Protein Tyrosyl Phosphatases in T Cell Activation: Implication for Human Immunodeficiency Virus Transcriptional Activity

Michel Ouellet, Benoit Barbeau, and Michel J. Tremblay

Centre de Recherche en Infectiologie, Hôpital CHUL, Centre Hospitalier Universitaire de Québec, and Département de Biologie Médicale, Faculté de Médecine, Université Laval, Ste-Foy (QC), Canada, G1V 4G2

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The protein tyrosine phosphatases (PTPs) superfamily is a large group of enzymes showing a wide diversity of structure and biological functions. Their implication in the regulation of signal transduction processes is critical for homeostasis and efficient cellular activation. Disturbance of the delicate balance between protein tyrosine kinase and protein tyrosine phosphatase activities is at the heart of a large number of diseases. Control of cellular activation is especially important for human immunodeficiency virus type 1 (HIV-1) since this retrovirus requires activated T cells in order to replicate efficiently. Identification of PTPs implicated in signaling pathways leading to upregulation of HIV-1 gene transcription therefore contributes to the general understanding of cellular factors needed for strong HIV-1 replication and progression to AIDS. The use of bisperoxovanadium compounds as potent, specific, and
highly purified PTP inhibitors releases HIV-1 from PTP control and strongly increases HIV-1 gene expression. These inhibitors can thus be used to study signal transduction mechanisms regulated by PTP activity that are important for HIV-1 replication and provide new and interesting therapeutic avenues for the efficient control of this debilitating retroviral infection. © 2003, Elsevier Science (USA).

I. Structure and Function of Protein Tyrosine Phosphatases in T Cells

A. Historic and General Overview

Phosphorylation of tyrosine residues was first discovered more than 20 years ago as a key element in signal transduction events. These findings were based on several studies of viral gene products implicated in oncogenesis such as the abelson murine leukemia virus protein v-Abl and the avian Rous sarcoma virus protein v-Src. Since then, numerous studies on the regulation of tyrosine phosphorylation and its effects on cellular processes have been published describing an impressive number of kinases, phosphatases, as well as crucial domains implicated in protein–protein interactions mediated by phosphorylated tyrosine residues.

The first specific in vivo protein tyrosine phosphatase (PTP) activity was reported in 1981 (1, 2) and partially purified in 1982 (3) from human epidermoid carcinoma cells. Complete purification, characterization, and cloning of the first human PTP from placental tissues was performed by Tonks et al. in 1988 (4–6); this PTP was subsequently named PTP1B. Using the signature motif of the PTP1B catalytic site, Charbonneau and Tonks searched for other PTPs from proteins whose functions were unknown. Their search led to CD45, a protein highly expressed on all leukocytes with a molecular weight ranging from 180 to 220 kDa and two intracellular domains of nearly 150 amino acids showing, respectively, 33% and 40% homology with the catalytic site region of PTP1B (7). On this basis, CD45 was first identified as a putative PTP and, upon isolation and characterization, its specific PTP activity was confirmed (8, 9). Since then, an impressive array of new PTPs were cloned, characterized, and studied showing a wide range of expression, regulation mechanisms, cellular distribution, and functions.

The PTP superfamily encompasses all enzymes sharing a common PTP phosphohydrolase catalytic mechanism implicating a cysteiny-phosphate enzyme intermediate. This group of enzymes includes three families of proteins: the tyrosine-specific phosphatases, the dual-specific phosphatases (DSPs), and the low-molecular-weight PTPs (LMPTPs). Tyrosine-specific
PTP can either be receptor-like such as CD45 and PTPα or intracellular such as PTP1B and SHP-1.

Receptor-like PTPs generally show one or two PTP domains, one transmembrane region, and an extracellular putative ligand-binding domain. Intracellular PTPs express only one PTP domain usually surrounded by one or many regulatory or interaction domains such as SH2, SH3, or PH domains, to name just a few. Dual-specific phosphatases can dephosphorylate many types of phosphorylated residues besides phosphotyrosines. Some can dephosphorylate phosphoserines, phosphothreonines, or both whereas others can even dephosphorylate phospholipids such as phosphatidylinositol-3-phosphate (PI3P). The dual-specificity phosphatase family includes members such as Pac-1, MKP3, Cdc25, and the more distantly related PI3P phosphatases PTEN and myotubularin. Finally, LMPTPs arise from differential splicing of a single polymorphic gene, giving a fast (F) and a slow (S) isoform (or A and B), as well as another smaller isoform that has been described only recently (isoform C) (10). Three codominant alleles of this gene are found in the majority of the population (*A, *B, and *C), each having a unique combination of three single-nucleotide polymorphisms (SNPs) (11) that modifies their enzymatic activity as well as their splicing ratio between the F and S isoforms (12).

The complexity of PTP functions as both negative and positive regulators of signaling pathways was initially underestimated as many researchers believed that PTPs acted as housekeeping enzymes with little or no specificity. However, in the past decade, as more and more studies were published on the roles of PTP in development, cancer, and general regulation of signal transduction, the scientific community was impelled to reassess its views on the importance of these seemingly trivial enzymes.

B. From Structure to Function and Back Again

With the first draft of the human genome completed and easy access to sequencing data from the internet, structure–function studies of proteins in general led to huge progress in our understanding of cellular mechanisms that were unthought of only 5 years ago. The field of PTPs, being relatively in its infancy, was one of those that benefited the most from the incredible amount of information provided by DNA databases and gene sequencing. Sequence alignment of the 113 human PTPs either revealed interesting conserved motifs that confirm or supplement previous structure–function studies of the PTP catalytic mechanism and regulation or permit us to focus on less conserved regions that could have some importance for substrate specificity or subcellular localization.

The most highly conserved motif of the PTP superfamily of proteins is a stretch of seven amino acids referred to as the PTP signature motif and is composed of a cysteine and an arginine separated by five variable amino acids
Depending on the specificity of the PTP, this motif can be further refined as HCSAGXGR for the tyrosine-specific PTPs and HCXAGXSR for the DSPs of the MKP family (see Table I for an alignment of the amino acids corresponding to the catalytic region and WPD loop of selected PTPs). This sequence corresponds to the core of the catalytic site cleft and is surrounded by three loops in tyrosine-specific PTPs that provide residues needed for catalysis and substrate specificity: the WPD loop, the phosphotyrosine recognition loop, and the Q loop \((13, 14)\). The sequence of the WPD loop (residues 179–187 in PTP1B) is generally conserved and contains a general acid catalyst (either aspartic acid “D” or glutamic acid “E”) implicated in the dephosphorylation reaction \((15)\). The phosphotyrosine recognition loop corresponds to amino acids KNRY (residues 43–46 in PTP1B) and is responsible for phosphotyrosine (pTyr) specificity by defining the depth of the catalytic site cleft. DSPs do not contain such a loop and thereby show a shallower and more open catalytic site able to dephosphorylate shorter phosphoserine and phosphothreonine residues or even lipid substrates \((16)\). LMPTPs also lack the pTyr recognition loop but remain exclusively tyrosine-specific. This is probably due to the peculiar conformation of the catalytic site and WPD loop of this family of enzymes. The Q loop contains a critical glutamine residue (Gln-262 in PTP1B) that is responsible for the correct positioning of a water molecule for nucleophilic attack onto the phosphocysteine intermediate of the PTP, thereby returning the enzyme to its native state \((15)\). The mechanism of pTyr dephosphorylation begins with a nucleophilic attack by the \(S_{\gamma}\) atom of the catalytic cysteine on the phosphorus atom of the pTyr. The general acid catalyst present in the WPD loop protonates the phenolic oxygen and thus facilitates the cleavage of the \(P-O\) bond and subsequent formation of a cysteinyl-phosphate intermediate. This transient intermediate is then hydrolyzed by a water molecule that has been activated and correctly positioned by the glutamine residue of the Q loop.

