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Upregulation of 11β-hydroxysteroid dehydrogenase 1 in lymphoid organs during inflammation in the rat

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ABSTRACT

Glucocorticoids exert anti-inflammatory and immunomodulatory effects that may be regulated in part by the activities of the glucocorticoid-activating and -inactivating enzymes, 11β-hydroxysteroid dehydrogenase type 1 (11HSD1) and type 2 (11HSD2), respectively. Previous studies have demonstrated that inflammatory bowel diseases in humans and experimental animals upregulate 11HSD1 and downregulate 11HSD2. We investigated whether proinflammatory cytokines modulate colonic 11HSDs as well as whether lymphoid organs exhibit any 11HSD response to inflammation. Colon tissue explants exposed to tumor necrosis factor α exhibited an upregulation of 11HSD1 mRNA whereas interleukin 1β downregulated 11HSD2 mRNA. Experimental colitis induced by the intracolonic administration of 2,4,6-trinitrobenzenesulfonic acid stimulated 11HSD1 activity not only in the colon but also in mesenteric lymph nodes and the spleen. Analysis of mRNA for 11HSD1 in colon-draining lymph nodes and the spleen showed that inflammation upregulates the expression of this enzyme in mobile lymphoid cells similar to the intraepithelial and lamina propria leukocytes isolated from the colon. It is inferred that inflammation stimulates the reactivation of glucocorticoids in lymphoid organs and in gut-associated lymphoid tissue.

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1. Introduction

Inflammation is a first-line host defense mechanism of the innate immune system for removing invading pathogens and repairing damaged tissues. In response to inflammation, the increase in circulating proinflammatory cytokines triggers a feedback loop to the hypothalamic-pituitary-adrenal (HPA) axis, which stimulates the synthesis and secretion of glucocorticoids by the adrenal gland [1]. Glucocorticoids elicit pleiotropic responses that not only counterregulate inflammation due to immunosuppressive effects but also modulate immunity by regulating the Th1/Th2 pathways differently [2] and reprogramme the differentiation of macrophages to a highly phagocytic phenotype [3]. Many studies have reported the activation of the HPA axis after both acute and chronic inflammatory stress, including colitis. Depending on the type and duration of the stressors, the associated response is characterized by a dramatic increase in the plasma level of glucocorticoids due to released cytokines such as tumor necrosis factor α (TNF-α) and interleukin 1β (IL-1β) followed by an increase in plasma ACTH [1]. The resulting increased serum glucocorticoids have a well-documented suppressive effect on colitis [4].

However, glucocorticoid bioavailability in target tissues not only depends on systemic production controlled by HPA and the ratio of free and bound glucocorticoids transported in the blood, but also on the level of these hormones in the target cells, which is determined by the local metabolism of glucocorticoids catalyzed by 11β-hydroxysteroid dehydrogenase (11HSD) [5]. Two enzymes are responsible for the interconversion of cortisol/cortisone and corticosterone/11-dehydrocorticosterone. 11HSD1 acts in vivo predominantly as a reductase, catalyzing the regeneration of active glucocorticoids and thereby promoting the activation of glucocorticoid receptors in target tissues. In contrast, 11HSD2 is an exclusive dehydrogenase that oxidizes the biologically active steroids, cortisol and corticosterone to their inactive 11-oxo derivatives [6]. Thus, 11HSD2 decreases the local concentration of cortisol or corticosterone in some cells, whereas 11HSD1 increases it via the reduction of cortisone and 11-dehydrocorticosterone.

Exposure to proinflammatory stimuli such as TNF-α and IL-1β increases 11HSD1 in some cells, while inducing a decrease in 11HSD2 in others [7–11], though there is some controversy

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regarding intravascular inflammation [12]. These findings led to the hypothesis that changes in 11HSD activity induced by proinflammatory cytokines might contribute to the feedback regulation of inflammation [8,10,13]. This hypothesis is also supported by the observation that patients with inflammatory bowel diseases [14,15] and animals with experimental colitis [16–19] downregulate colonic 11HSD2 and upregulate 11HSD1. However, it is not currently known whether proinflammatory cytokines alter 11HSDs in the colon or whether inflammation modulates glucocorticoid metabolism in lymphoid organs. Only thermal stress has been recently shown to modulate 11HSD1 expression in thymus [20]. Our objectives in this study were therefore to determine the effect of TNF-α and IL-1β on colonic 11HSD1 and 2 and to investigate the effect of inflammation associated with colitis on 11HSD1 in the spleen and mesenteric lymph nodes (MLN), i.e. in secondary lymphoid organs that are known to express 11HSD1 [21,22].

