Quantitative expression of RIG-like helicase, NOD-like receptor and inflammasome-related mRNAs in humans and mice

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Abstract

The cell-type-, organ- and species-specific expression of the surface and endosomally located Toll-like receptors are well described but little is known about the respective expression profiles of cytosolic pattern recognition molecules. We therefore determined the mRNA expression levels of 15 cytosolic pattern recognition molecules in 11 solid organs of human and mice. Human organs revealed lower mRNA levels of most molecules as in spleen but at least 2-fold higher were inflammasome-related NOD, leucine-rich repeat and pyrin domain-containing protein 1–3 (NLRP1–3) and -12 in brain, LGP2, retinoic acid-inducible gene I (RIG-I) and NLRP10 in liver, NLRP10 in small intestine, LGP2, RIG-I, NAIP, NLRP2 and -3 in testis and RIG-I, NLRP2 and -10 in muscle. In mice, most organs also expressed lower mRNA levels compared with spleen. Only NLRP6 in liver, NAIP and NLRP6 in small intestine, LGP2, nucleotide-binding oligomerization domain 1 (NOD1), NLRP1, -2, -6, -10 and -12 in colon and MDA5, RIG-I, NLRC4, NOD1, -2, NLRP1, -2, -6, -10 and -12 mRNA levels in kidney were higher. Resting human and mouse monocytes and T cells expressed most molecules and produced IL-1β and CCL5/RANTES upon activation. However, murine monocytes strongly up-regulated, whereas human monocytes down-regulated receptor expression upon activation. These data suggest that the cell-type-, organ- and species-specific expression and regulation need to be considered in the design and interpretation of related studies.

Keywords: infection, inflammasome, NOD-like receptors, pattern recognition receptors

Introduction

The integrity of multicellular organisms depends on their capacity to recognize potentially harmful dangers, to sense danger signals and to rapidly trigger appropriate defense mechanisms that prevent or minimize tissue damage (1). Different families of danger recognition receptors recognize pathogen- or endogenous danger-associated molecular patterns, i.e. the Toll-like receptors (TLRs) (2), the retinoic acid-inducible gene (RIG)-like helicases (RLHs) (3), the nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) (4) or inflammasome-related NOD, leucine-rich repeat and pyrin domain-containing proteins (NLRPs) (5, 6). Our understanding of the functional roles of these molecules in host defense and danger control largely derives from defined human or murine cell populations or from inducible disease models in knockout mice. But protein functions that had been proposed by murine disease models do not always reliably predict their roles in humans. It has been thought that this general limitation may be less relevant in the area of innate immunity because pattern recognition receptor (PRR) genes show large homologies among species and their origin dates long back in the evolution of multicellular organisms (7). However, (i) mice express PRR that do not exist in humans and vice versa, e.g. humans lack functional TLR11 (8) or mice lack TLR8 (9); (ii) humans and mice differ in the cell-type-specific expression of certain PRR, e.g. TLR9 being expressed by murine but not by human macrophages (10); and (iii) mice and humans show different functional significancies of PRR or their signaling adapters, e.g. MyD88-deficient mice but not human infants are highly susceptible to infection (11). While the expressions of the endosomal and surface TLRs have been extensively characterized (12, 13), little of such data are available on the cytosolic PRRs (14). We hypothesized similar species-specific differences for the expression of RLHs, NLRs and inflammasome-related molecules and therefore determined their mRNA expression profiles in human and mouse organs and leukocytes.
Methods

Human solid organ and leukocyte cDNA and preparation of leukocytes for in vitro experiments

