Vertebrates and invertebrates initiate a series of defence mechanisms following infection by Gram-negative bacteria by sensing the presence of lipopolysaccharide (LPS), a major component of the cell wall of the invading pathogen. In humans, monocytes and macrophages respond to LPS by inducing the expression of cytokines, cell-adhesion proteins, and enzymes involved in the production of small proinflammatory mediators. Under physiopathological conditions, LPS exposure can lead to an often fatal syndrome known as septic shock. Sensitive responses of myeloid cells to LPS require a plasma protein called LPS-binding protein (LBP), or to a combination of LPS and LBP, by monitoring expression of a receptor gene driven by the NF-kB-responsive enhancer of the E-selectin gene. Although neither LPS nor LBP treatment alone caused significant gene activation, addition of both LPS and LBP resulted in a substantial induction of reporter gene activity in cells expressing TLR2, but not in the parental 293 cells. Furthermore, LPS and LBP induced NF-kB activity in TLR2-expressing cells with kinetics similar to those observed in myeloid and non-myeloid cells. Activation of NF-kB by LPS/LBP in 293-TLR2 cells does not require de novo protein synthesis, because pretreatment with cycloheximide does not inhibit the response. Both the membrane-bound, glycosylphosphatidyl inositol (GPI)-linked CD14 (mCD14), which is present on myeloid cells, and soluble CD14 (sCD14), which is present in plasma, enhance the response.

**Figure 1** Expression pattern of TLR2. a, Northern analysis of human multiple immune tissues hybridized with a TLR2 probe. PBL, peripheral blood leukocytes. b, Quantitative polymerase chain reaction with reverse transcription (RT-PCR) was used to determine the relative expression of TLR2 in PBL, T cells, macrophages (MΦ), and LPS-stimulated macrophages (MΦ + LPS).

**Toll-like receptor-2 mediates lipopolysaccharide-induced cellular signalling**

Ruey-Bing Yang, Melanie R. Mark, Alane Gray, Arthur Huang, Ming Hong Xie, Min Zhang, Audrey Goddard, William I. Wood, Austin L. Gurney & Paul J. Godowski

Department of Molecular Biology, Genentech, South San Francisco, California 94080-4990, USA

Correspondence and requests for materials should be addressed to S.N. (e-mail: snaru@mfour.med.

letters to nature

19. Morley, R. M., Cohn, C. A., Kluger, M. J. & Vander, A. J. Temperature regulation in biotemetered physiological conditions, LPS exposure can lead to an often fatal syndrome known as septic shock. Sensitive responses of myeloid cells to LPS require a plasma protein called LPS-binding protein. In humans, monocytes and macrophages respond to LPS by inducing the expression of cytokines, cell-adhesion proteins, and enzymes involved in the production of small proinflammatory mediators. Under physiopathological conditions, LPS exposure can lead to an often fatal syndrome known as septic shock. Sensitive responses of myeloid cells to LPS require a plasma protein called LPS-binding protein and the glycosylphosphatidylinositol-anchored membrane protein CD14. However, the mechanism by which the LPS signal is transduced across the plasma membrane remains unknown. Here we show that Toll-like receptor 2 (TLR2) is a signalling receptor that is activated by LPS in a response that depends on the interleukin-1 receptor (IL-1R) pathway and is enhanced by CD14. A region in the intracellular domain of TLR2 with homology to a portion of the IL-1 receptor (IL-1R) pathway that is implicated in the activation of the IL-1 receptor–associated kinase is required for this response. Our results indicate that TLR2 is a direct mediator of signalling by LPS. In *Drosophila*, the Toll receptor participates in establishing the dorsoventral pattern in the embryo and inducing an antifungal immune response in the adult fly. Toll is a type I transmembrane protein containing an extracellular domain with leucine-rich repeats and a cytoplasmic domain with sequence homology to the interleukin-1 receptor (IL-1R) and several plant disease-resistance proteins. Activation of Toll leads to induction of genes through induction of the NF-kB pathway. In humans, five homologues of *Drosophila* Toll, designated as Toll-like receptors (TLRs), have been identified. A constitutively active version of one human TLR (Toll-like receptor homologue) can activate NF-kB and induce expression of inflammatory cytokines and co-stimulatory molecules, suggesting that human TLRs participate in the innate immune response and signal the activation of adaptive immunity.

