Identification of the scavenger receptors SREC-I, Cla-1 (SR-BI), and SR-AI as cellular receptors for Tamm-Horsfall protein

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Abstract: Tamm-Horsfall protein (THP) is expressed exclusively in the kidney and constitutes the most abundant protein in urine. An important role for THP in antibacterial host defense but also in inflammatory disorders of the urogenital tract has been suggested. In line with this, THP has been shown recently to potently activate macrophages and dendritic cells (DC) via the toll-like receptor 4 (TLR4) pathway. We show here that THP interacts specifically with surface structures on DC and provides evidence that they are distinct from TLR4. Using retroviral expression cloning, we have identified one such receptor as the scavenger receptor (SR) expressed by endothelial cells I (SREC-I). In addition, we found that two other receptors for acetylated low-density lipoprotein (AcLDL), namely scavenger receptors AI (SR-AI) and Cla-1 (SR-BI), also serve as receptors for THP. SREC-I/THP interaction is of high affinity (16.8±6.8 nM), whereas Cla-1 and SR-AI have lower affinities for THP (396 nM±114 nM and 802 nM±157 nM, respectively). The interaction of THP with these molecules is fully blocked by AcLDL. However, AcLDL only partially blocks binding of THP to DC, and a series of experiments did not support a role in DC activation for SR interacting with THP and AcLDL. Thus, our data point to the existence of additional receptors for THP, which mediate TLR4-dependent DC activation. Interaction and up-take of THP by SR might play an important role in local host defense and could contribute to inflammatory kidney diseases associated with THP-specific antibody responses. *J. Leukoc. Biol.* 83: 131–138; 2008.

Key Words: *dendritic cell · renal host defense · expression cloning*

INTRODUCTION

Tamm-Horsfall protein (THP) is a heavily glycosylated protein with a molecular weight of ~94 kDa. It is expressed exclusively in the thick, ascending limb of the Henle's loop in the kidney and is cleaved from its GPI-linked anchor to be secreted into the urine. It is the most abundant protein in normal human urine, present at 30–50 mg/24 h, which indicates an important physiological role in the urinary tract. THP interacts with type I-fimbriated *Escherichia coli* and prevents interaction of these bacteria with cellular receptors [1]. THP-deficient mice are impaired in their capability to clear *E. coli* and other bacteria from the urinary bladder [2–4]. Although normally expressed at the luminal surface of renal tubular epithelial cells and excreted into the urine, its aberrant presence was also detected at the basolateral surface and in interstitial infiltrates in several inflammatory kidney diseases [5]. Anti-THP immune responses in humans afflicted with renal diseases and tubulointerstitial nephritis in THP-challenged animals point to an important role for THP in the pathogenesis of inflammatory kidney diseases [6–8]. Recently, it has been shown that THP can potently activate dendritic cells (DC), a cell type that plays a key role in linking innate and adaptive immunity. DC activation by THP was dependent on the TLR4 pathways, and MyD88 and TLR4-deficient animals failed to raise THP-specific antibodies in response to i.v. challenge with THP [9]. We reasoned that interaction of THP with DC receptors is required to mediate these effects. In this work, we show that although DC do not express detectable amounts of TLR4 on their surface, THP binds specifically to these cells. Furthermore, expressing TLR4 on 293T cells did not result in enhanced binding of THP, also indicating that receptors other than TLR4 must mediate interaction of THP with DC. Using retroviral expression cloning, we identified the scavenger receptor (SR) expressed by endothelial cells I (SREC-I) to serve as a cellular THP receptor. In addition, two other SRs, namely Class A SRI

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(SR-AI) and the Class B SR CD36 and lysosomal integral membrane protein type II analogous-1 (Cla-1; SR-BI), also bound THP. We tested whether the interaction of THP with SRs is involved in DC activation. Our results do not support a role for these molecules in this process. Furthermore, we show that interaction of THP with cell lines expressing SRs is fully blocked by acetylated low-density lipoprotein (AcLDL), whereas AcLDL only partially blocks interaction of THP with DC. Thus, our data point to the existence of additional receptors for THP, which mediate the activation of DC. Nevertheless, THP-SR interaction might be important for renal immunity and could play a role in inflammatory kidney conditions.

MATERIALS AND METHODS

Antibodies and reagents

THP was purchased from Accurate Chemical and Scientific Corp. (Westbury, NY, USA). For binding studies, THP was biotinylated using Biotin-X-NHS (Calbiochem, Darmstadt, Germany) following a standard protocol. THP labeled with the fluorochrome Oregon Green™ (THP-OG) was generated using a FluoReporter OG 488 protein-labeling kit (Invitrogen, Carlsbad, CA, USA), following the manufacturer's instructions. Binding studies using THP or AcLDL were performed in PBS containing 0.5% BSA and 0.05% NaN_3. AcLDL was purchased from Biomedical Technologies (Stoughton, MA, USA), Alexa Fluor 488 AcLDL from Invitrogen, LPS from *E. coli* serotype 0127-B8, and polymyxin B (PMB) from Sigma Chemie (Deisenhofen, Germany). mAb directed against CD68, Cla-1, and TLR4 were obtained from BD PharMingen (Palo Alto, CA, USA) and a mAb to CD83 (clone HB15) was from Caltag (Burlingame, CA, USA). Goat antibodies to SR-AI were from Santa Cruz Biotechnology (Santa Cruz, CA, USA), and goat antibodies to SREC-I and lectin-like oxidized LDL (Ox-LDL) receptor-1 (LOX-1) were from R&D Systems (Minneapolis, MN, USA). mAb specific for CD36 and CD14 were produced at our institute. PE-labeled anti-mouse and anti-goat Ig antibodies were purchased from Jackson ImmunoResearch (West Grove, PA, USA).