2. Materials and methods

2.1. TNBS-induced colitis, colonic explants and preparation of cell suspensions

Colitis was induced in male Wistar rats (Inst. Physiol., Czech Acad. Sci., Prague) by the intracolonic administration of 2,4,6-trinitrobenzenesulfonic acid (TNBS) as described previously [19]. Control animals received physiological saline instead of TNBS. All rats were killed on day 7 after TNBS or saline administration. The colon, draining MLN, and spleen were harvested and used immediately for the measurement of enzyme activity and isolation of distinct cell types. Intraepithelial (IEL) or lamina propria leukocytes (LPLs) were isolated as described previously [19,23]. Splenic and MLN cell suspensions were prepared in RPMI 1640 medium by pressing the organs through nylon gauze (mesh size 45 μm). Erythrocytes were depleted from the spleen cell suspension by lysis in ACK buffer (150 mM NH_4Cl, 10 mM $KHCO_3$, 0.1 mM EDTA, pH 7.4) and the remaining cells were fractionated by passage through nylon wool (Kisker, Steifurt, Germany) column according to Hathcock [24]. Briefly, after 45 min incubation of the column containing cell suspension at 37 °C, the T-cell-enriched fraction of splenocytes (STF) was collected by allowing the medium with the non-adherent, effluent cells to drain completely. The column was then eluted by warm RPMI 1640 three times to remove remaining non-adherent cells, the wool was placed in cold RPMI 1640, loosed and the adherent cells were released by gentle shaking. The cell suspension was centrifuged and the B-cell-enriched fraction of splenocytes (SBF) was separated from non-lymphoidal cells by a discontinuous Histopaque 1077 (Sigma) gradient [25]. In some experiments the lymph nodes (LNs) of thoracic group were also harvested (a mix of mediastinal LNs, tracheobronchal LNs and superficial LNs of neck region).

Explants of Wistar rat colons were performed as described previously [26]. Briefly, the healthy animals were decapitated, the distal colon removed aseptically and maintained in Dulbecco's modified Eagle's medium, treated with TNF-α (10 ng/ml) or IL-1β (10 ng/ml) for 24 or 48 h and then total RNA was extracted. The values of measured transcripts were not significantly different between explants cultured in vitro for 24 or 48 h and hence the data of 24- and 48-h incubations were pooled for further statistical analysis.

2.2. Immunofluorescence and flow cytometry

In order to characterize the cells of isolates prepared from colon, spleen and MLN we performed phenotypic analysis of the isolated leukocytes by flow cytometry. Freshly isolated cells resuspended in buffer containing 1× PBS/0.1% gelatin (Sigma)/0.01% sodium azide (Sigma) were placed into 96-well plates and stained by antibody dye mix in the dark for 20 min at 4 °C. Monoclonal antibodies conjugated to different fluorochromes were used. Leukocytes were stained with anti-rat CD45-Alexa Fluor 488 (clone OX-1, BioLegend, San Diego, CA, USA), macrophages with anti-rat macrophage marker-phycoerythrin (clone HIS36, eBioscience, San Diego, CA, USA) and granulocytes with anti-rat granulocyte marker-biotin (clone HIS48, eBioscience, San Diego, CA, USA) antibodies. B cells, T cells and T_H and T_C subsets of T cells were stained with anti-rat CD45RA-fluorescein isothiocyanate (clone OX-33, BD PharMingen, San Diego, CA, USA), anti-rat CD3-biotin (clone G4.18, BD PharMingen), anti-rat CD4-phycoerythrin-Cy5 (clone OX-35, BD PharMingen), and anti-rat CD8a-phycoerythrin (clone OX-8, BD PharMingen) antibodies. Lymphocytes of the NK cell lineage were labeled via their triggering receptor NKR-P1 with anti-rat CD161-fluorescein isothiocyanate (clone 10/78, BioLegend). To detect biotinylated antibody Qdot 605 Streptavidin (Invitrogen, Carlsbad, CA, USA) was used for the secondary detection. The stained cells were washed twice with PBS buffer, resuspended and applied to LSRII cytometer (BD Immunocytometry Systems, San Jose, CA, USA) equipped with 4 lasers (405, 488, 561, 633 nm), HTS loader and FlowJo 9.2. (Tree Star Inc., Ashland, OR, USA). The results represent the percentage of the positively stained cells in the total cell population.

2.3. RNA extraction, reverse transcription, and quantitative real-time PCR

Total RNA was extracted and mRNA levels were analyzed as described previously [27,28]. Briefly, to determine the expression of 11HSD1, 11HSD2, TNF-α, IL-1β, cyclooxygenase 2 (COX-2) and the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) TaqMan Gene Expression Assays were used and the transcripts quantified on an ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Foster City, USA). Target gene probes labeled with FAM/TAMRA were used in conjunction with GAPDH probe (VIC/MGB) as the normalization factor. Gene-specific calibration curves were generated from serial dilutions of standard cDNA and the mRNA levels of all genes of interest were normalized to the reference gene (GAPDH).

