Molecular and Morphological Characterization of Inflammatory Infiltrate in Rosacea Reveals Activation of Th1/Th17 Pathways

Timo Buhl[1,2,3,7], Mathias Sulk[3,4,7], Pawel Nowak[4,7], Jörg Buddenkotte[4], Ian McDonald[2], Jérôme Aubert[5], Isabelle Carlavan[5], Sophie Déret[5], Pascale Reiniche[5], Michel Rivier[5], Johannes J. Voegel[5] and Martin Steinhoff[2,3,6]

Rosacea is a common chronic inflammatory skin disease of unknown etiology. Our knowledge about an involvement of the adaptive immune system is very limited. We performed detailed transcriptome analysis, quantitative real-time reverse-transcriptase–PCR, and quantitative immunohistochemistry on facial biopsies of rosacea patients, classified according to their clinical subtype. As controls, we used samples from patients with facial lupus erythematosus and healthy controls. Our study shows significant activation of the immune system in all subtypes of rosacea, characterizing erythematotelangiectatic rosacea (ETR) already as a disease with significant influx of proinflammatory cells. The T-cell response is dominated by Th1/Th17-polarized immune cells, as demonstrated by significant upregulation of IFN-γ or IL-17, for example. Chemokine expression patterns support a Th1/Th17 polarization profile of the T-cell response. Macrophages and mast cells are increased in all three subtypes of rosacea, whereas neutrophils reach a maximum in papulopustular rosacea. Our studies also provide evidence for the activation of plasma cells with significant antibody production already in ETR, followed by a crescendo pattern toward phymatous rosacea. In sum, Th1/Th17 polarized inflammation and macrophage infiltration are an underestimated hallmark in all subtypes of rosacea. Therapies directly targeting the Th1/Th17 pathway are promising candidates in the future treatment of this skin disease.

Journal of Investigative Dermatology (2015) **135,** 2198–2208; doi:10.1038/jid.2015.141; published online 14 May 2015

INTRODUCTION

Rosacea is a common, chronic inflammatory skin disease with poorly understood etiology. On the basis of its symptoms such as facial erythema, chronic inflammation, and fibrosis (Powell, 2005), complex pathomechanisms involving dysregulation in the immune, vascular, and nervous systems, as well as barrier function impairments, are suggested (Yamasaki *et al.*, 2007; Steinhoff *et al.*, 2011, 2013; Wollina, 2014).

The knowledge about rosacea immunology is still very limited, and most studies focused on the involvement of the vascular or innate immune system. Environmental rosacea trigger factors (e.g., UV, temperature changes, spicy food, exercise, "stress", demodex) or an altered skin microbiome activates (directly or indirectly) pattern recognition receptors such as Toll-like receptors (TLRs), which stimulate the release of antimicrobial peptides (among them the cathelicidin LL-37) or cytokines, for example (Steinhoff *et al.*, 2013). In rosacea, an increased expression of TLR2, LL-37, and kallikrein 5 (the protease that cleaves the cathelicidin precursor protein into LL-37) has been observed (Yamasaki and Gallo, 2011; Brown *et al.*, 2014), and inhibition of kallikrein 5 has been linked to improvement of the erythema and inflammatory papules in rosacea (Two *et al.*, 2014). In addition, cells of the innate immune system, among them macrophages, mast cells, and neutrophils, were described as part of the cellular infiltrate in rosacea (Marks and Harcourt-Webster, 1969; Jansen and Plewig, 1997; Schwab *et al.*, 2011).

Little is known about the adaptive immune system in rosacea. It was shown recently that in other inflammatory skin diseases such as acne, psoriasis, or atopic dermatitis, the

[1]*Department of Dermatology, Venereology and Allergology, University Medical Center Göttingen, Göttingen, Germany;* [2]*Charles Institute for Translational Dermatology, University College Dublin, Dublin, Ireland;* [3]*Department of Dermatology and Surgery, University of California, San Francisco, California, USA;* [4]*Department of Dermatology, University of Münster, Münster, Germany;* [5]*Molecular Dermatology, Research Department, Galderma R&D, Sophia Antipolis, France and* [6]*Department of Dermatology, University of San Diego, USA*

Correspondence: Martin Steinhoff, Charles Institute for Translational Dermatology, University College Dublin, Belfield, Dublin 4, Ireland or Johannes J. Voegel, Molecular Dermatology, Research Department, Galderma R&D, Sophia Antipolis, France. E-mail: martin.steinhoff@ucd.ie or johannes.voegel@galderma.com

[7]*These authors contributed equally to this work.*

Abbreviations: ETR, erythematotelangiectatic rosacea (rosacea I); IHC, immunohistochemistry; LE, lupus erythematosus; PPR, papulopustular rosacea (rosacea II); PhR, phymatous rosacea (rosacea III); qRT–PCR, quantitative real-time reverse-transcriptase–PCR; RI, rosacea type I (ETR); RII, rosacea type II (PPR); RIII, rosacea type III (PhR); Th1, T helper cell type 1; Th2, T helper cell type 2; TLR, Toll-like receptor; Th17, T helper cell type 17

Received 5 January 2015; revised 11 March 2015; accepted 18 March 2015; accepted article preview online 7 April 2015; published online 14 May 2015

adaptive immune system is anticipated to contribute to the inflammatory processes (Palau *et al.*, 2013; Suarez-Farinas *et al.*, 2013; Kelhala *et al.*, 2014). Only very few studies exist, which investigate a role of the adaptive immune system in rosacea and show that the cellular infiltrate in rosacea also contains T cells and B cells (Rufli and Buchner, 1984; Aloi *et al.*, 2000; Smith *et al.*, 2007; Brown *et al.*, 2014). With regard to T cells, $CD4^+$ T cells are reported to be more frequently found than $CD8^+$ T cells in papulopustular rosacea (PPR) and in nonpustular rosacea (Georgala *et al.*, 2001; Brown *et al.*, 2014). However, these studies are only based on histological findings mainly in pustular rosacea, and they do not investigate and distinguish between different cutaneous rosacea subtypes simultaneously, and they do not correlate histological findings with cellular pathways.

Therefore, the aim of this study was to characterize the inflammatory infiltrate of the three cutaneous rosacea subtypes comprehensively. Moreover, we performed whole transcriptome expression analysis and confirmed the results with quantitative real-time reverse-transcriptase–PCR (qRT–PCR) and quantitative immunohistochemistry of rosacea patient samples, as compared with healthy skin and facial lupus erythematosus (LE).

