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Corticosteroids shift the Toll-like receptor response pattern of primary-isolated murine liver cells from an inflammatory to an anti-inflammatory state

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Abstract

***Objective*: Only little is known about the mechanisms of action of corticosteroids in the treatment of inflammatory liver diseases. As there is increasing evidence that stimulation of the innate immune system plays an important pathogenetic role in these conditions, we hypothesized that steroids may interfere with the activation of the Toll-like receptor (TLR) system of the liver. *Methods*: To test this hypothesis, murine non-parenchymal liver cells (Kupffer cells, liver sinusoidal endothelial cells) and primary hepatocytes were stimulated with TLR 1–9 ligands in the presence or absence of dexamethasone. Expression of pro- and anti-inflammatory cytokines was determined by quantitative reverse transcription–PCR or ELISA, respectively. Nuclear factor 'kappa-light-chain-enhancer' of activated B-cells (NF-κB) activation was assessed by western blot analysis. *Results*: TLR agonists induced the expression of pro- [tumor necrosis factor-α (TNF-α), IL-6, IL-1β, IFN-β] and anti-inflammatory cytokines [IL-10, transforming growth factor-β (TGF-β)], which was differentially modulated by steroid treatment. TNF-α and IL-6 expression was suppressed by dexamethasone, while IL-10 but not TGF-β was enhanced after TLR stimulation. IFN-β production induced by TLR 4 agonists but not TLR 3 agonists was inhibited by dexamethasone. TLR expression itself was down-regulated by steroid treatment in a cell type-specific manner. These effects were associated with suppression of the TLR-mediated activation of NF-κB. *Conclusions*: TLR signaling is modulated by corticosteroids in a cell type-specific fashion resulting in down-regulation of TLR expression, suppression of pro-inflammatory and up-regulation of anti-inflammatory cytokines. This represents an as yet unknown mechanism of action for corticosteroids that may at least in part explain their therapeutic effects in inflammatory liver diseases.**

Keywords: autoimmune disease, dexamethasone, Kupffer cells, liver sinusoidal endothelial cells, toll-like receptors

Introduction

Glucocorticoids (GC) are widely used for the therapy of autoimmune liver diseases (1). They have anti-inflammatory properties including modulation of cytokine production and dendritic cell (DC) differentiation. This results in the generation of cells that are incapable of inducing efficient immune responses and reducing the capability of DCs to stimulate T cells (2).

While GC are the treatment of choice for some inflammatory liver diseases including autoimmune hepatitis and acute allograft rejection, the mechanisms of action in these conditions and their possible inhibitory effects upon the activation of the local innate immune system in particular are still not well understood (3, 4).

Toll-like receptors (TLRs) play an important role in the pathogenesis of inflammatory liver diseases. As a key component of the innate immune system, TLRs recognize different pathogen-associated molecular patterns from infectious agents but also endogenous damage-associated molecular patterns (DAMPs) during inflammatory reactions (5). It has been suggested that an inappropriate activation of TLRs is involved in the pathogenesis of autoimmune disorders. Stimulation of TLR 9 by DAMPs triggers polyclonal B-cell proliferation and antibody secretion, which results in the production of autoantibodies (6, 7). Furthermore, it was hypothesized that TLR activation protects autoreactive

cells against apoptosis and leads to the loss of self-tolerance (8, 9).

In this paper, we assessed the effects of corticosteroids upon the TLR-dependent activation of primary-isolated murine liver cells. We studied hepatocytes, liver sinusoidal endothelial cells (LSEC) and Kupffer cells (KC) as these cell types represent by far the largest populations in this organ (10).

Our data show that steroids are potent modulators of the TLR-mediated activation of these cells resulting in suppression of pro-inflammatory and induction of anti-inflammatory cytokines which can, at least in part, explain their mechanism of action in the treatment of inflammatory liver diseases.

Methods

Materials

Dexamethasone was purchased from Sigma (Heidenheim, Germany). The ligands for TLR 1–9 were obtained from Invivogen (Toulouse, France).

Animals

C57/Bl6 mice were generated, bred and kept under 12 h dark/light cycles in the animal facilities at the University Hospital of Essen. Mice were fed *ad libidum* and received human care according to the institution's guidelines.

Isolation and culture of non-parenchymal liver cells (NPC) and hepatocytes.

NPCs (KC, LSEC) and hepatocytes were prepared from 8 to 12 week-old C57/Bl6 mice as described recently (11, 12). The purity of these cell types was exemplarily determined and ranged between 92 and 99% (data not shown).