2.4. Measurement of 11HSD1 activity

11HSD1 reductase activity was measured in homogenates of MLN and spleen. The tissue was homogenized in an ice-cold buffer containing 200 mM sucrose and 10 mM TRIS/HCl (pH 8.5) (1:9 w/v) in a Polytron homogenizer (Kinematica AG, Littau, Switzerland). Homogenates were centrifuged at 400 × g for 10 min at 4 °C to remove cell debris and the supernatant was used for further analysis. 11HSD1 was measured via a radiometric conversion assay of $[^3H]$11-dehydrocorticosterone to $[^3H]$corticosterone and the steroids were quantified by HPLC [27]. Briefly, 11HSD1 activity was measured in incubation buffer containing 50 mM TRIS, 100 mM KCl, 0.8 mM NADPH, 1 mM glucose-6-phosphate and 2 U glucose-6-phosphate dehydrogenase in the presence of Complete Protease Inhibitor Cocktail (Roche) (pH 8.5). The reaction was initiated by the addition of 11-dehydrocorticosterone and $[^3H]$11-dehydrocorticosterone to reach the final concentration of 65 nM. Based on preliminary studies designed to determine appropriate protein concentration and the linear reaction velocity of 11-oxoreductase activity, the enzyme assays were performed using 2 (MLN) or 6 mg (spleen) of sample protein incubated for 2 or 1 h, respectively, at 37 °C in 1 ml of final volume.

Table 1
Phenotype comparison of cell populations isolated from control and TNBS-treated rats.

Antigen detected	Treatment	LPL	IEL	MLN	STF	SBF
% of cells						
$CD45^+$	CTRL	81 ± 2	79 ± 9	97 ± 1	98 ± 1	97 ± 1
	TNBS	83 ± 5	73 ± 6	98 ± 0	87 ± 5	97 ± 0
$HIS36^+$	CTRL	4 ± 1	1 ± 0	0 ± 0	1 ± 0	2 ± 0
	TNBS	1 ± 0	1 ± 0	0 ± 0	1 ± 0	2 ± 1
$HIS48^+$	CTRL	5 ± 1	12 ± 4	1 ± 0	1 ± 0	4 ± 1
	TNBS	15 ± 6*	23 ± 15	1 ± 0	3 ± 1	8 ± 2
% of lymphocytes						
$CD3^+$	CTRL	25 ± 2	22 ± 6	67 ± 3	67 ± 4	23 ± 2
	TNBS	50 ± 6**	19 ± 5	60 ± 6	67 ± 2	19 ± 2
$CD3^-CD45RA^+$	CTRL	45 ± 1	45 ± 0	23 ± 1	13 ± 3	59 ± 2
	TNBS	26 ± 3**	30 ± 3*	22 ± 1	17 ± 2	62 ± 4
$CD3^-CD45RA^-$	CTRL	28 ± 1	42 ± 3	6 ± 2	12 ± 2	14 ± 2
	TNBS	26 ± 5	43 ± 3	13 ± 6	13 ± 2	17 ± 2
$CD161^+$	CTRL	1 ± 0	1 ± 0	0 ± 0	5 ± 2	4 ± 1
	TNBS	2 ± 1	1 ± 0	0 ± 0	4 ± 1	5 ± 2
% of T cells						
$CD3^+CD4^+$	CTRL	84 ± 1	82 ± 2	72 ± 2	65 ± 4	63 ± 1
	TNBS	64 ± 4**	75 ± 5	76 ± 5	58 ± 2	67 ± 5
$CD3^+CD8^+$	CTRL	13 ± 0	14 ± 2	25 ± 1	31 ± 4	33 ± 1
	TNBS	30 ± 4**	23 ± 4	21 ± 5	38 ± 2	28 ± 2

Data are given as means ± SEM of 3 independent experiments. CTRL, control untreated rats; TNBS, rats with TNBS-colitis; LPLs, lamina propria leukocytes; IELs, intraepithelial leukocytes; MLN, mobile cells of mesenteric lymph nodes; STF, T-cell-enriched fraction of splenocytes; SBF, B-cell-enriched fraction of splenocytes. Each value is shown as the percentage of positive fluorescence. Differences from control cell populations are indicated by asterisk.

* $P < 0.05$.