To determine whether human TLRs are involved in LPS-induced cell activation, we first investigated the expression of TLRs in immune tissues. TLR2 was found to be expressed in all lymphoid tissues examined, with the highest expression in peripheral blood leukocytes (Fig. 1a). Expression of TLR2 messenger RNA is enriched in monocytes/macrophages, the primary CD14-expressing and LPS-responsive cells. TLR2 mRNA is upregulated upon stimulation of isolated monocytes/macrophages with LPS (Fig. 1b), as has been reported for CD14 (refs 14, 15). We therefore investigated whether TLR2 could be involved in LPS-mediated cellular signalling.

We isolated a stable population and a stable clone of human embryonic kidney 293 cells that express an epitope-tagged version of TLR2 (gD-TLR2) (Fig. 2b). We tested the response of these cells to LPS prepared from *Escherichia coli* strain 055:B5, to LPS-binding protein (LBP), or to a combination of LPS and LBP, by monitoring expression of a reporter gene driven by the NF-kB-responsive enhancer of the E-selectin gene. Although neither LPS nor LBP treatment alone caused significant gene activation, addition of both LPS and LBP resulted in a substantial induction of reporter gene activity in cells expressing TLR2, but not in the parental 293 cells (Fig. 2a). Furthermore, LPS and LBP induced NF-kB activity in TLR2-expressing cells (Fig. 2c) with kinetics similar to those observed in myeloid and non-myeloid cells. Activation of NF-kB by LPS/LBP in 293-TLR2 cells does not require *de novo* protein synthesis, because pretreatment with cycloheximide (Fig. 2c) or actinomycin D (not shown) does not inhibit the response.

Both the membrane-bound, glycosylphosphatidyl inositol (GPI)-linked CD14 (mCD14), which is present on myeloid cells, and soluble CD14 (sCD14), which is present in plasma, enhance the response.

**Figure 1** Expression pattern of TLR2. a, Northern analysis of human multiple immune tissues hybridized with a TLR2 probe. PBL, peripheral blood leukocytes. b, Quantitative polymerase chain reaction with reverse transcription (RT-PCR) was used to determine the relative expression of TLR2 in PBL, T cells, macrophages (MΦ), and LPS-stimulated macrophages (MΦ + LPS).
After 24h, transfected cells were stimulated with LPS for 6h in the serum-free medium (bottom).

Vector control 293-MSCV (open symbols) or 293-TLR2 pop1 cells (closed symbols) were transfected with the reporter plasmid, and either a CD14 expression vector (+mCD14; triangles) or vector control (–mCD14; squares).

Figure 2 TLR2 mediates LPS-induced signalling. a, 293 cells stably expressing TLR2 acquire LPS responsiveness. We transiently transfected a population of clones expressing gD.TLR2 (293–TLR2 pop1), or a single clone expressing gD.TLR2 (293–TLR2 clone 1), or control cells (293–MSCV) with pGL3.ELAM.tk and then stimulated them with LPS for 6h with or without LBP in serum-free medium. Activation of the ELAM enhancer was measured as described in Methods. Results were obtained from two independent experiments. No stimulation was observed using the control reporter plasmid that lacked the ELAM enhancer (data not shown). Expression of the reporter plasmid was equivalent in untreated cells or cells treated with LBP alone (data not shown). b, Western blot showing expression of epitope-tagged TLR2 in 293 cells. c, Time course of TLR2-dependent LPS-induced activation and translocation of NF-κB. Nuclear extracts were prepared from cells treated with LPS (10 μg ml⁻¹) and LBP for the indicated times (top), or cells pretreated with cycloheximide (CHX) for 1h then stimulated with LPS for 1h in the presence of LBP in serum-free medium (bottom). d, Effect of mCD14 on NF-κB activation by TLR2. Vector control 293–MSCV (open symbols) or 293–TLR2 pop1 cells (closed symbols) were transfected with the reporter plasmid, and either a CD14 expression vector (+mCD14; triangles) or vector control (–mCD14; squares). After 24h, transfected cells were stimulated with LPS for 6h in the presence of LBP in serum-free medium. Data are representative of three independent experiments.

