THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 272, No. 48, Issue of November 28, pp. 30215–30220, 1997
Printed in U.S.A.

No Evidence for Involvement of Mouse Protein-tyrosine Phosphatase-BAS-like Fas-associated Phosphatase-1 in Fas-mediated Apoptosis*

(Received for publication, July 15, 1997)

Edwin Cuppen‡, Shigekazu Nagata§, Bé Wieringa‡, and Wiljan Hendriks‡¶

From the ‡Department of Cell Biology and Histology, Institute of Cellular Signaling, University of Nijmegen, P. O. Box 9101, 6500 HB Nijmegen, The Netherlands and the §Department of Genetics, Osaka University Medical School, 2-2 Yamada-oka, Suita, Osaka 565, Japan

Recently, one of the PDZ domains in the cytosolic protein-tyrosine phosphatase Fas-associated phosphatase-1 (FAP-1)/protein-tyrosine phosphatase-BAS (PTP-BAS) was shown to interact with the carboxyl-terminal *t*S-L-V peptide of the human Fas receptor (Sato, T., Irie, S., Kitada, S., and Reed, J. C. (1995) *Science* 268, 411–415), suggesting a role for protein (de)phosphorylation in Fas signaling. To investigate whether this interaction is conserved in mouse, we performed yeast two-hybrid interaction experiments and transfection studies in mouse T cell lines. For the corresponding PDZ motif in the mouse homologue of FAP-1/PTP-BAS, protein-tyrosine phosphatase-BAS-like (PTP-BL), only an interaction with human but not with mouse Fas could be detected. Presence of the *t*S-L-V motif proper, which is unique for human Fas, rather than the structural context of its carboxyl terminus, apparently explains the initially observed binding. To test for functional conservation of any indirect involvement of PTP-BL in Fas-mediated signaling, we generated T lymphoma cell lines stably expressing mouse or human Fas receptor with and without PTP-BL. No inhibitory effect of PTP-BL was observed upon triggering apoptosis using mouse or human Fas-activating antibodies. Together with the markedly different tissue expression patterns for PTP-BL and Fas receptor, our findings suggest that protein-tyrosine phosphatase PTP-BL does not play a key role in the Fas-mediated death pathway.

Many signaling pathways depend upon the reversible assembly of protein complexes to evoke temporally and spatially distinct effects. Small modular protein domains like Src homology 2 (SH2) and SH3 domains and phosphotyrosine-binding domains, were found to play an important role in signal transduction cascades (1–3). Recently, it has become clear that PDZ motifs have a similar role (4). PDZ motifs (acronym for postsynaptic density protein PSD-95, *Drosophila* discs-large tumor suppressor DlgA, and the tight junction protein ZO-1) are now identified in a wide variety of proteins in bacteria (5) and lower and higher eukaryotes (6), many of which are localized at specific regions of cell-cell contact, such as tight, septate, and synaptic junctions (7). For example, PDZ motifs present in membrane-associated guanylate kinase family members have been found to interact with the carboxyl terminus of diverse transmembrane proteins (8), thereby inducing clustering of these proteins at specialized cell-cell junctions (9). Recent experiments demonstrate that PDZ motifs from different types of proteins can recognize unique carboxyl-terminal peptide motifs (10–12). As these interactions have been identified by screenings of peptide libraries, the *in vivo* relevance for most of them remains to be demonstrated. PTP-BAS (13), also known as hPTP1E (14) and PTPL1 (15) in humans, is one of the few PDZ-containing proteins known so far that contain an active catalytic protein domain, *i.e.* a carboxyl-terminal protein-tyrosine phosphatase domain. Recently, this protein was found to directly associate via one of its PDZ motifs to the carboxyl-terminal regulatory domain of Fas and was termed FAP-1[1] for Fas-associating phosphatase-1 (16). This association points to a possible link between reversible tyrosine phosphorylation and the apoptotic death cascade mediated by Fas. Activation of the Fas receptor (17), a member of the tumor necrosis factor receptor superfamily (18), induces very rapid physiological and morphological changes in many cell types (19). The Fas receptor itself does not contain a catalytic protein domain, but in the activated conformation it recruits several proteins to the death-inducing signaling complex (20), which can trigger several proteolytic cascades and signaling pathways (21). Direct association of a protein-tyrosine phosphatase could have important modulatory effects on Fas signaling, and indeed a correlation between FAP-1 expression and (partial) resistance to Fas-mediated apoptosis has been noted in the human system (16).

The second PDZ motif of the five PDZ motifs as present in FAP-1 (referred to as the third out of six PDZ motifs in Refs. 16 and 10) and the carboxyl-terminal 15 amino acids of Fas were found to be necessary and sufficient for the observed direct association between FAP-1 and Fas (16). Indeed, screenings of oriented peptide libraries employing the second PDZ motif of FAP-1 revealed a consensus binding sequence of *t*(S/T)-X-(V/I/L) (10, 12) that is present in the Fas carboxyl terminus (*t*S-L-V).

Puzzled by the considerable sequence divergence displayed by the carboxyl terminus of Fas (Fig. 1), we set out to study whether FAP-1's presumed role in Fas signaling was evolutionary conserved. We found that mouse Fas does not interact with the mouse homologue of PTP-BAS/FAP-1, known as PTP-BL (22) or RIP (23). Furthermore, studies using transformed mouse cell lines failed to demonstrate any role for PTP-BL in

* This work was supported by Dutch Cancer Society Grant NUKC 95-900 (to W. H.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¶ To whom correspondence should be addressed: Dept. of Cell Biology and Histology, Institute of Cellular Signaling, University of Nijmegen, P. O. Box 9101, 6500 HB Nijmegen, The Netherlands. E-mail: w.hendriks@celbi.kun.nl.

[1] The abbreviations used are: FAP-1, Fas-associated phosphatase-1; PTP-BAS, protein-tyrosine phosphatase-BAS; PTP-BL, protein-tyrosine phosphatase-BAS-like; *t*, carboxyl-terminal peptide; PBS, phosphate-buffered saline.

the regulation of Fas-mediated signaling. Our results show that the interaction between the Fas receptor and PTP-BL is not conserved between mice and men.

