and whether a direct association is another mechanism to inactivate the receptor.

The phenotype of *me* mice suggests that SHP functions to regulate activation and differentiation of multiple hematopoietic lineages including macrophages, neutrophils, lymphocytes, and erythrocytes. Thus, SHP does not exclusively regulate the BCR but other receptors as well. Indeed, SHP has been demonstrated to associate with the IL-3 and erythropoietin receptors and c-*kit* (31–33). Taken together, the data suggests that SHP has a fundamental role in the negative regulation of many immunoreceptors and that it accounts for the

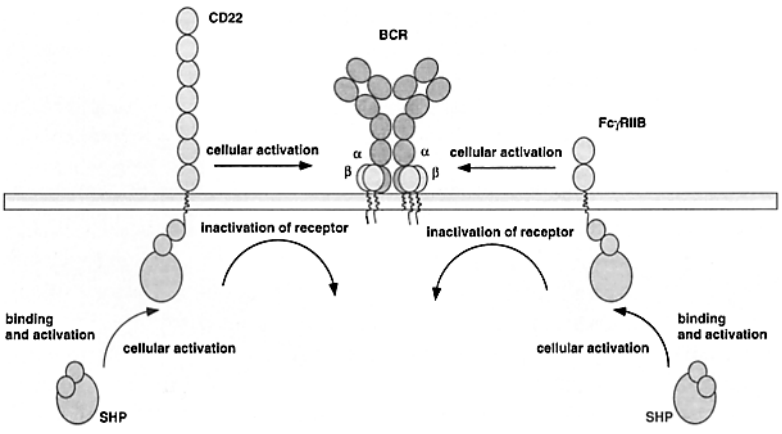

Figure 2. A potential model for the inactivation of BCR signaling. BCR ligation results in the tyrosine phosphorylation of CD22 and FcγRIIB cytoplasmic domain. SHP binds via the SH2 domains to phosphotyrosine sites in CD22 and FcγRIIB, resulting in the localization and activation of phosphatase activity. Upon binding of SHP, association of either CD22 or FcγRIIB with the BCR terminates signaling.

severity of the disease in *me* mice. The recruitment and activation of SHP is necessary for the termination of signal transduction for many immunoreceptors, including antigen, cytokine, and growth factor receptors. It is interesting that the transmembrane PTPase, CD45 is critical for the activation of lymphocyte Src family members and is necessary for antigen-induced signaling. Apparently, for antigen receptors, there are PTPases at the beginning and end of the activation process!

I thank my colleagues for helpful discussions and Drs. John Cambier, Jason Cyster, and Chris Goodnow for providing preprints and discussing unpublished observations.

Address correspondence to Dr. Matthew L. Thomas, Howard Hughes Medical Institute and Department of Pathology, Box 8118, Washington University School of Medicine, 660 S. Euclid Avenue, St. Louis, MO 63110.

Received for publication 13 March 1995.

References

1. Weiss, A., and D.R. Littman. 1994. Signal transduction by lymphocyte antigen receptors. *Cell.* 76:263–274.
2. Cambier, J.C., C.M. Pleiman, and M.R. Clark. 1994. Signal transduction by the B cell antigen receptor and its coreceptors. *Annu. Rev. Immunol.* 12:457–486.
3. Gold, M.R., and A.L. DeFranco. 1994. Biochemistry of B lymphocyte activation. *Adv. Immunol.* 55:221–295.

3a. Doody, G.M., L.B. Justement, C.C. Delibrias, R.J. Matthews, J. Lin, M.L. Thomas, and D.T. Fearon. 1995. Restriction of B lymphocyte activation by CD22 and the protein tyrosine phosphatase SHP. *Science (Wash. DC).* In press.

