First publ. in: Allergy ; 67 (2012), 2. - pp. 227-234

http://dx.doi.org/10.1111/j.1398-9995.2011.02749.x

# Local glucocorticoid production in the mouse lung is induced by immune cell stimulation

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**To cite this article:** Hostettler N, Bianchi P, Gennari-Moser C, Kassahn D, Schoonjans K, Corazza N, Brunner T. Local glucocorticoid production in the mouse lung is induced by immune cell stimulation.

**Keywords**
11beta-Hsd1; asthma; glucocorticoids; inflammation; lung.

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## Abstract

**Background:** Glucocorticoids (GC) are potent anti-inflammatory and immunosuppressive steroid hormones, mainly produced by the adrenal glands. However, increasing evidence supports the idea of additional extra-adrenal sources of bioactive GC. The lung epithelium is constantly exposed to a plethora of antigenic stimuli, and local GC synthesis could contribute to limit uncontrolled immune reactions and tissue damage.
**Methods:** Expression of steroidogenic enzymes and GC synthesis in *ex vivo* organ cultures was studied in mouse lung tissue after *in vivo* stimulation of immune cells.
**Results:** Mouse lung tissue was found to express steroidogenic enzymes required for the synthesis of corticosterone from cholesterol and to synthesize corticosterone in large quantities after immune cell activation by anti-CD3 antibody, lipopolysaccharide, or TNFα. In marked contrast, ovalbumin-induced allergic airway inflammation failed to promote lung GC synthesis. Although the lung expresses all steroidogenic enzymes necessary for *de novo* synthesis of corticosterone from cholesterol, functional data indicated that inactive serum-derived dehydrocorticosterone is converted to active corticosterone by 11β-hydroxysteroid dehydrogenase 1.
**Conclusion:** Our results support the notion that local GC synthesis represents a novel immunoregulatory mechanism to limit uncontrolled immune responses in the lung and indicate that defective local steroidogenesis may contribute to the pathogenesis of allergic airway inflammation.

The extensive epithelial surface of the lung mucosa is in direct contact with the plethora of pathogens of outside world. This prerequisite for efficient gas exchange represents a critical site of infection, which requires a specialized immune system that efficiently combats harmful infections while being tolerogenic toward most innocuous inhaled particles. Lung epithelial cells themselves are linking the innate and adaptive immune systems and may either initiate or counterbalance uncontrolled immune reactions to minimize lung damage (1, 2). However, chronic immune cell stimulation by allergens may develop into a hyper-responsiveness with uncontrolled allergic lung inflammation.

Allergic asthma is a disease with increasing prevalence in the modern world that can be controlled by inhalative glucocorticoid therapy (3, 4). Glucocorticoids (GC) are potent anti-inflammatory steroid hormones, which are also produced endogenously in the adrenal cortex by a cascade of steroidogenic enzymes. In mice, the bioactive GC corticosterone is increasingly produced upon systemic stimulation by inflammatory cytokines, such as IL-1β, IL-6, and TNFα (5, 6). However, owing to differential exposure to inflammatory triggers, the requirement of immunoregulatory GC may strongly diverge in different tissues. Thus, regulation of local GC concentrations may be important for the maintenance of

**Abbreviations:**
BAL, bronchoalveolar lavage; GC, glucocorticoids; HPA, hypothalamus–pituitary–adrenal (axis); LPS, lipopolysaccharide; LRH-1, Liver receptor homolog-1, Nr5a2; OVA, ovalbumin; RIA, radioimmunoassay.

homeostasis. Although the adrenal glands are the predominant source of endogenous GC, there is accumulating evidence for GC synthesis at extra-adrenal sites, such as the brain, vascular tissue, intestine, skin, and thymus (7, 8). We could previously show that extra-adrenal GC synthesis in the intestine represents an important negative feedback mechanism on local immune cell activation (9, 10). Additionally, several organs can increase local active GC amounts via the expression of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), which is able to locally interconvert the inert serum metabolite dehydrocorticosterone into active corticosterone (11).

In this study, we analyzed the capability of the lung to locally produce GC in response to inflammatory stimuli. As the mucosal surface of the lung has much in common with the intestinal mucosa, it is tempting to speculate that the lung may similarly represent an extra-adrenal source of immunoregulatory steroids. Our results demonstrate for the first time that the lung is a potent source of corticosterone, which may have important implications in the local regulation of immune responses and airway hypersensitivity, such as allergies and asthma.

## Materials and methods

### Cell lines and culture conditions

The human embryonic kidney cell line HEK 293T (CRL-11268) and human lung adenocarcinoma cell line H2009 (CRL-5911) were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA) and cultured in complete Iscove's Modified Dulbecco's Medium (IMDM, Sigma-Aldrich, Buchs, Switzerland) as described before (10).