Binding and internalization assays

Binding experiments using THP or AcLDL were done in PBS supplemented with 1% BSA. Bound, biotinylated THP (THP-bio) was detected using PE-labeled streptavidin (SA; BD PharMingen). For uptake experiments, SREC-I expressing murine thymoma cell lines Bw5147 (referred to as Bw cells throughout this work) were incubated with THP-bio (5 μg/ml) or THP-OG (10 μg/ml) in culture medium for 20 min, and following a washing step, cells were resupended in culture medium and kept on ice or incubated at 37°C (60 min or 200 min). Following an additional washing step with PBS, SA-PE was added to the cells incubated with THP-bio to label surface resident THP-bio as described above.

Cell culture

The cell lines Bw and AKRI were maintained in RPMI-1640 medium supplemented with 2 mM L-glutamine, 100 U/ml penicillin, and 10% FBS (Invitrogen; endotoxin below 1 Eu/ml). 293T cells were maintained in IMDM containing 2 mM glutamine, 100 U/ml penicillin, and 10% FBS. PBMC were isolated from heparinized whole blood of healthy volunteer donors by standard density centrifugation with Ficoll-Paque (Pharmacia Biotech, Uppsala, Sweden). Subsequently, monocytes were isolated by MACS using CD14 mAb. Monocyte-derived DC (referred to as DC throughout this work) were generated by culturing monocytes in RPMI 1640 containing 10% FBS in the presence of human recombinant (hr)GM-CSF (50 ng/ml) and hrIL-4 (100 U/ml) for 7–8 days. Both reagents were gifts from the Novartis Research Institute (Vienna, Austria). For DC maturation experiments, LPS, THP, AcLDL, or combinations of AcLDL and THP were added to cultures of immature DC on Day 5 at the indicated concentrations, and cells were analyzed 2 days later. In some experiments, PMB (final concentration, 20 μg/ml) was added to block LPS-mediated DC activation.

Generation of cell lines expressing SRs or TLR4

The coding sequences of human CD36, CD68, SR-AI, Cla-1, LOX-1, TLR4, and myeloid differentiation protein 2 (MD-2) were PCR-amplified from a cDNA expression library generated from human DC using appropriate primers encoding restriction endonuclease recognition sites. The PCR products were cloned into the retroviral expression vector pBMN [10]. The integrity of the resulting retroviral expression constructs was confirmed by DNA sequencing. The Bw cell line was then transduced retrovirally in parallel with pBMN plasmids encoding CD36, CD68, SR-AI, Cla-1, and LOX-1 and for control purposes, with a vector containing the lacZ gene (pBMN-Z). For retroviral transduction, 293T cells were cotransfected with the pEAK12-*gag-pol* vector. The supernatants were supplemented with polybrene (5 μg/ml), sterile-filtered, and used to spin-infect the Bw cell line as described [11].

To test binding of THP to TLR4-expressing cells, the 293T cell line was transiently transfected with a TLR4 plasmid or cotransfected with plasmids encoding TLR4 and MD-2 using the $CaCl_2$ transfection protocol. Analysis of THP-bio binding to TLR4 was done in the presence of cell culture medium containing 10% FBS.

Identification of THP receptors by retroviral expression cloning

A cDNA library generated from monocyte-derived DC (described in detail in ref. [11]) was expressed in the AKRI cell line. The transfected cell pool was subjected to two rounds of FACS with biotinylated THP (50 μg/ml) following an additional selection step of MACS using biotinylated THP in conjunction with SA conjugated with paramagnetic beads (Miltenyi Biotech, Bergisch Gladbach, Germany). For detection of bound, biotinylated THP, SA-PE was used. From the cell pool obtained, single cell clones were established by limiting dilution culturing. Genomic DNA was prepared from THP-reactive, single cell clones using Puregene (Gentra Systems, Minneapolis, MN, USA), following the manufacturer's instructions. The retrovirus-encoded cDNA inserts were PCR-amplified from genomic DNA with the oligonucleotide primers Ban1b 5′-GACCATCCTCTAGACTGCCGGATC-3′ and Ban2b 5′-CATTCCCCCCTTTTTCTGGAGACTAAATAAAATC-3′, specific for the flanking, retroviral sequences. The Long-PCR system (Invitrogen) was used for the PCR amplification under standard conditions. The obtained PCR products were gel-purified and cloned into the retroviral expression plasmid pBMN. Selected plasmids and pEAK12-*gag-pol* vector were cotransfected into 293T cells. Bw cells were transduced with the resulting culture supernatants and analyzed for reactivity with THP-bio. Plasmid DNA from selected clones was used for sequence analysis (MWG Biotech AG, Germany).