RESULTS

$CD4^+$ T helper cells dominate the immune cell infiltrate in erythematotelangiectatic rosacea (ETR) and PPR

Our study reveals strong levels of T-cell involvement in all three rosacea subtypes (Figure 1 and Supplementary Figure S1 online), reaching a maximum of activation in PPR (PPR > phymatous rosacea (PhR) > ETR). At the transcriptome level, strong upregulation of CD3G, CD3D, and CD247 (=CD3Z), which transcribe the CD3 subunits of the αβ T-cell receptor (Wang and Reinherz, 2012), can be observed for all rosacea stages. In addition, this correlates well with increased expression of the T-cell activation– and proliferation–associated genes LCK, MICB, VAV1, and LAG3 (Casati *et al.*, 2006; Gomez-Rodriguez *et al.*, 2007; Cerboni *et al.*, 2009; Salmond *et al.*, 2009). We also observe identical patterns in genes encoding for costimulatory molecules for T-cell activation (such as CD80, CD86, TNFSF14), as well as the proinflammatory cytokine IL-1β (del Rio *et al.*, 2010). For confirmation of our transcriptome data, we performed a qRT–PCR study on a total of 68 immunological markers for each rosacea subtype. We globally obtained a good agreement between transcriptome and qRT–PCR results (Supplementary Table S1 online). In support, quantitative immunohistochemistry (IHC) verifies a significant increase of $CD4^+$ cells in all subtypes, with highest CD4 counts in PPR. Interestingly, $CD4^+$ cells in PPR were mainly distributed around hair follicles, whereas $CD4^+$ cells were found to be predominantly localized in the perivascular region in the other subtypes of rosacea. Although expression of CD4 was only moderately elevated in all rosacea stages (30–40% higher than controls) based on the Affymetrix analysis, the results were always significant (false discovery rate <0.002 in all three stages). Immunohistochemical staining for CD8 showed spare cells in all three subtypes with a trend toward higher expression in PPR, but without reaching statistical significance. At the transcriptome level, CD8A was significantly elevated in all rosacea forms (PPR > PhR > ETR), whereas the increase of CD8B did not reach significance.

Immune response in rosacea displays Th1/Th17 polarization

Detailed transcriptome analysis of the induced T-cell response genes revealed significantly higher gene expression levels for the Th1-signature cytokines IFN-γ and tumor necrosis factor-α (Figure 2). This finding is consistent with higher messenger RNA expression levels for the Th1-related transcription factors signal transducer and activator of transcription 1 and 4 (Oestreich and Weinmann, 2012). Corresponding results were obtained for cellular receptors associated with Th1-immune responses such as IL12RB1 and CCR5 (Turner *et al.*, 2007). We confirmed these results by IHC and found a largely higher staining for IFN-γ, especially in PPR.

Besides the Th1-polarized immune response in rosacea, we also found induction of Th17-associated genes (Figure 2). The genes coding for Th17-signature cytokines IL17A and IL22 were significantly elevated (again PPR > PhR > ETR). The same was true for the genes IL6, tumor necrosis factor, IL20, and CCL20, which induce IL-17/IL-22 production, and for the Th17-associated transcription factor signal transducer and activator of transcription 3 (Lyakh *et al.*, 2008; Rutz *et al.*, 2013). These results were verified by significant IL-17 immunostaining in all subtypes, with highest staining in PPR patients. Neither transcriptome nor IHC analysis verified an involvement of Th2-associated genes of the cytokine IL-4 (Supplementary Figure S2 and S3 online). Finally, no upregulation of genes were found associated with activation of regulatory T cells (unaltered expression of FOXP3, IL10, CCR4, and CCR8, depicted in Supplementary Figure S3 online).

Recruitment and activation of macrophages and neutrophils differ between rosacea subtypes

To define an involvement of macrophages in rosacea, we analyzed messenger RNA expression levels of CD68, CD14, CD163, and MSR1. The finding of significant upregulation of these macrophage markers in PPR was supported by identical upregulation of ITGB2 and ITGAM messenger RNA (Figure 3), genes that encode proteins that form the macrophage-1 antigen (or integrin $\alpha_M\beta_2$). Several other important activators of macrophages (and other inflammatory immune cells) were also significantly upregulated at the transcriptome level, once more following the pattern PPR > PhR > ETR: TLR1, TLR4, CHI3L1/YKL-40, ARG1 (Rathcke and Vestergaard, 2006; Gordon and Martinez, 2010; Gallego *et al.*, 2011). Immunohistochemical staining for CD68 confirmed our transcriptome data, demonstrating highly significant increases of $CD68^+$ cells in all subtypes (PPR: $P<0.001$, ETR and PhR: $P<0.05$). In particular in PPR, $CD68^+$ cells were vastly organized as granulomatous areas and interfollicularly (Figure 3), whereas ETR macrophages showed a more perivascular diffuse pattern similar to $CD4^+$ cells.

Next, we analyzed RNA levels and distribution of neutrophils in all three subtypes of rosacea. Transcriptome

a

GENE_SYMBOL	TITLE	R I vs HV	R II vs HV	R III vs HV
T-cell component				
CD3G	CD3g molecule gamma (CD3-TCR complex)	3.9	7.5	4.5
LCK	Lymphocyte-specific protein tyrosine kinase	3.5	6.9	4.5
CD3D	CD3d molecule Delta (CD3-TCR complex)	3.2	6.4	3.7
CD8A	CD8a molecule	2.4	5.5	3.2
CD247	CD247 molecule	2.3	3.8	3
T-cell activation				
IL1B	Interleukin 1 beta	2.4	5.4	3.2
MICB	MHC class I polypeptide-related sequence B	1.9	4	2.4
CD86	CD86 molecule	*1.5*	2.6	*1.4*
VAV1	Vav 1 guanine nucleotide exchange factor	1.6	2.4	1.8
TNFSF14	Tumor necrosis factor (lignad) superfamily member 14	1.8	2.2	1.7
LAG3	Lymphocyte-activation gene 3	*1.5*	2.2	1.8

