Interaction between glucocorticoids and Toll-like receptor 2: regulatory and cooperative effects of glucocorticoids on cutaneous cells

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by

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Abbreviation

TLR	Toll-like receptor
PRR	pathogen-recognition receptor
PAMP	pathogen associated molecular pattern
DAMP	danger (or damage) associated molecular pattern
IL	interleukin
LPS	lipopolysaccharide
CD36	a class B scavenger receptor
LTA	lipoteichoic acid
MALP-2	macrophage-activating lipopeptide 2
PGN	peptidoglycan
TIR	Toll/IL-1 receptor
MyD88	myeloid differentiation primary response gene 88
Mal	MyD88 adapter-like
TIRAP	TIR adaptor protein
IκB	inhibitor of kappa B
IKK	IκB kinase
IRAK 4	interlukin-1 receptor-associated kinase 4
TRAF6	tumor necrosis factor receptor-associated factor 6
NF-κB	nuclear factor-κB
MAPK	mitogen-activated protein kinase
TRIF	TIR-domain-containing adapter-inducing interferon-β
HEK	human embryonic kidney
NOD	nucleotide-binding oligomerization domain

Th	T helper cell
IFN	interferon
MLST	multilocus sequence typing
GC	glucocorticoid
HPA	hypothalamus-pituitary-adrenal
CRH	corticotropin-releasing hormone
CRH-R	CRH-receptor
POMC	proopiomelanocortin
ACTH	adrenocorticotropin
11βHSD	11β-hydroxysteroid dehydrogenase enzyme
MC	mineralocorticoid
GR	glucocorticoid receptor
Hsp	heat-shock protein
GRE	glucocorticoid response element
nGRE	negative GRE
MKP-1	MAPK phosphase-1
DUSP1	dual specificity protein phosphatase-1
GILZ	GC-induced leucine zipper
AP-1	activator protein-1
TTP	tristetrapolin
STAT	signal transducer and activator of transcription
SAA	serum amyloid A
NLRP3	NACHT, LRR and PYD domains-containing protein 3 inflammasome
THP-1	human monocytic cell line
RT4/31	bladder papilloma cells

KB	oral epidermal carcinoma cells
Hela	cervical carcinoma cells
ECV304	umbilical cord endothelial cells
MRC5	fetal lung fibroblasts
Dex	dexamethasone
Ref	reference
PBMC	peripheral blood mononuclear cells
FPRL1	formyl peptide receptor-like 1
P2X7	a purinergic receptor
HUVEC	human umbilical vein endothelial cells
G-CSF	granulocyte colony-stimulated factor
MCP-1	monocyte chemoattractant protein-1
MMP	matrix metalloproteinase
TNF	tumor necrosis factor
RA	rheumatoid arthritis
COPD	chronic obstructive pulmonary disease
IA	inflammatory arthritis
IBD	inflammatory bowel diseases
CAD	coronary artery disease
MOI	multiplicity of infection
SEAP	secreted embryonic alkaline phosphatase
qPCR	quantitative polymerase chain reaction
siRNA	small interfering ribonucleic acid

Contents

1. INTRODUCTION

1.1. Toll-like receptors

The discovery of Toll-like receptors (TLRs) in the mid-1990s changed the concept of innate immunity from simply an unsophisticated initiator of adaptive immunity into a specific system to detect foreign pathogens. As germline-encoded pathogen-recognition receptors (PRRs), TLRs detect pathogen-associated molecular patterns (PAMPs) from microorganisms and danger-associated molecular patterns (DAMPs) from damaged tissue [1]. To date, more than 10 members of the TLR family have been identified in mammals. TLR1-10 are conserved in humans and mice additionally express TLR11-13.

Before the discovery of TLRs, immunologists have focused on revealing the molecular basis for innate immunity. After the identification of the first relevant molecule interleukin (IL)-1 receptor in mammals and the cell surface protein Toll in *Drosophila melanogaster*, TLR4 was the first human TLR homologue being cloned and studied in great details. As the receptor for Gram-negative bacteria-derived lipopolysaccharide (LPS), TLR4 is found at both the plasma membrane and the endosomes. Other cell surface TLRs include TLR1, TLR2, TLR5, TLR6 and TLR11, whereas TLR3, TLR7-TLR9 and TLR13 localize to the endosomes. Once TLRs being identified, the corresponding ligands were soon resolved. The exogenous TLR ligands are derived from a wide range of microbes, including LPS, lipoproteins, flagellin and nucleic acids [2]. More recently, host-derived molecules, ranging from released intracellular proteins to inflammatory mediators and oxidatively modified lipids, were found to serve as endogenous ligands of TLR2 and TLR4 [3].

1.1.1. TLR2 heterodimers

This work focuses on TLR2, the gene of which was identified, molecular characterized and cloned in 1998 at the same time with TLR1 [4], and one year later TLR6 was discovered as a new member sharing 69% sequence homology to TLR1 [5]. TLR2 recognizes a wide spectrum of PAMPs derived from bacteria, fungi and viruses in the form of homodimers or heterodimers (*Table 1-1*).

Bacterial species, such as Gram-positive *Listeria monocytogenes* [6] and Gram-negative *Burkholderia pseudomallei* are sensed by the host via TLR2 [7]. Although TLR2 homodimers have been proposed, the existence has not been proven by crystal structure studies. TLR2 was confirmed to heterodimerize with TLR1 or TLR6, specifically sensing different bacterial components and their analogs. TLR2/TLR1 or TLR2/TLR6 heterodimers distinguish triacylated lipopeptides (e.g. Pam_3CSK_4) or diacylated lipopeptides (e.g. Pam_2CSK_4), respectively [8]. TLR2 is also able to cooperate with CD14 to increase cellular responses [9]. Moreover, a member of the scavenger receptor type B family, CD36 may also participate to TLR2 heterodimers [10]. Interestingly, CD36 mediates recognition of certain TLR2 agonists via cooperation with TLR2/TLR6 heterodimers [11]. In contrast, TLR2/TLR1 signaling in the brain requires CD36 [12]. Additionally, TLR2/TLR10 heterodimers have been reported to sense triacylated lipopeptides [13], however, TLR10 was recognized as an anti-inflammatory PRR suppressing TLR2 signaling [14].

The recruitment of these TLRs or non-TLR receptors by TLR2 facilitates the detection of numerous, different structures of lipoproteins present in various pathogens, while it also increases the complexity of mechanistic studies. Therefore, the recognition principle of TLR2 homo- or heterodimers is still a field worth further investigating.

Table 1-1 Bacterial ligands of TLR2 [15].

Ligand	**Origin**	**TLR**
Heat-killed bacteria	e.g. *Listeria monocytogenes*	TLR2/?
Lipoteichoic acid (LTA)	Gram-positive bacteria	TLR2/?
Lipoarabinomannan	Mycobacteria	TLR2/TLR1
Lipomannan	Mycobacteria	TLR2/?
Porins	Gram-negative bacteria	TLR2/?
Triacylated lipoproteins (Pam_3CSK_4)	Gram-positive bacteria	TLR2/TLR1
Diacylated lipoproteins (Pam_2CSK_4, FSL-1, MALP-2)	Gram-negative bacteria, Mycoplasma	TLR2/TLR6
Peptidoglycan (PGN)*	Gram-positive bacteria	TLR2/?

"?": not determined. "*": disputed. TLR2 activation by PGN was attributed to lipopeptide contamination [16]. However, PGN extracted from mutant *S. aureus* lacking lipopeptides was able to activate TLR2 [17]. MALP-2, macrophage-activating lipopeptide 2.

TLR2 heterodimers have different ligand-binding pockets and may signal via distinct pathways [18]. The structural studies of TLR2 heterodimers in complex with certain ligands have demonstrated the mechanisms by which these heterodimers distinguish certain lipoproteins. Owing to the high homology to IL-1 receptor, the cytoplasmic regions of TLRs are named Toll/IL-1 receptor (TIR) domain. The extracellular domain contains leucine-rich repeats and is divided into three domains: N-terminal, central and C-terminal domain. TLR2 heterodimers share an "m"-shaped structure with the two N-terminal domains stretch outward to the opposite direction and the two C-terminal domains converge in the middle. In the TLR2/TLR1/Pam_3CSK_4 structure, the two ester-bound lipid chains of Pam_3CSK_4 are inserted into a pocket in TLR2, while the amide-bound lipid chain binds a hydrophobic channel in TLR1 [19]. The amide-bound lipid chain needs minimal eight carbons to induce responses. Hence, Pam_2CSK_4's lacking of the third chain decides that it cannot induce a stable dimerization of TLR2/TLR1. In the TLR2/TLR6/Pam_2CSK_4 complex, the binding to the lipid channel of TLR6 is disabled by two phenylalanines. However, the enhanced interface of the hydrophobic area in TLR2/TLR6 compensates the missing amide lipid interaction [20].

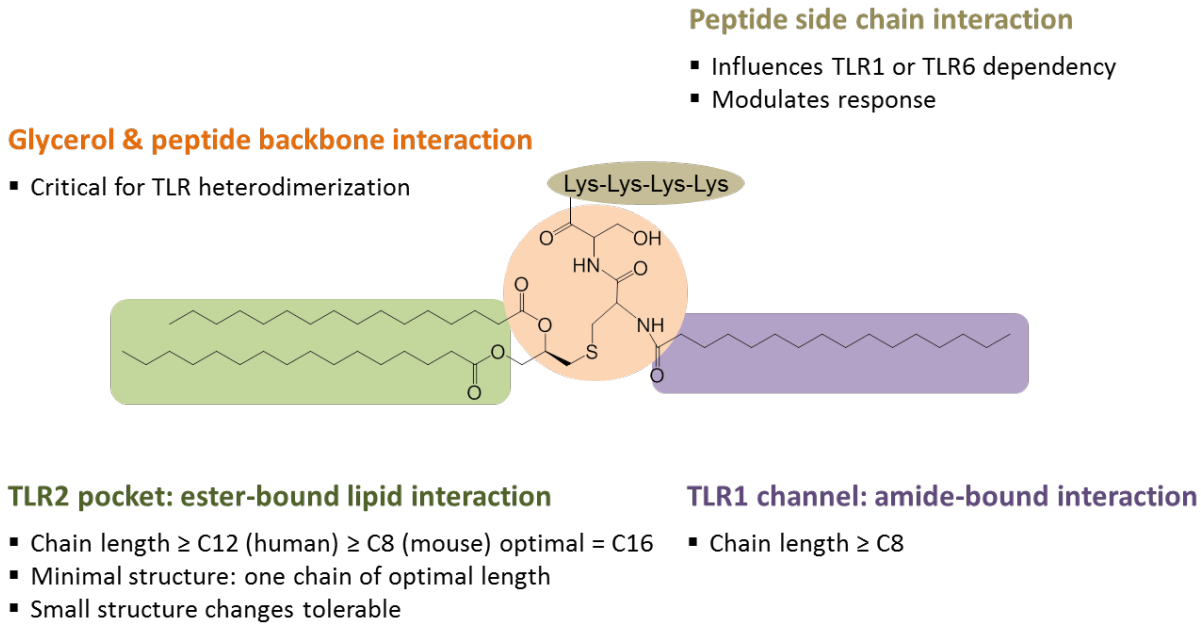

Figure 1-1

Summarized recognition pattern by TLR2/TLR1 or TLR2/TLR6 heterodimers (modified from [21]).

Comparison of human and mouse TLR2 sequences revealed 84% identical residues in the intracellular domains and 65% within the extracellular domains. Despite of the similarity of the backbone structures, the side chains of human and mouse TLR2 are different in sequence. The differences are responsible for the changes in the shape of the lipid-binding pocket in mouse and human TLR2. For instance, the activation of human TLR2 needs longer lipid chains (*Figure 1-1*). Therefore, an agonist for mouse TLR2 may not be able to activate the human counterpart [19, 20, 22]. Since most of the TLR ligands were defined in knockout mice, confirmation and further investigation are required in human cells.

1.1.2. TLR2 signaling

Upon ligand binding to TLRs, the receptor-ligand interaction triggers the tightening of the interface between the intracellular TIR domains, thereby initiating signaling. TLR2 signaling depends on the recruitment and association of adaptor molecules which also have a TIR domain. As a key mediator, myeloid differentiation primary response gene 88 (MyD88) is involved in TLR signaling of nearly all TLRs. It is a 33-kDa protein containing a C-terminal death domain and an N-terminal TIR. MyD88mediates TLR2 signaling and MyD88 adapter-like (Mal, also named TIRAP) is involved in bridging MyD88 to the TLR2-ligand complex [23, 24]. Mal-MyD88 leads to the activation of serine/threonine kinase interleukin-1 receptor-associated kinase 1/4 (IRAK 1/4), which in turn, interacts with tumor necrosis factor receptor-associated factor 6 (TRAF6). The IRAK-TRAF complex then mediates the activation of the inhibitor of kappa B (IκB) kinase (IKK) complex which phosphorylates IκB-α, leading to its degradation, allowing the disassociation and translocation of nuclear factor−κB (NF-κB) into the nucleus. IRAK-TRAF-mediated mitogen-activated protein kinase (MAPK) cascades also control NF-κB transcriptional activation [25]. Ultimately, pro-inflammatory cytokines, chemokines and other inflammatory mediators are produced (*Figure 1-2*).

Different from TLR4 signaling, TLR2 signaling was thought to lack TIR-domain-containing adapter-inducing interferon-β (TRIF)-dependent and MyD88-independent pathways, due to the observation that NF-κB was not activated by macrophage-activating lipopeptide 2 (MALP-2) in MyD88-deficient macrophages [26].

TLR2 plays a crucial role in immunity, which is further supported by the findings that TLR2 deficiency in mice causes more susceptibility and severely impaired host resistance to bacterial infection [27]. In addition, TLR2 polymorphisms have been associated with disease course and susceptibility [15].

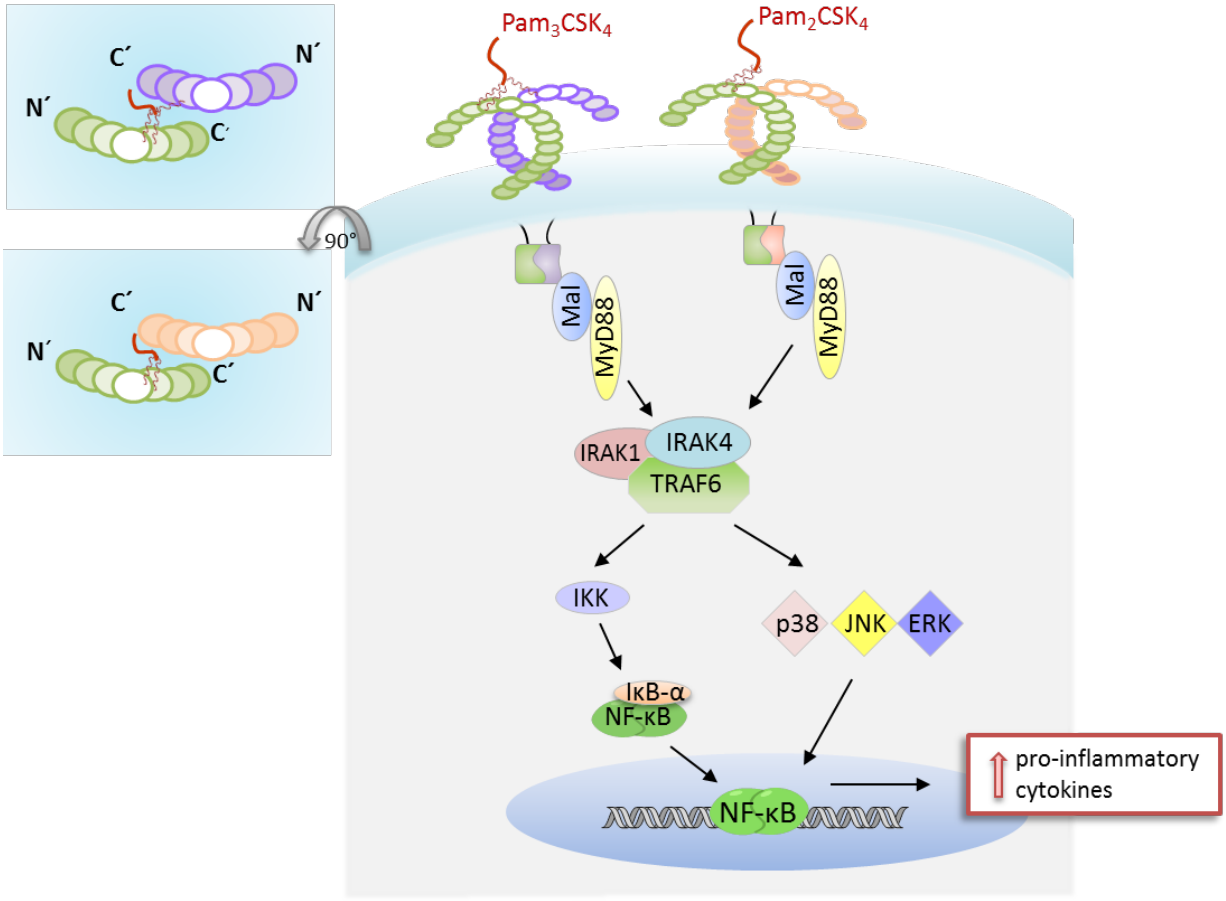

Figure 1-2

TLR2 signaling upon ligand binding. Pam_3CSK_4 and Pam_2CSK_4 are recognized by TLR2/TLR1 and TLR2/TLR6, respectively, and signal via a MyD88-dependent pathway. The figures on the left show the top view of TLR2 heterodimer recognition. MyD88 leads to the activation of IL-1R-associated kinase 1/4 (IRAK1/4) and TNFR-associated factor 6 (TRAF6), which in turn, activates mitogen-activated protein kinases (MAPKs) p38, JNK and ERK, or interacts with the inhibitor of kappa B (IκB) kinase (IKK) complex. IKK complex phosphorylates IκB-α, initiating its degradation, allowing NF-κB translocate to the nucleus. NF-κB activation results in the production of pro-inflammatory cytokines.

As the consequence of TLR2 activation, production of cytokines and chemokines initiates the recruitment of immune cells. For example, TLR2-MyD88 signaling modulates the phagocytosis of *L. monocytogenes* [28]. TLR2 activation also triggers antimicrobial defenses such as synthesis of antimicrobial peptides. Recognized by TLR2, *Propionibacterium acnes* induces the expression of beta-defensin-2 in human keratinocytes [29]. These results ultimately help to resolve microbial invasion, but may cause damage to the host, such as overwhelming inflammation that is seen in sepsis.

1.1.3. TLR2 modulators

Given the crucial role of TLR recognition and signaling in the immune response, efforts have been made in exploring modulators for clinical therapies, particularly for infection and cancer. A selective TLR2 agonist, SMP-105, cell wall skeleton component of *Mycobacterium bovis* BCG Tokyo showed anti-tumor activities in mice [30]. A TLR2-specific monoclonal antibody, OPN-305, is granted for clinical trials for the prevention of reperfusion injury associated with organ transplantation. Positive results have been received after phase I [31] and information from phase II is estimated in June 2018. TLR2/TLR6 agonist MALP-2 was used in patients with pancreatic carcinoma in phase I/II trial [32].

In recent years, small molecules targeting TLR2 signaling have been intensively investigated by Yin's group. CU-T12-9 has been proven to activate TLR2/TLR1 signaling in animal cells and transfected human embryonic kidney (HEK) cells [33], and CU-CPT22 has shown selective TLR2/TLR1 antagonistic effects in mouse cells [34]. However, the data from our group demonstrated CU-CPT22 failed to be selective for TLR2 heterodimers in human cells [35].

1.2. Skin

As a crucial interface encountering invading microorganisms, skin is equipped with PRRs to recognize pathogens in order to defend the host, including TLRs and a less reported family of cytoplasmic receptor, nucleotide-binding oligomerization domain (NOD)-like receptors [36]. Skin is also the primary sensing organ perceiving external stressors. Besides its pivotal immune functions in maintaining a homeostatic barrier,

skin is also defined as a balanced ecosystem for the host and colonized microorganisms including viruses, bacteria and fungi.

Skin is composed of three major structural compartments: epidermis, dermis and hypodermis (*Figure 1-3*). In humans, skin formation takes place within the first two weeks of gestational age. Epidermis originates from the ectoderm, so does the brain. Both structures are processed during neurulation, followed with later populated epidermis, hair follicles and sebaceous glands, neural crest-derived melanocytes and Merkel cells, as well as neural tube cells-derived central nerve system.

1.2.1. Cutaneous cells and their roles in immune system

Keratinocytes comprise more than 90% of epidermal cells and play a crucial role as a protective barrier against pathogens, water loss and physical or chemical insults. An organized differentiation process enables keratinocytes to achieve their functions. They undergo either self-replication (basal layer) or gradual differentiation towards the skin surface and form spinous, granular and cornified layers. Other cell populations in the epidermis function in concert with keratinocytes including pigment-producing melanocytes, bone marrow-derived Langerhans cells and sensory Merkel cells.

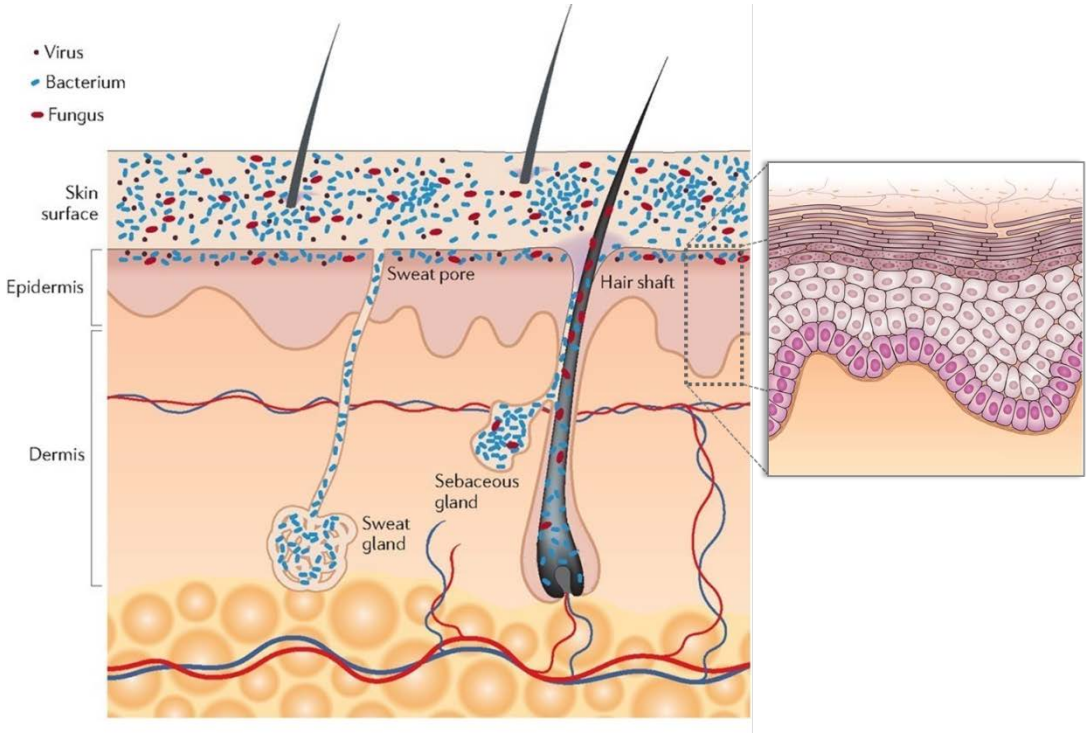

Figure 1-3

Schematic of skin histology viewed in cross-section (modified from [37, 38])**.**

Dermal fibroblasts, the major cell type in the dermis, are responsible for the production and repair of extracellular matrix. Besides the critical role as structural components in wound healing [39], they have been implicated in the interaction with inflammatory cells [40]. In the skin microenvironment, fibroblasts modulate immune responses by conditioning local cytokine and chemokine release, which brought the concept that fibroblasts can be a clinical target in chronic inflammation [41].

Given that skin is an important site where host-pathogen interactions take place, cutaneous cells are expected to express TLRs. Expression of TLR1, 2, 3, 5, 6, 9 and 10 in keratinocytes has been evidenced and functional TLR2 heterodimers have been identified in human keratinocytes. These TLRs respond to their ligands by NF-κB activation and cytokine production [42]. For example, *Staphylococcus aureus* TLR2-dependently induces the production of IL-8, nitric oxide and other chemokines in keratinocytes [43]. Moreover, as keratinocytes differentiate as they grow towards the skin surface, the pattern of TLR expression also varies [44]. Under inflammatory diseased conditions, enhanced TLR2 expression distributes towards the basal epidermis [45]. Fibroblasts express all 10 TLRs and respond to most ligands by releasing pro-inflammatory cytokines [46, 47]. To gain better understanding of skin responses to pathogens, the expression and regulation of TLR2 in different skin layers seem worth investigating.