Human solid organ prenormalized cDNAs derived from poly(A)-selected DNase-treated RNAs purified from pools of healthy human tissues were obtained from Clontech (Mountain View, CA, USA). As only a single pool was available for each organ, no studies on biological replicates allowing statistics could be performed. According to Clontech, all human samples were purchased and imported in accordance with all local laws and regulations. Human CD14+ monocytes and CD3+ T cells were isolated from human blood samples provided by 18- to 40-year-old healthy men and women who were tested to be negative for HIV, hepatitis B virus and hepatitis C virus. Further exclusion criteria were manifest infections during the last 4 weeks, fever, symptomatic allergies, abnormal blood cell counts, increased liver enzymes or medication of any kind except vitamins and oral contraceptives. The study was approved by the Ethics Committee of Klinikum der Universität München and written informed consent was obtained from all blood donors. Cells were collected fromuffy coats by Ficoll-Hypaque density gradient centrifugation (Beckman Coulter, Krefeld, Germany). CD14+ monocytes and CD3+ T cells were positively selected by immunomagnetic separation (Miltenyi, Bergisch Gladbach, Germany) and total RNA was prepared as described (15). Isolated cells were re-suspended in RPMI 1640 containing 10% FCS (Gibco, Karlsruhe, Germany), 100 U ml⁻¹ penicillin and 100 µg ml⁻¹ streptomycin and seeded at 1 x 10⁶ cells per well; 1 ml per well in 24-well plates at 37°C in a humidified atmosphere of 5% CO₂ in air. For activation, the culture medium was supplemented with 2 µl ml⁻¹ pokeweed mitogen (PWM) and 5 µg ml⁻¹ ConA, both from Sigma-Aldrich (Taufkirchen, Germany), for 3 days. Cytokine production was determined in cell culture supernatants using commercial ELISA kits for human IL-1β (BD, San Diego, CA, USA), murine IL-1β (BD), human CCL5/RANTES (R&D Systems, Wiesbaden-Nordenstadt, Germany) and murine CCL5/RANTES (R&D Systems). Supernatants were collected after 24 h of stimulation with 1 µg ml⁻¹ 5'-tri-DAP, 50 µg ml⁻¹ tri-DAP, 50 µg ml⁻¹ iE-DAP, 100 µg ml⁻¹ urate crystals or 1 µg ml⁻¹ ultrapure LPS (all from Invivogen, San Diego, CA, USA). Agonists were treated with polymyxin (Invivogen) to block residual LPS contamination. RNA and DNA agonists were transfected in the presence of lipofectamine (Invivogen).

Mouse solid organ and leukocyte cDNA and preparation of leukocytes for in vitro experiments

Six-week-old and 6-month-old C57BL/6 mice were obtained from Charles River (Sulzfeld, Germany) and housed in groups of five mice with a 12 h dark/light cycle and unlimited access to food and water. Mice were bled from the retro-orbital venous plexus under isoflurane anesthesia. Other groups of C57BL/6 mice were killed by cervical dislocation and solid organs were prepared under sterile conditions. The study was approved by the Regierung von Oberbayern. Solid organ samples were snap frozen in liquid nitrogen. CD14+ monocytes and CD3+ T cells were prepared as the respective human cells. Total RNA was prepared from organs and cells, quality checked and reverse transcribed into cDNA. For PCR, independent pools of five samples each were used. ELISA for murine IL-1β (BD) was performed as with human cells.

Quantitative real-time reverse transcription–PCR

PRR mRNA expression in human and mouse solid organ and human and mouse leukocyte cDNA was quantified by real-time reverse transcription (RT)–PCR using 18s ribosomal RNA and GAPDH as housekeeper genes as described (16). SYBR Green Dye detection system was used for quantitative real-time PCR on LightCycler 480 (Roche, Mannheim, Germany). All the technical steps were performed according to The Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines (17). Gene-specific primers (300 nM; Metabion, Martinsried, Germany) were used as listed in supplementary Table 1 (available at International Immunology Online). Controls consisting of ddH2O were negative for target and housekeeper genes. Primers were designed to be cDNA specific and to target possibly all known transcripts of gene of interest. In silico specificity screen (BLAST) was performed. The lengths of amplicons were between 80 and 130 bp. The kinetics of the PCR amplification (efficiency) was calculated for every set of primers. The efficiency-corrected quantification was performed automatically by the LightCycler 480 based on relative standard curves describing the PCR efficiencies of the target and the reference gene. To reduce the risk of false-positive crossing point (Cp), the high confidence algorithm was used. All the samples that during the amplification reaction did not rise above the background fluorescence (Cp or quantification cycle Cq) till 35 cycles were described as not detected (n.d. in the figures). The melting curve profiles were analyzed for every sample to detect eventual unspecific products or primer dimers.