** $P < 0.01$.

2.5. Immunohistochemistry

Rat colon specimens were fixed in 10% formaldehyde, embedded in paraffin and cut into 5-μm sections. The formalin-fixed paraffin-processed tissue sections were dewaxed in xylene and rehydrated. The endogenous peroxidase activity was inhibited by treatment with 0.3% hydrogen peroxide in methanol for 30 min. To unmask the antigen sites, the sections were treated with Target Retrieval Solution (Dako, Glostrup, Denmark) and then the rabbit polyclonal antibody to 11HSD1 (Cayman Chemical, Ann Arbor, MI, USA) was applied at a dilution of 1:100 for 1 h at room temperature. To determine the specificity of the primary antibody, the 11HSD1 blocking peptide composed of amino acids 78–92 (Cayman Chemical) was used in a ratio 1:1. The bound primary antibody was detected using EnVision+ System (Dako, Glostrup, Denmark) applied for 30 min and the brown color reaction was developed by incubation the sections with diaminobenzidine for 5 min. Finally, the sections were counterstained with hematoxylin, dehydrated and mounted. Rat liver samples containing high levels of 11HSD1 were used as positive controls; tissue sections incubated without primary antibody represented the negative controls.

2.6. Statistical analysis

Data were expressed as means ± SEM and analyzed using ANOVA followed by post hoc Newman–Keuls test using the package Statistica v.6 (StatSoft Inc., Tulsa, OK). Statistical significance was assessed at $P < 0.05$.

3. Results

3.1. Phenotype analysis of isolated cells

Phenotype of freshly isolated cells was determined using flow cytometry. To determine the phenotype of these cells, an aliquot of each sample was stained with anti-CD45, a pan-leukocyte marker, and analyzed by FACS (Table 1). With the exception of IEL the isolates contained leukocytes in the range of 81% (LPL isolate) to 98% (MLN cells). The mean percentage of macrophages was very low in all isolates, only the percentage of granulocytes was significantly increased in LPL prepared from TNBS-treated rats. In MLN cell suspension, the majority of cells represented T cells ($CD3^+$), whereas other phenotypes were found less and TNBS treatment did not change the cell population profile (Table 1). In the contrary to MLN, the isolates of LPL and IEL showed higher percentage of total B cells ($CD45RA^+$) and less of T cells. Within the T cell populations of LPL, IEL, MLN and splenocytes the majority of cells represented T helpers (T_H; $CD3^+CD4^+$) whereas T suppressor/cytotoxic (T_C; $CD3^+CD8^+$) cells were less frequent. The ratio of T_H and T_C cells was approximately 2:1 in isolated splenocytes and 3:1 in MLN and IEL and was not changed in TNBS-treated rats. In contrast, this treatment resulted in the pronounced decrease of T_H and increase of T_C cells in LPL whereas in IEL this decrease did not reach significance. Using CD191 antibody, NK cells were identified in very small numbers in all isolates. The analysis of IEL and LPL also revealed large population of $CD3^-CD45RA^-$ lymphocytes (Table 1). A small proportion of these cells were NK cells but the remainder was probably the undefined population of lymphocyte precursors that have not yet acquired marker characteristics of mature cells.

3.2. Colitis is associated with upregulation of 11-reductase activity and 11HSD1 mRNA in MLN and spleen

Inflammation is known to modulate 11HSDs [28]. Since TNBS-colitis was previously found to upregulate 11HSD1 activity and mRNA expression in rat colon [15,18], we initially examined whether colitis also modulates 11HSD1 in lymphoid organs. As summarized in Fig. 1 inflammation significantly upregulated 11-reductase activity. To investigate the differences between individual cell types, we separated the cells and measured the levels of 11HSD1 transcript. 11HSD1 mRNA transcript was found in both splenic stroma and STF fraction of splenocytes that contained almost 70% of T cells and only 13–17% of B cells. In this fraction but not in splenic stroma colitis significantly elevated 11HSD1 (Fig. 2). Trace levels of 11HSD1 transcript were observed also in some samples of B-cell-enriched fraction of splenocytes (SBF)

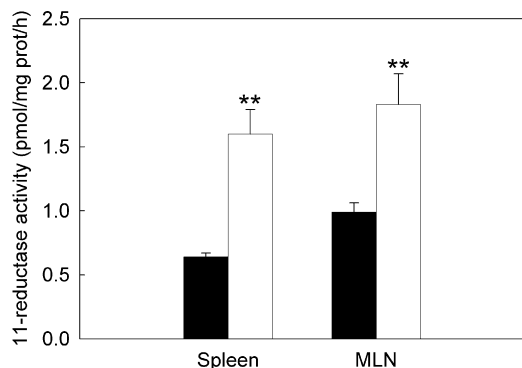

Fig. 1. Effect of colitis on 11-reductase activity in spleen and mesenteric lymph nodes (MLN). The bars represent control healthy rats (*filled bars*) and rats with TNBS-colitis (*open bars*). Results are presented as means ± SEM ($n = 15$). Statistically significant difference: $^{**}P < 0.01$.