TLRs have been implicated in cutaneous immune responses against various skin infections, such as infections caused by *S. aureus*, *Mycobacterium leprae*, *Candida albicans*, *Propionibacterium acnes* and viruses (e.g. herpes simplex and varicella-zoster). Mostly, TLR2 is involved and contributes to the pathophysiology of common skin diseases, including atopic dermatitis, psoriasis, leprosy and acne vulgaris [48]. Therefore, further understanding the role of TLR2 in skin cells may reveal new insights and, potentially, treatments for skin disorders.

1.2.2. Acne vulgaris

Acne vulgaris is a chronic skin disease mainly found on face, upper chest and back [49]. Undoubtedly, acne affects most population during some time in their lives. Prevalence studies showed that acne is not only a teenage disease but also persists into

adulthood, termed acne tarda [50]. Acne affects psychological health of individuals due to multiple features: acne distributes highly visible skin and acne peaks around puberty, an age vital for building confidence. Although the disease itself is not life-threatening, it causes psychological impairment leading to anxiety, depression, decreased self-esteem, social withdrawal and even suicide [49].

The severity of acne is graded into mild, moderate and severe [49]. Treatment should be tailored individually according to the type of acne, its severity, the patient's ability to use the treatment and their psychological state [51]. Topical treatment with benzoyl peroxide, retinoid, antibiotics or a combination is often used to control mild to moderate acne. Oral antibiotics or isotretinoin are more effective for severe acne although limited by side effects [52].

Many studies have addressed the mechanisms of acne. While the precise etiology and pathogenesis of acne are still unclear, there is a consensus on the four steps crucial for the formation of acne lesions: increased sebum production; inflammatory mediators causing skin inflammation; alteration of keratinization leading to comedone development; follicular colonization by *Propionibacterium acnes*. Genetic factors, living habits, stress and many other factors were also suggested to contribute to acne [49, 53]. The exact order of the steps and the interaction among them and with other factors remain to be elucidated.

1.2.3. Propionibacterium acnes

Acne is frequently found at the area with high density of pilosebaceous units where sebaceous glands connect to hair follicles and produce lipid-rich substance sebum. The relatively anoxic environment in sebaceous glands encourages the growth of anaerobic microorganisms such as *Propionibacterium* species. Correspondingly, *Propionibacterium* species are the dominant organisms in sebaceous areas [38].

P. acnes is a Gram-positive, anaerobic-aerotolerant bacterium and is involved in the pathogenesis of acne. While *P. acnes* is considered a commensal bacterium residing predominantly in the pilosebaceous follicles on human skin, emerging researches have implicated it as an opportunistic pathogen related to a wide range of inflammatory conditions on both skin and non-skin sites [54].

The role of *P. acnes* in acne vulgaris was first mentioned in 1896 and is well accepted. Due to the capability to initiate inflammatory responses, the organism is considered to be involved in the inflammatory phase of acne. *P. acnes*-induced proinflammatory cytokines have been found in immune and non-immune cells associated with innate and adaptive immune responses through distinct mechanisms, e.g. IL-1β production by monocytes [55, 56] or sebocytes [57] via NLRP3 inflammasone activation, IL-8 and TNF secretion in TLR2-activated keratinocytes [58], IL-17A and IFN-γ release by promoting T helper (Th) 17 and Th17/Th1 responses in T-cells [59].

Since *P. acnes* is present on the skin of the majority of people irrespective of the existence of acne lesions, chronic inflammatory acne was not defined as an infectious disease. One speculation is that *P. acnes* only triggers the disease when it meets favorable physiological conditions, or as demonstrated recently that *P. acnes* produces short-chain fatty acid to break immune tolerance and promote immune responses [60]. Another possibility is strain-dependent differences in pathogenic potential, based on the finding that certain strains were highly acne-associated [61].

P. acnes was originally classified by agglutination test into serotype I or II in 1970s [62]. While still occasionally used, such typing methods have generally been replaced by more efficient and precise genetic methods [63]. More recently, the difference between type I and II was confirmed by phylogenetic analysis of nonribosomal housekeeping genes [64], and type III was newly identified [65]. Multilocus sequence typing (MLST) is one of the most accepted methods, by which *P. acnes* strains fall into phylotypes IA_1, IA_2, IB, IC, II and III [66]. Given the emerging clinical differences in disease association and antibiotic resistance between types, *P. acnes* typing is more and more important [63]. In 1980s, it has been noticed that *P. acnes* type I is more related to acne than type II. Later, type IA_1 and IC bacteria were found highly associated with acne lesions, while type III bacteria were in healthy skin, IB and II were overrepresented in infections of other organs [67]. Strain ATCC 11827, ATCC 6919 and ATCC 11828 are used in most of the studies belonging to type IA_1, IA_1 and II, respectively.

TLR2 expressing cells in inflammatory acne lesions suggested the importance of local TLR2 responses [68]. Furthermore, *P. acnes* is recognized by TLR2 and keratinocytes

express functional TLR2 [69]. A study in TLR-knockout mice elucidated that *P. acnes*-induced IL-6 production in peritoneal macrophages was inhibited when TLR2 but not TLR1 or TLR6 was knocked out. The authors suggested that in mice and possibly humans, TLR2 homodimers instead of heterodimers recognize *P. acnes* [68]. However, as mentioned in 1.1.1, human TLR2 heterodimers have differences in the recognition binding pocket, which raises the question whether *P. acnes* is recognized by human cells in a different way than by mouse cells.

1.3. Glucocorticoids

Glucocorticoids (GCs) work on nearly every part in the body by modulating the metabolism of carbohydrate, fat and protein under normal physiological conditions. Excess endogenous GCs are released when the body is under psychological stress. These stress signals are perceived by the brain and trigger stress hormone production. Upon hypothalamus-pituitary-adrenal (HPA) axis activation, hypothalamus produces corticotropin-releasing hormone (CRH) which acts on CRH-receptors (CRH-Rs) located on the pituitary gland to induce the secretion of adrenocorticotropin (ACTH). ACTH is transported through the bloodstream and leads to the secretion of steroid GCs by the adrenal cortex. The major natural glucocorticoid produced by the human body is cortisol. 11β-hydroxysteroid dehydrogenase enzymes (11βHSD1 and 11βHSD2) are responsible for the interconversion of active (cortisol) and inactive (cortisone) GCs. Apart from being the effector of the HPA axis, skin developed an equivalent peripheral HPA system that expresses CRH, CRH-Rs and ACTH [70]. They enable skin cells to execute local GCs synthesis.

In the 1940s, GCs were found to exert potent anti-inflammatory properties and since then both natural and synthetic GCs have been used worldwide as anti-inflammatory drugs [71]. The earliest GC clinically used is cortisone, but prolonged treatment showed mineralocorticoid (MC) effects. Nowadays, minimal MC affinity analogues are applied clinically, such as dexamethasone, prednisone and beclomethasone. Lacking MC receptor activation, dexamethasone, as a glucocorticoid agonist (GRs), is the most commonly used GC in experimental studies. Prednisone is often used systemically for autoimmune diseases and inflammatory conditions, e.g. allergic disorders, arthritis,

ulcerative colitis and asthma. Hydrocortisone has more MC activity and is used as a cream or lotion for skin disorders. The GC/MC potency ratio of dexamethasone and prednisone relative to hydrocortisone is 30/0 and 4/0.8, respectively. Although GCs remain the mainstays in clinical treatment, with emerging new findings there still seems to be oversimplification of GCs effects on immune processes [72].

1.3.1. Glucocorticoid receptors

GCs convey the signals through binding to GRs that exist in the cytosol of almost all eukaryotic cells. Inactive GRs are in complex with chaperone proteins, including heat-shock protein (Hsp) 90, Hsp70 and several immunophilins [73]. Once bound to their ligands (GCs), GRs are dissociated with chaperones and rapidly translocate to the nucleus as an activated transcription factor [74]. GR homodimers repress or activate transcription by binding to glucocorticoid response elements (GREs) on DNA or transcription modulators (*Figure 1-4*). GCs directly bind to GREs or negative GREs (nGREs) and recruit co-regulator proteins, which in turn results in the transactivation or transrepression of target genes, respectively. For example, GRs enhance the expression of anti-inflammatory proteins, e.g. MAPK phosphase-1 (MKP-1, also named dual specificity protein phosphatase-1 (DUSP1)) which inhibits MAPK pathways [75]; GILZ (GC-induced leucine zipper) mediating NF-κB, activator protein-1 (AP-1) and MAPK inhibition; tristetrapolin (TTP), also known as zinc finger protein 36 homolog, that inhibits the expression and destabilizes pro-inflammatory cytokines [76]. GRs also physically interact with or "tether" other transcription factors, e.g. NF-κB, STATs (signal transducer and activator of transcription) and AP-1. By binding to GREs, GRs exert composite regulation of neighbor transcription factors, or without GRE binding, GRs prevent transcription factors from inducing immune responses. The most well established mechanisms of the repressing effects are GR/NF-κB and GR/AP-1 antagonisms. Aside from inducing IκBα to inhibit NF-κB, GRs physically interact with NF-κB or AP-1 by tethering, thereby impairing their capacities to induce pro-inflammatory cytokines. Notably, repression is not the only relationship with target genes by tethering. Studies have revealed that tethering is involved in the activation of target genes as well. STAT family members, for example, are modulated by GRs differentially via tethering, including enhanced STAT3 and STAT5 but reduced STAT6

activity. Additionally, GC-GR complexes can undergo a less known non-genomic regulation via direct interaction with signal transduction pathways, e.g. negative interference with MAP kinase pathways [77].

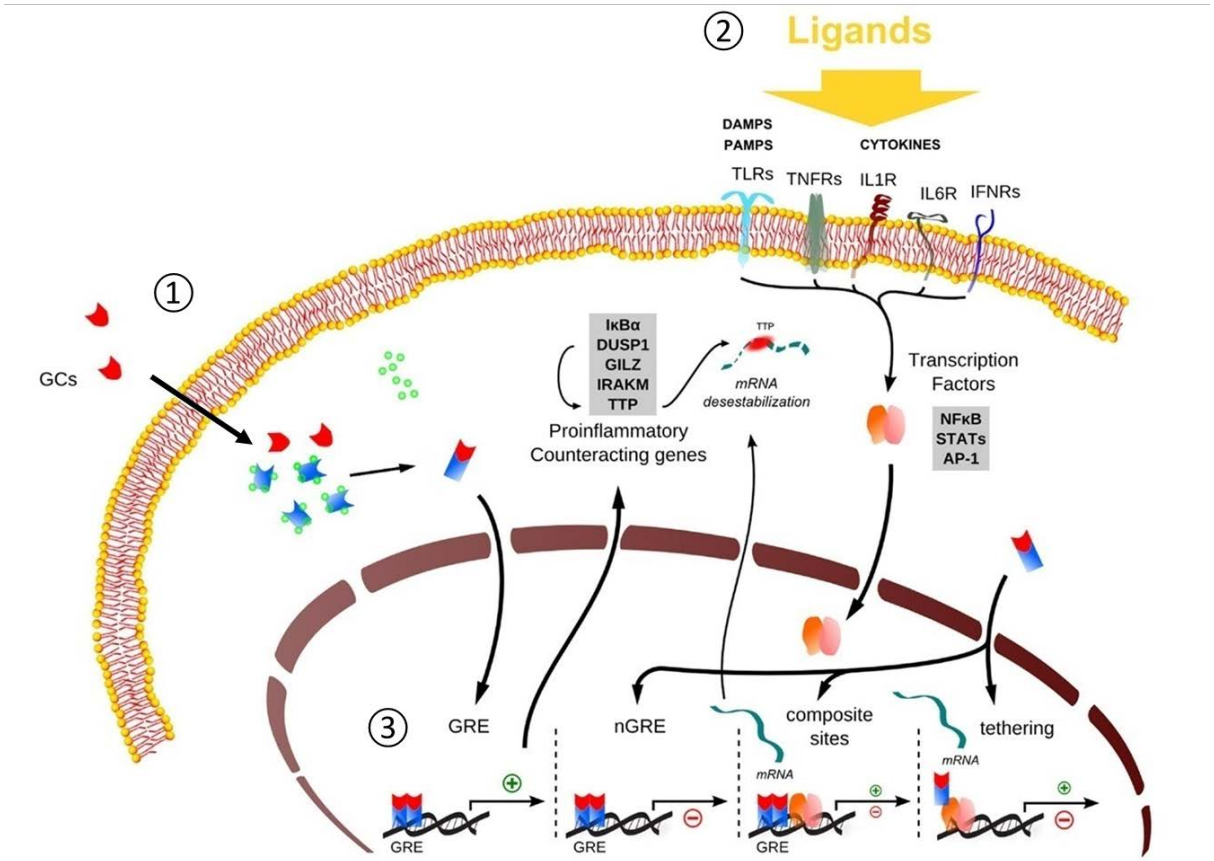

Figure 1-4

Glucocorticoid signaling under normal and inflammatory conditions. (Modified from [78]). 1. Glucocorticoids (GCs) bind to glucocorticoid receptor (GR) and dissociate chaperones. GC-GR complexes translocate into nucleus and lead to transcription regulation. 2. Inflammatory ligands recognized by respective receptors initiate communication between ligand-induced transcription factors and GC-GR complexes. 3. Four GC-GR complex mediated mechanisms: GC response element (GRE)-dependent transactivation e.g. IκBα, DUSP1, GILZ, IRAKM and TTP; negative GRE (nGRE)-dependent repression; transcription factor-recruited GRE binding to composite sites or directly interaction with transcription factors (tethering).

1.3.2. Pro-inflammatory effects of glucocorticoids and related skin disorders

In the past decades, emerging studies have highlighted the pro-inflammatory actions of GCs [79]. These effects appear to be tissue-specific and were found in the central nerve system as well as in the periphery. Some key factors were identified to contribute to the pro-inflammatory effects: low concentrations of GCs, acute stimulation and GC treatment prior to inflammation [80]. In contrast to the general accepted suppressive effects of GCs, several reports have shown various aspects that GCs are not strictly immunosuppressive *(Figure 1-5)*.

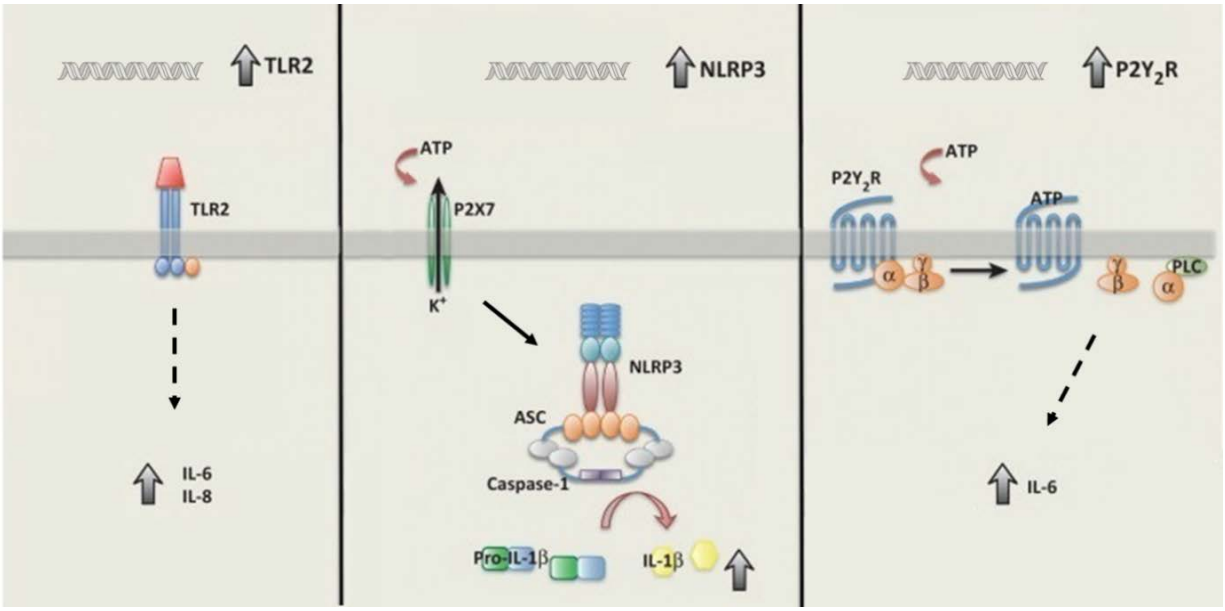

Figure 1-5

The pro-inflammatory aspects of glucocorticoid signaling (Modified from [81]). Glucocorticoids (GCs) induce the expression of TLR2, which leads to enhanced production of IL-6 and IL-8. GC-induced intracellular pattern-recognition receptor, NLRP3, increases IL-1β secretion via ATP activated inflammasome. GCs also upregulate the expression of the purinergic receptor, $P2Y_2R$, thereby contributes to IL-6 production.

A microarray study surprisingly revealed that more genes were co-regulated than antagonistically regulated by GC and TNF. Among 311 inflammatory disease genes, 210 genes were co-regulated [82]. In specific cases, GCs have been found to enhance TLR2 expression and further increase TLR2 expression in combination with bacteria or pro-inflammatory cytokines [83, 84]. Interestingly, the expression of an intracellular PRR NLRP3 was positively modulated by GCs. This effect sensitized macrophages to ATP, a common DAMP secreted by necrotic or damaged cells, which resulted in increased pro-inflammatory cytokine secretion [85]. Furthermore, GCs also elevate the expression of the purinergic receptor $P2Y_2R$ in human microvascular endothelial cells, so that subsequent signaling and IL-6 production are enhanced upon ATP stimulation [86].

Topical corticosteroids are widely used since 1951 in dermatological diseases. However, *in vivo* or clinically, topical steroids are commonly associated with systemic or/and cutaneous side effects. In terms of adverse effects on skin, steroid atrophy, steroid acne and symptoms assembling dermatitis or rosacea are frequently documented [87-89]. A study elucidated a connection between glucocorticoid treatment and acne vulgaris in human keratinocytes. The authors attributed GC-induced acne to GC-enhanced TLR2 expression and demonstrated a MAPK p38 mediated signal mechanism [90]. Although it seems reasonable to link increased TLR2 expression to a pro-inflammatory consequence, when looking into details at the related studies (*Table 1-2*), GC-induced functional TLR2 signaling has not been demonstrated. Moreover, considering the global anti-inflammatory effects, a pro-inflammatory mediator involved in GC-induced disease conditions is still elusive.

To mimic GC-induced skin disorder conditions, stimulation with Dex and *P. acnes in* keratinocytes appears to be a suitable experimental model. Microarray studies in GC-treated keratinocytes [91] and acne lesions [92] indicate that genes encoding serum amyloid A (SAA), among others, would be worth examining in Dex and *P. acnes*-stimulated keratinocytes. This would help to further decipher GC-induced TLR2 expression and signaling, as well as to identify co-regulated genes associated with acne.

Table 1-2 Studies related to glucocorticoid-induced TLR2 expression.

Cell type	Stimulation	Main finding	Ref
Hela cells (cervix epithelial cell line)	Dex + NTHi	Dex-induced MPK-1 inhibits p38 MAPK, which leads to the enhancement of TLR2 expression via negative regulation by p38 MAPK. Overexpressed TLR2 increases NTHi-induced pro-inflammatory cytokine release.	[82, 93]
Hela cells	Dex + IL-1β	Dex and IL-1β enhances TLR2 expression through MPK-1-dependent downregulation of JNK and p38 MAPK.	[83]
A549 cells (lung epithelial cell line)	Dex ± TNF	Dex, TNF and the combination upregulate TLR2 expression. NF-κB, GR and STAT are involved.	[94]
Keratinocytes	Dex ± TNF/IL-1α/*P. acnes*	Dex alone or in combination with TNF, IL-1α or *P. acnes* increases TLR2 expression. The underlying mechanism is MPK-1-dependent downregulation of p38 MAPK.	[89]

Dex, Dexamethasone; NTHi, nontypeable *Haemophilus influenza*; non MKP-1, MAPK phosphatase-1; Ref, Reference.

1.4. Serum amyloid A

SAA is a family of 104-112 amino acid apolipoproteins. Four members of SAAs are divided into two groups: A-SAA and C-SAA. The 'A' refers to acute phase and consists of SAA1 and SAA2. SAA1/2 are evolutionarily conserved and expressed in all studied vertebrates. The expression and production of SAA1/2 are markedly increased in response to tissue injury, infection and trauma [95]. SAA3 was implicated in tumor metastasis in mice [96] and has been recently detected in humans at rather low concentration [97]. SAA4 is also termed C-SAA referring to constitutive existence in the blood circulation and its production is barely induced [98].

1.4.1. The induction of SAA1/2 *in vitro*

Production of SAA1/2 can be experimentally induced by stimuli such as pro-inflammatory cytokines IL-1, TNF, IL-6 and LPS. As anti-inflammatory drugs, GCs have also been shown to induce SAA1/2 secretion in hepatocytes or extrahepatic cells

[95]. The extrahepatic induction of SAA1/2 by UV irradiation, pro-inflammatory cytokines or in combination of GCs (dexamethasone) is summarized in **Table 1-3**. In terms of TLR ligand-induced SAA1/2 production, it has only been demonstrated for LPS, a TLR4 ligand. Moreover, LPS-induced SAA1/2 has not been reported in other human cells than THP-1 cells. These studies showed SAA gene expression in THP-1 monocytic cells in stimulation of LPS alone or combined with dexamethasone [99, 100]. The mechanistic studies have identified a number of transcription factors including C/EBP, STAT3, NF-κB, which are differently involved cell-specifically and stimuli-dependently. The induction of SAA1/2 by various TLR2 ligands in different human cells remains to be investigated. Additionally, SAA1/2 may be the missing link to connect GC-induced TLR2 to GC-induced skin diseases.

Table 1-3 The induction of SAA1/2 in extrahepatic cells.

Cell type	Stimulation	SAA*		SAA1/2		Ref
		mRNA	Protein	mRNA	Protein	
THP-1 cells	Dex ± LPS	+				[100]
Keratinocytes	UV irradiation				SAA1	[101]
KB cells	Dex ± IL-6/TNF			SAA1/2		[102]
	Dex ± IL-1/TNF			SAA1/2		[103]
RT4/31, KB, Hela, ECV304, MRC5	Dex + IL-1β ± TNF/IL-6	+				[104]
AoSMC	Dex ± IL-1	+	+			[105]
Choriocarcinoma cells	IL-1α ± IL-1β			SAA1/2		[106]

"*": not specified which SAA type produced.

THP-1, human monocytic cell line; RT4/31, bladder papilloma cells; KB, oral epidermal carcinoma cells; Hela, cervical carcinoma cells; ECV304, umbilical cord endothelial cells; MRC5, fetal lung fibroblasts; AoSMC, Aortic smooth muscle cells; Dex, Dexamethasone; Ref, Reference.

1.4.2. The function of SAA1/2

Increased SAA production has been found in rheumatoid arthritis, atherosclerosis, Crohn's disease and diabetes [107]. This indicates that SAA1/2 play crucial roles in inflammatory disorders. Plasma SAA1/2 are mainly synthesized and released in the liver to reach a high plasma level during acute phase responses. The function in lipid metabolism has been intensively investigated. In addition to SAA-HDL association,

SAA1/2 can also bind to *Escherichia coli* and *Pseudomonas aeruginosa* and act as an opsonin for macrophages and neutrophils [108]. Interestingly, SAA1 is an endogenous TLR2 ligand [109] and as shown in **Table 1-4**, tissue-derived SAA1/2 function via several receptors and elicit predominantly pro-inflammatory effects with cytokine and chemokine inducing capacities.

Table 1-4 The function of SAA1/2 in extrahepatic cells.

Receptor	Pathway	Cell type	Product	Related disease
TLR2	NF-κB	Dermal fibroblasts	IL-6	Systemic sclerosis [110]
	?	PBMC	G-CSF	RA [111]
	?	Myoblasts C2C12 (mouse)	IL-6	COPD [112]
	NF-κB	HEK293	IL-8, MCP-1, etc	RA [113]
TLR4	NF-κB	Fibroblasts	IL-6, IL-8, IL-1β	Skin inflammation [114]
	MAPK	Dermal fibroblasts	MMP-1	Skin ageing [101]
FPRL1	ERK, NF-κB	Monocytes	MCP-1	Atherosclerosis [115]
	NF-κB	THP-1	MMP-9	Inflammation [116]
	NF-κB, AP-1	HUVEC	MCP-1	Atherosclerosis [117]
	?	Fibroblast-like synoviocytes	MMP-1, MMP-3	IA [118]
P2X7	NLRP3	Keratinocytes	IL-1β	Skin disease [119]
	NLRP3	Macrophages	IL-1β, TNF	Amyloidosis [120]
CD36	JNK, ERK	HEK293	IL-8	Atherosclerosis [121]
?	NF-κB	Intestinal epithelial cells	IL-8	IBD [122]
	MAPK, NF-κB	Rheumatoid synoviocytes	IL-6	RA [123]
	NLRP3, MAPK	Neutrophils	IL-1β, TNF	Inflammation [124, 125]
	MAPK	PBMC	IL-1β, IL-6, etc	CAD [126]

"?": not determined.