Western blot

The cells or solid organs were lysed in 1 ml lysis buffer [50 mM Tris–HCl, pH 7.5, 150 mM NaCl, 100 mM sodium orthovanadate, 0.5% sodium deoxycholate, 4% NP-40, 2% Triton X-100, 5 mM EDTA and 300 mM sucrose proteases inhibitor tablets Complete (Roche, Penzberg, Germany)] and centrifuged for 45 min at 30 000 × g. The supernatant was tested for protein concentration. Extracted proteins were incubated in 2× loading buffer for 5 min at 95°C (15 µg protein isolate per lane), resolved by 10% SDS-PAGE and transferred to an Immobilon-P membrane (Millipore, Eschborn, Germany). After blocking with 5% milk, the membrane was incubated with a goat polyclonal anti-human or anti-mouse Zbp1 antibody (1:500, E13 or K14, respectively; Santa Cruz Biotechnology) overnight in Tris-buffered saline. Immune complexes were visualized using a peroxidase-conjugated donkey anti-goat IgG antibody (1:20 000; Dianova, Hamburg, Germany) for 1 h in 5% milk (18). For Nod1, β-actin and GAPDH (Cell Signaling) western blotting, the membrane was blocked with 5% BSA and incubated with the secondary anti-rabbit antibodies (Cell Signaling).

Statistical analysis

Data were expressed as mean ± SEM. Comparison between groups was performed using univariate analysis of
Results

Cytosolic PRR mRNA expression in adult human tissues

We used real-time RT-PCR to assess the mRNA expression levels of the following innate pattern recognition molecules in human solid organs: LGP2, MDA5, RIG-I, ZBP1, C2TA, NLRC4, NOD1, NOD2, NAIP, NLRP1-3, -6, -10 and -12. Most of these molecules were constitutively expressed in human spleen (Fig. 1a). ZBP1, NLRP2, -3, -6, -10 and -12 mRNAs were detectable but their levels were low in spleen. In human thymus, the mRNA levels of all aforementioned molecules were lower as in spleen except that of LGP2 and RIG-I. The mRNA levels of most cytosolic signaling molecules were higher in solid organs as compared with spleen, except for the following: human brain revealed much higher levels of NLRP1-3, -6 and -12, liver revealed higher expression levels of LGP2, RIG-I and NLRP10; NLRP10 only of small intestine. LGP2, RIG-I, NLRC4, NLRP2 and NLRP3 mRNA levels were higher in testis and RIG-I, NOD1, -2, NAIP, NLRP1, -2, -10 and -12 in muscle. Thus, human organs display an organ-specific PRR mRNA expression pattern.

Cytosolic innate PRR mRNA expression in adult murine tissues

Next, we determined the mRNA expression levels of the same pattern recognition molecules in the same 11 murine organs from 6-week- and 6-month-old C57BL/6 mice. The mRNA expression profiles from both age groups were almost identical. All molecules were constitutively expressed in mouse spleen but the mRNA levels of NOD2, NLRP1, NLRP2, -6, -10 and -12 were low (Fig. 1b). In all other solid organs, all PRR mRNA levels were much lower as in spleen except for the following: NALP6 mRNA levels were higher in mouse liver, small intestine, colon and kidney. This was most obvious in kidney and colon that also expressed higher levels of NLRP1, -2, -10 and -12 as well as LGP2 and NOD1. MDA5, RIG-I, NLRC4 and NOD2 were higher in kidney only. Figure 1(c) compares the organ-specific PRR mRNA expression in humans and mice where white (human) and black (murine) bars indicate the x-fold induction versus respective spleen mRNA levels. The graph illustrates many discordant relative mRNA expressions between the two species. For example, higher mRNA levels of NLRP1-3, NLRP6 and NLRP12 in brain and lung were observed in humans but not in mice. The relative NLRP6 mRNA expression in mouse liver, small intestine, colon and kidney was higher as in humans. Murine colon and kidney expressed higher levels of NLRP2, -6, -10 and -12 as compared with humans. NOD1 but not NOD2 mRNA expression was higher in murine than in human colon. Muscle also displayed discordant relative mRNA expression patterns in humans and mice. We conclude that the mRNA expression levels of cytosolic pattern recognition molecules differ in human and mouse organs, especially those of the NLRPs in brain, lung, colon, kidney, testis and muscle.