but most of them had CT out of the dynamic range of 11HSD1-specific calibration curve and were below the lower detection limit. It seems therefore that the trace levels of 11HSD1 might indicate a contamination of SBF with T cells. Similar to spleen, colitis upregulated 11HSD1 expression also in the mobile but not stromal lymphoid cells of MLN (Fig. 2). In contrast, the effect of colitis was not found in thoracic lymphatic nodes (CTRL: 100 ± 6% (6) vs. TNBS: 112 ± 10% (6), NS). Taken together, these findings indicate that inflammation might increase the production of corticosterone from 11-dehydrocorticosterone not only in the inflamed colon as described earlier, but also in some lymphoid tissues.

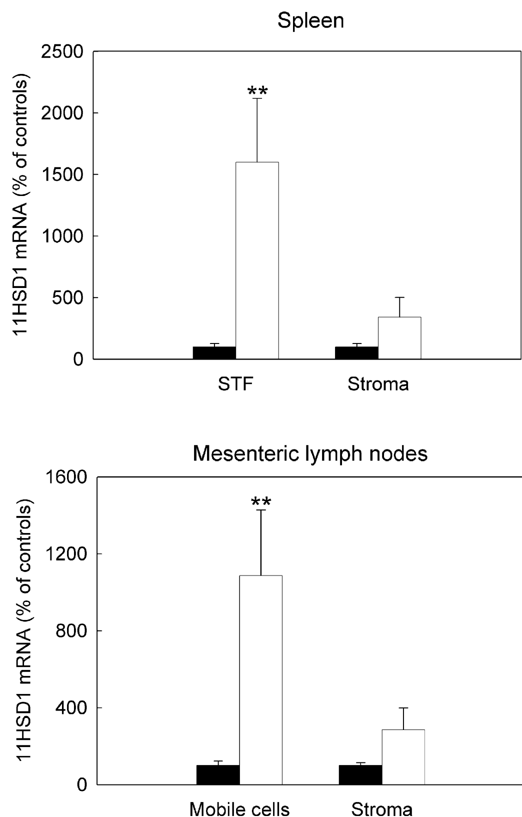

Fig. 2. Effect of colitis on expression of 11HSD1 in spleen and mesenteric lymph nodes. The bars represent control healthy rats (*filled bars*) and rats with TNBS-colitis (*open bars*); STF, T-cell-enriched fraction of splenocytes. The mean ratio of 11HSD1 mRNA/GAPDH mRNA reached in STF and MLN stroma of control animals the value 0.005 and in mobile MLN cells 0.008, respectively. Results are presented as means ± SEM ($n = 5$–9). Statistically significant difference: $^{**}P < 0.01$.

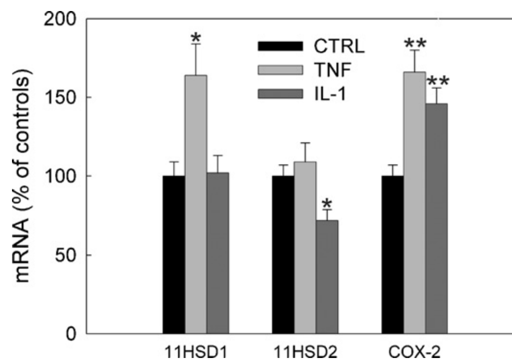

Fig. 3. Expression of 11HSD1, 11HSD2 and cyclooxygenase-2 in rat colonic explant culture system. CTRL, untreated control tissue; TNF, tumor necrosis factor α-treated tissue; IL-1, interleukin 1β-treated tissue; COX-2, cyclooxygenase-2. Results are presented as means ± SEM (number of dishes: 46 in controls and 20–25 in cytokine treated tissues), statistically significant difference: $^{*}P < 0.05$; $^{**}P < 0.01$.

3.3. TNF-α and IL-1β modulate expression of colonic 11HSDs

To assess whether proinflammatory cytokines upregulate the expression of 11HSD1 and downregulate the expression of 11HSD2 in the colon similar to colitis [16,19], colonic explant cultures were incubated in the presence of TNF-α or IL-1β. Treatment with TNF-α caused a significant increase in the level of 11HSD1 mRNA expression but had no effect on 11HSD2 mRNA. In contrast, IL-1β suppressed 11HSD2 mRNA without any significant effect on 11HSD1 mRNA (Fig. 3). To eliminate the possibility that TNF-α and IL-1β did not activate proinflammatory processes in colonic explants, the expression of COX-2 was also measured. As shown in Fig. 3, COX-2 mRNA levels were increased in the presence of both TNF-α and IL-1β.