4. Cyster, J.G., and C.C. Goodnow. 1995. Protein tyrosine phosphatase 1C negatively regulates antigen receptor signaling in B lymphocytes and determines thresholds for negative selection. *Immunity.* 2:13–24.
5. D'Ambrosio, D., K.H. Hippen, S.A. Minskoff, I. Mellman, K.A. Siminovitch, G. Pani, and J.C. Cambier. 1995. PTP1C recruitment and activation by FcγRIIB1: a molecular basis for negative signal cooperativity with the B cell antigen receptor. *Science (Wash. DC).* In press.
6. Pani, G., M. Kozlowski, J.C. Cambier, G.B. Mills, and K.A. Siminovitch. 1995. Identification of the tyrosine phosphatase PTP1C as a B cell antigen receptor–associated protein involved in the regulation of B cell signalling. *J. Exp. Med.* 181:XXX–XXX.
7. Shultz, L.D., P.A. Schweitzer, T.V. Rajan, T. Yi, J.N. Ihle, R.J. Matthews, M.L. Thomas, and D.R. Beier. 1993. Mutations at the murine motheaten locus are within the hematopoietic cell protein-tyrosine phosphatase (*Hcph*) gene. *Cell.* 73:1445–1454.
8. Tsui, H.W., K.A. Siminovitch, L. de Souza, and F.W.L. Tsui. 1993. Motheaten and viable motheaten mice have mutations in the haematopoietic cell phosphatase gene. *Nature Genet.* 4: 124–129.
9. Feng, G.-S., C.-C. Hui, and T. Pawson. 1993. SH2-containing phosphotyrosine phosphatase as a target of protein-tyrosine kinases. *Science (Wash. DC).* 259:1607–1611.
10. Vogel, W., R. Lammers, J. Juang, and A. Ullrich. 1993. Activation of a phosphotyrosine phosphatase by tyrosine phosphorylation. *Science (Wash. DC).* 259:1611–1614.
11. Zhao, Z., P. Bouchard, C.D. Diltz, S.-H. Shen, and E.H. Fischer. 1993. Purification and characterization of a protein tyrosine phosphatase containing SH2 domains. *J. Biol. Chem.* 268:2816–2820.
12. Adachi, M., M. Sekiya, T. Miyachi, K. Matsuno, Y. Hinoda, K. Imai, and A. Yachi. 1992. Molecular cloning of a novel protein-tyrosine phosphatase SH-PTP3 with sequence similarity to the *src*-homology region 2. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 314:335–339.
13. Freeman, Jr., R.M., J. Plutzky, and B.G. Neel. 1992. Identification of a human src homology 2-containing protein-tyrosine-phosphatase: a putative homolog of *Drosophila* corkscrew. *Proc. Natl. Acad. Sci. USA.* 89:11239–11243.
14. Matthews, R.J., D.B. Bowne, E. Flores, and M.L. Thomas. 1992. Characterization of hematopoietic intracellular protein tyrosine phosphatases: description of a phosphatase containing an SH2 domain and another enriched in proline-, glutamic acid-, serine-, and threonine-rich sequences. *Mol. Cell. Biol.* 12:2396–2405.
15. Perkins, L.A., I. Larsen, and N. Perrimon. 1992. *corkscrew* encodes a putative protein tyrosine phosphatase that functions to transduce the terminal signal from the receptor tyrosine kinase torso. *Cell.* 70:225–236.
16. Plutzky, J., B.G. Neel, and R.D. Rosenberg. 1992. Isolation of a src homology 2-containing tyrosine phosphatase. *Proc. Natl. Acad. Sci. USA.* 89:1123–1127.
17. Yi, T., J.L. Cleveland, and J.N. Ihle. 1992. Protein tyrosine phosphatase containing SH2 domains: characterization, preferential expression in hematopoietic cells, and localization to human chromosome 12p12-p13. *Mol. Cell. Biol.* 12:836–846.
18. Shen, S.-H., L. Bastien, B.I. Posner, and P. Chrétien. 1991. A protein-tyrosine phosphatase with sequence similarity to the SH2 domain of the protein-tyrosine kinases. *Nature (Lond.).* 352:736–739.
19. Shultz, L.D. 1991. Hematopoiesis and models of immunodeficiency. *Semin. Immunol.* 3:397–408.
20. Amigorena, S., C. Bonnerot, J.R. Drake, D. Choquet, W. Hunziker, J.-G. Guillet, P. Webster, C. Sautes, I. Mellman, and W.H. Fridman. 1992. Cytoplasmic domain heterogeneity and functions of IgG Fc receptors in B lymphocytes. *Science*