Cytosolic innate PRR mRNA expression in human CD14+ monocytes and CD3+ T cells

We next analyzed the basal PRR mRNA expression levels of human CD14+ monocytes and CD3+ T cells. The basal PRR mRNA expression profiles were mostly comparable in CD14+ and CD3+ cells as both expressed mRNA of all PRR except NLRP6 and NLRP10 (Fig. 2). To study the regulation of PRR expression in pro-inflammatory environments, the cells were stimulated with PWM/ConA. CD14+ cells significantly induced the RNA helicases LGP2, RIG-I and MDA5 as well as NLRP3 but down-regulated NLRC4, NAIP and NLRP12 (Fig. 2). In CD3+ cells, only MDA5 was induced and ZBP1, C2TA, NLRC4, NAIP, NLRP1, NLRP3 and NLRP12 were down-regulated. Together, human CD14+ monocytes and CD3+ T cells express similar patterns of cytosolic PRR mRNAs but show a cell-type-specific regulation pattern upon stimulation with PWM/ConA.

Cytosolic innate PRR mRNA expression in mouse CD14+ monocytes and CD3+ T cells

The basal PRR mRNA expression profiles were mostly comparable in murine CD14+ and CD3+ cells (Fig. 2). The mRNA expression levels in murine resting leukocytes were mostly similar to that of the respective human cells. However, in contrast to human CD14+ cells, PWM/ConA stimulation strongly elevated the mRNA expression levels of LGP2, MDA5, RIG-I and especially of ZBP1, NOD1 and NLRP3 in murine CD14+ cells. This effect was limited to some induction of ZBP1 in murine CD3+ cells. All other PRR mRNAs were rather suppressed by PWM/ConA stimulation, a phenomenon similar to what we observed in human CD3+ cells. Thus, human and murine CD14+ monocytes differ in their regulation of most PRR mRNA upon PWM/ConA activation. Human and murine CD3+ cells differ in terms of the regulation of ZBP1.

PRR agonists regulate PRR mRNAs in human CD14+ monocytes and CD3+ T cells

Next, we studied the potential of classical PRR agonists to modulate PRR mRNA expression in human CD14+ monocytes and CD3 T cells. We used 3P-RNA as a specific ligand for RIG-I, dAdT DNA-ligating cytosolic DNA sensors including ZBP1, tri-DAP activating NOD1, MDP activating NOD2, monosodium urate (MSU) crystals activating the inflammasome via NLRP3. LPS was used as a positive control activating TLR4 on the cell surface. As a consistent finding, all stimuli strongly induced the mRNA levels of the cytosolic nucleic acid sensors in both cell types (Fig. 3a). In contrast, C2TA, NLRC4, NAIP and NLRP12 were rather down-regulated. We conclude that human CD14+ monocytes and CD3+ T cells regulate their PRR mRNA similarly upon various triggers of which the induction of cytosolic nucleic acid sensors is most prominent.