Figure 3 Domain function of TLR2 in signalling. a, TLR2 constructs. TLR2-WT is the full-length epitope-tagged form of TLR2. TLR2-Δ1 and -Δ2 represent truncations of 13 or 141 amino acids at the C terminus, respectively. CD4–TLR2 contains amino acids 1–205 of human CD4 fused to the transmembrane and intracellular domains of TLR2. ECD, extracellular domain; TM, transmembrane region; ICD, intracellular domain. b, C-terminal residues critical for IL-1R and TLR2 signal transduction. Residue numbers are shown to the right of each protein. Arrow indicates the position of the TLR2-Δ1 truncation. Asterisks indicate residues essential for IL-1R signalling7; I, identical amino acid; colon dots, conservative changes.

293 cells were transiently transfected with pGL3.ELAM.tk and response to LPS and LBP. 293 cells were transiently transfected with pGL3.ELAM.tk and expression vectors encoding TLR2 or TLR2 variants, as indicated. Cells were also transfected with a CD14 expression plasmid (+mCD14) or with a control expression plasmid that lacks any cDNA insert (–mCD14). Expression of the TLR2 variants was confirmed by western blotting using either anti-gD or CD4 antibody (bottom). The luciferase assay was done as described in Methods. Data were from duplicate experiments. d, Inhibition of endogenous LPS responsiveness by TLR2-Δ2. LPS-responsive U373 cells, transfected with reporter, mCD14 and vector alone or TLR2-Δ2 constructs, were stimulated with LBP and either 5 or 10 ng ml⁻¹ of LPS from E. coli K12 (strain LCD25) for 6h. The luciferase activity (average of duplicate wells) was determined and expressed as fold induction compared with unstimulated U373 cells transfected with reporter, mCD14 and control vector.
responsiveness of cells to LPS. We found that 293 cells express little or no CD14 on their cell surface (data not shown). However, transient transfection of 293 cells with mCD14 increased the sensitivity and magnitude of TLR2-mediated LPS responsiveness (Fig. 2d).

The intracellular domain of TLR2 is important in signalling the LPS response because variants with carboxy-terminal truncations of either 13 (TLR2-Δ1) or 141 amino acids (TLR2-Δ2) were defective for induction of the reporter in transiently transfected 293 cells (Fig. 3a, c). The region of the intracellular domain deleted in TLR2-Δ1 is homologous to a region of the IL-1R intracellular domain that is required for association with the IL-1R-associated kinase IRAK (Fig. 3b). The extracellular domain (ECD) of TLR2 is also required for responsiveness to LPS because a TLR2 variant in which the ECD of TLR2 was replaced with a portion of the ECD of CD4 failed to respond to LPS (Fig. 3a, c).

We investigated whether TLR2-Δ2 acted as a dominant-negative receptor to inhibit the 'endogenous' response of U373 astrocytoma cells, which express the cell-adhesion molecule ELAM and IL-6 in response to LPS. We found that the ELAM reporter construct was induced in U373 cells by low concentrations of LPS, that this response was not further modulated by expression of full-length TLR2 (data not shown), and that expression of TLR2-Δ2 in these cells inhibited the LPS response (Fig. 3d). Expression of the ELAM reporter construct in U373 cells is also induced by the tumour-necrosis factor TNF-α, which activates NF-κB through the TNF receptor. However, this response was not inhibited by co-expression of TLR2-Δ2 (data not shown). Also, TLR2-Δ2 forms heterodimers with TLR2, but not with the IL-1 receptor, in 293 cells (R.-B.Y., manuscript in preparation). Taken together, these results indicate that TLR-Δ2 is a specific inhibitor of LPS-mediated NF-κB activation.