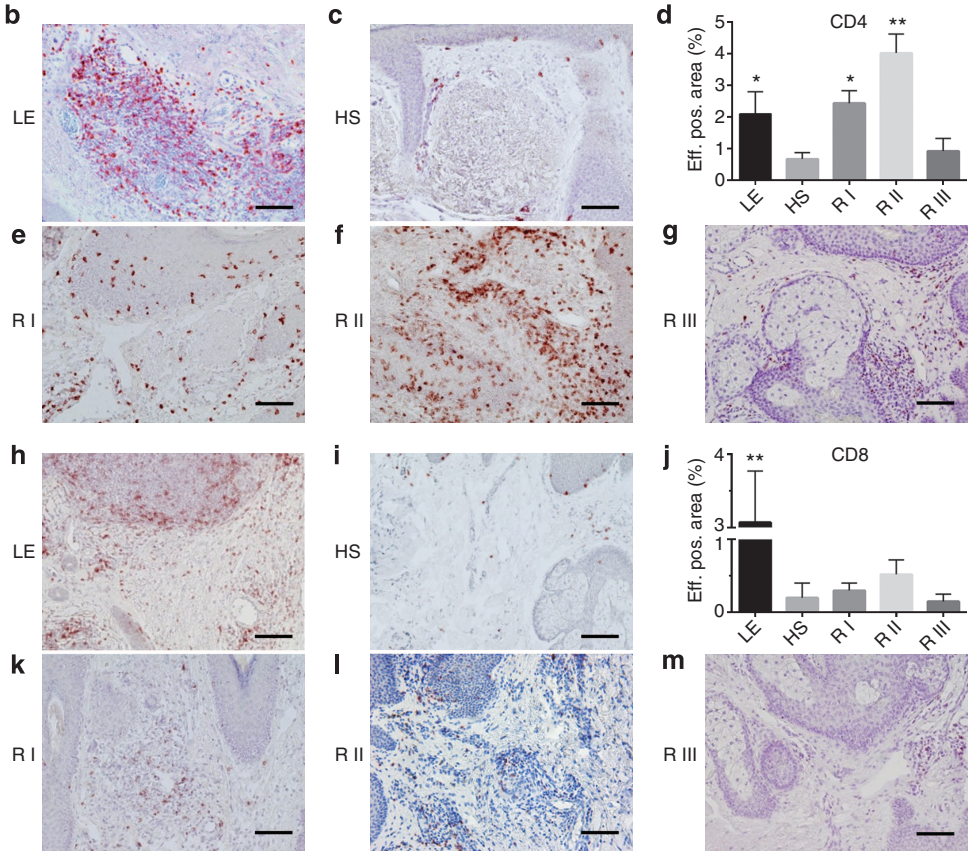


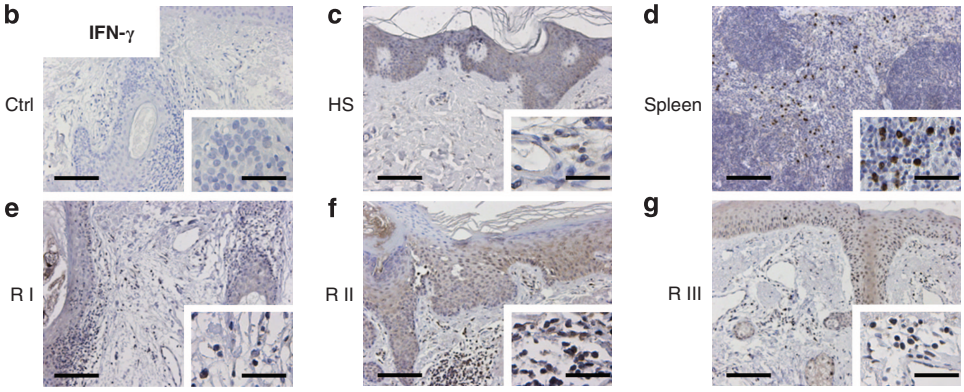
Figure 1. Rosacea shows activation and proliferation of T cells, which is mainly owing to CD4$^+$ T helper cells, but not CD8$^+$ cytotoxic T cells. The table (**a**) depicts genes that are involved in T-cell activation and proliferation and significantly modulated in at least one of the rosacea subtypes (full list of gene sets in Supplementary Figure S1 online). Fold modulations in bold face type depict false discovery rate (FDR) < 0.05; fold modulations in italic type depict FDR > 0.05. The upper graphs depict representative pictures (**b**,**c**,**e**–**g**) and morphometric analysis (**d**) of CD4$^+$ cells in each rosacea subgroup (erythematotelangiectatic rosacea/rosacea type I (RI), papulopustular rosacea/rosacea type II (RII), phymatous rosacea/rosacea type III (RIII)), lupus erythematosus (LE) patients, and healthy skin (HS), all comprising $n \geq 5$ patients with five representative areas per slide (eff.pos.area = effective positive area). The lower graphs and pictures display the results for CD8$^+$ cells (**h**–**m**). All images are ×200 magnification (bar = 100 μm).

Figure 2. The T helper cell subset is polarized toward a Th1/Th17 immune response. The upper table (**a**) shows Th1 cell–related genes significantly modulated in at least one of the rosacea subtypes. Fold modulations in bold face type depict false discovery rate (FDR) < 0.05; fold modulations in italic type depict FDR > 0.05. Representative pictures of IFN-γ staining by immunohistochemistry (IHC) are depicted below (**b**–**g**), with results shown in each rosacea subgroup (erythematotelangiectatic rosacea/ rosacea type I (RI), papulopustular rosacea/rosacea type II (RII), phymatous rosacea/rosacea type III (RIII)), healthy skin (HS), murine spleen, and controls without primary antibody. The lower table (**h**) displays significant alterations of Th17 cell–related gene expression only and corresponding IHC for IL-17A (**i**–**n**). All images are ×200 magnification (bar = 100 μm); inserts taken from the same slide located in the lower right corner are ×1,000 magnification (bar = 20 μm). More detailed gene lists including nonaltered Th2 and regulatory T-cell gene sets, as well as IHC for noninvolved IL-4 in rosacea, are displayed in Supplementary Figure S2 and S3 online.

analysis of CXC chemokines (Figure 3) shows significant upregulation of CXCL8 messenger RNA (formerly known as IL-8 or neutrophil chemotactic factor), one of the most potent chemotactic molecules for neutrophils. Similarly, other chemotactic chemokines such as CXCL1, CXCL2, CXCL5, and CXCL6 were found to be upregulated (Scapini *et al.*, 2000). Although neutrophils were found in pustular regions of PPR and PhR patients by immunohistochemistry,

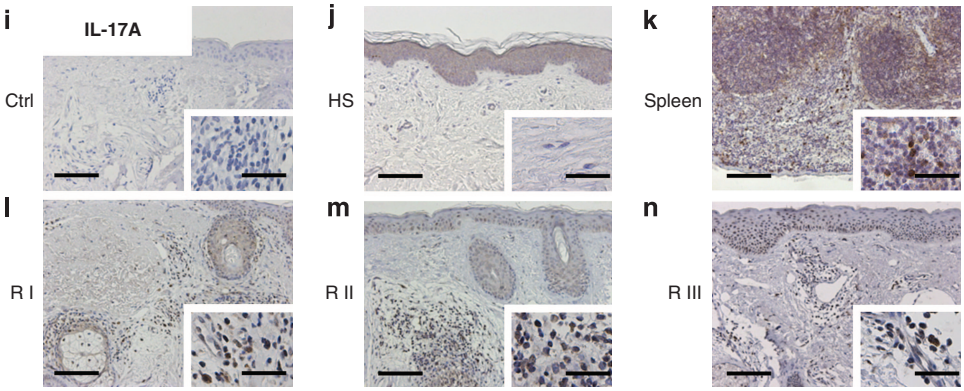
a

GENE_SYMBOL	**Th1** TITLE	R I vs. HV	R II vs. HV	R III vs. HV
Th1 activation				
STAT1	Signal transducer and activator of transcription 1	2.5	6.5	3.6
STAT4	Signal transducer and activator of transcription 4	*1.4*	2.5	*1.6*
IL18	Interleukin 18 (interferon-gamma-inducing factor)	–1.6	–2.5	–1.8
Cyto released				
IFNG	Interferon. Gamma	*1.8*	4.1	*2.0*
TNF	Tumor necrosis factor	1.4	2.1	1.6
Receptors				
IL12RB1	Interleukin 12 receptor. Beta 1	2.2	4.7	2.6
CCR5	Chemokine (C–C motif) receptor 5 (gene/pseudogene)	3.0	6.6	4.3



h

GENE_SYMBOL	**Th17** TITLE	R I vs. HV	R II vs. HV	R III vs. HV
Th17 activation				
IL6	Interleukin 6 (interferon. beta 2)	*1.1*	2.7	*1.4*
STAT3	Signal transducer and activator of transcription 3 (acute-phase response factor)	1.6	2.4	1.5
RORC	RAR-related orphan receptor C	*–1.4*	–2.4	*–1.9*
Cyto released				
IL17A	Interleukin 17A	*1.3*	2.4	2.0
IL22	Interleukin 22	*1.6*	4.4	*1.2*
IL6	Interleukin 6 (interferon. beta 2)	*1.1*	2.7	*1.4*
TNF	Tumor necrosis factor	*1.4*	2.1	*1.6*
CCL20	Chemokine (C-C motif) ligand 20	4.3	6.7	4.2
IL20	Interleukin 20	*1.5*	2.4	1.4
Receptors				
CD28	CD28 molecule	1.7	3.2	2.0
CD8A	CD8a molecule	2.4	5.5	3.2
ICAM1	Intercellular adhesion molecule 1	1.7	3.4	1.8
ICOS	Inducible T-cell co-stimulator	1.6	2.9	1.7
TLR4	Toll-like receptor 4	*1.2*	2.0	*1.0*



we found no evidence for an involvement of neutrophils in ETR.