FPRL1, formyl peptide receptor-like 1; P2X7, a purinergic receptor; CD36, a class B scavenger receptor; NF-κB, nuclear factor κB; AP-1, activator protein 1; MAPK, mitogen activated protein kinases including p38, JNK and ERK; NLRP3, NACHT, LRR and PYD domains-containing protein 3 inflamasome; PBMC, peripheral blood mononuclear cells; HEK293, human embryonic kidney cell line; HUVEC, human umbilical vein endothelial cells; IL, interleukin; G-CSF, granulocyte colony-stimulated factor; MCP-1, monocyte chemoattractant protein-1; MMP, matrix metalloproteinase; TNF, tumor necrosis factor; RA, rheumatoid arthritis; COPD, chronic obstructive pulmonary disease; IA, inflammatory arthritis; IBD, inflammatory bowel diseases; CAD, coronary artery disease.

1.5. Aim of this work

Mammalian cells recognize a broad spectrum of bacteria via TLR2 and initiate a cascade of signaling pathways in response. Although glucocorticoids (GCs) are the most widely used immunosuppressive drugs, the paradoxical induction of skin diseases by GCs warrants further investigation. This thesis first aims to identify which TLRs are involved in the recognition of *Propionibacterium acnes* in human cells. The second objective was to study the crosstalk between GCs and TLR2 focusing on molecular mechanisms of steroid-induced skin inflammation. Studies on different modulators targeting TLR2 heterodimers, their downstream pathways, GC- and *P. acnes*-induced transcription factors may provide perspectives into mechanisms and application of the clinical drug candidates.

The specific aims were as follows:

- To determine *P. acnes* is recognized by TLR2 homo- or heterodimers in human cells, HEK-Blue hTLR2 cells were used as well-established systems to monitor TLR2 signaling. Primary human keratinocytes were also applied, as *P. acnes*-triggered TLR2 recognition and activation in keratinocytes initiates comedogenesis. To complete the picture of *P. acnes* recognition and effects on human cells, the subsequent signaling pathways were examined additionally.
- Although studies indicate the involvement of GC-increased TLR2 expression in keratinocytes, it remains unclear whether enhanced TLR2 expression leads to functional signaling. In addition, very little is known about the regulation of GCs on TLR2 heterodimer partners, TLR1 and TLR6. Even less is known about a pro-inflammatory mediator which might be induced by GCs or in combination with inflammatory factors such as TNF or bacteria. Therefore, the regulation of TLR2, TLR1 and TLR6 by GCs was determined. TLR2 signaling molecules were examined whether GC-enhanced TLR2 is able to induce downstream pathways. Moreover, the expression of TLR2 and SAA1/2 were evaluated in the presence of GCs and *P. acnes* to gain a better understanding in GC-induced skin diseases.

2. RESULTS

2.1. Recognition of *Propionibacterium acnes* by human TLR2 heterodimers

The manuscript has been published in *International Journal of Medical Microbiology*:

Qi Su, Maria Grabowski, Günther Weindl. Recognition of *Propionibacterium acnes* by human TLR2 heterodimers. *Int J Med Microbiol* 307 (2017) 108–112.

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The following contributions have been made:

Design of experiments: Su Q (60 %), Grabowski M and Weindl G

Practical, experimental part: Su Q (80 %) and Grabowski M

- TLR2 activation by synthetic ligands in HEK-Blue cells: Grabowski M
- All other experiments: Su Q

Data analysis: Su Q (80 %), Grabowski M and Weindl G

Interpretation of results: Su Q (75 %), Grabowski M and Weindl G

Writing of manuscript: Su Q (80 %), Grabowski M and Weindl G

2.2. Cell type-specific regulatory effects of glucocorticoids on cutaneous TLR2 expression and signaling.

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Qi Su, Anja Pfalzgraff, Günther Weindl. Cell type-specific regulatory effects of glucocorticoids on cutaneous TLR2 expression and signalling. *J Steroid Biochem Mol Biol* 171 (2017) 201-208.

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The following contributions have been made:

Design of experiments: Su Q (50 %), Pfalzgraff A and Weindl G

Practical, experimental part: Su Q (90 %) and Pfalzgraff A

- Supportive work in *P. acnes* culture: Pfalzgraff A
- All other experiments: Su Q

Data analysis: Su Q (80 %), Pfalzgraff A and Weindl G

Interpretation of results: Su Q (70 %), Pfalzgraff A and Weindl G

Writing of manuscript: Su Q (80 %), Pfalzgraff A and Weindl G

2.3. Glucocorticoids and Toll-like receptor 2 cooperatively induce acute-phase serum amyloid A

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Design of experiments: Su Q (80 %) and Weindl G

Practical, experimental part: Su Q (100 %)

Data analysis: Su Q (90 %) and Weindl G

Interpretation of results: Su Q (80 %) and Weindl G

Writing of manuscript: Su Q (80 %) and Weindl G

Glucocorticoids and Toll-like receptor 2 cooperatively induce acute-phase serum amyloid A

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Abstract

Serum amyloid A (SAA) is a highly conserved acute-phase protein and extrahepatic produced SAA1/2 contributes to cutaneous inflammation. Prolonged systemic or topical treatment with glucocorticoids can provoke skin diseases such as steroid-induced acne. Glucocorticoids increase Toll-like receptor 2 (TLR2) expression, however, an inflammatory mediator linked to this side effect remains elusive. We report that TLR2 agonists in combination with dexamethasone substantially increase SAA expression and production in human keratinocytes and epithelial cells. Dexamethasone-mediated SAA1 induction depends on the glucocorticoid receptor (GR). In response to *Propionibacterium acnes*, TLR2-activated signal transducer and activator of transcription 3 (STAT3) and nuclear factor κB (NF-κB) signaling pathways are critically involved in dexamethasone-induced SAA1 production. The formation of transcription factor complexes between GR or p300 and phospho-STAT3, was confirmed by co-immunoprecipitation in dexamethasone- and *P. acnes*-stimulated keratinocytes. Furthermore, dexamethasone and *P. acnes*-increased TLR2 and mitogen-activated protein kinase phosphatase-1 (MKP-1) contribute to induction of SAA1 and 2. Likewise, tumor necrosis factor (TNF) induces SAA1 in combination with dexamethasone. GR, transcription factors STAT3 and NF-κB, but not MKP-1, mediate TNF- and dexamethasone-induced SAA1. Conclusively, we provide evidence that glucocorticoids promote SAA1 production under infectious and sterile inflammatory conditions which may provide significant insights to the pathogenesis of steroid-induced acne.

Key words: serum amyloid A, glucocorticoids, Toll-like receptor 2, skin, transcription factors

1. Introduction

Serum amyloid A (SAA) is a family of apolipoproteins mainly synthesized in the liver. Extrahepatic production of SAA has been demonstrated in other cells including macrophages, neutrophils and smooth muscle cells [1]. Humans express two groups of different isoforms: acute-phase SAA (A-SAA: SAA1 and SAA2) and constitutive SAA (c-SAA: SAA4). During the acute phase of inflammation SAA1/2 are secreted rapidly, and the levels positively correlate with the severity of inflammatory diseases such as rheumatoid arthritis and osteoarthritis [2]. Thus, SAA is suggested as a clinical marker for disease progression. SAAs elicit their functions through binding to multiple proteins and receptors [1]. More recently the role of SAA1 in cutaneous inflammation has been described. Excessive ultraviolet irradiation-induced SAA1 is recognized by Toll-like receptors (TLRs) and promotes expression of proinflammtory cytokines and matrix metalloproteinase-1 in fibroblasts [3, 4]. Moreover, increased SAA1 levels in psoriatic epidermis contribute to IL-1β secretion via inflammasome activation [5]. In contrast, the molecular mechanisms modulating SAA expression are poorly understood. Previous studies suggest cell-type dependent involvement of CCAAT/enhancer binding proteins (C/EBP), signal transducers and activators of transcription (STAT) 3 and NF-κB [6, 7].

As a side effect, prolonged systemic or topical treatment with glucocorticoids may cause acne or exacerbate existing acne lesions [8]. This clinical observation is paradoxical to the well-known immune suppression effect of glucocorticoids. Glucocorticoids induce TLR2 expression [9] and signaling in keratinocytes [10]. *Propionibacterium acnes* contributes to inflammation in acne vulgaris by activation of TLR2 heterodimers in human cells [11], however, an inflammatory mediator linked to TLR2 signaling in the presence of *P. acnes* and glucocorticoids is still missing. Interestingly, glucocorticoids alone or in combination with

proinflammatory cytokines induce SAA1/2 production in non-hepatic cell lines [12-14] and SAA2 expression is upregulated in acne lesions [15].

In the present study, we aimed to examine the expression of SAA1/2 in primary human keratinocytes stimulated with glucocorticoids under infectious and sterile inflammatory conditions and characterized the underlying molecular mechanisms modulating SAA1 production.

2. Materials and Methods

2.1. Cell culture

All donor and patient samples were obtained after written informed consent and only anonymized samples were used for the experiments. All experiments were performed in accordance with relevant guidelines and regulations and were approved by the ethics committee of the Charité - Universitätsmedizin Berlin, Germany. For primary cultures, normal human epidermal keratinocytes and dermal fibroblasts were isolated from human juvenile foreskin and cultured as described [16, 17]. Keratinocytes were grown in keratinocyte basal medium (KBM; Lonza, Basel, Switzerland) supplemented with insulin, hydrocortisone, human epidermal growth factor and bovine pituitary extract (keratinocyte growth medium, KGM) as provided by the manufacturer. Fibroblasts, the immortalized keratinocyte cell line HaCaT (CLS Cell Lines Service, Eppelheim, Germany) passage 45-52 and the oral epithelial cell line TR146 were cultured in Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 (Sigma-Aldrich, Steinheim, Germany), 10% fetal calf serum (FCS, Biochrom AG, Berlin, Germany), and 5 mM l-glutamine (PAA Laboratories, Pasching, Austria). Cells were maintained at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air. HEK-Blue hTLR2 cells were obtained from InvivoGen (Toulouse, France). HEK cells passage 10-15 were cultured as described [11]. The cell lines were regularly tested negative

for mycoplasma contamination (Venor GeM Classic Mycoplasma PCR detection kit, Minerva Biolabs, Berlin, Germany).

2.2. Cell stimulation

Primary cells from the third passage were used and pooled from at least three donors to reduce donor-specific properties. 1.8×10^5 (6-well) or 7×10^4 (12-well) cells were seeded into culture plates (TPP, Trasadingen, Switzerland) and incubated in growth medium for 24 h. Before stimulation, keratinocytes, fibroblasts, HaCaT and TR146 cells were washed with phosphate-buffered saline (PBS; Sigma-Aldrich) and KBM (without hydrocortisone) or basal medium without FCS and antibiotics was added for 24 h. Cells were washed and stimulated with 10 ng/ml TNF (eBioscience, San Diego, USA), or different inhibitors (Ro 31-8220, mifepristone (RU486), pimozide, S3I-201, BAY 11-7082 and CU-CPT22; all from Sigma-Aldrich) in the presence or absence of dexamethasone (Sigma-Aldrich). The TLR2 ligands Pam_3CSK_4 (TLR2/1; 1 μg/ml), Pam_2CSK_4 (TLR2/6; 1 μg/ml), and HKLM (TLR2; 1×10^8 cells/ml), as well as anti-hTLR2-IgA (clone B4H2), anti-hTLR6-IgG (clone C5C8) and isotype-matched control antibodies were purchased from Invivogen (San Diego, USA). The vehicles used were ethanol (dexamethasone, RU486), DMSO (S3I-201, pimozide, CU-CPT22, BAY 11-7082), and cell culture grade water (TLR ligands, TLR antibodies). Final vehicle concentrations in cell culture were below 1% (v/v). Vehicle controls showed no significant difference to nonstimulated controls (data not shown) and cell viability of primary keratinocytes was at least 75% in the presence of the inhibitors (Fig. S1). HEK-Blue cells were stimulated in Opti-MEM (ThermoFisher Scientific, Darmstadt, Germany). At the end of the experiments, cell culture supernatants were collected and SEAP production was determined [11].

2.3. ELISA

The cell culture supernatant was assayed for IL-8 (ELISA-Ready Set Go; eBioscience) and SAA1 (DuoSet, R&D Systems, Wiesbaden, Germany) by using commercially available ELISA kits.

2.4. RNA isolation and quantitative RT-PCR

Total RNA isolation, cDNA synthesis and quantitative RT-PCR (qPCR) were performed as described [16]. Primers (synthesized by TIB Molbiol, Berlin, Germany) with the following sequences were used: *YWHAZ*, *MKP1* and *TLR2* as published previously [18], *SAA1* 5'-CCTGGGCTGCAGAAGTGATCAGCGA-3' and 5'-AGTCCTCCGCACCATGGCCAAAGAA-3', and *SAA2* 5'-AGCCAATTACATCGGCTCAG-3' and 5'-ATTTATTGGCAGCCTGATCG-3'. Fold difference in gene expression was normalized to the housekeeping gene *YWHAZ*.

2.5. Bacteria

Propionibacterium acnes (ATCC 11827; DSMZ, Braunschweig, Germany) was cultured as described previously [10, 11]. Bacteria were heat-inactivated at 95 °C for 10 min.

2.6. Co-immunoprecipitation

Nuclear extracts were prepared from keratinocytes following the protocol (Nuclear Extract Kit, Active Motif, La Hulpe, Belgium). Total protein content was determined by using the BCA Protein Assay kit (Pierce, Thermo Scientific) and an equivalent amount was used for co-immunoprecipitation. Lysates were immunoprecipitated with rabbit anti-phospho-Stat3 (Tyr705) (D3A7) antibody (Sepharose Bead conjugate) or rabbit anti IgG isotype control (Sepharose Bead conjugate) (NEB, Germany) and incubated overnight at 4 °C. Samples were

washed 5 times with IP buffer (Thermo Scientific). Immunoprecipitated proteins were eluted with standard SDS-PAGE sample buffer in the presence of DTT, boiled and analysed by Western blot. The non-immunoprecipitated nuclear extracts served as loading control (10% input).

2.7. Western Blotting

Cells were lysed and prepared as described previously [19]. After gel electrophoresis and blotting, membranes were blocked with 5% bovine serum albumin (BSA; Sigma-Aldrich) for 1h at 37 °C, membranes were incubated with rabbit anti-phospho-Stat3 (Tyr705) (D3A7), rabbit anti-stat3 (79D7), rabbit anti-phospho-NF-κB p65 (Ser536) (93H1), rabbit anti-Acetyl-CBP (K1535)/ p300 (K1499), rabbit anti-glucocorticoid receptor (D6H2L), rabbit anti-TLR2 (D7G9Z) and rabbit anti-β-actin (13E5) (all 1:1000, from NEB) over night at 4 °C and incubated with anti-rabbit horseradish-peroxidase (HRP)-conjugated secondary antibody (NEB; 1:1000) for 1 h. Then blots were developed with SignalFire ECL reagent or Elite ECL reagent (NEB) and visualized by PXi Touch gel imaging system (Syngene, Cambridge, UK). All western blots were performed at least three times independently and the results shown are from one representative experiment. Quantifications were obtained by densitometric analysis using ImageJ version 1.46r and normalized to the respective β-actin loading controls.

2.8. RNA interference

siRNA duplexes for TLR2 and a nonsilencing control siRNA duplex (synthesized by Eurofins Genomics, Ebersberg, Germany) were tested for knockdown efficiency. The siRNA target sequences were as follows: TLR2: 5′-GGCUUCUCUGUCUUGUGAC-3′ and control: 5′-UUCUCCGAACGUGUCACGU-3′. TLR2 and control siRNA stock solutions were prepared according to the manufacturer's instructions. Keratinocytes were incubated with 100 nM

siRNA and TransIT-X2 transfection reagent (Mirus, Darmstadt, Germany) for 24 h prior to stimulation. Knockdown was confirmed by qRT-PCR and western blot.

2.9. Statistical analysis

Data are depicted as means + SD. Statistical significance of differences was determined by 2-tailed, unpaired Student's *t*-tests, one-way or two-way analysis of variance (ANOVA) followed by Bonferroni or Dunnett post-hoc analysis and considered significant at $P \leq 0.05$. Statistical analysis was performed using GraphPad Prism 6.0 (GraphPad software, San Diego, USA).

3. Results

3.1. A-SAAs are induced by dexamethasone and the combination of dexamethasone and P. acnes

We first confirmed the suppressive effect on IL-8 production by glucocorticoids in primary human keratinocytes stimulated with *P. acnes* (Fig. S2). In agreement with microarray data in dexamethasone (Dex) stimulated keratinocytes [20], we found Dex increased gene expression of SAA1 and SAA2. Most strikingly, Dex elevated SAA1 expression after 2 h and more than 100-fold after 24 h in *P. acnes*-costimulated keratinocytes (Fig. 1A). TNF-induced SAA1 expression could also be further enhanced by Dex in keratinocytes after 24 h (Fig. 1A,B). Similar results were obtained for SAA2 mRNA levels.

Dex alone induced SAA1 production whereas SAA1 levels were not detected in the presence of *P. acnes*. Importantly, the combination of Dex and *P. acnes* significantly induced SAA1 in a concentration- and time-dependent manner (Fig. 1C). Similarly, the combination of Dex with TNF increased SAA1 secretion in primary keratinocytes (Fig. 1D) and the HaCaT

keratinocyte cell line (Fig. 1E). In contrast to primary keratinocytes, co-stimulation of HaCaT cells with Dex and *P. acnes* did not further modulate SAA1 protein levels.

3.2. Dexamethasone-mediated SAA1 induction is dependent on the glucocorticoid receptor

To elucidate the mechanism of SAA1 enhancement by co-stimulation of Dex and *P. acnes* or TNF, the glucocorticoid receptor (GR) antagonist RU486 was used. RU486 inhibited Dex-induced SAA1 production in primary keratinocytes (Fig. 2A) and the oral epithelial cell line TR146 which showed high constitutive SAA1 levels (Fig. 2B). Likewise, in combination with *P. acnes* or TNF, SAA1 levels were almost completely suppressed in the presence of RU486 in keratinocytes (Fig. 2C) and in primary human dermal fibroblasts when stimulated with Dex and TNF (Fig. 2D). These findings indicate that Dex-mediated SAA1 production during inflammation depends on the GR.

3.3. STAT3 and NF-κB are essential for the induction of SAA1

We next assessed the molecular mechanisms of SAA1 induction following Dex and *P. acnes*-stimulation. Since STAT3 acts in synergy with GR for transcriptional increase of acute phase proteins [21], we investigated whether *P. acnes* activates STAT3. In *P. acnes*-stimulated keratinocytes rapid phosphorylation of NF-κB p65 and subsequent STAT3 tyrosine phosphorylation which peaked at 1 h was observed (Fig. 3A). NF-κB phosphorylation was blocked by the indirect IκB-α kinase inhibitor BAY 11-7082 and STAT3 activation by the specific inhibitor S3I-201 [22] and BAY 11-7082 (Fig. 3B). Previously, we demonstrated the prerequisite of TLR2 heterodimers, and in particular TLR2/6-heterodimers, for the recognition of *P. acnes* in human cells [11]. In the presence of the TLR2 antagonist CU-CPT22 and TLR2 and TLR6 neutralizing antibodies, *P. acnes*-mediated NF-kB and STAT3 phosphorylation was suppressed (Fig. 3B). Control antibodies failed to inhibit activation of the transcription factors (data not shown). We next sought to determine whether the two

transcription factors are involved in the regulation of SAA1 production in human keratinocytes as demonstrated in HepG2 cells [7]. The STAT3 inhibitors S3I-201 and pimozide [23] as well as BAY 11-7082 significantly abrogated SAA1 production induced by Dex and *P. acnes* (Fig. 3C) or TNF (Fig. 3D) indicating that STAT3 and NF-κB are essential for SAA1 expression. Significant inhibition of SAA1 production by S3I-201 and BAY 11-7082 was also demonstrated in TR146 cells (Fig. 3E). STAT3 and NF-κB p65 were reported to form a complex with p300 driving the transcription of SAA1 [7]. Thus, nuclear extracts were immunoprecipitated with anti-phospho-STAT3 (Tyr705) antibodies, and the immunoprecipitates were blotted with p300, phospho-NF-κB p65 and GR antibodies. Co-immunoprecipitation confirmed that phospho-STAT3 formed transcription factor complexes with p300 and GR but not phospho-NF-κB in the nucleus when keratinocytes were stimulated with Dex and *P. acnes* (Fig. 3F).

3.4. Dexamethasone and P. acnes-mediated SAA1 production depends on TLR2

Since Dex-induced TLR2 expression [10] facilitates recognition of *P. acnes* via TLR2 which may contribute to increased SAA expression, we next assessed the effects of other well-known TLR2 ligands on SAA1 secretion. Pam_3CSK_4 (TLR2/1), Pam_2CSK_4 (TLR2/1) and heat-killed *Listeria monocytogenes* (TLR2) all increased SAA1 secretion in combination with Dex (Fig. 4A). To confirm *P. acnes* induced SAA1/2 expression is mediated by TLR2, *P. acnes* plus Dex-induced SAA1 and SAA2 levels were determined in the presence of the TLR2 antagonist CU-CPT22. CU-CPT22 significantly reduced SAA1 production in *P. acnes* and Dex-costimulated cells and unexpectedly in TNF and Dex-costimulated cells (Fig. 4B and D). To confirm that CU-CPT22 has no inhibitory effect on TNF signaling, HEK-Blue hTLR2 cells, which endogenously express TNF receptors, were stimulated with TNF in the presence of CU-CPT22. TNF-induced NF-κB activation in HEK-Blue hTLR2 cells was not inhibited by CU-CPT22 (Fig. 4C). In addition, siRNA technology was used to further confirm the

contribution of TLR2. Gene expression was reduced by at least 75% (data not shown) and protein levels were strongly decreased compared to control siRNA (Fig. S3). Gene knockdown of TLR2 did not affect the expression of TLR1 and TLR6 (data not shown). Silencing of TLR2 significantly suppressed Dex and *P. acnes*-induced SAA1 production (Fig. 4E), however, SAA1 levels were reduced only by 30% relative to control probably due to Dex and *P. acnes*-increased TLR2 expression [10].

3.5. SAA1 expression and production induced by dexamethasone and P. acnes is mediated by MKP-1

Glucocorticoid-induced TLR2 expression is mediated via deactivation of mitogen-activated protein (MAP) kinases in keratinocytes [9]. Our previous study confirmed that glucocorticoids cooperate with *P. acnes* in enhancement of TLR2 expression via negative regulation of the JNK MAPK pathway by MAPK phosphatase 1 (MKP-1) [11]. Thus, we investigated the involvement of MKP-1 in the induction of SAA1/2 in keratinocytes. MKP-1 was increased by Dex and further upregulated by the combination of Dex and *P. acnes* (Fig. 5A). Ro 31-8220, an MKP-1 inhibitor, strongly inhibited Dex and *P. acnes*-induced SAA1 secretion (Fig. 5B). SAA1, SAA2 and TLR2 gene expression was also decreased by Ro 31-8220. Differently, TNF and Dex-induced SAA1 and SAA2 appeared not to be mediated by MKP-1 (Fig. 5C-E), although MKP-1 expression is increased by TNF and Dex [9].

4. Discussion

We demonstrate that keratinocytes, fibroblasts and epithelial cells produce high amounts of SAA1 when stimulated with glucocorticoids and TLR2 ligands. SAA1 induction depends on the GR and is driven by activation of STAT3 and NF-κB in response to TLR2-mediated recognition of *P. acnes*. Furthermore, glucocorticoids and *P. acnes* coregulate TLR2 and MKP-1 expression which contributes to SAA1 production.

Direct induction of SAA1/2 by TLR ligands has been demonstrated for lipopolysaccharide (LPS) in human cell lines [24]. LPS-induced SAA mRNA expression was further increased in the presence of Dex in THP-1 cells [25]. In the present study, TLR2 ligands including *P. acnes* showed strong SAA1/2 induction in cooperation with Dex, and GR is essential in SAA1 production. These findings are of high relevance with respect to the pathogenesis of steroid-induced acne, where glucocorticoids induce skin inflammation despite of its immune suppression effect.

In the past decade, emerging inflammatory functions of SAA1/2 have been described focusing on the cytokine-like properties and downstream signaling mechanisms [26]. SAA1 induce IL-1β release in keratinocytes, monocytes and macrophages through P2X7 receptor-mediated NLRP3 inflammasome activation [5, 27]. In dermal fibroblasts, SAA1 stimulate IL-6 production via TLR2 [28]. Although SAA1 has been claimed to activate TLR4, further confirmation is needed due to possible LPS contamination in rhSAA1 [26]. In the present study, keratinocytes produced 30 ng/ml SAA1 which is more than sufficient to induce proinflammatory cytokines in fibroblasts [3].