PRR agonists regulate PRR mRNAs in murine CD14+ monocytes and CD3+ T cells

When we performed the same experiments in murine CD14+ monocytes and CD3+ T cells, the induction of the cytosolic nucleic acid sensors by all stimuli was only observed in CD3+ cells (Fig. 3b). In CD14+ monocytes, this phenomenon was limited to stimulation with LPS, 3P-RNA and dAdT DNA. Also the NLRPs were differently regulated in CD14+ and
Fig. 1. PRR mRNA expression in adult human and mouse tissues. (a) Quantitative real-time PCR analysis was performed on prenormalized cDNAs derived from poly(A)-selected DNase-treated RNAs purified from pools of healthy human tissues as described in Methods. (b) Real-time PCR was performed on pooled cDNAs derived from five adult 6-week-old C57BL/6 mice as described in Methods. Biological replicates were quantified in triplicates and normalized to the respective GAPDH mRNA level. The results in the table within the figure are expressed relative to the respective expression level of each transcript in spleen. In the table, red shades indicate higher and green shades indicate lower mRNA levels as compared with the respective mRNA levels in spleen. The spleen mRNA levels are illustrated in the histogram on top of the table. (c) The respective relative human (white bars) and murine (black bars) PRR mRNA levels from Fig. 1(a and b) are illustrated. The x-axis marks a ratio of 1; hence, positive values indicate stronger expression in humans and negative values indicate stronger expression in mice. The y-axis marks the fold-change in each direction. Note that the scale of the y-axis is different for each organ. Data in (b) and (c) are mean ± SEM.
CD3+ T cells. MSU crystals potently induced the mRNA levels of many PRR in CD3+ but not in murine CD14+ cells. We conclude that human and murine CD14+ monocytes and CD3+ T cells show significant differences in the regulation of PRR mRNAs upon various immune triggers.

Stimulation of human and murine CD14+ monocytes and CD3+ T cells with PRR agonists

Next, we determined the capacity of human and murine CD14+ monocytes and CD3+ T cells to induce IL-1β and CCL5/RANTES secretion after 24 h upon activation...
with the aforementioned PRR agonists. Resting human CD14+ and CD3+ cells displayed comparable levels of constitutive IL-1β release that significantly increased upon activation with each of agonists tested (Fig. 4). The IL-1β levels reached after stimulation were higher in CD3+ T cells as compared with CD14+ cells. However, when the same type of stimulation experiments was performed in CD14+ monocytes and CD3+ T cells that had been prestimulated with PWM/ConA, the increase of IL-1β release was no longer observed (Fig. 4). Performing identical experiments in murine CD14+ monocytes and CD3+ T cells revealed different results in two ways. First, the overall release of IL-1β

Fig. 2. PRR mRNA expression in human and mouse CD14+ monocytes and CD3 T cells upon activation with PWM/ConA. Real-time PCR was performed on cDNAs derived from human or murine resting (black bars) or activated (white bars) CD14+ monocytes and CD3+ T cells as indicated. Cells were activated with PWM/ConA as described in Methods. Biological replicates were analyzed in triplicates and normalized to the housekeeper genes. Data represent mean ± SEM. *P < 0.05 versus respective resting cells.
was significantly lower in the murine cells (Fig. 4). Second, prestimulating the cells with PWM/ConA rather enhanced the IL-1β release for most of the PRR agonists (Fig. 4). CCL5/RANTES production was different. Most of the PRR agonists (except for cytosolic nucleic acids) induced CCL5/RANTES production in resting human and murine cells (Fig. 5). PWM/ConA prestimulation strongly induced CCL5/RANTES secretion so that significant additional effects by PRR agonist exposure could not be observed. Together, human and murine CD3$^+$ cells and CD14$^+$ cells consistently induce IL-1β and CCL5/RANTES upon PRR activation in their naive state while PWM/ConA prestimulation strongly induces CCL5/RANTES and rather reduces IL-1β secretion.