Knowledge concerning this catalytic mechanism helped to develop phosphatase mutants devoid of activity but still able to interact with their substrates \((17)\). These modified phosphatases are called substrate-trapping mutants and allow the identification of potential substrates of any given PTP \((18)\). Mutations of the catalytic cysteine to serine or the general acid catalyst aspartic acid to alanine abrogate enzyme activity while maintaining the enzyme’s ability to interact with its substrate. One important difference between the two types of generated mutants relates to the extent of the enzyme–substrate interaction since the C→S mutation will not allow the formation of the cysteinyl-phosphate intermediate whereas the D→A mutation will effectively trap the substrate covalently bonded with the enzyme. Moreover, the D181A/Q262A (Q loop) double mutant of PTP1B has been reported to be a much more powerful substrate-trapping mutant than the D181A or C215S
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Consensus: p d h c r

*a* Amino acid sequence of WPD loop is inverted
mutant. This new form of substrate-trapping mutant will certainly lead to as yet undiscovered substrates of PTP1B and many other PTPs (19).

PTP regulation is critical for signal transduction to be mediated in an orderly manner within the cell. Because the isolated catalytic domain of most PTPs demonstrates activity, it is essential that a tight control over the enzymatic activity of these proteins is maintained and efficient inhibition of their phosphatase activities is thus required. One such mechanism of PTP regulation relies on specific hindrance of substrate access to the catalytic site either by intramolecular or intermolecular interactions. Regulation of the SH2 domain containing PTPs such as SHP-1 and SHP-2 occurs via an intramolecular interaction between their N-terminal SH2 domain and their C-terminal catalytic region (20, 21). Recognition of a phosphorylated tyrosine by the SH2 domain induces a conformational change that allows access to the catalytic site, leading to PTP activation. Conversely, it was observed that homodimerization of PTPα, a receptor-type PTP, led to a drastic inhibition of its activity (22). It was further shown that a two-residue insertion within the wedge connecting α1' with α2'' in the amino-terminal helix–turn–helix portion of PTPα participates at the dimeric interface of PTPα and inserts itself into the catalytic site with the obvious effect of inhibiting both enzymes (23). This feature has been reported for CD45 (24, 25) and the physiological effects of such dimerization-induced inhibition were studied using mice expressing a CD45 protein with an E613R amino acid substitution that obstructs CD45 homodimerization (26). Such mice displayed lymphoproliferation and severe autoimmune nephritis with autoantibody production causing premature death, demonstrating the importance of CD45 regulation through homodimerization.

Various other mechanisms of PTP regulation exist such as inducible expression following stimulation (HePTP, CD148), control of subcellular localization by sequestration (Cdc25, TC-PTP, PTP1B) or interaction domains (LyPTP, PTP-PEST, PTP-MEG1), and even conformational changes of the catalytic site following specific substrate binding (MKP3). These regulatory mechanisms contrive to ensure a homeostatic cellular environment allowing the cell to respond appropriately to any type of natural stimuli implicating the balance of tyrosine phosphorylation. Most of our knowledge regarding PTP implication in development and organogenesis comes from naturally occurring mutants or gene-knockout studies on mice. The motheaten (me/me) and motheaten viable (me'/me') mice express a mutant SH2-domain PTP 1 (SH-PTP1 also called HCP, PTP1C, or SHP-1) enzyme resulting in a splicing defect leading to abrogated (me/me) or reduced (me'/me') PTP activity. These mutant mice show immunodeficiency, hyperproliferative disorders, stunted growth, and skin lesions and normally die at 2–15 weeks of age of severe pneumonitis associated with accumulations of lymphocytes, granulocytes, and macrophages in the lungs (27, 28). This phenotype reflects the hematopoietic
cell-restricted expression of SHP-1 and shows that a functional SHP-1 enzyme is critical for the efficient development of immunity after birth.

CD45 gene-knockout experiments (LCA, T200) revealed a positive role of this PTP for mature thymocyte development and for T and B cell antigenic responses (29). CD45 is indeed implicated in keeping a pool of p56Lck conformationally active through dephosphorylation of its inhibitory tyrosine-505 residue, thereby permitting T cell receptor (TCR) signal transduction to occur (30, 31). It was later shown that CD45 could in fact dephosphorylate both positive (39) and negative (505) regulatory tyrosines of p56Lck and thus had negative effects on TCR signal transduction despite its needed presence during the initiation of an antigenic stimulation-dependent cascade (32). Recently, CD45 has been described as a negative regulator of another important signaling pathway, i.e., the JAK/STAT pathway. Indeed, CD45 regulates cytokine signaling through dephosphorylation of multiple JAK kinases and was reported to control interleukin-3, erythropoietin, and interferon-mediated antiviral responses in vivo (33). This novel function of CD45 is an exciting discovery that will lead to a better understanding of the T and B cell developmental and functional defects observed in CD45−/− mice.

SH2-domain protein tyrosine phosphatase 2 (SH-PTP2, also called Syp, PTP1D, or SHP-2) is another ambivalent PTP that can exert positive or negative regulation of signal transduction processes depending on the context. Disruption of the two alleles of SHP-2 in mice leads to midgestational embryonic lethality and chimeric animals harboring SHP-2−/− and wild-type (WT) cells demonstrate multiple developmental defects such as short hind legs, aberrant limb features, split lumbar vertebrae, abnormal rib patterning, and pathological changes in the lungs, intestines, and skin. Furthermore, no SHP-2−/− cellular progenitors for erythroid or myeloid cells were detected in the fetal liver and bone marrow of chimeric animals and hematopoiesis was defective in SHP-2−/− yolk sacs (34). Using the RAG2-deficient blastocyst complementation technique, Qu et al. (35) were able to further demonstrate the importance of SHP-2 in hematopoiesis since these chimeric mice had no detectable mature T and B cells, serum immunoglobulin M, or even Thy-1(+) and B220(+) precursor lymphocytes. Collectively, these studies and others implicating SHP-2 in epidermal growth factor receptor (EGFR) (35), CXCR4 (36), and CCR5 (37) signaling present strong evidence that SHP-2 plays a positive role in tissue differentiation, body organization, hematopoiesis, and chemotaxis. Nevertheless, studies performed with other signaling models also depict SHP-2 as a negative regulator of signal transduction. In particular, SHP-2 binding to the SHP-2-interacting transmembrane adapter (SIT) (38), the cytoplasmic molecular adapter Grb2-associated binder 2 (Gab2) (39), and the cytotoxic T lymphocyte antigen-4 (CTLA-4) (40, 41) is essential for the negative effects of these proteins on TCR/CD28-mediated signal
transduction. Although the exact contribution of SHP-2 on TCR signaling is still unclear, some studies suggested positive effects of this PTP on TCR-mediated induction of the Ras/MAPK pathway \((42, 43)\). It is possible that depending on the nature and strength of the antigenic stimuli, SHP-2 could contribute differently to the outcome of the signaling cascade.

The T cell PTP (TC-PTP, also called MPTP, PTP-2, or PTP-S) was identified shortly after the original purification of PTP1B and, although ubiquitously expressed, showed very high expression levels in placental and hematopoietic tissues. Two isoforms of this PTP are expressed from differentially spliced transcripts yielding a 45-kDa nuclear isoform and a 48-kDa endoplasmic reticulum isoform \((44)\). Homozygous disruption of this gene led to a normal Mendelian distribution of this gene although all homozygous mutant mice died at 3–5 weeks of age, displaying runting, diarrhea, enlarged spleen, and lymphadenopathy \((45)\). Although the number of immature thymocytes was largely reduced, only a slight reduction in the number of mature T cells could be observed in the thymus, spleen, and lymph nodes of these animals. Interestingly, peripheral T cells from TC-PTP\(^{-/-}\) mice failed to proliferate and secrete interleukin (IL)-2 upon TCR multimerization, although TC-PTP overexpression was shown to have no effect on membrane proximal TCR-mediated signaling and IL-2 transcription. TC-PTP is indeed thought to act downstream of protein kinase C (PKC) and calcium to positively regulate the TCR signaling cascade. Its effects on other signaling pathways is still ambiguous since TC-PTP was reported to positively affect platelet-derived growth factor (PDGF)-induced NF-κB activation and progression through G\(_1\) \((46)\) whereas it was also described as being a negative regulator of EGFR-induced PI3K activation \((47)\).

More recently, TC-PTP was also shown to be a negative regulator of cytokine signaling by dephosphorylating both Janus protein tyrosine kinases (JAK) 1 and 3 \((48)\) as well as the signal transducer and activator of transcription (STAT) 1 \((49)\). These studies help to explain why a deficiency in this particular PTP leads to such a defect in B cell development and T and B cell functions since its absence disrupts a variety of signal transduction pathways.

