Unique pattern of expression and inhibition of IL-1 signaling by the IL-1 receptor family member TIR8/SIGIRR

Nadia Polentarutti[1], Giselle Penton Rol[1, 2], Marta Muzio[1, 3], Daniela Bosisio[1, 4], Marco Camnasio[1, 5], Federica Riva[1, 6], Carla Zoja[7], Ariela Benigni[7], Susanna Tomasoni[7], Annunciata Vecchi[1], Cecilia Garlanda[1], Alberto Mantovani[1, 8, 9]

[1] Dept. Immunology and Cell Biology, Mario Negri Institute for Pharmacological Research, Milan, 20157, Italy
[2] Current affiliation : Centro de Ingenieria Genetica y Biotecnologia, Havana, Cuba
[3] Current affiliation : Dept. Pharmacology, Discovery Research Oncology, Pharmacia corp., Nerviano, Italy
[4] Current affiliation : Inst. for Research in Biomedicine, Bellinzona, Switzerland
[5] Current affiliation : MolMed S.p.A. Milan, Italy
[6] Current affiliation : Dept. Animal Pathology, Hygiene and Public Health, Faculty of Veterinary Medicine, University of Milan, Milano, Italy
[7] Dept. Molecular Medicine, Mario Negri Institute for Pharmacological Research, Bergamo, Italy
[8] Centro IDET, Institute of General Pathology, Università degli Studi di Milano, Milan, 20100, Italy
N.P. and G.P.R. equally contributed to this project

Correspondence: Alberto Mantovani, Dept. Immunology and Cell Biology, Mario Negri Institute for Pharmacological Research, via Eritrea 62, Milan, 20157, Italy. Tel : 39 2 39014493. Fax : 39 2 39014596. E-mail : mantovani@marionegri.it

ABSTRACT. TIR8, also known as single Ig IL-1R-related molecule (SIGIRR), is a member of the IL-1 receptor family. The present study was designed to investigate the expression and function of TIR8. TIR8 was mainly expressed in mouse and human epithelial tissues such as kidney, lung and gut. Resting and activated T and B lymphocytes and monocytes-macrophages expressed little or no TIR8, with the exception of the mouse GG2EE macrophage line. In the kidney, the organ with highest mRNA levels, TIR8 expression was confined to epithelial cells and, *in situ*, to tubular epithelium. A variety of signals failed to regulate TIR8 expression, but LPS reduced TIR8 mRNA transcripts. An NF-kB driven reporter system was used to investigate the function of TIR8. TIR8 did not activate NF-kB expression alone or in concert with IL-1R1. In contrast, TIR8 inhibited signaling from the IL-1R complex. Inhibition required the intracellular portion of TIR8 but the extracellular domain was dispensable for blocking activity. Thus, TIR8 is a unique member of the IL-1R family, with a distinct pattern of epithelial expression, including the kidney and mucosae, and an inhibitory function on IL-1 signaling.

Keywords: TIR8; SIGIRR; IL-1R/TLRs; expression; signal transduction regulation

INTRODUCTION

Interleukin-1 receptors (IL-1Rs) and Toll-like receptors (TLRs) are members of a large superfamily of phylogenetically conserved proteins involved in innate immunity and inflammation [1, 2]. The common characteristics of the members of this family is the presence, in their cytoplasmic region, of a conserved sequence, called Toll/IL-1R (TIR) domain, which is involved in the activation of a stereotypical signaling pathway leading to nuclear factor kB (NF-kB) translocation to the nucleus and activation of protein kinases such as p38 mitogen-activated protein kinase and JNK [3]. The family is subdivided into two subfamilies, depending on the structure of the extracellular region: TLRs bear leucine-rich repeats in the extracellular domain, whereas IL-1Rs bear Ig-like domains. TLRs play an essential role in the detection of microbes through the recognition of well identified and specific pathogen-associated molecular patterns [1], and IL-1Rs subfamily includes the receptors and the accessory proteins for IL-1 and IL-18. However, in both subfamilies several members remain orphan receptors with still unknown ligands and functions. The orphan receptor T1/ST2 is a member of the IL-1R family, preferentially expressed on T helper type 2 cells, that appears to be involved in the regulation of Th2 cell function [4, 5]. Among mammalian TLRs, TLR10 does not yet have a characterized ligand [2].

TIR8, also called single Ig IL-1R-related molecule (SIGIRR), is an orphan receptor belonging to the IL-1R subfamily, identified by our group in 1998 (Accession number: AF113795) and by others [6], by searching in EST databases for TIR domain-containing sequences. TIR8 full length cDNA predicted a 409 amino acid-long protein with unique and interesting characteristics: TIR8 is composed of a single Ig domain in its extracellular region (amino acids 17-112), a transmembrane domain (amino acids 117-139), an intracellular, conserved TIR domain (amino acids 166-305), and a unique, characteristic 95 amino acid-long tail which differentiates TIR8 from other IL-1/TLR superfamily members.