TLR stimulation

KC, LSEC and hepatocytes were stimulated with TLR 1–9 ligands [Pam3CSK4 (1 μg ml^{-1}), HKLM (108 cells ml^{-1}), poly I:C (100 μg ml^{-1}), LPS (10 μg ml^{-1}), Flagellin (1.5 μg ml^{-1}), FSL-1 (1 μg ml^{-1}), ssRNA40 (10 μg ml^{-1}), Gardiquimod (10 μg ml^{-1}) and ODN1826 (2.5 μM) with or without pre-treatment of 0.1 mM dexamethasone for 24 h, respectively. Nuclear protein extracts were extracted 30 min after stimulation was started, whereas total RNA was isolated after 8 h and supernatants were harvested 24 h after exposure to TLR ligands.

RNA isolation and real-time PCR

Total RNA was isolated and purified using the Qiazol™ solution (Qiagen, Hilden, Germany) and the RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions. Quantitative reverse transcription (RT)–PCR was performed with the QuantiTect SYBR Green RT-PCR Kit (Qiagen) using 0.1–0.3 μg of total RNA. GC-induced leucine zipper (GILZ), IFN-β, IL-6, tumor necrosis factor (TNF-α), IL-1β and IL-10 expression was detected by commercially available primer sets (QuantiTec Primer Assay; Qiagen; sequences are not given by the manufacturer). The calculated copy numbers were normalized to the housekeeping gene β-actin which was detected with the sense primer 5′-AAATCGTGCGTGACATCAAA-3′ and the anti-sense primer 5′-CAAGAAGGAAG GCTGGAAAA-3′.

Protein isolation

Cellular proteins were extracted using NE-PER® Nuclear and Cytoplasmic Extraction Reagents (PIERCE, Rockford, IL, USA) according to the manufacturer's instructions. Nuclear proteins were used to examine the activation status of nuclear factor 'kappa-light-chain-enhancer' of activated B cells (NF-κB).

Western blot

Western blot analysis was performed as described previously (13). The following specific antibodies were used: anti β-actin (Sigma) and anti-NF-κB (p65) (Cell signaling, Danvers, MA, USA). Signal intensities were visualized, quantified and normalized to β-actin using the Pieper photo system FK 7512-IQ (software: Phoretix Grabber 2.0 plus Phoretix 1D Advanced 4.01; Biostep, Jahnsdorf, Germany).

ELISA

Supernatants from KCs or LSECs pre-treated with dexamethasone and stimulated with various TLR agonists were tested for the secretion of IL-10 or TNF-α, respectively. ELISAs were performed according to the manufacturer's instructions (Quantikine Mouse IL-10 & TNF-α Immunoassay Kits; R&D Systems, Wiesbaden, Germany).

Statistical analysis

Data were expressed as mean ± SEM. Differences between any two groups were determined by Wilcoxon's test, $P < 0.05$ was considered to be statistically significant.

Results

Dexamethasone response in NPCs

Initially, dose–response experiments were performed with LSEC to determine the optimal concentration of dexamethasone. The cells were stimulated with different concentrations of dexamethasone ranging from 0.01 to 1 mM for 24 h. Then, RNA was isolated and the expression of the GILZ was measured by quantitative RT–PCR, which is a sensitive parameter for the effect of steroids (14). Our data indicated that 100 nM dexamethasone induced the highest GILZ expression levels (fold change 7.1 ± 0.8) and was therefore considered to be the optimal concentration for induction of an effective steroid response in NPCs (Fig. 1). Higher dexamethasone concentrations were associated with cytotoxic effects as indicated by a loss of β-actin (−74.6% ± 5.2) and Glycerinaldehyd-3-phosphat-Dehydrogenase (−73.3% ± 4.9) gene expression (data not shown).

Modulation of TLR expression by dexamethasone in murine NPCs and hepatocytes

To investigate the effect of dexamethasone on TLR signaling, we initially determined steroid-induced changes in TLR expression. Therefore, murine NPC and primary hepatocytes were stimulated with dexamethasone for 24 h. Changes in TLR 1–9 expression were determined by quantitative RT–PCR. The basal expression of the TLR 1–9 is shown in Table 1. In general, TLR 1–4 reached higher expression levels than the TLR 5–9. LSEC showed higher

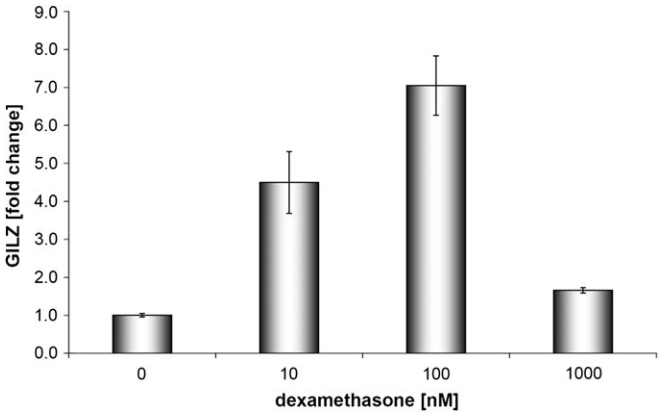

Fig. 1. Dexamethasone-induced dose response in LSEC. LSEC were stimulated with different concentrations of dexamethasone ranging from 0.01 to 1 mM for 24 h. RNA was isolated and the expression of the GILZ was determined by quantitative RT–PCR.