Gene knockout mice of many more PTPs were studied and showed various phenotypic abnormalities such as neuronal defects (PTP\(_{α\, -}\) and PTP\(_{δ\, -}\)), impaired mammary gland development (LAR\(^{-/-}\) and PTP\(_{α\, -}\)), and increased insulin sensitivity (LAR\(^{-/-}\) and PTP1B\(^{-/-}\)) but none of these PTPs had any obvious effect on T cell development, repertoire selection, or function \((50)\) and so will not be discussed further.

Other PTPs implicated in T cell signal transduction include the hematopoietic PTP (HePTP, also called BPTP or LC-PTP), CD148 (DEP-1, HPTP \(η\)), and the proline–glutamic acid–serine–threonine (PEST)-type phosphatases LyPTP (PEP, Lyp) and PTP-PEST. HePTP is an early response gene \((51)\) encoding a PTP that negatively regulates ERK and p38 MAPK activation but not
JNK (52), thereby influencing AP-1 and ATF-2 transcription factor activation and IL-2 production through cooperation with NFAT and regulation of c-fos transcription.

Overexpression of catalytically inactive HePTP effectively leads to increased IL-2 production in T cells (53) and disruption of the human 1q32.1 locus encoding for HePTP was reported in non-Hodgkin lymphomas and chronic lymphoproliferative disorders (54). CD148 is expressed at low levels in naive T lymphocytes and its expression is highly upregulated following antigenic stimulation (55, 56). This PTP controls membrane proximal TCR-mediated signal transduction by dephosphorylating the linker for activation of T cells (LAT) molecular adapter and phospholipase Cγ (PLCγ) after prolonged TCR engagement (57). LyPTP interacts through its proline-rich region with the PTK Csk (58) and cooperates with this inhibitory kinase to negatively regulate the p56lck and p59fynsrc family kinases (59, 60). The mouse homolog of LyPTP was indeed shown to be able to dephosphorylate the positive regulatory tyrosine phosphorylation residue 394 of p56lck whereas Csk phosphorylated the negative regulatory tyrosine phosphorylation residue 505, therefore completely inhibiting this critical PTK for TCR signaling (60). In support of this model, expression of a substrate-trapping mutant (D195A) of LyPTP effectively reduced TCR/CD28-induced transcriptional activity (61). On the other hand, PTP-PEST is a quite unique PTP in its role as a negative regulator of both integrins and TCR signaling-mediated cytoskeleton rearrangement. These functions correlate with its ability to dephosphorylate important molecular adapters such as PSTPIP (62) and Cas (63), tyrosine kinases such as Pyk2 and Fak, and generally cause the inactivation of the Ras/MAPK pathway (64).

Despite sharing a common catalytic mechanism, little sequence homology can be observed between DSPs and PTPs. Besides the obvious absence of the phosphotyrosine recognition loop in DSPs, the WPD loop displays important modifications such as a one or two amino acid insertion between the proline and the general acid catalyst and the replacement of the tryptophan (W) for an isoleucine (I), a valine (V), or even a tyrosine (Y). These alterations of the WPD loop could provide better interactions with shorter phosphoamino acids (e.g., serine or threonine) and thus reflect the broader substrate specificity of DSPs. In addition, modifications of the CX5R signature motif can, in some instances, induce a radical change of substrate specificity. PTEN and the myotubularin family of proteins are such examples as they are very poor PTPs but excellent phosphatidylinositol-3-phosphatases. PTEN shows greatest specificity toward the dephosphorylation of the D3 position of PI(3,4,5)P3 but can also dephosphorylate PI3P and PI(3,4)P2 albeit at a much lower rate (65). This specificity is conferred by positively charged amino acids near the WPD loop (YPFEDH) and in the CX5R signature motif (CKAGKGR) that accommodate
the negative charges of the D1 and D5 phosphate moieties of PI(3,4,5)P3 (66). These positively charged amino acids are absent from the signature motifs of all the myotubularin family members and are replaced by negatively charged aspartic acid residues (CSDGWDR). These residues electrostatically repulse D4 and D5 phosphate moieties of multiply phosphorylated phosphatidylinositols thus explaining the restricted specificity of this family of enzymes for PI3P (67).

Functional studies of DSPs immediately identified an important class of enzymes that is a critical regulator of the MAP/ERK pathway. DSPs of the MKP family are able to efficiently dephosphorylate the activating TXY motif of the ERK, p38, and JNK family of MAP kinases with varying specificity. The first identified human member of this family is MKP-1, an oxidative stress-inducible 39-kDa protein that is more than 95% homologous to the vaccinia virus H1 (VH1) phosphatase (68). The finding that disruption of the homologous gene (3CH134) in mice results in phenotypically and histologically normal fertile mice with no defects in the activation of the MAP/ERK pathway (69) is surprising but could be explained by functional overlap between the 11 MKPs presently known. Most MKPs show little specificity, being able to dephosphorylate ERK, JNK, or p38 family members with little discrimination (70). Nevertheless, some members can be quite specific; MKP3 and MKP-X (71), for example, bind and dephosphorylate ERK1 and ERK2 only (72, 73). As mentioned earlier, their catalytic site is conformationally fully active only when bound to ERK enzymes, thus demonstrating an ingenious mechanism of regulation (74).

MKPs have been reported to have an important role in cell proliferation, hypertrophy, and differentiation. Several studies using muscle cell lines implicate MKP-1 and MKP-3 in muscle differentiation and MKP-1 was also reported to regulate cardiac myocyte hypertrophy, arterial smooth muscle cell proliferation, as well as proliferation of glomerular mesangial cells implicated in proliferative glomerulonephritis (75). In T lymphocytes, multiple MKPs have been reported to downregulate the TCR-mediated ERK signal transduction pathway. Recently, VHX (also called MKPX), a 19-kDa VHR homolog constitutively expressed in peripheral blood T lymphocytes, was overexpressed in a T lymphoid cell line and was shown to suppress TCR-mediated Erk2 phosphorylation and NFAT/AP-1 transcriptional activation (76). This report and another concerning VHR itself (77) clearly demonstrate the implication of these small MKPs in the constitutive regulation of the ERK pathway, first ensuring that only a strong stimulus can lead to gene expression and second that this gene expression is downregulated in a timely fashion after removal of the stimulus. In a search for proteins that can interact with the intracytoplasmic tail of CD28, Marti et al. isolated MKP6, another MKP that regulates TCR/CD28 signaling (78). Expression of this MKP is strongly induced following
antigenic stimulation of peripheral blood T lymphocytes and negatively regulates IL-2 secretion induced by TCR/CD28 costimulation in a highly specific fashion. It is important to point out that these studies made use of overexpression systems, substrate-trapping mutants, and mutated receptors, experimental strategies that can bias our understanding of the fine tuning of MKP/MAPK interplay. Gene disruption of MKPs and probably multiple knockout experiments will be needed to more clearly show the individual and cooperative effects of each MKPs on signal transduction in a physiological setting and their overall effects on development, organogenesis, and cellular functions.

PTEN and the myotubularin family of phosphatidylinositol-3-phosphatases have been the subject of several studies in the past few years. In particular, the implication of PTEN in cancer has propelled this atypical PTP to the center of research efforts of many scientists, thereby pioneering the development of a whole new field, that of the phosphatidylinositol-3-phosphatases. Production of PI(3,4,5)P₃ by the action of PI3K on PI(4,5)P₂ in the cellular membrane is an early event following many hormone and growth factor receptor ligation as well as TCR/CD28 costimulation (79). The presence of PIP₃ in the membrane provides a signal for PH domain-containing signaling effectors leading to their translocation to the membrane where they become activated either by a PIP₃- or a phosphorylation-induced conformational change (80). Important effectors of the PI3K pathway are PDK1 and PKB/Akt, which are serine/threonine kinases implicated in cell growth and survival signaling (79).