The activation of the signaling cascade leading to the production of proteins related to inflammation and immunity by IL-1/TLRs is tightly regulated. An uncontrolled or deregulated activation of these receptors can be detrimen-

tal as they mediate potentially devastating local and systemic inflammatory reactions. For the IL-1 system, the control is exerted at different levels, extracellularly and intracellularly: IL-1R antagonists are pure polypeptide antagonists [7, 8] and the IL-1RII, which lacks a signaling domain, both in membrane-bound or released form, binds IL-1, preventing its interaction with a signaling receptor complex. In addition, it forms a dominant, negative non-signaling complex with the accessory protein (AcP) [9-11]. A negative regulation of signal transduction is also exerted by IRAK-M [12], one member of the IL-1 receptor-associated kinase (IRAK) family, which lacks kinase activity and regulates the dissociation of IRAK-1 and IRAK-4 from the adaptor protein MyD88, and by MyD88s [13], a spliced form of MyD88, which prevents IRAK-4 recruitment.

In this report, we describe the unique pattern of expression of TIR8 in murine and human tissues and cells, and we show, using an NF-kB-driven reporter system with full length TIR8 or truncated molecules and chimeric proteins, that TIR8 and, in particular, its intracellular domain, exerts a negative regulatory effect on IL-1R signaling.

METHODS

Animals

Male and female, 8-10 week-old C57Bl/6 mice were obtained from Charles River Laboratories (Calco, Italy). When indicated, C57Bl/6 mice were injected intravenously (i.v.) with 1 µg lipopolysaccaride (LPS) (*E. coli* O55:B5, Sigma, St. Luis, MO, USA) in 0.2 ml saline and sacrified 6 hours later for tissue mRNA extraction.

Procedures involving animals and their care conformed with institutional guidelines, in compliance with national (4D.L. N.116, G.U., suppl. 40, 18-2-1992) and international law and policies (EEC Council Directive 86/609, OJ L 358,1,12-12-1987; NIH Guide for the Care and Use of Laboratory Animals, US National Research Council 1996). All efforts were made to minimize the number of animals used, and their suffering.

Cell cultures

Murine peritoneal macrophages and neutrophils (PMN) were collected from the peritoneal cavity, 5 days and 4 hours respectively, after the injection of 1 ml 3% sterile thioglycollate (Difco, Detroit, MI, USA). Bone marrow-derived macrophages were generated by culturing bone marrow cells for 5 or 7 days, in the presence of 20%, L929 cell-conditioned medium. Murine macrophagic cell lines GG2EE [14] and J774 (ATCC TIB 67) were cultured in RPMI 1640 medium (Biochrome, Berlin, Germany), with 10% FCS (Hyclone Laboratories, Logan, UT, USA). The murine endothelial cell line 1G11 was isolated and cultured as described [15].

Circulating human monocytes, lymphocytes and polymorphonuclear cells were obtained from peripheral blood of healthy donors. Cells were separated by Percoll (Pharmacia, Uppsala, Sweden) gradient centrifugation (> 95% pure as assessed by morphology), as described [16]. Monocyte-derived macrophages were obtained from freshly isolated monocytes after incubation for 5 days in RPMI 1640 complete medium supplemented with 40% autologous serum as described [17]. NK cells were obtained through a Ficoll (Biochrome, Berlin, Germany) gradient, monocyte depletion, and discontinuous Percoll (Pharmacia) gradient [18]. Large B cells were prepared from tonsils as described [19]. Human umbilical vein endothelial cells were obtained as described [20]. HK-2 cells, a human proximal tubular cell line, were obtained from the ATCC (Rockville, MD, USA) and grown in DMEM/F-12 plus 5% fetal calf serum (FCS) supplemented with L-glutamine (2 mmol/L), penicillin (100 U/ml), streptomycin (100 µg/ml), 3,3',5-triiodo-L-thyronine (5 pg/ml), hydrocortisone (5 ng/ml), prostaglandin E1 (5 pg/ml), epidermal growth factor (10 ng/ml), insulin (5 µg/ml), transferrin (2.5 µg/ml) and sodium selenite (3.3 ng/ml), as previously described [21]. For the experiments, cells were pre-incubated with serum-free DMEM/F12 for 24 hours. To investigate TIR8/SIGIRR mRNA expression, confluent HK-2 were exposed for 24 hours to medium alone (control) or TNF-α. Primary cultures of mesangial cells were obtained from collagenase-treated isolated human glomeruli and cultured as previously described [22]. For the experiments, confluent mesangial cells were incubated for 6 hrs at 37°C in RPMI 1640 medium with 10% FCS.

Northern blot analysis

Total RNA was isolated from mouse tissues, and mouse and human cells by the guanidine isothiocyanate method. Total RNA (10 µg) was analyzed by elecrophoresis through 1% agarose/formaldehyde gels, followed by Northern blot transfer to Gene Screen Plus membrane (New England Nuclear, Boston, MA, USA). Expression analysis of human TIR8 was performed using a commercial filter (Human Multiple Tissue Expression Array, Clontech, Palo Alto, CA, USA) with polyA RNA from several human tissues. Human and mouse TIR8 full length c-DNAs were labeled with [α-^{32}P]dCTP (3000 Ci/mmol; Amersham, Buckinghamshire, UK), using the Megaprime DNA labeling system (Amersham). Membranes were pretreated and hybridized in 50% formamide (Merck, Rahway, NJ, USA) with 10% dextran sulfate (Sigma), 1% sodium dodecyl sulfate (SDS; Merck), 1 M NaCl, and 100 µg/mL salmon sperm DNA at 42 °C for 16 hours, washed twice with 2X SSC (1X SSC; 0.15 M NaCl; 0.015 M sodium citrate), and 1% SDS at 60 °C for 30 minutes, and finally repeatedly washed with 0.1X SSC at room temperature. Membranes were exposed, at-80 °C, to autoradiography. RNA transfer to membranes was checked by UV irradiation, as shown in each figure.