3.4. Colitis increases mRNA expression of 11HSD1 together with proinflammatory genes in colonic leukocytes and MLNs

Since colitis is characterized by mucosal injury, an infiltration of immune cells and increased production of proinflammatory cytokines, we next examined whether there is any correlation between the mRNA expression of proinflammatory genes and 11HSD1. The results of RT-PCR showed that the mRNA expression of TNF-α and IL-1β, well characterized pivotal proinflammatory cytokines, was significantly increased in both LPLs (not shown) and IELs similar to COX-2 mRNA. As shown in Fig. 4, inflammation increased the expression of TNF-α sixfold, IL-1β thirtyfold and COX-2 sevenfold, respectively. Compared with these cells, the MLNs had a more upregulated expression of TNF-α mRNA and COX-2 mRNA, but there was no difference in the level of IL-1β transcript between healthy rats and animals with colitis (Fig. 4). The upregulated expression of markers of inflammation was accompanied by an increased expression of 11HSD1. Fig. 5 shows a strong effect of colitis on 11HSD1 in IEL and particularly in LPLs. 11HSD1 mRNA in MLNs was also increased, but remained at a lower level than in IELs and LPLs, which may indicate that the cells of the effector regions of the mucosal immune system [30] exhibit a higher activation of the glucocorticoid regenerating system.

3.5. Immunohistochemistry of colonic 11HSD1 in control and TNBS-treated rats

The cellular distribution of 11HSD1 within numerous rat tissues, including stomach, has been described previously [31,32] but its immunohistochemical detection has not been reported for colon. We used therefore rabbit polyclonal antibody to detect 11HSD1 specific immunostaining in colon of control and TNBS-treated rats.

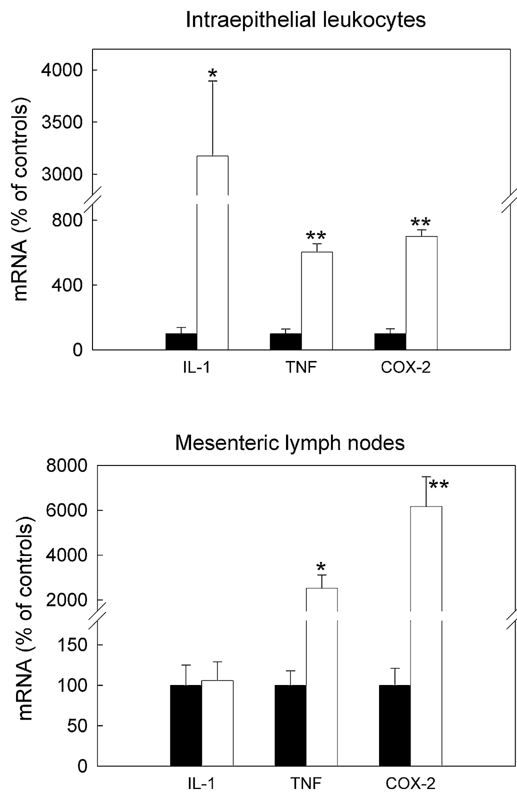

Fig. 4. Expression of proinflammatory markers in intraepithelial leukocytes and mesenteric lymph nodes. Colitis upregulated the transcripts for interleukin 1β (IL-1), tumor necrosis factor α (TNF) and cyclooxygenase-2 (COX-2). The bars represent control healthy rats (*filled bars*) and rats with TNBS-colitis (*open bars*). Results are presented as means ± SEM (n = 9–12). Statistically significant difference: $^{*}P < 0.05$; $^{**}P < 0.01$.

Typical immunohistochemical staining of 11HSD1 is shown in photomicrographs on Fig. 6. In normal liver tissue, used as a positive control, we observed a diffuse cytoplasmic immunopositivity with intense staining concentrated around the central vein (Fig. 6A). This staining pattern was absent if the primary antibody was preabsorbed with the blocking peptide (Fig. 6B). Immunostaining in colon was localized to both epithelial and mesenchymal tissue (Fig. 6C and E). In case that blocking peptide was used, the granular cytoplasmic positivity of the epithelium as well as mesenchymal positivity was almost completely abrogated and only irregularly distributed nonspecific nuclear immunostaining along the crypt