LPS is a complex glycolipid consisting of the proximal hydrophobic lipid A moiety, the distal hydrophilic O-antigen polysaccharide region, and the core oligosaccharide that joins the lipid A and O-antigen structures. There is considerable diversity in the O-antigen structures from different Gram-negative bacteria. The lipid A portion of LPS is sufficient for LPS responses, whereas treatments that remove the fatty-acid side chains from the lipid A moiety inactivate LPS. We compared the potency of LPS prepared from various Gram-negative bacteria, lipid A alone, and LPS that had been 'detoxified' by alkaline hydrolysis. LPS isolated from Escherichia coli serotype LCD25 was nearly two orders of magnitude more potent than the serologically distinct Escherichia coli 055:B5 LPS (Fig. 4a). Using 293 cells that stably express both TLR2 and mCD14, we detected activation of the ELAM reporter using as little as 400 pg ml⁻¹ LCD25 LPS (Fig. 4b). LPS prepared from Salmonella minnesota R595 was also a potent inducer of TLR2 activity (Fig. 4a). TLR2 was activated by lipid A alone, whereas there was no response to 'detoxified LPS', even at concentrations as high as 10 μg ml⁻¹ (Fig. 4a, and data not shown).

We tested whether TLR2 binds LPS by using a soluble form of the TLR2 extracellular domain (TLR2-Fc) and ³H-labelled LPS in vitro. ³H-LCD25 LPS bound to the TLR2-Fc fusion protein but not to fusion proteins containing the ECDs of several other receptors (Fig. 4c). This binding was specifically competed by cold LCD25 LPS but not by detoxified LPS. Preliminary analysis of the binding of LPS to TLR2-Fc suggests that the Kₐ is relatively low (500–700 nM) and that the kinetics of association are slow (data not shown). Other proteins, such as LBP, may enhance binding of LPS to TLR2 in vivo, much like LBP acts to transfer LPS from its free, aggregated (micellar) form to CD14. Nevertheless, TLR2-Fc could be used to immunodeplete LPS, as assayed by the inhibition of LPS-induced TNF-α release from primary macrophages (Fig. 4d).

LPS treatment of macrophages leads to the expression of several inflammatory cytokines. Likewise, expression of TLR2 in 293 cells resulted in a >100 fold-induction of IL-8 mRNA in response to LPS/LBP, whereas detoxified LPS was inactive in this assay (Fig. 5).

Figure 4 High potency of E. coli K12 LPS (LCD25) and its binding to TLR2. a, Dose–response curve for activation of the ELAM reporter gene by different types of LPS in 293-TLR2 cells. b, Activation of TLR2 by low concentrations of LPS in 293 stably expressing both TLR2 and mCD14. c, Specific interaction of ³H-LPS (LCD25) with the extracellular domain of TLR2. We observed specific binding to TLR2-Fc, but not to Fc alone, or fusion proteins containing the extracellular domains of tyrosine kinase receptors Rse, Axl, Her2 or Her4. Binding to TLR2-Fc was specifically competed with cold LCD25 LPS, but not with detoxified LPS. d, Inhibition of LPS-induced TNF-α production by TLR2-Fc. Heparinized human whole blood was exposed to LCD25 LPS preincubated with buffer alone ('None') or with RSE-Fc or TLR2-Fc fusion protein. TNF-α levels were measured by enzyme-linked immunosassay. Unstimulated whole blood released no detectable TNF-α (<15 pg ml⁻¹).
CD4/TLR2 chimaera was constructed by PCR and contained amino acids 1–205 (the signal peptide and two immunoglobulin-like domains) of human CD4 fused to amino acids 588–784 (the transmembrane and intracellular domain) of human TLR2 with a linker-encoded valine at the junction of the CD4 and TLR2 sequences. The pGL3-ELAM.tkt reporter plasmid contained the sequence 5′-GCT ACC TCC TGA CAT GAT GTT AAT AAG CAT CTT GGA ATT TTG TAT TTC GGA GAA AGT TGG TAT GAG AAC AAT TGG GCC CGG GCG TCC GAG AAC CAC CAC TCT CCT GGA ATT TTG GAT CTG TCT GCC TGA ACC TCA AGG CTC CAC CTC GAA TAT TAA GGT GAC GGC TGT GCG GTC GCT GAA CAC CGA GGA ACC CAG GGA CCA ACC AGC TT-3′ inserted between the SacI and HindIII sites of the luciferase reporter plasmid pGL3 (Promega). The C-terminal epitope tag version of LBP (LBP-Flag) was constructed by PCR through the addition of an AscI site in place of the native stop codon and the subcloning of this fragment into pRK5-Flag, resulting in the C-terminal addition of amino acids GRADYKDDDDK.

