Regulation of Src Family Kinases Involved in T Cell Receptor Signaling by Protein-tyrosine Phosphatase CD148

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CD148 is a receptor-like protein-tyrosine phosphatase known to inhibit transduction of mitogenic signals in non-hematopoietic cells. Similarly, in the hematopoietic lineage, CD148 inhibited signal transduction downstream of T cell receptor. However, it also augmented immunoreceptor signaling in B cells and macrophages via dephosphorylating C-terminal tyrosine of Src family kinases (SFK). Accordingly, endogenous CD148 compensated for the loss of the main SFK activator CD45 in murine B cells and macrophages but not in T cells. Hypothetical explanations for the difference between T cells and other leukocyte lineages include the inability of CD148 to dephosphorylate a specific set of SFKs involved in T cell activation or the lack of CD148 expression during critical stages of T cell development. Here we describe striking differences in CD148 expression between human and murine thymocyte subsets, the only unifying feature being the absence of CD148 during the positive selection when the major developmental block occurs under CD45 deficiency. Moreover, we demonstrate that similar to CD45, CD148 has both activating and inhibitory effects on the SFKs involved in TCR signaling. However, in the absence of CD45, activating effects prevail, resulting in functional complementation of CD45 deficiency in human T cell lines. Importantly, this is independent of the tyrosines in the CD45 C-terminal tail, contradicting the recently proposed phosphotyrosine displacement model as a mechanism of SFK activation by CD148. Collectively, our data suggest that differential effects of CD148 in T cells and other leukocyte subsets cannot be explained by the CD148 inability to activate T cell SFKs but rather by its dual inhibitory/activatory function and specific expression pattern.

Protein tyrosine phosphorylation plays an important role in transducing many cellular signals. The tyrosine phosphorylation status of individual proteins is regulated by opposing actions of protein-tyrosine kinases and PTPs. The effects of PTP activity on signal transduction can be both stimulatory and inhibitory, depending on the individual PTP specificity. In TCR signaling, some PTPs (e.g. SHP-1) have a largely negative impact on signal propagation, whereas the CD45 PTP appears to have a dual function. It enables the activation of SFKs by dephosphorylating their inhibitory C-terminal phosphotyrosine (Tyr-505 in Lck and Tyr-528 in Fyn) and is, thus, indispensable for the initiation of TCR signal transduction. However, CD45 also plays a direct or indirect role in the dephosphorylation of SFKs at their catalytic sites (Tyr-394 for Lck), which negatively influences their activity (1). Another PTP expressed in the immune system, CD148, also appears to produce both activating and inhibitory effects.

CD148 (DEP-1, PTPRI) is an R3 family receptor-like PTP with a large highly N-glycosylated extracellular segment containing multiple fibronectin III-like repeats, a transmembrane domain, and a single intracellular protein-tyrosine phosphatase domain followed by a short C-terminal tail containing three conserved tyrosine residues (1–5).

CD148 is a broadly expressed PTP found in non-hematopoietic tissues such as epithelia and fibroblasts as well as in leukocytes (2, 4, 6). In peripheral blood, CD148 is expressed on all white blood cell populations, including T cells, and platelets in humans (5–8). CD148 is further up-regulated on T cells after activation with mitogens (5, 6, 8). Less is known about CD148 expression on thymocytes. Immunohistochemical staining of human thymi revealed that CD148-positive T cells are localized in medulla, suggesting its expression in mature thymocytes (5, 9). This was supported by flow cytometry that detected CD148 only on CD3-positive thymocytes (5). However, examination of CD148 expression at defined stages of thymocyte development has not been reported.

Similar to human cells, the majority of murine peripheral leukocyte subsets are also CD148-positive. However, naïve T cells are a notable exception, exhibiting very weak CD148 expression (10). This also seems to be reflected in contrasting data obtained with murine and human thymi as only mouse...
Expression and Function of CD148 in T Cells

CD4^−/CD8^− double negative thymocytes, representing the earliest stage of T cell thymic development, exhibited low level CD148 positivity (10). A wide range of evidence suggests that CD148 acts as a tumor suppressor in non-hematopoietic tissues (11–17), likely by dephosphorylating and negatively regulating receptor tyrosine kinases (16, 18–21) and/or downstream signal transducers (13, 22). On the other hand, CD148 also plays an activating role via dephosphorylation of the inhibitory tyrosine in Src (19, 23).

CD148 function has been studied in hematopoietic lineage cells by using CD148^+/− mice, where partially blocked B cell receptor and macrophage FcR signaling was observed (24). Double-deficient CD148^−/− CD45^−/− mice revealed overlapping functions of these PTPs in B cells and macrophages. Double-deficient cells were unable to signal via B cell receptor and FcR due to the inactivation of SFKs, which were hyperphosphorylated at their inhibitory tyrosines (24). In platelets, which do not express CD45, CD148 inactivation alone was sufficient to block signaling through glycoprotein VI and αIIbβ3 integrin, again most likely due to the inability of CD148-deficient platelets to activate SFKs (25).

It has been speculated that conserved tyrosine residues in the C-terminal tail of CD148 may be critical for SFK activation by CD148 (26). In members of R4 PTP family, similar tyrosines have been shown to play a major role in this process via displacing SFK inhibitory tyrosine from the SH2 domain, thus making it available for dephosphorylation (27). However, this model has never been tested on any R3 family member including CD148.

In T cells, no specific effect of CD148 deficiency was observed on wild type or CD45^−/− genetic backgrounds in mice (1, 24). However, this may be explained by the lack of CD148 on murine thymocytes (beyond DN stage) as well as naïve T cells. The function of CD148 in human T cells has been studied using two approaches; that is, CD148 cross-linking with a specific antibody and CD148 ectopic expression in CD148 negative Jurkat T cell line. Cross-linking of CD148 induced calcium influx and tyrosine phosphorylation in human peripheral blood leukocytes (7, 28) and enhanced peripheral T cell proliferation induced by anti-CD3 antibody in vitro (7, 8). Although it is not clear how the cross-linking affects CD148 function, these data clearly show that modulation of endogenous CD148 activity and/or localization impacts peripheral T cell signaling. Forced expression of CD148 in Jurkat cells inhibited both proximal and distal effects of TCR engagement probably via inhibition of LAT and PLCγ1 phosphorylation (29–31). This was observed even at physiologically relevant levels of CD148 expression (29). Taken together, studies to date suggest that CD148 is a negative regulator of TCR signaling, which is in apparent contradiction with data obtained from other leukocyte populations.