Accumulation of PIP₃ caused by PTEN malfunction or absence leads to reduced sensitivity to apoptotic signals and uncontrolled proliferation, hallmarks of cancerous cells. Indeed, PTEN is a tumor-suppressor gene located on chromosome 10q23 found mutated in a large number of tumor tissues and human cancer cell lines (65). Interestingly, germ line mutations of PTEN are also a major factor for the development of Cowden and Bannayan–Zoana syndromes, hereditary diseases characterized by increased risks of thyroid and breast cancers (81, 82). In T lymphocytes, PTEN negatively regulates TCR/CD28-induced PIP₃ production by PI3K and as such controls membrane localization of PH-domain-containing proteins such as the Tec family tyrosine kinases and Vav guanine nucleotide exchange factor, actin cytoskeleton rearrangement, and NFAT nuclear translocation, as well as apoptosis and cell cycle progression (83).

Finally, structural studies of the LMPTP family of PTP show a similar folding of their catalytic domains compared to the D1 phosphatase domains of tyrosine-specific PTP. Some important changes can easily be highlighted since the WPD loop is not located at the amino-terminal of the PTP signature motif, but is rather redirected to the carboxy-terminal end of this small protein, with an inverted sequence homologous to those of tyrosine-specific phosphatases. Moreover, the PTP signature motif shows a great level of variability
when compared to the tyrosine-specific and DSP families. Nevertheless, LMPTPs have been shown to be quite specific for phosphorylated tyrosine (84), probably due to the peculiar arrangement of the WPD loop and the residues surrounding the catalytic site. LMPTPs are presently studied much less frequently than other PTPs and little information regarding their regulation, implication in signal transduction pathways, and involvement in human diseases is available. Nevertheless, some groups have made LMPTPs their primary target and their studies have begun to shed some light on the functions of this group of small phosphatases.

Alternative splicing of exons 3 and 4 of the LMPTP gene located on chromosome 2p25 (85) generates three different isoforms of LMPTP (A, B, and C). Moreover, three codominant alleles are generally expressed in the population (*A, *B, and *C), each having a characteristic enzymatic activity and a specific expression ratio of A/B isoforms. Changes in the ratios between the A and B isoforms and the expression pattern of each allele have both been correlated with different clinical outcomes from patients suffering from a variety of diseases and conditions. Particular pairs of LMPTP alleles have thus been linked with predisposition to either allergies, asthma, diabetes, Alzheimer's disease, or myocardial hypertrophy (86). On a molecular level, LMPTP was shown to be involved in the regulation of signal transduction induced by PDGF (87), insulin (88), IL-4 (89), and antigenic stimulation (90, 91). Phosphorylation of LMPTP by src family kinases on tyrosines 131 and 132 (92) influences its catalytic activity and subcellular localization, respectively (93), thereby providing a regulatory mechanism that supports LMPTP activation, recruitment, or sequestration, depending on the initiating stimulus. Furthermore, alternative splicing of LMPTP generates enzymes with distinct configurations in their catalytic site pocket that could lead to discrete substrate specificity (isoforms A and B) or abolition of catalytic activity (isoform C) (10). In T cell signaling, LMPTP has been reported to increase TCR-mediated NFAT/AP-1 transcriptional activation and IL-2 production (90) and has further been shown to dephosphorylate ZAP-70 on its inhibitory tyrosine-292 residue (91). Dephosphorylation of this residue by a low level expression of LMPTP actually increased overall ZAP-70 tyrosine phosphorylation and kinase activity as well as downstream MAPK signaling following TCR stimulation. Antigenic stimulation could thus lead to activation of LMPTP by src family kinases, which would in turn help to fully activate ZAP-70 by dephosphorylating an inhibitory tyrosine residue, akin to the CD45/p56^ck^ regulatory mechanism.

C. Activation vs. Inhibition: A Fragile Equilibrium

Rapid activation of protein tyrosine kinases (PTKs) following antigenic stimulation shifts the delicate balance of protein tyrosine phosphorylation and leads to a sharp increase in the level of tyrosine phosphorylated proteins
within minutes. Protein tyrosine phosphatases that are normally able to counteract PTK activity within an unstimulated cell are suddenly overwhelmed by this swift augmentation of PTK activity and an accumulation of proteins phosphorylated on specific tyrosine residues occurs. This posttranslational modification can induce changes in the subcellular localization and/or in the activation state of these proteins, thus generating a signal transduction cascade that leads to specific gene expression. T lymphocyte activation is therefore a function of the balance between these two counteracting activities and either an increase of PTK activity or a decrease of PTP activity can cause cellular activation. Such is the premise behind the use of PTP inhibitors to study T cell signaling pathways and the effects of cellular activation on a variety of diseases.

The very first PTP inhibitor discovered was sodium orthovanadate (Na$_3$VO$_4$), which was already known as a potent inhibitor of Na$^+$/K$^+$- (94) and Ca$^{++}$-dependent (95) ATPases as well as alkaline phosphatases (96). Na$_3$VO$_4$, having a molecular structure similar to a phosphate molecule, competitively inhibits PTPs by hindering substrate access to the catalytic site. Interestingly, the potency of Na$_3$VO$_4$ to induce tyrosine phosphorylation in intact cells was rather poor, whereas H$_2$O$_2$, another early PTP inhibitor, could result in a significant increase in the amount of tyrosine phosphorylated proteins when added to living cells. Before the mechanism of action of H$_2$O$_2$ on PTPs was discovered, studies using both inhibitors demonstrated that Na$_3$VO$_4$ and H$_2$O$_2$ synergized and induced a much more potent tyrosine phosphorylation of proteins than either inhibitor alone or even physiological stimulation of the cells. Such effect was later demonstrated to result from the reaction of H$_2$O$_2$ on Na$_3$VO$_4$ to produce a vanadium peroxide or pervanadate, a very potent PTP inhibitor. Although H$_2$O$_2$ nonspecifically oxidizes the catalytic cysteine of PTPs, pervanadate specifically targets PTPs due to its peculiar structure and is the subject of a nucleophilic attack by the catalytic cysteine of the enzyme, leading to the oxidation of the residue and subsequent deactivation of the enzyme (97).

Pervanadate is widely used because it is easily produced and this compound can quite potently induce protein tyrosine phosphorylation without much toxicity. In its very nature, however, pervanadate is a highly unstable molecule and its composition is a heterogeneous mixture of many differently oxidized forms of vanadate that each has specific biological effects. These are the reasons why Alan Shaver and Barry Posner at McGill University endeavored to purify particular compounds and study their insulinomimetic effects. Addition of various ancillary ligands to a pervanadate solution and purification by $^{51}$V and $^3$H nuclear magnetic resonance led to the purification of highly stable molecules called bisperoxovanadium (bpV) compounds (Fig. 1), some of which were at the time the most potent and specific PTP inhibitors available (98).

The discovery that PTP1B$^{-/-}$ mice showed increased resistance to obesity-induced diabetes and better sensitivity to insulin (99, 100) sparked huge
interest in discovering a specific PTP1B inhibitor that could be used clinically for the treatment of type II diabetes in humans. Different approaches yielded peptide-based as well as non-peptide-based compounds able to specifically interact and inhibit PTP1B with remarkable efficiency. Although peptide-based compounds usually show interesting inhibitory potential, many inherent problems remain for efficient delivery in a clinical setting. Non-peptide-based compounds thus seem to be the preferential avenue of research. Because most PTPs share a high degree of similarity in their catalytic site region, new approaches had to be designed to achieve sufficient specificity. Bidentate inhibitors were thus designed as a mean to generally target PTPs through a region that recognizes the catalytic site configuration and then to target one specific PTP through a more variable region surrounding the catalytic site (reviewed in 16). Some of these investigations revealed attractive candidates such as the bis-aryldifluorophosphonate family of inhibitors and some heavily modified versions of benzylxyoxacetic acid and salicylic acid molecules. These compounds inhibit PTP1B at submicromolar concentrations, are cell membrane permeable, and demonstrate insulinomimetic properties in vivo.