**Stable cell lines/pools.** 293 human embryonic kidney cells were grown in LGDMEM/HAM’s F12 (50:50) medium supplemented with 10% FBS, 2 mM glutamine, and penicillin/streptomycin. The vector control line, 293-MSGCV, was generated by stable transfection of 293 cells with the empty expression cassette. For stable expression of gD.TLR2, cells were transfected with the gD.TLR2 expression vector and selected for puromycin resistance at a final concentration of 1 μg ml⁻¹. A stable pool of cells (293-TLR2 prop 1) was isolated by FACS using an antibody raised against the gD tag. Both the pool and the single-cell clone (293-TLR2 clone 1) were characterized by FACS and western blot analyses as described. A stable cell line expressing both CD14 and TLR2 was constructed by transfecting 293-TLR2 pop 1 with a CD14 expression vector containing a neomycin-selectable marker. A clone resistant to both neomycin and puromycin was isolated; expression of both gD-TLR2 and CD14 was verified by FACS and western blot analyses.

**Luciferase reporter assay and electrophoretic mobility shift assay (EMSA).** 293 parental or stable cells (2 × 10⁵ cells per well) were seeded into six-well plates, and transfected on the following day with the expression plasmids, together with 0.5 μg of the luciferase reporter plasmid pGL3-ELAM.tkt and 0.05 μg of the Renilla luciferase reporter vector as an internal control. After 24 h, cells were treated with either LPS, LBP or both LPS and LBP for 6 h, and the reporter gene activity was measured. Data are expressed as relative luciferase activity by dividing firefly luciferase activity with that of Renilla luciferase. For EMSA, nuclear extracts were prepared and used in a DNA-binding reaction with a 5′-[³²P]-radio-labelled oligonucleotide sequence containing a consensus NF-κB binding site (Santa Cruz Biotechnology, sc-251). The identity of NF-κB in the complex was confirmed by supershift with antibodies against NF-κB (data not shown). U373 cells were grown in 12-well plates (8 × 10⁵ cells per well) as described. Cells were transfected with 0.1 μg of reporter, 0.01 μg of Renilla control, 0.05 μg of pcDNA expression vector and 0.5 μg of either control or TLR2-Δ2 expression plasmid using DOPSER reagent (Boehringer-Mannheim). Cells were treated with LPS and extracts prepared as described above.

**RNA expression.** The tissue northern blot was purchased from Clontech and hybridized with a probe encompassing the extracellular domain of TLR2. Polyadenylated mRNA was isolated from 293 cells or 293-TLR2 cells and northern blots were probed with a human IL-8 cDNA fragment. TLR2 expression was determined using quantitative PCR using real time “Taqman” technology and analysed on a Model 770 sequence detector (Applied Biosystems) essentially as described. Forward and reverse primers, 5′-GCG GAG AGG ATT TTT GGT GAT GTT AAT AAG CAT CTT GGA ATT TTG TAT TTC GGA GAA AGT TGG TAT GAG AAC AAT TGG GCC CGG GCG TCC GAG AAC CAC CAC TCT CCT GGA ATT TTG GAT CTG TCT GCC TGA ACC TCA AGG CTC CAC CTC GAA TAT TAA GGT GAC GGC TGT GCG GTC GCT GAA CAC CGA GGA ACC CAG GGA CCA ACC AGC TT-3′. The 3′ polylinker of the expression vector followed by ligation of oligonucleotide linkers: Δ1:5′-TCA GCC GTA AGC-3′ and 5′-GCC CGC TTA CGG C -3′; Δ2:5′-TAA CAA GTG TAA GCC-3′ and 5′-GCC CGC TTA AGC TTA TGC A-3′. The

