Tyrosine Phosphatases and the Antibody Response

Anthony L. DeFranco and Debbie A. Law

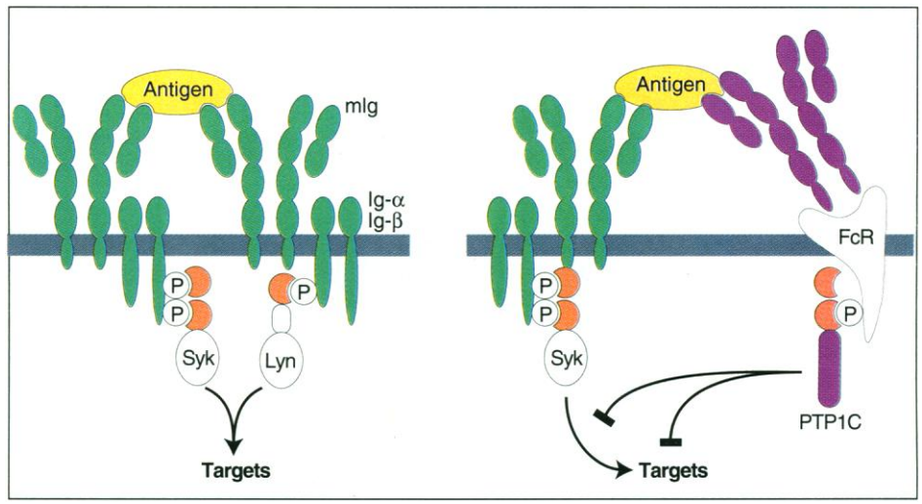
At the beginning of this century, Paul Ehrlich envisioned the consequences to the organism of attack by its own immune system. We now know that this does occur—an immune attack on a limited number of self components characterizes the autoimmune diseases. The most striking violation of Ehrlich's "horror autotoxicus" occurs in an autoimmune strain of mice called *motheaten* in which there is vigorous autoantibody production against many self components. In 1993, it was discovered that the two independently arising *motheaten* strains both exhibit mutations in the gene for an SH2-containing protein tyrosine phosphatase variously called PTP1C, HCP, SHP, and SH-PTP1 (*1*, *2*). PTP1C is expressed in cells of all hematopoietic lineages, and the defects in *motheaten* mice are correspondingly extensive (*3*). Nonetheless, it is clear that one of the defects in these mice is the production of large amounts of self-reactive antibodies. This suggests that the PTP1C phosphatase may regulate in some way the activation of B lymphocytes, the cells that make antibodies.

In this week's issue of *Science*, D'Ambrosio and colleagues elucidate a mechanism by which PTP1C regulates antibody production (*4*). The activation of B cells generally begins when the antigen receptor on these cells, composed of a membrane form of immunoglobulin complexed with two other polypeptides, immunoglobulin α (Ig-α) and Ig-β, binds antigen and transmits this information to the inside of the cell. The antigen receptor signals by activating at least two types of intracellular protein tyrosine kinases, Syk and several members of the Src-family of tyrosine kinases (*5*) (see figure). These kinases then phosphorylate and activate a number of signaling components including phospholipase Cγ2, phosphatidylinositol 3-kinase, Shc, rasGAP, and Vav. Once specific antibody has been produced, it can combine with the antigen to form an immune complex. Antigen in this form does not activate B cells because B cells express a receptor for the constant portion of IgG molecules, FcγRIIB1, that interferes with signal transduction by the antigen receptor when immune complexes bring this receptor into the region of the antigen receptor (*6*). This inhibition is manifested by a decrease in phosphatidylinositol 4,5-bisphosphate hydrolysis (*7*) and prevention of calcium influx from outside the cell (*8*, *9*).

Recently, it has been shown that the inhibitory ability of FcγRIIB1 is contained in a 13–amino acid sequence in its cytoplasmic domain and that a tyrosine in this sequence is required (*10*). Moreover, coligation of FcγRIIB1 with the antigen receptor leads to phosphorylation of this tyrosine residue. D'Ambrosio and co-workers (*4*) have synthesized this phosphopeptide, linked it to beads, and used this as an affinity matrix to isolate proteins capable of binding to it. Three prominent binding proteins from B cells were detected. One of these proteins turns out to be PTP1C. The binding of PTP1C to FcγRIIB1 was clearly seen in lysates from B cells stimulated with a ligand that brought this receptor into a complex with the antigen receptor and not by ligands that stimulated only the antigen receptor. Moreover, the tyrosine phosphorylation of FcγRIIB1 and the binding of PTP1C to it required the presence of the previously identified tyrosine in the cytoplasmic domain of this receptor.