### Animals

C57Bl/6 and Balb/c mice were used in experiments between 6 and 12 weeks of age. TNFR1$^{-/-}$, TNFR2$^{-/-}$, TNFR1/2$^{-/-}$, and LRH1$^{+/-}$ mice were described elsewhere (12–14). Adrenalectomized C57Bl/6 mice, obtained from Harlan Laboratories (Host, the Netherlands), were used after a recovery period of 2 weeks, and drinking water was supplemented with 0.9% NaCl. All experiments were performed according to ethical guidelines of the Cantonal Veterinary Office of the State of Bern.

### Detection of gene expression by RT-PCR

RNA isolation, reverse transcription, and PCR were performed as previously described (9). The following primers were used for conventional PCR: Cyp11a1 forward 5′-CCAGGCCAACATTACCGAGAT-3′ and reverse 5′-GAC TTCAGCCCGCAGCAT-3′; Cyp21 forward 5′-AACCACTG GTCCATCCAAATCT-3′ and reverse 5′-TCTTCGTCTTTG CCATCCCT-3′; Hsd3b3 forward 5′-CATCGTGAAAGCAC ATGGTC-3′ and reverse 5′-ATACTGGGTTGGCTGTGGA G-3′; Cyp11b1 forward 5′-GCTGGGACAGTCCTCAATGT-3′ and reverse 5′-ACGTGGAAGGATTTCAGCAC-3′; Hsd11b1 forward 5′-CAGAAATGCTCCAGGGAAAGAA-3′ and reverse 5′- GCAGTCAATACCACATGGGC-3′; and Gapdh forward 5′-AGGTCGGTGTGAACGGATTTG-3′ and reverse 5′-TG TAGACCATGTAGTTGAGGTCA-3′. Annealing temperatures were 60°C for Cyp11a1 and Hsd3b3, 57°C for Cyp11b1, and 53°C for Hsd11b1, and touchdown PCR with temperatures between 72 and 57°C was performed for Cyp21 and Gapdh. Quantitative enzyme expression analysis and internal normalization with Gapdh were performed with commercial primers from Qiagen (Hombrechtikon, Switzerland) as previously described (15).

### Animal experiments

C57Bl/6 wild-type and gene-deficient mice were i.p. injected with either 20 μg of anti-CD3ε (clone 145-2c11), 100 μg of lipopolysaccharide (LPS) (from *Salmonella enterica* serotype enteridis, cell culture grade; Sigma-Aldrich), or 1–3 μg of TNFα (Peprotech, London, UK). After 3 h, mice were killed, and serum and organs were collected for further analysis.

### Lung *ex vivo* organ cultures and corticosterone radioimmunoassay

Lung *ex vivo* cultures were performed as previously described for intestinal tissue (16). Corticosterone from lung culture supernatant was measured by a previously described radioimmunoassay (RIA) (9). Glucocorticoid synthesis was defined as nanograms of corticosterone produced per gram tissue weight and calculated as the difference between samples cultured without and with metyrapone (metyrapone-blockable corticosterone synthesis) to correct for contamination with serum GC.

### Glucocorticoid bioassay

Glucocorticoids synthesis was additionally assessed with a GC receptor–mediated bioassay (10). Briefly, HEK 293T cells were transfected with a GC receptor SVGR1 expression construct and a luciferase reporter construct containing GC response elements GRE2tk-LUC and β-galactosidase for normalization. Luciferase activity was assessed from cells that were exposed to either corticosterone standard or lung culture supernatant.

### Allergic airway inflammation

To induce an allergic airway inflammation, Balb/c mice were sensitized on day 0 and day 7 by i.p. injection of 100 μg of chicken egg ovalbumin (OVA grade V; Sigma-Aldrich) and 2 mg of aluminum hydroxide (Alum; Sigma-Aldrich). On day 14, mice were challenged in an aerosol chamber with 3% OVA in PBS or PBS only for 30 min using a PariBoy SX Nebulisor (Pari, Sternberg, Germany). Twenty four hours after challenge, mice lungs were lavaged two times with 1 ml of PBS by an intratracheal venflon cannula (22G; Beckton Dickinson, Helsingborg, Sweden), and lavage cells were spun on glass slides and stained with Hemacolor (Merck, Darmstadt, Germany).

### Immunohistochemistry

Formalin-fixed and paraffin-embedded lung tissue sections were stained using a polyclonal anti-mouse 11β-HSD1 antibody (ab39364 1 : 600; Abcam, Cambridge, UK) or isotype control and a secondary biotinylated antibody. Vectastain ABC-kit (Vector Laboratories, Burlingham, CA, USA) was used to convert DAB (Sigma-Aldrich) into a colored product. Nuclei were counterstained by Meyer hemalum solution (Merck).