---

**Methods**

**Reagents.** ³H-labelled, unlabelled LCD25 and S. minnesota R595 LPS were from List Biochemicals; all other LPS were from Sigma. LBP was supplied as serum-free conditioned medium from 293 cells transfected with a human LBP expression vector. The TLR2-Fc fusion protein was produced by a baculovirus system and purified as described.

**Construction of expression plasmids.** A cDNA encoding human TLR2 was cloned from a human fetal lung library. The predicted amino-acid sequence matched that previously published, except for a Glu-to-Asp substitution of amino acid 726. The N-terminal epitope tag version of TLR2 (gD.TLR2) was constructed by adding an XhoI restriction site immediately upstream of leucine at position 17 (the first amino acid of the predicted mature form of TLR2) and at amino acid 726. The N-terminal epitope tag version of TLR2 (gD.TLR2) was constructed by adding an XhoI restriction site in place of the native stop codon and the subcloning of this fragment into pRK5-Flag, resulting in the C-terminal addition of amino acids GRADYKDDDDK.

**Somatic cell lines/pools.** 293 human embryonic kidney cells were grown in LGDMEM/HAM’s F12 (50:50) medium supplemented with 10% FBS, 2 mM glutamine, and penicillin/streptomycin. The vector control line, 293-MSGCV, was generated by stable transfection of 293 cells with the empty expression cassette. For stable expression of gD.TLR2, cells were transfected with the gD.TLR2 expression vector and selected for puromycin resistance at a final concentration of 1 μg ml⁻¹. A stable pool of cells (293-TLR2 prop 1) was isolated by FACS using an antibody raised against the gD tag. Both the pool and the single-cell clone (293-TLR2 clone 1) were characterized by FACS and western blot analyses.

**Luciferase reporter assay and electrophoretic mobility shift assay (EMSA).** 293 parental or stable cells (2 × 10⁵ cells per well) were seeded into six-well plates, and transfected on the following day with the expression plasmids, together with 0.5 μg of the luciferase reporter plasmid pGL3-ELAM.tkt and 0.05 μg of the Renilla luciferase reporter vector as an internal control. After 24 h, cells were treated with either LPS, LBP or both LPS and LBP for 6 h, and the reporter gene activity was measured. Data are expressed as relative luciferase activity by dividing firefly luciferase activity with that of Renilla luciferase. For EMSA, nuclear extracts were prepared and used in a DNA-binding reaction with a 5′-³²P-labelled oligonucleotide sequence containing a consensus NF-κB binding site (Santa Cruz Biotechnology, sc-251). The identity of NF-κB in the complex was confirmed by supershift with antibodies against NF-κB (data not shown). U373 cells were grown in 12-well plates (8 × 10⁵ cells per well) as described. Cells were transfected with 0.1 μg of reporter, 0.01 μg of Renilla control, 0.05 μg of pcDNA expression vector and 0.5 μg of either control or TLR2-Δ2 expression plasmid using DOPSER reagent (Boehringer-Mannheim). Cells were treated with LPS and extracts prepared as described above.