To better understand the role of CD148 in human T cell development and function, we analyzed the expression of CD148 during T cell development in mice and humans as well as the ability of CD148 to activate SFKs in human T cells. We describe striking differences in CD148 expression on different human and mouse thymocyte subsets. Moreover, we show that CD148 is capable of SFK activation and complements CD45 deficiency in human T cells.

EXPERIMENTAL PROCEDURES

Antibodies—For Western blotting and cell activation, the following antibodies were used anti-phosphotyrosine (clone 4G10, mouse origin (Upstate Biotechnologies, Lake Placid, NY), human Lck and human Fyn (rabbit, kindly provided by A. Veillette, Clinical Research Institute of Montreal, Canada), human Lck (mouse, Exbio, Vestec, Czech Republic), human Erk2 (rabbit, Santa Cruz Biotechnology, Santa Cruz, CA), Jurkat TCRβ (clone C305 (32)), human CD3 (MEM92, mouse, Exbio), human LAT (rabbit, kindly provided by L. Samelson, Center for Cancer Research, Bethesda, MA), human PLCγ1, Myc tag (mouse), phospho-p42/44 MAPK (Erk1/2) (Thr-202/Tyr-204), phospho-Src family (Tyr-416), phospho-Src (Tyr-527), non-phospho-Src (Tyr-527, all three chicken Src numbering), phospho-Lck (Tyr-505), phospho-PLCγ1 (Tyr-783) (rabbit, Cell Signaling, Danvers, MA), phospho-LAT (Tyr-191, rabbit, Upstate Biotechnology). For flow cytometry, mouse antigens were CD148 (clone 8A1-1, hamster (10)), TCRβ-FITC, CD8α-PB, CD11b-APC, CD11c-APC, Thy1.1-FITC (eBioscience, San Diego, CA), TCRγ-Alexa Fluor 680, CD45-FITC, NK1.1-APC, CD19-DyLight 647, CD45-PB (Exbio), CD19-PB, CD4-PerCP/Cy5.5 (BioLegend, San Diego, CA). For flow cytometry, human antigens were CD148-PB (clone 143–41, R&D Systems, Minneapolis, MN), TCRαβ-PB (BioLegend), CD19-APC, CD44-PB, CD69-APC, CD1a-APC, CD8-Alexa Fluor 700 (Exbio), CD27-FITC, CD45RA-PE-Cy7, CD3-APC-H7, CD34-PerCP-Cy5.5 (BD Biosciences), CD4-ECD (Immunotech, Marseille, France), and CD45-PO (PO, Pacific Orange (Invitrogen)). Secondary antibodies were goat anti-mouse-HRP IgG-specific (Sigma), goat anti-rabbit-HRP (Bio-Rad), goat anti-mouse-HRP light chain specific (Jackson ImmunoResearch, West Grove, PA), goat anti-mouse-IRDye 680 and goat anti-rabbit-IRDye 800CW (LI-COR Biosciences, Lincoln, NE), goat anti-hamster-DyLight 549 (Rockland, Gilbertsville, PA), donkey anti-goat-DyLight 549 (Jackson ImmunoResearch), and goat anti-mouse-Alexa Fluor 488 (Invitrogen). Monoclonal antibodies against human CD148 were generated by standard techniques. Briefly, F1 (BALB/c × B10.A) hybrid mice were immunized intrasplenically with the bacterially expressed and purified antigen (Clontech Laboratories, Mountain View, CA) His-tagged N-terminal fragment (aa 36–452) of human CD148. Hybridomas were prepared and selected by standard techniques using Sp2/0 myeloma cells as fusion partners.

dDNA Constructs, Cloning, and Mutagenesis—The construct encoding the Myc-tagged version of human CD148 was generated using fusion PCR to insert the Myc-tag coding sequence (EQKLISEEDL) downstream of the leader peptide between amino acids Gly-38 and Thr-39 of the CD148 precursor protein, and the resulting product was cloned into MSCV-IThy1.1 vector (NotI/Sall), kindly provided by P. Marrack (National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, Bethesda, MD), and generated by restriction cloning. 2YF and 3YF mutants, where tyrosines 1311/1320 or 1311/1320/1335, respectively, were replaced with phenylalanines, were generated with the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA) according to the manufacturer’s instruc-
Expression and Function of CD148 in T Cells

A similar procedure was also used to generate substrate trapping mutant of CD148 where aspartate 1205 was replaced with alanine. The CD148-HP1 chimera consisting of aa 1–1018 of CD148 (containing the Myc tag described above) followed by aa 214–595 from SHP-1 was generated using fusion PCR. Myc-CD45 in MSCV-IThy1.1 encoding a protein composed of CD148 signal peptide (aa 1–38), Myc tag, and CD45RABC-coding sequence was generated from the Myc-CD148 construct by replacing all of the CD148 coding sequence downstream of the Myc tag with aa 26–1304 of CD45RABC. All the constructs were verified by sequencing.

Cell Lines and Primary Cells—JS-7 (33), Jurkat with inducible CD148 TetOn expression (29), CD45+ HPB-ALL (34) (kindly provided by P. Beverley, The Jenner Institute, Compton, UK), and J45.01 (35) T cell lines and Phoenix Amphi cells (Origene, Rockville, MD) were cultivated in RPMI 1640 or DMEM, respectively, supplemented with 10% FBS, 2 mM glutamine, 20 μg/ml gentamycin, 50 μg/ml streptomycin, and 10^4 units/ml penicillin at 37 °C in 5% CO2. Murine blood and thymi were collected from healthy C57Bl/6j mice 2–16 weeks old (obtained from Institute of Physiology AS CR, Prague, Czech Republic) or from fresh blood of healthy donors or their guardians and in accordance with local ethical guidelines and declaration of Helsinki. They were also approved by the Institutional Review Board and Animal Care and Use Committee of Institute of Molecular Genetics as well approved by the Institutional Review Board of University Hospital in Motol, Prague.

Cell Activation—JS-7 cells were activated with 4 μg/ml soluble C305 antibody. The activation was stopped by rapid cell lysis in SDS-PAGE sample buffer. To analyze CD69 up-regulation, cells were activated overnight by plate-bound C305 antibody. CD69 expression was analyzed by flow cytometry.