![Fig. 3. PRR mRNA expression in human and mouse CD14$^+$ monocytes and CD3$^+$ T cells upon activation with PRR agonists. Real-time PCR was performed on cDNAs derived from human (a) or murine (b) CD14$^+$ monocytes and CD3$^+$ T cells that had been exposed to medium only or to any of the listed PRR agonist as described in Methods. Biological replicates were analyzed in triplicates and normalized to the housekeeper genes. The tables within the figure illustrate the ratio of the respective PRR mRNA expression level with each transcript in medium-treated cells. In the table, red shades indicate higher and green shades indicate lower mRNA levels as compared with the respective mRNA levels from cells kept in medium. The baseline mRNA levels are illustrated in the histogram on top of the table. 3P-RNA, 5'-triphosphate RNA complexed with cationic lipid; dAdT, double-stranded dAdT DNA complexed with cationic lipid; tri-DAP, meso-diaminopimelic acid.](https://academic.oup.com/intimm/article-abstract/22/9/717/786068)
Discussion

Pattern recognition molecules are a central element of tissue homeostasis (19) and rapid responses to infectious as well as non-infectious dangers to tissue integrity (2, 20). Because little is known about the respective expression profiles of the cytosolic pattern recognition molecules, we determined the organ-specific expression of RLHs and NLRs in humans and mice.

In a previous report, Zarember et al. (13) described the mRNA expression of TLRs in human organ cDNA samples and found TLRs generally to be much less expressed in solid organs as compared with spleen which related to the number of immune cells in these organs. Consistent with this observation and using human organ cDNA from the same source, we found the mRNA levels of most cytosolic pattern recognition molecules in human heart, small intestine, colon and kidney to be lower as compared with spleen. However, some organs expressed higher levels of selected receptors as compared with spleen, e.g. NLRP1-3, -6 and -12 in brain, LGP2, RIG-I and NLRP10 in liver, LGP-2, RIG-I, NAIP, NLRP2, -3 and -10 in testis and RIG-I, NOD1, -2, NLRP1, -2, -10 and -12 in muscle. Some of these findings are consistent...
Fig. 4. Exposure of human and mouse CD14+ monocytes and CD3+ T cells to PRR agonists. CD14+ and CD3+ cells were isolated by magnetic beads from human and murine peripheral blood leukocytes and stimulated with 1 μg/ml 5′-triphosphate RNA, 6 μg/ml dAdT DNA, 10 μg/ml MDP, 50 μg/ml tri-DAP, 50 μg/ml iE-DAP, 100 μg/ml MSU crystals or 1 μg/ml LPS as described in Methods. Cells used were either in the resting state (black bars) or had been pre-activated (white bars) with PWM/ConA as described in Methods. IL-1β was measured in cell culture supernatants by ELISA after 24 h of stimulation. Data represent mean ± SEM from three experiments each analyzed in duplicate. *P < 0.05 versus medium, #P < 0.05 versus resting cells.

with previous studies like the expression of NLRP10 in human kidney, muscle and liver while we did not find much of NLRP10 expression in human brain and heart as reported by northern blot results using different sources of human material (21). RNA expression profiling for NLRP3 also varies between different reports and our own data, which all use their own sources of murine organ samples and different detection systems. While Anderson et al. (22) found NLRP mRNA to be highly expressed in skeletal muscle and skin, Wang et al. (21) reported NLRP3 mRNA expression in lung, liver, kidney and colon. Unfortunately, immunohistochemical studies that validate mRNA expression at the protein level and inform about the cellular distribution of cytosolic PRR in human tissues are mostly lacking. We also observed that protein expression levels do not always correspond to tissue mRNA expression that may relate to different mRNA and protein kinetics. However, whether or not single PRR have specific roles in human organ-specific pathology can only be concluded from functional studies usually performed in mice. As one example, brain NLRP1 expression was shown
to originate from pyramidal neurons and oligodendrocytes (23) and brain NLRP3 expression from microglia (24), which corresponds to our finding of high mRNA expression levels of NLRP1 and -3 in human brain. However, NLRP3 is expressed and functionally important in mouse brains because β-amyloid fibrils activate murine brain microglia to produce neurotoxic factors via the NLRP3-inflammasome pathway, a potentially novel pathomechanism of Alzheimer plaque formation (24). As another example, murine lungs trigger inflammation via NLRP3 in response to silica crystals (25), although we found NLRP3 mRNA levels in murine lungs to be rather low (as compared with spleen). Generally, we have to admit that low mRNA levels do not exclude an important contribution of the gene to homeostasis or disease as, e.g. NOD2 was not highly expressed in the intestine but it is functionally relevant in inflammatory bowel disease (4, 26).