SAA1 secretion was differentially regulated in cell types by dexamethasone or in combination with *P. acnes* and TNF, respectively. Dexamethasone-stimulated primary keratinocytes produced SAA1, but neither fibroblasts nor HaCaT cells. When combined with *P. acnes* or TNF, keratinocytes and TR146 epithelial cells both substantially enhanced SAA1 production. Keratinocytes are amongst the first cells to encounter exogenous pathogens. Thus, largely enhanced SAA1 production by glucocorticoids in the presence of *P. acnes* likely leads to inflammation by activation of fibroblasts, sebocytes and chemotactic recruitment of inflammatory cells. A relatively low induction of SAA1 in Dex and *P. acnes*-stimulated HaCaT cells may be explained by the observation that Dex failed to upregulate TLR2 expression in HaCaT keratinocytes [10].

Activated STAT3 interacts with ligand-bound GR by forming a transactivating complex [29]. In our experimental setting, STAT3 Tyr705 phosphorylation is induced by *P. acnes*, which appears to be downstream of NF-κB phosphorylation [30]. In the nucleus, the complex binds to glucocorticoid-responsive elements (GREs) with STAT3 serving as co-activator without direct association with its DNA binding motif [7]. Relatedly, a functional GRE has been described in the SAA1 promoter [31]. Here, we present the formation of complexes between either GR or p300 with STAT3 in bacteria and glucocorticoid-stimulated cells. Although SAA1 and SAA2 have equivalent consensus functional binding sites for NF-κB in their promoters [31] and the formation of a complex with STAT3, NF-κB p65 and p300 at the NF-κB response element has been reported under sterile inflammation [7], we failed to detect phospho-NF-κB p65 in the complex induced by Dex and *P. acnes*. This discrepancy may be explained by transient or lower binding of phospho-STAT3 with phospho-NF-κB than with GR. Alternatively, phospho-STAT3 acts as a downstream effector of NF-κB signaling, which is supported by the observation that *P. acnes* alone induced phospho-NF-κB and phospho-STAT3 but not SAA1 secretion. In addition, we can not rule out the contribution of other transcription factors and transcriptional coactivators such as CCAAT/enhancer binding proteins [6, 7].

It is established that STAT3 Tyr705 is required for nuclear translocation of STAT3 and Ser727 is crucial for its mitochondrial functions [32]. Since STAT3 Ser727 is involved in transcription activation and association with p300 [7], it would be worth investigating whether *P. acnes* can also induce Ser727 phosphorylation. Aside from STAT3, other STATs might play a role in the induction of SAA1. SAA1 production was reduced in the presence of the antipsychotic drug pimozide which also inhibits STAT5 and STAT1 [33]. Yet, both STAT3 inhibitors S3I-201 and pimozide inhibited SAA1 secretion indicating that targeting STAT3 may have therapeutic potential not only in the treatment of psoriasis [34] but also steroid-induced acne.

In terms of TLR2, this work brings new insights into the multiple roles of TLR2 under Dex and *P. acnes*-stimulated conditions. First, TLR2 forms heterodimers with TLR1 and TLR6 to recognize *P. acnes* and initializes TLR2 signaling [10, 11]. Subsequently, phosphorylation of NF-κB and STAT3 are induced. The phosphorylated transcription factors then translocate into the nucleus where they interact with dexamethasone-bound GR dimers, eventually inducing the transcription of SAA1. Second, TLR2 expression is increased by Dex and *P. acnes* resulting in an enhanced TLR2 recognition and signaling [10]. Third, as one of the receptors for SAA1, TLR2 may mediate SAA1 induction orchestrating a network of cytokines and chemokines [24]. Unexpectedly the TLR2 antagonist CU-CPT22 blocked TNF- and Dex-induced SAA1 production without influencing TNF signaling, indicating that SAA1 induced its own expression via TLR2 in an autocrine/paracrine manner. In addition, *P. acnes*-induced TNF [35] may act as an endogenous molecule contributing to SAA1 production. Besides, considering the more notable difference of SAA1 expression between dexamethasone alone and in combination with *P. acnes* on protein than gene levels, post-transcriptional modifications may contribute to SAA induction [36].

Aside from TLR2 being a key role in SAA1 induction as noted above, MKP-1 appears to be crucial in the crosstalk between glucocorticoids and TLRs. Consistent with increased MKP-1 expression in dexamethasone- and *P. acnes*-stimulated keratinocytes [9], we demonstrate that SAA1 expression is induced via upregulation of MKP-1 expression. *P. acnes* alone showed no effect but promoted SAA1 transcription and production through TLR2 signaling. Thus, our work presents yet another aspect of MKP-1 resulting from crosstalk between glucocorticoids and TLR2 signaling [37]. Mechanistically, GR and p300 form a protein complex to induce MKP-1 transcription by recruitment to MKP-1 GRE [38]. However, further studies are needed to elucidate the mechanism of increased MKP-1 transcription in the presence of TLR2 ligands.

In conclusion, we propose that glucocorticoids facilitate recognition of commensal bacteria by TLR2 leading to enhanced TLR2 signaling [10] and upregulation of SAA1/2 which promotes inflammatory responses.

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Conflict of interest

The authors declare no financial or commercial conflict of interest.

References

[1] K.K. Eklund, K. Niemi, P.T. Kovanen, Immune functions of serum amyloid A, Crit Rev Immunol 32(4) (2012) 335-48.

[2] M. Connolly, R.H. Mullan, J. McCormick, C. Matthews, O. Sullivan, A. Kennedy, O. FitzGerald, A.R. Poole, B. Bresnihan, D.J. Veale, U. Fearon, Acute-phase serum amyloid A regulates tumor necrosis factor alpha and matrix turnover and predicts disease progression in patients with inflammatory arthritis before and after biologic therapy, Arthritis Rheum 64(4) (2012) 1035-45.

[3] S. Han, S.P. Jin, J.H. Oh, E.Y. Seo, C.H. Park, H.S. Yoon, D.H. Lee, J.H. Chung, Serum amyloid A1 secreted from UV-irradiated keratinocytes induces matrix metalloproteinase-1 in fibroblasts through toll-like receptor 4, Exp Dermatol 25(7) (2016) 526-31.

[4] S. Han, J.H. Oh, C.Y. Shin, H.S. Yoon, D.H. Lee, J.H. Chung, Serum amyloid A1 is induced by UV irradiation and detected by toll-like receptor 4 to causes skin inflammation, J Dermatol Sci 84(1) (2016) 107-110.

[5] N. Yu, S. Liu, X. Yi, S. Zhang, Y. Ding, Serum amyloid A induces interleukin-1beta secretion from keratinocytes via the NACHT, LRR and PYD domains-containing protein 3 inflammasome, Clin Exp Immunol 179(2) (2015) 344-53.

[6] Y. Kumon, T. Suehiro, D.J. Faulkes, T. Hosakawa, Y. Ikeda, P. Woo, J.D. Sipe, K. Hashimoto, Transcriptional regulation of serum amyloid A1 gene expression in human aortic smooth muscle cells involves CCAAT/enhancer binding proteins (C/EBP) and is distinct from HepG2 cells, Scand J Immunol 56(5) (2002) 504-511.

[7] K. Hagihara, T. Nishikawa, Y. Sugamata, J. Song, T. Isobe, T. Taga, K. Yoshizaki, Essential role of STAT3 in cytokine-driven NF-kappaB-mediated serum amyloid A gene expression, Genes Cells 10(11) (2005) 1051-63.

[8] G. Plewig, A.M. Kligman, Induction of acne by topical steroids, Arch. Derm. Forsch. 247(1) (1973) 29-52.

[9] M. Shibata, M. Katsuyama, T. Onodera, R. Ehama, J. Hosoi, H. Tagami, Glucocorticoids enhance Toll-like receptor 2 expression in human keratinocytes stimulated with Propionibacterium acnes or proinflammatory cytokines, J Invest Dermatol 129(2) (2009) 375-82.

[10] Q. Su, A. Pfalzgraff, G. Weindl, Cell type-specific regulatory effects of glucocorticoids on cutaneous TLR2 expression and signalling, J Steroid Biochem Mol Biol 171 (2017) 201-208.

[11] Q. Su, M. Grabowski, G. Weindl, Recognition of Propionibacterium acnes by human TLR2 heterodimers, Int J Med Microbiol 307(2) (2017) 108-112.

[12] D.M. Steel, F.C. Donoghue, R.M. O'Neill, C.M. Uhlar, A.S. Whitehead, Expression and regulation of constitutive and acute phase serum amyloid A mRNAs in hepatic and non-hepatic cell lines, Scand J Immunol 44(5) (1996) 493-500.

[13] C.F. Thorn, Z.Y. Lu, A.S. Whitehead, Regulation of the human acute phase serum amyloid A genes by tumour necrosis factor-alpha, interleukin-6 and glucocorticoids in hepatic and epithelial cell lines, Scand J Immunol 59(2) (2004) 152-8.

[14] C.F. Thorn, Z.Y. Lu, A.S. Whitehead, Tissue-specific regulation of the human acute-phase serum amyloid A genes, SAA1 and SAA2, by glucocorticoids in hepatic and epithelial cells, Eur J Immunol 33(9) (2003) 2630-9.

[15] N.R. Trivedi, K.L. Gilliland, W. Zhao, W. Liu, D.M. Thiboutot, Gene array expression profiling in acne lesions reveals marked upregulation of genes involved in inflammation and matrix remodeling, J Invest Dermatol 126(5) (2006) 1071-9.

[16] G. Weindl, F. Castello, M. Schäfer-Korting, Evaluation of anti-inflammatory and atrophogenic effects of glucocorticoids on reconstructed human skin, Altern Lab Anim 39(2) (2011) 173-87.

[17] F.M. Bätz, W. Klipper, H.C. Korting, F. Henkler, R. Landsiedel, A. Luch, U. von Fritschen, G. Weindl, M. Schäfer-Korting, Esterase activity in excised and reconstructed human skin--biotransformation of prednicarbate and the model dye fluorescein diacetate, Eur J Pharm Biopharm 84(2) (2013) 374-85.

[18] G. Weindl, J.R. Naglik, S. Kaesler, T. Biedermann, B. Hube, H.C. Korting, M. Schaller, Human epithelial cells establish direct antifungal defense through TLR4-mediated signaling, J Clin Invest 117(12) (2007) 3664-72.

[19] S. Hamidi, M. Schäfer-Korting, G. Weindl, TLR2/1 and sphingosine 1-phosphate modulate inflammation, myofibroblast differentiation and cell migration in fibroblasts, Biochim Biophys Acta 1841(4) (2014) 484-94.

[20] O. Stojadinovic, B. Lee, C. Vouthounis, S. Vukelic, I. Pastar, M. Blumenberg, H. Brem, M. Tomic-Canic, Novel genomic effects of glucocorticoids in epidermal keratinocytes: inhibition of apoptosis, interferon-gamma pathway, and wound healing along with promotion of terminal differentiation, J Biol Chem 282(6) (2007) 4021-34.

[21] L. Lerner, M.A. Henriksen, X. Zhang, J.E. Darnell, Jr., STAT3-dependent enhanceosome assembly and disassembly: synergy with GR for full transcriptional increase of the alpha 2-macroglobulin gene, Genes Dev 17(20) (2003) 2564-77.

[22] K. Siddiquee, S. Zhang, W.C. Guida, M.A. Blaskovich, B. Greedy, H.R. Lawrence, M.L. Yip, R. Jove, M.M. McLaughlin, N.J. Lawrence, S.M. Sebti, J. Turkson, Selective chemical probe inhibitor of Stat3, identified through structure-based virtual screening, induces antitumor activity, Proc Natl Acad Sci U S A 104(18) (2007) 7391-6.

[23] W. Zhou, M.K. Chen, H.T. Yu, Z.H. Zhong, N. Cai, G.Z. Chen, P. Zhang, J.J. Chen, The antipsychotic drug pimozide inhibits cell growth in prostate cancer through suppression of STAT3 activation, Int J Oncol 48(1) (2016) 322-8.

[24] M. De Buck, M. Gouwy, J.M. Wang, J. Van Snick, P. Proost, S. Struyf, J. Van Damme, The cytokine-serum amyloid A-chemokine network, Cytokine Growth Factor Rev 30 (2016) 55-69.

[25] S. Bozinovski, M. Uddin, R. Vlahos, M. Thompson, J.L. McQualter, A.S. Merritt, P.A. Wark, A. Hutchinson, L.B. Irving, B.D. Levy, G.P. Anderson, Serum amyloid A opposes lipoxin A(4) to mediate glucocorticoid refractory lung inflammation in chronic obstructive pulmonary disease, Proc Natl Acad Sci U S A 109(3) (2012) 935-40.

[26] R.D. Ye, L. Sun, Emerging functions of serum amyloid A in inflammation, J Leukoc Biol 98(6) (2015) 923-9.

[27] K. Niemi, L. Teirila, J. Lappalainen, K. Rajamaki, M.H. Baumann, K. Oorni, H. Wolff, P.T. Kovanen, S. Matikainen, K.K. Eklund, Serum amyloid A activates the NLRP3 inflammasome via P2X7 receptor and a cathepsin B-sensitive pathway, J Immunol 186(11) (2011) 6119-28.

[28] S. O'Reilly, R. Cant, M. Ciechomska, J. Finnigan, F. Oakley, S. Hambleton, J.M. van Laar, Serum amyloid A induces interleukin-6 in dermal fibroblasts via Toll-like receptor 2,

interleukin-1 receptor-associated kinase 4 and nuclear factor-kappaB, Immunology 143(3) (2014) 331-40.

[29] Z. Zhang, S. Jones, J.S. Hagood, N.L. Fuentes, G.M. Fuller, STAT3 Acts as a Co-activator of Glucocorticoid Receptor Signaling, Journal of Biological Chemistry 272(49) (1997) 30607-30610.

[30] F. Baran-Marszak, M. Boukhiar, S. Harel, C. Laguillier, C. Roger, R. Gressin, A. Martin, R. Fagard, N. Varin-Blank, F. Ajchenbaum-Cymbalista, D. Ledoux, Constitutive and B-cell receptor-induced activation of STAT3 are important signaling pathways targeted by bortezomib in leukemic mantle cell lymphoma, Haematologica 95(11) (2010) 1865-72.

[31] C.F. Thorn, A.S. Whitehead, Differential glucocorticoid enhancement of the cytokine-driven transcriptional activation of the human acute phase serum amyloid A genes, SAA1 and SAA2, J Immunol 169(1) (2002) 399-406.

[32] J.A. Meier, A.C. Larner, Toward a new STATe: the role of STATs in mitochondrial function, Semin Immunol 26(1) (2014) 20-8.

[33] E.A. Nelson, S.R. Walker, E. Weisberg, M. Bar-Natan, R. Barrett, L.B. Gashin, S. Terrell, J.L. Klitgaard, L. Santo, M.R. Addorio, B.L. Ebert, J.D. Griffin, D.A. Frank, The STAT5 inhibitor pimozide decreases survival of chronic myelogenous leukemia cells resistant to kinase inhibitors, Blood 117(12) (2011) 3421-9.

[34] K. Miyoshi, M. Takaishi, K. Nakajima, M. Ikeda, T. Kanda, M. Tarutani, T. Iiyama, N. Asao, J. DiGiovanni, S. Sano, Stat3 as a therapeutic target for the treatment of psoriasis: a clinical feasibility study with STA-21, a Stat3 inhibitor, J Invest Dermatol 131(1) (2011) 108-17.

[35] G.M. Graham, M.D. Farrar, J.E. Cruse-Sawyer, K.T. Holland, E. Ingham, Proinflammatory cytokine production by human keratinocytes stimulated with Propionibacterium acnes and P. acnes GroEL, Br J Dermatol 150(3) (2004) 421-8.

[36] C.M. Uhlar, A.S. Whitehead, Serum amyloid A, the major vertebrate acute-phase reactant, Eur J Biochem 265(2) (1999) 501-23.

[37] Y. Chinenov, I. Rogatsky, Glucocorticoids and the innate immune system: crosstalk with the toll-like receptor signaling network, Mol Cell Endocrinol 275(1-2) (2007) 30-42.

[38] L.E. Shipp, J.V. Lee, C.Y. Yu, M. Pufall, P. Zhang, D.K. Scott, J.C. Wang, Transcriptional regulation of human dual specificity protein phosphatase 1 (DUSP1) gene by glucocorticoids, PLoS One 5(10) (2010) e13754.

Figure legends

Fig. 1. Dexamethasone and the combination of dexamethasone and *P. acnes* induce A-SAAs. (A-B) Primary human keratinocytes were stimulated with 0.1 µM dexamethasone (Dex), *P. acnes* (MOI 500) or both for 2 or 24 h. Gene expression levels of *SAA1* and *SAA2* were determined by qPCR. mRNA expression values are normalized to *YWHAZ* and relative to control cells (assigned as 1.0). Mean + SD (n = 3). **P* ≤ 0.05, ***P* ≤ 0.01, one-way ANOVA followed by Bonferroni posttest in comparison with control cells or as indicated. (C) Keratinocytes were stimulated with (left) *P. acnes* (MOI 500), different concentration of Dex or the combination for 24 h; (right) *P. acnes* (MOI 500), 0.1 µM Dex or the combination for different time points. SAA1 secretion was assessed by ELISA. SAA1 secretion was not detected in cells stimulated with vehicle control (ethanol) or *P. acnes*. Mean ± SD (n = 3). ****P* ≤ 0.001, two-way ANOVA followed by Bonferroni posttest in comparison with Dex-stimulated cells. (D) Keratinocytes were stimulated with 0.1 µM Dex, 10 ng/ml TNF or the combination of Dex and TNF for 24 h. (E) HaCaT cells were stimulated with 0.1 µM Dex, *P. acnes* (MOI 500), the combination of Dex and *P. acnes*, 10 ng/ml TNF or the combination of Dex and TNF for 24 h. ELISA was performed to determine SAA1 protein levels. Mean + SD

(n = 3). *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, one-way ANOVA followed by Bonferroni posttest in comparison with control cells or as indicated.

Fig. 2. Dexamethasone-mediated SAA1 induction is dependent on the glucocorticoid receptor. Keratinocytes (A) or TR146 cells (B) were pre-incubated with 1 µM RU486 for 30 min and then stimulated with 0.1 µM dexamethasone (Dex) for 24 h. Keratinocytes (C) or primary human fibroblasts (D) were pre-incubated with 1 µM RU486 for 30 min and then stimulated with the combination of 0.1 µM Dex and 500 MOI *P. acnes* or 10 ng/ml TNF for 24 h, respectively. SAA1 production was quantified by ELISA. Mean + SD (n = 3-4). *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, one-way ANOVA followed by Bonferroni posttest in comparison with control cells or as indicated.

Fig. 3. STAT3 and NF-κB are essential for the induction of SAA1. (A) Keratinocytes were stimulated with 500 MOI *P. acnes* for the indicated time. (B) Keratinocytes were pre-incubated with 50 µM S3I-201 (S3I), 10 µM CU-CPT22 (CU), 5 µM BAY 11-7082 (BAY), 1 µg/ml anti-TLR2, anti-TLR6, a combination of anti-TLR2 and anti-TLR6 antibodies and then stimulated with 500 MOI *P. acnes* for 1 h. Protein levels of phospho-STAT3 (Tyr705), STAT3 and phospho-NF-κB were assessed by western blot analysis. β-actin served as control. Relative protein expression was quantified with ImageJ. Mean + SD (n = 3). The relative fold change of phospho-STAT3 or phospho-NF-κB p65 (*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$) was compared to nonstimulated cells, one-way ANOVA followed by Dunnett posttest in comparison with control cells (A) or *P. acnes*-stimulated cells (B). (C-D) Keratinocytes were pre-incubated with the indicated concentrations of S3I, pimozide or BAY and then stimulated with 0.1 µM Dex combined with 500 MOI *P. acnes* (C) or 10 ng/ml TNF (D) for 24 h. (E) TR146 cells were pre-incubated with the indicated concentrations of S3I or BAY and then

stimulated with a combination of 0.1 µM Dex and 500 MOI *P. acnes* for 24 h. SAA1 levels were assessed by ELISA. Mean + SD (n = 3). ***$P \leq 0.001$, one-way ANOVA followed by Bonferroni posttest in comparison with control cells or as indicated. (F) Keratinocytes were stimulated with 0.1 µM Dex combined with 500 MOI *P. acnes* for 2 h. Nuclear extracts were immunoprecipitated with bead-conjugated anti-phospho-STAT3 (Tyr705) or anti-IgG antibody. Phospho-STAT3 and co-immunoprecipitated p300, phospho-NF-κB p65 and GR were assessed by western blot analysis. Input shows results obtained from nuclear extracts without immunoprecipitation. Data are representative of 3 independent experiments.

Fig. 4. Dexamethasone and *P. acnes*-mediated SAA production is TLR2-dependent. (A) Keratinocytes were stimulated with 1 µg/ml Pam_3CSK_4, 1 µg/ml Pam_2CSK_4, 10^8 cells/ml heat-killed *Listeria monocytogenes* (HKLM) or in combination with 0.1 µM Dex for 24 h. SAA1 levels were evaluated by ELISA. Mean + SD (n = 3). **$P \leq 0.01$, ***$P \leq 0.001$, Student's t-test was used to compare the groups. (B) Keratinocytes were pre-incubated with the indicated concentrations of CU-CPT22 and then stimulated with 0.1 µM Dex combined with 500 MOI *P. acnes* or 10 ng/ml TNF for 24 h. (C) HEK-Blue hTLR2 cells were pre-incubated with 10 µM CU-CPT22 and then stimulated with 10 ng/ml TNF for 24 h. SEAP production indicating NF-κB activation was detected by QUANTI-Blue and OD was quantified at 640 nm. Mean + SD (n = 3-4). ***$P \leq 0.001$, one-way ANOVA followed by Bonferroni posttest in comparison with control cells. (D) TR146 cells were pre-incubated with 10 µM CU-CPT22 and then stimulated with 0.1 µM Dex combined with 500 MOI *P. acnes* for 24 h. SAA1 secretion was quantified by ELISA. Mean + SD (n = 3). *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, one-way ANOVA followed by Bonferroni posttest in comparison with control cells or as indicated. (E) After siRNA transfection, keratinocytes were washed and stimulated with 0.1 µM Dex plus 500 MOI *P. acnes* for 24 h. SAA1 production was

determined by ELISA and levels in cells incubated with control siRNA were set to 100%. Mean + SD (n = 3). *$P \leq 0.05$, one-sample t test in comparison with cells incubated with control siRNA.

Fig. 5. SAA1 expression induced by dexamethasone and *P. acnes* is mediated by MKP-1. (A) Keratinocytes were stimulated with 0.1 µM Dex, *P. acnes* (MOI 500) or both for 2 h. Gene expression levels of *MKP1* were determined by qPCR. mRNA expression values are normalized to *YWHAZ* and relative to control cells (assigned as 1.0). Mean + SD (n = 3-4). *$P \leq 0.05$, **$P \leq 0.01$, one-way ANOVA followed by Bonferroni posttest in comparison with control cells or as indicated. (B) Keratinocytes were pre-incubated with 1 µM Ro 31-8220 and then stimulated with 0.1 µM Dex combined with 500 MOI *P. acnes* or 10 ng/ml TNF for 24 h. SAA1 secretion was determined be ELISA. Mean + SD (n = 3-4). *$P \leq 0.05$, **$P \leq 0.01$, one-way ANOVA followed by Bonferroni posttest in comparison with control cells or as indicated. (C-E) Keratinocytes were pre-incubated with 1 µM Ro 31-8220 and then stimulated with 0.1 µM Dex combined with 500 MOI *P. acnes* or 10 ng/ml TNF for 24 h. Gene expression levels of *SAA1*, *SAA2* and *TLR2* were determined by qPCR. mRNA expression values are normalized to *YWHAZ* and relative to control cells (assigned as 1.0). Mean + SD (n = 3-5). *$P \leq 0.05$, **$P \leq 0.01$, one-way ANOVA followed by Dunnett posttest compared as indicated.