Table 1. Basal expression of TLR 1–9 in primary-isolated liver cells

Gene	KC	LSEC	Heps
TLR1	++	+++	±
TLR2	++	+++	+++
TLR3	++	+++	+++
TLR4	++	+++	+
TLR5	±	±	++
TLR6	+	+	+
TLR7	+	+	+
TLR8	+	+	±
TLR9	±	±	±

TLR 1–9 copy numbers were determined by quantitative RT–PCR and normalized to 100 000 copies of β-actin. Normalized copy number is represented by the following symbols: +++, >10 000; ++, <10 000; +, <5000; ±, <1000.

expression levels for most of the TLRs compared with KC and hepatocytes.

In KC, expression levels of TLR 1, -2, -3, -6 and -9 were profoundly suppressed, while expression of TLR 5, -7 and -8 was enhanced, whereas TLR 4 expression was not affected. In LSEC, similar patterns of TLR expression could be shown with profound suppression of TLR 1, -2, -3, -4, -6 and -9, while TLR 5 and -7 were up-regulated and TLR 8 was not affected. In hepatocytes treated with dexamethasone, however, TLR 1, -2 and -9 expression was suppressed, whereas enhanced expression levels could be shown for TLR 3, -4, -6, -7 and -8, while TLR 5 expression was unaffected (Fig. 2).

TLR-mediated activation of KC and LSEC resulted in elevated expression of TNF-α, IL-6, IL-1β and IL-10 (Table 2). Compared with KC, LSEC showed higher TLR-induced expression of TNF-α but not IL-6. In contrast, KC showed higher IL-10 expression levels after TLR activation. In hepatocytes, TLR signaling was assessed by induction of IL-1β and IL-10. TLR-induced expression levels of IL-1β did not differ between NPC and hepatocytes, except for TLR 8 and -9 activation. In addition, hepatocytes showed similar IL-10 expression after TLR 1, -2, -4 and TLR 6–8 activation compared with KC.

Dexamethasone modulates TLR signaling in KCs

KC were stimulated with dexamethasone for 24 h, followed by stimulation with TLR 1–9 ligands for 8 h. Changes in gene expression of selected cytokines were determined by quantitative RT–PCR. Treatment with TLR 1–9 ligands except TLR 5 resulted in enhanced expression of the pro-inflammatory cytokines TNF-α and IL-6. The highest expression levels of TNF-α were induced by stimulation of TLR 2, -3 and -4. Maximum expression of IL-6 was induced by TLR 3 und TLR 4 ligands. Pre-treatment with dexamethasone significantly suppressed TNF-α expression by about 50–80%, whereas TLR-induced IL-6 expression was completely suppressed to basal levels (except for TLR 4 and -9 stimulation) (Fig. 3A). In addition, the gene expression of immunocyte-specific IL-1β was measured upon TLR 1–9 activation. Dexamethasone induced a strong suppression in IL-1β expression comparable to the IL-6 expression pattern (data not shown).

Furthermore, stimulation of TLR 1–9, except TLR 5, led to enhanced expression of the anti-inflammatory cytokine IL-10. The most potent induction of IL-10 was induced after TLR 4 stimulation compared with the control. Pre-treatment with dexamethasone caused a further increase of IL-10 production after stimulation of TLR 1–9, except TLR 8 (Fig. 3A). In comparison with IL-10 neither TLR 1–9 stimulation nor dexamethasone, pre-treatment had any effects on transforming growth factor (TGF-β) expression in these cells (data not shown).

Dexamethasone modulates TLR signaling in LSECs

Stimulation of TLR 1–9, except TLR 5, led to enhanced TNF-α, IL-6 and IL-10 expression in LSEC. Pre-treatment with dexamethasone resulted in 30–80% suppression of TNF-α expression after stimulation of TLR 1–9. IL-6 expression was affected by dexamethasone pre-treatment likewise. Suppression of TLR-induced IL-6 expression by dexamethasone ranged from 30 to 90% (Fig. 3B). The gene expression pattern of IL-1β in LSEC was determined likewise. TLR 1–9 ligands differently induced IL-1β expression. The dexamethasone pre-treatment induced a strong suppression in IL-1β expression comparable to IL-1β expression in KC (data not shown).