These observations suggest the model depicted in the figure: An antigen receptor–stimulated tyrosine kinase phosphorylates the FcγRIIB1 molecule if FcγRIIB1 is brought into a complex with the antigen receptor by the extracellular ligand. This phosphorylation leads to binding of PTP1C. Moreover, this binding appears to increase the phosphatase activity of PTP1C by approximately fivefold (*4*). Thus, the presence of FcγRIIB1 attracts and activates the phosphatase PTP1C adjacent to the antigen receptor signaling complex, which contains activated tyrosine kinases. This could interfere with antigen receptor signaling in several ways. For example, PTP1C may dephosphorylate Ig-α and Ig-β and thereby interfere with binding of tyrosine kinases to the activated receptor or it may dephosphorylate the activation sites of the kinases themselves. Alternatively, PTP1C might act selectively on particular signaling targets (such as phospholipase Cγ2) or on adaptor proteins needed for phosphorylation of particular targets. In any case, it is quite likely that the ability of FcγRIIB1 to attract PTP1C to the antigen receptor accounts for the ability of this receptor to inhibit B cell activation.

PTP1C also negatively regulates the function of the B cell antigen receptor under conditions that likely do not engage FcγRIIB1 (*11*), indicating the presence of additional inhibitory mechanisms for this phosphatase. PTP1C also appears to negatively regulate c-Kit, the CSF-1 receptor, two transmembrane tyrosine kinase receptors, as well as the interleukin-3 receptor



The phosphatase PTP1C inhibits B cell signaling. Antigen that does not also engage FcγRIIB1 (**left**) cross-links antigen receptors, causing phosphorylation of tyrosines in the cytoplasmic domains of Ig-α and Ig-β, binding and activation of Syk- and Src-family tyrosine kinases such as Lyn, and subsequent phosphorylation of signaling targets. If the antigen is present in the form of an immune complex (**right**), then FcγRIIB1 is brought into the region of the cross-linked antigen receptors by the extracellular ligand. The antigen receptor–activated tyrosine kinases are thought to phosphorylate a cytoplasmic tyrosine of the Fc receptor, which promotes binding of PTP1C. The exact mechanism by which PTP1C then inhibits antigen receptor signaling remains to be determined.

The authors are at the G.W. Hooper Foundation and in the Department of Microbiology and Immunology and Department of Biochemistry and Biophysics, University of California at San Francisco, San Francisco, CA 94143–0552, USA.

and the erythropoietin receptor, two members of the hematopoietin/cytokine receptor family (*12–15*). These observations are likely to explain the excessive erythropoiesis and the leukocyte hyperactivity seen in the *motheaten* mice.

One of the most surprising aspects of the emerging inhibitory role of PTP1C is that it contrasts with evidence for a positive signaling role of the highly related PTP1D tyrosine phosphatase (also called Syp, SH-PTP2, and PTP2C). PTP1D also contains two SH2 domains and a phosphatase domain, but in contrast to the restricted hematopoietic distribution of PTP1C, PTP1D is widely expressed. A number of lines of evidence indicate that PTP1D has a positive role in signaling induced by transmembrane tyrosine kinase receptors such as the platelet-derived growth factor receptor. PTP1D plays a positive role by acting as an adaptor that can bind to autophosphorylated receptors via its SH2 domains, become tyrosine phosphorylated by these receptors, and then serve as a binding site for the Grb-2–SOS complex that activates Ras (*16*, *17*). The result is that PTP1D plays a positive role in receptor activation of Ras. It seems likely that the phosphatase activity of PTP1D also contributes to receptor signaling, as suggested by the inhibitory effects of overexpressing a catalytically inactive mutant of PTP1D (*18*) and by microinjection experiments (*19*). Interestingly, the *Drosophila melanogaster corkscrew* gene product has the same overall structure as PTP1C and PTP1D, but clearly has closer sequence similarity to PTP1D. Functionally, the *corkscrew* product also is more like PTP1D, as genetic experiments indicate that it acts positively downstream of the *Torso* receptor tyrosine kinase to specify terminal structures of the fly embryo (*20*). It is not known whether this developmental function of *corkscrew* requires tyrosine phosphatase activity or whether it uses the adaptor function. In any case, it is clear that the SH2-containing protein tyrosine phosphatases are critical, both positively and negatively, in regulating signaling by receptors that use tyrosine phosphorylation to mediate their intracellular effects.