EXPERIMENTAL PROCEDURES

Two-hybrid Interaction Trap—Plasmid DNAs and the yeast strain used for the interaction trap assay were provided by Dr. Roger Brent and colleagues (Massachusetts General Hospital, Boston) and used as described (24). The individual PDZ motifs of PTP-BL were cloned in the pEG202 bait vector by amplifying PTP-BL sequences using specific primers containing convenient restriction sites for cloning purposes. This results in LexA fusion proteins containing amino acids 1076–1177 (BL-PDZ-I), 1352–1450 (BL-PDZ-II), 1471–1601 (BL-PDZ-III), 1756–1855 (BL-PDZ-IV), and 1853–1946 (BL-PDZ-V) of PTP-BL. The carboxyl-terminal 60 residues of mouse Fas were cloned in the prey vector pJGBr2 by subcloning a 599-base pair *Pvu*II-*Dra*I restriction fragment from pBOS-EA (25), and for the human Fas carboxyl terminus (40 amino acids), this was done by subcloning a 120-base pair *Bgl*II-*Apa*I restriction fragment from pCMV-hFAS (a kind gift of Dr. Anderson, Cambridge, MA). Construction of the control baits is described elsewhere[2] (11). Two-hybrid interactions were analyzed by plating yeast containing reporter, bait, and prey constructs on minimal medium agar plates containing 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside and lacking tryptophan, histidine, uracil, and leucine and subsequent incubation at 30 °C for 4 days.

The relative strength of two-hybrid interactions was determined by measuring the β-galactosidase activity in crude yeast lysates. For this, a single yeast colony was inoculated in 1.5 ml of minimal medium lacking histidine, tryptophan, and uracil and containing 2% galactose and 1% raffinose. After overnight growth at 30 °C until the culture reached $A_{600\,nm} \approx 1.0$, the cells were pelleted and resuspended in 0.2 ml of ice-cold buffer (0.1 M NaCl, 10 mM Tris-HCl, pH 7.0, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride). About 100 μl of acid-washed glass beads (425–600 μm, Sigma) were added, and the suspension was vortexed six times for 30 s alternated with 30-s incubations on ice. Lysates were centrifuged for 5 min with 14,000 rpm at 4 °C. Supernatants were transferred to fresh tubes and used for protein concentration determination (26). β-Galactosidase activity was measured by adding 10 μl of lysate to 200 μl of freshly prepared ONPG buffer (100 mM phosphate buffer, pH 7.0, 1 mM MgCl, 10 mM KCl, 50 mM β-mercaptoethanol, 0.35 mg/ml *o*-nitrophenyl-β-D-galactopyranoside (Sigma)) in microtiter plates. Reactions were incubated at 30 °C and the $A_{420\,nm}$ was monitored for 1 h on a CERES UV900C MTP-photometer (Bio-Tek). The specific β-galactosidase activity (arbitrary units) was calculated using the initial linear part of the $A_{420\,nm}$/min curves and normalized for the protein concentration in the sample. Every sample was measured in duplicate, and for each bait-prey combination, five independent colonies were assayed.

Cell Lines and Cell Culture—Mouse T cell lymphoma WR19L cell line (ATCC TIB52) was grown in RPMI 1640 medium containing 10% fetal calf serum. Transformants expressing mouse or human Fas and PTP-BL were established by electroporation as described (17). Briefly, 1×10^7 WR19L cells in 0.5 ml of K-PBS (30.8 mM NaCl, 120.7 mM KCl, 8.1 mM Na_2PO_4, 1.46 mM KH_2PO_4) were cotransfected with 2.5 μg/ml *Pvu*I linearized pcDNA3 (Invitrogen) or pcDNA-BL (full-length PTP-BL cDNA (22) cloned in pcDNA3) and 25 μg/ml *Vsp*I linearized pEF-F58 (17) or pBOS-EA (25) by electroporation at 290 V with a capacitance of 960 μF using a Gene Pulser (Bio-Rad). Cells were cultured in 40 ml of RPMI, 10% fetal calf serum in 96-well microtiter plates (0.1 ml/well) for 2 days, and neomycin-resistant clones were then selected in medium containing G418 (900 μg/ml). Cells were analyzed for PTP-BL and Fas receptor expression after 14 days of selection.

Western Blot Detection of PTP-BL—WR19L or derived transformed cells were lysed in buffer (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 5 mM EDTA, 0.5% Nonidet P-40) for 1 h on ice and centrifuged for 10 min at 10,000 × *g*. Amounts of lysate containing 25 μg of protein were loaded in each lane of a 4% (w/v) polyacrylamide mini-gel (Bio-Rad), and following electrophoresis, proteins were blotted onto nitrocellulose (Amersham Corp.). The blot was blocked for 30 min in 5% non-fat dry milk in PBST (PBS containing 0.05% Tween 20) and subsequently incubated for 1 h with affinity-purified polyclonal antibody directed against the carboxyl-terminal catalytic PTPase domain of PTP-BL[2] in a one to thousand dilution in PBST containing 1% normal goat serum. After subsequent washings, the blot was incubated for 1 h with peroxidase-conjugated goat anti-rabbit (20.000 × diluted, Pierce), washed, and labeled bands were visualized using chemiluminescent substrate (Pierce) according to the manufacturer's instructions.

Flow Cytofluorometric Analysis of Surface-bound Fas Receptor—Expression of Fas antigen was analyzed by incubating 5×10^5 cells in 100 μl of IF buffer (PBS, 1% bovine serum albumin, 0.1% sodium azide) containing 0.5 μg/ml hamster monoclonal antibody Jo2 (27) for detection of mouse Fas or mouse monoclonal antibody CH-11 (28) for detection of human Fas, for 30 min on ice. The cells were washed twice with IF buffer and incubated with fluorescein isothiocyanate-conjugated goat α-hamster-Ig (Jackson ImmunoResearch, diluted 1:25 in IF buffer) or fluorescein isothiocyanate-conjugated Rabbit α-mouse-Ig (Dako, diluted 1:50 in IF buffer). Cells were washed and resuspendend in 0.5 ml of IF buffer and analyzed on a flow cytofluorometer (Coulter).