### Steroidogenic enzyme assays

Enzymatic activities of P450C11 or 11β-HSD1 in lung tissue were assessed by a previously described enzymatic conversion assay (17). Briefly, perfused lung pieces were put in culture supplemented with $^{3}$H-labeled 11-deoxycorticosterone or 11-dehydrocorticosterone (0.5 μCi; American Radiolabeled Chemicals, St Louis, MO, USA) and incubated up to 16 h. Extracted radioactive steroids were spotted on a silica gel thin-layer chromatography plate (Macherey-Nagel, Oensingen, Switzerland). Deoxycorticosterone was separated from its metabolites using dichloromethane/methanol/$H_2O$ (150 : 10 : 1), whereas dehydrocorticosterone conversion products were separated in chloroform/ethanol (9 : 1). Radioactivity of steroid spots was measured with a TRI-Carb 2800TR liquid scintillation analyzer (Perkin Elmer, Schwerzenbach, Switzerland).

### Luciferase reporter analysis

The murine Cyp11a1 promoter luciferase reporter construct (Cyp11a1-Luc) and expression plasmid for mouse LRH-1 have been described previously (18, 19). Nur77 mouse expression plasmid was provided by Barbara Osborne (University of Massachusetts, USA). H2009 cells were transiently transfected using Effectene transfection reagent (Qiagen), β-galactosidase transfection was used for internal normalization, and cells were either control-treated or stimulated by 3 ng/ml phorbol myristate acetate (PMA; Enzo Life Science, Lausen, Switzerland).

### Statistical Analysis

Student's *t*-test was performed on Prism5 software (GraphPad Software, La Jolla, CA, USA) to define significant differences between two experimental groups. A *P*-value of $<0.05$ was regarded significant and marked as ★.

## Results

### Steroidogenic enzymes are expressed in lung tissue

Glucocorticoids can be synthesized *de novo* from cholesterol via a cascade of steroidogenic enzymes or via reactivation from inactive serum dehydrocorticosterone by 11β-HSD1 (Fig. 1A). Here, we assessed the expression of the complete GC-synthesizing enzymatic machinery in the lung by conventional RT-PCR in comparison with the intestine, which was previously shown to express all steroidogenic enzymes necessary for *de novo* synthesis of corticosterone (Fig. 1B) (9). In contrast to the intestine, most enzymes were constitutively expressed in the lung, and only the rate-limiting enzyme P450scc, encoded by Cyp11a1, was induced upon immune cell activation, promoted by injection of an anti-CD3 antibody (Fig. 1B). These results were confirmed by quantitative RT-PCR analysis of the lung (Fig. 1C–F).

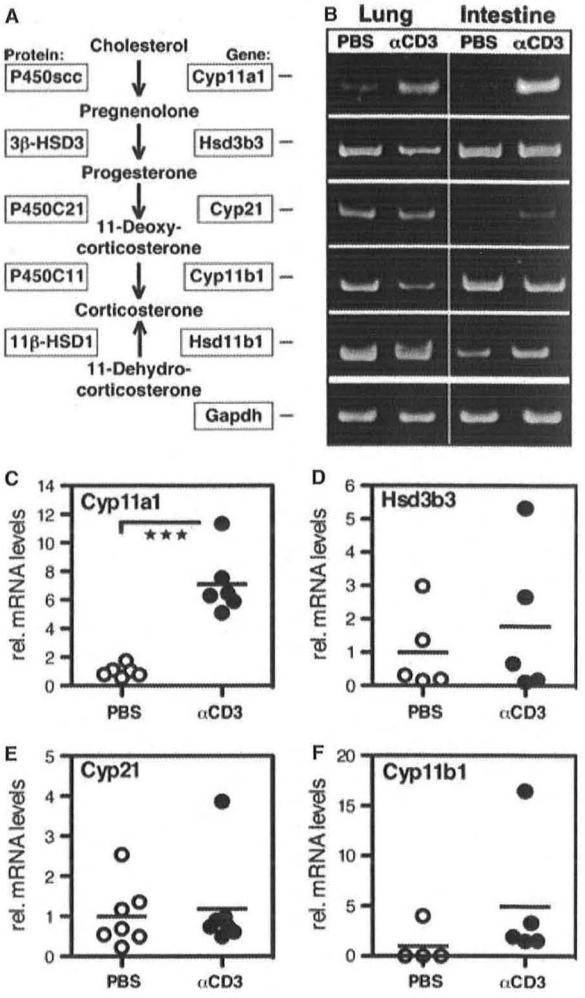


**Figure 1** Expression of steroidogenic enzymes in lung tissue. (A) Simplified scheme of the mouse glucocorticoids biosynthesis pathway. (B) Detection of steroidogenic enzyme gene expression by conventional RT-PCR in lung and intestinal tissue of PBS-injected mice and mice treated for 3 h with anti-CD3 (αCD3, 20 μg) antibody. Gapdh was used as loading control. (C–F) Changes in Cyp11a1 (C), Hsd3b3 (D), Cyp21 (E), and Cyp11b1 (F) in lung tissue from control or anti-CD3-treated mice were quantified by quantitative PCR. Relative expression levels were normalized to mean expression levels of control mice. Gapdh was used for internal normalization. Each dot represents expression values of one mouse; 4–7 mice were analyzed per group. $^{***}P < 0.005$.