Affinity measurements

To assess the affinity of the THP-SR interaction, Bw cells expressing SREC-I, Cla-1, or SR-AI were stained with various concentrations of THP-bio, as indicated. To obtain dissociation constant (K_D) values 1/mean fluorescence intensity (MFI) of the different measurement points were plotted as a function of 1/concentration of THP-bio in a Lineweaver-Burk diagram for each receptor. Using linear regression analysis, the intercept of the line obtained with the x-axis represents $-1/K_D$; the intercept with the y-axis 1/maximal velocity (V_{max}); and the slope is K_D/V_{max}.

NF-κB luciferase reporter assay

A 293T cell line stably coexpressing TLR4 and MD-2 was transduced to stably express THP-binding SRs or GFP. The cell lines obtained were seeded at the same density in a 24-well plate and transiently transfected with a NF-κB luciferase reporter construct (20 ng/well, Stratagene, La Jolla, CA, USA). Cells were stimulated 24 h post-transfection with THP (15 μg/ml), LPS (1 μg/ml), or no stimulus. Eighteen hours later, cells were lysed with 200 μl lysis buffer according to the luciferase assay system protocol (Promega, Madison, WI, USA). Samples (20 μl) were transferred in a 96-well microlite plate (Dynex, Chantilly, VA, USA). After automatic injection of 50 μl luciferase assay reagent, samples were read in a plate-reading luminometer (Luminoscan RS, Labsystems Franklin, MA, USA) for 10 s with a 2-s delay.

RESULTS

THP activates human DC

THP has been described recently to be a potent, endogenous activator of innate immune cells. DC activation by THP has been shown to be TLR4-dependent [9]. Immature DC were incubated with different concentrations of THP and LPS for 48 h, and DC maturation was assessed by analyzing expression of CD83, a well-established and sensitive marker for DC activation. In contrast to LPS, THP-mediated DC activation could not be inhibited by PMB, indicating that THP itself and not a contamination with LPS is responsible for the observed effect (**Fig. 1**).

THP interacts with a surface structure on DC

To analyze the interaction of THP with human DC, we performed binding studies using biotinylated THP at different concentrations. Bound THP-bio was detected by SA-PE. In these experiments, we found THP to bind to DC (**Fig. 2A**). Binding of THP to DC was saturable and could be blocked completely by excess of unlabeled THP (Fig. 2B), pointing to a specific receptor-ligand interaction. This binding was not influenced significantly by the activation status of the DC, indicating that the expression level of the receptor structures is not regulated by DC-maturation signals (data not shown). As TLR4 is involved in activation of DC by THP, we tested whether THP binds directly to this receptor. However, neither 293T cells transfected with TLR4 plasmid nor cells cotransfected with TLR4 and its coreceptor MD-2 showed a higher capacity to bind THP-bio compared with control-transfected cells (Fig. 2C, and data not shown). In addition, TLR4 was not detectable on the surface of DC by flow cytometry (Fig. 2D), thus further ruling out that binding of THP to DC is mediated by TLR4.

Identification of THP receptors by retroviral expression cloning

To identify DC surface molecules involved in the interaction with THP, we used retroviral expression cloning. A cDNA

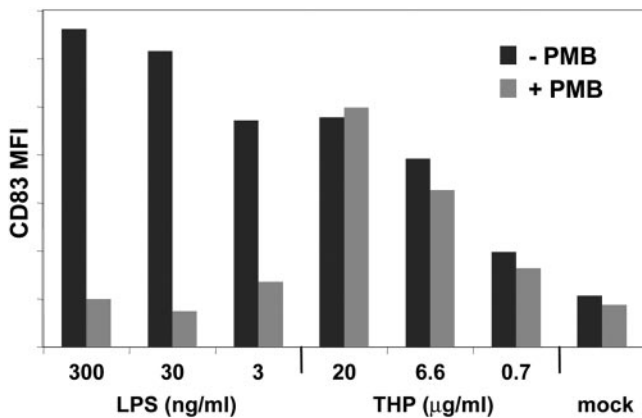

Fig. 1. THP activates DC. Immature DC were incubated with the indicated concentrations of LPS or THP in the absence or presence of PMB (20 μg/ml). Following 48 h of incubation, DC maturation was determined by assessing CD83 expression using flow cytometry. One experiment representative of four independently performed is shown.