In contrast, mouse colon was rich in NOD1 mRNA consistent with its role in maintaining the colonic epithelial barrier that prevents intramural inflammation and tumorigenesis in mice (27).

Human and mouse kidneys both revealed mRNA expression of RIG-I. Renal RIG-I immunostaining localizes to glomerular mesangial cells but not to podocytes or tubular epithelial cells in nephritic kidneys (28). These immunohistochemical data were confirmed by functional studies on RIG-I in human glomerular mesangial cells (29). 3P-RNA injected intra-peritoneally localized to the glomerulus compartment of the mouse kidney and aggravated glomerulonephritis in lupus-prone mice suggesting a role for RIG-I in mediating viral RNA-triggered glomerular inflammation (30). In addition, murine kidney was particularly rich in many of the other cytosolic PRR. Nothing is yet known about potential roles of the

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**Fig. 5.** Exposure of human and mouse CD14+ monocytes and CD3+ T cells to PRR agonists. CD14+ and CD3+ cells were isolated and stimulated as outlined in the legend of Fig. 4. Cells used were either in the resting state (black bars) or had been pre-activated (white bars) with PWM/ConA as described in Methods. CCL5/RANTES was measured in cell culture supernatants by ELISA after 24 h of stimulation. Data represent mean ± SEM from three experiments each analyzed in duplicate. *P < 0.05 versus medium, **P < 0.05 versus resting cells.
NODs and NLRPs in renal pathology. In contrast, MDA5 was recently shown to mediate viral RNA recognition and type I IFN production in glomerular endothelial cells, a mechanism that could contribute to viral glomerulonephritis (31).

The basal mRNA expression profiles were surprisingly comparable in CD14+ and CD3+ cells, which is in contrast to the leukocyte sub-population-specific expression of human TLRs (12). This may reflect the universal importance of detecting the entry of pathogens into the intracellular cytosol that either represents invasive infection or rupture of the endosomal compartment as previously shown in crystal-induced inflammation (4, 25, 32). Both represent ultimate states of danger to cell integrity that are beyond the roles of TLRs to recognize extracellular or phagocytosed pathogens and signal appropriate immune responses (14). This is further supported by the observation that resting murine and human CD14+ monocytes and CD3 T cells share the expression of the cytosolic nucleic acid sensors, NOD1, NOD2, NLRP1 and NLRP3 and that cells of both species respond to exposure with respective receptor agonists. Obviously, the basal expression of RLRs and NLRs in monocytes and T cells is conserved among these two vertebrate species. Our findings on the regulation of these receptors by PRR agonists are in part inconsistent with previous reports that should relate to the different cell types studied as well as different agonist doses and time of analysis (33, 34). For example, we may have missed an induction of NLRP3 mRNA by LPS in murine monocytes due to our RNA harvest after 24 h of stimulation that was previously shown to peak at 1 h (33). Interestingly, mice and human CD14+ monocytes revealed significant differences in terms of PRR regulation upon stimulation with PWM/ConA. While stimulation did not much affect PRR expression in human monocytes, PWM/ConA strongly up-regulated the mRNA levels of LGP2, MDA5, RIG-I, ZBP, NOD1 and NLRP3. This effect was specific to murine CD14+ monocytes. This is in line with significant differences of the cell-type-specific TLR functions in mice and humans TLRs. For example, TLR9 expression is restricted to human plasmacytoid dendritic cells (and B cells) while being expressed in various murine monocyte/macrophages phenotypes (10). Other TLRs are only present in one of the two species like TLR8 in humans (9) or TLR11 in mice (8). Other TLRs are only present in one of the two species like TLR8 in humans (9) or TLR11 in mice (8).

Supplementary data
Supplementary data are available at International Immunology Online.

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