Biochemical Procedures—To obtain whole cell SDS lysates, cells in serum-free RPMI or PBS (50 million/ml) were lysed by adding equal volume of 2× concentrated SDS-PAGE sample buffer followed by sonication. These lysates were used to quantify the phosphorylation status of SFKs by immunoblotting using IRDye 680- or IRDye 800CW-conjugated secondary antibodies, Odyssey Infrared Imaging System (LI-COR), and AIDA image analyzer software (Raytest, Straubenhardt, Germany). For analysis of other phosphorylation events, standard peroxidase-based ECL techniques and exposure to x-ray films was used. For immunoprecipitation experiments, 7.5 x 10^7 cells were lysed in 1.5 ml of ice-cold laurylmaltoide lysozyme buffer (1% dodecylmaltoside, 1 mM Pefabloc, 5 mM iodoacetamide, 1 mM sodium orthovanadate, 100 mM NaCl, 50 mM NaF, 10% glycerol v/v, 20 mM Tris, pH 7.5) and incubated for 30 min on ice. Nuclei and debris were removed by centrifugation, and the resulting lysate was subjected to immunoprecipitation with anti-Myc antibody (3.3 μg/ml) followed by Protein A/G PLUS-Sepharose (Santa Cruz Biotechnology). Immunoprecipitates were eluted with SDS-PAGE sample buffer and subjected to immunoblotting. For substrate trapping experiments with Myc-CD148 D1205A, ice-cold phosphate buffer without phosphatase inhibitors (0.2% dodecylmaltoside, 1 mM Pefabloc, 50 mM NaCl, 10% glycerol v/v, 100 mM phosphate, pH 7.5) was used for cell lysis.

Flow Cytometry—Cells lines were stained with the indicated antibodies. Murine leukocytes were stained in PBS, 1% BSA, 20% mouse serum for 30 min on ice. The 8A-1 hamster antibody recognizing mouse CD148 gives only a very low signal using standard staining procedure (10). Thus, we used a sequential double secondary antibody staining (goat anti-hamster DyLight 549 and donkey anti-goat DyLight 549) to increase the specific signal intensity. Human peripheral blood mononuclear cells were stained in PBS, 1% BSA, 20% human AB serum on ice for 30 min. Human thymocytes were stained for 30 min in PBS with 2 mM EDTA at 4 °C. For calcium response measurements, JS-7 cells and their derivatives were loaded with 2 μg/ml Fluor-4 dye (Invitrogen) and analyzed by flow cytometry at 37 °C for 30 s at rest and then activated with 0.2 μg/ml C305 antibody, and the fluorescence was followed for another 3–4 min. The calcium response index was calculated as the percentage of cells with Fluor-4 fluorescence higher than the 95th percentile of resting cells during the time interval between 10 and 20 s. An LSR II (BD Biosciences) flow cytometer was used for analysis of surface markers. A FACScalibur (BD Biosciences) flow cytometer was used for calcium flux measurements. Data were analyzed using FlowJo software (TreeStar, San Carlos, CA).

Cell Sorting and Quantitative RT-PCR—Thymocytes from 2 mice (4–6 weeks old) were pooled and stained with the indicated antibodies in Hanks’ balanced salt solution, 25% goat serum on ice for 45 min, and sorted using an Influx cell sorter (BD Biosciences). Of αβT cell developmental stages (gated as CD45+/CD11b−/CD11c−/CD19−/NK1.1−/γδTCR−), 0.5–2 x 10^5 cells of DN (CD3−/CD4−/CD8−), iSP (CD3+/CD4−/CD8−), DP (CD4+/CD8+), SP8 (CD3+/CD4−/CD8−), and SP4 (CD3+/CD4+/CD8−) subpopulations were collected. RNA was isolated using a Quick-RNA MiniPrep kit (Zymo Research Corp., Irvine, CA). Reverse transcription was performed with RevertAid reverse transcriptase (Fermentas, Thermo Fisher Scientific, Waltham, MA) using a combination of random pentadecamer and anchored oligo(dT)20 primers. Quantitative PCR was performed using a LightCycler 480 SYBR Green I Master chemistry (Roche Applied Science) in duplicate with following primers: CD148-for 5′-ctgatgtctgcacagagaca-3′, CD148-rev ctcagctggctgaggctt, Actb-forward cctgcttctgctgtggctt, Actb-rev cacaggacatcagagac, Tbp-forward gcggctgggtgctaggtt, Tbp-rev gggtatccacaccactgta, Tubb2A-forward aaccaaggctggctgctctgcttgt, Tubb2A-rev tggcagagctccttagtta, Hprt1-forward 5′-ctccctcagacgagcccttt, Hprt1-rev cctccgctatcagactgaatc, Eef1a-forward acagcatggagccagcaaat, dEef1a-revers rev aggagccctttcccatctc. Primer efficiencies were determined on diluted cDNA from DN cells. Relative mRNA amounts were calculated from measured Ct.
Expression and Function of CD148 in T Cells

Values, and primer efficiencies with DN mRNA levels were arbitrarily set as 1. CD148 mRNA level was normalized to a geometric mean of all five or four (excluding Eef1a1) reference genes.

Retroviral Infection—Phoenix Ampho cells were transfected with retroviral MSCV-1Thyl.1 vector using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions. Virus-containing supernatant was supplemented with 10 μg/ml Polybrene (Sigma) and added to J7 cells. The mixture was centrifuged for 90 min (1250 × g). Positive cells were sorted by auto-MACS Pro Separator (Miltenyi, Bergisch Gladbach, Germany) after staining with an anti-Thy-1.1-FITC antibody on ice and subsequently with anti-FITC microbeads (Miltenyi) at 4°C.

RNA Interference—CD148 knockdown in J7 cells was performed by electroporation (700 V/cm, 60 ms) of 5 × 10^5 cells with 100 pmol of siRNA1 (ID s230207, Ambion, Austin, TX) or siRNA2 (ID s230208) in 100 μl of Opti-MEM media (Ambion) using a BTX electroporator (Harvard Apparatus, Holliston, MA). Cells were used for analysis 2 days after electroporation.

Statistics—For calculation of statistical significance, a two-tailed Student’s t test (unequal variance) was used.

RESULTS

CD148 Is Differentially Expressed on Human and Murine T Cells—Published data on CD148 expression appeared to be partially inconsistent. Although CD148 was not detected on murine peripheral T cells (10), human T cells were repeatedly shown to be CD148-positive (5–7). Moreover, murine thymocytes were almost CD148-negative with a weak signal only at early stages (10). In contrast, the limited data available on human thymus suggested that the regulation of CD148 expression on human thymocytes may be very different, with CD148 expression appearing at the later stages of T cell development (5, 9). However, no detailed subset analysis has been carried out.