Figure 1

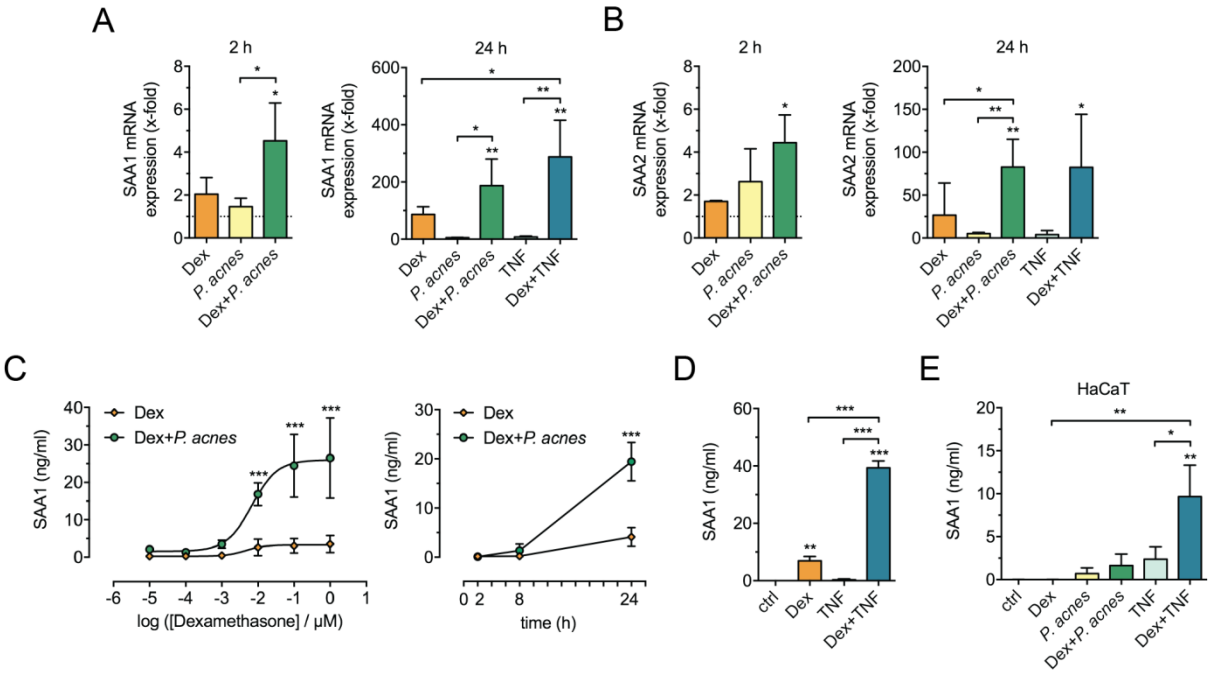

A
2 h
24 h
SAA1 mRNA expression (x-fold)
Dex
P. acnes
Dex+P. acnes
TNF
Dex+TNF
B
SAA2 mRNA expression (x-fold)
C
SAA1 (ng/ml)
Dex
Dex+P. acnes
log ([Dexamethasone] / µM)
time (h)
D
ctrl
E
HaCaT

Figure 2

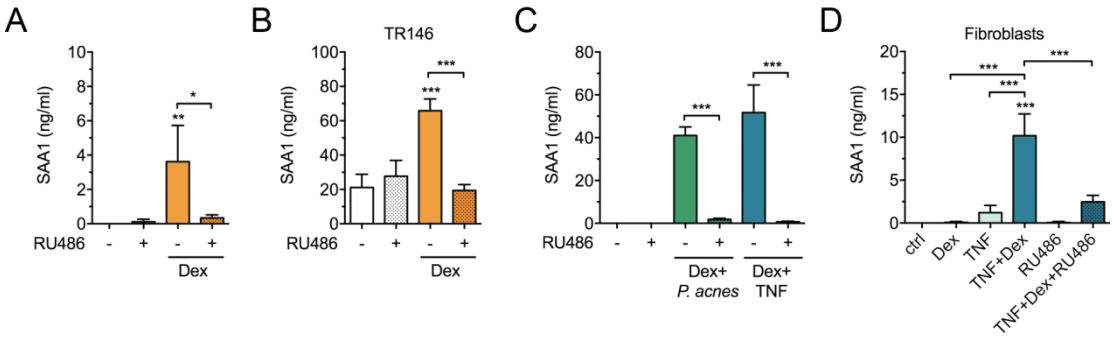
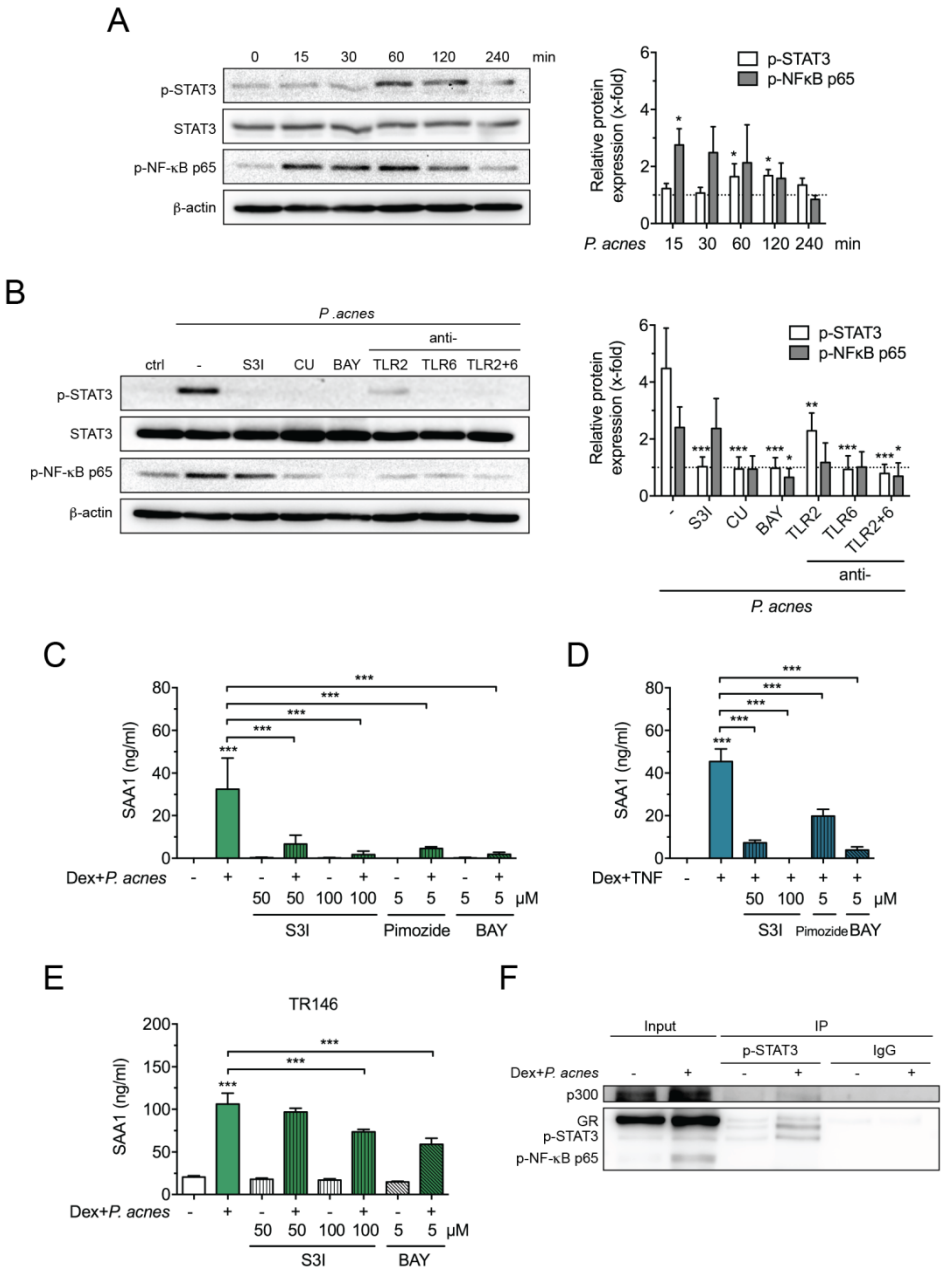

A
SAA1 (ng/ml)
RU486
Dex
B
TR146
SAA1 (ng/ml)
RU486
Dex
C
SAA1 (ng/ml)
RU486
Dex+ P. acnes
Dex+ TNF
D
Fibroblasts
SAA1 (ng/ml)
ctrl
Dex
TNF
TNF+Dex
RU486
TNF+Dex+RU486

Figure 3


A
0 15 30 60 120 240 min
p-STAT3
STAT3
p-NF-κB p65
β-actin
p-STAT3
p-NFκB p65
Relative protein expression (x-fold)
P. acnes 15 30 60 120 240 min
B
P .acnes
anti-
ctrl - S3I CU BAY TLR2 TLR6 TLR2+6
p-STAT3
STAT3
p-NF-κB p65
β-actin
p-STAT3
p-NFκB p65
Relative protein expression (x-fold)
- S3I CU BAY TLR2 TLR6 TLR2+6
anti-
P. acnes
C
SAA1 (ng/ml)
Dex+P. acnes - + - + - + - + - + - + μM
50 50 100 100 5 5 5 5
S3I Pimozide BAY
D
SAA1 (ng/ml)
Dex+TNF - + + + + + μM
50 100 5 5
S3I Pimozide BAY
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TR146
SAA1 (ng/ml)
Dex+P. acnes - + - + - + - + - + μM
50 50 100 100 5 5
S3I BAY
F
Input
IP
p-STAT3
IgG
Dex+P. acnes - + - + - +
p300
GR
p-STAT3
p-NF-κB p65

Figure 4

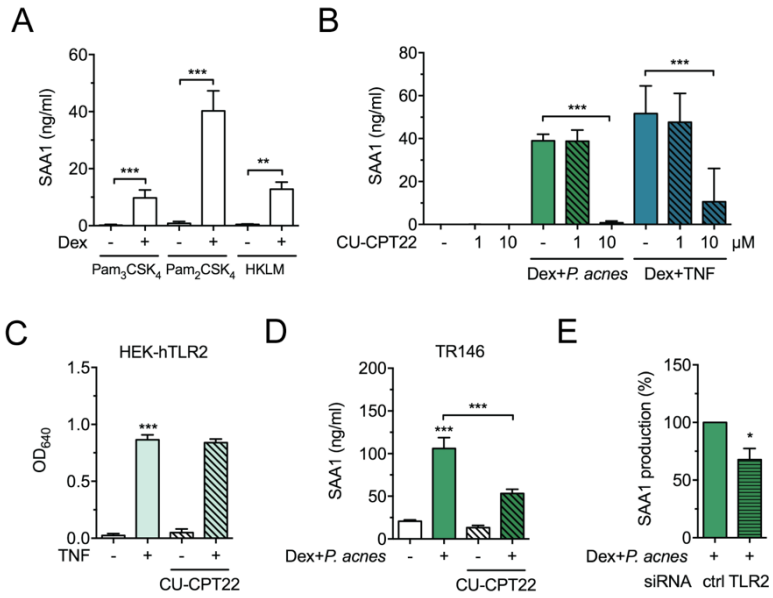
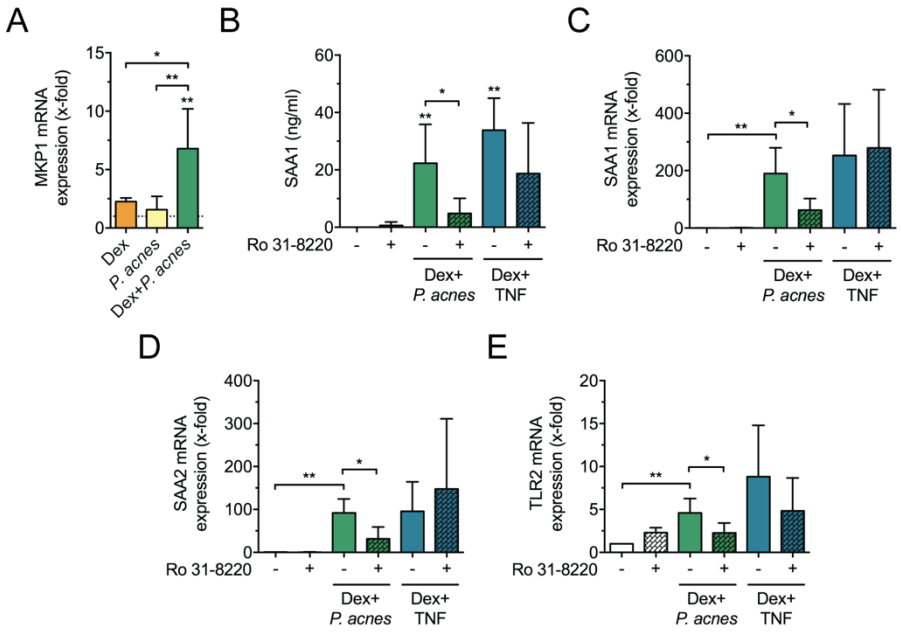

A
SAA1 (ng/ml)
60
40
20
0
***
***
**
Dex - + - + - +
Pam3CSK4 Pam2CSK4 HKLM
B
SAA1 (ng/ml)
80
60
40
20
0
***
***
CU-CPT22 - 1 10 - 1 10 - 1 10 µM
Dex+P. acnes Dex+TNF
C
HEK-hTLR2
OD640
1.5
1.0
0.5
0.0
***
TNF - + - +
CU-CPT22
D
TR146
SAA1 (ng/ml)
200
150
100
50
0
***
***
Dex+P. acnes - + - +
CU-CPT22
E
SAA1 production (%)
150
100
50
0
*
Dex+P. acnes + +
siRNA ctrl TLR2

Figure 5


A
MKP1 mRNA expression (x-fold)
15
10
5
0
Dex
P. acnes
Dex+P. acnes
B
SAA1 (ng/ml)
60
40
20
0
Ro 31-8220
Dex+ P. acnes
Dex+ TNF
C
SAA1 mRNA expression (x-fold)
600
400
200
0
Ro 31-8220
Dex+ P. acnes
Dex+ TNF
D
SAA2 mRNA expression (x-fold)
400
300
200
100
0
Ro 31-8220
Dex+ P. acnes
Dex+ TNF
E
TLR2 mRNA expression (x-fold)
20
15
10
5
0
Ro 31-8220
Dex+ P. acnes
Dex+ TNF

Supplementary data

Glucocorticoids and Toll-like receptor 2 cooperatively induce acute-phase serum amyloid A

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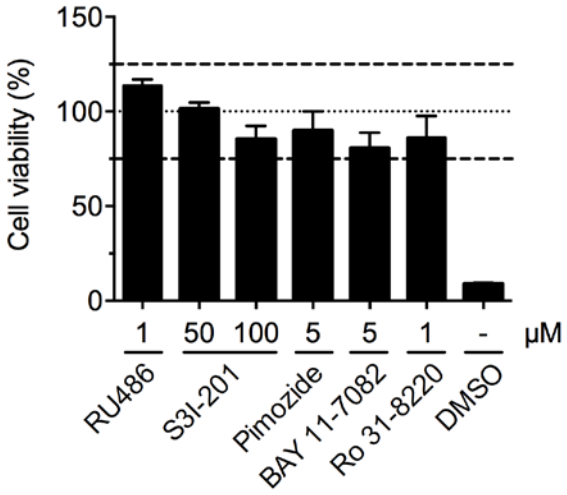

Fig. S1. Inhibitors do not affect cell viability of primary human keratinocytes. Cells were stimulated with indicated concentrations of inhibitors for 24 h and cell viability was evaluated by MTT assay. 5% (v/v) DMSO served as control. Dashed lines indicate 75, 100, and 125% relative to nonstimulated control cells. Mean + SD (n = 3-4).

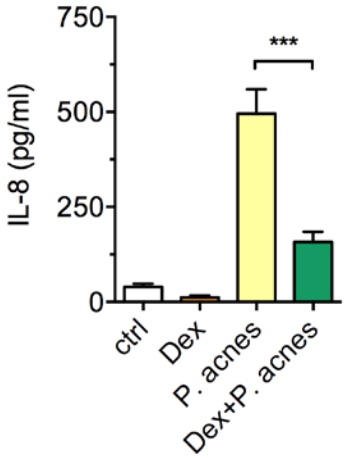

Fig. S2. Dexamethasone inhibits *P. acnes*-induced IL-8 production. Keratinocytes were stimulated with 0.1 µM Dex, *P. acnes* (MOI 500) or the combination of Dex and *P. acnes* for 24 h. ELISA was performed to determine IL-8 protein levels. Mean + SD (n = 3). ***$P \leq 0.001$, one-way ANOVA followed by Bonferroni posttest.

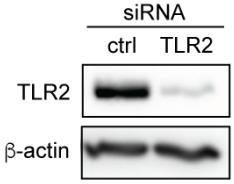

Fig. S3. siRNA transfection downregulates TLR2 expression in primary keratinocytes. Cells were transfected with 100 nM TLR2 or control siRNA, respectively. After 24 h TLR2 protein expression was analyzed by western blot analysis. β-actin served as control. Data are representative of 3 independent experiments.

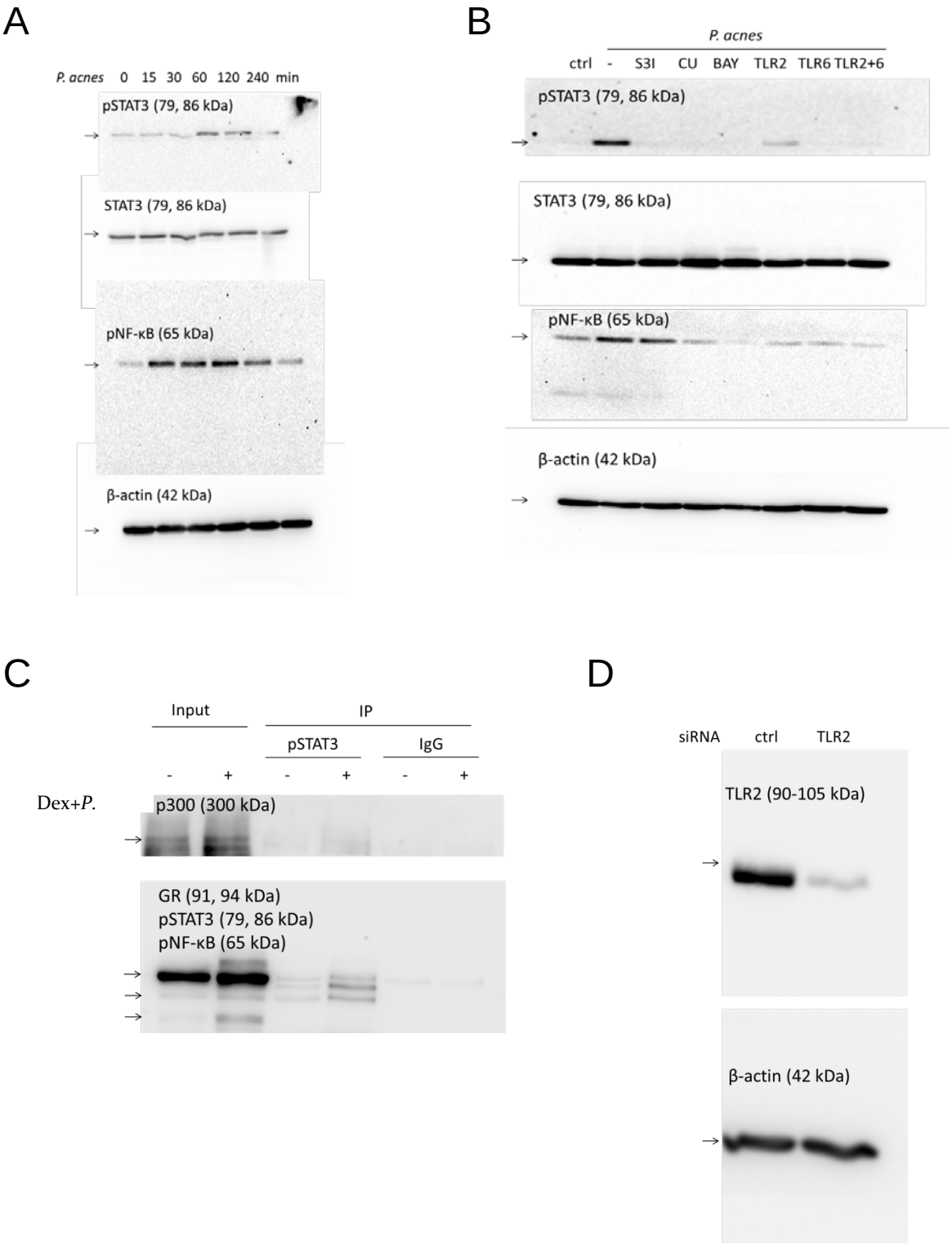

Fig. S4. Whole uncropped images of the original western blots. (A) Complete western blots of pSTAT3, STAT3, pNF-κB and β-actin shown in Fig. 3A. (B) Complete western blots of pSTAT3, STAT3, pNF-κB and β-actin shown in Fig. 3B. STAT3 and β-actin levels were determined on the same blots after membrane stripping. (C) Complete western blots of p300, GR, pSTAT3 and pNF-κB after immunoprecipitation shown in Fig. 3F. (D) Complete western blots of TLR2 and β-actin shown in Fig. S3.

3. DISCUSSION

3.1. Human TLR2 heterodimers recognize *P. acnes*

The species-specific recognition of *P. acnes* by human TLR2 homo- or heterodimers was investigated with HEK-Blue cells and primary human keratinocytes. As expected, differences between mice and human cells in terms of bacterium recognition by TLR2 dimers have been identified. This work demonstrates that TLR1 and TLR6 contribute to NF-κB and AP-1 activation in human cells and the recognition of live or heat-inactivated bacteria appears to be predominantly mediated by TLR2/6 heterodimers.

3.1.1. *P. acnes* recognition by TLR2 heterodimers in HEK cells

Two acne-associated strains of *P. acnes* [61] activated NF-κB-inducible SEAP production in HEK-Blue-hTLR2 cells, but not HEK-Blue Null1 cells. The activation was reversed dose-dependently by TLR2, TLR1 and TLR6 neutralizing antibodies and CU-CPT22. The combination of anti-TLR1 and anti-TLR6 antibodies completely abrogated activation of SEAP by *P. acnes*. These results revealed a *P. acnes*-induced TLR2 heterodimer-dependent NF-κB activation in HEK cells. No difference was observed between these two strains, while notably, compared with heat-inactivated *P. acnes*, live *P. acnes* activated a lower level of SEAP induction and the blocking effects of antibodies and antagonists were less pronounced. Bacteria preparation methods vary in studies, including culture condition, temperature and duration of heat inactivation, and should be taken into consideration. Heat inactivation may lead to the release of cytoplasmic compounds which are recognized by TLR2 dimers. Supportive of this notion is the finding that cell-free extracts of *P. acnes* induce IL-8 production in sebocytes [127]. Besides, the cell culture environment is unfavorable for anaerobic *P. acnes*, which probably influences the activity of live bacteria.

Along with the species-specific *P. acnes* recognition, mouse TLR2/1 specific modulator, CU-CPT22 [34], failed to be selective towards human TLR2 heterodimers. This further highlighted the distinct recognition pattern and modulation between humans and mice.

3.1.2. TLR2 heterodimers mediate NF-κB activation in keratinocytes

The etiological role of *P. acnes* has been extensively discussed or reviewed in the literature [54]. Whether *P. acnes* is a harmless skin commensal or opportunistic pathogen is still under discussion. To support the latter opinion, the arguments include the beneficial effect of antimicrobial therapy and emerging evidence showing *P. acnes*' impact on the innate and adaptive immune system. Furthermore, new studies suggested that although *P. acnes* can be found on the skin of virtually everyone, each individual is often colonized with heterogeneous communities of strains. Acne-associated type IA was found to be the most predominantly used experimental strain, which is also the type applied in the present work. We evaluated the capability of type IA *P. acnes* to activate NF-κB in human primary keratinocytes. *P. acnes* increased phospho-NF-κB p65, and in line with the findings in HEK cells, TLR2/6 heterodimers seem to be the main receptor.

Downstream signaling pathways are following with cytokine secretion, including follicular keratinocytes-produced IL-8, the gene expression of which is one of the most highly expressed genes in acne lesions [91]. TLR2 antagonist CU-CPT22 potently decreased *P. acnes*-induced IL-8 secretion and gene expression. Consistent with previous studies, TLR2 antibodies reduced IL-8 secretion by 50 % in keratinocytes [29]. However, no reduction was found in the presence of TLR1 and TLR6 antibodies. These data indicate the involvement of TLR6-independent IL-8 regulation. It is tempting to speculate that endogenous expressed CD36 in keratinocytes mediates IL-8 secretion as a co-receptor for TLR2. Additionally, the lower concentrations of TLR antibodies may be insufficient to reduce IL-8, limited by reduced cell viability with higher concentrations.

The ubiquitous existence of *P. acnes* on human skin can not only be explained by the fact that the host "supports" its presence by supplying nutrients, favorable anaerobic environment and producing antimicrobial substances which kill other species, but also as a result of their special metabolic capacities helping them to survive and compete other microorganisms. They produce short-chain fatty acids (SCFAs) such as propionate and acetate as metabolic products, which suppress the growth of other bacteria. Furthermore, as demonstrated recently, *P. acnes*-produced SCFAs can break

immune tolerance of keratinocytes to innate immune ligands. The underlying mechanism is that these SCFAs inhibit histone deacetylase activity, thereby modulating epigenetic events and promoting the inflammatory response to TLR2 ligands [60].