In situ *hybridization*

The mouse TIR8 antisense and sense RNA probes were prepared and labeled by *in vitro* transcription using digoxigenin-labeled uridine triphosphate (Roche, Milan, Italy) [23]. A 317 bp mTIR8 cDNA was cloned into the BamHI/XbaI sites of the pcDNA3 vector between T7 and SP6 promoters. Fragments of renal cortex were fixed in Dubosq-Brazil, dehydrated in alcohol, and embedded in paraffin. Sections were cut at 4 µm and processed as described. Briefly, after permeabilization with proteinase K (40 µg/ml, Sigma, St. Louis, MO, USA), the sections were hybridized with the RNA probes at the final concentration of 0.4 ng/µl in 2X standard sodium citrate (SSC), 10% dextran sulfate, 1X Denhardt's solution, 20 mM Vanadyl

Ribonucleoside Complex (Invitrogen, Life Technologies, Milan, Italy), 0.1 M sodium phosphate, and incubated overnight in a moist chamber at 45 °C. After being washed in 0.2X SSC and blocked with a buffer blocking solution (50 mg/ml skimmed dried milk, 150 mM NaCl in 100 mM Tris HCL, pH 7.8) at room temperature for 30 min, the sections were incubated with anti-digoxigenin antibody conjugated with alkaline phosphatase (Roche) at the dilution of 1:1000 for 45 min at 37 °C. Colorimetric detection with nitro blue tetrazolium salt and 5-bromo-4-chloro-3-indolyl phosphate (Roche) was then performed, and the sections were mounted in 60% glycerol and examined by light microscopy. The negative control included a hybridization step with the sense probe.

Biological reagents

All reagents contained < 0.125 units/ml of endotoxin as checked by Limulus amebocyte lysate assay (Microbiological Associates, Walkersville, MD, USA).

LPS was used at 100 ng/ml. Human recombinant IFN-γ was a kind gift of Institut Roussel-Uclaf (Paris, France). Human recombinant TNF-α (BASF/Knoll, Ludwigshafen, Germany) was used at 500 U/ml. Human recombinant IL-1β was a kind gift from Dr. J. E. Sims (Immunex, Seattle, WA, USA). Human recombinant IL-4 was from Schering-Plough (Kenilworth, NJ, USA).

Expression vectors and transfection

Full-length murine TIR8 cDNA (TIR8-1300) and truncated molecules with the novel cytoplasmic domain (TIR8-950) or the cytoplasmic region (TIR8-450) deleted were generated by PCR. The primers used were for TIR8-1300: 5'-agcaggtgtctgtgacatggcccc (forward) and 5'-gtcctttcctcacaccggggtcc (backward); for TIR8-950: 5'-agcaggtgtctgtgacatggcccc (forward) and 5'-tcattgagggtctccctccaccggcctg (backward); for TIR8-450: 5'-agcaggtgtctgtgacatggcccc (forward) and 5'- tcaggtcatctccaccacccggtaagt (backward). Chimeric molecules were generated by PCR, amplifying and ligating the extracellular and the transmembrane domains of the murine AcP, and the full length (AcP-out-TIR8-in) or truncate intracellular domains of mTIR8 (AcP-out-TIR8-D-in and AcP-out). Murine AcP, amplified by PCR, was used as control. Primers used were: for the full length mAcP: 5'-ggaattcaggacttctgtggtatttgatg (forward) and 5'-gaagatcttcagccagtaaacatggtaaac (backward); for the truncated mAcP (AcP-out): 5'-ggaattcaggacttctgtggtatttgatg (forward) and 5'-ccatcgatggccagtaaacatggtaaaccac (backward); for full length intracellular domain of mTIR8 (TIR8-in): 5'-ccatcgatgctttggtaccaagacacttaccgg (forward) and 5'-gtcctttcctcacaccggggtcc (backward); for the truncated intracellular domain of mTIR8 (TIR8-Δ-in): 5'-ccatcgatgctttggtaccaagacacttaccgg (forward) and 5'-tcattgagggtctccctccaccggcctg (backward). Constructs EcoRI-XbaI were inserted, into the polylinker of the mammalian expression vector pFlag-CMV-1 (Eastman Kodak Co., Rochester, NY, USA). The human IL-1RI was a gift from J. E. Sims (Immunex).

Reporter genes pCMV-β-galactosidase (0.2 μg) and ELAM-NF-kB-luciferase (0.1 μg), pFlag-CMV-1 empty vector (1 μg) or the expression vectors mentioned (1 μg) were used in co-transfection experiments. 293 (human embryonic kidney) cells were grown in DMEM (Biochrome, Berlin, Germany) with 10% FCS. Sixty per cent confluent cells were co-transfected by the calcium-precipitated method with the indicated expression vectors. After 48 hours, cells were lysed with 100 μl of reporter lysis buffer (Promega, Medison, WI, USA), and luciferase activity was measured with a Promega kit and a luminometer. β-galactosidase activity was determined by a colorimetric method to normalize transfection efficiency as described [24]. When indicated, cells were stimulated with 100 ng/ml of IL-1β and lysed 6 hours later. Data shown are from one out of two to five independent experiments with similar qualitative results.