Fig. 1. Molecular structure of bisperoxovanadium compounds. These powerful PTP inhibitors are based on a vanadium molecule surrounded by two oxo groups (O=O) and one oxyanion (O\(^{-}\)). Ancillary ligands (L, L\('\)) are added during the production process and are responsible for the stability and specificity of these compounds compared to pervanadate. These ligands are associated with the vanadium core through its outer coordination sphere and allow purification of bpV molecules by \(^{3}\)H and \(^{51}\)V nuclear magnetic resonance yielding more than 95% pure compounds.
Specific inhibitors of individual PTPs implicated in TCR signal transduction have yet to be developed. This is why nonspecific inhibitors such as pervanadate and bpV compounds are still widely used as a general mean of increasing protein tyrosine phosphorylation and studying signaling events mediated by this post translational modification. In T cells, pervanadate was reported to increase p56^Lck, p59^Fyn, and ZAP-70 PTK activity (101, 102), augment intracellular calcium concentrations (103), and activate transcription factors such as NFAT (103), NF-κB (103, 104), and AP-1 (103). These signaling cascades led to increased IL-2 production (101) and surface expression of activation markers such as CD25 and CD69 (102, 103). Moreover, another work demonstrated that pervanadate treatment of T lymphocytes promoted their attachment to fibronectin via enhanced tyrosine phosphorylation of paxillin (105). Interestingly, the mechanism of activation of the NF-κB transcription factor following pervanadate treatment is quite unusual. NF-κB is normally sequestered in the cytoplasm by a family of natural inhibitory proteins, termed IκB (IκBo being the most studied), that are degraded by the proteasome in a ubiquitin-dependent fashion after phosphorylation of two serine residues by specific IκB kinases. Upon IκBo degradation, NF-κB is free to translocate to the nucleus where it can increase gene expression (reviewed in 106). Pervanadate-induced NF-κB activation does not, however, require IκBo degradation and is instead dependent on phosphorylation of IκBo on the tyrosine residue 42 (104, 107). Tyrosine phosphorylation is believed to induce a conformational change of IκBo that permits NF-κB translocation to the nucleus, DNA binding, and transcriptional activation even if IκBo is still bound to the NF-κB dimer. This model could not be confirmed by another research group who made use of proteasome inhibitors and showed that degradation was indeed essential for pervanadate-induced NF-κB activation (108). This discrepancy could be explained by the fact that they used a macrophage cell line instead of T lymphocytes, raising the interesting possibility that NF-κB activation without proteolytic degradation of IκBo could depend on specific factors expressed by different cell types. In a physiological setting, this mode of activation was proposed for NF-κB activation following reoxygenation of hypoxic T cells, which also induces Tyr-42 phosphorylation (104). Later, src family kinase p56^Lck and syk family kinase ZAP-70 were shown to be important for pervanadate-induced NF-κB activation via IκBo tyrosine phosphorylation, although IκBo does not seem to be a direct target of either PTKs (109).

Bisperoxovanadium compounds, as a purer and more stable form of pervanadate, are thought to have a more restricted effect on T cell signal transduction events. The ancillary ligands of the different bpV compounds alter the induced changes in the levels of tyrosine phosphorylation and can even provoke different patterns of phosphorylated tyrosine as determined by
antiphosphotyrosine Western blot analysis of whole stimulated cell extracts (110). Moreover, some compounds are definitely more toxic than others. Our group made use of a bpV compound having a picolinic acid ancillary ligand (bpV[pic]) because of its very low toxicity in T cells at a concentration that leads to higher tyrosine phosphorylation in comparison to other compounds having slightly different ancillary ligands i.e., hydroxypicolinic acid [HOpic] and bipyridine [bipy] (unpublished observations). Another research group also demonstrated that different bpV compounds affect insulin receptor signaling and overall inhibition of phosphatase activity in distinct ways (98). For these reasons, we believe that different bpV compounds might show some specificity toward individual PTPs and lead to activation of different signaling cascades. We also observed that the window of effectiveness of these compounds in T cells is somewhat restricted since concentrations lower than 2 μM do not lead to transcription factor activation and concentrations higher than 30 μM are deleterious to the cells. The bisperoxovanadium compound bpV[phen] was shown to increase interferon-γ (IFN-γ) sensitivity and NO production of murine macrophages, which resulted in a better clearing of Leishmania-infected macrophages in vitro and in vivo (110, 111). In PC12 and HeLa cells, monoperoxovanadium compounds were shown to induce JNK activation and downregulate MKP-1 expression (112, 113) whereas bpV compounds were reported to activate phospholipase D and all MAP kinases (ERK, JNK, and p38) in endothelial cells (114). These effects can be added to the classic insulinomimetic properties of the bpV compounds and indicate that PTP inhibition induces an increased activation state in most cell types.

In the next section, we will describe our past observations concerning bpV-induced activation of transcription factors, production of cytokines, and expression of adhesion molecules and their general effects on human immunodeficiency virus type 1 (HIV-1) transcriptional activity and replication.

II. Regulation of HIV-1 Replication by PTPs

A. Signaling Pathways in T Cells Activated by Bisperoxovanadium Compounds

The phenomenon of T cell activation has been addressed in lengthy detail in the past few years, but important issues have still been left unanswered. It is nonetheless accepted from several biochemical studies that TCR-mediated signaling is a very complex phenomenon and involves multiple protein–protein interactions and phosphorylation events (Fig. 2). In our laboratory, for several years now, we have been investigating the role of PTP in the activation of
Fig. 2. Protein tyrosine phosphatases in T cell receptor and cytokine receptor signaling pathways. Signaling cascades leading to activation of inducible transcription factors such as NF-κB, NFAT, AP-1, and STAT depend on efficient tyrosine phosphorylation of membrane-proximal and -distal elements. Protein tyrosine phosphatases are thus implicated in negative regulation of most signaling pathways, although more and more positive roles are being described.
transcription factors and specific cellular genes through the use of the bpV compounds.

Our experiments were conducted using established Jurkat T cells and well-characterized Jurkat variants and were confirmed in other T cell lines and/or primary T cells. Our experiments have revealed that numerous genes can be activated in T cells in the presence of these PTP inhibitors. Hence, upon incubation of Jurkat cells or peripheral blood mononuclear cells (PBMC) with bpV compounds, we have shown a strong induction of several genes such as ICAM-1, COX-2, IL-4, IL-10, tumor necrosis factor-α (TNF-α), and INF-γ (115 and unpublished results). In addition, chemokines such as MIP-1α and RANTES have been observed to be upregulated by these same inhibitors (unpublished results). Most of these results have been determined by both reverse transcription-polymerase chain reaction (RT-PCR) and RNase protection assays and more extensive analyses have been performed on the positive transcriptional modulation of ICAM-1 and COX-2 (115 and unpublished results). Importantly, IL-2 has been shown to be transcriptionally upregulated either by bpV alone or in combination with anti-CD3 or anti-CD28 antibodies, phorbol ester [i.e., phorbol myristate acetate (PMA)], or ionomycin (116). Our results are thus consistent with previous reports obtained through the use of the pervanadate PTP inhibitor, in which similar induction of IL-2 production was measured with or without the addition of other activating agents (101–103, 117).

Over the past few years, we have accumulated a substantial amount of data on the cascade leading to the activation of cellular genes induced by bpV molecules in T cells. These studies led us toward a general overview of the bpV-oriented signaling cascade, but most of our effort was focused on induction of the ubiquitous mammalian transcription factor NF-κB (118 and unpublished results). Nevertheless, several elements in the NF-κB signaling pathway have also been shown to be involved in bpV-dependent activation of NFAT as well as in upregulation of ICAM-1 (115, 116). It was shown that signaling cascades that were induced following bpV treatment and TCR/CD28 engagement share a vast array of common effectors. Our current working model actually points to a membrane proximal initiation of the activation process but without an obligatory presence of the TCR (116, 118). Some of the required cellular factors include the PTKs p56lck and ZAP-70 as well as an increase in calcium mobilization (116, 118). This is based on the use of Jurkat T cell lines that are deficient in either PTK (119, 120) or are defective in the process of capacitative calcium entry (120). Recently, the p36LAT molecular adapter, the kinases IKKα, IKKβ, IKKe, COT, and NIK, and different categories of PKC were found to be key cellular players in the bpV-induced cascade (unpublished results). These data can be compared to the signaling pathway previously described by Imbert et al. that is mediated by pervanadate in Jurkat cells (103).
For example, p56Lck, ZAP-70, and calcium influx in addition to Vav and Shc tyrosine phosphorylation were all found to be part of the signaling cascade leading to NF-κB nuclear translocation. This group of researchers proposed that pervanadate-mediated NF-κB activation was due to two distinct but coupled pathways. The explanation for this discrepancy is unknown but could relate to the use of different PTP inhibitors (i.e., pervanadate vs. bpV) or a different technical strategy to measure NF-κB activation. One interesting and important distinction between the activated pathway leading to NFAT activation versus the one resulting in induction of NF-κB relates to CD45. Although we have conclusively shown that this PTP was required for the activation of NF-κB by bpV molecules (as for pervanadate) (103 and unpublished data), CD45 does not seem to be essential for NFAT induction (116). These data might indicate the peculiar characteristics of signal transducers needed for the activation of different transcription factors in T cells.