### The lung produces bioactive corticosterone *in situ*

Expression of the complete steroidogenic cascade suggested that the lung is capable of secreting corticosterone to the supernatant of *ex vivo*-cultured lung tissue. Even under basal conditions, corticosterone released into the supernatant could be detected by radioimmunoassay (RIA), and GC synthesis was further enhanced after stimulation by i.p. injection with a T-cell-activating anti-CD3 antibody or macrophage-activating LPS (Fig. 2A,C). To discriminate between *in situ*-produced lung corticosterone and serum contamination, lung GC synthesis was blocked by metyrapone, an inhibitor of 11β-hydroxylase (P450C11), which also weakly inhibits the enzymes P450scc and 11β-HSD1 (20, 21). Consequently, the metyrapone-blockable fraction of detected GC represents bona fide *in situ* lung biosynthesis (Fig. 2B,D) and will be illustrated in subsequent figures as metyrapone-blockable GC.

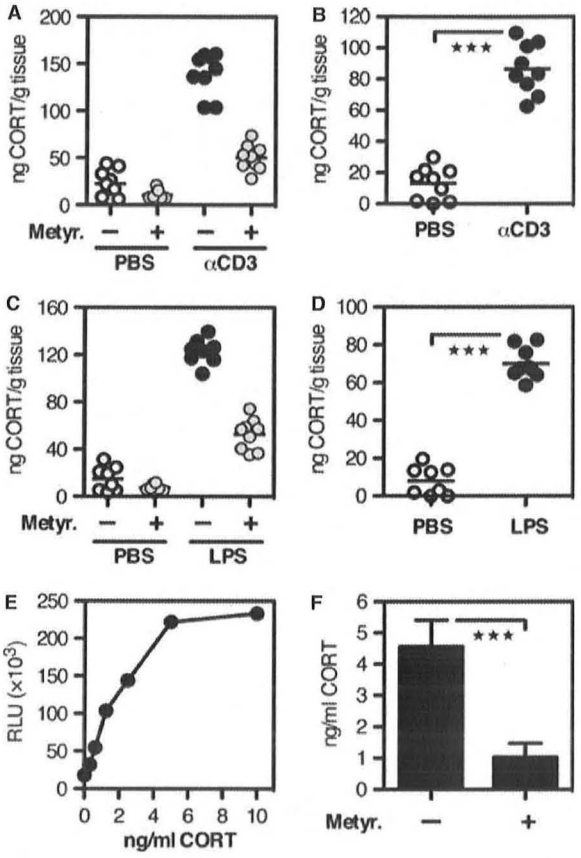


**Figure 2** *In situ* corticosterone synthesis in lung tissue. (A–D) Mice were i.p. injected with PBS control, anti-CD3 (αCD3, 20 μg) antibody, or lipopolysaccharide (LPS) (100 μg) for 3 h, lung tissue was cultured *ex vivo* in the presence or absence of metyrapone (Metyr., 250 μg/ml) for 5 h, and corticosterone (CORT) released into the supernatant was measured by RIA. (B, D) Illustration of metyrapone-blockable *in situ* lung corticosterone synthesis after anti-CD3 (B) or LPS injection (D) by subtraction of metyrapone-blocked values from unblocked values. Dots represent values of individual mice ($n$ = 9). (E–F) HEK-293T cells were transfected with GR and a luciferase reporter construct (GRE-LUC). Luciferase activity (RLU) was measured after cells have been stimulated with CORT (E) or lung culture supernatant from anti-CD3 stimulated mice in the presence or absence of metyrapone (F). Bars show mean values of six mice ± SD. ***$P < 0.005$.

Synthesis of bioactive corticosterone in the lung was confirmed in a GC bioassay. HEK 293T cells were transfected with GC receptor (GR) and a GR response element luciferase reporter (GRE-LUC). Addition of increasing concentrations of corticosterone (Fig. 2E) or lung culture supernatant (Fig. 2 F) to the reporter cells resulted in increased luciferase activity, which was abolished when lung tissue was cultured in the presence of metyrapone.

### Lung GC synthesis in a model of allergic airway inflammation

As our findings demonstrate that immune cell activation by anti-CD3 or LPS injection promotes local GC synthesis, we further aimed at investigating lung steroidogenesis during an antigen-dependent allergic airway inflammation. Because GC are well known for their therapeutic potential in inflammatory lung disease such as asthma, locally produced lung GC might act as a putative counter-regulatory mechanism. Figure 3A illustrates the protocol used to sensitize mice to OVA. Upon challenge with OVA aerosol, massive infiltration of neutrophils and eosinophils into the alveolar space and peribronchiolar tissue could be observed (Fig. 3C,E) in comparison with control-treated mice (Fig. 3B,D). Analysis of GC synthesis in lung tissue showed a peak induction at 24 h after OVA challenge (Fig. 3F), coinciding with maximal eosinophil infiltration. However, lung GC production remained rather at low levels, and no significant induction could be detected with larger numbers of mice analyzed (Fig. 3G). Similarly, only a slight increase in lung GC concentrations after OVA challenge could be detected in bronchoalveolar lavage (BAL) (Fig. S1A).