When analyzed by flow cytometry, human blood T cells exhibited substantial levels of CD148, albeit somewhat lower than B cells. In contrast, CD148 was hardly detectable on murine blood T cells (Fig. 1A). To compare signals given by two different antibodies (to human and murine CD148), we calculated the T cell intensity as a percentage of the B cell mean fluorescence intensity (Fig. 1B). Thus, the expression of CD148 on peripheral T cells relative to B cells was much higher in humans than in mice. Next, we analyzed the expression of CD148 during particular thymocyte developmental stages in mice and humans by flow cytometry (supplemental Figs. S1 and S2). An improved staining procedure enabled us to measure the expression of murine CD148 more reliably than before (see “Experimental Procedures”). In mice, CD148 is expressed at the DN stage but sharply drops through the DP stage to the SP stage cells, which exhibited only very low CD148 amounts (Fig. 2, A and C). Staining of human thymocytes revealed a completely inverse pattern as DN cells were CD148-negative and only a relatively low number of DPs displayed CD148 positivity (Fig. 2, B and D). On the other hand, a substantial percentage of SP cells exhibited high CD148 expression.

To rule out the possibility that the loss of anti-CD148 staining on murine thymocytes during the development could be caused by the loss of the epitope on CD148 (e.g. due to alternative glycosylation or splicing), we performed quantitative RT-PCR on sorted thymic populations. Using primers specific for conserved intracellular part of CD148 molecule, we detected the highest level of CD148 mRNA in DN cells with a gradual decline during the maturation, thus confirming the flow cytometry data (Fig. 2E).

Completely different CD148 expression in thymus between mice and humans provides an explanation for differences in CD148 expression in peripheral T cells. Loss of CD148 during transition of murine thymocytes to DP and SP stages is consistent with CD148 negativity of mature peripheral T cells. In contrast, human thymocytes gain CD148 expression at later thymocyte developmental stages and retain it in the periphery.

CD148 Is Expressed on Mature CD1a−, CD27+, CD44+ Thymocytes in Humans—DP and SP cells in human thymus contained both CD148-negative and -positive cells (Fig. 2, B and D); thus, we performed analysis of thymic CD148 expression in more detail. CD1a is a marker of cortical immature thymocytes. Its expression is lost during maturation of single-positive cells and is accompanied with transition to a terminal thymocyte stage (36). Expression of CD148 negatively correlated with expression of CD1a in the whole thymus (Fig. 3A), suggesting that CD148 could be expressed exclusively by the most mature human thymocytes. Further analysis was focused on DP and SP cells (excluding iSPs). The maturation status of these cells was assessed based solely on the expression of CD1a and CD44 (36–38). This approach simplified the gating process as well as the data representation in the manner complementary to the strategy used in Fig. 2. Development from early DP cells into mature SPs starts at CD44−CD1a−/stage progresses through a CD44+CD1a−/stage and terminates at CD44+CD1a−/stage (Fig. 3B). Classification according to CD44 and CD1a expression correlated well to DP/SP transition. CD44−CD1a− cells consisted mainly of DP cells, and the CD44+CD1a+ population contained comparable numbers of DP and SP, predominantly CD4−SP, and finally, CD44+CD1a− thymocytes represented almost exclusively SP cells, highly positive for CD3 (Fig. 3C and not shown). Cells expressing markers of successful positive selection, CD27 and CD69, appeared at CD44+CD1a− stage.
and constituted a majority of CD44^{+}CD1a^{-} cells (Fig. 3C). Interestingly, CD148 up-regulation was delayed after CD27 and CD69 markers as CD148 was rarely expressed before the cells reached a CD44^{+}CD1a^{-} terminal stage (Fig. 3C). Expression of CD45RA splice isomorph followed a similar kinetics as CD148. Interestingly, CD45RA and CD148 exhibited rather an inverse correlation on mature CD44^{+}CD1a^{-} thymocytes (of three) is shown. A, staining of the entire thymocyte pool with CD148 and CD1a shows inverse correlation between the expressions of these two surface proteins. B, CD1a versus CD44 stainings of SP + DP thymocyte pool (CD4^{+} and/or CD8^{+}) shows three subsets representing three maturation stages from CD1a^{+}CD44^{-} (here termed I) through CD1a^{+}CD44^{+} (II) to CD1a^{-}CD44^{+} (III). C, shown is expression of CD4 versus CD8 and CD148 versus CD27, CD69, or CD45RA of particular maturation stages gated as shown in B.

Thus, CD148 is expressed mainly on CD44^{+}CD1a^{-} SP thymocytes, representing the terminal stage of thymic T cell development. CD148 was also detected on an unusual subpopulation of DP thymocytes characterized by CD44^{+}CD1a^{-} mature phenotype.

FIGURE 3. CD148 is expressed exclusively on mature thymocytes in humans. Human thymocytes were investigated by polychromatic flow cytometry after staining with antibodies to CD1a, CD3, CD4, CD8, CD44, CD45, and CD27 or CD45RA or CD69. A representative thymus (of three) is shown. A, staining of the entire thymocyte pool with CD148 and CD1a shows inverse correlation between the expressions of these two surface proteins. B, CD1a versus CD44 staining of SP + DP thymocyte pool (CD4^{+} and/or CD8^{+}) shows three subsets representing three maturation stages from CD1a^{+}CD44^{-} (here termed I) through CD1a^{+}CD44^{+} (II) to CD1a^{-}CD44^{+} (III). C, shown is expression of CD4 versus CD8 and CD148 versus CD27, CD69, or CD45RA of particular maturation stages gated as shown in B.

FIGURE 2. CD148 expression is down-regulated in mice but up-regulated in humans during thymic αβ T cell development. A, murine thymocytes from 2–16-week-old mice were stained with antibodies to CD4, CD8, CD11c, CD19, CD148, αβ TCR, γδ TCR, and NK1.1. Cells of non-T cell lineage were gated out, and the remaining cells were divided into five developmental stages: DN, iSP, DP, SP8, and SP4 (see supplemental Fig. 1 for details). CD148 fluorescence intensity (solid black line) as well as background signal (gray) of particular thymocyte subpopulations from a representative thymus (of five) is shown. B, human thymocytes were stained with antibodies to CD3, CD4, CD8, CD34, CD45, and CD148. Thymocytes were divided into five developmental stages similarly as for mouse thymus: DN, iSP, DP, SP8, and SP4 (supplemental Fig. 2). CD148 fluorescence intensity as well as the background signal of particular thymocyte subpopulations from a representative thymus (of six) is shown. C, quantification of CD148 expression in murine T cell subsets is shown as mean fluorescence intensity because CD148 was homogenously expressed in all individual subpopulations. Data are the mean ± S.D., n = 3. D, some human subsets (DP and SP) exhibited a bimodal distribution of CD148 signal; therefore, the percentage of CD148-positive cells at a particular developmental stage is shown. Data are the mean ± S.D., n = 3. E, murine thymocytes from 4–6-week-old mice were stained with antibodies to CD4, CD8, CD11a, CD11b, CD19, CD45, αβ TCR, γδ TCR, and NK1.1 and FACSc-sorted. RNA was isolated and subjected to RT-quantitative PCR with primers specific for CD148, actinβ, tubulinβ2A, TATA-box binding protein, HPRT1, or eEF-1α. The relative amount of CD148 mRNA was determined after normalization using the geometric mean of mRNA levels of all used reference genes. The CD148 mRNA level in DN subpopulation was arbitrarily set as 1. Data are the mean ± S.D., n = 3.
Expression and Function of CD148 in T Cells