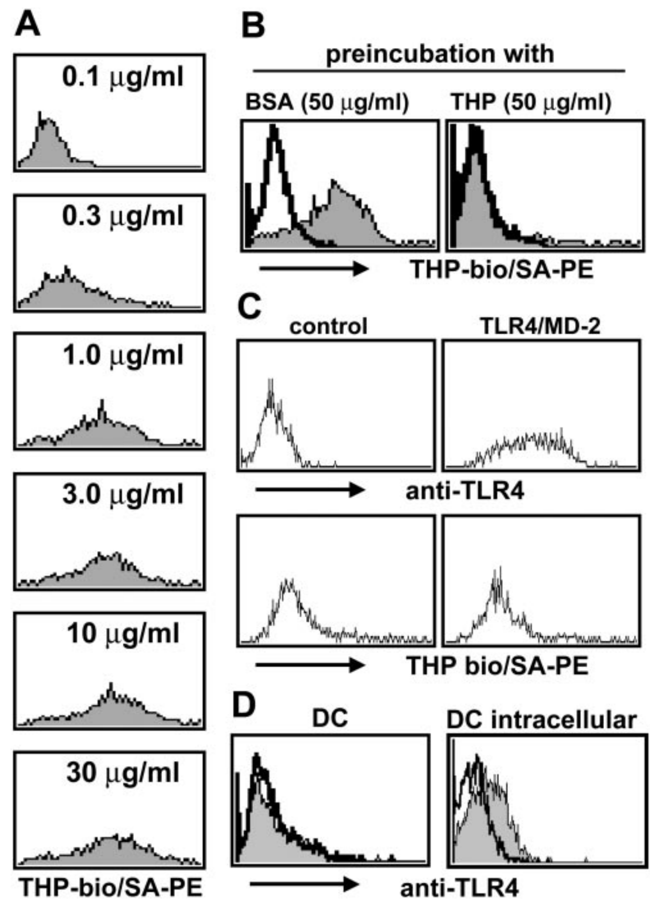

Fig. 2. THP interacts with receptors on DC. (A) Binding of indicated concentrations of THP-bio to DC was analyzed by flow cytometry using SA-PE as a secondary reagent. (B) Binding of THP-bio to DC is blocked by excess of unlabeled THP. DC were preincubated with unlabeled THP or BSA as indicated and then probed with THP-bio (gray histograms) or BSA-bio (bold lines). (C) THP-bio does not bind to TLR4/MD-2-expressing 293T cells, which were cotransfected with plasmids encoding human TLR4 and MD-2 (TLR4/MD-2) or were mock-transfected (control). Forty-eight hours post-transfection, cells were probed with a TLR4 mAb (upper panels) or with THP-bio using SA-PE as a secondary reagent (lower panels). (D) TLR4 cannot be detected on the surface of DC. Immature DC were incubated with TLR4 antibodies (gray histograms) or isotype control antibodies (histograms shown as bold lines). For intracellular detection of TLR4, cells were first fixed and permeabilized and then incubated with TLR4 antibodies (gray histogram) or isotype control antibodies (histogram shown as bold lines). All experiments shown were repeated at least three times with similar outcome.

library, derived from DC described previously [11–13], was expressed in the murine thymoma cell line AKRI. The target cell pool was then stained with THP-bio as a primary reagent, and cells binding THP-bio were labeled with a SA-PE conjugate, subjected to FACS (**Fig. 3A**) and expanded for an additional selection step. After two rounds of FACS and an additional selection step using MACS, the majority of the obtained cells reacted strongly with THP (Fig. 3B). Single cell clones were established from the reactive cell pool, and the retroviral cDNA inserts were retrieved from THP-binding clones by PCR. Figure 3C shows retroviral inserts from two different cell clones. A 3.8-kb retroviral cDNA product, which was present in both clones, was cloned into a retroviral expression vector and expressed in Bw cells. This cDNA encodes a THP recep-

tor, as it conferred THP reactivity to cells transduced with the resulting construct (Fig. 3D). The DNA insert was sequenced and found to be identical with SREC-I, a type F SR [14, 15]. SREC-I has been described previously to be present on endothelial cells, and there are no data about the expression of SREC-I on DC. To assess expression of SREC-I on DC, we probed them with antibodies to this molecule and found immature DC and LPS-activated DC to express SREC-I on their surface (Fig. 3E). SRs are known to mediate cellular uptake of their ligands, and this could also be seen with THP: incubation of SREC-I transductants at 37°C resulted in a strong and time-dependent reduction of THP-bio on the surface of these cells, indicating that SREC-I mediates internalization of bound THP (Fig. 3F). To rule out that the reduction of detected THP-bio at 37°C is a result of a loss of binding at this temperature rather then cellular uptake, we also analyzed the interaction of THP labeled directly with a fluorescent dye (THP-OG, Fig. 3F). We found that THP-OG binding is not reduced following incubation at 37°C, demonstrating that the reduction of THP-bio on the cell surface is a result of internalization via SREC-I.