RESULTS

Expression of TIR8 mRNA in murine tissues and cells

To study TIR8 mRNA expression in murine tissues and leukocyte subpopulations, we performed Northern blot analysis of total RNA using radiolabeled mTIR8 cDNA as probe.

Northern blot analysis of total mRNA extracted from murine tissues (Figure 1A) revealed a particularly strong result for murine TIR8 in the kidney, colon and lung; a weaker result was also present in the liver, thymus and spleen. Almost no signal was present in the brain, heart, testis and skeletal muscle.

To specifically analyze TIR8 expression in leukocyte populations, we recovered PMN and macrophages from the peritoneal cavity of thioglycollate-treated mice. We obtained bone marrow- (BM-) derived macrophages by culturing total bone marrow cells with 20% L929 cell-conditioned medium for 5 or 7 days, and lymphocytes from splenocytes depleted of adherent cells. Thioglycollate-elicited peritoneal macrophages and BM-derived macrophages cultured for 5 or 7 days expressed a very faint signal for TIR8 (Figure 1B). A weak signal was also present in thioglycollate-elicited peritoneal PMN. Even after a long exposure of the blots, no signal for TIR8 was evident in lymphocytes isolated from the spleen (not shown). Two different macrophagic cell lines were also analyzed: GG2EE expressed high levels of the messenger, whereas J774 showed a weaker signal. Finally, the endothelial cell line 1G11 expressed low amounts of TIR8 mRNA in basal conditions.

These results suggested a preferential expression of murine TIR8 in tissues with an epithelial component, whereas in leukocytes TIR8 expression was very faint.

Expression of TIR8 mRNA in human tissues and cells

To analyze TIR8 expression in human tissues, a multiple tissue expression array was hybridized with a radiolabelled hTIR8 probe. As shown in Figure 2A, a signal was present essentially in all the organs analyzed, indicating that hTIR8 has a ubiquitous expression, but a particularly strong message was found in the organs of the digestive tract, in the kidney, in the liver and other organs with an epithelial component and in lymphoid organs. Among the cell lines present in the array, the leukocytic cell lines exhibited a faint signal for TIR8, whereas a colorectal adenocarcinoma line expressed high levels of the messenger. In fetal tissues, the hybridization signal was particularly strong in the kidney, in the spleen and in the lung.

We next analyzed TIR8 expression in human leukocytes: we separated fresh human monocytes, T lymphocytes, NK

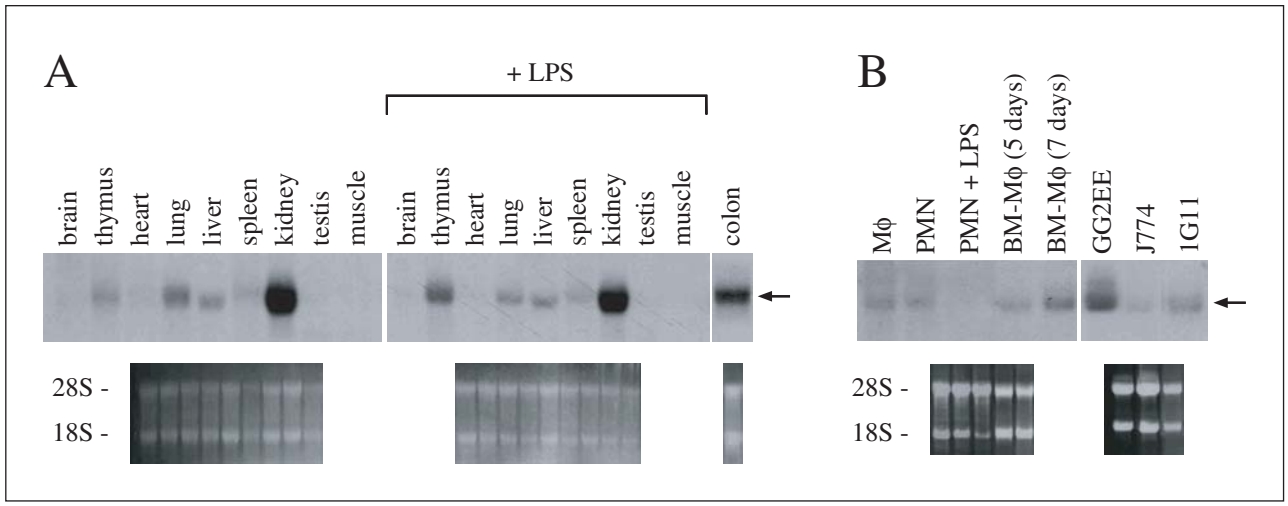

Figure 1

TIR8 gene expression in murine tissues and immunocompetent cells.

A: Total RNA was extracted from tissues of untreated or LPS-injected mice as indicated in Methods and analyzed by Northern blot. Specific TIR8 transcripts are indicated by the arrow. The lower part of the panel shows the ethidium bromide staining after RNA transfer to the membrane. Ten μg of total RNA were used in each lane.