Assay for Apoptotic Response—Mouse WR19L wild type or transformed cells were seeded at 1×10^5 cells/ml in a 24-well tissue culture plate and cultured at 37 °C. Cell death was induced by addition of anti-Fas monoclonal antibody (Jo2 for mouse Fas or CH-11 for human Fas expressing cells) to a final concentration ranging from 10^{-9} to 10^{-3} mg/ml. After incubation at 37 °C for 1 to 20 h, dead cells were stained by adding propidium iodine to a final concentration of 4 μg/ml and incubating at room temperature for 10 min. The percentage of dead cells was determined by flow cytofluorometric analysis (Coulter).

Northern Blotting—Total RNA from several tissues was prepared using the guanidium isothiocyate-phenol-chloroform extraction method (29). 15 μg of RNA was loaded on a 1% formamide agarose gel, and after electrophoresis the RNA was transferred to nylon membrane according to standard procedures (30). Complete PTP-BL (22) and mouse Fas (25) cDNAs were radioactively labeled by random priming and used as probes for hybridization.

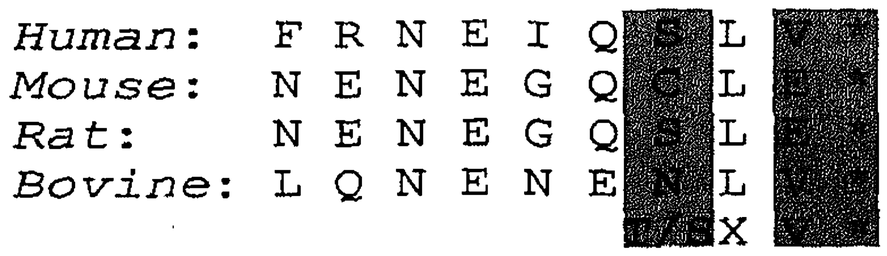


FIG. 1. **Sequence comparison of the Fas receptor carboxyl terminus from different species.** The last 9 amino acids of human (accession number M67454), mouse (accession number A46484), rat (accession number JC2395), and bovine Fas receptor (accession number U34794) are shown and aligned with the proposed PDZ binding motif *t*(T/S)-X-V (*asterisks* indicate the carboxyl terminus).

RESULTS

No Interaction between Mouse Fas Receptor and PTP-BL—The large difference between species that is obvious in the carboxyl-terminal Fas receptor sequence (Fig. 1) prompted us to test the interaction between Fas receptor and PTP-BL using the two-hybrid interaction trap (24). Baits were generated containing either the complete array of PDZ motifs or individual PDZ motifs of PTP-BL and were assayed for interaction with both mouse and human Fas carboxyl termini. An interaction of the ensemble of five PDZ motifs of PTP-BL (BL-PDZ-I-V) with the human Fas receptor could be observed, but no interaction occurred with mouse Fas receptor (Fig. 2). Assays employing single PDZ motifs only revealed an interaction for BL-PDZ-II and human Fas receptor, but this was rather weak. No interaction of mouse Fas receptor with any of the PTP-BL PDZ motifs tested was observed, nor did any of the baits activate transcription of the β-galactosidase reporter in the presence of an empty prey vector (Fig. 1).

Finally, two nonrelated domains, the nNOS PDZ motif, found to recognize the carboxyl-terminal *t*G-(D/E)-X-V motif (11, 31), and the RIL PDZ motif,[2] did not interact with mouse or human Fas receptor. Another motif, the second PDZ motif of PSD95/SAP90, known to recognize *t*(T/S)-X-V (32, 33), did interact only with the human Fas receptor (not shown, Ref. 11).

Generation of Stable PTP-BL and Fas Transformants—To study any functional involvement of PTP-BL in Fas-mediated apoptosis, we generated stable transformants of the mouse T cell lymphoma WR19L cell line. This cell line does not express PTP-BL (Fig. 3 C) or endogenous Fas receptor as identical flow

[2] E. Cuppen, H. Gerrits, B. Pepers, B. Wieringa, and W. Hendriks, submitted for publication.

