Protein tyrosine phosphatases as negative regulators of the immune response

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Abstract
In this mini-review, we provide an overview of those PTPs (protein tyrosine phosphatases) that are relevant to the immune response, highlighting the function of a number of intracellular and transmembrane PTPs that have been identified as having important negative regulatory roles on distinct aspects of host immunity.

PTP (protein tyrosine phosphatase) phosphatasome
Reversible protein phosphorylation is recognized as a control mechanism that pervades all aspects of leucocyte cell physiology. In particular, tyrosine phosphorylation, although accounting for only 0.01% of the total phospho amino acid content of cells [1], has a profound impact on the proliferation, differentiation, activation, adhesion, secretion and motility of leucocytes [2]. The induction of tyrosine phosphorylation on a range of immune cell receptors such as antigen, cytokine and integrin receptors and their downstream intracellular signalling mediators is catalysed by the PTKs (protein tyrosine kinases) [2] and the subsequent hydrolysis of the phosphate moiety from phosphotyrosine residues is catalysed by the PTPs [3]. The annotation of the human genome has established that the protein phosphatase family of enzymes or phosphatasome includes 38 classical tyrosine-specific PTPs that can be further classified into 21 transmembrane and 17 intracellular members [4,5]. A minimum of 22 PTPs are expressed in the myeloid and lymphoid branches of the immune system with a number of PTPs demonstrating patterns of expression predominantly in leucocytes (Table 1). Essentially, each different type of leucocyte expresses a distinct profile of PTPs. The transmembrane PTPs are characterized by the possession of extracellular immunoglobulin, fibronectin, MAM (meprin/A5 antigen/PTPµ) and carbonic anhydrase domains fused to intracellular PTP domains [4,5]. The extracellular domains of the PTPs have been implicated in soluble ligand binding, homophilic adhesion interactions, extracellular matrix binding and dimerization, although for many the exact functions of these domains in the context of leucocytes remain to be elucidated [4,5]. Most of the transmembrane PTPs encode molecules with two PTP domains but with the catalytic activity restricted to the membrane-proximal domain and the second PTP domain playing a regulatory role [4,5]. Likewise, the intracellular PTPs possess additional motif(s) or domain(s) juxtaposed to the PTP domain with the non-catalytic domains primarily implicated in protein–protein and protein–phospholipid interactions [4,5]. These non-catalytic domain interactions serve to locate the individual PTPs to specific subcellular locations or to regulate PTP activity [4,5]. Importantly, all 38 PTPs are conserved between mouse and human, which has facilitated their functional characterization [4,5].

PTPs and the innate immune response
Dendritic cells and macrophages have been reported to express significant levels of a number of transmembrane PTPs including PTPζ, PTPµ, PTPρ, LAR (leucocyte antigen-related) and GLEPP1 (glomerular epithelial protein 1) [6] but the function of these PTPs on myeloid cell behaviour remains to be elucidated. However, the intracellular PTP, TC-PTP, which was originally described in T-cells, has been established to have a critical role in influencing myeloid cell differentiation and function. TC-PTP-deficient mice have been reported to develop a severe systemic inflammatory disease as demonstrated by chronic myocarditis, gastritis and nephritis [7]. An extensive mononuclear cell infiltrate into non-lymphoid tissues has been detected in these mice correlating with elevated levels of interferon-γ, TNF-α (tumour necrosis factor-α), IL-12 (interleukin-12) and nitric oxide [7]. A recent further examination of the molecular basis for the inflamed phenotype of TC-PTP-deficient mice has indicated that TC-PTP can act directly on the CSF-1 (colony-stimulating factor 1) receptor to regulate CSF-1 signalling and thereby influence mononuclear macrophage development [8]. In addition, TC-PTP can dephosphorylate Src family PTKs downstream of the TNF-α receptor in order to affect the inflammatory response [9].

The phenotype of the SHP-1 (Src homology 2 domain-containing protein tyrosine phosphatase 1)-deficient mouse is a further striking illustration of the capacity
Table 1 | PTPs expressed by leucocytes