IL-10 expression increased after stimulation of TLR 1–4 and TLR 6 with maximum levels after TLR 4 stimulation. Dexamethasone pre-treatment further increased the expression level of IL-10 (Fig. 3B). TGF-β expression was not altered after TLR stimulation (data not shown).

Dexamethasone modulates TLR signaling in hepatocytes

Stimulation of primary-isolated hepatocytes by TLR agonists led to enhanced expression of IL-1β and IL-10 (Table 2). Maximum IL-1β expression was achieved after stimulation of TLR 4 and -5. The strongest induction of IL-10 expression was detected after exposure to TLR 2 and -8 ligands. Dexamethasone pre-treatment suppressed TLR 1–9-induced IL-1β expression about 80–100% (Fig. 3C). In contrast, TLR-induced IL-10 expression was not affected by dexamethasone pre-treatment.

In hepatocytes, adverse drug application was exemplarily performed by adding dexamethasone 6 h after TLR stimulation. While suppression of pro-inflammatory genes was quantitatively lower as compared with the initial experimental setting, the

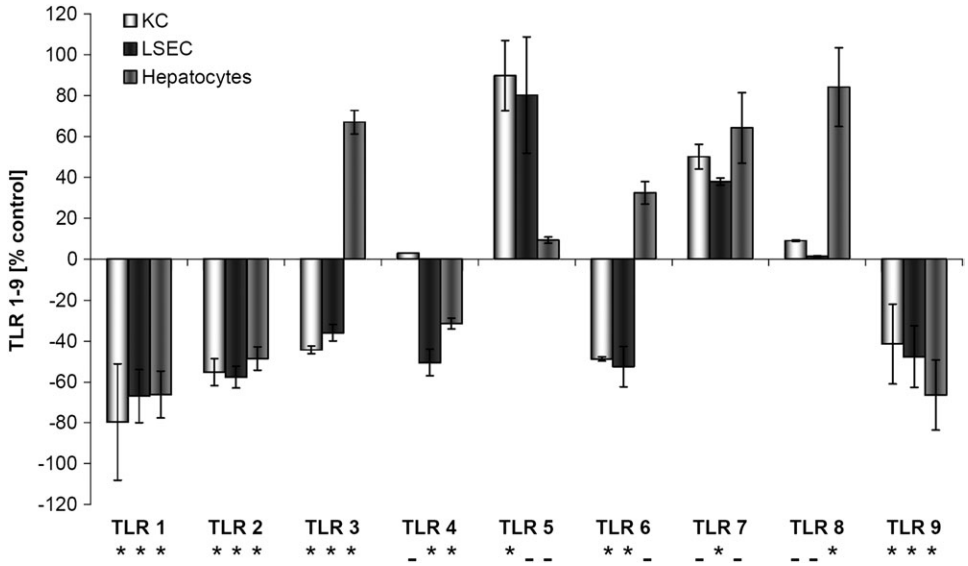

Fig. 2. Modulation of TLR expression by dexamethasone in murine liver cells. KC, LSEC and primary-isolated hepatocytes were cultured with or without dexamethasone (0.1 mM) for 24 h. RNA was extracted and TLR 1–9 expression was measured by quantitative RT–PCR. Copy numbers were normalized against β-actin (×/100 000 β-actin). Dexamethasone-induced changes were determined by comparison with the untreated control (% control). Significant results ($P < 0.05$) are marked by an asterisks. Data are shown as mean values ± SEM.

Table 2. TLR-mediated expression of TNF-α, IL-6, IL-1β and IL-10 in primary-isolated liver cells

	TNF-α		IL-6		IL-1β			IL-10		
	KC	LSEC	KC	LSEC	KC	LSEC	Heps	KC	LSEC	Heps
w/o	±	+	±	±	±	+	±	±	±	±
TLR1	++	++	+	++	+++	+++	+++	+	±	+
TLR2	++	+++	±	+	+++	+++	+++	+	±	++
TLR3	++	+++	++	++	+	+++	++	+	±	±
TLR4	++	+++	++	+	+++	+++	+++	+	+	+
TLR5	+	++	±	±	+	+++	+++	+	±	±
TLR6	++	++	+	+	+++	+++	+++	+	±	+
TLR7	++	++	±	+	+++	+++	+++	+	±	+
TLR8	++	++	±	+	++	+++	±	+	±	++
TLR9	+	++	±	+	+++	+++	+	++	±	+

Copy numbers were determined by quantitative RT–PCR and normalized to 100 000 copies of β-actin. Normalized copy number is represented by the following symbols: +++, >30 000; ++, <20 000; +, <5000; ±, <1000.

dexamethasone-induced increased expression of the anti-inflammatory cytokine IL-10 was much more profound under these conditions. Both experimental settings efficiently shifted a pro-inflammatory cytokine profile to an anti-inflammatory one (data not shown).