Using immunohistochemistry (CD1a) and transcriptome analysis, no significant cell enhancement or RNA upregulation was observed for Langerhans cells in all three rosacea subtypes (Supplementary Figure S5 online). Distribution and transcriptome analysis of mast cells has recently been published (Schwab *et al.*, 2011).

Involvement of B cells and plasma cells in the regulation of immune responses in rosacea

Transcriptome analysis revealed significantly increased RNA expression levels of the B-cell marker MS4A1 (CD20) in PPR and PhR (Figure 4, Supplementary Figure S6 online). Similar results were obtained for the PAX5 gene, which encodes the transcription factor B-cell lineage–specific activator protein, and for the expression of CD24, which functions on activated B cells as a T-cell costimulator for $CD4^+$ T-cell clonal expansion (Fang *et al.*, 2010; Medvedovic *et al.*, 2011). We also found up to 170-fold upregulated genes involved in different parts of the light and heavy chains for immunoglobulins. Interestingly, all these genes reached the highest expressions in PhR (Figure 4). Because rearrangement and combinatorial procedures of immunoglobulins are especially complex in molecule assembly, these results need to be evaluated carefully. With IHC, we found highly significant increases in $CD79a^+$ cells in all rosacea subtypes, with highest cell numbers in PhR. Of note, $CD79a^+$ cells were often localized perivascularly or perifollicularly, or grouped as pseudofollicles in the papillar dermis. CD20 immunostaining showed an identical increase of positive cells in ETR and PPR, but dropped to almost baseline expression in PhR. As CD79a positivity is retained in plasma cells, whereas this late stage of B-cell differentiation shows no CD20 expression, it may be concluded that most of the $CD79a^+$ cells in PhR are in fact immunoglobulin-producing plasma cells (Chu and Arber, 2001). Analysis of several genes roughly differentiating antigen-dependent (e.g., CD28) and antigen-independent (e.g., IL6, IL7) B-cell activation did not show a distinct pattern of activation (Figure 4).

Chemokine expression patterns support the Th1/Th17 polarization of the T-cell response

As the family of chemokines appears redundant and their ligand–receptor relationships are promiscuous for many cell types, interpretation of immune cell and immune system polarization behavior by chemokines needs to be done with caution (Zlotnik and Yoshie, 2012). Moreover, as ligands of the Th1 cell–associated receptor CXCR3 and the Th2 cell–associated CCR3 are reciprocally natural antagonists, these ligands can form mutually exclusive microenvironments that are in favor of either Th1 or Th2 cell differentiation (Loetscher *et al.*, 2001). Among all chemokines, we found the highest expression levels for the CXCR3 ligands CXCL9, CXCL10, CXCL11, and CXCL13 (which are also induced by IFN-γ). In contrast, CCR3 ligands such as CCL3, CCL5, CCL11, and CCL13 were only modestly upregulated or unchanged compared with healthy controls (Figure 5).

The also highly expressed CCL18 acts as an antagonist to CCR3 (Nibbs *et al.*, 2000). Th17 cells express CCR6, which is only known to be activated by the highly elevated CCL20 and by β-defensin-2 (Singh *et al.*, 2008). Activation of macrophages and neutrophil granulocytes is also reflected by the increased expression profile of the receptors CCR1, CCR2, CCR5, CCR7 (macrophages and monocytes), and CXCR2 (neutrophils), and their corresponding ligands CCL2, CCL3, CCL4, CCL5, CCL8, CCL19, and CXCL1, CXCL2, CXCL5, CXCL6, respectively (Gerber *et al.*, 2011). In contrast, CCL27 and IL-37 are the most significantly downregulated transcripts among all cytokines and chemokines, emphasizing again the importance of Th1/Th17 involvement in the pathophysiology of rosacea. CCL27 is involved in cutaneous homing of Th2 cells in atopic dermatitis, it is downregulated in psoriasis plaques, and IFN-γ has been demonstrated to be the responsible component for downregulation of CCL27 in keratinocytes (Nomura *et al.*, 2003; Riis *et al.*, 2011). IL-37 (IL-1F7) suppresses immune responses, and its protective activity affects a broad spectrum of Th1/Th17 proinflammatory insults, placing this cytokine as a new member in the portfolio of anti-inflammatory cytokines such as IL-10 or TGF-β (Nold *et al.*, 2010). Thus, the considerable downregulation of the anti-inflammatory IL-37 may result in increased inflammation in rosacea. In summary, although a vast majority of altered cytokines and chemokines reach their maximum of upregulation and downregulation in PPR patients, significant changes in the RNA expression levels for cytokines/chemokines can be observed in all three cutaneous subtypes.

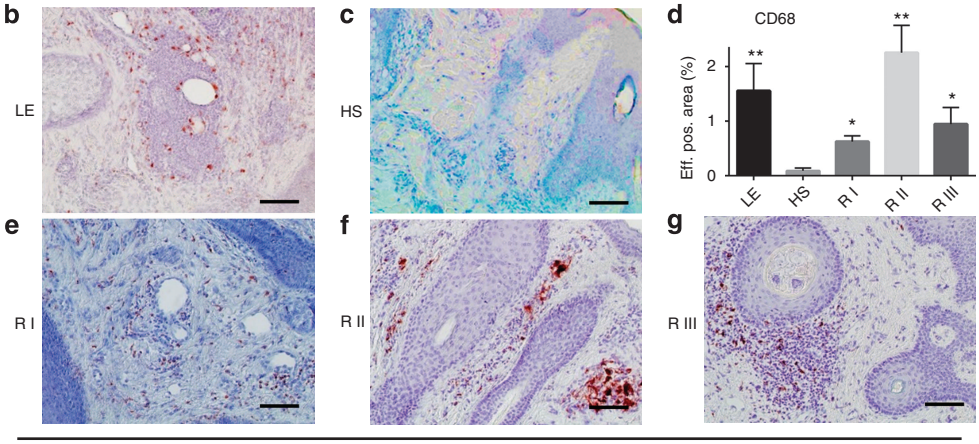
DISCUSSION

Although of importance, a detailed characterization of the inflammatory infiltrate and the associated cytokine/chemokine profiles in the three skin subtypes of rosacea has not been performed yet. Here, we comprehensively analyzed the inflammatory infiltrate of the three rosacea subtypes (ETR, PPR, and PhR) in comparison with healthy skin. In addition, we characterized the inflammation hallmarks of rosacea and LE, a facial chronic inflammatory autoimmune disease of different origin. Our combined transcriptome and immunohistochemistry analysis demonstrates Th1/Th17 dominance in all three subtypes (Figures 1 and 2), as well as the presence of macrophages (Figure 3) and mast cells in all subtypes (Schwab *et al.*, 2011). In contrast, B cells or neutrophils can only be found in PPR and PhR with a specific staining pattern. In contrast, activation of Langerhans cell–associated genes or cell numbers could not be observed. Together, these observations suggest the involvement of the innate and adaptive immune system in the pathophysiology of rosacea.