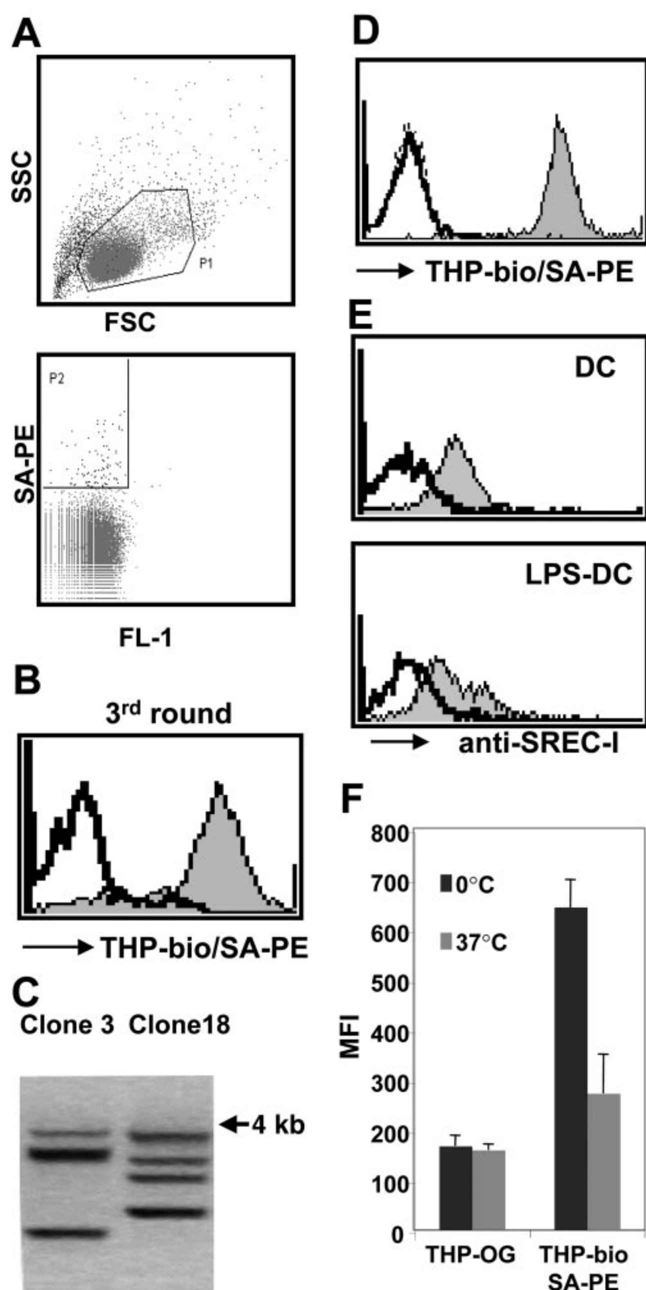

Fig. 3. Identification of THP receptors by retroviral expression cloning. (A) THP-reactive cells were isolated from a retroviral library, derived from human DC, expressed in the AKRI cell line by FACS. Gates used for selection of THP-binding cells in the first round of FACS are shown. SSC, Side-scatter; FSC, forward-scatter; FL-1, fluorescence channel 1. (B) The reactivity of THP-bio with the cell pool obtained after three rounds of selection. Cells were probed with THP-bio (gray histogram) or BSA-bio (bold histogram) following a staining step with SA-PE. (C) PCR-amplified retroviral inserts from two different THP-binding single cell clones established from the selected cell pool. The 3.8-kb band, which was obtained in both products, was cloned in a retroviral expression vector and subjected to DNA sequence analysis. (D) Cells expressing SREC-I show strong binding of THP. The 3.8-kb cDNA (encoding SREC-I) was expressed in AKRI cells and analyzed for THP binding [gray histogram, THP-bio; bold line, BSA-bio; dotted line, secondary reagent (SA-PE) only]. (E) SREC-I is expressed on immature DC and LPS-matured DC. Binding of SREC-I antibodies (gray histograms) or isotype control antibodies (histogram shown as a bold line) was detected with PE-labeled secondary antibodies. (F) SREC-I mediates cellular uptake of bound THP. Surface THP-bio was detected following incubation on ice (0°C) or at 37°C, as indicated on SREC-I transductants using SA-PE. To rule out that loss of binding rather than uptake is responsible of the reduction of surface THP-bio following incubation at 37°C, cells were incubated directly with fluorescein-labeled THP (THP-OG) under the same conditions. Cells were analyzed by flow cytometry, and MFI is shown. One experiment representative of three independently performed is shown.

THP is recognized by SRs SREC-I, SR-AI, and Cla-1

We were interested to test whether SREC-I shares the specificity for THP with other SRs. Therefore, we generated retroviral expression constructs encoding the human SRs CD36, CD68, SR-AI, Cla-1, and LOX-1 and expressed them in Bw cells (**Fig. 4**). When probing the resultant cells with THP-bio, we found that in addition to SREC-I, SR-AI and Cla-1 are receptors for this molecule (Fig. 4). The interaction of THP-bio with these cells was dose-dependent and was blocked in the presence of excess of unlabeled THP (Fig. 4). SRs are defined by their specificity for modified LDL (AcLDL or Ox-LDL). When probing cells expressing SRs with Alexa Fluor 488 AcLDL, we found that AcLDL and THP interacted with the same receptors. Binding of THP to cells expressing SREC-I, SR-AI, and Cla-1 was blocked in the presence of AcLDL, which indicates that THP and AcLDL share binding sites on these receptors (Fig. 4).

Determination of equilibrium K_D for THP receptors

To assess the affinity of THP-SR interaction, we performed saturation assays over a wide range of concentrations of labeled THP (**Fig. 5A**). Binding of THP-bio to SR-expressing cells was saturable and could be blocked completely by excess of unlabeled THP over the whole concentration range analyzed (Fig. 4, and data not shown). These results point to a classical receptor-ligand interaction of THP with its receptors. K_D values for the binding of THP to the SR-expressing cells were