The anti-inflammatory shift in TLR signaling induced by dexamethasone was also found on the protein level. In KC, dexamethasone enhanced IL-10 production after stimulation of TLR 1–8 (Fig. 3D), while TLR-induced TNF-α expression in LSECs was profoundly in the presence of dexamethasone (Fig. 3E).

TLR 4- but not TLR 3-induced IFN-β expression is suppressed by dexamethasone pre-treatment

NPC and hepatocytes were stimulated with dexamethasone for 24 h, followed by stimulation with TLR 1–9 ligands for 8 h. Changes in IFN-β expression were determined by quantitative RT–PCR. Poly I:C and LPS treatment led to significantly enhanced IFN-β expression in KC and LSEC. IFN-β expression increased >1000-fold after stimulation of TLR 3 and ~200-fold after TLR 4 stimulation (Fig. 4A and B). Dexamethasone had no effect upon TLR 3-induced IFN production. On the contrary, TLR 4-induced expression of IFN-β was totally abrogated after pre-treatment with dexamethasone. In hepatocytes, induction of IFN-β was only found after TLR 3- but not TLR 4 stimulation which was not affected by dexamethasone (Fig. 4C).

Dexamethasone specifically inhibits TLR 1–9-mediated NF-κB activation

To determine the effect of dexamethasone on TLR signaling, NF-κB activation was examined by western blot analysis. Primary hepatocytes were stimulated with dexamethasone for 24 h, followed by stimulation with TLR 1–9 ligands for 30 min. A strong NF-κB activation (fold increase > 4) could be shown after stimulation of TLR 1, -2, -5 and -6, whereas TLR 3, -4, -7 and -9 ligands induced lower NF-κB activation (fold increase > 2) signals (Fig. 5A and B). Pre-treatment with dexamethasone had selective effects on TLR-induced NF-κB activation. TLR 1-, TLR 2-, TLR 3-, TLR 4-, TLR 7- and TLR 9-induced NF-κB activation was inhibited by 60–85%, whereas TLR 5-, TLR 6- and TLR 8-mediated NF-κB activation was unaffected by dexamethasone (Fig. 5A and C).

Discussion

In this paper, we analyzed the effects of steroid treatment on TLR-mediated activation of the innate immune system of the liver in murine NPC and hepatocytes. Our data indicate that steroids lead to suppression of TLR-induced production of pro-inflammatory cytokines (TNF-α, IL-6, IL-1β and IFN-β),