**RNA expression.** The tissue northern blot was purchased from Clontech and hybridized with a probe encompassing the extracellular domain of TLR2. Polyadenylated mRNA was isolated from 293 cells or 293-TLR2 cells and northern blots were probed with a human IL-8 cDNA fragment. TLR2 expression was determined using quantitative PCR using real-time “Taqman” technology and analysed on a Model 770 sequence detector (Applied Biosystems) essentially as described. Forward and reverse primers, 5′-GCG GAG AGG ATT TTT GGT GAT GTT AAT AAG CAT CTT GGA ATT TTG TAT TTC GGA GAA AGT TGG TAT GAG AAC AAT TGG GCC CGG GCG TCC GAG AAC CAC CAC TCT CCT GGA ATT TTG GAT CTG TCT GCC TGA ACC TCA AGG CTC CAC CTC GAA TAT TAA GGT GAC GGC TGT GCG GTC GCT GAA CAC CGA GGA ACC CAG GGA CCA ACC AGC TT-3′. The 3′ polylinker of the expression vector followed by ligation of oligonucleotide linkers: Δ1:5′-TCA GCC GTA AGC-3′ and 5′-GCC CGC TTA CGG C -3′; Δ2:5′-TAA CAA GTG TAA GCC-3′ and 5′-GCC CGC TTA AGC TTA TGC A-3′. The
Amino-acid transport by heterodimers of 4F2hc/CD98 and members of a permease family

Luca Mastroberardino*,†, Benjamin Spindler*,‡, Rahel Pfeiffer*, Patrick J. Skelly*, Jan Loffing†, Charles B. Shoemaker‡ & François Verrey†

Institutes of *Physiology and ‡Anatomy, University of Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland †Department of Immunology and Infectious Diseases, Harvard School of Public Health, 665 Huntington Avenue, Boston, Massachusetts 02115, USA
† These authors contributed equally to this work

Amino-acid transport across cellular plasma membranes depends on several parallel-functioning (co-)transporters and exchangers. The widespread transport system L accounts for a sodium-independent exchange of large, neutral amino acids, whereas the system y′L exchanges positively charged amino acids and/or neutral amino acids together with sodium23. The molecular nature of these transporters remains unknown, although expression of the human cell-surface glycoprotein 4F2 heavy chain (4F2hc; CD98 in the mouse) is known to induce low levels of L- and/or y′L-type transport4. This glycoprotein is found in activated lymphocytes, together with an uncharacterized, disulfide-linked lipophilic light chain with an apparent relative molecular mass of 40,000 (M, 40K).11 Here we identify the permease-related protein E16 (ref. 12) as the first light chain of 4F2hc and show that the resulting heterodimeric complex mediates L-type amino-acid transport. The homologous protein from Schistosoma mansoni, SPRM1, also associates covalently with coexpressed 4F2hc glycoprotein, although it induces amino-acid transport of different substrate specificity. The coexpression of 4F2hc is required for surface expression of these permease-related light chains, which belong to a new family of amino-acid transporters that form heterodimers with cell-surface glycoproteins.

While searching for early aldosterone-regulated gene products, we cloned a complementary DNA (ASUR4) from A6 kidney cells. This cDNA encodes a highly lipophilic, permease-related protein that migrates in SDS–polyacrylamide gel electrophoresis (PAGE) as a band of ~40K (theoretical M, 55.5K; in vitro translation not shown) (EMBL accession number Y12716)13. The protein ASUR4 shows 26% identity with the yeast methane permease MUP-1 (ref. 14) and 40% identity with the membrane protein SPRM1 of the platyhelminth S. mansoni (GenBank accession number L25068; P.I.S. and C.B.S., manuscript in preparation). The highest degree of identity found in the database is with the human E16 protein, a partial cDNA of which had originally been cloned because it is induced in activated lymphocytes2. The predicted amino-acid sequence of the full-length coding cDNA of E16 (P.I.S., B.M. Rosenthal and C.B.S., manuscript in preparation; GenBank accession number AFO7866) indicates that it is the human homologue of ASUR4 (79% identity; sequence comparison is available as Supplementary information). The hydrophathy and TMpred plots of ASUR4, E16 and SPRM1 are very similar and support a 12-transmembrane-domain topology (Fig. 1) (ISREC TMpred server)13.