CD148 Dephosphorylates Src-family Kinases in T Cells—The positive role in signal transduction and selective ability of endogenous murine CD148 to complement CD45 deficiency in B cells and macrophages but not in T cells (24) could be explained by the lack of CD148 expression in DP stage where the major developmental block in CD45-deficient mice was observed. Alternatively, CD148 could play an opposite role in T cells. This hypothesis is supported by the observation that forced expression of physiologically relevant levels of CD148 in the human T cell line Jurkat resulted in inhibition of T cell signaling (29, 31). To determine whether CD148 is capable of activation of SFKs involved in TCR signaling, we ectopically expressed CD148 in JS-7 cells (33). JS-7 is a T cell line derived from Jurkat and was chosen because it does not express CD45 (33) and (like majority of leukemic T cell lines but in contrast to human peripheral T cells) contains only a low level of CD148 (supplemental Fig. 3), which results in defects in TCR signaling in these cells.

We retrovirally transduced JS-7 to express N-terminal Myc-tagged wild type CD148 (CD148-WT) and the C1239S phosphatase-dead mutant of CD148 (CD148-CS). The proteins were surface-localized with proper transmembrane orientation (supplemental Fig. 3). Interestingly, Myc-CD148-WT or CS co-precipitated with Lck and Fyn (Fig. 4A), suggesting an interaction of CD148 with these SFKs in T cells, independent of CD148 phosphatase activity.

To assess the effect of CD148 expression on the activation state of SFKs in resting cells, we monitored phosphorylation status of SFKs using specific antibodies recognizing Lck phosphorylated at Tyr-505, Src phosphorylated at Tyr-530, or Src non-phosphorylated at Tyr-530 (numbers correspond to human Src protein) via immunoblotting. The latter two antibodies also stain an Src-related kinase Fyn when phosphorylated or non-phosphorylated at Tyr-528 (not shown); therefore, we detected an aggregate pool of Fyn and Src proteins (Fyn + Src) phosphorylated or not at their C-terminal inhibitory tyrosines. Expression of CD148-WT, but not catalytically inactive CS mutant, was associated with reduced phosphorylation of Tyr-505 on Lck (Fig. 4, B, C, and E). Similarly, Fyn and/or Src were also hypophosphorylated at the respective tyrosines in CD148-WT-expressing cells as revealed by the anti-pSrc and anti-non-pSrc antibody stainings (Fig. 4, B, D, and E). Staining with two complementary antibodies to non-phosphorylated and phosphorylated Src enabled us to estimate the ratio of phosphorylated Fyn + Src molecules in resting JS-7 cells and its change after CD148 expression (supplemental Fig. 4). Approximately one-third of Fyn + Src molecules were non-phosphorylated at C-terminal tyrosine in JS-7 cells. The expression of CD148 changed the amount of non-phosphorylated forms of Fyn + Src nearly to one-half of the total Fyn + Src pool. Lck Tyr-505 phosphorylation seemed to be even more affected by CD148 expression (supplemental Fig. 4). Accordingly, TCR-induced Erk phosphorylation was higher in CD148-WT-expressing cells as revealed by the anti-pSrc and anti-non-pSrc antibody stainings (Fig. 4, B, C, and E).

Activation via anti-TCR antibody induced a global increase of protein tyrosine phosphorylation in JS-7 cells that was substantially enhanced by expression of either CD148-WT or CD45 but not CD148-CS (Fig. 5A). CD148 and CD45 also induced phosphorylation of the activation loop tyrosines in SFKs both in resting and TCR-stimulated cells (Fig. 5B). Accordingly, TCR-induced Erk phosphorylation was higher in cells expressing CD148-WT or CD45 (Fig. 5C). Calcium increase was also positively regulated by CD148 and CD45 in JS-7 cells after the TCR engagement (Fig. 5D). CD69, an activation marker whose expression depends on Ras signaling pathways in T cells, was used for analysis of long term effects of TCR...
stimulation. In agreement with the amplification of the proximal signaling pathways, CD69 expression was enhanced by CD148 and CD45 in activated but not resting cells (Fig. 5E).

The low but detectable level of CD148 in JS-7 cells allowed us to further reduce CD148 expression using small interfering RNA (Fig. 5F). Silencing of CD148 reduced the outcome of TCR triggering measured as the number of CD69 up-regulating cells (Fig. 5G).

Examination of the response of JS-7 cells to TCR activation provided us with solid evidence that CD148 is able not only to globally decrease the C-terminal tyrosine phosphorylation of SFKs, but it can positively regulate those SFKs involved in TCR signal transduction. A similar role of CD148 in promoting TCR signaling was observed in two other CD45-deficient T cell lines also; that is, Jurkat cell-derived J45.01 and CD45−HPB-ALL, a cell line unrelated to Jurkat cells (Fig. 6).

CD45 Activity Determines the Net Effect of CD148 on LAT and PLCγ1 Phosphorylation after TCR Triggering—An inhibitory function of CD148 in the CD45-sufficient Jurkat T cell line was demonstrated previously using doxycycline-inducible expression. It has been shown that in these cells, CD148-WT, but not the CS mutant, inhibits TCR-induced phosphorylation of LAT and PLCγ1 (Fig. 6).