Identification of the PTP(s) that is (are) directly involved in bpV-mediated activation of specific transcription factors such as NFAT remains of high interest. Based on previous experiments aimed at defining intracellular second messengers implicated in bpV-dependent induction of NFAT, we postulated that SHP-1 could be one of the important PTPs being targeted by the bpV inhibitors (116). Indeed, using a Jurkat cell line stably expressing a dominant-negative form of SHP-1, we observed a significant increase in both basal and PMA/ionomycin-induced levels of nuclear NFAT. Furthermore, SHP-1 has been previously observed to act as a strong negative regulator of T cell activation through its action on CD4 (or CD8)-associated PTKs (121, 122) and other numerous factors involved in the TCR-dependent signaling cascade, most of which seem to be shared with the cascade induced by bpV compounds. Although no such similar analysis has been performed with pervanadate, it is very likely that it should also be the case for this PTP inhibitor. Surprisingly, a study has suggested that activation of T cells by pervanadate does not require SHP-1, although the responsible PTP has yet to be determined (123). It should be pointed out that their conclusion was based on the use of SHP-1⁻/⁻ “motheaten” mouse thymocytes with the assessment of tyrosine phosphorylation in membrane preparation, which differs from our experimental model. Further studies will be required to explain these discrepant results.

B. Transcription Factors Activated by Bisperoxovanadium Molecules

The powerful activation of numerous genes following PTP inhibition is linked to activation of a high number of transcription factors. Indeed, CREB, AP-1, NFAT, NF-κB, and STAT1 have all been identified as being negatively
regulated by PTPs on the basis of their sudden activation following intracellular PTP inhibition. A certain amount of data has been obtained pointing to specific underlying mechanisms for some of these activated transcription factors. First, as indicated above, NF-κB has been demonstrated by our group to be activated by the bpV PTP inhibitors \((118, 124)\). In fact, through several investigations, we have confirmed its involvement in the induction of promoter activity of several cellular genes such as ICAM-1 and COX-2 \((115\text{ and unpublished results})\). On the basis of transfection studies with a dominant-negative mutants of IκBα and immunoprecipitation experiments \((124)\), activation of NF-κB is thought to occur through the classic pathway requiring degradation of the IκBα repressor that is preceded by phosphorylation of serines-32 and -36 and ubiquitination \((124)\). Another group has also obtained similar results in terms of the requirement of serine phosphorylation and degradation of IκBα for NF-κB activation by bpV compounds \((125)\). Our recent findings that the IKKα and IKKβ kinases are important elements in the bpV-dependent signaling cascade further support the classic mode of NF-κB activation (see above). It is peculiar that the mode of NF-κB activation observed with the bpV compounds differs from what has been reported with pervanadate, resulting in the activation of NF-κB in T cells independently of IκBα degradation \((104)\). Several studies have further demonstrated the absence of IκBα degradation following hypoxia or oxidative stress conditions in T cells and correlated the activation of NF-κB with tyrosine phosphorylation of IκBα \((107, 109)\). Although we believe that the signaling cascade induced by both inhibitors mostly bears convergent elements, some differences are likely to be discerned due to the method of preparation of either molecules. In a more explicit manner, in addition to the pervanadate compound itself, we would argue that IκBα inactivation might also be induced via other intermediary by-products resulting from the mixture of both H₂O₂ and Na₃ VO₄ unique to this PTP inhibitor preparation. Such compounds would be in limited amount in the bpV preparations as these products are purified \((98)\).

Similar investigations have further shown that the bpV compounds were extremely potent in inducing NFAT translocation and activation and that activation was typically sensitive to the immunosuppressors cyclosporin A and FK506 \((116)\). These results also suggested that the activation process of NFAT through our PTP inhibitors occurred by a classic mode of activation of NFAT. As such, calcium entry was found to be critical for the bpV-mediated NFAT activation when tested in capacitative calcium entry-deficient Jurkat cells. The impact of the bpV inhibitor on NFAT activation in Jurkat cells has also been highlighted in another study performed by Ehring and co-workers \((126)\). Further analysis by supershift assays demonstrated that the bpV-activated NFAT complex was mainly constituted of the NFAT1 family member. No studies have yet been performed with other PTP inhibitors, although it is
likely that pervanadate also induces NFAT since, as stated above, this compound induces IL-2 expression, which is dependent on NFAT activation \( (101-103, 117) \). It should be stated that vanadate and hydrogen peroxide were, however, shown to activate NFAT in mouse fibroblast cell lines and vanadium was shown to be a potent inducer of NFAT activation \( (127) \).

In addition to these two factors, several other studies have provided evidence that a number of other transcription factors are turned on by the bpV compounds. The results that we have obtained on NFAT activation induced by the addition of the bpV products lead us to to propose that AP-1 might also be activated as well \( (116) \). Indeed, numerous studies have shown that NFAT often requires the AP-1 complex next to its binding site in order to modulate gene expression \( (128) \). The NFAT-binding sites located in the plasmid used in our investigation come from the IL-2 promoter and are highly dependent of their proximal AP-1-binding site. In support of this speculation, we have indeed demonstrated that AP-1 complexes are more abundant in the nucleus of bpV-treated cells as determined in electromobility shift assays (EMSA) (unpublished results). Activation of fos gene expression and of the AP-1 complex had in addition been reported earlier by other groups upon activation of T cells by pervanadate \( (102, 103) \). In Jurkat cells, this laboratory has also presented results demonstrating that the bpV inhibitors were efficient in allowing nuclear translocation of the STAT1 transcription factor \( (115) \). Although these results were mostly presented in the context of the bpV-mediated activation of ICAM-1 gene expression, EMSA experiments have indeed indicated an increase in the level of phosphorylated nuclear STAT1. On the basis of previous studies, this information is consistent with the importance of tyrosine phosphorylation on the STAT protein generated by the activation of the associated JAK kinases \( (129) \). Previous results in a different cell context have also demonstrated that pervanadate induced STAT translocation \( (130) \).

Finally, more recently, our group has obtained results indicating that bpV compounds are good inducers of CREB activation (unpublished results). This activation was found to be enhanced when bpV compounds were added in the presence of forskolin, a well-known activator of adenylate cyclase and thus a strong activator of CREB. Furthermore, unpublished results from our team has provided evidence that the bpV compounds were strong modulator of the human T cell leukemia virus type 1 transcription and that this activation was mainly orchestrated by the CREB transcription factor. Our results are in line with a previous report that stated a negative modulatory role of PTP on the transcriptional activation of cAMP-responsive elements \( (131) \). More data are needed to understand the link between PTPs and CREB activation, especially with respect to the phosphorylation state of the serine residue 133 of the CREB factor, a requirement for its activation.
C. Modulation of HIV-1 Gene Expression by PTPs

Gene expression of HIV-1, as for all retroviruses, is a process initiated from its promoter region located in the 5' long terminal repeat (LTR). Several regulatory elements span the entire length of the promoter, some of which have been demonstrated to play key roles in the process of HIV-1 gene expression. One of the most important segments for HIV-1 transcription remains the TAR sequence, which, in the RNA form, produces a stable secondary structure bound by the viral transactivator Tat protein and the cellular component P-TEFb comprised of cyclin T1 and the cyclin-dependent kinase CDK9. On a more cellular perspective, HIV-1 gene expression is also highly dependent on cellular transcription factors known to be activated upon T cell activation. Some of these cellular transcription factors bind to a segment of the LTR referred to as the enhancer region. Although several reports have indicated that this enhancer region binds different transcription factors, NF-κB remains the most studied and surely the most important cellular factor in the regulation of HIV-1 following T cell activation. However, NFAT is another factor that has recently been brought to the attention of the scientific community. Indeed, this factor specifically interacts with the HIV-1 enhancer region and several studies have provided strong evidence that this factor can upregulate HIV-1 gene transcription in the context of immortalized T cell lines and primary T cells. Although a great deal needs to be learned as to the mechanistic action of NFAT on HIV-1 transcription, several studies support the notion of a potential synergistic action between NF-κB and NFAT. Interestingly, both NF-κB and NFAT are normally cytoplasmic in resting T cells. Upon T cell activation, via distinct cascades, these factors become active and nuclear. Whereas NF-κB acts alone as a homodimer or heterodimer on gene transcription, the monomer NFAT largely depends on neighboring transcription factors to permit expression of targeted genes. It is now well established that HIV-1 LTR activity and thus virus replication are markedly induced upon T cell activation. Because both NF-κB and NFAT are activated upon T cell activation and are central for the expression of cytokine genes following T cell activation, it thus should come as no surprise that these factors are also important in promoting HIV-1 transcription.

