The hematopoietic tyrosine phosphatase (HePTP) is predominantly expressed in thymocytes and T lymphocytes and at lower levels in other hematopoietic cells. Expression of the gene is enhanced by the T cell growth factor interleukin-2, suggesting a role for HePTP in T cell proliferation or differentiation. We report that HePTP blocks T cell antigen receptor (TCR)-induced transcriptional activation of a reporter gene driven by a nuclear factor of activated T cells (NFAT)/AP-1 element taken from the interleukin-2 gene promoter. This effect was specific to HePTP and was abolished by a mutation (C270S) that impaired its phosphatase activity. Co-expression of HePTP also reduced TCR-induced activation of the mitogen-activated protein kinase Erk2 and the TCR-induced appearance of phosphorylated Erk. In contrast, HePTP did not affect the activation of the N-terminal c-Jun kinase, Jnk. Together these findings suggest that HePTP plays an active negative role in TCR signaling by dephosphorylating one or several signaling molecules between the receptor and the mitogen-activated protein kinase pathway.

One of the earliest biochemical events seen in T lymphocytes triggered through the T cell antigen-receptor (TCR) is an enhanced, but transient, phosphorylation of a number of cellular proteins on tyrosine residues. Inhibition of this event by pharmacological agents prevents T cell activation as measured by both functional read-outs and biochemical assays (1, 2). It has become evident that several protein tyrosine kinases (PTKs) and the CD45 protein tyrosine phosphatase (PTPase) play crucial roles (reviewed in Ref. 3) and that the TCR-induced cascade of transient tyrosine phosphorylation events depends on a dynamic interplay between these and, presumably, many additional PTKs and PTPases. In addition to CD45 (4–7), only three other PTPases have been implicated in T cell activation, namely SHP1 (8, 9), SHP2 (10), and the low molecular weight PTPase, LMPTP-B (11). The hematopoietic protein tyrosine phosphatase (HePTP) was originally cloned from human T lymphocytes (12–14). The gene is expressed in thymus and at lower levels in spleen, but not in nonhematopoietic tissues. The exon/intron structure of the HePTP gene (15) is quite similar to that of the phosphatase domains of human CD45. In contrast to CD45, however, the 38-kDa HePTP consists of only a single PTPase domain, which occupies the C-terminal three-fourths of the enzyme and is preceded by a ~70-amino acid noncatalytic N terminus. Presently, very little is known about the physiological function of HePTP, but some indications may be derived from the findings that its gene maps to chromosome 1q32.1 (15), which is a site for frequent chromosomal abnormalities in preleukemic myeloproliferative disease (16, 17), and that gene amplification and overexpression of HePTP have been reported for leukemic cells (18). In addition, expression of HePTP in NIH3T3 cells resulted in altered cell morphology and decreased contact inhibition (13). Together, these findings suggest a role for HePTP in the regulation of cell proliferation, survival, or differentiation. This possibility is supported by the up-regulation of HePTP mRNA in T cells in response to interleukin-2 (IL-2) (18), and by the rapid phosphorylation of HePTP on tyrosine in RBL-2H3 mast cells stimulated through their FceRI (19).

Since HePTP is preferentially expressed in T cells and may be involved in the regulation of cell proliferation, we decided to investigate the involvement of this enzyme in TCR-induced T cell activation. We report that HePTP had a strong inhibitory effect on the transcriptional activation of a reporter gene driven by three tandem nuclear factor of activated T cells (NFAT)/AP-1 elements derived from the 5′ IL-2 gene promoter, while a catalytically inactive C270S mutant HePTP did not. HePTP also reduced TCR-induced activation of the mitogen-activated protein kinase (MAPK) Erk2, but not of the N-terminal c-Jun kinase (Jnk). Based on these findings we suggest that HePTP plays a negative role in TCR signaling by acting on signaling molecules upstream of MAPK and transcriptional activation of the IL-2 gene.
RESULTS

Cloning of HePTP and Characterization of the HePTP Antisera—The coding region of HePTP was amplified by the polymerase chain reaction technique using oligonucleotide primers corresponding to both ends of the open reading frame and with human HePTP (clone HEPTP2761 PBSKKM from B. Zanke) as the template. The 1019-base pair amplification product was cloned into the pEF-HA vector and sequenced. The obtained nucleotide sequence was 100% identical to the published sequence (12). Transient expression of the pEF-HA-HePTP construct in COS or J-TAg cells resulted in the appearance of a ~40-kDa (the tag adds ~2 kDa) protein that was both immunoblotted and immunoprecipitated with the anti-HA tag mAbs 12CA5 and 16B12. The expression plasmid was also well expressed in Jurkat, JCaM1, and particularly well in J-TAg cells. Very similar expression levels were seen with the catalytically inactive C270S mutant of HePTP. Fig. 1d shows a PTPase assay of anti-tag immunoprecipitates obtained from 20 × 10^6 J-TAg cells transfected with empty pEF-HA vector or the HePTP constructs or another PTPase TCPTP and its inactive (C216S) mutant. As expected, the wild-type enzymes had easily measurable catalytic activity, while the inactive enzymes did not. The amounts of wild-type and mutated proteins were similar (Fig. 1d, inset).