**Figure 5.** CD148 complements CD45 deficiency of JS-7 cells. JS-7 cells transduced with CD148-WT, CD148-CS inactive mutant, or CD45 and non-transduced cells (NT) were analyzed for intracellular signaling responses after TCR triggering (A–E), and the effects of CD148 knockdown on TCR signaling in JS-7 cells were examined (F and G). A, transgenic JS-7 cells and non-transduced cells were stimulated with 4 μg/ml anti-TCR-specific antibody for 30 s and immuno-blotted after lysis. Anti-phosphotyrosine (pY) antibody was used to detect overall tyrosine phosphorylation in activated and non-activated cells. Re-probing the membrane with anti-Lck rabbit antibody served as a loading control. The phosphorylated bands in the non-stimulated samples probably represent Src family kinases that migrate in the corresponding molecular weight range. B, transgenic JS-7 cells and non-transduced cells were stimulated with 4 μg/ml anti-TCR-specific antibody for 30 s or left non-stimulated and immunoblotted after lysis. The blots were stained with the antibody to the activation loop tyrosine of SFKs (Tyr(p)-416 (pY416), numbered according to chicken Src). Re-probing the membrane with antibody to total-Lck served as a loading control. C, transgenic JS-7 cells and non-transduced cells were stimulated with 4 μg/ml anti-TCR specific antibody for 1 min and immunoblotted after lysis. Anti-phospho-Erk1/2 (Thr(P)-202/Tyr(P)-204 (pErk)) antibody was used to detect Erk activation in stimulated and non-stimulated cells. Re-probing the membrane with anti-Erk2 antibody was used as a loading control. D, JS-7 cells ectopically expressing CD148-WT (solid black line), CD148-CS mutant (dashed black line), or CD45 (dashed gray line) and non-transduced JS-7 cells (solid gray line) were analyzed by flow cytometry after Fluo4 loading. Anti-TCR antibody (200 ng/ml) was added 30 s after beginning the measurement. One representative experiment (of four) is shown. E, transduced JS-7 cells and non-transduced cells were activated via immobilized anti-TCR antibody overnight and examined for CD69 expression. Black bars represent the CD69 signal of non-stimulated cells (including autofluorescence), and white bars represent CD69 up-regulation after TCR stimulation. Data are the mean ± S.D. Data originate from triplicates from one representative experiment (of five). F, shown are the effects of CD148 silencing by electrosporation of specific interfering RNA oligonucleotides on CD148 surface level in JS-7 cells. G, CD148 silenced and control JS-7 cells were examined for up-regulation of CD69 after plate-bound anti-TCR antibody stimulation via flow cytometry. *, p < 0.005. Data are the mean ± S.D. Data originate from triplicates from one representative experiment (of four).
Expression and Function of CD148 in T Cells

FIGURE 6. CD148 complements CD45 deficiency in two additional T cell lines. J45.01 cells (A) and CD45-HPB-ALL (B) were transduced with N-terminal Myc-tagged CD148-WT. Expression of CD148 was verified by extracellular anti-Myc staining followed by flow cytometry analysis. Calcium influx was measured by flow cytometry after Fluo4 loading. Anti-TCR antibody (2.5 μg/ml, for J45.01) or anti-CD3 antibody (40 μg/ml, for CD45-HPB-ALL) was added 30 s after beginning the measurement. One representative experiment (of three) is shown.

of LAT and PLCγ1, leading to a hypothesis that these components of TCR signal transduction pathways are direct substrates of CD148 (29). We used the same Jurkat clones inducibly expressing CD148 WT or the inactive C12395 mutant (Fig. 7A), and we observed a similar negative effect of CD148 on LAT and PLCγ1 phosphorylation (Fig. 7B). In contrast, expression of CD148 in CD45-deficient JS-7 cells resulted in substantially enhanced phosphorylation of both LAT and PLCγ1 after TCR triggering (Fig. 7C). These results suggest that the positive effects of CD148 on SFKs over-balanced the potential negative effect on LAT and PLCγ1 phosphorylation in JS-7 cells.

CD148 Dephosphorylates Both C-terminal and Activation Loop Tyrosine in T Cells—We induced expression of CD148-WT or CD148-CS in the CD45-sufficient Jurkat cells and monitored changes in the phosphorylation status of Lck using the Odyssey infrared imaging system. Both tyrosines were significantly hypophosphorylated after the induction of CD148 wild type but not CS mutant expression (Fig. 7, D and E). This indicated that CD148 was able to dephosphorylate the C-terminal inhibitory tyrosine of Lck in T cells even in the presence of endogenous CD45. More importantly, our data indicate that the SFK activation loop phosphotyrosines are also substrates for CD148.

To further confirm that Lck is a direct substrate of CD148, we generated a substrate trapping D1205A (CD148-DA) mutant that covalently binds its substrates but is unable to catalyze the dephosphorylation reaction (21). Immunoprecipitation from cells transduced with CD148-WT and CD148-DA revealed that similar amounts of Lck co-precipitated with both constructs (Fig. 7F). However, the Lck co-precipitated with the trapping mutant exhibited much higher level of phosphorylation at both activating and inhibitory residues, indicating that both tyrosines were trapped by the mutant and protected from dephosphorylation. Collectively, these data suggest that both activatory and inhibitory tyrosines of SFKs are substrates of CD148.

Selectivity of CD148 PTP Domain Does Not Depend on the Presence of the C-terminal Tyrosines—CD148 contains three tyrosines near its C terminus, one of which (Tyr-1320) is conserved not only among different vertebrate species but also among members of R3 subtype of receptor-like PTPs (26). C-terminal Tyr-789 of unrelated phosphatase PTPRA plays a crucial role in binding and recognition of Fyn in a phosphorylation-dependent manner (27). Although flanked by different residues than PTPRA Tyr-789, the C-terminal tyrosines corresponding to CD148 Tyr-1320 in three R3 subtype members were reported to bind Fyn after pervanadate-induced phosphorylation (40). To study the role of CD148 C-terminal tyrosines in recognition and dephosphorylation of SFKs, we generated Y1311F/Y1320F double tyrosine mutant (CD148–2YF) and Y1311F/Y1320F/Y1335F triple tyrosine mutant (CD148–3YF) (supplemental Fig. 5). Surprisingly, both CD148 tyrosine mutants were able to rescue TCR signaling in JS-7 cells, similarly to the wild type phosphatase (Fig. 8, A–C). To further study the mechanism by which CD148 interacts with SFKs, we generated a chimeric receptor-like PTP that contained extracellular, transmembrane, and submembrane parts of CD148 and the phosphatase domain of another phosphatase, SHP-1. CD148/SHP1 chimera-transduced JS-7 cells (supplemental Fig. 5) were unable to rescue signaling as measured in a number of different assays (Fig. 8, A, B, and D). Moreover, TCR-induced CD69 up-regulation was slightly weakened by the expression of the chimera, suggesting it was catalytically functional with an SHP-1-like inhibitory effect (Fig. 8B).