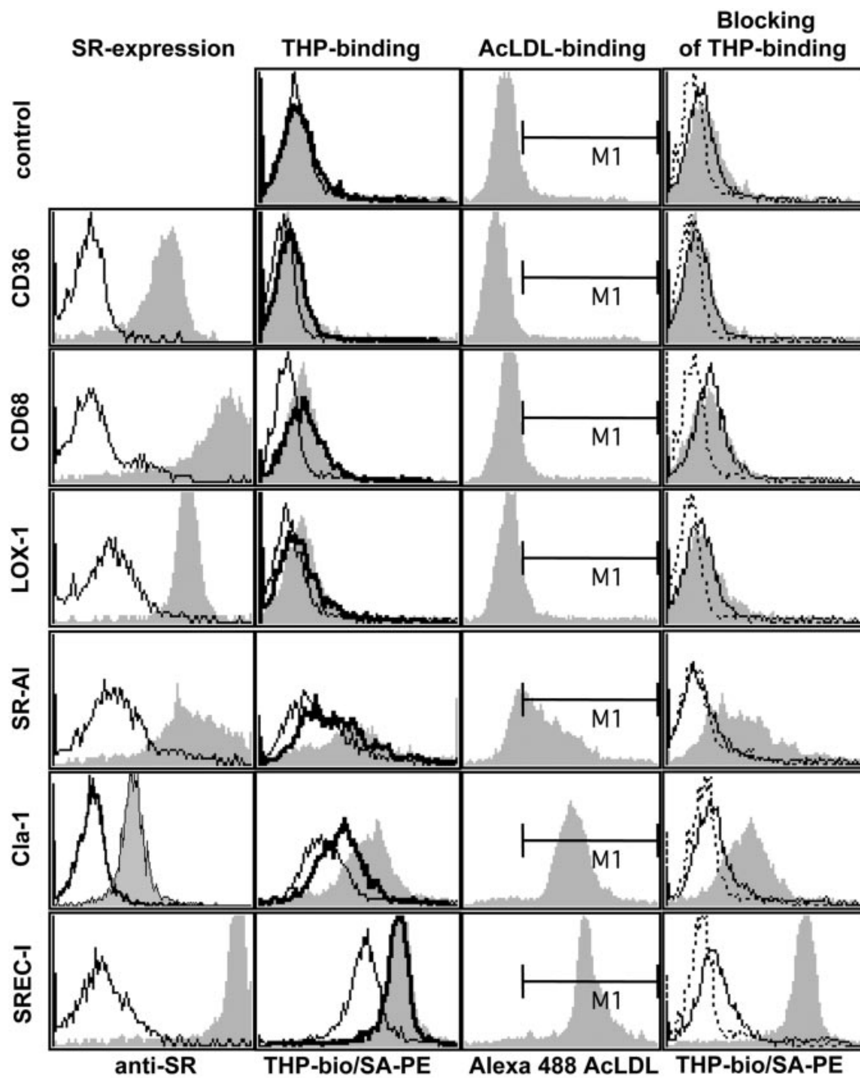

Fig. 4. THP and AcLDL appear to have the same binding sites on the SRs SREC-I, Cla-1, and SR-AI. (Left panel) Bw cells expressing the human SRs CD36, CD68, SREC-I, SR-AI, Cla-1, and LOX-1 were probed with antibodies to these molecules. Bound antibody was detected by FACS using appropriate fluorophor-labeled secondary reagents (gray histograms). The thin lines represent reactivity of these antibodies with mock-transduced, control Bw cells. (Left middle panel) Interaction of THP-bio with cells expressing SRs and mock-transduced control cells. Bound THP-bio was detected by FACS using SA-PE as a secondary reagent; thin line, 1 μg/ml THP-bio; bold line, 5 μg/ml THP-bio; gray histogram, 10 μg/ml THP-bio. (Right middle panel) Interaction of SRs with Alexa Fluor 488-labeled AcLDL (10 μg/ml). M1, . (Right panel) Interaction of THP-bio with SRs is blocked by unlabeled AcLDL or THP. Interaction of THP-bio (5 μg/ml) with mock-transduced cells or cells expressing SRs is shown; gray histogram, no competitor; thin line, binding of THP-bio in the presence of AcLDL (100 μg/ml); dotted line, interaction of THP-bio in the presence of unlabeled THP (100 μg/ml). The experiments shown are representative of at least three independently performed.

determined using the Lineweaver-Burk equation (Fig. 5B). SREC-I was found to interact with THP at the highest affinity (16.8 nM±6.8 nM, n=3), and Cla-1 and SR-AI had lower affinities for THP (396 nM±114 nM, n=3; 802 nM±157 nM, n=3, respectively).

THP receptors distinct from SRs might mediate DC activation by THP

Using transductants expressing SRs, we could show competition between AcLDL and THP for receptor binding (Fig. 4). In addition to SREC-I, SR-AI is expressed on DC, and we found that these cells express high levels of Cla-1 encoding mRNA (data not shown). We performed a number of experiments to test whether SRs are involved in the activation of DC by THP. For this purpose, we added THP or AcLDL and LPS as a control to DC differentiation cultures on Day 5. However, in contrast to THP or LPS, even the presence of high concentrations of AcLDL did not result in activation of these cells, as tested by the induction of the activation marker CD83, 48 h later (**Fig. 6A**). We also analyzed whether AcLDL could interfere with THP-mediated DC maturation. For this purpose, we added THP together with excess of AcLDL to immature DC on Day 5 and analyzed the induction of CD83 on Day 7. In these experiments, we could not observe any inhibition of the THP-mediated DC activation (Fig. 6A). As the presence of high concentrations of AcLDL, which were able to completely block interaction of THP with the SRs, were ineffective in inhibiting DC activation by THP, an involvement of SRs in this process appears unlikely. This hypothesis is supported by the results of a NF-κB reporter assay using 293 cells: presence of LPS but not THP resulted in NF-κB reporter activation in cells expressing TLR4/MD-2. Coexpressing SREC-I with TLR4 and MD-2 still did not result in NF-κB reporter activation in the presence of THP (Fig. 6B). Taken together, these observations indicate that interaction of THP with SRs is not involved in DC activation and that these cells must express additional THP receptors. We therefore analyzed binding of THP-bio to DC in the presence of excess of unlabeled THP or AcLDL. We found that in contrast to unlabeled THP, which blocked THP-bio binding completely, AcLDL blocked interaction of THP with DC only partially, indicating that these cells express additional receptors for THP, which are distinct from the SRs (Fig. 6C). In contrast, AcLDL completely blocks binding of THP to transductants expressing SRs (Fig. 4). Thus, although we have identified the first cellular receptors for THP, our results point to additional THP-