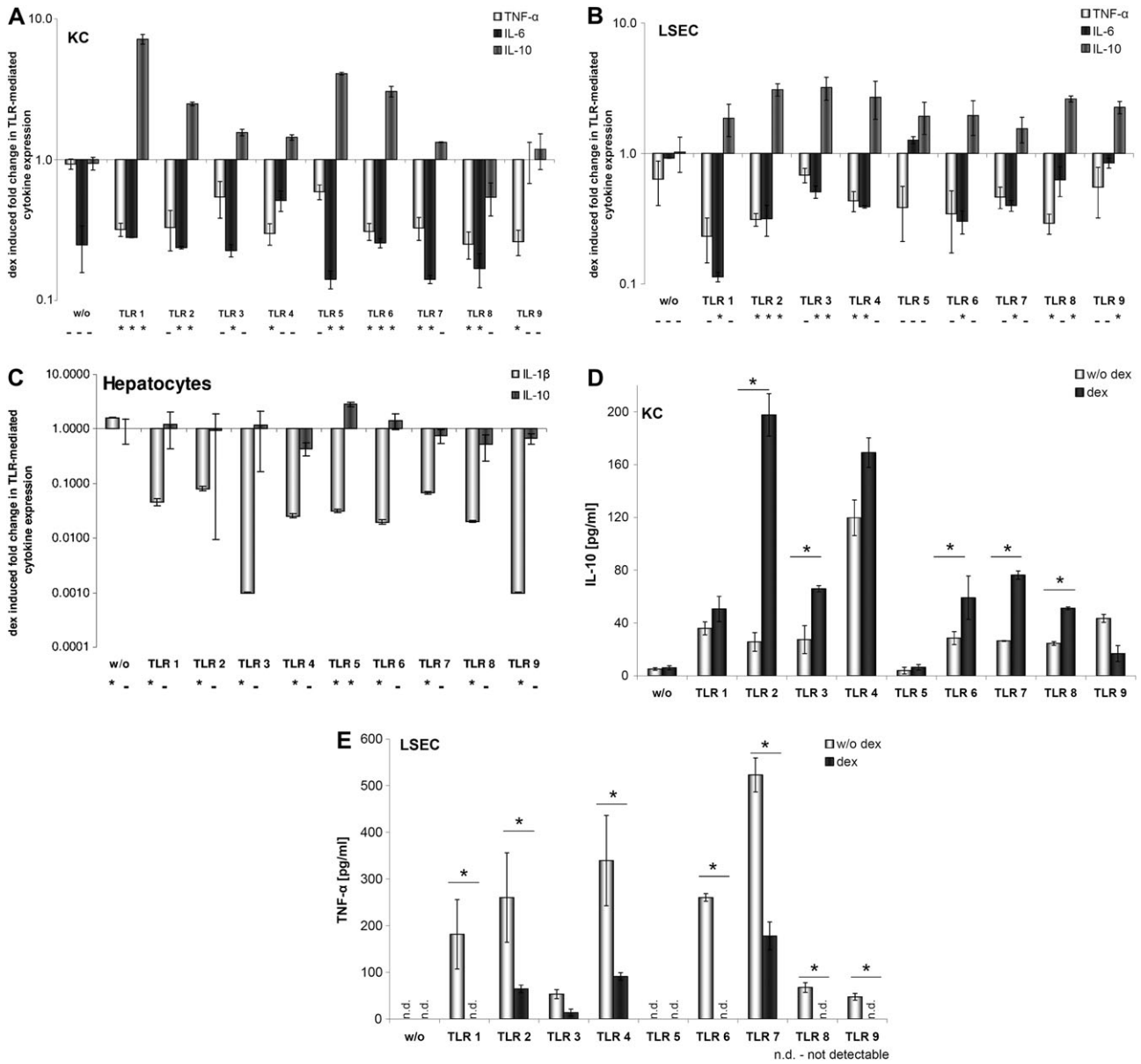

Fig. 3. Dexamethasone modulates TLR 1–9 signaling in murine liver cells. KC (A), LSEC (B) and hepatocytes (C) were cultured with or without dexamethasone (0.1 mM) for 24 h followed by stimulation with TLR 1–9 ligands for 8 h. RNA was extracted and gene expression was determined by quantitative RT–PCR. TLR ligand-induced expression of TNF-α, IL-6, IL-1β and IL-10 with or without dexamethasone pre-treatment was assessed. Copy numbers were normalized against β-actin (×/100 000 β-actin). All experiments were performed in triplicate. Data are shown as mean values (fold change no dexamethasone versus dexamethasone) ± SEM. Supernatants from KCs or LSECs pre-treated with dexamethasone for 24 h and then stimulated with various TLR agonists for additional 24 h were tested for the secretion of IL-10 (D) or TNF-α (E), respectively. Significant results ($P < 0.05$) are marked by an asterisks.

while the induction of the anti-inflammatory cytokine IL-10 but not TGF-β is enhanced in a cell type-specific manner. This is associated with suppression of NF-κB activation in these cells.

Taken together, these data improved the knowledge of the mechanisms of action that are the basis for the anti-inflammatory and immunosuppressive effects of corticosteroids in inflammatory liver diseases. Battacharyya *et al.* demonstrated that Toll/interleukin-1 receptor-like domain-containing adapter-inducing IFN-β (Trif), Myeloid differentiation primary response gene (88) (MyD88) or both adapter molecules specify the capability of GC to inhibit different mitogen-activated protein kinases to suppress inhibitor of κB (IκB) degradation or to activate Janus kinase (JNK). Battacharyya *et al.* identified transforming growth factor-activated kinase 1 as a novel target for GC that mediates these anti-inflammatory action in innate immunity signaling pathways (15, 16). This diversity of mechanism is consistent with our data, assuming a cell type-specific and TLR-specific action of dexamethasone.

The pro-inflammatory cytokines TNF-α and IL-1β function as positive regulators of NF-κB. Therefore, their suppressed expression level during dexamethasone treatment additionally inhibits NF-κB activation (17).