Although little is known about *P. acnes*-derived TLR2 ligands, proteins e.g. GroEl, Dnak, lipoglycans as well as peptidoglycans have been suggested to be TLR2 ligands [29, 68]. To further identify the *P. acnes* ligands, a comprehensive proteome study of *P. acnes* strain ATCC 6919 may offer good starting materials. It covers proteins from all fractions of *P. acnes*, including cell surface, secreted and intracellular proteins [128].

3.1.3. Implications for *P. acnes*-associated diseases

TLR2 heterodimers may be a potential therapeutic target for the treatment of inflammatory acne vulgaris. TLR2 heterodimer antagonists such as CU-CPT22 may have wide spectrum inhibitory effects, since the predicted antagonist binding site is only located in TLR2 [35]. Modification of these modulators to increase the competitive capability is likely leading to more specific antagonists.

Polymorphisms in genes encoding TLR1, TLR2, TLR6, and their signaling adaptor protein Mal/TIRAP affect susceptibility to diseases [129]. For example, polymorphisms in TLR1, TLR2 and TLR6 increase susceptibility to skin infections caused by *Staphylococcus aureus*, *Enterococcus faecalis*, etc. [130]. Polymorphisms in TLR2 Arg677Trp and Arg753Gln showed no association with acne in Caucasian subjects [131], in contrast, TLR2 Arg753Gln genetic polymorphisms are involved in acne pathogenesis in Chinese Han patients [132]. The role of TLR1 or TLR6 polymorphisms is not yet uncovered. Given the necessity of TLR1/6 in *P. acnes* recognition, it is presumably that polymorphisms in TLR1 or TLR6 gene play roles in *P. acnes* infection.

In addition, *P. acnes* is associated with non-skin site diseases ranging from inflammation, post-operative and device-related infections to prostate cancer [133]. This study may have implications for general *P. acnes*-mediated conditions. More precise approaches investigating closer related *P. acnes* strains should be taken into account, for instance, type IB and II are more often isolated from deep tissue

infections. Thus, using strains account for distinct diseases may bring insights to a better understanding of host recognition and immune responses.

3.2. Glucocorticoids regulate & interact with TLR2

The emerging "pro-inflammatory" or stimulatory effects have challenged the exclusive anti-inflammatory properties of GCs in recent years. Mimicking glucocorticoid-induced skin infections, especially acne, GCs were found to upregulate TLR2 expression and enhance TLR2-MyD88-TRAF6 signaling in a cell-specific manner. GCs' ability to interact with TLR2 ligands and corporately induce serum amyloid A 1/2 was also demonstrated.

3.2.1. Glucocorticoids cell type-specifically increase TLR2 expression

This work intended to elucidate the skin-site regulatory effects of GCs on TLR expression, focusing on the modulation of TLR2, TLR1 and TLR6 expression in different skin cells. Interestingly, among basal keratinocytes, differentiated keratinocytes and fibroblasts, notable GC-enhanced TLR2 expression was only observed in basal keratinocytes [45]. Likewise, in inflammatory skin diseases TLR2 expression was found highly increased towards the basal epidermis. This indicates that regarding TLR2 upregulation basal keratinocytes are more responsive to both GCs and inflammation.

Taking one step closer to inflammation, TNF and *P. acnes* were used to mimic inflammatory conditions. Although regarded as antagonistic regulators, GCs and TNF or *P. acnes* further increase TLR2 expression. The TLR2 promotor contains GREs and due to a concomitant activation of cytokine-induced pro-inflammatory transcription factors and GRs, opened chromatin sites allow GRs to access to GREs and elicit transcriptional responses [94]. Cytokines promote the transcriptional process of GCs by unwinding local DNA. The GC-responsive changes take place as a result of gene regulation by tethering or gene regulation at composite sites [90].

Consistent with previous studies, Dex induced MKP-1 expression in keratinocytes, which in turn inhibits MAPK pathways. Under *P. acnes*-induced inflammatory conditions, GC-increased TLR2 expression is negatively regulated by the JNK MAPK

pathway (*Figure 3-1*). TNF-induced TLR2 upregulation is differently mediated via p38 MAPK, which reflects activation of different signaling pathways by TNF and *P. acnes*.

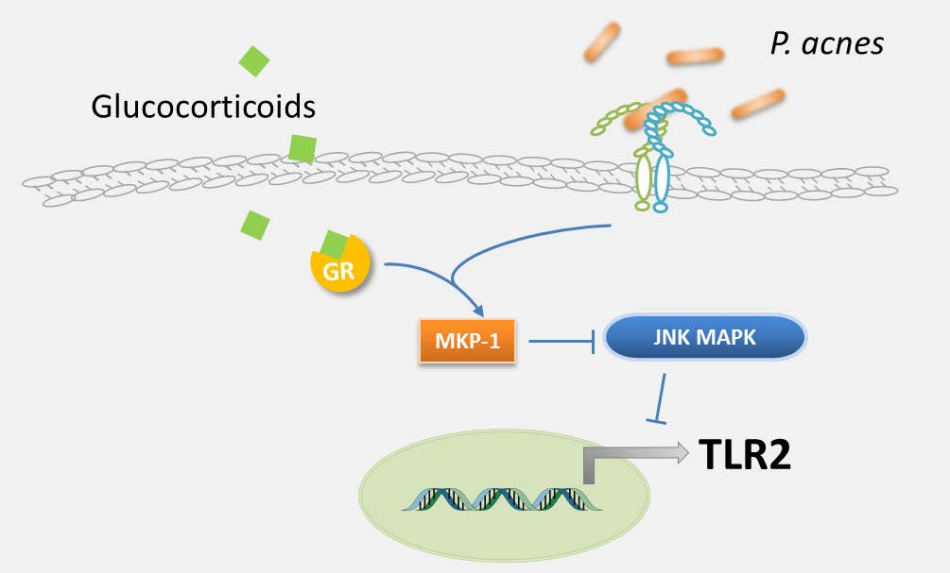

Figure 3-1

GCs and P. acnes-induced TLR2 expression undergoes MKP-1 induction, deactivating JNK MAPK pathway which negatively regulator of TLR2 expression.

3.2.2. Glucocorticoid-decreased TLR1 and TLR6 expression

Although TLR1 and TLR6 are heterodimer partners for TLR2, their expression profiles are relatively less characterized. Unexpectedly, TLR1 and TLR6 are differently regulated by GCs. The expression of both TLR1 and TLR6 are reduced by GCs or in combination with of TNF. Given this observation, GCs are presumably reinforcing host immunity by upregulating TLR2 [56]. To prevent overwhelming TLR2 signaling impairing the homeostasis, TLR1 and TLR6 expression are oppositely regulated to balance TLR2 responses. Notably, the expression ratio of TLR1: TLR2: TLR6 is approximately 10: 40: 1 in keratinocytes [134]. Therefore, regulating the expression of TLR1 and TLR6 is an efficient control over TLR2 signaling.

3.2.3. Glucocorticoid-induced TLR2 signaling

As one of the key experiments in this study, co-immunoprecipitation revealed that GC-enhanced TLR2 expression is functional to mediate signaling through MyD88 recruitment. MyD88 was revealed to form a large signaling complex (called

Myddosome) by hierarchical recruitment, consisting of death domains of six MyD88, four IRAK4 and four IRAK2 or the related IRAK1 [135]. Assembly of these Myddosome complexes allows the phosphorylation and activation of kinase domains of IRAKs (*Figure 3-2*). Once phosphorylated, IRAK1/2 leaves the complex and interacts with TRAF6 to elicit downstream signaling [136]. Increased TRAF6 protein levels indicate initiation of TLR2 signaling pathways in GC-treated cells upon TLR2 activation.

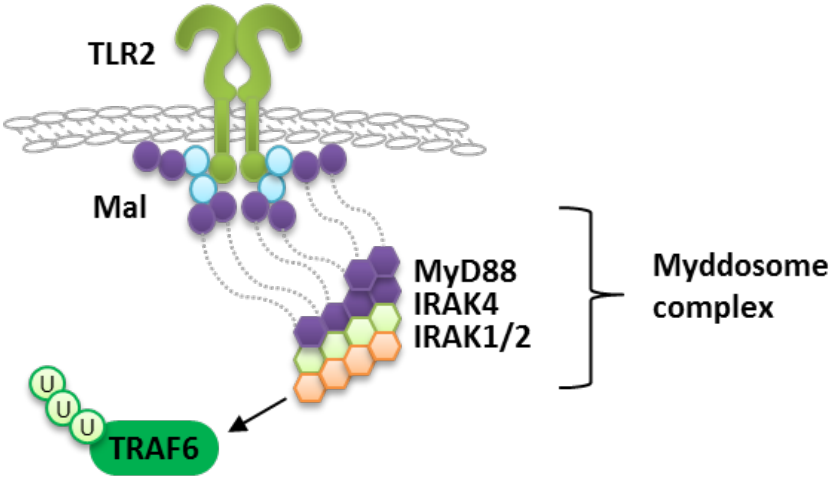

Figure 3-2

Formation of the Myddosome complex and initiation of TRAF6 ubiquitination. Activated TLR2 dimers recruit Mal, which in turn recruit Myddosome complex containing MyD88, IRAK4 and IRAK1/2 and subsequently activate TRAF6.

The present study demonstrates that GC-increased TLR2 expression is functional to initiate TLR2 signaling, which seems to be TLR2 homodimer-specific but not TLR2/1 or TLR2/6 heterodimer-mediated. This is likely due to GC-downregulated TLR1 and TLR6 expression. Although TLR2-MyD88-TRAF6 pathway is activated, subsequent signaling might be blocked by GCs through inducing negative regulators. For example, GCs induce IRAK-M as a critical negative competitor of MyD88-IRAK1/2 activation [137]. This warrants further investigation to identify potential inflammatory mediators downstream of TLR2-MyD88-TRAF6 signaling.

3.2.4. GC-induced SAA1/2 and its mechanisms

To induce steroid acne *in vivo* GCs are applied on the back of healthy adults [88]. In the present study, such regimen (dexamethasone + *P. acnes*) may mimic *in vivo*

inflammatory conditions where GCs are topical applied to skin areas rich of resident *P. acnes*. Of high relevance to the pathogenesis of steroid acne, *P. acnes* leads to strong SAA1/2 induction in combination with GCs in primary human keratinocytes. Accumulating data have demonstrated the cytokine-like properties of SAA1/2, such as inducing IL-1β in keratinocytes [119], neutrophils [124], monocytes and macrophages [120]. Although it is less likely that dermal fibroblasts encounter *P. acnes in vivo*, keratinocyte-produced SAA1 is able to stimulate pro-inflammatory cytokines in fibroblasts [101]. Taken together, it is tempting to speculate that topical treatment of GCs can induce local inflammation in the presence of *P. acnes*.

The expression of SAA1/2 in keratinocytes stimulated with GCs under TNF-induced sterile inflammatory conditions was assessed. Likewise, GCs induced SAA1/2 in cooperation with TNF. Interestingly, regarding the underlying molecular mechanisms, both similarities and differences have been observed between *P. acnes* and TNF corporately modulating SAA1/2 production with GCs.

First, both the induction of SAA1 by GCs alone or combined with TNF/*P. acnes* are strictly GR-dependent. Second, transcription factors STAT3 and NF-κB are involved in SAA1 production. *P. acnes* activates NF-κB phosphorylation and induces subsequent STAT3-Tyr705 phosphorylation which interacts with ligand-bound GR by forming a transactivation complex. In the nucleus, the complex binds to GREs in the SAA1 promoter with STAT3 and p300 serving as co-activators (*Figure 3-3*). Although the hypothesis proposed a complex with STAT3, NF-κB p65 and p300 binding to NF-κB response element in SAA1/2 promotors, phospho-NF-κB was not detected in the complex. Probably, low-affinity or transient interactions of phospho-STAT3 with phospho-NF-κB hampered the detection. Overexpression with tagged protein of interests may facilitate the detection. Alternatively, phospho-NF-κB only functions as an upstream mediator of phospho-STAT3 contributing to SAA1 transcription. To confirm these assumptions, methods such as electrophoretic mobility shift assay or chromatin immunoprecipitation assay could be considered to examine the binding of transcription factors to the SAA1 promotor. The contribution of other transcription factors and transcriptional coactivators is not excluded, e.g. CCAAT/enhancer binding

proteins. In addition, the characterization of underlying mechanisms also uncovered new aspects of the involvement of TLR2, which will be discussed later.

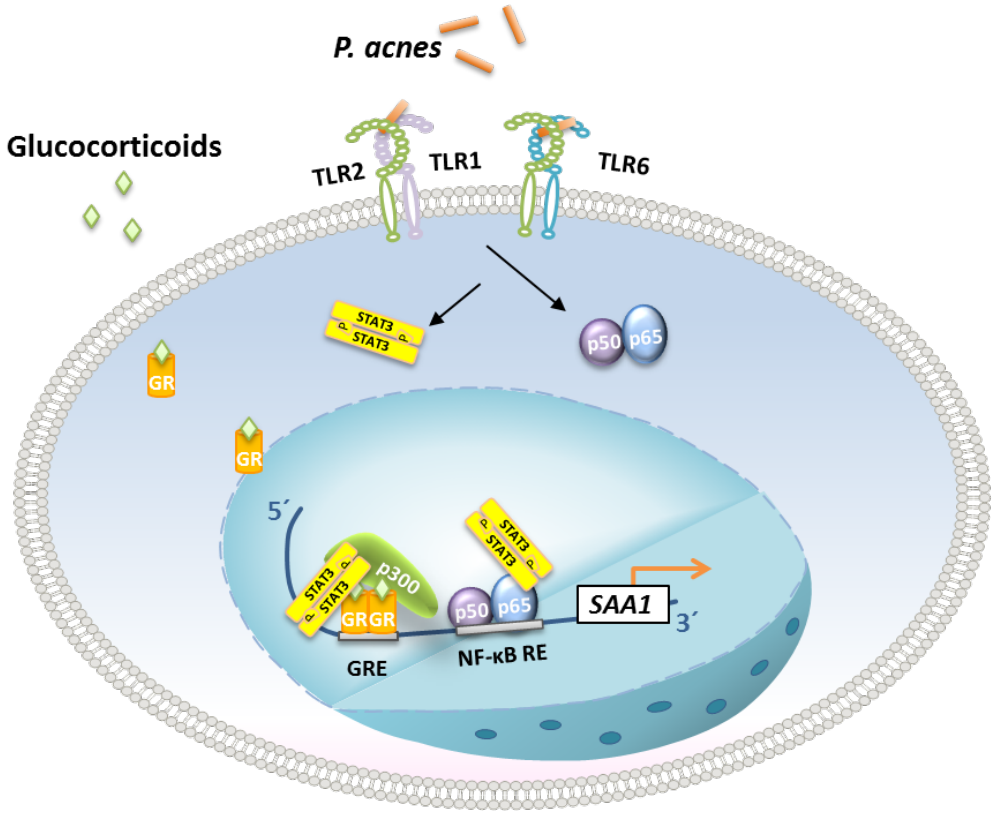

Figure 3-3

Proposed mechanisms of Glucocorticoids corporately induction of SAA1 with P. acnes.

There is considerable heterogeneity between cell types in respect to SAA1/2 induction. GCs-stimulated primary keratinocytes, but neither fibroblasts nor HaCaT cells produced SAA1. In the presence of *P. acnes* or TNF, keratinocytes and TR146 epithelial cells both substantially enhanced SAA1 production. A relatively low induction of SAA1 in GCs and *P. acnes*-treated HaCaT keratinocytes may be linked to the cell type-specific regulation of TLR2 expression by GCs. The findings that GCs failed to induce TLR2 expression in HaCaT cells indicate a possible connection to the weak SAA1 induction [10]. Furthermore, because of the biochemical defects of MAPK pathways in HaCaT cells, the differences observed in HaCaT cells suggest a possible contribution of MAPK pathways in SAA1/2 secretion, which requires further studies.

Further *in vitro* studies of SAA1 expression could consider using combinations of different cytokines or in combination with GCs. These approaches could be designed to accurately reflect the sequential mobilization of individual cytokines at different stages of the *in vivo* inflammatory cascade. In addition to the cooperation of TNF with Dex to induce SAA1 production, IL-1β seems to be a good candidate to be investigated to extend the understanding of multifactor-involved local skin situations.

3.2.5. The role of TLR2 in the immune-GC crosstalk

The role of TLR2 has been extended in different aspects. Here, TLR2 modulation has implicated in a positive feedback loop when keratinocytes encounter *P. acnes*. Skin has been long termed as a peripheral site expressing the genes essential for the synthesis of corticosteroids. The skin site GC synthesis organizes skin immunity independently from the central HPA axis. For example, *P. acnes*-induced local CRH expression may promote inflammatory responses in keratinocytes [138, 139]. Moreover, several major metabolic enzymes participate in the modulation of local GC synthesis. A corresponding observation in this study is that *P. acnes* upregulated the expression of 11β-hydroxysteroid dehydrogenase (11βHSD) type 1, a GC activating enzyme. Conceivably, the feedback mechanism leads to endogenous GC-induced TLR2 enhancement in keratinocytes, which in turn reinforces the recognition of *P. acnes* (*Figure 3-4*). The precise role of topical applied GCs is likely to exacerbate the feedback pathways, since 11βHSD1 is synergistically increased by IL-1α and GCs in epithelial cells [140].

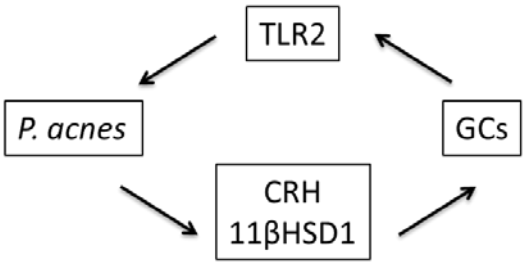

Figure 3-4

TLR2 upregulation as a component of positive feedback loop upon exposure to P. acnes. In the proposed model, *P. acnes* may lead to skin site glucocorticoid (GC) synthesis by induction of corticotropin-releasing hormone (CRH) and 11β-hydroxysteriod dehydrogenase enzyme (11βHSD1). GC-upregulated TLR2 maintains this circle as pattern recognition receptor for *P. acnes*.

3.2.6. The role of TLR2 in terms of SAA1/2 induction

The consequences of GC-immunity interaction is no longer simply considered as upregulation of TLR2 expression. Here more aspects of TLR2 have been demonstrated and discussed. TLR2 heterodimerizes with TLR1/6 to recognize *P. acnes* and then triggers the phosphorylation of NF-κB and STAT3. Phosphorylated transcription factors translocate into the nucleus and interact with GC-bound GRs, thereby inducing the transcription of SAA1/2. Furthermore, the combination of GCs and *P. acnes* increase TLR2 expression allowing enhanced TLR2 recognition and signaling. In addition, as an endogenous TLR2 ligand, SAA1 may induce a network of cytokines and chemokines, as well as the production of its own (*Figure 3-5*).

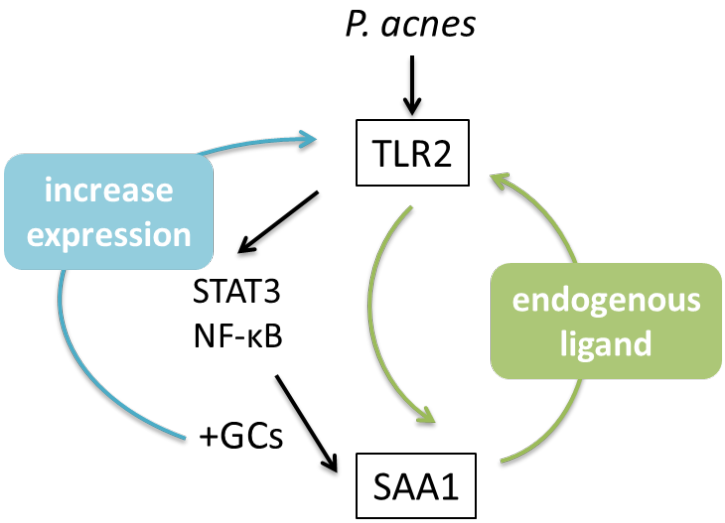

Figure 3-5

Schematics of TLR2-mediated GCs and P. acnes-induced SAA1. The model depicts the roles of TLR2 in SAA1 induction: TLR2 recognizes *P. acnes,* triggers STAT3-NF-κB activation and induces SAA1 in corporation with GCs; GCs and *P. acnes*-increased TLR2 expression leads to enhanced signaling; acing as ligand for TLR2, SAA1 may maintain its own induction.

Controversy exists concerning GC-decreased TLR1/6 expression and GCs plus *P. acnes*-induced SAA1 production, since TLR2 heterodimers are essential for *P. acnes* recognition. In fact, these two seemingly disparate processes take place in different time-windows. At the first moment when GCs and *P. acnes* are applied to

keratinocytes, GCs bind to GRs and *P. acnes* induces NF-κB and STAT3 phosphorylation, leading to SAA1 transcription within 2 hours, while GC-induced TLR1/6 downregulation needs at least 6 hours. Additionally, post-transcriptional mechanisms customize the translational efficiency of SAA1/2 including regulation of mRNA stability, control over ribosome migration, and secondary cytokine cascades affect the post-transcriptional fate of SAA1/2 mRNA [141].

Finally, there are some directions for future studies. For instance, this study only demonstrated the mRNA levels of SAA2, while SAA2 was noticed to be one of the most upregulated genes in acne lesions. Besides, *P. acnes*-induced STAT3 phosphorylation is an incomplete topic requiring further investigation. In light of the more knowledge of it, STAT3 is possible to be a therapeutic target to redirect skin inflammation. Moreover, since STAT3 is activated through phosphorylation of both tyrosine and serine, it is still unknown whether *P. acnes* induces phospho-STAT3-Ser727, and if so, how phosphorylated STAT3-Ser contributes to SAA1 and as to the entire *P. acnes*-triggered immune responses.

4. CONCLUSIONS & FUTURE DIRECTIONS

These results demonstrate that TLR1 and TLR6 contribute to the detection of *P. acnes* in human cells. Further research may focus on the identification of *P. acnes*-derived ligands and the discovery of targeting compounds as potential options for treatment of inflammatory acne lesions.

Given the presented cell-specific regulatory effects of GCs on TLR expression and the cooperation with *P. acnes* in SAA1/2 induction, it is reasonable to link the findings to GC-induced acne. *In vivo* confirmation of the SAA1/2 levels in steroid acne could ensure the implication of GC-primed TLR2 enhancement and GC-TLR2-cooperated SAA1/2 induction in clinical conditions. Furthermore, analysis of the underlying mechanisms suggests interaction between GRs and *P. acnes*-induced STAT3 activation. Thus, it would be interesting to know whether *P. acnes* induces not only tyrosine but also serine phosphorylation; if so, whether phospho-STAT3-Ser727 functions as co-transcription factor or mediates mitochondrial regulation. STAT3 appears to play an important role in pro-inflammatory responses to bacteria. However, *P. acnes*-induced cytokine production as a result of STAT3 activation has remained poorly characterized, such as *P. acnes*-induced IL-1β secretion in monocytes and IL-17A release in T cells. Further approaches may determine whether targeting STAT3 is a therapy for acne.

5. SUMMARY

Topical treatment of glucocorticoids can provoke skin diseases such as acne vulgaris. This clinical observation has drawn attention to the paradoxical effect of Glucocorticoids. Glucocorticoid-enhanced Toll-like receptor (TLR) 2 expression has been suggested to play a role in steroid-induced skin inflammation. However, the modulatory effects of glucocorticoids on TLR2 expression and signaling in various cutaneous cells are still largely unknown. Furthermore, whether the cooperative effect of glucocorticoids with the Gram-positive bacterium *Propionibacterium acnes*, which is thought to contribute to the pathogenesis of acne, leads to the induction of pro-inflammatory mediators remains to be determined.

TLR2 recognition mediates host responses to *P. acnes*. Distinct from *P. acnes* recognition by TLR2 homodimers in mice, the present thesis demonstrates that TLR2 heterodimers is a prerequisite in human cells. This species-specific recognition of *P. acnes* by TLR2 heterodimers might be exploited as drug targets for the control of inflammatory responses.

Studies on the regulatory effects of glucocorticoids revealed keratinocyte-specific modulation of TLR2 expression, as dexamethasone increased TLR2 expression mainly in undifferentiated and less in calcium-induced differentiated keratinocytes but not in HaCaT cells or fibroblasts. Concurrent applied with TNF (tumor necrosis factor) or *P. acnes,* glucocorticoids further increase TLR2 in keratinocytes. Unlike under TNF-induced inflammatory conditions, glucocorticoid-enhanced TLR2 expression is negatively regulated by JNK (c-Jun N-terminal kinase) MAPK (mitogen-activated protein kinase) pathways under *P. acnes* stimulation. In contrast to increased TLR2 expression, glucocorticoids reduce TLR1 and TLR6 in the absence or presence of TNF presumably to maintain homeostasis of TLR2 signaling. In addition, glucocorticoid-induced TLR2 expression can result in functional TLR2 signaling in response to TLR2 ligands, i.e. glucocorticoid pre-stimulation increases MyD88 (myeloid differentiation primary response gene 88) recruitment and expression of adaptor protein TRAF6 (tumor necrosis factor receptor-associated factor 6).