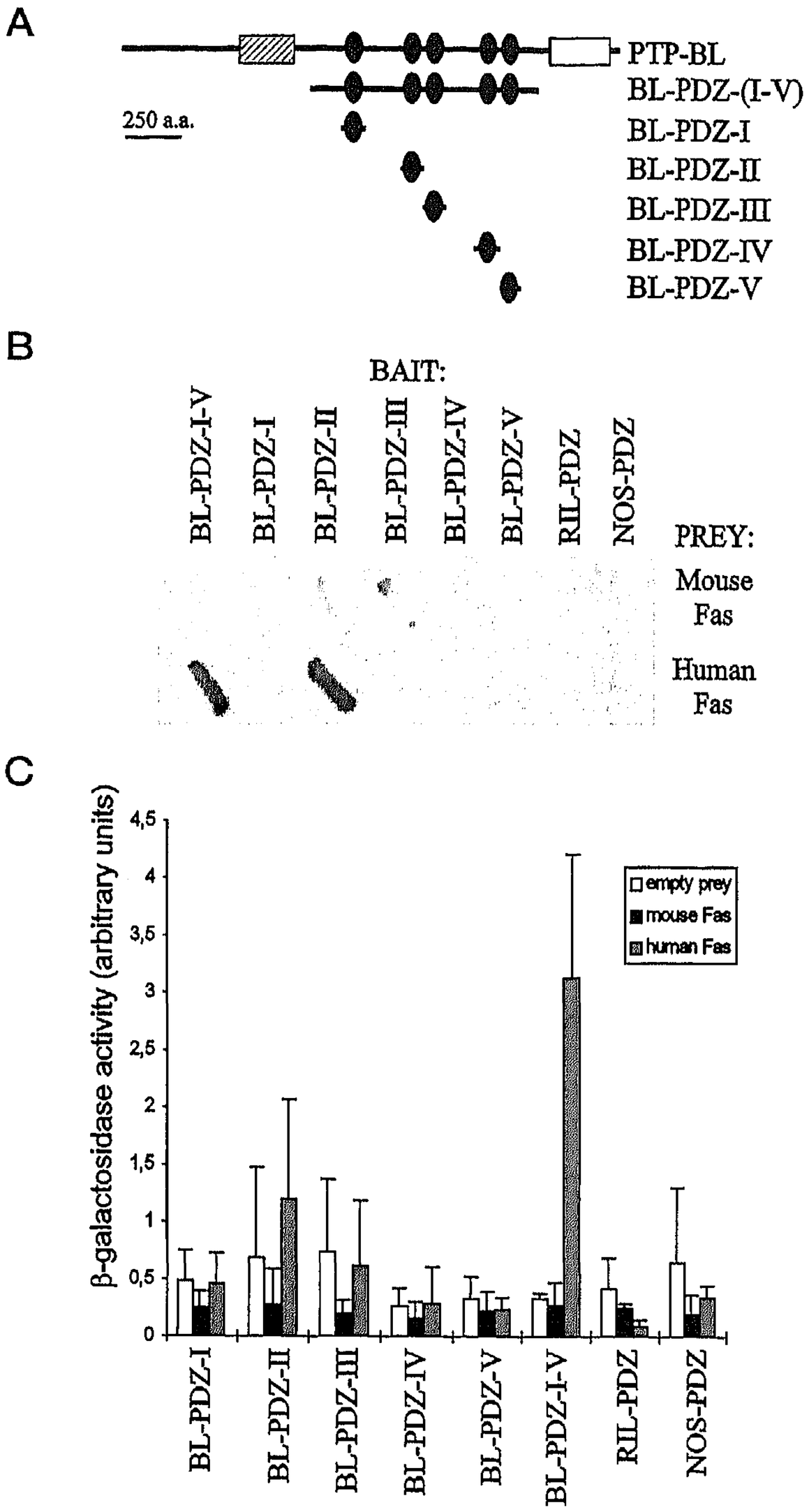
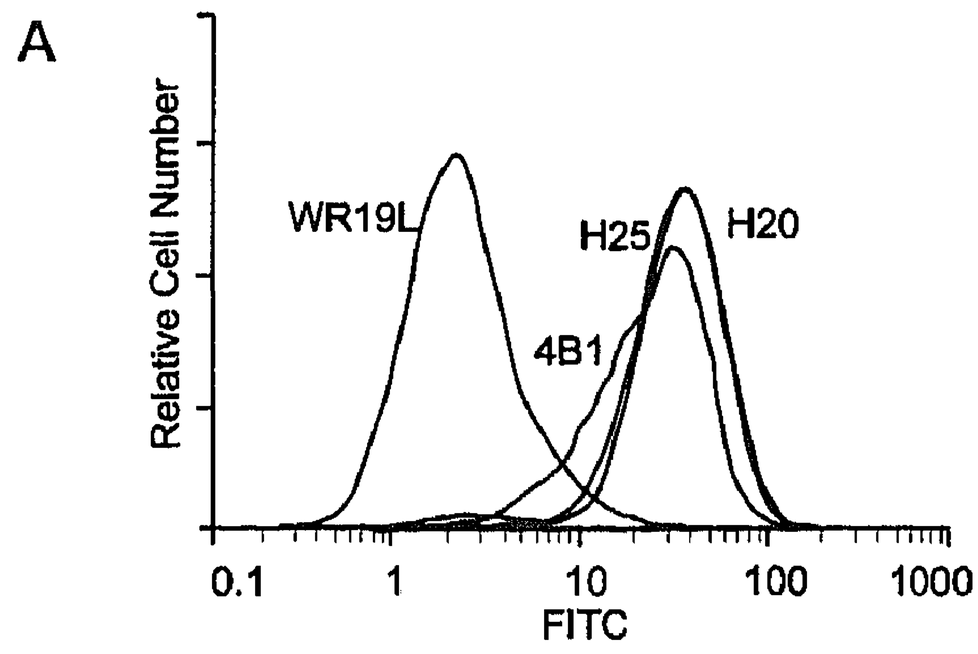
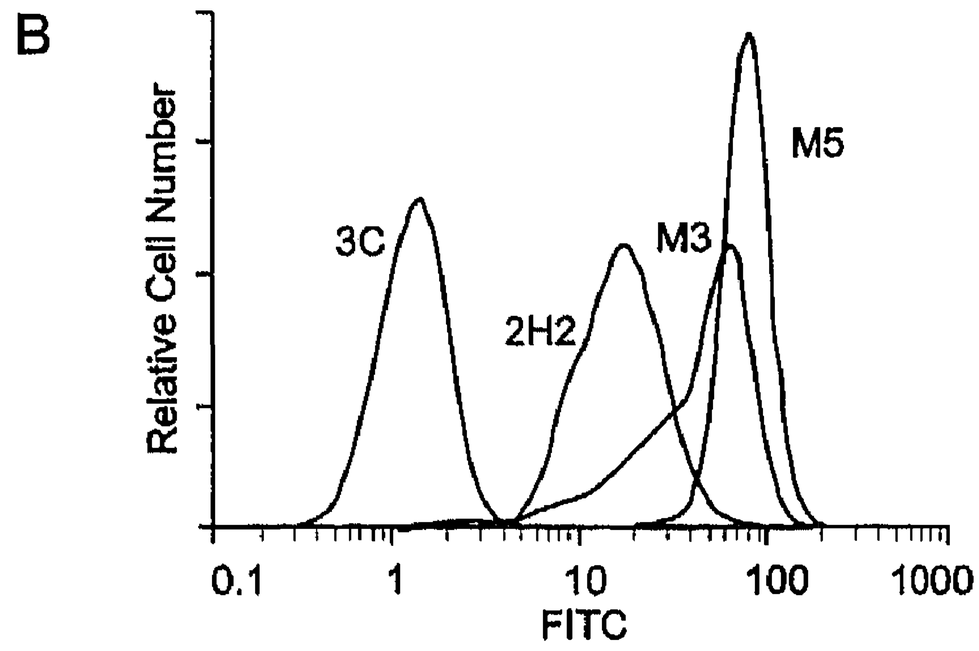


FIG. 2. **Two-hybrid analysis of the interaction between PTP-BL PDZ motifs and the carboxyl terminus of mouse and human Fas receptor.** *A*, schematic overview of the domain structure of PTP-BL and the protein domains used for the two-hybrid interaction analysis. *B*, 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside assay plate for a two-hybrid interaction in yeast between PTP-BL PDZ motifs as baits and mouse or human Fas receptor carboxyl termini as prey. *C*, semi-quantitative analysis of the interaction strength between different PDZ motifs and mouse and human Fas receptor carboxyl termini using the two-hybrid interaction trap. The interaction strength for mouse Fas receptor (*black bar*), human Fas receptor (*gray bar*), and empty prey vector (*open bar*) as a control, with the various PDZ baits, were determined by measuring the β-galactosidase activity in crude yeast lysates. *Error bars* indicate the standard deviation ($n = 5$).