<table>
<thead>
<tr>
<th>Leucocyte expression</th>
<th>Role</th>
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<tbody>
<tr>
<td><strong>Transmembrane PTPs</strong></td>
<td></td>
</tr>
<tr>
<td>CD45</td>
<td>Exclusively all leucocytes</td>
</tr>
<tr>
<td>RPTPα</td>
<td>All leucocytes</td>
</tr>
<tr>
<td>RPTPε</td>
<td>All leucocytes, high level of expression in monocytes/macrophages and granulocytes; cytoplasmic isoform expressed in monocytes</td>
</tr>
<tr>
<td>RPTPµ</td>
<td>Monocytes/macrophages and DCs</td>
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<tr>
<td>RPTPρ</td>
<td>Monocytes/macrophages and DCs</td>
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<tr>
<td>RPTPκ</td>
<td>Monocytes/macrophages and DCs</td>
</tr>
<tr>
<td>CD148</td>
<td>All leucocytes</td>
</tr>
<tr>
<td>GLEPP1</td>
<td>Monocytes/macrophages and DCs</td>
</tr>
<tr>
<td>LAR</td>
<td>T-cells and DCs</td>
</tr>
<tr>
<td><strong>Intracellular PTPs</strong></td>
<td></td>
</tr>
<tr>
<td>PTP1B</td>
<td>All leucocytes</td>
</tr>
<tr>
<td>TC-PTP</td>
<td>All leucocytes</td>
</tr>
<tr>
<td>PTPH1</td>
<td>All leucocytes</td>
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<tr>
<td>PTP-MEG1</td>
<td>All leucocytes</td>
</tr>
<tr>
<td>PTP-MEG2</td>
<td>All leucocytes, high level of expression in neutrophils</td>
</tr>
<tr>
<td>PEZ</td>
<td>T-cells</td>
</tr>
<tr>
<td>LC-PTP</td>
<td>Exclusively all leucocytes</td>
</tr>
<tr>
<td>Lyp</td>
<td>Exclusively all leucocytes</td>
</tr>
<tr>
<td>PTP-HSCF</td>
<td>Haemopoietic progenitors and mature T-cells</td>
</tr>
<tr>
<td>PTP-BAS</td>
<td>T-cells</td>
</tr>
<tr>
<td>HD-PTP</td>
<td>T-cells</td>
</tr>
<tr>
<td>PTP-PEST</td>
<td>All leucocytes, high level of expression in NK (natural killer) cells</td>
</tr>
<tr>
<td>SHP-2</td>
<td>All leucocytes</td>
</tr>
<tr>
<td>SHP-1</td>
<td>All leucocytes</td>
</tr>
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</table>

of PTPs to regulate the innate immune response [10]. The motheaten mouse suffers from a severe autoreactive disease process resulting from the infiltration of neutrophils and macrophages into peripheral tissues [10]. The motheaten mice succumb to an inflammatory pathology that includes haemorrhagic pneumonitis accompanied by the accumulation of expanded myeloid cell populations within the alveoli of the lungs by day 21 after birth [10]. Furthermore, an infiltration of neutrophils into the skin epidermis leading to the loss of hair follicles provides an explanation for the name of this spontaneous mouse mutant [10]. Bone marrow granulocytes from an allelic variant of motheaten termed viable motheaten exhibit increased proliferation to granulocyte CSF [11] and similarly the proliferation of motheaten macrophages is enhanced in response to GM-CSF (granulocyte/macrophage CSF) [12]. Nevertheless, the most profound effects of SHP-1 in myeloid cells appear to be manifest in the adhesive properties of these cells. SHP-1-deficient macrophages demonstrate an increased adhesion and spreading that is also accompanied by dysregulated detachment [13]. Likewise, motheaten bone marrow neutrophils are hyperadherent to protein-coated plastic and demonstrate decreased chemotaxis that may be attributable to decreased de-adhesion [14]. These changes in macrophage and neutrophil function most likely account for most of the lesions that form the pathology of the motheaten mouse.

PTPs and lymphocyte function

Both transmembrane and intracellular PTPs have been implicated in regulating the function of T- and B-lymphocytes [15]. With regard to B-cells, the transmembrane PTP, CD45, has been demonstrated to be critical for B-cell proliferation following BCR (B-cell receptor) ligation [16]. Furthermore, in the absence of CD45, B-cells fail to up-regulate anti-apoptotic Bcl-2 (B-cell lymphocytic-leukaemia proto-oncogene 2) family members following BCR ligation, resulting in their apoptosis [17]. Hence, while the
immunization of bone-marrow chimaeric mice, in which CD45 deficiency has been restricted to the B-cell lineage, results in the formation of germinal centres, these centres do not persist [17]. CD45 therefore appears to act as a positive effector at distinct stages in a B-cell life history and with limited evidence so far of significant negative roles for this receptor in the B-cell lineage. In contrast, B-cells from motheaten mice hyperproliferate in response to stimuli through the BCR [18]. Furthermore, an examination of responses initiated by a transgenic BCR recognizing HEL (hen’s-egg lysozyme) has revealed an exaggerated intracellular Ca\(^{2+}\) elevation in motheaten B-cells culminating in cell death when encountering soluble HEL, an effect not seen in the normal B-cells [19].

In T-cells, CD45 has been shown to dephosphorylate the negative regulatory phosphotyrosine at the C-terminus of the PTKs, Lck and Fyn [16]. However, the function of CD45 in T-cells is complex and additional negative roles for the receptor have been deduced from use of a mutant Lck transgene encoding a phenylalanine substitution for the negative regulatory phosphotyrosine. When introduced into a CD45-deficient genetic background, the Lck transgene universally leads to the development of thymomas in mice that correlates with the increased phosphorylation of the autophosphorylation site on Lck [20]. Hence, CD45 is believed to have dual positive and negative roles in regulating the Src-like PTKs in T-cells [21].

In addition to CD45, another transmembrane PTP capable of dephosphorylating PTKs in T-cells is PTP\(\alpha\) [22]. PTP\(\alpha\)-deficient thymocytes show increased phosphorylation of a number of phosphoproteins under basal conditions, correlating with an increase in the activity of the PTK, Fyn [22]. PTP\(\alpha\) is present in lipid rafts and Interestingly the alteration in Fyn activity appears to be restricted to lipid rafts in PTP\(\alpha\)-deficient thymocytes [22].