(Wash. DC). 256:1808–1812.
21. Muta, T., T. Kurosaki, Z. Misulovin, M. Sanchez, M.C. Nussenzweig, and J.V. Ravetch. 1994. A 13-amino-acid motif in the cytoplasmic domain of FcγR11B modulates B-cell receptor signalling. *Nature (Lond.).* 368:70–73.
22. Leprince, C., K.E. Draves, R.L. Geahlen, J.A. Ledbetter, and E.A. Clark. 1993. CD22 asssociates with the human surface IgM-B-cell antigen receptor complex. *Proc. Natl. Acad. Sci. USA.* 90:3236–3240.
23. Peaker, C.J.G., and M.S. Neuberger. 1993. Association of CD22 with the B cell antigen receptor. *Eur. J. Immunol.* 23:1358–1363.
24. Munro, S., B.J.E.G. Bast, K.J. Colley, and T.F. Tedder. 1992. The B lymphocyte surface antigen CD75 is not an α-2,6-sialytransferase but is a carbohydrate antigen, the production of which requires the enzyme. *Cell.* 68:1003.
25. Pezzutto, A., B. Dörken, G. Moldenhauer, and E.A. Clark. 1987. Amplification of human B cell activation by a monoclonal antibody to the B cell-specific antigen CD22, Bp 130/140. *J. Immunol.* 138:98–103.
26. Pezzutto, A., P.S. Rabinovitch, B. Dörken, G. Moldenhauer, and E.A. Clark. 1988. Role of the CD22 human B cell antigen in B cell triggering by anti-immunoglobulin. *J. Immunol.* 140:1791–1795.
27. Pei, D., B.G. Neel, and C.T. Walsh. 1993. Overexpression, purification, and characterization of SHPTP1, Src homology 2-containing protein-tyrosine-phosphatase. *Proc. Natl. Acad. Sci. USA.* 90:1092–1096.
28. Townley, R., S.-H. Shen, D. Banville, and C. Ramachandran. 1993. Inhibition of the activity of protein tyrosine phosphatase 1C by its SH2 domains. *Biochemistry.* 32:13414–13418.
29. Pei, D., U. Lorenz, U. Klingmüller, B.G. Neel, and C.T. Walsh. 1994. Intramolecular regulation of protein tyrosine SH-PTP1: a new function for Src homology domains. *Biochemistry.* 33: 15483–15493.
30. Sugimoto, S., T.J. Wandless, S.E. Shoelson, B.G. Neel, and C.T. Walsh. 1994. Activation of the SH2-containing protein tyrosine phosphatase, SH-PTP2, by phosphotyrosine-containing peptides derived from insulin receptor substrate-1. *J. Biol. Chem.* 269:13614–13622.
31. Yi, T., and J.N. Ihle. 1993. Association of hematopoietic cell phosphatase with c-Kit after stimulation with c-Kit ligand. *Mol. Cell. Biol.* 13:3350–3358.
32. Yi, T., A.L.-F. Mui, G. Krystal, and J.N. Ihle. 1993. Hematopoietic cell phosphatase associates with the interleukin-3 (IL-3) receptor β chain and down-regulates IL-3 induced tyrosine phosphorylation and mitogenesis. *Mol. Cell. Biol.* 13:7577–7586.
33. Yi, T., J. Zhang, O. Miura, and J. Ihle. 1995. Hematopoietic cell phosphatase associates with erythropoietin (Epo) receptor after epo-induced receptor tyrosine phosphorylation: identification of potential binding sites. *Blood.* 85:87–95.