B: Total RNA was extracted from freshly isolated (Mf, macrophages; PMN, neutrophils) or cultured (BM-mf, bone marrow-derived macrophages) immunocompetent cells, macrophage cell lines (GG2EE and J774) and an endothelial cell line (1G11). When indicated, PMN were stimulated with LPS for 4 hours. Northern blot analysis was performed as indicated in panel A.

cells and PMN from peripheral blood of healthy donors. B cells were prepared from tonsils. Macrophages were obtained by culturing monocytes with 40% autologous serum for 5 days. As shown in Figure 2B, we found a strong signal in freshly isolated or cultured NK cells and an extremely weak signal in monocytes, macrophages and PMN. Circulating B cells, large B cells from tonsils and resting or PHA-activated lymphocytes did not express the transcript. Finally, human umbilical vein endothelial cells exhibited a faint signal in basal conditions.

Regulation of TIR8 expression by inflammatory stimuli

To determine whether inflammatory conditions could somehow modulate TIR8 mRNA levels, mice were treated with 1 μg LPS i.v. and their organs were excised and analyzed by Northern blot 6 hours after the inoculum. As shown in Figure 1A, after LPS administration, the expression of TIR8 was partially down-modulated in all the organs that expressed the transcript in basal conditions. A partial down-modulation of TIR8 expression was also evident in human PMN and monocytes upon treatment with LPS.

Given the low expression of TIR8 in leukocytes in resting conditions, or upon activation with LPS, we treated human monocytes with a series of different pro- and anti-inflammatory stimuli to investigate the regulation of its expression. As shown in Figure 2C, the expression of TIR8 in human monocytes was not modulated by any of the stimuli analyzed (IL-1β, IFN-γ, TNF-α, IL-4). A similar assay was performed with murine thioglycollate-elicited peritoneal macrophages, using a series of cytokines (IL-1β, IFN-γ, TNF-α, IL-4), microbial moieties (LPS, peptidoglycan, poly IC, CpG) or heat inactivated microbes (*Candida albicans, Aspergillus fumigatus, Pseudomonas aeruginosa, Staphilococcus aureus*, zymosan) to activate the cells, but we failed to observe up-regulation of TIR8 mRNA expression in this cell type under these conditions (not shown).

TIR8 expression in the kidney

Following the initial observations of the particularly high expression of TIR8 mRNA in the whole kidney, both in human and mouse, it was of interest to investigate the cellular component expressing TIR8 in this organ. Murine cortical and medullar kidney were analyzed separately by Northern blot. As shown in Figure 3A, a strong signal was found in both parts of the organ.

To further investigate this aspect, we performed *in situ* hybridization in murine kidney sections with a radiolabelled, antisense-oriented riboprobe encoding mTIR8 and a sense-oriented negative probe as control. As shown in Figure 3B, upper panel, only tubular cells exhibited a strongly positive hybridization signal. In contrast, glomerular cells did not exhibit any positive signal.

To confirm this results in human kidney, a human cell line of mesangial origin and a tubular cell line (HK2) were analyzed by Northern blot. As shown in Figure 3C, hybridization was restricted to the tubular cell line HK2. An inflammatory cytokine, TNF-α did not modulate TIR8 expression in HK2 cells.

TIR8 exerts a negative role in IL-1-induced NF-kB activation

To investigate the function of TIR8, an NF-kB-driven reporter system was used. Two hundred and ninety three cells were co-transfected with a NF-kB responsive construct and different expression constructs encoding the full length TIR8 (TIR8-1300), or the truncated versions lacking the novel intracellular domain (TIR8-950) or all the intracellular portion of the molecule (TIR8-450) (Figure 4A). In contrast with what has been observed with other IL-1R family members, such as IL-1RI or AcP e.g. [25], forced over-expression of these constructs did not activate NF-kB expression, nor did it modulate IL-1RI-induced NF-kB activation (not shown). In contrast, when cells were treated with IL-1, full length TIR8 (TIR8-1300) inhibited IL-1RI-induced NF-kB activation (Figure 4B); interest-