The full-length open reading frame of human HePTP was also subcloned into the prokaryotic expression vector pGEX-2T (with a glycine kinker) and the recombinant fusion protein was expressed, purified by glutathione-Sepharose 4B chromatography, cleaved by thrombin, purified, and used for immunization of two rabbits. The resulting antisera were highly reactive against a 38-kDa endogenous protein in Jurkat T cells (Fig. 1a) and a 40-kDa protein in T cells or COS cells transfected with the pEF-HA-HePTP construct (Fig. 1, b and c). The polyclonal anti-HePTP antisem also precipitated enzymatically active HePTP from 20 × 10^6 Jurkat T cells: the colorimetric assays gave an A 0.009 and 0.011. The corresponding values for 20 × 10^6 JCaM1 cells were 0.423 and 0.369 versus 0.008 and 0.011.

HePTP Suppresses TCR-induced Activation of an NFAT/ AP-1 Element from the IL-2 Promoter—Activation of T cells through the TCR plus accessory molecules leads to the transcriptional activation of the IL-2 gene, followed by production and secretion of IL-2. Subsequently, this lymphokine binds to specific high-affinity receptors present mainly on activated T cells and drives the cells through the cell cycle, resulting in the clonal proliferation of T cells. TCR-induced activation of the IL-2 gene is conveniently measured by transfecting T cells with a luciferase reporter construct driven by elements from the IL-2 gene 5' promoter.

First, we transfected J-TAg cells with the NFAT/AP-1-luc reporter together with HePTP or empty pEF/H vector. Two days after transfection the cells were treated with OKT3 for 6 h, lysed, and the activity of the induced luciferase measured. As can be seen in the upper panel of Fig. 2., co-expression of HePTP strongly reduced the capacity of the TCR/CD3 to activate NFAT/AP-1. This result was obtained in three independent experiments (duplicate determinations in each). However, due to the SV40 large T antigen, these cells express high levels of transiently transfected constructs, making the system potentially prone to artifacts. Therefore, we next used JCaM1 cells, in which pEF-based constructs are expressed at levels close to the endogenous amounts of signaling proteins (25). These cells also lack endogenous Lck (23) and have very low levels of Syk (26) and do not respond to anti-CD3 mAbs unless either kinase is re-expressed. First, we transfected these cells...
with Lck alone or in combination with wild-type HePTP or catalytically inactive HePTP-C270S together with the NFAT/AP-1-luc reporter. Two days after transfection the cells were treated with OKT3 for 8 h, lysed, and the luciferase activity measured. As shown in the lower panel of Fig. 2., expression of Lck restored the capacity of the TCR/CD3 to activate NFAT/AP-1. In comparison, co-transfection of active HePTP reduced the activation of the reporter construct to less than 10%, while the C270S mutant did not have any significant effect. This result was obtained in four independent experiments (duplicate determinations in each), and control blots showed that HePTP and Lck were similarly expressed in all cases (Fig. 2, lower panel inset).

To determine whether the effect of HePTP was specific for the co-expression with Lck, we utilized our recent observation that expression of Syk in JCaM1 cells also enables the TCR to induce a normal activation of the NFAT/AP-1 reporter gene (25). When HePTP was co-transfected with Syk and NFAT/AP-1-luc, there was a very substantial reduction in the NFAT activation induced by OKT3 compared with cells transfected with Syk and NFAT-luc alone. The average reduction from eight determinations was 83.5% with a total block in many experiments. In contrast, the catalytically inactive HePTP-C270S augmented the activation of the reporter somewhat, but this augmentation was variable and was not seen in all experiments. Therefore, we felt that it was important to compare these effects of wild-type and C270S-mutated HePTP with another number of other PTPases. As can be seen in Fig. 3, SHP1, TCPTP, PTP36, VHR, LMPTP (all in the same pEF vector), and the c-Cbl protooncogene all had a slight stimulatory effect on the OKT3-induced Syk-mediated NFAT/AP-1 activity. Thus, the strong inhibition by HePTP is unique to this PTPase. We also used a positive control in these experiments, namely Vav, which we have earlier shown to synergize with Syk in mediating TCR-induced NFAT/AP-1 activation (27). Co-expression of Vav with Syk augmented the activation of the reporter construct about 20-fold. This suggests that the slight stimulatory effect of HePTP-C270S, the control PTPases, and c-Cbl are insignificant. Note that Syk is equally expressed in all samples and that the HA-tagged PTPases are all expressed at comparable levels, and most importantly that wild-type and C270S-mutated HePTP are present at equal amounts.