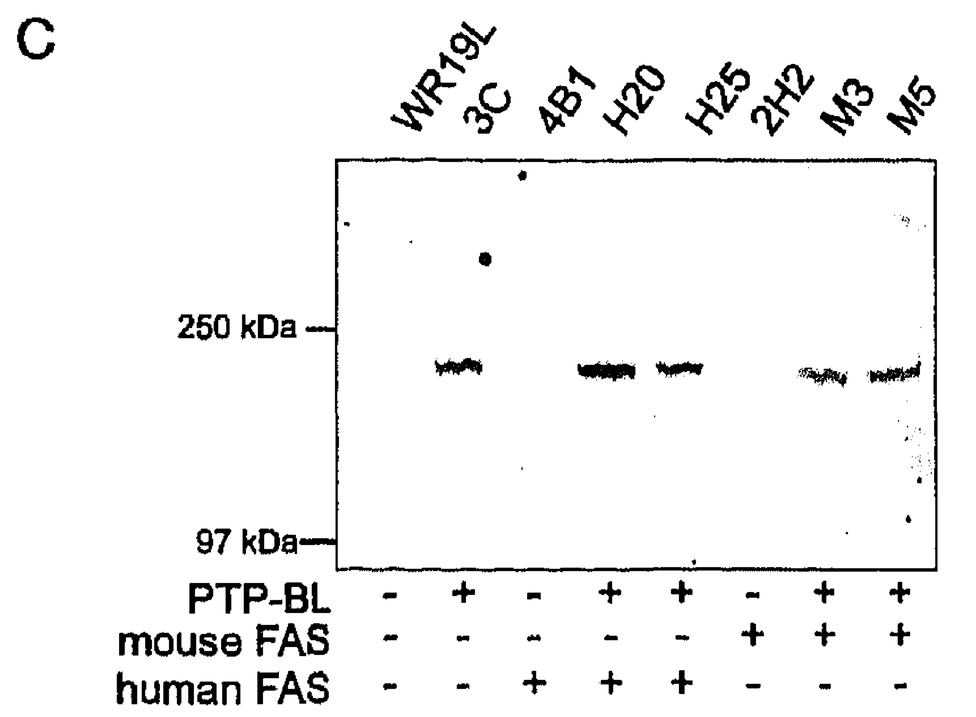


FIG. 3. **Characterization of transformed WR19L cells.** Surface expression of human Fas receptor (*A*) and mouse Fas receptor (*B*) in WR19L transformants was detected using specific monoclonal antibodies and analyzed by flow cytofluorometry. PTP-BL expression in the transformants was analyzed by Western blotting (*C*). Equal amounts of protein from total lysates were loaded on a 4% polyacrylamide gel, blotted, and PTP-BL was detected using an affinity-purified polyclonal antiserum (α-BL-PTP). Absence (−) or presence (+) of PTP-BL, mouse, and human Fas receptor in the different transformants is indicated at the *bottom*.

cytometric staining profiles were obtained with (Fig. 3*A*) and without the primary anti-Fas antibody (not shown). Expression plasmids were introduced by electroporation and protein expression of Fas receptor and PTP-BL were analyzed by flow cytofluorometric analysis and Western blotting, respectively. No endogenous Fas receptor could be detected in the wild type WR19L cell line. Stable transformants were obtained expressing human Fas receptor in combination with (H2O and H25) or without (4B1) PTP-BL (Fig. 3, *A* and *C*). Similarly transformants were generated expressing mouse Fas receptor with (M3 and M5) or without (2H2) PTP-BL (Fig. 3, *B* and *C*). In addition a control line expressing PTP-BL only was generated (3C) (Fig. 3, *B* and *C*). Transformants H2O, H25 and M3, M5 express about equal amounts of surface-bound Fas receptor, as measured by flow cytofluorometric analysis (Fig. 3, *A* and *B*), but express different amounts of PTP-BL as determined by Western blotting (Fig. 3*C*).

Time Response to Antibody-induced Fas-mediated Apoptosis—Apoptosis was triggered in the human Fas receptor transformants by adding monoclonal anti-Fas IgM (CH-11, 250 ng/ml), and apoptosis was measured at fixed time points by propidium iodine uptake and flow cytofluorometric analysis. As expected, the wild type cell line WR19L and the transformant that expresses PTP-BL only (3C), which both lack Fas receptor