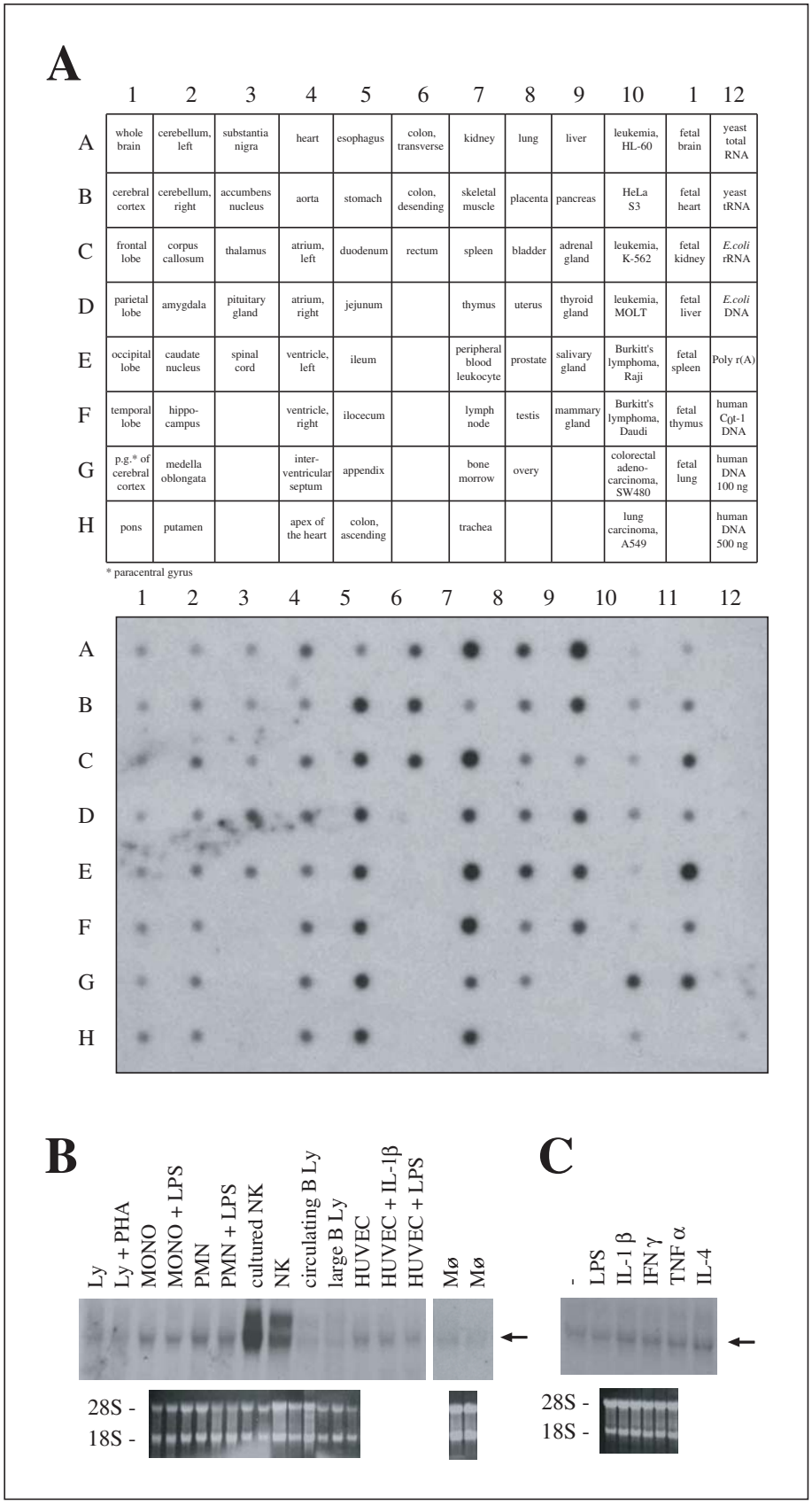

Figure 2

TIR8 gene expression in human tissues and immunocompetent cells.

A: A human multiple tissue expression array was hybridized with a radiolabelled hTIR8 probe.

B: Total RNA was extracted from freshly isolated (Ly, lymphocytes; mono, monocytes, PMN, neutrophils; NK, natural killer) or cultured (Mf, macrophages) immunocompetent cells and endothelial cells (HUVEC, human umbilical vein endothelial cells) in the absence or presence of the indicated stimuli for 4 hours and analyzed by Northern blot. Specific TIR8 transcripts are indicated by the arrow. The lower part of the panel shows the ethidium bromide staining after RNA transfer to the membrane. Ten μg of total RNA were used in each lane.

C: Monocytes were treated with the indicated stimuli for 4 hours. After incubation total RNA was extracted and analyzed by Northern blot as described in panel B.