Recently, the involvements of a dysfunctional innate immune system and a dysregulated neuro-immune communication network controlling vascular responses have been discovered as important contributors in the pathophysiology of rosacea. In early rosacea (ETR), a malicious vasodilation to

a

GENE_symbol	Title	R I vs. HV	R II vs. HV	R III vs. HV
Macrophage component				
ITGB2	Integrin. beta 2	2.3	4.3	2.4
CD68	CD68 molecule	1.5	2.2	*1.5*
ITGAM	Integrin. alpha M	1.8	2.0	1.8
CD14	CD14 molecule	2.2	3.5	2.0
CD163	CD163 molecule	2.3	4.2	2.2
MSR1	Macrophage scavenger receptor 1	1.8	2.6	1.9
Macrophage activation				
CHI3L1	Chitinase 3-like 1 (cartilage glycoprotein-39)	6.2	4.0	5.7
TLR1	Toll-like receptor 1	1.7	3.0	*1.6*
ARG1	Arginase. liver	1.9	2.3	2.1
TLR4	Toll-like receptor 4	*1.2*	2.0	*1.0*



h

GENE_symbol	Title	R I vs. HV	R II vs. HV	R III vs. HV
IL8	Interleukin 8	*1.8*	17.2	4.0
CXCL2	Chemokine (C–X–C motif) ligand 2	*1.5*	6.0	*2.0*
CXCL1	Chemokine (C–X–C motif) ligand 1 (melanoma growth Stimulating activity. alpha)	*1.3*	4.9	2.7
CXCL6	Chemokine (C–X–C motif) ligand 6 (granulocyte Chemotactic protein 2)	*1.2*	4.7	3.0
CXCL5	Chemokine (C–X–C motif) ligand 5	*1.1*	3.3	*1.2*

Figure 3. Recruitment and activation of macrophages and neutrophils differ between rosacea stages. The upper table (**a**) depicts monocyte/macrophage-related genes significantly modulated in at least one of the rosacea subtypes, which are involved in activation and proliferation (full list of gene sets in Supplementary Figure S4 online). Fold modulations in bold face type depict false discovery rate (FDR) < 0.05; fold modulations in italic type depict FDR > 0.05. The graphs depict representative pictures (**b**,**c**,**e**–**g**) and morphometric analysis (**d**) of $CD68^+$ cells in each rosacea subgroup (erythematotelangiectatic rosacea/rosacea type I (RI), papulopustular rosacea/rosacea type II (RII), phymatous rosacea/rosacea type II (RIII)), lupus erythematosus (LE) patients, and healthy skin (HS), all comprising $n \geq 5$ patients with five representative areas per slide (eff.pos.area = effective positive area). The lower table (**h**) displays neutrophil-associated genes with significant modulation.

normally innocuous physical stimuli (sunlight, temperature changes, spices) and associated imbalanced neuro-immune circuits may be important for the characteristic flushing responses of rosacea patients (Schwab *et al.*, 2011; Steinhoff *et al.*, 2011; Sulk *et al.*, 2012). A detailed analysis of the inflammatory mediators and cells that are involved in innate and adaptive immunity in rosacea patients, however, is so far lacking.

We show that the adaptive immune system is broadly and significantly activated, which interestingly starts as early as in ETR, and increases further in PPR. We identified the increased T-cell population as consisting of predominantly $CD4^+$ T helper cells, and our transcriptome data revealed an upregulation of Th1- and Th17-polarizing gene sets. We confirmed these findings by immunohistochemistry for the signature cytokines IFN-γ and IL-17A. Of note, the highest increase for all genes involved was observed in PPR, followed by PhR and ETR (both with roughly comparable changes). We observed an identical pattern of activation in macrophages and neutrophils (PPR > PhR > ETR). Together with our data that mast cells and mast cell–associated genes are increased in all three subtypes of rosacea, our studies indicate a critical role of Th1/Th17 and mast cells, as well as for macrophages in rosacea pathophysiology.

Previous studies on T cells in rosacea have been very limited in number or subtypes, and were exclusively histology based. One recent study compared T-cell subsets in nonpustular rosacea vs. LE patients with immunohistochemistry (Brown *et al.*, 2014). They found a T cell–rich lymphocytic infiltrate in rosacea with a ratio of $CD4^+/CD8^+$

a

GENE_symbol	Title	R I vs. HV	R II vs. HV	R III vs. HV
Antibodies				
IGHG1/ IGHG2/IGHM/	Immunoglobulin heavy constant gamma 1/constant gamma 2/constant mu/variavle 4–31	45	73	170
IGKC	Immunoglobulin kappa conatant	11	19	38
IGJ	Immunoglobulin J polypeptide	13	19	29
IGK@ / IGKC /	Immunoglobulin kappa locus/immunoglobulin kappa Constant	4.2	6.8	17
IGLJ3	Immunoglobulin lambda joining 3	2.2	2.9	7.9
IGH@/ IGHA1/IGHA2/ IGHD /IGHG1/IGHG3/ IGHG4/IGHM/IGHV3–23 / IGHV1–31	Immunoglobulin heavy locus	2.0	2.5	4.2
B-cell activation				
MS4A1	Membrane-spanning 4-domains subfamily A member 1	2.6	5.7	6.9
CD24	CD24 molecule	1.8	3.9	2.9
CD28	CD28 molecule	1.7	3.2	2.0
IL6	Interleukin 6 (interferon beta 2)	*1.1*	2.7	*1.4*
PAX5	Paired box 5	1.7	2.4	2.7
FASLG	Fas ligand (TNF superfamily member 6)	1.5	2.0	1.6
IL7	Interleukin 7	–2.0	–2.1	–2.5