References and Notes

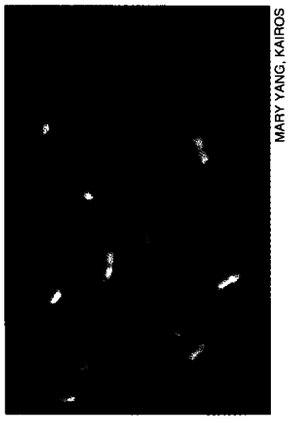
1. L. D. Schultz *et al.*, *Cell* **73**, 1445 (1993).
2. H. W. Tsui, K. A. Siminovitch, L. de Souza, F. W. L. Tsui, *Nature Genet.* **4**, 124 (1993).
3. L. D. Schultz and C. L. Sidman, *Annu. Rev. Immunol.* **5**, 367 (1987).
4. D. D'Ambrosio *et al.*, *Science* **268**, 293 (1995).
5. M. R. Gold and A. L. DeFranco, *Adv. Immunol.* **55**, 221 (1994).
6. J. V. Ravetch, *Cell* **78**, 553 (1994).
7. M. K. Bijsterbosch and G. G. B. Klaus, *J. Exp. Med.* **162**, 1825 (1985).
8. H. A. Wilson *et al.*, *J. Immunol.* **138**, 1712 (1987).
9. D. Choquet *et al.*, *J. Cell Biol.* **121**, 355 (1993).
10. T. Muta *et al.*, *Nature* **368**, 70 (1994).
11. J. G. Cyster and C. C. Goodnow, *Immunity* **2**, 13 (1995).
12. Y.-G. Yeung, K. L. Berg, F. J. Pixley, R. H. Angeletti, E. R. Stanley, *J. Biol. Chem.* **267**, 23447 (1992).
13. T. Yi, A. L.-F. Mui, G. Krystal, J. N. Ihle, *Mol. Cell. Biol.* **13**, 7577 (1993).
14. T. Yi and J. N. Ihle, *ibid.*, p. 3350.
15. T. Yi, J. Zhang, O. Miura, J. N. Ihle, *Blood* **85**, 87 (1995).
16. W. Li *et al.*, *Mol. Cell. Biol.* **14**, 509 (1994).
17. A. M. Bennett, T. L. Tang, S. Sugimoto, C. T. Walsh, B. G. Neel, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 7335 (1994).
18. K. L. Milarski and A. R. Saltiel, *J. Biol. Chem.* **269**, 21239 (1994).
19. S. Xiao *et al.*, *ibid.*, p. 21244.
20. N. Perriman, *Cell* **74**, 219 (1993).

21. We thank J. Richards and S. Harmer for helpful comments on the manuscript.

Green Fluorescent Pets

Douglas C. Youvan

Fifteen years ago, when the genes for the photosynthetic light-harvesting proteins were found, my colleagues in molecular biology asked the following question: Why can't we use these proteins as in vivo fluorescent tags, and wouldn't that be a lot better than attaching β-galactosidase to proteins and having to supply substrate for visualization? The answer was straightforward: Sure, but the light-harvesting proteins need associated pigments for their signal. You'll also have to express and regulate 20 other genes for pigment biosynthesis!



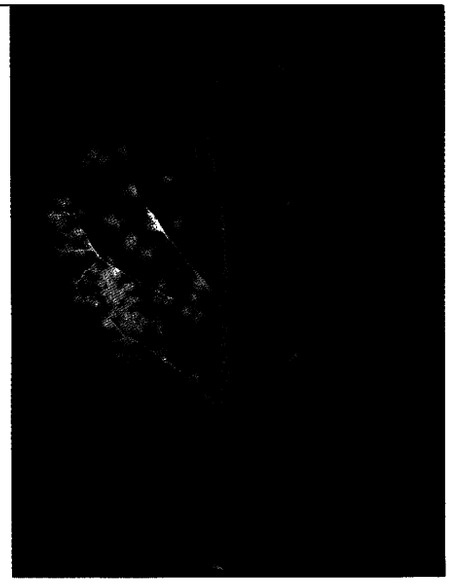
MARY YANG, KAIROS

From a crayon box of *Escherichia coli* to...

Green fluorescent protein (GFP) (*1*) gets around this problem by autocatalytically forming a fluorescent pigment from natural amino acids present in the nascent protein. GFP works across the phyla—from glowing tobacco mosaic virus with GFP-infected leaves to lighted sensory neurons in worms (*2*). To these add fluorescent zebrafish eyes, flies, yeast, and yes, possibly people—if engraftment with GFP-tagged stem cells in bone marrow becomes possible.

GFP engineering is rapidly creating a crayon box of different colors, from wild-type green to blue emitters and red-shifted excitation derivatives. Fluorescence resonant energy transfer (FRET) experiments on GFP-tagged proteins to measure distances between proteins are right around the corner. Protein-protein interactions are already assessed with GFP in the yeast two-hybrid system. But, by using different colored GFP derivatives there is the possibility of performing FRET in single cells to visualize the interaction of tagged proteins. Add to this the tools of fluorescence-activated cell sorting and confocal microscopy, and one has the makings of a methodology on par with the polymerase chain reaction.

The real fun will start in the near future when methodologies begin to collide.



CURTIS HOLT, SCRIPPS

...tobacco mosaic virus–infected leaves.

FRET-GFP technology might be brought together with combinatorial chemistry and screening technologies with high throughput. With the help of specifically engineered cell types, this could lead to a massively parallel strategy for finding new drugs. Hundreds of thousands of chemicals could be screened, with each cell behaving as a separate instrument.

References

1. J. G. Morin and J. W. Hastings, *J. Cell Physiol.* **77**, 313 (1971).
2. M. Chalfie, Y. Tu, G. Duskirchen, W. W. Ward, D. C. Prasher, *Science* **263**, 802 (1994).

The author is with the Palo Alto Institute of Molecular Medicine, Mountain View, CA 94043, USA.