Given that PTP inhibitors are highly potent T cell activators, we and others have conducted experiments addressing the potential regulation of HIV-1 transcription by these same compounds. An initial study demonstrated that the classic PTP inhibitor vanadate had the potential to positively modulate HIV-1 LTR activity but that, upon the addition of hydrogen peroxide (and thus formation of pervanadate), this upregulation was greatly improved. It has further been indicated that the inhibition of PTP by pervanadate permitted
an important increase in the release of the viral capsid p24 protein in the supernatant and LTR activation in Jurkat T and myelocytic U937 and THP-1 cell lines. Our group was interested in confirming these results in other T cell lines and with our own bpV PTP inhibitors. Upon incubation of various T cell lines, we have indeed observed an important induction in HIV-1 LTR activity, which was independent of the strain of origin of the LTR (124). This induction was also paralleled by an increase in supernatant-associated reverse transcriptase activity when latently HIV-1-infected T cell lines were treated with the bpV compounds. With the use of different types of available bpV compounds, we have also established, through intracellular flow cytometric analyses, that there was an almost perfect correlation between the extent of intracellular phosphotyrosine content and LTR-mediated luciferase reporter gene expression (98). Through transfection of constructs containing a 5′ LTR mutated in its NF-κB-binding sites, we have then determined that the NF-κB factor played an important role in this activation process, as expected, given the fact that these inhibitors are strong inducers of NF-κB translocation and activation.

This latter investigation also provided evidence that the activation of the HIV-1 LTR by bpV molecules involved a transcription factor that was different from NF-κB. Upon analysis with the cyclosporin A and FK506 inhibitors, mutated LTR constructs, and dominant-negative mutants, this factor was proven to be NFAT (116). Using EMSA experiments, we have in fact demonstrated

**Figure 3.** Regulatory elements of the HIV-1 LTR. The HIV-1 LTR is composed of multiple binding sites for transcription factors, most of which are located in the U3 segment. Upstream to the TATA box and the three Sp1-binding sites, the enhancer region operates on HIV-1 gene transcription. In the model depicted at the bottom of the figure, the two transcription factors NF-κB and NFAT are shown to bind independently on the enhancer region and are thought to act synergistically on gene expression. In this model, we speculate that this actual synergy acts via the occupancy of each κB repeat by NFAT and NF-κB.
the formation of an NFAT-related complex on the HIV-1 enhancer. As determined in other EMSA experiments, we have also demonstrated that NFAT1 was the major NFAT family member binding to the HIV-1 enhancer region upon induction with the bpV compounds. Concerns over the overrepresentation of NFAT1 as a component of HIV-1 gene regulation by bpV compounds were raised in relation to a previous report suggesting a negative role of this NFAT member in HIV-1 transcription (140). In this regard, numerous studies including our own have finally demonstrated that like NFAT2, NFAT1 can act positively on HIV-1 gene expression (136, 137, 141). However, other NFAT family members might also be active in the upregulation of HIV-1 transcription induced by bpVs, although this has yet to be shown. These activation patterns are in agreement with the powerful activating potential of PTP inhibitors on HIV-1 transcription likely resulting from an obligatory synergistic action of NFAT and NF-κB. Interestingly, an AP-2-like binding site has been identified between the two NF-κB binding site repeats (133) and could permit the binding of another transcription factor and thereby help NFAT to modulate HIV-1 transcription.

These analyses have thus helped in assessing the important role played by PTPs in the control of HIV-1 transcription and could suggest that their activity permits a certain state of latency to exist in the gene expression of HIV-1. We have recently extended these studies to determine the modulation of LTR regions from different HIV-1 clades by bpV molecules. These studies have demonstrated potent activation of these different clades of HIV-1 LTRs upon bpV treatment. Interestingly, the induction accounted by the bpV compounds was mostly concordant with the number of NF-κB-binding sites present in the enhancer. Indeed, LTRs from HIV-1 clade E isolates typically bear only one repeat (142–144) and were those that were the least activated following bpV-mediated PTP inhibition, as determined by reporter gene transfection studies (unpublished results). In addition, EMSA analyses revealed that the enhancer region from clade E LTR offered the weakest enhancer context for the binding of both NFAT and NF-κB when compared to the enhancer region from the other HIV-1 clade LTRs.

We have also looked at the impact of bpV compounds on the transcriptional control of the HIV-2 LTR, since this LTR also bears an enhancer region with a single NF-κB-binding site. Surprisingly, our results have shown that the HIV-2 LTR was more potently activated by bpV than any of the other HIV-1 clade LTRs. Probing the binding factors on the HIV-2 enhancer indicated weak binding of both NFAT and NF-κB to this region. It is known that the regulation of the HIV-2 LTR is highly dependent on the presence of a second enhancer sequence located upstream of the classic NF-κB-containing enhancer (145–147). Transcription factors, such as Elf-1 and DEK, bind in this region (148, 149) and might also be relevant in the induction of the HIV-2 LTR.
by our PTP inhibitors. Again, such an assumption would argue that several
transcription factors are activated by our PTP inhibitors and can subsequently
contribute to the modulation of HIV-1 (or HIV-2) gene expression.

With respect to the involvement of other transcription factors in bpV-
mediated LTR activation, CREB should be considered as another candidate.
Indeed, in T cell lines, CREB has been demonstrated to contribute to the
regulation of HIV-1 gene expression following treatment with agents that in-
crease intracellular cAMP concentrations such as forskolin and prostaglandin
E$_2$ (PGE$_2$) (150). This modulation of the HIV-1 LTR is suggested to act via
the previously described C/EBP-binding sites (151, 152). Because this factor
is activated by bpV compounds, it might also be involved in HIV-1 LTR acti-
vation by these inhibitors. Further investigations will need to be conducted
to validate these speculations.