To investigate whether this lack of local GC induction was attributable to the genetic background of the mice used for OVA experiments (Balb/c), we compared lung GC synthesis of C57Bl/6 and Balb/c mice. Neither anti-CD3 injection nor OVA challenge resulted in significant differences in maximal lung corticosterone synthesis between the two mouse strains (Fig. S1B,C). As the adjuvant alum triggers a Th2 response with increased serum and BAL levels of IL-4 and IL-5 and unchanged levels of IFNγ (Fig. S2A–C), a shift toward a Th1 response might be more favorable to trigger lung GC synthesis (16). Therefore, C57Bl/6 mice were immunized with incomplete Freund adjuvant and CpG DNA, but no significant increase in OVA-induced lung GC synthesis could be observed (Fig. S1D). Thus, allergic airway inflammation fails to induce a comparable increase in lung steroidogenesis as seen after anti-CD3 or LPS stimulation, despite the presence of a strong inflammatory response.

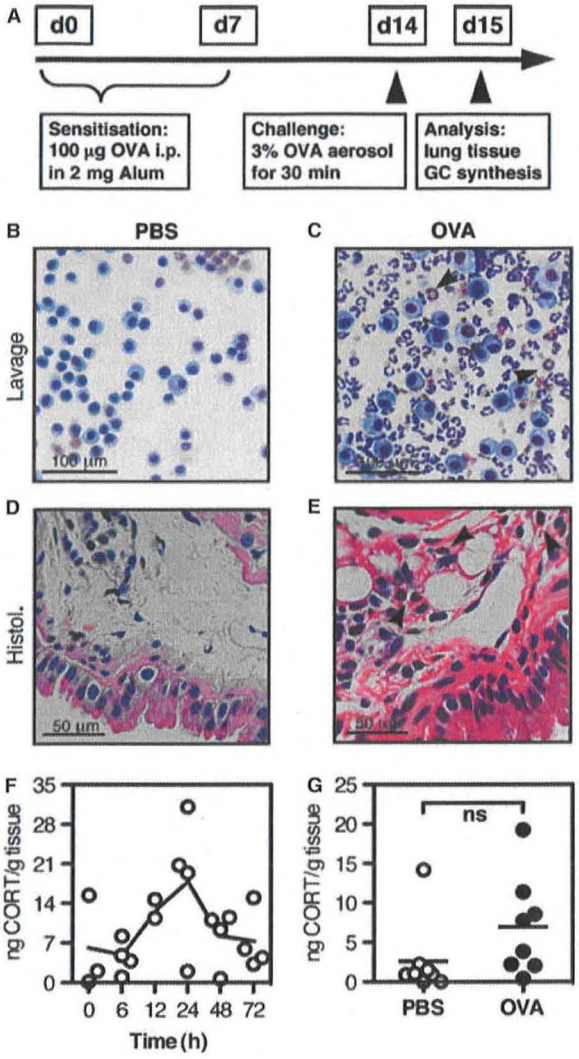


**Figure 3** Induction of lung glucocorticoids synthesis in a model of allergic airway inflammation. (A) A descriptive scheme for the induction of airway hypersensitivity to ovalbumin (OVA) antigen is shown. (B–C) Cytospin preparations of bronchoalveolar lavage cells from control (B) and OVA-sensitized (C) mice 24 h after aerosol challenge. Arrowheads indicate eosinophils. (D–E) H&E-stained lung tissue sections showing leukocyte infiltration in control-treated (D) and OVA-challenged (E) mice. (F) Detection of metyrapone-blockable corticosterone by RIA in the supernatant of *ex vivo* lung tissue culture at various time points after OVA challenge. Values of individual mice lungs are shown (2–4 per time point); mean values are connected by the black line. (G) Metyrapone-blockable lung corticosterone synthesis 24 h after OVA challenge compared to PBS controls. Each dot represents the measurement from one mouse ($n$ = 8).

### Role of TNFα in lung GC synthesis

The pro-inflammatory cytokine TNFα has been previously identified as a key inducer of intestinal steroidogenesis, as the absence of TNFα leads to a complete lack of intestinal GC synthesis and consequently to an exacerbation of experimental colitis (15, 16). Anti-CD3 and LPS injections both lead to a strong increase in serum TNFα levels, which declined rapidly 2 h after injection (Fig. S3A,B). In marked contrast and despite the strong inflammatory response, no increase in serum TNFα and only minimal levels of TNFα in BAL were seen in OVA-treated mice (Fig. S3C). To assess whether lack of TNFα production could explain differences in lung GC synthesis, we treated mice directly i.p. with TNFα. While TNFα increased dose-dependently serum GC levels (Fig. 4A), injection of TNFα promoted *in situ* lung GC synthesis even more strongly, comparable to levels observed after anti-CD3 injection (Fig. 4B). However, anti-CD3 injection into TNFR1- or TNFR2-deficient mice, or mice lacking both TNF receptors, did not result in reduced lung GC synthesis (Fig. 4C,D). Thus, while TNFα can induce lung GC synthesis, other factors might have redundant effects and compensate for missing TNFα signaling.