Effect of HePTP on TCR-induced Erk2 Activation—As the IL-2 promoter activation experiments above suggest that HePTP may play a role in TCR-induced T cell activation somewhere between the TCR and the IL-2 gene, we next tested a more receptor-proximal read-out for TCR signaling, namely the activation of the MAPK pathway. JCaM1 cells were transiently co-transfected with a Myc-tagged Erk2 MAPK together with HePTP or HePTP-C270S. Two days after transfection, each sample was divided in half; one-half was left unstimulated while the other half was treated with anti-CD3 mAbs for 5 min at 37 °C. After cell lysis, the tagged Erk2 was immunoprecipitated with the 9E10 anti-c-Myc mAb, and after washing these precipitates were subjected to in vitro kinase assays with myelin basic protein as a substrate and the phosphorylation of the substrates was visualized by autoradiography. As can be seen in Fig. 4, the tagged Erk2 was not significantly activated in cells co-transfected with empty pEF/HA vector (lanes 1 and 2). Co-expression of Lck (lanes 3 and 4) enabled the anti-CD3 mAb to cause several-fold activation of Erk2. Furthermore, HePTP had a significant inhibitory effect on MAPK activation whereas HePTP-C270S augmented the effect. The amount of Erk2 was found to be similar in all the samples (Fig. 4, middle panel), and HePTP and HePTP-C270S were equally expressed in lanes 5–8 (lower panel). The expression of Lck was also similar in lanes 3–8 (not shown). This result was obtained in several independent experiments.

HePTP Also Blocks TCR-induced Phosphorylation of Endogenous Erk—To further verify that the effect of HePTP on Erk2 truly reflects an inhibitory effect on the intracellular mechanism of MAPK activation rather than a direct effect in the kinase assays or some other artificial event connected to the transfected Erk, we analyzed lysates from JCaM1 cells transfected with Lck with or without HePTP, HePTP-C270S, TCPTP, or TCPTP-C216S by immunoblotting with antibodies...
specific for the phosphorylated and activated form of Erk.

These blots (Fig. 5, upper panel) showed that phospho-Erk was only induced by OKT3 treatment in cells expressing Lck and that catalytically active HePTP reduced this induction. In contrast, the C270S mutant of HePTP, as well as the TCPTP constructs, did not. Control blots showed that all samples contained similar amounts of endogenous MAPK (second panel) and that HePTP and TCPTP were well and equally expressed (third panel), as was Lck (bottom panel). This result was obtained in three independent experiments.

HePTP Does Not Affect the Jnk Pathway—To further un-
HePTP in T Cell Activation

stand the negative effect of HePTP on NFAT/AP-1 activation, we decided to also study the effect of this PTPase on the Jnk kinase pathway, which is involved in the activation of c-Jun, a component of the AP-1 complex. Jurkat cells were transfected with empty vector, Jnk1 (in pEFl/HA), alone or together with HePTP. Two days after transfection, samples were treated with OKT3 and anti-CD28 (mAb 9.3) plus sheep anti-mouse Ig antibody or with the secondary antibody alone. 20 min later, the cells were lysed and the HA-tagged Jnk1 immunoprecipitated with the 12CA5 anti-HA mAb and subjected to in vitro kinase assays using glutathione S-transferase-c-Jun as a substrate. These experiments consistently failed to reveal any effect of HePTP despite good activation of Jnk1 and good expression of both Jnk1 and the PTPase (not shown). We conclude that the substrate(s) for HePTP are involved in MAPK regulation, but not in Jnk activation.

**DISCUSSION**

Reversible tyrosine phosphorylation is a critical mechanism for the regulation of signal transduction, cell growth, differentiation, and development. These processes involve the balanced action of both PTKs and PTPases, which also regulate each other in a complex manner. In T cells, the repertoire of PTKs involved in the earliest signaling events initiated by ligation of the TCR and its co-receptors is relatively well characterized. In contrast, the PTPases that regulate these PTKs and that counter their phosphorylation of key signaling molecules are largely unknown. The transmembrane receptor-like PTPase CD45 was the first PTPase implied in T cell activation (3–7). This enzyme dephosphorylates the C-terminal negative regulatory tyrosine in the Src family PTKs Lck (6, 7) and Fyn (28). This function of CD45 is not induced by TCR triggering, but occurs in a constitutive manner and is critical for T cell for activation, presumably by maintaining a large fraction of Lck and Fyn in a potentially active state (3) and by promoting a relatively high level of basal tyrosine phosphorylation of the activation motifs in the TCR-ζ and CD3 chains. Another PTPase, the SH2 domain-containing PTPase SHP1, has been implicated as a negative regulator of signaling from the TCR as well as many other hematopoietic receptors. The evidence for its direct participation in TCR triggering, however, is not compelling, and it may be that SHP1 is mainly involved in signaling by inhibitory receptors, such as p58KIR, p70KIR, FcγRIIB, and it may be that SHP1 is mainly involved in signal-

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Negative Regulation of T Cell Antigen Receptor Signal Transduction by Hematopoietic Tyrosine Phosphatase (HePTP)
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