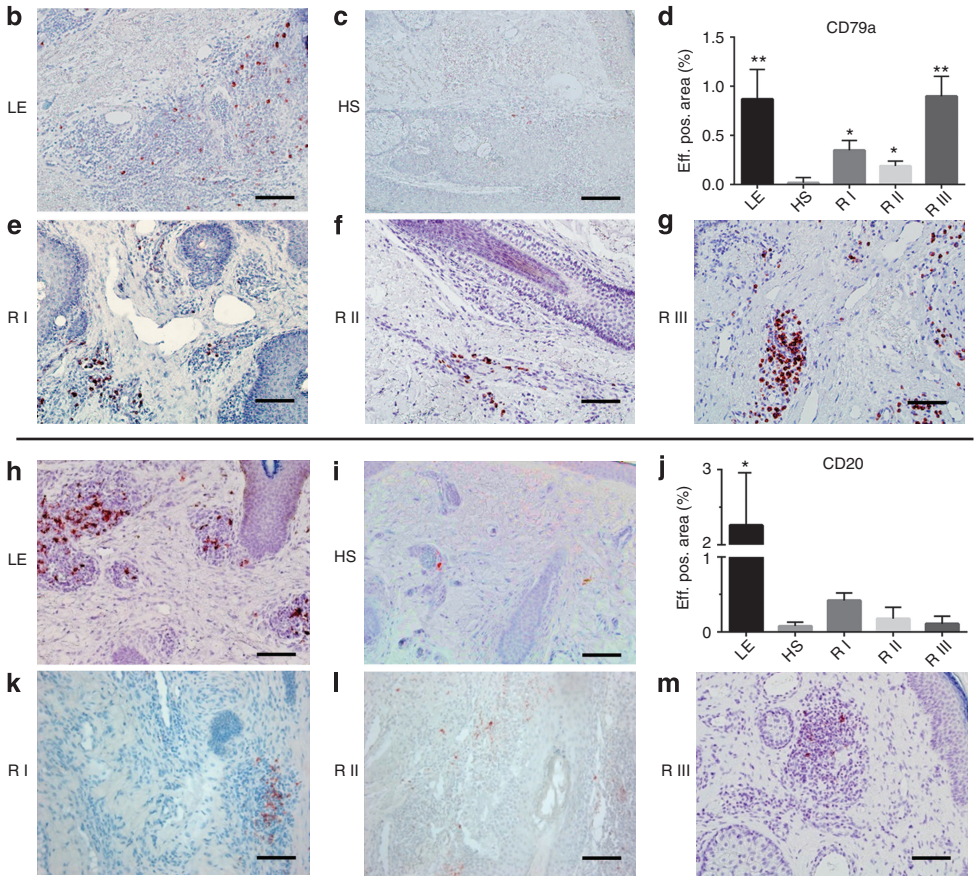


Figure 4. B cells and plasma cells are activated in the regulation of the immune response. The table (**a**) depicts B cell–related genes significantly modulated in at least one of the rosacea subtypes, which are involved in activation and proliferation (full list of gene sets in Supplementary Figure S5 online). Fold modulations in bold face type depict false discovery rate (FDR) < 0.05; fold modulations in italic type depict FDR > 0.05. The upper graphs depict representative pictures (**b**,**c**,**e**–**g**) and morphometric analysis (**d**) of CD79a$^+$ cells in each rosacea subgroup (erythematotelangiectatic rosacea/rosacea type I (RI), papulopustular rosacea/rosacea type II (RII), phymatous rosacea/rosacea type III (RIII)), lupus erythematosus (LE) patients, and healthy skin (HS), all comprising $n \geq 5$ patients with five representative areas per slide (eff.pos.area = effective positive area). The lower pictures (**h**,**I**,**k**–**m**) and graph (**j**) display the results for CD20$^+$ cells. All images are ×200 magnification (bar = 100 μm).

T cells of 2.80. This is in line with our detailed findings of a CD4$^+$ T cell–driven inflammatory response and their associated genes. Two other histological studies also describe an increase of CD4$^+$ over CD8$^+$ cells exclusively in PPR patients, and in ocular rosacea, respectively (Hoang-Xuan *et al.*, 1990; Georgala *et al.*, 2001). Our study, however, suggests a contribution of CD4$^+$ cells already in ETR, and also in PhR, although at different levels. In support, CD4$^+$ -associated genes are already significantly upregulated in ETR.

Of note, Th17 pathways were recently unveiled as being importantly involved in acne vulgaris (Agak *et al.*, 2014). In one transcriptome study on acne patients, the pattern of

GENE_symbol	Title	R I vs. HV	R II vs. HV	R III vs. HV
CXCL9	Chemokine (C-X-C motif) ligand 9	11	36	16
CXCL13	Chemokine (C-X-C motif) ligand 13	6.5	34	30
CCL18	Chemokine (C-C motif) ligand 18 (pulmonary and activation-regulated)	12	32	8.3
CXCL10	Chemokine (C-X-C motif) ligand 10	8.7	28	13
IL26	Interleukin 26	5.5	18	6.2
CXCL11	Chemokine (C-X-C motif) ligand 11	6.0	17	6.7
IL7R	Interleukin 7 receptor	4.1	11	4.4
CCL19	Chemokine (C-C motif) ligand 19	6.1	11	5.9
CXCR4	Chemokine (C X-C motif) receptor 4	5.1	8.9	4.8
IL36G	Interleukin 36. gamma	2.6	7.6	4.2
CCL8	Chemokine (C-C motif) ligand 8	2.6	6.9	*2.1*
CCL3 /CCL3L1 / CCL3L3	Chemokine (C-C motif) ligand 3 / chemokine (C-C motif) ligand 3-Likre 1/ chemokine (C-C motif) ligand 3-like 3	*2.1*	6.9	2.9
CCL20	Chemokine (C-C motif) ligand 20	4.3	6.7	4.2
CCR5	Chemokine (C-C motif) receptor 5 (gene/pseudogene)	3.0	6.6	4.3
IL8	Interleukin 8	*1.2*	6.5	*1.9*
CCL4	Chemokine (C-C motif) ligand 4	2.7	6.5	3.6
CXCL2	Chemokine (C-X-C motif) ligand 2	*1.5*	6.0	*2.0*
CCR7	Chemokine (C-C motif) receptor 7	2.1	5.8	2.9
CCR1	Chemokine (C-C motif) receptor 1	*1.8*	5.1	*2.0*
CSF2RB	Colony stimulating factor 2 receptor. beta. low-affinity (granulocyte-macrophage)	2.3	5.0	2.6
CXCL1	Chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity. alpha)	*1.3*	4.9	2.7
IL1B	Interleukin 1. beta	2.3	4.8	2.9
CXCL6	Chemokine (C-X-C motif) ligand 6 (granulocyte chemotactic protein 2)	*1.2*	4.7	3.0
IL12RB1	Interleukin 12 receptor. beta 1	2.2	4.7	2.6
CCL5	Chemokine (C-C motif) ligand 5	3.2	4.6	4.0
LTB	Lymphotoxin beta (TNF superfamily. member 3)	2.9	4.6	3.4
IL22	Interleukin 22	*1.6*	4.4	*1.2*
TNFSF13B	Tumor necrosis factor (ligand) superfamily. member 13b	2.1	4.3	1.9
IFNG	Interferon. gamma	*1.8*	4.1	*2.0*
CCR2	Chemokine (C-C motif) receptor 2	2.5	4.0	2.9
CCR1	Chemokine (C-C motif) receptor 1	*1.6*	4.0	*1.8*
IL13RA1	Interleukin 13 receptor. alpha 1	3.3	3.7	2.9
CSF2RA	Colony stimulating factor 2 receptor, alpha. low-affinity (granulocyte-macrophage)	1.6	3.3	1.8
CXCL5	Chemokine (C-X-C motif) ligand 5	*1.1*	3.3	*1.2*
CCL2	Chemokine (C-C motif) ligand 2	1.9	3.2	2.5
IL12RB2	Interleukin 12 receptor. beta 2	1.4	3.2	1.4
IL32	Interleukin 32	1.9	3.0	2.0
IL4R	Interleukin 4 receptor	1.9	3.0	2.1
CCRL2	Chemokine (C-C motif) receptor-like 2	1.5	2.8	1.5
IL6	Interleukin 6 (interferon. beta 2)	*1.1*	2.7	*1.4*
OSMR	Oncostatin M receptor	1.9	2.7	1.7
IL15	Interleukin 15	2.0	2.6	1.7
CXCR2	Chemokine (C-X-C motif) receptor 2	1.7	2.6	1.9
IL21R	Interleukin 21 receptor	*1.4*	2.6	1.5
XCL1 / XCL2	Chemokine (C-motif) ligand 1 /chemokine (C motif) ligand 2	*1.5*	2.5	*1.6*
CCRL1	Chemokine (C-C motif) receptor-like 1	–2.3	–2.6	–3.2
TNFRSF19	Tumor necrosis factor receptor superfamily. member 19	–2.2	–2.7	–2.5
IL20RB	Interleukin 20 receptor beta	–3.2	–4.0	–3.1
CCL27	Chemokine (C-C motif) ligand 27	–3.5	–5.7	–11.3
IL37	Interleukin 37	–4.6	–7.6	–5.6