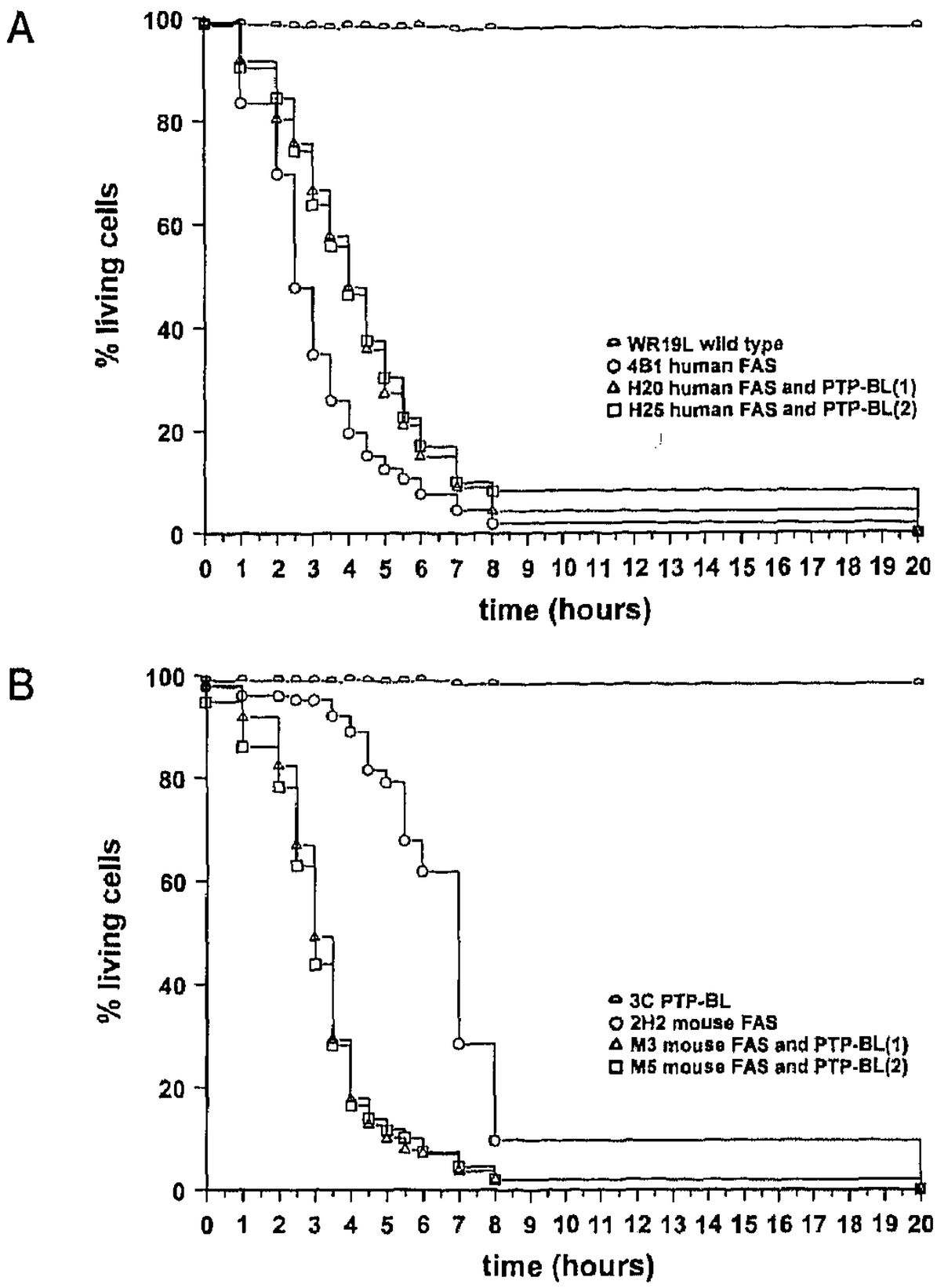


FIG. 4. **Time response curve of WR19L transformants upon antibody-induced Fas-mediated apoptosis.** Apoptosis mediated by the human Fas receptor (*A*) or the mouse Fas receptor (*B*) was induced by adding the monoclonal antibody CH-11 (250 ng/ml) or Jo2 (250 ng/ml), respectively. At fixed time points cells were withdrawn, and dead cells were stained by propidium iodine uptake and analyzed by flow cytofluorometry.

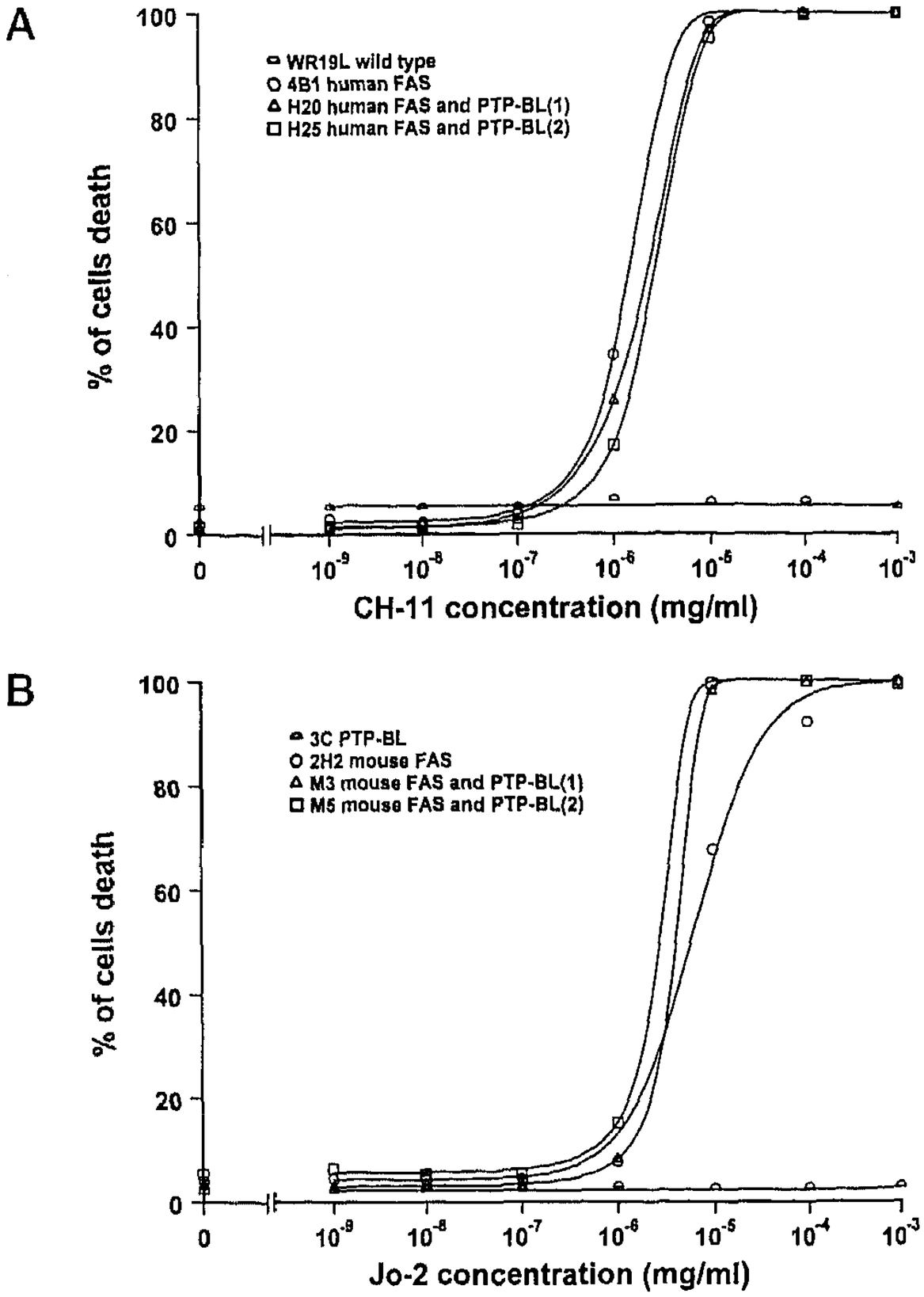


FIG. 5. **Dose response of WR19L transformants for antibody-induced Fas-mediated apoptosis.** Apoptosis was induced in WR19L transformants expressing human Fas receptor (*A*) and mouse Fas receptor (*B*) by adding increasing amounts of monoclonal antibody (final concentration ranging from 10^{-9} to 10^{-3} mg/ml). After 20 h dead cells were stained by propidium iodine uptake and analyzed by flow cytofluorometry.