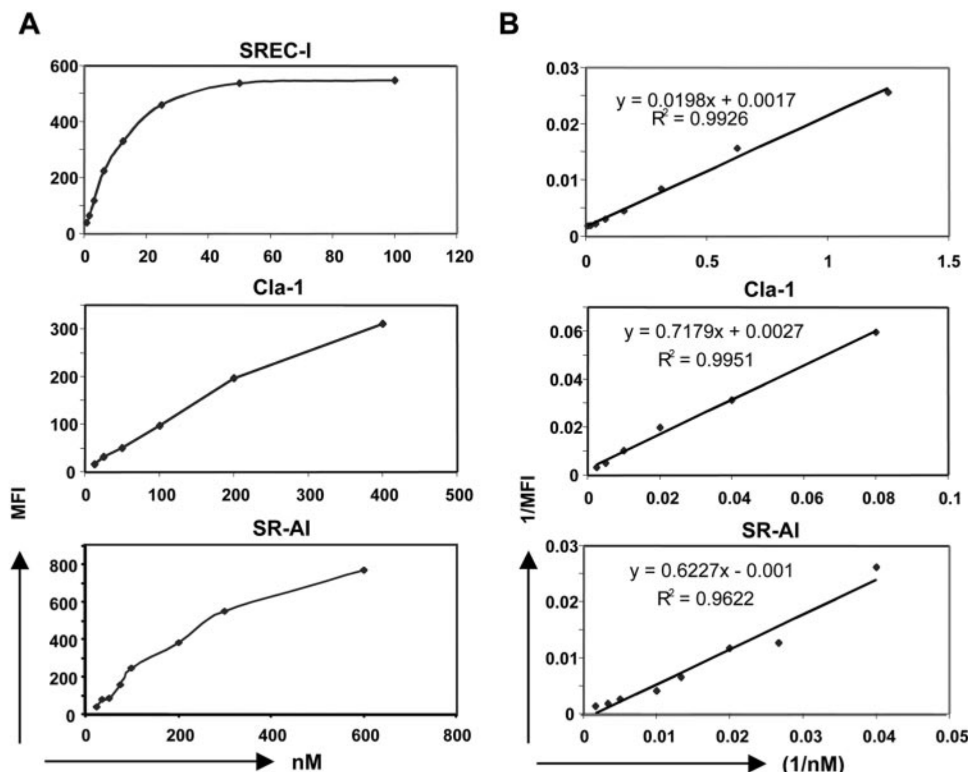

Fig. 5. Determination of equilibrium K_D for THP receptors. (A) Saturation curves of THP-bio binding to cells expressing SRs. Binding of THP-bio was measured by flow cytometry using SA-PE as a secondary reagent. One representative experiment of triplicate measurements is shown for each cell line. (B) Lineweaver-Burk diagrams (1/MFI is shown as a function of 1/THP-bio concentration, nM) of data depicted in A.

binding structures on DC, which are likely candidates to mediate DC activation via the TLR4 pathway.

DISCUSSION

THP is produced in the ascending loop of Henle, cleaved from its GPI anchor and secreted in the urine. Under normal physiological conditions, this protein might not interact with cells of the immune system, but by binding to bacteria, it plays an important role in host defense against these microorganisms. However, its aberrant presence was also described at the basolateral surface and in interstitial infiltrates in several inflammatory kidney diseases. Under such conditions, THP might interact with cells of the immune system, which eventually can result in a strong antibody response to this molecule, which is seen in individuals afflicted with inflammatory kidney diseases [6]. An important role for immune responses to THP under such circumstances is supported in studies that show that THP challenge can result in tubulointerstitial nephritis in rats [8]. Interaction of THP with cells of the immune system might underlie such mechanisms, and previous studies have shown that THP can interact with and activate human granulocytes and monocytes [16–18]. However, cellular receptors, which mediate this interaction, have not been identified to date. Recent findings, which show that THP can act as a potent activator of macrophages and DC [9, 19, 20], have prompted us to study the interaction of THP with this cell type. Binding studies using flow cytometry in conjunction with THP-bio gave evidence for interaction of THP with receptors on DC. Despite the fact that activation of DC by THP was shown to be dependent on the TLR4 pathway, TLR4 is an unlikely candidate to mediate such an interaction for two reasons: Although the TLR4 pathway is highly operative in these cells, TLR4 surface expression on DC is low; and cells expressing TLR4 and its coreceptor MD-2 do not show enhanced THP binding. Thus, other receptors must mediate binding of THP to DC, and to identify such molecules, we have screened a retroviral cDNA library derived from human DC expressed in AKRI cells with THP. Using this approach, we could identify the SR SREC-I as a receptor for THP. Furthermore, we found that two additional SRs, namely SR-AI and Cla-1, are also receptors for this molecule, whereas cells expressing CD36, CD68, or LOX-1 did not show enhanced THP binding compared with mock-transduced control cells. SREC-I, SR-AI, and Cla-1 but not CD36, CD68, and LOX-1 did bind Alexa Fluor 488-labeled AcLDL, and binding of THP to SRs could be fully blocked by AcLDL (Fig. 4). From these results, it could be inferred that THP and AcLDL share binding sites on SRs. It is interesting that although AcLDL competed strongly with THP for binding to SRs, it was unable to activate DC, and moreover, excess of AcLDL was completely ineffective in blocking THP-mediated DC activation (Fig. 6A). In line with these results in reporter assays, coexpression of THP-binding SRs and MD-2/TLR4 did not mediate NF-κB activation in the presence of THP (Fig. 6B). Furthermore, Säemann et al. [9] reported previously that HUVEC can be activated by LPS but not by THP, and we could confirm these findings (data not shown). We have analyzed the surface expression of SREC-I and SR-AI on HUVEC and found these molecules to be present in amounts comparable with those found on DC (data not shown). Taken together, these data point to the existence of additional THP receptors on DC, which are distinct from SRs that mediate TLR4-dependent activation of these cells. In support of this, binding of THP-bio