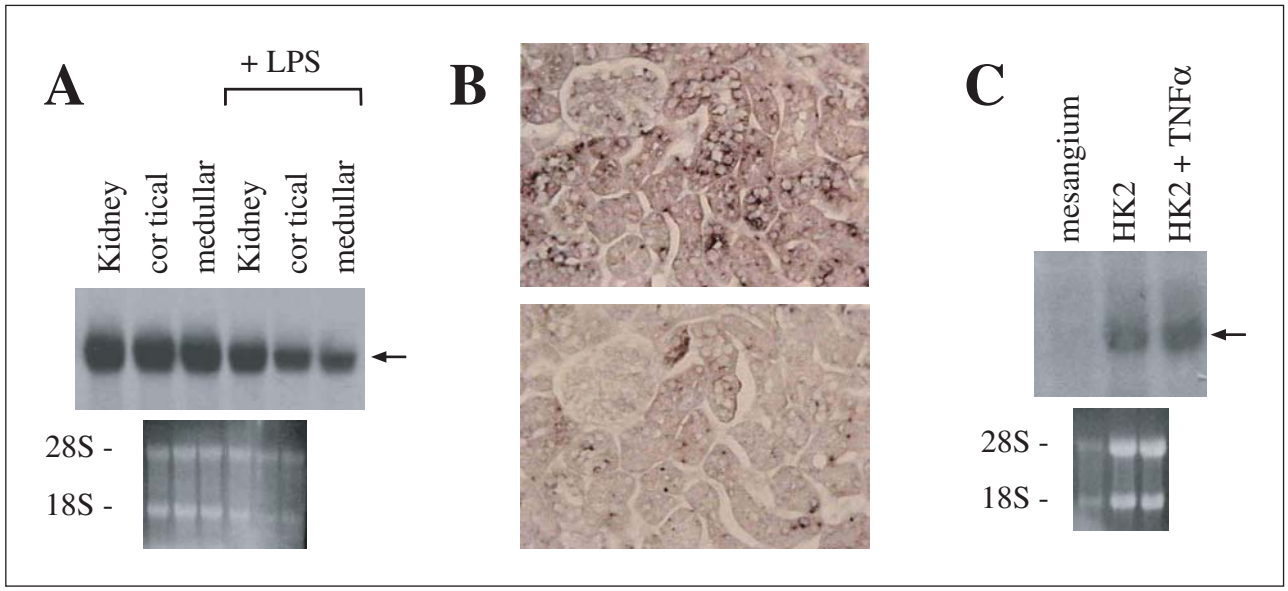

Figure 3

TIR8 mRNA expression in kidney.
A: Total RNA was extracted from total kidney and dissected cortical and medullar kidney of untreated or LPS injected mice and analyzed by Northern blot. Specific TIR8 transcripts are indicated by the arrow. The lower part of the panel shows the ethidium bromide staining after RNA transfer to the membrane. Ten μg of total RNA were used in each lane.
B: *In situ* hybridization analysis of TIR8 mRNA expression in murine kidney with a radiolabelled antisense-oriented probe (upper panel). A sense-oriented probe was used as negative control (lower panel).
C: Norther blot analysis of total RNA extracted from mesangial and tubular (HK2) cell lines. TIR8 transcripts are indicated by the arrow. The lower part of the panel shows the ethidium bromide staining after RNA transfer to the membrane. Ten μg of total RNA were used in each lane.

ingly, the inhibitory effect was lost when truncated TIR8 molecules, TIR8-950 and in particular TIR8-450, were co-transfected with IL-1RI (Figure 4B).

To study the role played by the extracellular and cytoplasmic domains of TIR8, we generated chimeric molecules by engineering distinct expression constructs encoding for the extracellular and transmembrane domains of the murine AcP and the full length or truncated versions of TIR8 (Figure 4C). Also in this case, in the absence of IL-1, ectopic expression of these constructs did not activate NF-kB (not shown). In the presence of IL-1, the chimeric molecule containing the full length intracellular domain of TIR8 (AcP-out-TIR8-in) inhibited NF-kB activation induced by the endogenous IL-1R complex (Figure 4D). The blocking effect was lost when the two truncated chimeric molecules were used (AcP-out-TIR8-D-in and AcP-out), in particular with the construct containing only the extracellular and transmembrane domain of AcP (AcP-out) (Figure 4D). We observed the same effect when IL-1RI was co-transfected and the cells were treated with IL-1β (Figure 4E). Under these conditions, NF-kB activation induced by ectopic IL-1RI was strongly inhibited by the chimeric molecule containing the full length TIR8 intracellular domain (AcP-out-TIR8-in) and the blocking effect was reduced when the truncated molecules (AcP-out-TIR8-D-in and AcP-out) were used.

All together, these results suggest that TIR8 did not activate NF-kB expression alone or in concert with IL-1R1. In contrast, TIR8 inhibited signaling from the IL-1R complex. Inhibition required the intracellular portion of TIR8 but the extracellular domain was dispensable for blocking activity.

DISCUSSION

The results presented here show that TIR8 (also known as SIGIRR), a member of the IL-1R family, has a unique pattern of expression *in vitro* and *in vivo*. In mouse and man, TIR8 was most prominently expressed in organs with an epithelial component, such as the liver, lung and gut, in agreement with a previous report [6]. Moreover, the kidney was the organ with the highest level of mRNA transcripts. Resting and stimulated T and B lymphocytes and mononuclear phagocytes generally had low levels of TIR8 mRNA. However, substantial expression in the mouse macrophage GG2EE cell line [14] raises the possibility that this receptor may be expressed at some stages in cells related to the myelomonocytic differentiation pathway. When expression in the kidney was further dissected, TIR8 mRNA was localized in the epithelial component, in particular in the proximal tubular cells, and not in mesangial cells.

A variety of pro- and anti-inflammatory signals failed to induce TIR8 expression in mononuclear phagocytes. However, TIR8 transcripts were down-regulated by *in vivo* administration of LPS. Other members of the IL-1R family are regulated by pro- and anti-inflammatory signals [9, 11, 26-28]. For instance, the orphan receptor T1/ST2 [4] is up-regulated by LPS [26]. Thus, the tissue and cellular expression of TIR8, as well as its regulation, are distinct from those of other members of the IL-1R family.

In an effort to identify the function of TIR8, a gene transfer/NF-kB reporter system was used. Under the experimental condition used (data presented here and data not shown), we have failed to identify a signaling function