### Regulation of steroidogenic enzyme expression by LRH-1

The nuclear receptor LRH-1 is known to be critically involved in transcriptional regulation of intestinal steroidogenic enzymes

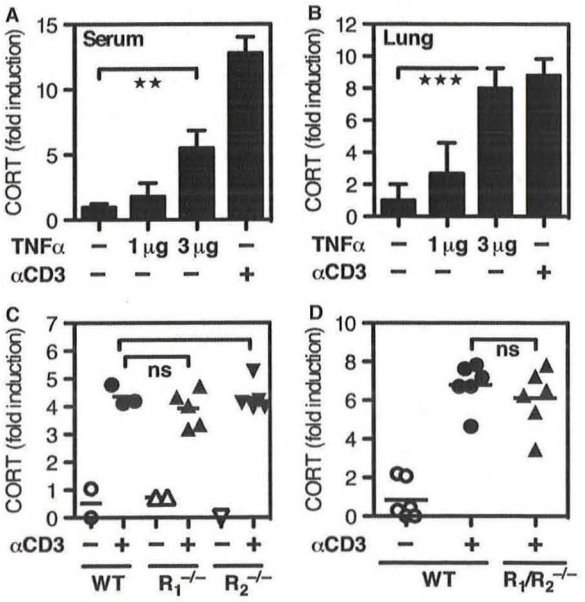


**Figure 4** TNFα is a redundant inducer of corticosterone synthesis in lung tissue. Mice were injected i.p. with 1 or 3 µg of mouse recombinant TNFα or 20 µg of anti-CD3 (αCD3). Corticosterone concentrations were measured by RIA in serum (A) or in *ex vivo* lung tissue cultures (B). Bars show mean values ± SD of $n$ = 5 mice for TNFα and $n$ = 3 mice for PBS and anti-CD3. (C–D) Wild-type, TNFR1$^{-/-}$ ($R_1^{-/-}$), TNFR2$^{-/-}$ ($R_2^{-/-}$), or TNFR1/2$^{-/-}$ ($R_1/R_2^{-/-}$) mice were injected with PBS control or anti-CD3 antibody, and metyrapone-blockable corticosterone synthesis in *ex vivo* lung tissue cultures was assessed. Dots represent values of individual mice, which are normalized to mean synthesis of untreated tissue. ns, not significant; $^{**}P < 0.01$; $^{***}P < 0.005$.

(14, 18). In comparison with intestinal mRNA expression, the lung expressed slightly reduced but comparable basal levels of LRH-1, which were further reduced in anti-CD3-treated mice (Fig. 5A). To investigate whether LRH-1 could be responsible for the increase in immune cell–induced Cyp11a1 expression (Fig. 1C), H2009 lung epithelial cells were transfected with LRH-1 expression plasmids and a Cyp11a1 promoter luciferase reporter construct (Fig. 5B). As reported earlier (22, 23), LRH-1 nicely induced Cyp11a1 promoter activity, which was further enhanced after LRH-1 activation by phorbol ester (PMA) (24). In contrast, transfection with the nuclear receptor Nur77 failed to induce Cyp11a1 promoter activity. Thus, as LRH-1 could represent a critical regulator of lung steroidogenesis, we further assessed lung GC synthesis in haploinsufficient LRH-$1^{+/-}$ mice previously shown to have impaired immune cell–induced intestinal steroidogenesis (18) (Fig. S4A,B). However, wild-type and LRH$1^{+/-}$ mice showed no differences in lung corticosterone synthesis or Cyp11a1 expression after anti-CD3 injection