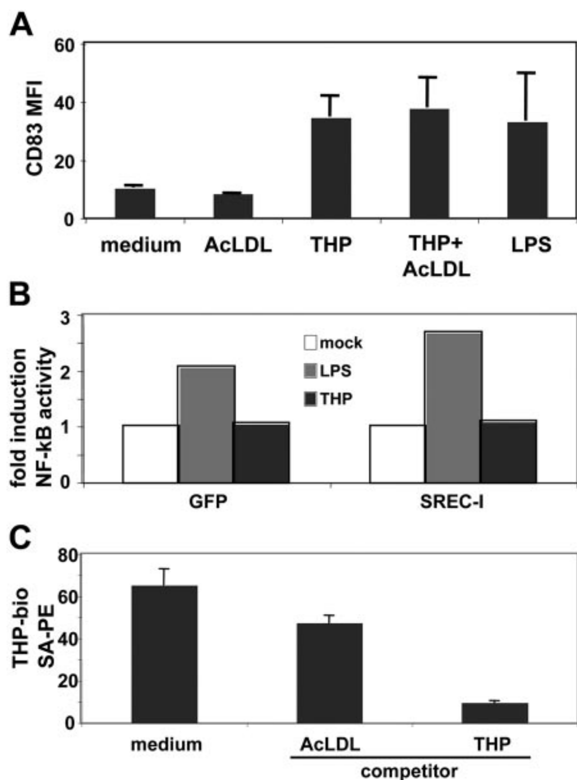

Fig. 6. Interaction of THP with SRs does not appear to mediate activation of DC. (A) AcLDL neither promote activation of human DC nor are able to block THP-mediated DC activation. AcLDL (50 μg/ml), THP (10 μg/ml), THP (10 μg/ml) in the presence of AcLDL (200 μg/ml), or LPS (100 ng/ml) was added to Day 5 immature DC, and their maturation state was assessed by analyzing CD83 expression on Day 7. (B) 293T cells coexpressing TLR4 and MD-2 and THP-binding SRs do not activate NF-κB pathways in the presence of THP. 293T cells stably expressing TLR4 and MD-2 and GFP or SREC-I were transiently transfected with a NF-κB reporter construct, and 24 h post-transfection, THP (15 μg/ml) or LPS (1 μg/ml) was added. Reporter activity was determined 18 h later and is shown as fold induction of untreated cells. (C) AcLDL only partially blocks binding of THP-bio to DC. THP-bio (10 μg/ml) binding to human DC in the absence of competitors and in the presence of unlabeled THP or AcLDL. Medium, (no competitor); THP, unlabeled THP at 100 μg/ml; AcLDL, AcLDL at 400 μg/ml. All experiments were repeated at least three times with similar outcome.

to DC is only inhibited partially by AcLDL, whereas THP fully blocked this interaction (Fig. 6C).

Our data do not support a function for SRs in the THP-mediated activation of DC, but as it is well established that SRs mediate efficient antigen uptake, they could play an important physiological role in the generation of the strong immune responses to THP in individuals afflicted with inflammatory kidney diseases. An intriguing theory is that THP-DC interaction happens at two distinct levels: by the interaction with SRs, which leads to antigen uptake, and by the interaction with a yet-unidentified receptor structure, leading to DC activation. It has been proposed previously that THP has a dual role as adjuvant and autoantigen [19]. The interaction of this molecule with two functionally distinct types of receptors could be an explanation for this dualism of THP function. The particular properties of the urogenital tract, which lacks typical physical barriers, necessitate specific mechanisms to combat infections [21]. The capability of THP to bind bacteria and activate professional APCs efficiently makes it an ideal defense molecule. Interaction of THP with SRs should lead to an efficient uptake of bound pathogens by these cells. Thus, our results point to an additional property by which THP could mediate efficient, local immune responses to invading pathogens.

In our current research, we are focusing on the identification of THP receptors, which mediate this DC activation. The characterization of such molecules would advance our understanding of immune response to THP and in addition, might give new insights in the biology of TLR4-dependent activation processes.

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