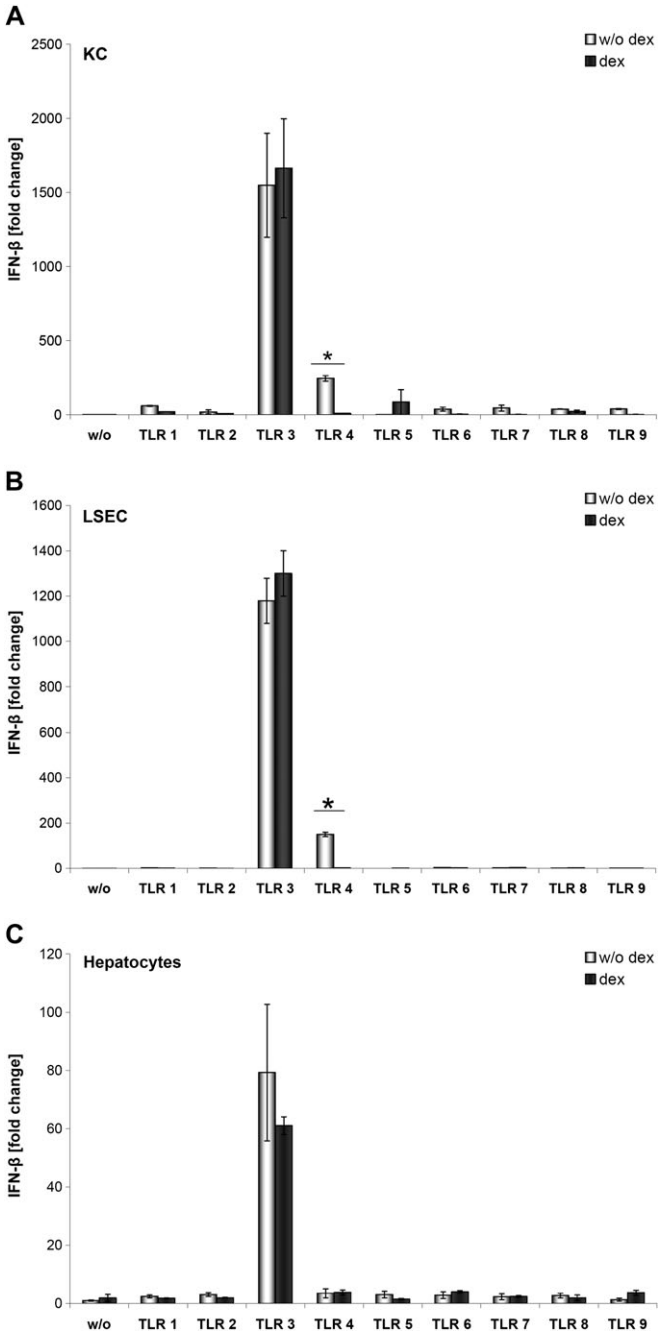

Fig. 4. Modulation of TLR-induced IFN-β expression by dexamethasone in murine liver cells. Murine NPC were cultured with or without dexamethasone (0.1 mM) for 24 h followed by stimulation with TLR 1–9 ligands for 8 h. RNA was extracted and gene expression was measured by quantitative RT–PCR. TLR ligand-induced fold changes in IFN-β expression and their modification by pre-treatment with dexamethasone were determined for KC (A), LSEC (B) and hepatocytes (C). Copy numbers were normalized against β-actin (×/100 000 β-actin). All experiments were performed in triplicate. Significant results ($P < 0.05$) are marked by an asterisks. Data are shown as mean values ± SEM.