Although the bpV compounds have permitted us to begin to understand
general PTP activity in relation to HIV-1 LTR activity, a better focus is needed
on the involvement of each individual PTP expressed in T cells on HIV-1 gene
expression. Hence, several studies from our group have recently been aimed at
obtaining a better understanding of the modulation of LTR activity by CD45.
Instead of PTP inhibitors, we used Jurkat cell lines (as well as the HPB-ALL
cell line) devoid of cell surface CD45 protein to assess the role of this PTP
alone. Because this PTP has been known to contribute positively to T cell activi-
vation, TCR-mediated activation of HIV-1 LTR depends on the presence of
CD45. However, in one study, it was reported that the absence of CD45 ex-
pression might lead to an upregulation of basal HIV-1 gene transcription
(153). In our laboratory, experiments performed on CD45-positive versus
CD45-negative Jurkat cells has revealed that induction with the combination
of the activating agents PMA and ionomycin provided a better activation of
the HIV-1 LTR in CD45-negative cells than in the parental cell line (CD45-
positive cells) (136). Further analyses have demonstrated that this concurred
with a more intense activation of the NFAT transcription factor, which trans-
lated into a stronger NFAT complex bound to the HIV-1 enhancer. In this
study, we have also initiated experiments addressing the pathways responsible
for this higher state of NFAT activation in a CD45-negative cell line. Results
suggest that this increase is due to a greater level of calcium mobilization,
CD3ζ, and ZAP-70 tyrosine phosphorylation, thereby positioning the differ-
ences in NFAT activation level proximally to the membrane. A recent study,
conducted this time in the monocytic cell line U937, has shown that the differ-
entiation of this cell line by PMA was downregulated by the expression of
CD45, an observation that considerably resembles our findings in the Jurkat
cell line model (154). It was further reported that the kinase PKCδ was the
modulated enzyme that was responsible for this CD45-dependent phenome
non. Whether PKCδ or another PKC is responsible for the CD45
FIG. 4. Working model of bpV-induced gene expression in CD4+ T lymphocytes. (A) Untreated or resting T cells display a hypophosphorylated p36LAT due to high constitutive PTP activity and low PTK activity. (B) Upon bpV treatment, PTP activity is abrogated and PTK activity is sufficient to lead to p36LAT phosphorylation and the recruitment of SLP-76 possibly via Grb2-like molecular adapters. SLP-76 could localize Tec family PTK such as Itk to the membrane, which activation is dependent on phosphorylation of its activation loop by p56lck. Upon activation, Itk could phosphorylate p36LAT-associated PLCγ and lead to catalysis of PIP2 to IP3 and diacylglycerol (DAG), two critical second messengers required for calcium mobilization and PKC activation, respectively. Following emptying of the intracellular calcium stores, capacitative entry of calcium induces a high and sustained intracellular concentration of calcium ions and activate calcium-sensitive enzymes such as PKCα, Ca2+/calmodulin kinases (CaMK), and the serine/threonine phosphatase calcineurin, which will activate NFAT. Activation of PKCβ through tyrosine phosphorylation by p56lck and binding of DAG could induce IKK complex activation directly or via TPL-2/Cot and NIK and lead to phosphorylation and degradation of IκB and

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downregulation of NFAT activation in Jurkat cells has yet to be determined. Our data raise an important issue given that HIV-1 had previously been demonstrated to inhibit the PTP activity of CD45 (153, 155, 156). Based on this, it might be postulated that TCR-independent activation of HIV-1-infected T cells might be more efficient in activating HIV-1 gene expression and that this bypass mechanism could help the HIV-1 virus to replicate actively in the absence of antigenic stimulation. More recent studies from our group on the role of CD45 in HIV-1 LTR regulation have centered on the specific modulation by the different existing CD45 isoforms. This was based on the still unknown explanation for the preferential HIV-1 replication observed in CD4+ memory T cells (expressing the CD45RO isoform) in comparison to CD4+ naive T cells (expressing the CD45RABC isoform). Through the use of Jurkat cell lines selectively expressing either isoform, we have shown that a greater level of HIV-1 replication occurred in the CD45RO-expressing Jurkat cells following stimulation (137). The results from this study have further revealed that the CD45RO isoform in Jurkat T cells permits a higher level of activation of the HIV-1 LTR following stimulation by a variety of activating agents. This enhanced LTR activity in CD45RO-positive cells was not only suppressed by cyclosporin A and FK506 but was also paralleled by a greater level of NFAT activation. This study has thus provided a working model to explain the preferential replication observed in memory T cells where a stronger expression of the HIV-1 viral genome would be anticipated in these same cells in comparison to the naive T cells. The implication of NFAT agrees with a previous suggestion by Spina et al. that a cellular factor should be the element responsible for this higher level of replication efficiency characterizing the memory T cells (157).

In summary, results obtained from our group and others indicate that PTPs are important for downregulating HIV-1 gene expression. These results lead to the suggestion that PTPs might thus be important assets in the process of HIV-1 latency. A recent study by Kinoshita and colleagues has further suggested that NFAT could also help in permitting completion of the replication cycle of HIV-1 in T cells (158). In this case, a PTP that could inhibit this transcription factor could also be an important hindrance in the replicative cycle of HIV-1. Another issue that should be considered regarding the expression of the HIV-1 viral genes concerns Tat-dependent LTR activation, highly dependent on the CDK9/cyclin T1 complex. Because this complex is activated by T cell-activating agents, it is likely that PTP inhibitors would also contribute to the upregulation of HIV-1 LTR activation in the context of full length proviral DNA via an

subsequent NF-κB nuclear translocation and transcriptional activation. DAG can also activate RasGRP, which will lead to the Ras/MAPK pathway and AP-1 activation. CREB activation through bpV compounds is weak, but it synergizes strongly in the presence of cAMP-inducing agents such as forskolin and prostaglandins.
increment in the Tat-transactivating potential. The impact of PTP on HIV-1 gene expression remains an important issue and needs further studies. A more focused approach in which individual PTPs are addressed should bring new and interesting information as to their role in HIV-1 replication.

III. Conclusions and Perspectives

Our observations regarding bpV-mediated activation of T lymphocytes points toward a global model in which PTP inhibition initiates signal transduction pathways that are highly similar to those induced upon antigenic costimulation (Fig. 4). These signaling cascades result in activation of multiple transcription factors known to be implicated in the regulation of gene expression following antigenic recognition. These transcription factors are also important for the life cycle of HIV-1 since virus replication occurs mainly in macrophages and activated T cells that express every cellular factors required to complete the replicative cycle of the virus. In the context of a highly active antiretroviral therapy (HAART) that is aimed at controlling viral replication, increasing HIV-1 gene expression could thus help to provide new cellular targets to the immune system or at least ensure a continuous stimulation of virus-specific CD4 and CD8 T cells that could help in the long term control of this infection. Previous attempts at using immunomodulators in conjunction with HAART were aimed at the restoration of lost immune system functions and provided mitigated results. We instead would like to propose the use of non-protein immunomodulators such as purified bpV compounds in combination with the efficient HAART cocktail to drive HIV-1 gene expression and ensure a strong immune response against targets that normally express very low levels of viral proteins. Moreover, the use of small and stable molecules ensures a better dissemination of the immunomodulator in the organism while limiting the risks of developing autoimmune reactions and allergies. We are convinced however that inhibition of a limited set of PTPs can lead to the activation of the cellular factors needed to induce HIV-1 gene expression. Identification of these PTPs could direct our researches toward the development of specific inhibitors that could more specifically enforce HIV-1 gene expression, thereby limiting the establishment of viral reservoirs and promoting the development of a strong immune response against viral proteins. In addition, inhibiting a small subset of PTPs could help to limit undesirable side effects of such drugs while retaining full potency towards HIV-1 gene expression. Identification of PTPs implicated in the regulation of HIV-1 gene transcription is under way. We have already identified CD45 as a major negative regulator of HIV-1 gene expression due to its repression of NFAT (136, 137) but its role in bpV-mediated transcriptional activation of
HIV-1 is nonetheless a positive one since NF-κB activation from TCR-oriented signals depends on the expression of CD45 (103 and unpublished results). We have also examined SHP-1 as a potential candidate (116) and many more PTPs are currently under investigation. Of particular interest is LyPTP which, in synergy with Csk, strongly represses TCR-mediated p56\textsuperscript{ck} activation and could thus be an important target for inhibition that would ultimately lead to strong activation of transcription factors such as NFAT and NF-κB that are critical for initiation of HIV-1 replication.

In a more fundamental setting, inhibition of PTP in T cells by bpV compounds is a useful tool to study signal transduction pathways controlled by tyrosine phosphorylation events. These compounds are an inexpensive and efficient way to evaluate tyrosine phosphorylation-dependent protein-protein interactions in a physiological setting, to investigate conformational changes induced by tyrosine phosphorylation \textit{in vivo} and even to study genes that are controlled by PTPs. We are thus currently performing experiments using DNA microarray technology and two-dimensional polyacrylamide gel electrophoresis to identify genes and proteins that are expressed in primary T lymphocytes following PTP inhibition by bpV compounds. It would also be interesting to determine whether bpV compounds can also inhibit DSPs and LMPTPs. Experiments using immunoprecipitation techniques and phosphatase assays are presently conducted to address this issue, which could have strong implications on this research field. Since bpV molecules are stable and display low toxicity in T cells at concentrations where they are the most efficient, such compounds clearly represent useful assets in the vast collection of cellular activators that are now available. Overall, a better understanding of the key cellular players implicated in the delicate balance of protein tyrosine phosphorylation will certainly provide us with new and interesting targets for therapeutic intervention to combat HIV-1.

**References**


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