Figure 5. Chemokine expression patterns support the Th1/Th17 polarization of the T-cell response. In all, 50 most upregulated and downregulated cytokines and chemokines, respectively, as well as their receptors, are depicted, sorted by expression in rosacea type II (RII). Fold modulations in bold face type depict false discovery rate (FDR) < 0.05; fold modulations in italic type depict FDR > 0.05.

upregulated genes was widely comparable to the pattern that we observed in rosacea (Kelhala *et al.*, 2014). Kistowska *et al.* (2015) have demonstrated that *Propionibacterium acnes* has a central role in driving the Th17/Th1 response in acne vulagris. Hypothetically, microorganisms may also be involved in polarizing the Th1/Th17 immune response in rosacea, although the role of microorganisms has been a matter of considerable debate in recent years. Several studies link the microbes *Demodex folliculorum*, *Bacillus oleronius*, *Helicobacter pylori*, *Staphylococcus epidermidis*, *Chlamydia pneumoniae,* and *Bartonella quintana* to rosacea pathophysiology (Holmes, 2013; Murillo *et al.*, 2014). Underlying mechanisms may be increased by bacterial loads and/or hyperreactivity of the patients' immune system. Nevertheless, the causative role of microorganisms (and especially *Demodex spp.* and/ or their associated microbiota) remains unclear in rosacea as of now. Although reduction of facial *Demodex* colonization has been achieved in multiple clinical trials on rosacea, a higher reduction of *Demodex* colonization did not directly translate into better clinical outcome in these patients (Kocak *et al.*, 2002).

It has been well recognized over the past years that dysregulation of the innate immune response contributes to rosacea pathophysiology significantly. The antimicrobial pro-protein cathelicidin and its fragment LL-37 were found to be

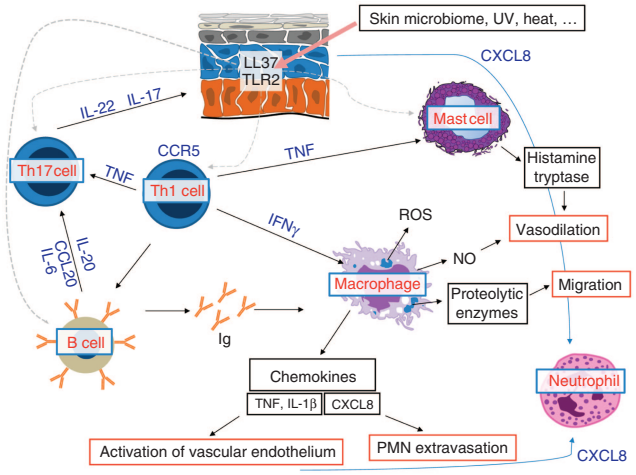


Figure 6. Hypothesis on involvement of the immune system in rosacea. This cartoon depicts our hypothesis how the complex network of soluble and cellular components of the innate and acquired immune system may interact for the generation of the Th1/Th17-polarized immune response in rosacea.

elevated and abnormally processed (Yamasaki *et al.*, 2007), and pattern-recognition receptors (TLR2, NALP3) were upregulated (Yamasaki *et al.*, 2011; Casas *et al.*, 2012). As the kallikrein (KLK) serine proteases 5 and 7 are major activators of antimicrobial peptides such as cathelicidin, the abundance of KLK5 in rosacea is responsible for processing the excess cathelicidins into aberrant forms, which fuels the course of the disease (Yamasaki *et al.*, 2007). Interestingly, it was recently shown that IL-17 can also contribute to the induction of LL-37 expression levels in keratinocytes (Chen *et al.*, 2013; Sakabe *et al.*, 2014), and therefore Th17 cytokines may also lead to or enhance the dysregulation of LL-37 expression.

Besides polarization of Th1/Th17-dominated immune responses, we also demonstrated upregulation of macrophage- and neutrophil-associated gene sets, as well as the influx of macrophages and their perifollicular and perivascular localization by IHC. This adds up well to our recently published observations on upregulation of mast cells in all rosacea subtypes, reaching a maximum in PPR (Schwab *et al.*, 2011). We did not observe any significant activation or expansion of Langerhans cells by transcriptome data, quantitative PCR, and IHC in all rosacea substages.

With regard to B cells, we found an unexpectedly massive upregulation of immunoglobulin-associated gene sets in all subgroup analyses, and IHC confirmed a significant increase in plasma cells, but not of $CD20^+$ B cells (Figure 4). Plasma cells and antibody production showed the highest involvement in PhR at the gene level, and strikingly already ETR patients revealed a significant increase in plasma cell numbers. Although the increase of plasma cells has been acknowledged in earlier studies, only limited research has focused on their pathophysiological role in rosacea (Smith *et al.*, 2007; Cribier, 2013). Thus, the role of plasma cells in rosacea pathophysiology remains highly speculative at this time. Interestingly, the bacterium *B. oleronius* can be isolated from a *D. folliculorum* mite extracted from a PPR patient (Lacey *et al.*, 2007). It was demonstrated that two highly immunogenic proteins from *B. oleronius* were capable of mounting an inflammatory response in PPR patients. Of note, the same reactivity to *B. oleronius* was already found in ETR patients, and also confirmed in patients with ocular rosacea (O'Reilly *et al.*, 2012; Jarmuda *et al.*, 2014). These promising findings warrant further verification, and proof of clinical improvement in patients with decreased numbers of *B. oleronius* is still lacking. Alternatively, autoimmune processes associated with molecular mimicry of antigens from microbiota and skin cells cannot be excluded, and should be more considered in the future.

In conclusion, our study showed significant involvement of the immune system in all subtypes of rosacea, characterizing even ETR as a chronic disease with significant inflammatory activity. The T-cell response is dominated by a Th1/Th17-polarized immune response, as we verified by correlating enhanced expression profiles and staining for signature cytokines including IFN-γ and IL-17. Chemokine expression patterns also support the Th1/Th17 polarization of the T-cell response, and recruitment, as well as activation, of macrophages or neutrophils, which is already present in ETR and reaches a maximum in PPR. Our current hypothesis of the complex interplay of the different skin cells in rosacea is depicted in Figure 6. We also provide evidence for a vast activation of antibody-producing plasma cells, beginning already in ETR, followed by a crescendo pattern toward PhR. Thus, an altered adaptive, as well as innate, immunity is the hallmark in the development of rosacea. Therapies targeting the Th1/Th17 pathway are promising candidates for an optimized treatment of this frequent, chronic skin disease.