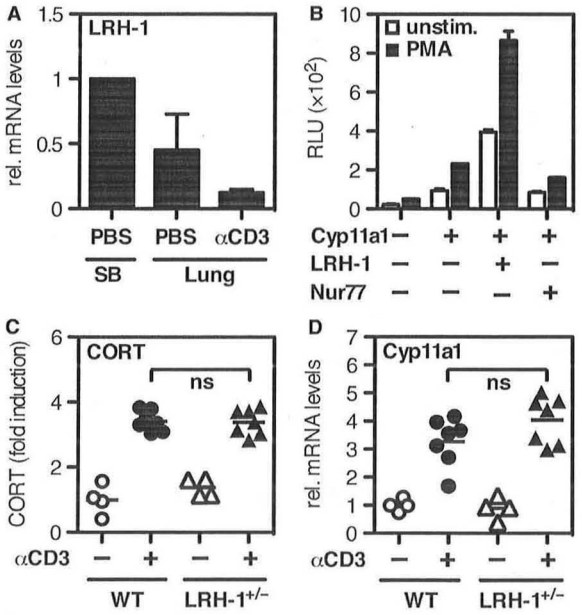


**Figure 5** The nuclear receptor LRH-1 is not required for the regulation of lung steroidogenesis. Relative expression levels of lung LRH-1 (A) in control and anti-CD3 (αCD3)-treated mice were determined by quantitative RT-PCR, in comparison with small bowel (SB). Bars show mean values of five samples ± SD. (B) H2009 cells were transfected with a Cyp11a1-LUC construct and expression plasmids for LRH-1 or Nur77 as negative control. Cells were either control-treated or stimulated by 3 ng/ml phorbol ester (PMA). Bars show mean values of four samples ± SD. (C–D) Wild-type (WT) and LRH-$1^{+/-}$ mice were injected i.p. with PBS control or anti-CD3, and metyrapone-blockable corticosterone synthesis in *ex vivo* lung cultures (C) and lung Cyp11a1 expression (D) were analyzed. Dots represent values of individual mice ($n$ = 4–7 per group). Values were normalized to mean basal values of wild-type mice. ns, not significant.

(Fig. 5C,D). Thus, extra-adrenal GC synthesis in the lung appears to be regulated differently than in the intestine and not to depend on LRH-1.

### Role of 11β-HSD1-dependent GC reactivation *vs de novo* synthesis

As LRH-1, which is a potent inducer of Cyp11a1 and Cyp11b1 expression (18), does not appear to play a role in lung GC synthesis (Fig. 5C,D) and Cyp11b1 expression is less inducible by inflammatory triggers than in the intestine (Fig. 1F) (15), lung *de novo* GC synthesis from cholesterol seems unlikely and rather 11β-HSD1-mediated reactivation of adrenal-derived dehydrocorticosterone might represent the relevant source of GC. To test the dependency of lung GC synthesis on adrenal metabolites, such as dehydrocorticosterone, we injected anti-CD3 into control or adrenalectomized animals. Surprisingly, adrenalectomy not only eliminated serum GC but also prevented anti-CD3-induced local lung GC synthesis (Fig. 6A,B). To further investigate the mechanism of GC synthesis, we assessed the conversion of the $^{3}$H-labeled precursors dehydrocorticosterone and deoxycorticosterone into $^{3}$H-corticosterone in *ex vivo* lung cultures of anti-CD3-stimulated mice using thin-layer chromatography (Fig. 6C,D). While deoxycorticosterone supplementation only resulted in an increase in aldosterone production but no P450C11-dependent corticosterone accumulation (Fig. 6C), dehydrocorticosterone was mainly metabolized into corticosterone (Fig. 6D). Further analysis of 11β-HSD1 protein expression by immunohistochemistry showed strong staining in lung epithelial cells of anti-CD3-injected mice in comparison with isotype control (Fig. 6 E,F). The predominant role of lung GC reactivation was further supported by much higher Hsd11b1 mRNA levels in the lung than in intestinal tissue (Fig. 6G). We conclude from these experiments that the lung produces increased levels of corticosterone after immune cell stimulation via an 11β-HSD1-mediated conversion of inactive serum dehydrocorticosterone.

## Discussion

Our data suggest that the lung senses inflammatory responses and induces local reactivation of active corticosterone to resolve inflammatory processes and limit tissue damage. It is feasible to believe that immune cell–induced local GC synthesis may be a common feature of mucosal surfaces to protect the vital functions of these tissues, as both lung and intestine represent extra-adrenal sources of GC (9, 15, 16). Nonetheless, mucosal tissues are also highly specialized with a characteristic set of immune cells and have evolved different strategies to maintain tissue homeostasis (25). Similarly, we could show substantial differences in lung and intestinal GC synthesis. Although the lung expresses the complete steroidogenic enzyme cascade for *de novo* synthesis, functional analyses revealed that the predominant GC synthesis pathway is LRH-1-independent reactivation of serum dehydrocorticosterone via 11β-HSD1, in contrast to the intestine.