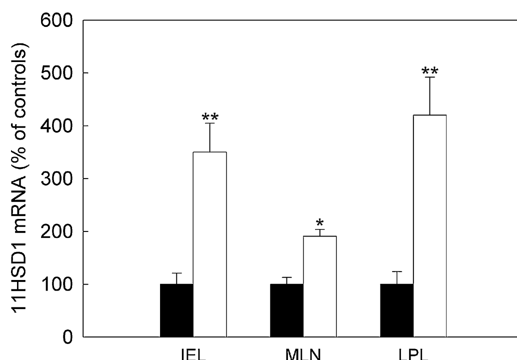

Fig. 5. Effect of colitis on expression of 11HSD1 in mesenteric lymph nodes and intraepithelial and lamina propria leukocytes. The bars represent control healthy rats (*filled bars*) and rats with TNBS-colitis (*open bars*). IELs, intraepithelial leukocytes; MLN, mesenteric lymph nodes; LPLs, lamina propria leukocytes. Results are presented as means ± SEM (n = 10–13). Statistically significant difference: $^{*}P < 0.05$; $^{**}P < 0.01$.

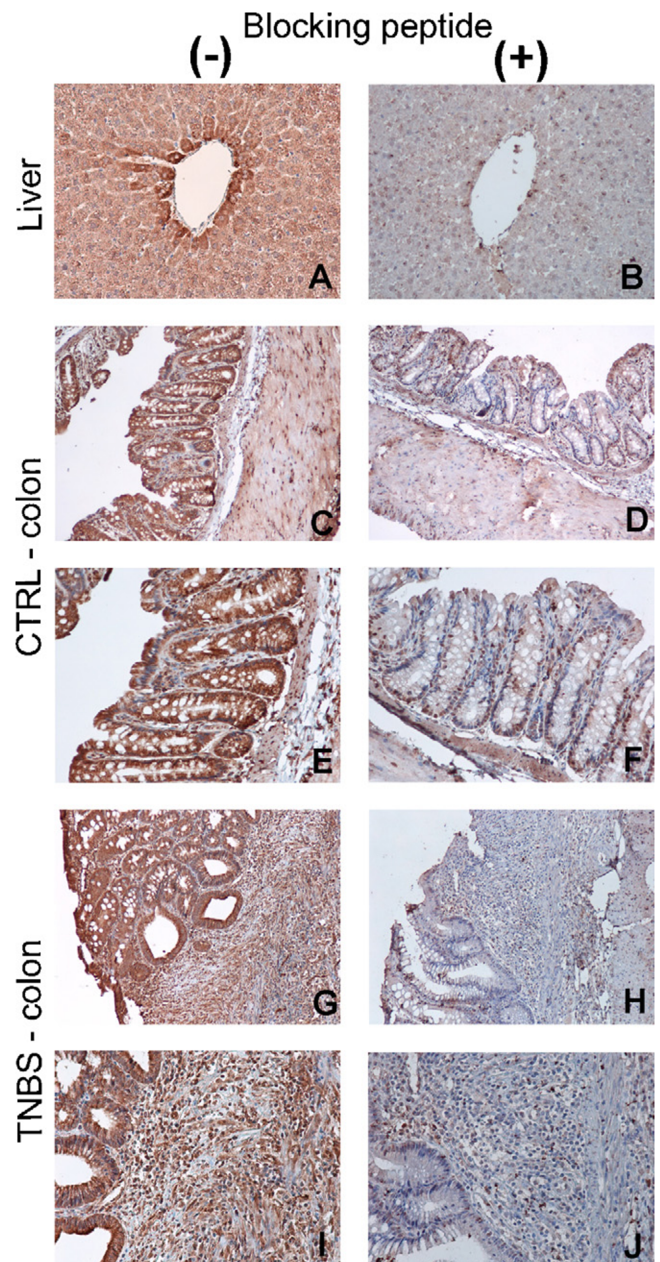

Fig. 6. Immunohistochemical detection of 11HSD1 in TNBS-colitis, normal colon and liver (positive control) with or without blocking peptide preincubation. (A and B) Liver sample with central vein region (×200); (C and D) normal colon tissue (×100), (E and F) normal colon epithelium (×200), (G and H) TNBS-colitis with ulcer margin (×100), (I and J) inflammatory infiltrate in TNBS-colitis (×200).

was visible (Fig. 6D and F). TNBS colitis was associated with strong immunopositivity not only in the regenerative epithelium, but also in reactive stromal cells and infiltrating immune cells (Fig. 6G and I). Staining was markedly decreased when primary antibody was incubated with blocking peptide (Fig. 6H and J). Although we have found immunoreactivity in colonic epithelium, it is not obvious whether this staining reflects 11HSD1 or false positive reactivity. First, the purified rat colonocytes (unpublished results) did not give positive signal for 11HSD1 mRNA. Second, Western blot stained with this polyclonal antibody gave not only the strong band of 11HSD1 protein detected in the expected molecular mass range of approximately 32 kDa but also one more band (∼140 kDa) whose origin is unknown (data not shown). Third, Whorwood et al. [33] localized NAD^{+}-dependent 11β-oxidase activity exclusively

to surface and crypt epithelial cells whereas $NADH^+$-dependent 11β-oxidase activity localized to nonepithelial cells in the lamina propria.