The intracellular PTP, PEP (Pro-Glu-Ser-Thr)-enriched PTP), [23] has been implicated in regulating TCR (T-cell receptor) activation [24] and PEP-deficient mice demonstrate a striking splenomegaly and enlarged lymph nodes after 6 months of age resulting from increased numbers of T-cell within the effector/memory pool [25]. PEP has been reported to dephosphorylate Src-like PTKs [26] so it is surprising that the T-cell defects in PEP-deficient mice appear restricted to the effector/memory population. One possible explanation for this observation is that PEP is one of three structurally related PTPs and that PTP-PEST [27] or PTP-HSCF (PTP from haemopoietic stem cell fraction) [28] might compensate for loss of PEP in naïve T-cells.

The generation of T-lymphocytes deficient for the intracellular PTP, PTP-MEG2 (PTP from megakaryocyte-2), has revealed an additional aspect of immune function, namely cytokine secretion that can be impacted upon by PTPs, although in this context the PTP is acting as a positive regulator [29]. In the absence of PTP-MEG2, T-cells fail to proliferate in response to TCR triggering due to a defect in the formation of mature secretory vesicles containing IL-2 [29]. The release of other cytokines in response to TCR stimulation is similarly affected. PTP-MEG2 is targeted to the membrane of secretory vesicles via its Sec14p homology phospholipid-binding domain where it dephosphorylates a critical regulator of vesicle fusion, N-ethylmaleimide-sensitive factor [30].

Studies on SHP-1-deficient T-cells from motheaten and viable motheaten mice have affirmed repeatedly that SHP-1 has negative regulatory roles on T-cell function [31]. Primarily, SHP-1-deficient T-cells are hyper-responsive to TCR stimulation. The cardinal responses of T-cells to TCR engagement are up-regulation of a limited number of cell-surface receptors, secretion of IL-2 and proliferation. Thymocytes from motheaten or viable motheaten mice, when stimulated with anti-TCR/CD3 antibodies, proliferate at a 3–5-fold higher level than control thymocytes and also secrete increased amounts of IL-2 [32–35]. Consistent with lowered thresholds for TCR activation, SHP-1 deficiency leads to increased positive selection of thymocytes in mice expressing MHC Class I or II restricted transgenic TCRs [31,35–37]. In addition, analyses of peripheral T-cells from mice expressing transgenic TCRs reveal an increased sensitivity and maximal proliferation of SHP-1-deficient T-cells in response to stimulation with cognate peptide [35–37]. However, the cellular basis for the hyperproliferative phenotype of motheaten T-cells has not been fully explained. Recent results we have obtained utilizing motheaten T-cells expressing a class I restricted TCR, F5, have demonstrated that a greater proportion of motheaten compared with control naïve CD8\(^{+}\) T-cells undergo cell division when activated by peptide pulsed APCs (antigen-presenting cells). Significantly, there is an increased likelihood of TCRs on SHP-1-deficient compared with control T-cells binding to peptide/MHC ligands on APCs that correlates with a greater proportion of SHP-1-deficient T-cells capable of forming stable conjugates with APCs. These combined observations may explain, at least in part, the hyper-responsiveness of motheaten T-cells to TCR-triggered stimulation and point to a possible effect of SHP-1 on regulating the actin cytoskeleton in T-cells (Figure 1). Importantly, there is a significantly enhanced in vivo expansion and CTL (cytolytic T-lymphocyte) capacity generated in mice receiving adoptively transferred SHP-1-deficient compared with control naïve CD8\(^{+}\) T-cells, highlighting the validity of potentially targeting SHP-1 expression by siRNA (small interfering RNA) and pharmacological means in order to boost human CD8\(^{+}\) T-cell function.

**PTPs and disorders of the immune system**

Given the central importance of tyrosine phosphorylation to leucocyte differentiation and function, it is not surprising that PTPs have been implicated in several human immune disorders or produce profound immunophenotypes when their expression is ablated in the mouse by homologous gene recombination.

Lck and Fyn PTK activity is required for antigen receptor signalling in lymphocyte development [38] and loss of expression of the prototypic receptor PTP, CD45, has been reported in several patients with severe combined immunodeficiency.
Figure 1 | Possible mechanism of SHP-1 action in T-cells

SHP-1 is recruited and activated at the plasma membrane by phosphorylated ITIMs (immunoreceptor tyrosine-based inhibitory motifs) on inhibitory receptors. Active SHP-1 dephosphorylates a putative tyrosine-phosphorylated regulator or component of the actin cytoskeleton, thereby facilitating T-cell spread on the surface of the APC and increasing the likelihood of TCR engagement of MHC ligand and T-cell–APC conjugate formation. Arp2/3, actin-related protein 2/3; ICAM-1, intercellular cell-adhesion molecule 1; LFA-1, leucocyte function antigen 1; SH2, Src homology 2; Wasp, Wiskott–Aldrich syndrome protein.

References

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