Further approaches examined the cooperative effects of glucocorticoids with *P. acnes* in terms of pro-inflammatory mediator release. Serum amyloid A (SAA) 1/2 have been implicated in numerous inflammatory disorders and can be induced by pro-inflammatory cytokines, lipopolysaccharide and glucocorticoids. The present study demonstrates that glucocorticoids and *P. acnes* cooperate in SAA1/2 induction, as a result of *P. acnes*-activated NF-κB (nuclear factor-κB) and STAT3 (signal transducer and activator of transcription 3) in complex with glucocorticoid-bound glucocorticoid receptors and transcription factor p300. Glucocorticoids and *P. acnes* synergistically increase MAP kinase phosphatase-1, thereby mediating TLR2 enhancement as well as SAA1/2 induction. Furthermore, TNF-induced sterile inflammation interacts with glucocorticoids to increase SAA1/2 which is also contributed by NF-κB and STAT3 activation. These findings are of high relevance to the pathogenesis of glucocorticoid-induced acne.

Collectively, the results bring novel insights into the crosstalk between glucocorticoids and TLRs. In respect of TLR2, *P. acnes* is sensed by TLR2 heterodimers and activates NF-κB and STAT3 interacting with glucocorticoid receptors, which in turn induces SAA1/2. The combination of glucocorticoids and *P. acnes* enhance TLR2 expression and signaling that probably contributes to increased TLR2 recognition. As an endogenous TLR2 ligand, SAA1 might function through increased and activated TLR2 to induce further SAA1 production, as well as a network of inflammatory responses. In light of these findings, discovering small molecules targeting TLR2 dimers and STAT3 are potential therapeutic strategies for the treatment of *P. acnes*-induced acne and steroid acne.

6. ZUSAMMENFASSUNG

Die topische Applikation von Glukokortikoiden kann Hauterkrankungen wie Akne vulgaris hervorrufen. Diese klinische Beobachtung hat auf den paradoxen Effekt der Glukokortikoide aufmerksam gemacht. Die durch Glukokortikoide hochregulierte Expression von Toll-like Rezeptor (TLR) 2 soll eine Rolle bei steroid-induzierten kutanen Entzündungen spielen. Dennoch sind die modulierenden Effekte von Glukokortikoiden auf TLR2 Expression und Signalwege in verschiedenen kutanen Zellen noch weitgehend unbekannt. Zusätzlich gilt es aufzuklären, ob der kooperative Effekt von Glukokortikoiden mit dem Gram-positiven Bakterium *Propionibacterium acnes*, das an der Akne-Pathogenese beteiligt sein soll, zur Induktion von pro-inflammatorischen Mediatoren führt.

Die körpereigene Immunantwort auf *P. acnes* wird über Erkennung durch TLR2 vermittelt. Diese Arbeit zeigt, dass im Unterschied zur Erkennung von *P. acnes* in Mäusen durch TLR2 Homodimere, die Erkennung in menschlichen Zellen durch TLR2 Heterodimere erfolgt. Diese Spezies-spezifische Erkennung von *P. acnes* durch TLR2 Heterodimere stellt daher einen potenziellen therapeutischen Ansatzpunkt dar, um entzündliche Reaktionen zu kontrollieren.

Versuche zu regulatorischen Effekten von Glukokortikoiden zeigten eine keratinozyten-spezifische Modulation der TLR2 Expression, da Dexamethason die TLR2 Expression hauptsächlich in undifferenzierten und weniger in Calcium-induzierten differenzierten Keratinozyten erhöhte, dagegen aber nicht in HaCaT Zellen oder Fibroblasten. Noch stärker erhöhen Glukokortikoide die TLR2 Expression in Keratinozyten zusammen mit TNF (Tumornekrosefaktor) oder *P. acnes*. Anders als bei Entzündungsprozessen, die durch TNF hervorgerufen werden, wird die durch Glukokortikoide erhöhte Expression von TLR2 bei gleichzeitiger Stimulation mit *P. acnes* negativ reguliert durch JNK (c-Jun N-terminale Kinase) MAPK (Mitogen-aktivierte Proteinkinase) Signalwege. Andererseits verringern Glukokortikoide die TLR1 und TLR6 Expression in Ab- oder Anwesenheit von TNF - vermutlich, um die TLR2 Signaltransduktion im Gleichgewicht zu halten. Zusätzlich kann die durch Glukokortikoide hervorgerufene TLR2 Expression als Reaktion auf TLR2 Liganden zu

funktioneller TLR2 Signaltransduktion führen. So steigert eine Vorstimulation mit Glukokortikoiden die Rekrutierung von MyD88 (myeloid differentiation primary response gene 88) und Expression des Adaptorproteins TRAF6 (Tumornekrosefaktor Rezeptor-assoziierter Faktor 6).

Ein weiterer Ansatz war die Untersuchung kooperative Effekte von Glukokortikoiden mit *P. acnes* bezüglich der Freisetzung pro-inflammatorischer Mediatoren. Serum Amyloid A (SAA) 1/2 soll eine Rolle in zahlreichen entzündlichen Erkrankungen zukommen. Sie können beispielsweise durch pro-inflammatorische Zytokine, Lipopolysaccharide und Glukokorticoide induziert werden. Die vorliegende Arbeit zeigt, dass Glukokortikoide und *P. acnes* bei der SAA1/2-Induzierung zusammenwirken, was aus *P. acnes*-aktiviertem NF-κB und STAT3 (signal transducer and activator of transcription) im Komplex mit Glukokortikoid-gebundenem Glukokortikoid-Rezeptor und dem Transkriptionsfaktor p300 resultiert. Glukokortikoide und *P. acnes* steigern synergistisch die MAP Kinase Phosphatase-1, wobei sie sowohl Hochregulierung von TLR2 als auch die Induzierung von SAA1/2 vermitteln. Darüber hinaus kann auch eine sterile Entzündung, ausgelöst durch TNF, mit Glukokortikoiden interagieren, um SAA1/2 zu erhöhen. Auch hier sind NF-κB und STAT3 Aktivierung involviert. Diese Ergebnisse sind hoch relevant für die Pathogenese der Glukokortikoid-induzierten Akne.

Zusammengefasst werden in dieser Arbeit neue Einblicke in das Zusammenspiel von Glukokortikoiden und Toll-like-Rezeptoren gegeben. *P. acnes* wird durch TLR2 Heterodimere detektiert und aktiviert NF-κB und STAT3 im Zusammenspiel mit Glukokortikoidrezeptoren, was wiederum SAA1/2 induziert. Die Kombination aus Glukokortikoiden und *P. acnes* erhöht die TLR2 Expression und Signaltransduktion, was vermutlich zur gesteigerten TLR2 Erkennung beiträgt. Als endogener Ligand könnte SAA1 über Erhöhung und Aktivierung von TLR2 wirken, um weiterhin die SAA1 Produktion und ein Netzwerk aus entzündlichen Reaktionen zu induzieren. Unter Berücksichtigung dieser Ergebnisse dürfte die Entdeckung von kleinen Molekülen, die TLR2 Dimere und STAT3 als Zielstrukturen besitzen, potenzielle therapeutische Strategien für die Behandlung von *P. acnes*-induzierter Akne und Steroidakne darstellen.

References

1. S. Akira, K. Takeda, T. Kaisho, Toll-like receptors: critical proteins linking innate and acquired immunity, Nature Immunology 2(8) (2001) 675-680.
2. L.A.J. O'Neill, D. Golenbock, A.G. Bowie, The history of Toll-like receptors-redefining innate immunity, Nature Reviews Immunology 13(6) (2013) 453-460.
3. C. Erridge, Endogenous ligands of TLR2 and TLR4: agonists or assistants?, Journal of Leukocyte Biology 87(6) (2010) 989-999.
4. F.L. Rock, G. Hardiman, J.C. Timans, R.A. Kastelein, J.F. Bazan, A family of human receptors structurally related to Drosophila Toll, Proceedings of the National Academy of Sciences 95(2) (1998) 588-593.
5. O. Takeuchi, T. Kawai, H. Sanjo, N.G. Copeland, D.J. Gilbert, N.A. Jenkins, K. Takeda, S. Akira, TLR6: A novel member of an expanding Toll-like receptor family, Gene 231(1–2) (1999) 59-65.
6. T.H. Flo, Ø. Halaas, E. Lien, L. Ryan, G. Teti, D.T. Golenbock, A. Sundan, T. Espevik, Human Toll-like receptor 2 mediates monocyte activation by *Listeria monocytogenes*, but not by group B *Streptococci* or lipopolysaccharide, The Journal of Immunology 164(4) (2000) 2064-2069.
7. W.J. Wiersinga, C.W. Wieland, M.C. Dessing, N. Chantratita, A.C. Cheng, D. Limmathurotsakul, W. Chierakul, M. Leendertse, S. Florquin, A.F. de Vos, N. White, A.M. Dondorp, N.P. Day, S.J. Peacock, T. van der Poll, Toll-like receptor 2 impairs host defense in Gram-negative sepsis caused by *Burkholderia pseudomallei* (melioidosis), PLOS Medicine 4(7) (2007) 1268-1280.
8. K. Farhat, S. Riekenberg, H. Heine, J. Debarry, R. Lang, J. Mages, U. Buwitt-Beckmann, K. Röschmann, G. Jung, K.-H. Wiesmüller, A.J. Ulmer, Heterodimerization of TLR2 with TLR1 or TLR6 expands the ligand spectrum but does not lead to differential signaling, Journal of Leukocyte Biology 83(3) (2008) 692-701.
9. T. Nakata, M. Yasuda, M. Fujita, H. Kataoka, K. Kiura, H. Sano, K. Shibata, CD14 directly binds to triacylated lipopeptides and facilitates recognition of the lipopeptides by the receptor complex of Toll-like receptors 2 and 1 without binding to the complex, Cellular Microbiology 8(12) (2006) 1899-1909.
10. M. Triantafilou, F.G.J. Gamper, R.M. Haston, M.A. Mouratis, S. Morath, T. Hartung, K. Triantafilou, Membrane sorting of Toll-like receptor (TLR)-2/6 and TLR2/1 heterodimers at the cell surface determines heterotypic associations with CD36 and intracellular targeting, Journal of Biological Chemistry 281(41) (2006) 31002-31011.
11. K. Hoebe, P. Georgel, S. Rutschmann, X. Du, S. Mudd, K. Crozat, S. Sovath, L. Shamel, T. Hartung, U. Zahringer, B. Beutler, CD36 is a sensor of diacylglycerides, Nature 433(7025) (2005) 523-527.
12. T. Abe, M. Shimamura, K. Jackman, H. Kurinami, J. Anrather, P. Zhou, C. Iadecola, Key role of CD36 in Toll-Like Receptor 2 signaling in cerebral ischemia, Stroke; a journal of cerebral circulation 41(5) (2010) 898-904.
13. Y. Guan, D.R.E. Ranoa, S. Jiang, S.K. Mutha, X. Li, J. Baudry, R.I. Tapping, Human TLRs 10 and 1 share common mechanisms of innate immune sensing but not signaling, The Journal of Immunology 184(9) (2010) 5094-5103.
14. M. Oosting, S.C. Cheng, J.M. Bolscher, R. Vestering-Stenger, T.S. Plantinga, I.C. Verschueren, P. Arts, A. Garritsen, H. van Eenennaam, P. Sturm, B.J. Kullberg, A.

Hoischen, G.J. Adema, J.W.M. van der Meer, M.G. Netea, L.A.B. Joosten, Human TLR10 is an anti-inflammatory pattern-recognition receptor, Proceedings of the National Academy of Sciences of the United States of America 111(42) (2014) 4478-4484.
15. L. de Oliviera Nascimento, P. Massari, L. Wetzler, The role of TLR2 in infection and immunity, Frontiers in Immunology 3(79) (2012) 1-17.
16. L.H. Travassos, S.E. Girardin, D.J. Philpott, D. Blanot, M.A. Nahori, C. Werts, I.G. Boneca, Toll-like receptor 2-dependent bacterial sensing does not occur via peptidoglycan recognition, EMBO Reports 5(10) (2004) 1000-1006.
17. M.A. Müller-Anstett, P. Müller, T. Albrecht, M. Nega, J. Wagener, Q. Gao, S. Kaesler, M. Schaller, T. Biedermann, F. Götz, Staphylococcal peptidoglycan co-localizes with Nod2 and TLR2 and activates innate immune response via both receptors in primary murine keratinocytes, PLOS ONE 5(10) (2010) 1-13.
18. J. van Bergenhenegouwen, T.S. Plantinga, L.A.B. Joosten, M.G. Netea, G. Folkerts, A.D. Kraneveld, J. Garssen, A.P. Vos, TLR2 & Co: a critical analysis of the complex interactions between TLR2 and coreceptors, Journal of Leukocyte Biology 94(5) (2013) 885-902.
19. M.S. Jin, S.E. Kim, J.Y. Heo, M.E. Lee, H.M. Kim, S.G. Paik, H. Lee, J.O. Lee, Crystal structure of the TLR1-TLR2 heterodimer induced by binding of a tri-acylated lipopeptide, Cell 130(6) (2007) 1071-1082.
20. J.Y. Kang, X. Nan, M.S. Jin, S.J. Youn, Y.H. Ryu, S. Mah, S.H. Han, H. Lee, S.-G. Paik, J.O. Lee, Recognition of lipopeptide patterns by Toll-like receptor 2-Toll-like receptor 6 heterodimer, Immunity 31(6) (2009) 873-884.
21. J.Y. Kang, J.O. Lee, Structural biology of the Toll-like receptor family, Annual Review of Biochemistry 80(1) (2011) 917-941.
22. Grabiec, G. Meng, S. Fichte, W. Bessler, H. Wagner, C.J. Kirschning, Human but not murine Toll-like receptor 2 discriminates between tri-palmitoylated and tri-lauroylated peptides, Journal of Biological Chemistry 279(46) (2004) 48004-48012.
23. T. Horng, G.M. Barton, R.A. Flavell, R. Medzhitov, The adaptor molecule TIRAP provides signalling specificity for Toll-like receptors, Nature 420(6913) (2002) 329-333.
24. M. Yamamoto, S. Sato, H. Hemmi, H. Sanjo, S. Uematsu, T. Kaisho, K. Hoshino, O. Takeuchi, M. Kobayashi, T. Fujita, K. Takeda, S. Akira, Essential role for TIRAP in activation of the signalling cascade shared by TLR2 and TLR4, Nature 420(6913) (2002) 324-329.
25. B. Verstak, K. Nagpal, S.P. Bottomley, D.T. Golenbock, P.J. Hertzog, A. Mansell, MyD88 adapter-like (Mal)/TIRAP interaction with TRAF6 is critical for TLR2- and TLR4-mediated NF-κB proinflammatory responses, Journal of Biological Chemistry 284(36) (2009) 24192-24203.
26. T. Kawai, O. Takeuchi, T. Fujita, J.-i. Inoue, P.F. Mühlradt, S. Sato, K. Hoshino, S. Akira, Lipopolysaccharide stimulates the MyD88-independent pathway and results in activation of IFN-regulatory factor 3 and the expression of a subset of lipopolysaccharide-inducible genes, The Journal of Immunology 167(10) (2001) 5887-5894.
27. M. Puliti, S. Uematsu, S. Akira, F. Bistoni, L. Tissi, Toll-Like Receptor 2 deficiency is associated with enhanced severity of group B Streptococcal disease, Infection and Immunity 77(4) (2009) 1524-1531.
28. Y. Shen, I. Kawamura, T. Nomura, K. Tsuchiya, H. Hara, S.R. Dewamitta, S. Sakai, H. Qu, S. Daim, T. Yamamoto, M. Mitsuyama, Toll-like receptor 2- and MyD88-dependent phosphatidylinositol 3-kinase and Rac1 activation facilitates the phagocytosis of

Listeria monocytogenes by murine macrophages, Infection and Immunity 78(6) (2010) 2857-2867.
29. I. Nagy, A. Pivarcsi, A. Koreck, M. Szell, E. Urban, L. Kemeny, Distinct strains of *Propionibacterium acnes* induce selective human beta-defensin-2 and interleukin-8 expression in human keratinocytes through Toll-like receptors, Journal of Investigative Dermatology 124(5) (2005) 931-938.
30. M. Murata, Activation of Toll-like receptor 2 by a novel preparation of cell wall skeleton from Mycobacterium bovis BCG Tokyo (SMP-105) sufficiently enhances immune responses against tumors, Cancer Science 99(7) (2008) 1435-1440.
31. M. Reilly, R.M. Miller, M.H. Thomson, V. Patris, P. Ryle, L. McLoughlin, P. Mutch, P. Gilboy, C. Miller, M. Broekema, B. Keogh, W. McCormack, J. van de Wetering de Rooij, Randomized, double-blind, placebo-controlled, dose-escalating phase I, healthy subjects study of intravenous OPN-305, a humanized anti-TLR2 antibody, Clinical Pharmacology and Therapeutics 94(5) (2013) 593-600.
32. J. Schmidt, T. Welsch, D. Jäger, P.F. Mühlradt, M.W. Büchler, A. Märten, Intratumoural injection of the Toll-like receptor-2/6 agonist 'macrophage-activating lipopeptide-2' in patients with pancreatic carcinoma: a phase I/II trial, British Journal of Cancer 97(5) (2007) 598-604.
33. K. Cheng, M. Gao, J.I. Godfroy, P.N. Brown, N. Kastelowitz, H. Yin, Specific activation of the TLR1-TLR2 heterodimer by small-molecule agonists, Science Advances 1(3) (2015) 1-12.
34. K. Cheng, X. Wang, S. Zhang, H. Yin, Discovery of small molecule inhibitors of the TLR1-TLR2 complex, Angewandte Chemie (International ed. in English) 51(49) (2012) 12246-12249.
35. S. Bock, M.S. Murgueitio, G. Wolber, G. Weindl, Acute myeloid leukaemia-derived Langerhans-like cells enhance Th1 polarization upon TLR2 engagement, Pharmacological Research 105 (2016) 44-53.
36. H.D. de Koning, D. Rodijk-Olthuis, I.M.J.J. van Vlijmen-Willems, L.A.B. Joosten, M.G. Netea, J. Schalkwijk, P.L.J.M. Zeeuwen, A comprehensive analysis of pattern recognition receptors in normal and inflamed human epidermis: upregulation of Dectin-1 in psoriasis, Journal of Investigative Dermatology 130(11) (2010) 2611-2620.
37. G. Solanas, S.A. Benitah, Regenerating the skin: a task for the heterogeneous stem cell pool and surrounding niche, Nature Reviews Molecular Cell Biology 14(11) (2013) 737-748.
38. E.A. Grice, J.A. Segre, The skin microbiome, Nature Reviews Microbiology 9(4) (2011) 244-253.
39. L.E. Tracy, R.A. Minasian, E.J. Caterson, Extracellular matrix and dermal fibroblast function in the healing wound, Advances in Wound Care 5(3) (2016) 119-136.
40. S. Van Linthout, K. Miteva, C. Tschöpe, Crosstalk between fibroblasts and inflammatory cells, Cardiovascular Research 102(2) (2014) 258-269.
41. S.J. Flavell, T.Z. Hou, S. Lax, A.D. Filer, M. Salmon, C.D. Buckley, Fibroblasts as novel therapeutic targets in chronic inflammation, British Journal of Pharmacology 153(Suppl 1) (2008) 241-246.
42. A. Hari, T.L. Flach, Y. Shi, P.R. Mydlarski, Toll-like receptors: role in dermatological disease, Mediators of Inflammation 2010 (2010) 1-16.
43. M. Mempel, V. Voelcker, G. Köllisch, C. Plank, R. Rad, M. Gerhard, C. Schnopp, P. Fraunberger, A.K. Walli, J. Ring, D. Abeck, M. Ollert, Toll-Like receptor expression in human keratinocytes: nuclear factor κB controlled gene activation by *Staphylococcus*

aureus is Toll-like receptor 2 but not Toll-like receptor 4 or platelet activating factor receptor dependent, Journal of Investigative Dermatology 121(6) (2003) 1389-1396.
44. J.E. McInturff, R.L. Modlin, J. Kim, The role of Toll-like receptors in the pathogenesis and treatment of dermatological disease, Journal of Investigative Dermatology 125(1) (2005) 1-8.
45. R. Panzer, C. Blobel, R. Fölster-Holst, E. Proksch, TLR2 and TLR4 expression in atopic dermatitis, contact dermatitis and psoriasis, Experimental Dermatology 23(5) (2014) 364-366.
46. C. Yao, J. Oh, D.H. Lee, J. Bae, C.L. Jin, C. Park, J.H. Chung, Toll-like receptor family members in skin fibroblasts are functional and have a higher expression compared to skin keratinocytes, International Journal of Molecular Medicine 35(5) (2015) 1443-1450.
47. S. Hamidi, M. Schäfer-Korting, G. Weindl, TLR2/1 and sphingosine 1-phosphate modulate inflammation, myofibroblast differentiation and cell migration in fibroblasts, Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids 1841(4) (2014) 484-494.
48. L.S. Miller, Toll-like receptors in skin, Advances in dermatology 24 (2008) 71-87.
49. H.C. Williams, R.P. Dellavalle, S. Garner, Acne vulgaris, The Lancet 379 (2012) 361-372.
50. C.N. Collier, J.C. Harper, W.C. Cantrell, W. Wang, K.W. Foster, B.E. Elewski, The prevalence of acne in adults 20 years and older, Journal of the American Academy of Dermatology 58(1) (2008) 56-59.
51. J. Ayer, N. Burrows, Acne: more than skin deep, Postgraduate Medical Journal 82(970) (2006) 500-506.
52. S.K. Rathi, Acne vulgaris treatment : the current scenario, Indian Journal of Dermatology 56(1) (2011) 7-13.
53. S. Zahra Ghodsi, H. Orawa, C.C. Zouboulis, Prevalence, severity, and severity risk factors of acne in high school pupils: a community-based study, Journal of Investigative Dermatology 129(9) (2009) 2136-2141.
54. H. Brüggemann, The emerging role of propionibacteria in human health and disease, The human microbiota and chronic disease, John Wiley & Sons, Inc.2016, pp. 199-214.
55. M. Kistowska, S. Gehrke, D. Jankovic, K. Kerl, A. Fettelschoss, L. Feldmeyer, G. Fenini, A. Kolios, A. Navarini, R. Ganceviciene, J. Schauber, E. Contassot, L.E. French, IL-1beta drives inflammatory responses to *Propionibacterium acnes* in vitro and in vivo, Journal of Investigative Dermatology 134(3) (2014) 677-685.
56. M. Qin, A. Pirouz, M.-H. Kim, S.R. Krutzik, H.J. Garbán, J. Kim, *Propionibacterium acnes* induces IL-1β secretion via the NLRP3 inflammasome in human monocytes, Journal of Investigative Dermatology 134(2) (2014) 381-388.
57. Z.J. Li, D.K. Choi, K.C. Sohn, M.S. Seo, H.E. Lee, Y. Lee, Y.J. Seo, Y.H. Lee, G. Shi, C.C. Zouboulis, C.D. Kim, J.H. Lee, M. Im, *Propionibacterium acnes* activates the NLRP3 inflammasome in human sebocytes, Journal of Investigative Dermatology 134(11) (2014) 2747-2756.
58. W.H. Li, L. Zhang, P. Lyte, K. Rodriguez, D. Cavender, M.D. Southall, p38 MAP kinase inhibition reduces *Propionibacterium acnes*-induced inflammation in vitro, Dermatology and Therapy 5(1) (2015) 53-66.
59. M. Kistowska, B. Meier, T. Proust, L. Feldmeyer, A. Cozzio, T. Kuendig, E. Contassot, L.E. French, *Propionibacterium acnes* promotes Th17 and Th17/Th1 responses in acne patients, Journal of Investigative Dermatology 135(1) (2015) 110-118.