expression, do not undergo antibody-induced apoptosis (Fig. 4, *A* and *B*). The human Fas receptor-expressing transformant (4B1) undergoes rapid apoptosis, and within 8 h over 95% of the cells have permeable membranes and take up propidium iodine, indicative for the onset of apoptosis. The transformants expressing in addition PTP-BL (H2O and H25) undergo rapid apoptosis also, although at a slightly slower pace. However, cell lines with and without PTP-BL are indistinguishable when the time points at 20-h incubation are compared. Furthermore, no influence of the amount of PTP-BL protein expression is observed (H2O *versus* H25, Fig. 4*A*). These results indicate that PTP-BL expression has no major influence on human Fas-mediated apoptosis in mouse WR19L cells and doesn't protect against apoptosis.

A similar experiment was done with the mouse Fas receptor transformants by triggering apoptosis with the monoclonal antibody Jo2 (anti-mouse Fas). Apoptosis in the cell line expressing only mouse Fas receptor (2H2) is somewhat slower compared with human Fas-mediated apoptosis. This difference may be explained by the lower surface expression of the mouse Fas receptor on 2H2 (Fig. 3*B*) or may be due to clonal variations or subtle differences in the way these two monoclonal antibodies induce apoptosis.

Compared with the cell line expressing only mouse Fas receptor (2H2), the transformants that express PTP-BL as well as mouse Fas receptor (M3 and M5) undergo apoptosis more rapidly (Fig. 4*B*), but at a rate comparable with the human Fas-mediated apoptosis (Fig. 4*A*). Again, comparison of the read-out upon overnight incubation of the transformants with apoptosis inducing antibody revealed no effect of PTP-BL at all. Taken together these findings indicate that PTP-BL expression is not a significant modulatory factor in mouse or human Fas-mediated apoptosis.

Dose Response to Antibody-induced Fas-mediated Apoptosis—Perhaps the impact of PTP-BL on Fas-mediated apoptosis is much more subtle than what was proposed for the human counterpart. Therefore, we tested for the influence of PTP-BL expression on dose responsiveness by treating wild type WR19L cells and its transformants overnight with increasing amounts of apoptosis inducing antibody. Even at the highest concentration of antibody tested, cells without Fas receptor are refractory to undergo apoptosis (Fig. 5, *A* and *B*). The dose-dependent sensitivity for Fas-mediated apoptosis did not significantly differ between Fas receptor transformants with and without PTP-BL. Again, this argues against an inhibiting role of PTP-BL in Fas signaling. Moreover, the mouse Fas receptor expressing transformant seems to be slightly less sensitive without PTP-BL expression, even suggesting a small apoptosis-enhancing effect of PTP-BL.

Tissue Expression Patterns for PTP-BL and Fas Receptor—A Northern blot of total RNA from several mouse tissues was used to determine the tissue distribution of both Fas receptor and PTP-BL. The obtained patterns are quite distinct (Fig. 6). Expression of Fas receptor is very high in lung and thymus and

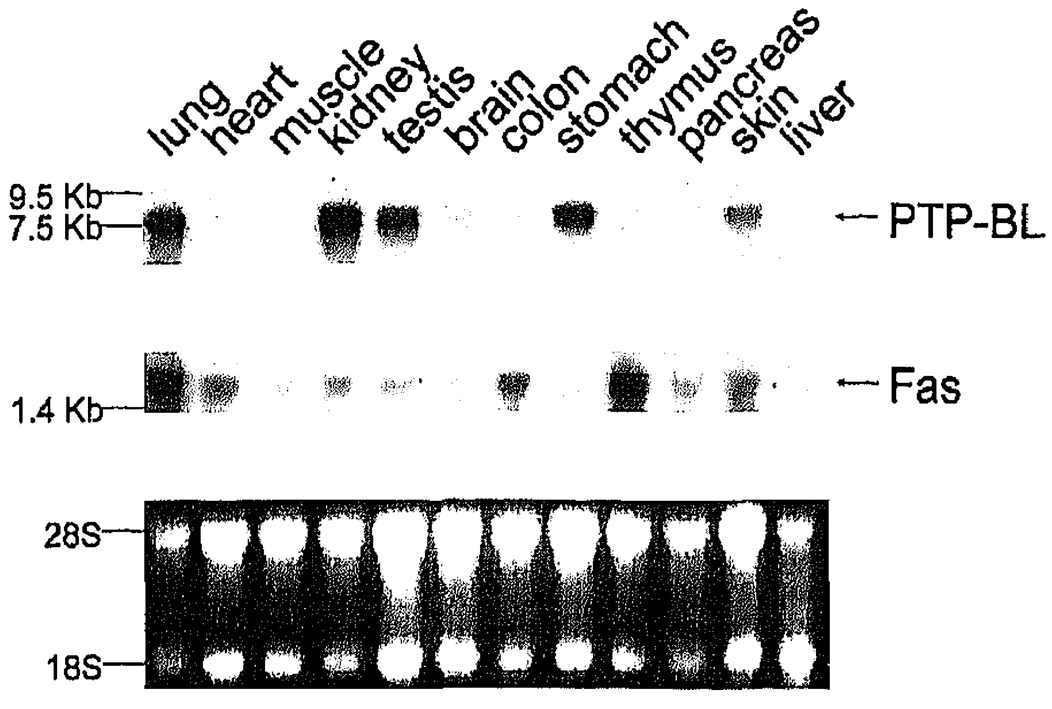


FIG. 6. **Tissue expression for PTP-BL and Fas receptor.** Northern blot analysis of PTP-BL (*upper panel*) and Fas receptor (*middle panel*) mRNA expression in different mouse tissues. The *lower panel* shows an ethidium bromide staining as a control for RNA loading.

ubiquitously at a low level in all tissues examined. PTP-BL, on the contrary, is expressed at a high level in lung, kidney, testis, stomach, and skin (Fig. 6).