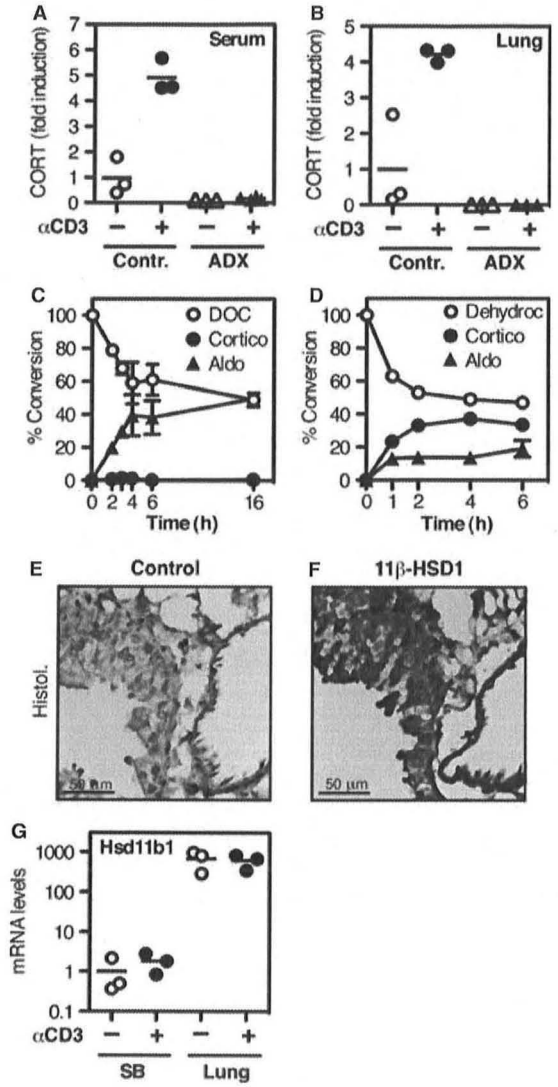


**Figure 6** Lung glucocorticoids synthesis is dependent on serum metabolites that can be reactivated by 11β-HSD1. (A–B) Control-treated and adrenalectomized (ADX) mice were injected with PBS or anti-CD3 antibody (αCD3), and serum (A) and metyrapone-blockable lung corticosterone (B) levels were measured by RIA. Values of individual mice normalized to mean levels of untreated control mice are shown, $n = 3$ per group. (C–D) Lung tissue of anti-CD3-stimulated mice was cultured for indicated time points with $^{3}$H-labeled deoxycorticosterone (DOC) (C) or $^{3}$H-dehydrocorticosterone (Dehydroc) (D), and steroid metabolites were separated by TLC. Values for deoxycorticosterone, dehydrocorticosterone, aldosterone, and corticosterone are indicated. Mean values of triplicated ± SD are shown. (E–F) Immunohistochemical analysis of 11β-HSD1 protein expression of lung tissue sections in comparison with isotype control. (G) Relative mRNA expression of Hsd11b1 in small bowel (SB) and lung tissue of control-treated and anti-CD3-injected mice, measured by quantitative PCR. Relative mRNA levels are shown as fold induced over basal SB expression levels. Mean (bar) and individual values are shown for three mice.

These findings correlate with previous reports where Hsd11b1 expression was detected shortly before the emergence of surfactants in the fetal lung as well as in adult lung tissue (26, 27).

Importantly, it has been reported that TNFα and other inflammatory cytokines, such as IL-1β, may induce Hsd11b1 expression (28). Because T-cell and macrophage activation both lead to the release of TNFα, but TNFR knockout mice showed no impaired lung GC synthesis, other factors might synergize in the induction of lung steroidogenesis. Although steroid regulatory mechanisms are highly complex and encompass innumerable factors, IL-1β represents a promising candidate for future analysis, as it often synergizes with TNFα and has been shown to play a central role in extra-adrenal GC synthesis in the human skin (29). Additionally, TNFα and IL-1β are both known inducers of systemic GC synthesis, and GC in turn regulate their expression in an efficient negative feedback network (6). On the other hand, there is little evidence that Th2-type cytokines are able to induce GC synthesis. In line with this notion, it was shown that a Th2 cytokine–mediated colitis failed to elicit local steroid production, whereas a Th1-type colitis strongly promoted intestinal GC synthesis (16).

Thus, during OVA-induced allergic airway inflammation, the predominant Th2 response may lack key signature cytokines to induce lung tissue steroidogenesis. As allergic diseases in humans are also characterized by a Th2 cytokine pattern, it is feasible to propose that defects in lung GC synthesis may contribute to the pathogenesis of asthma in human patients. In support of this idea is the observation that impaired production of adrenal GC is associated with increased allergic airway inflammation and susceptibility to asthma exacerbations (30). While we do not propose to use TNFα in the future to treat patients with asthma, specific activation of 11β-HSD1 to increase local GC concentrations and contain the inflammatory response may offer an attractive novel strategy for the treatment for allergic inflammatory disorders in the lung.

## Acknowledgments

We would like to thank Christoph Müller and Wilhelm Hofstetter for providing the TNFR1/2-deficient mice, Brigitte Frey, Mario Noti and Daniel Sidler for intellectual and technical advice, and Sam Okret for reagents. This work was supported by grants from the Julia and Gottfried Bangerter-Rhyner Foundation, the Swiss National Science Foundation, and AFF University of Konstanz to TB.

## Author contribution

NH performed most experiments and wrote the manuscript, PB contributed to experiments, CGM helped with the metabolic assays, DK contributed initial experiments, KS provided gene-deficient mice and helped with experiments, NC helped with the supervision of the study, and TB supervised the study and wrote the manuscript.

## Conflict of interest

The authors have no conflicting financial interests.

**Figure S1.** Further characterization of GC synthesis in OVA dependent airway hypersensitivity.

**Figure S2.** Th2 type cytokine pattern in serum and BAL after OVA challenge.

**Figure S3.** TNFα production in serum and BAL after immune cell stimulation by αCD3, LPS and OVA.

**Figure S4.** Steroidogenic enzyme expression in small intestinal tissue of LRH-1 heterozygous mice.

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