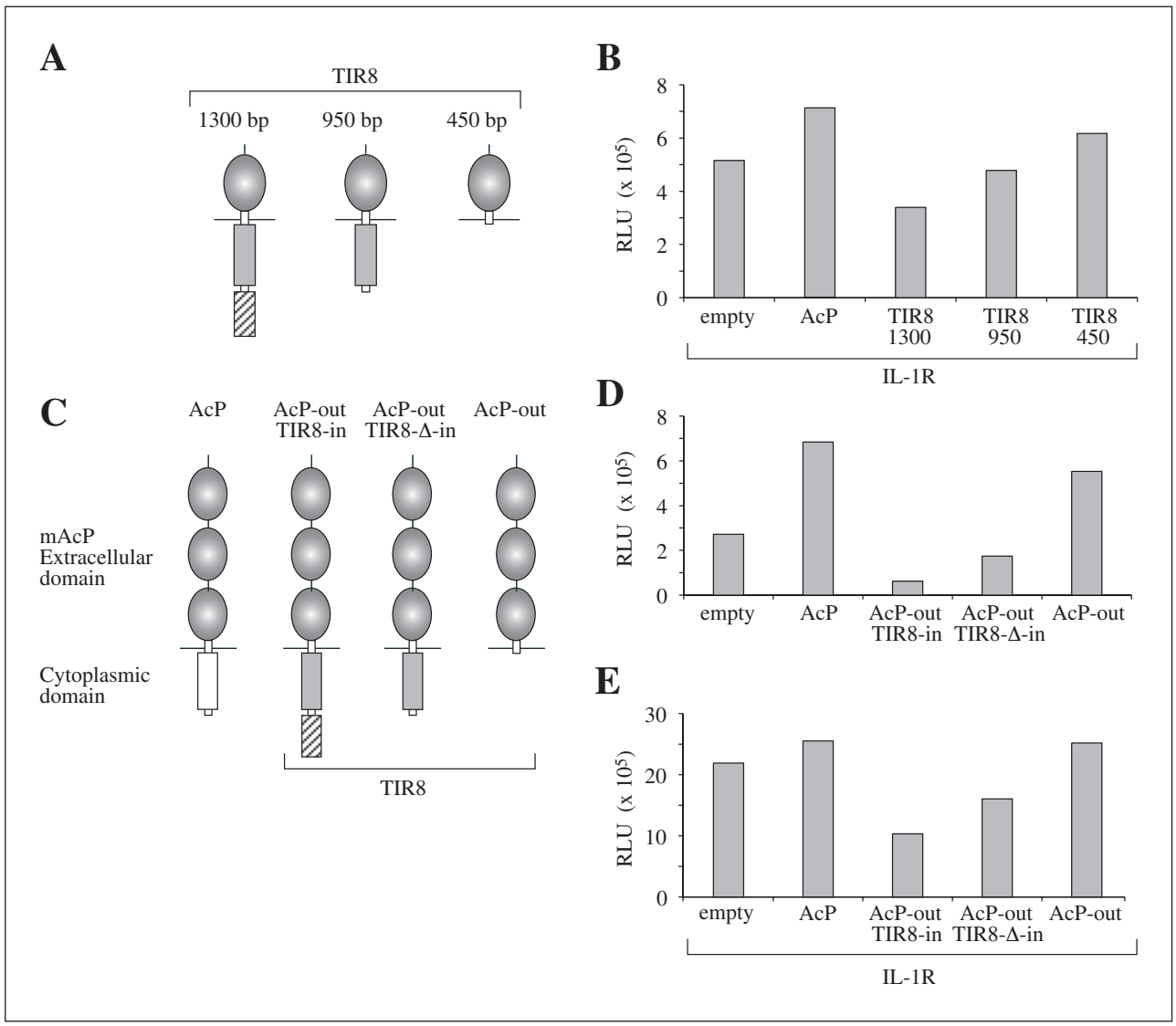

Figure 4

Inhibition of IL-1-induced NF-kB activation by TIR8.
A: Schematic representation of full length TIR8 (TIR8-1300) and truncated proteins with the novel intracytoplasmic tail (TIR8-950), or both the novel intracytoplasmic tail and the TIR domain deleted (TIR8-450), used in transient transfection studies shown in panel B.
B: NF-kB activation induced by the constructs indicated upon IL-1 treatment. Two hundred and ninety three cells were co-transfected with IL-1RI and either an empty vector, a positive control vector (AcP), full length TIR8 (TIR8-1300), or truncated versions of TIR8 (TIR8-950 and TIR8-450). NF-kB activity was measured by NF-kB reporter gene (luciferase) activity after normalization with β-galacto-sidase activity.
C: Schematic representations of chimeric proteins used in transfection studies shown in panels D and E. Chimeric molecules contained the extracellular and transmembrane domains of AcP and the full length TIR8 intracellular domain (AcP-out-TIR8-in) or a truncated version of the molecule missing the novel intracytoplasmic tail (AcP-out-TIR8-D-in), or only the AcP extracellular domain (AcP-out). AcP was used as positive control.
D: NF-kB activation induced by the indicated constructs upon IL-1 treatment. NF-kB activity was measured as reported in panel B.
E: NF-kB activation induced by the constructs indicated, co-transfected with IL-1RI upon IL-1 treatment. NF-kB activity was measured as reported in panel B.

for TIR8, in agreement with a previous report [6]. Unexpectedly, we found that TIR8 dampens IL-1R signaling. Inhibition of IL-1R-mediated NF-kB activation required the intracellular portion of TIR8, and was independent of the extracellular domain. During handling of this manuscript, a paper by Wald *et al.* [29] reported that TIR8/SIGIRR inhibits IL-1R and TLR signaling, possibly by trapping IRAK-1 and TRAF-6, key elements in the signaling cascade [25, 30-32]. Studies with gene targeted mice [29] (Garlanda *et al.*, unpublished data) show increased susceptibility of TIR8-deficient animals to selected inflammatory signals. Thus, TIR8 is a member of the IL-1R family with a unique pattern of expression and inhibitory function, which may serve to tune inflammation and innate immunity.

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