DISCUSSION

It is now well established that one of the functions of PDZ motifs is the recruitment of proteins via recognition binding of their carboxyl-terminal domain (9, 10). The preferred motif in this domain has an extremely simple consensus structure (10, 34). This raises the question how the cell can meet the criteria required for high specificity in binding interaction, necessary to achieve correct functioning of the cell signaling network. As a working hypothesis for the studies presented here, we assumed that there must be a strong evolutionary preference to conserve the optimal interaction avidity between individual PDZ domains and their preferred substrate.

Interestingly, the protein-tyrosine phosphatase PTP-BAS/FAP-1, which contains five PDZ motifs, was found to directly associate with the Fas receptor via its second PDZ motif. However, the carboxyl terminus of the Fas receptor, which is involved in this interaction, is not strongly conserved between species (Fig. 1). Furthermore, the amino acids demonstrated to be important for the interaction with PTP-BAS (10, 12) have been substituted in the mouse Fas receptor. To conserve the interaction, the second PDZ motif of the mouse homologue of PTP-BAS/FAP-1, PTP-BL, may have adapted to these changes. This is not very likely, since this motif is very homologous between human (13) and mouse (22) (96% identical) and bovine (GenBank™ accession number 915210) (98% identical). Alternatively, the PTP-BL PDZ-II may be tolerant in peptide recognition, allowing different peptides to bind. This, however, would affect high specificity constraints necessary in signaling networks.

Our results show that PTP-BL does interact with the human Fas receptor, but fails to interact with the mouse Fas receptor. This indicates that specific peptide recognition is conserved between species and that changes, as found in the carboxyl terminus of the Fas receptor abolishes the interaction. This last observation is in line with the tightly defined consensus motif for binding to the second PDZ domain of PTP-BAS/FAP-1, which was independently found to be *t*S/TxV/I (10) or *t*S/TxV/L/I (12). Furthermore, none of the other PDZ motifs of PTP-BL does interact with the carboxyl terminus of the mouse Fas receptor, indicating that PTP-BL does not directly interact with the Fas receptor *in vivo*. This is corroborated by immunoprecipitation experiments, which failed to detect PTP-BAS/FAP-1 or an approximately 250-kDa protein in the unstimulated or stimulated Fas receptor-containing complex (20, 35, 36).

However, any indirect involvement of the tyrosine phosphatase PTP-BL in Fas signaling cannot be excluded, although even the basal question if tyrosine phosphorylation is involved in modulating Fas signaling is not answered yet. For tyrosine kinases as well as tyrosine phosphatases, both stimulatory and inhibitory effects on Fas-mediated apoptosis have been reported. For example, the protein-tyrosine kinase Abl and the tyrosine phosphatase PTP-BAS/FAP-1 both were found to inhibit the process (16, 37), whereas another cytosolic tyrosine phosphatase, the hematopoietic cell phosphatase HCP/SHP-1, and the p59fyn kinase seem to potentiate the apoptotic signal induced by Fas (38, 39). The involvement of HCP/SHP-1 remains controversial (40). Furthermore, a rapid induction of tyrosine phosphorylation of multiple cellular proteins upon stimulation of Fas-mediated apoptosis is reported (41). This phosphorylation as well as the apoptotic process could be blocked by inhibitors of protein-tyrosine kinases in a concentration-dependent manner. Findings that a Jurkat cell variant, deficient in this elevation of tyrosine phosphorylation upon Fas ligation, still does undergo apoptosis are contradictory to these findings (42). Furthermore, the observed correlation between the levels of endogenous PTP-BAS/FAP-1 and resistance to Fas-mediated apoptosis in several cell lines (16) is not confirmed by others (20). Taken together, the involvement of tyrosine phosphorylation in general and certain protein-tyrosine kinases and phosphatases in particular in Fas signaling remains controversial. The reported effects may be secondary downstream effects and might be cell type-specific.

Functional studies using our model system revealed no obvious role for the protein-tyrosine phosphatase PTP-BL in the regulation of Fas-mediated apoptosis. A small inhibitory effect on Fas-mediated apoptosis was observed in transformants expressing human Fas receptor. In contrast, PTP-BL seems to enhance apoptosis in mouse Fas receptor expressing transformants. However, both effects are not dependent upon the expression level of PTP-BL, since transformants expressing equal amounts of Fas receptor on their surface, but expressing different amounts of PTP-BL, behave very similar. Clonal variations and differences in the amount of surface expression of Fas receptor for the transformants with and without PTP-BL may partially account for the differences observed. Furthermore, the small inhibitory effect of PTP-BL on human Fas-mediated apoptosis may be caused by its capability to interact with the carboxyl terminus of the human Fas receptor and thereby inhibiting the formation of a functional death-inducing signaling complex rather than its catalytic activity. This effect will not occur in the mouse system, because PTP-BL does not interact with the mouse Fas receptor. Nevertheless, no influence of PTP-BL expression could be observed after overnight incubation with apoptosis-inducing antibodies, clearly demonstrating that PTP-BL can not (partially) protect from Fas-mediated apoptosis.

These results, combined with the different RNA expression patterns make an important role for PTP-BL in the regulation of Fas-mediated apoptosis in mouse very unlikely. In mouse the negatively regulatory carboxyl terminus of the Fas receptor may bind other, so far unknown, proteins and perhaps in a PDZ-mediated fashion. Interestingly, we have observed that a deletion of the carboxyl-terminal region of human Fas potentiates Fas-induced apoptosis in mouse L929 cells (43). However, a similar deletion in mouse Fas did not show any effect.[3] Thus, the role of the Fas carboxyl terminus in regulating the apoptotic process remains unclear. Furthermore, the target molecules for the PTP-BL PDZ motifs are still to be determined and will aid in elucidating its biological function.

[3] S. Nagata, unpublished observations.

Acknowledgment—We thank Cor Jacobs for help with flow cytofluorometric analysis, Drs. R. L. Finley and R. Brent for providing the yeast two-hybrid interaction trap plasmids and yeast strain, and Dr P. Anderson for the human Fas receptor cDNA.

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