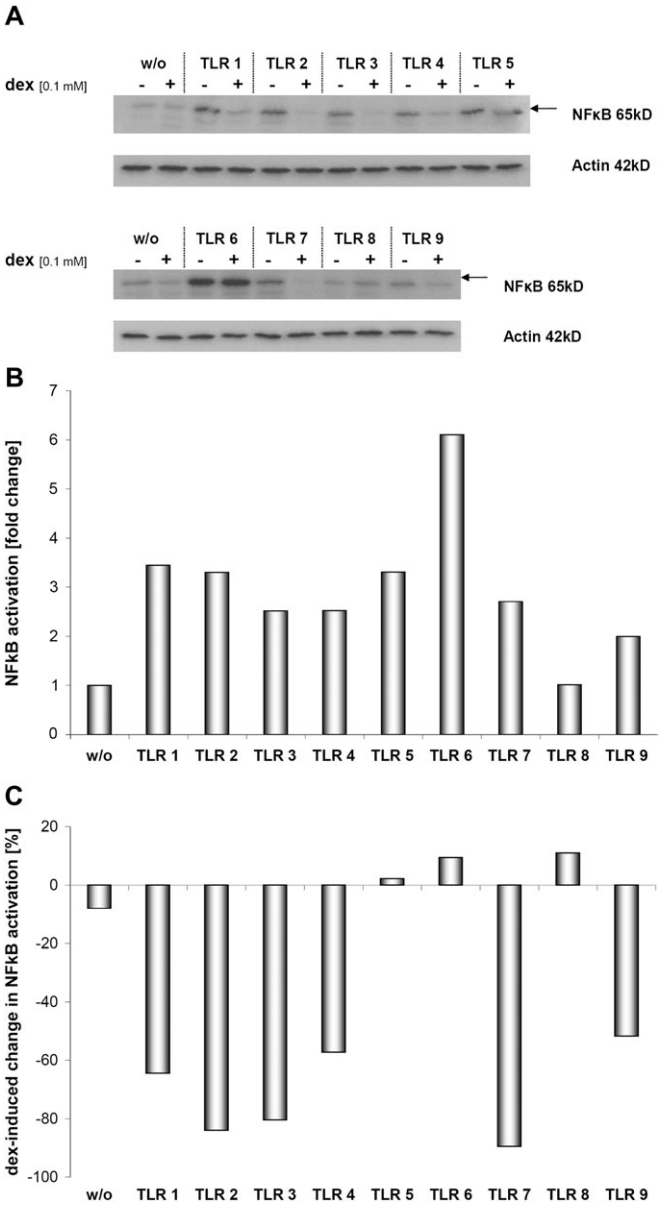

Fig. 5. Dexamethasone differently inhibits TLR-mediated NF-κB activation. Hepatocytes were cultured with or without dexamethasone (0.1 mM) for 24 h followed by stimulation with TLR 1–9 ligands for 30 min. Nuclear proteins were extracted and NF-κB activation was analyzed by western blot (A). The NF-κB signal intensity was quantified and normalized to β-actin. TLR 1–9-induced activation of NF-κB (B) and dexamethasone-mediated changes were determined (C).

Here, we additionally showed the increased anti-inflammatory shift induced by corticosteroid-mediated-enhanced IL-10 expression upon TLR 1–9 activation. The molecular mechanism of differential regulation of IL-10 has to be evaluated in the future.

The TLR system plays an important role in the pathogenesis of most inflammatory liver diseases. This includes chronic viral hepatitis (11,18,19,20,21) but also holds true for autoimmune disorders like primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC) and autoimmune hepatitis. Here, an inappropriate activation of several TLR has been described (22,23,24).

For PBC and PSC, an over-expression of TLR 3, -4 and -9 or TLR 4 and -9, respectively, has been demonstrated. As

a functional consequence, stimulation of the TLR system leads to significantly increased release of inflammatory cytokines in these patients (23,25,26,27,28).

Stimulation of TLR 3 during infection with lymphocyte choriomeningitis virus (LCMV) has been shown to induce autoimmune hepatitis (24). Furthermore, Sacher *et al.* (29). demonstrated that repeated stimulation of TLR 9-induced autoimmune hepatitis. Finally, TLR 4 activation plays a crucial role in the pathogenesis of end-stage liver disease (30,31,32).

In addition, the TLR system has important regulatory functions in ischemia and reperfusion (I/R) injury and liver transplantation, respectively. An important mechanism of I/R injury is the release of high-mobility group protein B1 (HMGB1) from damaged hepatocytes which in turn stimulates TLR 4 on NPC including KC (33,34,35). Thereby TLR 4 induces IFN-β expression though TRIF and Interferon regulatory factor 3 leading to liver injury (33, 36, 37). Interestingly, our data suggest that IFN-β release after TLR 4 stimulation is almost completely suppressed by dexamethasone.

Furthermore, TLR are involved in allorecognition after liver transplantation (30). Here, the TLR-driven MyD88-dependent signaling is important for DC maturation, $CD8^+$ alloimmune priming and subsequent T_h1-dependent alloimmunity (31). Deng *et al.* showed significantly elevated TLR 2 and TLR 4 expression levels in PBMC from liver transplantation recipients with acute rejection compared with those with normal liver function. Analogous to our data, steroids decreased the expression of TLR 2 and TLR 4 on monocytes which was associated with reduced liver injury (32). Our data, however, suggest that the therapeutic effect of steroids in acute allograft liver rejection may also involve suppression of TLR-mediated signaling of liver cells.

The primary target for GC is a specific intracellular GC receptor (GR) (38). The engagement of the GR by GC promotes its dissociation and translocation into the nucleus. It functions as transcription factor for GC response elements (39). Of interest, GC can directly induce the expression of IκB-α, the inhibitor of NF-κB in a cell type-specific manner (40, 41).

In contrast to activation via TLR 4, dexamethasone had no effect on TLR 3-induced IFN-β expression. This could be explained by the fact that the TLR 3 pathway is independent of MyD88 and NF-κB (42). Battacharyya *et al.* (15, 16) additionally described dexamethasone as adaptor molecule-specific modulator of downstream signaling. We have previously shown that TLR 3 and -4 agonists can induce IFN-β expression in NPC thereby suppressing Hepatitis C virus (HCV) replication in co-culture model (11, 18).

Steroid treatment against acute rejection after transplantation is associated with an increase in viral load and the severity of recurrence in HCV-positive patients. Two mechanisms for steroid-mediated effects upon HCV viremia have been proposed. It has been suggested that steroids may enhance viral replication directly (43,44,45), while indirect effects due to the suppression of antiviral adaptive immune responses directed against HCV may also be relevant (46, 47). It is unclear, however, whether the effects of steroids upon the innate immune system, as described here, may contribute to effects described above.

In conclusion, our data indicate that dexamethasone is a potent modulator of the innate immune system by shifting the response pattern of the TLR system from an inflammatory to an anti-inflammatory state.

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