Considering these data, we hypothesize that C-terminal tyrosines of CD148 are not necessary for interaction of CD148 with SFKs in T cells. However, the inability of CD148/SHP1 chimera to promote TCR signaling in JS-7 cells suggests some level of selectivity of CD148 and SHP1 catalytic domains or their proximal structures.

DISCUSSION

CD148 has been previously shown to play a positive role in surface receptor signal transduction via dephosphorylation of inhibitory tyrosines of SFKs in B cells, macrophages, platelets, and some non-hematopoietic tissues (19, 23–25). On the other hand, CD148 has been reported to act as a negative regulator of signal transduction in many non-hematopoietic biological systems as well as in TCR signaling in human T cell line Jurkat (29–31). Moreover, endogenous CD148 is obviously unable to rescue T cell development in CD45-deficient mice and humans (41–44). To bring more clarity to these somewhat contradictory functions, we carried out a more thorough analysis of CD148 expression during T cell development in mice and humans and also tested the ability of CD148 to positively regulate SFKs involved in TCR signal transduction. We found striking differences in CD148 expression between human and murine thymocytes as well as peripheral T cells. Moreover, we were able to show that CD148 had the ability to positively reg-
ulate Src family kinase functions mediating TCR signal transduction in human T cell lines.

CD45-deficient mice exhibit a severe developmental block during thymocyte development (1) that sharply contrasts with developmental consequences in B cells and macrophages (24). Three published cases of CD45-deficient patients revealed an indispensable role of CD45 in the development of mature peripheral T cells also in humans (41–43). Here we clearly show that although murine thymocytes lose CD148 expression at early stages, human thymocytes gain CD148 positivity at the terminal phase. However, both humans and mice express very low levels of CD148 at the DP stage when positive selection takes place and when the development is blocked in the absence of CD45, potentially explaining the inability of CD148 to compensate for the loss of CD45 in T cell development. The limited number of T cells that escape from thymus to the periphery in CD45-deficient mice and humans exhibit a strong functional defect (42, 44). However, the maturation status as well as the level of CD148 on these cells was not studied in the rare human cases.

Our data pointed to the restricted expression of CD148 to CD1a$^-$CD27$^+$CD44$^+$ terminal maturation stage of human thymocytes. CD1a is a commonly used diagnostic marker for T cell neoplasia. CD1a-positive cortical precursor T cell acute lymphoblastic leukemia cases were repeatedly reported to exhibit a better treatment response and survival prognosis in comparison with CD1a-negative T cell acute lymphoblastic leukemia both in children and adults (45, 46). Additionally, expression of CD1a correlated with susceptibility to in vitro induced apoptosis in childhood T cell acute lymphoblastic leu-

**FIGURE 7.** Effects of CD148 on phosphorylation of its potential substrates in TCR signal transduction pathway. A, expression of CD148-WT or C1239S mutant was induced with doxycycline (Dox) and analyzed by flow cytometry. B, expression of CD148-WT or CD148-CS was induced in Jurkat TetOn cells with doxycycline. Subsequently, the cells were stimulated with 4 μg/ml anti-TCR specific antibody for 30 s or left non-stimulated and immunoblotted after lysis. The phosphorylation status of LAT Tyr-191 and PLCγ1 Tyr-783 was detected with specific antibodies. Re-staining the membranes with antibodies to total LAT or PLCγ1, respectively, served as loading controls. C, non-transduced JS-7 cells (WT) and transgenic JS-7 cells expressing CD148-WT, CD148-CS, or CD45 were stimulated with 4 μg/ml anti-TCR-specific antibody for 30 s or left non-stimulated and immunoblotted after lysis. The phosphorylation status of LAT Tyr-191 and PLCγ1 Tyr-783 was detected with specific antibodies. Re-probing the membranes with antibodies to total LAT or PLCγ1, respectively, served as loading controls. D, Jurkat cells inducibly expressing CD148-WT or CD148-CS were lysed, and phosphorylation of Lck inhibitory tyrosine 505 and Lck activation loop tyrosine 394 was detected by antibodies to Lck Tyr(P)-505 and Src Tyr(P)-416, respectively. Total Lck was used as a loading control. One representative experiment (of three) is shown. E, relative change in phosphorylation of both key Lck tyrosines normalized to total Lck after the induction of CD148-WT or CS expression was quantified using the Odyssey infrared imaging system. The level of phosphorylation in cells untreated with doxycycline was arbitrarily set as 1 (black line). Data are the mean ± S.D. (n = 4 for WT and 3 for CS). *, p (WT versus CS) < 0.05; **, p (WT versus CS) < 0.01. F, JS-7 cells expressing Myc-CD148-WT or Myc-CD148-DA and non-transduced cells were lysed in phosphate buffer without phosphatase inhibitors and subjected to immunoprecipitation (IP) via anti-Myc-tag antibody. The precipitates were immunoblotted and stained with antibodies to total Lck, Tyr(P)-416 of Src (SFK pY416), and Tyr(P)-505 of Lck.
Expression and Function of CD148 in T Cells

The absence of CD148 on mature peripheral T cells is in agreement with the lack of any described T cell phenotype of CD148 knock-out mice (24). However, a role for CD148 in mouse T cell biology cannot be completely excluded, as murine T cells up-regulate CD148 after activation (10). CD148 expression in human mature thymocytes and peripheral T cells implies that the function of CD148 in human T cells cannot be easily uncovered using a mouse model. We showed that expression of either CD148 or CD45 promotes TCR signaling in CD45-deficient human T cell lines, which reveals a level of redundancy between CD148 and CD45 in T cells not appreciated so far. Unavailability of CD45- or CD148-deficient humans restricted our functional analysis to cell lines. However, because we observed similar effects of CD148 on TCR signaling in three different CD45-negative T cell lines as well as in a knockdown experiment, we believe that our observations revealed a general mechanism rather than a particular cell line and/or overexpression-specific effect and were sufficient to prove that CD148 is capable of activating a T cell-specific set of SFKs, most notably Lck. Although Lck is also expressed in other cell types, it is really critical only in T cells, and the effect of CD148 on the activity of this particular kinase has not been tested before. Our data thus broadened the spectrum of SFKs known to be activated by CD148 and suggest that selectivity of CD148 for specific members of this family may be limited.