60. J.A. Sanford, L.J. Zhang, M.R. Williams, J.A. Gangoiti, C.M. Huang, R.L. Gallo, Inhibition of HDAC8 and HDAC9 by microbial short-chain fatty acids breaks immune tolerance of the epidermis to TLR ligands, Science Immunology 1(4) (2016) 1-10.
61. S. Fitz-Gibbon, S. Tomida, B.H. Chiu, L. Nguyen, C. Du, M. Liu, D. Elashoff, M.C. Erfe, A. Loncaric, J. Kim, R.L. Modlin, J.F. Miller, E. Sodergren, N. Craft, G.M. Weinstock, H. Li, *Propionibacterium acnes* strain populations in the human skin microbiome associated with acne, Journal of Investigative Dermatology 133(9) (2013) 2152-2160.
62. J.L. Johnson, C.S. Cummins, Cell wall composition and deoxyribonucleic acid similarities among the anaerobic coryneforms, classical Propionibacteria, and strains of Arachnia propionica, Journal of Bacteriology 109(3) (1972) 1047-1066.
63. Y. Yu, J. Champer, H. Garbán, J. Kim, Typing of *Propionibacterium acnes*: a review of methods and comparative analysis, British Journal of Dermatology 172(5) (2015) 1204-1209.
64. A. McDowell, S. Valanne, G. Ramage, M.M. Tunney, J.V. Glenn, G.C. McLorinan, A. Bhatia, J.F. Maisonneuve, M. Lodes, D.H. Persing, S. Patrick, *Propionibacterium acnes* Types I and II Represent Phylogenetically Distinct Groups, Journal of Clinical Microbiology 43(1) (2005) 326-334.
65. A. McDowell, A.L. Perry, P.A. Lambert, S. Patrick, A new phylogenetic group of Propionibacterium acnes, Journal of Medical Microbiology 57(2) (2008) 218-224.
66. M. Kilian, C.F.P. Scholz, H.B. Lomholt, Multilocus sequence typing and phylogenetic analysis of *Propionibacterium acnes*, Journal of Clinical Microbiology 50(4) (2012) 1158-1165.
67. A. McDowell, E. Barnard, I. Nagy, A. Gao, S. Tomida, H. Li, A. Eady, J. Cove, C.E. Nord, S. Patrick, An expanded multilocus sequence typing scheme for *Propionibacterium acnes*: investigation of 'pathogenic', 'commensal' and antibiotic resistant strains, PLOS ONE 7(7) (2012) 1-14.
68. J. Kim, M.T. Ochoa, S.R. Krutzik, O. Takeuchi, S. Uematsu, A.J. Legaspi, H.D. Brightbill, D. Holland, W.J. Cunliffe, S. Akira, P.A. Sieling, P.J. Godowski, R.L. Modlin, Activation of Toll-like receptor 2 in acne triggers inflammatory cytokine responses, The Journal of Immunology 169(3) (2002) 1535-1541.
69. J.L. Selway, T. Kurczab, T. Kealey, K. Langlands, Toll-like receptor 2 activation and comedogenesis: implications for the pathogenesis of acne, BMC Dermatology 13(1) (2013) 1-7.
70. A.T. Slominski, P.R. Manna, R.C. Tuckey, On the role of skin in the regulation of local and systemic steroidogenic activities, Steroids 103 (2015) 72-88.
71. A.R. Clark, M.G. Belvisi, Maps and legends: The quest for dissociated ligands of the glucocorticoid receptor, Pharmacology & Therapeutics 134(1) (2012) 54-67.
72. D.W. Cain, J.A. Cidlowski, Immune regulation by glucocorticoids, Nature Reviews Immunology advance online publication 17 (2017) 233-247.
73. K.D. Dittmar, D.R. Demady, L.F. Stancato, P. Krishna, W.B. Pratt, Folding of the glucocorticoid receptor by the heat shock protein (hsp) 90-based chaperone machinery: the role of p23 is to stabilize receptor hsp90 heterocomplexes formed by hsp90 p60 hsp70, Journal of Biological Chemistry 272(34) (1997) 21213-21220.
74. K.A. Smoak, J.A. Cidlowski, Mechanisms of glucocorticoid receptor signaling during inflammation, Mechanisms of Ageing and Development 125(10-11) (2004) 697-706.
75. O. Kassel, A. Sancono, J. Krätzschmar, B. Kreft, M. Stassen, A.C.B. Cato, Glucocorticoids inhibit MAP kinase via increased expression and decreased degradation of MKP-1, The EMBO Journal 20(24) (2001) 7108-7116.

76. K. Smoak, J.A. Cidlowski, Glucocorticoids regulate tristetraprolin synthesis and posttranscriptionally regulate tumor necrosis factor alpha inflammatory signaling, Molecular and Cellular Biology 26(23) (2006) 9126-9135.
77. E. Ayroldi, L. Cannarile, G. Migliorati, G. Nocentini, D.V. Delfino, C. Riccardi, Mechanisms of the anti-inflammatory effects of glucocorticoids: genomic and nongenomic interference with MAPK signaling pathways, The FASEB Journal 26(12) (2012) 4805-4820.
78. A.M. Xavier, A.K.O. Anunciato, T.R. Rosenstock, I. Glezer, Gene expression control by glucocorticoid receptors during innate immune responses, Frontiers in Endocrinology 7(31) (2016) 1-8.
79. D. Cruz-Topete, J.A. Cidlowski, One hormone two actions: anti- and pro-inflammatory effects of glucocorticoids, Neuroimmunomodulation 22 (2015) 20-32.
80. S.F. Sorrells, R.M. Sapolsky, An inflammatory review of glucocorticoid actions in the CNS, Brain, behavior, and immunity 21(3) (2007) 259-272.
81. J.M. Busillo, J.A. Cidlowski, The five Rs of glucocorticoid action during inflammation: ready, reinforce, repress, resolve, and restore, Trends in Endocrinology & Metabolism 24(3) (2013) 109-119.
82. E.A. Lannan, A.J. Galliher-Beckley, A.B. Scoltock, J.A. Cidlowski, Proinflammatory actions of glucocorticoids: glucocorticoids and TNFα coregulate gene expression in vitro and in vivo, Endocrinology 153(8) (2012) 3701-3712.
83. A. Imasato, C. Desbois-Mouthon, J. Han, H. Kai, A.C. Cato, S. Akira, J.D. Li, Inhibition of p38 MAPK by glucocorticoids via induction of MAPK phosphatase-1 enhances nontypeable Haemophilus influenzae-induced expression of toll-like receptor 2, The Journal of biological chemistry 277(49) (2002) 47444-47450.
84. A. Sakai, J. Han, A.C. Cato, S. Akira, J.D. Li, Glucocorticoids synergize with IL-1beta to induce TLR2 expression via MAP kinase phosphatase-1-dependent dual inhibition of MAPK JNK and p38 in epithelial cells, BMC molecular biology 5(2) (2004) 1-16.
85. J.M. Busillo, K.M. Azzam, J.A. Cidlowski, Glucocorticoids sensitize the innate immune system through regulation of the NLRP3 inflammasome, The Journal of biological chemistry 286(44) (2011) 38703-38713.
86. Y. Ding, Z.G. Gao, K.A. Jacobson, A.F. Suffredini, Dexamethasone enhances ATP-induced inflammatory responses in endothelial cells, Journal of Pharmacology and Experimental Therapeutics 335(3) (2010) 693-702.
87. A. Abraham, G. Roga, Topical steroid-damaged skin, Indian Journal of Dermatology 59(5) (2014) 456-459.
88. K.H. Kaidbey, A.M. Kligman, The pathogenesis of topical steroid acne, Journal of Investigative Dermatology 62(1) (1974) 31-36.
89. G. Plewig, A. Kligman, Induction of acne by topical steroids, Archives of Dermatological Research 247(1) (1973) 29-52.
90. M. Shibata, M. Katsuyama, T. Onodera, R. Ehama, J. Hosoi, H. Tagami, Glucocorticoids enhance Toll-like receptor 2 expression in human keratinocytes stimulated with *Propionibacterium acnes* or proinflammatory cytokines, Journal of Investigative Dermatology 129(2) (2008) 375-382.
91. O. Stojadinovic, B. Lee, C. Vouthounis, S. Vukelic, I. Pastar, M. Blumenberg, H. Brem, M. Tomic-Canic, Novel genomic effects of glucocorticoids in epidermal keratinocytes: inhibition of apoptosis, interferon-γ pathway and wound healing along with promotion of terminal differentiation, Journal of Biological Chemistry 282(6) (2007) 4021-4034.

92. N.R. Trivedi, K.L. Gilliland, W. Zhao, W. Liu, D.M. Thiboutot, Gene array expression profiling in acne lesions reveals marked upregulation of genes involved in inflammation and matrix remodeling, Journal of Investigative Dermatology 126(5) (2006) 1071-1079.
93. T. Shuto, A. Imasato, H. Jono, A. Sakai, H. Xu, T. Watanabe, D.D. Rixter, H. Kai, A. Andalibi, F. Linthicum, Glucocorticoids synergistically enhance nontypeablehaemophilus influenzae-induced Toll-like receptor 2 expression via a negative cross-talk with p38 map kinase, Journal of Biological Chemistry 277(19) (2002) 17263-17270.
94. M.A. Hermoso, T. Matsuguchi, K. Smoak, J.A. Cidlowski, Glucocorticoids and tumor necrosis factor alpha cooperatively regulate Toll-like receptor 2 gene expression, Molecular and cellular biology 24(11) (2004) 4743-4756.
95. M. De Buck, M. Gouwy, J.M. Wang, J. Van Snick, P. Proost, S. Struyf, J. Van Damme, The cytokine-serum amyloid A-chemokine network, Cytokine & Growth Factor Reviews 30(2016) 55-69.
96. S. Hiratsuka, A. Watanabe, Y. Sakurai, S. Akashi-Takamura, S. Ishibashi, K. Miyake, M. Shibuya, S. Akira, H. Aburatani, Y. Maru, The S100A8-serum amyloid A3-TLR4 paracrine cascade establishes a pre-metastatic phase, Nature Cell Biology 10(11) (2008) 1349-1355.
97. T. Tomita, K. Ieguchi, T. Sawamura, Y. Maru, Human serum amyloid A3 (SAA3) protein, expressed as a fusion protein with SAA2, binds the oxidized low density lipoprotein receptor, PLOS ONE 10(3) (2015) 1-18.
98. A.S. Whitehead, M.C. de Beer, D.M. Steel, M. Rits, J.M. Lelias, W.S. Lane, F.C. de Beer, Identification of novel members of the serum amyloid A protein superfamily as constitutive apolipoproteins of high density lipoprotein, Journal of Biological Chemistry 267(6) (1992) 3862-3867.
99. S. Bozinovski, M. Uddin, R. Vlahos, M. Thompson, J.L. McQualter, A.-S. Merritt, P.A.B. Wark, A. Hutchinson, L.B. Irving, B.D. Levy, G.P. Anderson, Serum amyloid A opposes lipoxin A4 to mediate glucocorticoid refractory lung inflammation in chronic obstructive pulmonary disease, Proceedings of the National Academy of Sciences 109(3) (2012) 935-940.
100. B.K. Ray, A. Ray, Involvement of an SAF-like transcription factor in the activation of serum amyloid A gene in monocyte/macrophage cells by lipopolysaccharide, Biochemistry 36(15) (1997) 4662-4668.
101. S. Han, S.P. Jin, J.H. Oh, E.Y. Seo, C.-H. Park, H.S. Yoon, D.H. Lee, J.H. Chung, Serum amyloid A1 secreted from UV-irradiated keratinocytes induces matrix metalloproteinase-1 in fibroblasts through Toll-like receptor 4, Experimental Dermatology 25(7) (2016) 526-531.
102. C.F. Thorn, Z.Y. Lu, A.S. Whitehead, Regulation of the human acute phase serum amyloid A genes by tumour necrosis factor-α, interleukin-6 and glucocorticoids in hepatic and epithelial cell lines, Scandinavian Journal of Immunology 59(2) (2004) 152-158.
103. C.F. Thorn, Z.Y. Lu, A.S. Whitehead, Tissue-specific regulation of the human acute-phase serum amyloid A genes, SAA1 and SAA2, by glucocorticoids in hepatic and epithelial cells, European Journal of Immunology 33(9) (2003) 2630-2639.
104. D.M. Steel, F.C. Donoghue, R.M. O'Neill, C.M. Uhlar, A.S. Whitehead, Expression and regulation of constitutive and acute phase serum amyloid A mRNAs in hepatic and non-hepatic cell lines, Scandinavian Journal of Immunology 44(5) (1996) 493-500.

105. Y. Kumon, T. Suehiro, K. Hashimoto, J.D. Sipe, Dexamethasone, but not IL-1 alone, upregulates acute-phase serum amyloid A gene expression and production by cultured human aortic smooth muscle cells, Scandinavian Journal of Immunology 53(1) (2001) 7-12.
106. A. Kovacevic, A. Hammer, M. Sundl, B. Pfister, A. Hrzenjak, A. Ray, B.K. Ray, W. Sattler, E. Malle, Expression of serum amyloid A transcripts in human trophoblast and fetal-derived trophoblast-like choriocarcinoma cells, FEBS Letters 580(1) (2006) 161-167.
107. R.D. Ye, L. Sun, Emerging functions of serum amyloid A in inflammation, Journal of Leukocyte Biology 98(6) (2015) 923-929.
108. C. Shah, R. Hari-Dass, J.G. Raynes, Serum amyloid A is an innate immune opsonin for Gram-negative bacteria, Blood 108(5) (2006) 1751-1757.
109. N. Cheng, R. He, J. Tian, P.P. Ye, R.D. Ye, Cutting edge: TLR2 is a functional receptor for acute-phase serum amyloid A, The Journal of Immunology 181(1) (2008) 22-26.
110. S. O'Reilly, R. Cant, M. Ciechomska, J. Finnigan, F. Oakley, S. Hambleton, J.M. van Laar, Serum amyloid A induces interleukin-6 in dermal fibroblasts via Toll-like receptor 2, interleukin-1 receptor-associated kinase 4 and nuclear factor-κB, Immunology 143(3) (2014) 331-340.
111. R.L. He, J. Zhou, C.Z. Hanson, J. Chen, N. Cheng, R.D. Ye, Serum amyloid A induces G-CSF expression and neutrophilia via Toll-like receptor 2, Blood 113(2) (2009) 429-437.
112. S.L. Passey, S. Bozinovski, R. Vlahos, G.P. Anderson, M.J. Hansen, Serum amyloid A induces Toll-like receptor 2-dependent inflammatory cytokine expression and atrophy in C2C12 skeletal muscle myotubes, PLOS ONE 11(1) (2016) 1-17.
113. M. Connolly, P.R. Rooney, T. McGarry, A.X. Maratha, J. McCormick, S.M. Miggin, D.J. Veale, U. Fearon, Acute serum amyloid A is an endogenous TLR2 ligand that mediates inflammatory and angiogenic mechanisms, Annals of the Rheumatic Diseases 75(7) (2016) 1392-1398.
114. S. Han, J.-H. Oh, C.-Y. Shin, H.-S. Yoon, D.H. Lee, J.H. Chung, Serum amyloid A1 is induced by UV irradiation and detected by Toll-like receptor 4 to causes skin inflammation, Journal of Dermatological Science 84(1) (2016) 107-110.
115. H.Y. Lee, S.D. Kim, J.W. Shim, S.Y. Lee, H. Lee, K.-H. Cho, J. Yun, Y.-S. Bae, Serum amyloid A induces CCL2 production via formyl peptide receptor-like 1-mediated signaling in human monocytes, The Journal of Immunology 181(6) (2008) 4332-4339.
116. H.Y. Lee, M.K. Kim, K.S. Park, Y.H. Bae, J. Yun, J.I. Park, J.Y. Kwak, Y.S. Bae, Serum amyloid A stimulates matrix-metalloproteinase-9 upregulation via formyl peptide receptor like-1-mediated signaling in human monocytic cells, Biochemical and Biophysical Research Communications 330(3) (2005) 989-998.
117. H.Y. Lee, S.D. Kim, J.W. Shim, J. Yun, K. Kim, Y.-S. Bae, Activation of formyl peptide receptor like-1 by serum amyloid A induces CCL2 production in human umbilical vein endothelial cells, Biochemical and Biophysical Research Communications 380(2) (2009) 313-317.
118. R. O'Hara, E.P. Murphy, A.S. Whitehead, O. FitzGerald, B. Bresnihan, Local expression of the serum amyloid A and formyl peptide receptor-like 1 genes in synovial tissue is associated with matrix metalloproteinase production in patients with inflammatory arthritis, Arthritis & Rheumatism 50(6) (2004) 1788-1799.
119. N. Yu, S. Liu, X. Yi, S. Zhang, Y. Ding, Serum amyloid A induces interleukin-1β secretion from keratinocytes via the NACHT, LRR and PYD domains-containing protein 3 inflammasome, Clinical and Experimental Immunology 179(2) (2015) 344-353.

120. K. Niemi, L. Teirilä, J. Lappalainen, K. Rajamäki, M.H. Baumann, K. Öörni, H. Wolff, P.T. Kovanen, S. Matikainen, K.K. Eklund, Serum amyloid A activates the NLRP3 inflammasome via P2X7 receptor and a Cathepsin B-sensitive pathway, The Journal of Immunology 186(11) (2011) 6119-6128.
121. I.N. Baranova, A.V. Bocharov, T.G. Vishnyakova, R. Kurlander, Z. Chen, D. Fu, I.M. Arias, G. Csako, A.P. Patterson, T.L. Eggerman, CD36 is a novel serum amyloid A (SAA) receptor mediating SAA binding and SAA-induced signaling in human and rodent Cells, The Journal of biological chemistry 285(11) (2010) 8492-8506.
122. H.B. Jijon, K.L. Madsen, J.W. Walker, B. Allard, C. Jobin, Serum amyloid A activates NF-κB and proinflammatory gene expression in human and murine intestinal epithelial cells, European Journal of Immunology 35(3) (2005) 718-726.
123. T. Koga, T. Torigoshi, S. Motokawa, T. Miyashita, Y. Maeda, M. Nakamura, A. Komori, Y. Aiba, T. Uemura, H. Yatsuhashi, H. Ishibashi, K. Eguchi, K. Migita, Serum amyloid A-induced IL-6 production by rheumatoid synoviocytes, FEBS Letters 582(5) (2008) 579-585.
124. K. Migita, Y. Izumi, Y. Jiuchi, H. Kozuru, C. Kawahara, M. Nakamura, T. Nakamura, K. Agematsu, J. Masumoto, M. Yasunami, A. Kawakami, K. Eguchi, Serum amyloid a induces NLRP-3-mediated IL-1β secretion in neutrophils, PLOS ONE 9(5) (2014) 1-7.
125. E. Hatanaka, C.J. Furlaneto, F.P. Ribeiro, G.M. Souza, A. Campa, Serum amyloid A-induced mRNA expression and release of tumor necrosis factor-alpha (TNF-α) in human neutrophils, Immunology Letters 91(1) (2004) 33-37.
126. C. Song, K. Hsu, E. Yamen, W. Yan, J. Fock, P.K. Witting, C.L. Geczy, S.B. Freedman, Serum amyloid A induction of cytokines in monocytes/macrophages and lymphocytes, Atherosclerosis 207(2) (2009) 374-383.
127. Y.C. Huang, C.H. Yang, T.T. Li, C.C. Zouboulis, H.C. Hsu, Cell-free extracts of *Propionibacterium acnes* stimulate cytokine production through activation of p38 MAPK and Toll-like receptor in SZ95 sebocytes, Life Sciences 139 (2015) 123-131.
128. Y. Yu, J. Champer, J. Kim, Analysis of the surface, secreted, and intracellular proteome of Propionibacterium acnes, EuPA Open Proteomics 9 (2015) 1-7.
129. M. Noreen, M. Arshad, Association of TLR1, TLR2, TLR4, TLR6, and TIRAP polymorphisms with disease susceptibility, Immunologic Research 62(2) (2015) 234-252.
130. M.H.T. Stappers, Y. Thys, M. Oosting, T.S. Plantinga, M. Ioana, P. Reimnitz, J.W. Mouton, M.G. Netea, L.A.B. Joosten, I.C. Gyssens, TLR1, TLR2, and TLR6 gene polymorphisms are associated with increased susceptibility to complicated skin and skin structure infections, The Journal of Infectious Diseases 210(2) (2014) 311-318.
131. A. Koreck, K. Kis, K. Szegedi, V. Paunescu, R. Cioaca, R. Olariu, S. Negru, Z. Bata-Csorgo, L. Kemeny, A. Dobozy, M. Szell, TLR2 and TLR4 polymorphisms are not associated with acne vulgaris, Dermatology 213(3) (2006) 267-269.
132. L.M. Tian, H.F. Xie, T. Yang, Y.H. Hu, J. Li, W.Z. Wang, Association study of tumor necrosis factor receptor type 2 M196R and Toll-like receptor 2 Arg753Gln polymorphisms with acne vulgaris in a Chinese Han ethnic group, Dermatology 221(3) (2010) 276-284.
133. A. Perry, P. Lambert, *Propionibacterium acnes:* infection beyond the skin, Expert Review of Anti-infective Therapy 9(12) (2011) 1149-1156.
134. A.T. Vu, T. Baba, X. Chen, T.A. Le, H. Kinoshita, Y. Xie, S. Kamijo, K. Hiramatsu, S. Ikeda, H. Ogawa, K. Okumura, T. Takai, *Staphylococcus aureus* membrane and diacylated lipopeptide induce thymic stromal lymphopoietin in keratinocytes through the Toll-like

receptor 2-Toll-like receptor 6 pathway, Journal of Allergy and Clinical Immunology 126(5) (2010) 985-993.
135. S.C. Lin, Y.C. Lo, H. Wu, Helical assembly in the MyD88-IRAK4-IRAK2 complex in TLR/IL-1R signalling, Nature 465(7300) (2010) 885-890.
136. D.B. Conze, C.J. Wu, J.A. Thomas, A. Landstrom, J.D. Ashwell, Lys63-linked polyubiquitination of IRAK-1 is required for interleukin-1 receptor- and Toll-Like receptor-mediated NF-κB activation, Molecular and Cellular Biology 28(10) (2008) 3538-3547.
137. M. Miyata, J.Y. Lee, S. Susuki-Miyata, W.Y. Wang, H. Xu, H. Kai, K.S. Kobayashi, R.A. Flavell, J.-D. Li, Glucocorticoids suppress inflammation via the upregulation of negative regulator IRAK-M, Nature Communications 6 (2015) 1-12.
138. O. Isard, A.C. Knol, N. Castex-Rizzi, A. Khammari, M. Charveron, B. Dréno, Cutaneous induction of corticotropin releasing hormone by Propionibacterium acnes extracts, Dermato-endocrinology 1(2) (2009) 96-99.
139. B. Zbytek, A.T. Slominski, CRH mediates inflammation induced by lipopolysaccharide in human adult epidermal keratinocytes, The Journal of investigative dermatology 127(3) (2007) 730-732.
140. M.T. Rae, D. Niven, A. Ross, T. Forster, R. Lathe, H.O.D. Critchley, P. Ghazal, S.G. Hillier, Steroid signalling in human ovarian surface epithelial cells: the response to interleukin-1α determined by microarray analysis, Journal of Endocrinology 183(1) (2004) 19-28
141. C.M. Uhlar, A.S. Whitehead, Serum amyloid A, the major vertebrate acute-phase reactant, European Journal of Biochemistry 265(2) (1999) 501-523.

Publication Record

Original research articles

Pfalzgraff A, Heinbockel L, **Su Q**, Brandenburg K, Weindl G (2017) Synthetic anti-endotoxin peptides inhibit cytoplasmic LPS-mediated responses. 140: 64-72. Biochem Pharmacol. DOI: 10.1016/j.bcp.2017.05.015

Su Q, Pfalzgraff A, Weindl G (2017) Cell type-specific regulatory effects of glucocorticoids on cutaneous TLR2 expression and signaling. J Steroid Biochem Mol Biol 171: 201-208. DOI: 10.1016/j.jsbmb.2017.03.023

Su Q, Grabowski M, Weindl G (2017) Recognition of *Propionibacterium acnes* by human TLR2 heterodimers. Int J Med Microbiol 307 (2): 108–112. DOI: 10.1016/j.ijmm.2016.12.002

Pfalzgraff A, Heinbockel L, **Su Q**, Gutsmann T, Brandenburg K, Weindl G (2016) Synthetic antimicrobial and LPS-neutralising peptides suppress inflammatory and immune responses in skin cells and promote keratinocyte migration. Sci Rep 6: 31577. DOI: 10.1038/srep31577

Conferences (published abstracts)

Su Q, Grabowski M, Weindl G (2017) Recognition of *Propionibacterium acnes* by human TLR2 heterodimers. Naunyn-Schmiedebergs Arch Pharmacol 390 (Suppl 1): S68.

Su Q, Weindl G (2016) Dexamethasone synergistically enhances TNFα-induced TLR2 expression via inhibition of p38 MAPK and induces TRAF6 in human primary keratinocytes. Naunyn-Schmiedebergs Arch Pharmacol 389 (Suppl 1): S65.

Poster

Su Q, Grabowski M, Weindl G. Recognition of *Propionibacterium acnes* by human TLR2 heterodimers. DGPT, Heidelberg, Germany 2017.

Su Q, Weindl G. Dexamethasone synergistically enhances TNFα-induced TLR2 expression via inhibition of p38 MAPK and induces TRAF6 in human primary keratinocytes. DGPT, Berlin, Germany 2016.

Su Q, Weindl G. The regulatory effects of glucocorticoids on Toll-like receptor expression and signalling in skin cells. Tag der Pharmazie, Berlin, Germany 2016.

Su Q, Weindl G. Modulation of Toll-like receptor expression by dexamethasone in keratinocytes and fibroblasts. Tag der Pharmazie, Berlin, Germany 2014.