4. Discussion

Glucocorticoids are powerful modulators of inflammatory processes [1] whose overall effect on immune response may not only result from circulating glucocorticoids via the HPA axis, but also via the tissue-specific metabolism of glucocorticoids catalyzed by the enzymes 11HSD1 and 11HSD2. Data presented here suggest that 11HSD1 is likely to play an important role in the inflammation-associated regulation of immune cells. We have now demonstrated that inflammation increases 11HSD1 mRNA expression and 11-reductase activity in both lymphoid organs, particularly in their mobile cells, and in colonic lymphocytes. Inductive sites for mucosal immunity consist of mucosa-associated lymphoid tissue and the draining lymph nodes, whereas the effector histological compartments predominantly consist of the lamina propria and intraepithelial immune cells [30]. Our results therefore demonstrate that inflammation enhances 11HSD1 not only in effector but also in inductive sites of lymphoid tissue in which the triggering of naive immune cells and the generation of memory-effector cells take place, such as in MLNs. Elevated expression of TNF-α, IL-1β and COX-2 indicates an increased inflammatory state in the MLNs of TNBS-treated rats. Moreover, we also observed the upregulation of 11HSD1 in the T-cell-enriched fraction of splenocytes of TNBS-treated rats, whose splenic T-lymphocytes have been shown to induce colitis upon transfer to a naive recipient [34]. This upregulation of 11HSD1 is analogous to that in a previous study by Zhang et al. [22], showing a marked activation of 11HSD1 in splenic and lymph node $CD4^+$ T lymphocytes in vitro. Similarly, the activation of dendritic cells by bacterial lipopolysaccharide, a strong innate immunity activating signal, is associated with the upregulation of 11HSD1 mRNA expression and activity [35]. These findings support the hypothesis that inflammation provides lymphoid organs and immune cells with a higher capacity to reactivate active glucocorticoids and that 11HSD1 might represent a regulator of lymphocyte activity. Indeed, a direct correlation was found between 11HSD activity and the production of type 1 cytokines by T cells residing within certain lymphoid organs [21,22]. Moreover, two recent studies also showed that 11HSD1-mediated intracellular glucocorticoid amplification in activated macrophages modulates their TNF-α and IL-1β expression [36] and phagocytic activity [37]. The cell sub-population-associated increase of 11HSD1 in lymphoid organs during inflammation may somehow be typical for these organs, because arthritis has been shown recently to be also associated with elevated 11HSD1 in the mobile but not stromal cells of draining lymph nodes [28].

The mechanism by which colitis and other inflammatory diseases upregulate 11HSD1 has not yet been elucidated. Numerous in vitro studies have shown that cytokines are potent inducers of 11HSD1 in various cell culture systems [7–11,34] but not in all of them [12]. Similarly, our data indicate, that not only the colon [19] but also the MLNs and isolated colonic leukocytes (Fig. 3) of TNBS-treated rats exhibit an upregulation of TNF-α and IL-1β. In addition, we have shown that proinflammatory cytokines increase 11HSD1 and decrease 11HSD2 in colonic explants, and that these results are consistent with the data obtained in the inflamed colonic tissue of TNBS-treated rats [16,19]. The difference between 11HSD1 and 11HSD2 cannot be explained by the loss of epithelial cells during colitis, even if 11HSD2 is expressed predominantly in colonocytes [33], since the downregulation of 11HSD2 was not only observed in the inflamed colon of TNBS-treated rats, but also in explant cultures. In addition, the downregulation of 11HSD2 by TNF-α was recently described in colon adenocarcinoma cells [39]. The mechanism through which TNF-α and IL-1β affect 11HSD remains unknown. Only the recent data of Ignatova et al. [38] indicate the role of p38 MAPK-mediated regulation of 11HSD1 in the presence of TNF-α. Relatively little is known about 11HSD1, particularly in immune cells [29], but it is conceivable that cytokines modulate 11HSD1 transcription. In airway smooth muscle, IL-13 caused the upregulation of 11HSD1 via the activation of AP-1 coupled to MAPK signaling via the ERK1/2 and JNK pathways [40].

In conclusion, we propose that the inflammatory stress induced by colitis is associated with an increased basal availability of biologically inactive 11-dehydrocorticosterone to be converted to active corticosterone in lymphoid tissues and cells and that proinflammatory cytokines play a role in this process.

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