Moreover, we bring evidence that in addition to the C-terminal inhibitory phosphotyrosine, the activation loop phosphorylation in SFKs is also a substrate of CD148 in living cells. This finding is also supported by previous observations that recombinant CD148 phosphatase domain dephosphorylated activated tyrosine in SFKs is also a substrate of CD148 in living cells. Our initial observations seemingly contradicted previous work that described the inhibitory effects of ectopically expressed CD148 in CD45-sufficient Jurkat T cells (29, 31). In our hands, CD148 had inhibitory effects in CD45-expressing Jurkat, whereas at the same time we observed activating effects in the CD45-deficient Jurkat clone JS-7. The most plausible explanation was that CD148 could impact TCR signaling both in a positive and negative manner. In CD45-negative JS-7 cells, CD148 expression led to reduced phosphorylation of the inhibitory tyrosine in Lck accompanied by enhanced activity of Lck and autophosphorylation of the activating tyrosine. In contrast, in CD45-positive cells both key tyrosines were hypophosphorylated when CD148 was expressed, probably as a consequence of substantially higher phosphatase activity resulting from concerted action of both CD45 and CD148. According to Nika et al. (49), the activity of Lck is mainly determined by the phosphor-

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**FIGURE 8.** Catalytic domain of CD148 but not the C-terminal tyrosines is required for SFK recognition as a substrate. JS-7 cells transduced with CD148-WT, CD148–2YF mutant, CD148–3YF mutant, or CD148/SHP1 chimera and non-transduced cells (NT) were analyzed for intracellular signaling response after TCR triggering. A, transgenic JS-7 cells and non-transduced cells were stimulated with 4 μg/ml anti-TCR antibody for 30 s and immunoblotted. Anti-phosphotyrosine antibody was used to detect overall tyrosine phosphorylation in activated and non-activated cells. Re-probing the membrane with anti-Erk2 antibody was used as a loading control. B, transgenic JS-7 cells and non-transduced cells were analyzed by flow cytometry after plate-bound anti-TCR antibody overnight and examined for CD69 expression via flow cytometry. Black bars represent CD69 signals in non-stimulated cells (including autofluorescence), whereas white bars represent CD69 up-regulation after TCR stimulation. Data are the mean ± S.D. Data originate from triplicates from one representative experiment (of four). a.u., arbitrary units; Chim, chimera. C, JS-7 cells ectopically expressing CD148-WT (solid black line), CD148–2YF mutant (dashed gray line), and CD148–3YF mutant (solid gray line), and non-transduced JS-7 cells (dashed black line) were analyzed by flow cytometry after Fluo4 loading. Anti-TCR antibody (200 ng/ml) was added 30 s after beginning of the measurement. One representative experiment (of five) is shown. D, shown is the same experiment as in C with JS-7 cells ectopically expressing CD148-WT (solid black line) or CD148/SHP1 chimera (solid gray line) and non-transduced JS-7 cells (dashed black line). One representative experiment (of five) is shown.
ylation status of the activation loop tyrosine, thus explaining reduced kinase activity when both tyrosines are dephosphorylated. We concluded that CD148 influences the activity of Lck negatively via dephosphorylation of the activation loop phosphotyrosine and positively through dephosphorylation of the C-terminal phosphotyrosine. This is consistent with the inhibitory impact of CD148 on TCR signaling in CD45-positive T cells, and it is also very similar to the observed effects of altering the CD45 expression level in murine thymocytes (50, 51).

Thus, our data indicate that CD148 regulates SFKs in T cells in a similar manner as CD45 and suggest that the activity of CD45 is the decisive factor determining whether the net effect of CD148 expression is an enhancement or an inhibition of TCR signal transduction. Importantly, this can be dependent not only on the regulation of SFKs but also on direct dephosphorylation of other proteins, such as PLCγ or LAT, as suggested before (27).

Although the canonical TCR pathway has been intensively studied, less is known about TCR signaling in particular biological contexts, characterized by different T cell life stages of differentiation (e.g. thymic stages, naïve mature, activated, or memory T cells), T cells lineages (e.g. CD8+ or CD4+, Th1, Th2, Th17, or regulatory T cells), or conditions (tonic or ligand dependent). Importantly, CD148 expression and CD45 splicing differ among particular T cell subsets (our data and Refs. 6, 8, 10, and 44). Furthermore, it has been shown that CD45 differentially regulates basal and inducible TCR signaling in murine thymocytes (50). Thus, the effects of CD148 activity could vary substantially depending on particular T cell developmental stage, lineage, and other circumstances. Recently, several mouse genetic models with varied CD45 expression level or activity have been developed and intensively studied to improve the understanding of the complex behavior of CD45 in T cells (50, 51). Given the differential expression of CD148 on T cells together with the ability of CD148 to regulate SFKs in a similar manner as CD45, CD148 activity should be taken into account when applying such findings to humans.

Phosphorylation of a SFK C-terminal tyrosine inhibits the catalytic activity by stabilizing it in the closed conformation via intramolecular interaction with SH2 and SH3 domains. To explain how a PTP can access the nested phosphorytrosine, a phosphotyrosine displacement model was suggested (27). According to this model, a C-terminal tyrosine of the PTP gets phosphorylated and subsequently competitively binds to the SH2 domain of the SFK, resulting in the release of the closed conformation and access of the PTP to the phosphorylated C-terminal site of the kinase. Although the experimental evidence supporting this model comes from studies done on PTups of R4 subtype, other unrelated receptor-like phosphatases including CD148 also usually contain at least one tyrosine at their C-terminal region. Moreover, phosphatases PTPRO, SAP-1 (PTPRH), and VE-PTP (PTPRB) related to CD148 were shown to bind Fyn after pervanadate-induced phosphorylation via their C-terminal tyrosine, indicating the phosphotyrosine displacement model can be valid also for PTups of R3 subtype (26, 40). However, this model was never directly tested using a member of R3 family. Here we show that mutation of all three CD148 C-terminal tyrosines to phenylalanines does not inhibit its ability to enhance TCR signaling. These results led us to conclude that the phosphotyrosine displacement model for CD148 does not apply. On the other hand, the finding that the CD148/SHP-1 swap chimera, containing the SHP-1 catalytic domain, failed to enhance TCR signaling suggests a specific interaction mechanism between CD148 and SFKs.

Our investigation demonstrated that CD148 is able to activate SFKs involved in TCR signal transduction. This could be most clearly observed in CD45 deficient environment. In CD45-sufficient T cell line, the proactivatory effect on the Lck inhibitory tyrosine is overbalanced by dephosphorylating the activation loop tyrosine of Lck and/or other substrates essential for TCR signal transduction leading to the net inhibitory effect of CD148 (29, 31). Together with the analogous dual role of CD45 (50, 51), our study suggests that dual inhibitory/stimulatory function may be a common principle governing the signaling by different receptor-like PTPs. The net outcome of their action may depend on cellular or biochemical context.

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