MATERIALS AND METHODS

Tissue collection and patients selection

For Affymetrix microarray studies and qRT–PCR-analysis, we studied a group of 19 rosacea patients, of whom 7 were diagnosed with ETR, 6 with PPR, and 6 with PhR based on the classification system of the American Rosacea Society (Wilkin *et al.*, 2002). Skin biopsies were taken for all disease subtypes from the nasolabial fold of the patients with the aim to minimize differences based on biopsy localization. In addition, skin biopsies of 10 healthy individuals were investigated. Patients with systemic LE displaying a malar rash were identically biopsied from the nasolabial fold for comparison. Skin biopsies were obtained in local anesthesia after the patients gave their written consent. Permission for human studies was given by the Ethical Committee of the University of Münster, Germany, in accordance with the ethical standards of the 1964 Declaration of Helsinki Principles.

Supplement material

Additional materials and methods for this manuscript can be found in the supplement.

CONFLICT OF INTEREST

JA, IC, SD, PR, MR, and JJV are employees of Galderma. The remaining authors state no conflict of interest.

ACKNOWLEDGMENTS

We thank C Mess, C Menigot, S Corvaisier, and S Wise for excellent technical assistance. Furthermore, we highly appreciate the contribution of all patients in this study. This work was supported by grants of the German Research Foundation to TB (BU2991/1-1), MSu (SU803/1-1), MSt (IZKF, STE1014), and by the Dublin Skin and Cancer Hospital Charity (Ireland) to MSt.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

REFERENCES

Agak GW, Qin M, Nobe J *et al.* (2014) *Propionibacterium acnes* induces an IL-17 response in acne vulgaris that is regulated by vitamin A and vitamin D. *J Invest Dermatol* 134:366–73

Aloi F, Tomasini C, Soro E *et al.* (2000) The clinicopathologic spectrum of rhinophyma. *J Am Acad Dermatol* 42:468–72

Brown TT, Choi EY, Thomas DG *et al.* (2014) Comparative analysis of rosacea and cutaneous lupus erythematosus: histopathologic features, T-cell subsets, and plasmacytoid dendritic cells. *J Am Acad Dermatol* 71:100–7

Casas C, Paul C, Lahfa M *et al.* (2012) Quantification of Demodex folliculorum by PCR in rosacea and its relationship to skin innate immune activation. *Exp Dermatol* 21:906–10

Casati C, Camisaschi C, Rini F *et al.* (2006) Soluble human LAG-3 molecule amplifies the in vitro generation of type 1 tumor-specific immunity. *Cancer Res* 66:4450–60

Cerboni C, Ardolino M, Santoni A *et al.* (2009) Detuning CD8+ T lymphocytes by down-regulation of the activating receptor NKG2D: role of NKG2D ligands released by activated T cells. *Blood* 113:2955–64

Chen X, Takai T, Xie Y *et al.* (2013) Human antimicrobial peptide LL-37 modulates proinflammatory responses induced by cytokine milieus and double-stranded RNA in human keratinocytes. *Biochem Biophys Res Commun* 433:532–7

Chu PG, Arber DA (2001) CD79: a review. *Appl Immunohistochem Mol Morphol* 9:97–106

Cribier B (2013) Rosacea under the microscope: characteristic histological findings. *J Eur Acad Dermatol Venereol* 27:1336–43

del Rio ML, Lucas CL, Buhler L *et al.* (2010) HVEM/LIGHT/BTLA/CD160 cosignaling pathways as targets for immune regulation. *J Leukoc Biol* 87: 223–35

Fang X, Zheng P, Tang J *et al.* (2010) CD24: from A to Z. *Cell Mol Immunol* 7: 100–3

Gallego C, Golenbock D, Gomez MA *et al.* (2011) Toll-like receptors participate in macrophage activation and intracellular control of Leishmania (Viannia) panamensis. *Infect Immun* 79:2871–9

Georgala S, Katoulis AC, Kylafis GD *et al.* (2001) Increased density of Demodex folliculorum and evidence of delayed hypersensitivity reaction in subjects with papulopustular rosacea. *J Eur Acad Dermatol Venereol* 15: 441–4

Gerber PA, Buhren BA, Steinhoff M *et al.* (2011) Rosacea: The cytokine and chemokine network. *J Investig Dermatol Symp Proc* 15:40–7

Gomez-Rodriguez J, Readinger JA, Viorritto IC *et al.* (2007) Tec kinases, actin, and cell adhesion. *Immunol Rev* 218:45–64

Gordon S, Martinez FO (2010) Alternative activation of macrophages: mechanism and functions. *Immunity* 32:593–604

Hoang-Xuan T, Rodriguez A, Zaltas MM *et al.* (1990) Ocular rosacea. A histologic and immunopathologic study. *Ophthalmology* 97:1468–75

Holmes AD (2013) Potential role of microorganisms in the pathogenesis of rosacea. *J Am Acad Dermatol* 69:1025–32

Jansen T, Plewig G (1997) Rosacea: classification and treatment. *J R Soc Med* 90:144–50

Jarmuda S, McMahon F, Zaba R *et al.* (2014) Correlation between serum reactivity to Demodex-associated Bacillus oleronius proteins, and altered sebum levels and Demodex populations in erythematotelangiectatic rosacea patients. *J Med Microbiol* 63:258–62

Kelhala HL, Palatsi R, Fyhrquist N *et al.* (2014) IL-17/Th17 pathway is activated in acne lesions. *PLoS One* 9:e105238

Kistowska M, Meier B, Proust T *et al.* (2015) *Propionibacterium acnes* promotes Th17 and Th17/Th1 responses in acne patients. *J Invest Dermatol* 135: 110–8

Kocak M, Yagli S, Vahapoglu G *et al.* (2002) Permethrin 5% cream versus metronidazole 0.75% gel for the treatment of papulopustular rosacea. A randomized double-blind placebo-controlled study. *Dermatology* 205: 265–70

Lacey N, Delaney S, Kavanagh K *et al.* (2007) Mite-related bacterial antigens stimulate inflammatory cells in rosacea. *Br J Dermatol* 157: 474–81

Loetscher P, Pellegrino A, Gong JH *et al.* (2001) The ligands of CXC chemokine receptor 3, I-TAC, Mig, and IP10, are natural antagonists for CCR3. *J Biol Chem* 276:2986–91

Lyakh L, Trinchieri G, Provezza L *et al.* (2008) Regulation of interleukin-12/interleukin-23 production and the T-helper 17 response in humans. *Immunol Rev* 226:112–31

Marks R, Harcourt-Webster JN (1969) Histopathology of rosacea. *Arch Dermatol* 100:683–91

Medvedovic J, Ebert A, Tagoh H *et al.* (2011) Pax5: a master regulator of B cell development and leukemogenesis. *Adv Immunol* 111:179–206

Murillo N, Mediannikov O, Aubert J *et al.* (2014) Bartonella quintana detection in Demodex from erythematotelangiectatic rosacea patients. *Int J Infect Dis* 29:176–7

Nibbs RJ, Salcedo TW, Campbell JD *et al.* (2000) C-C chemokine receptor 3 antagonism by the beta-chemokine macrophage inflammatory protein 4, a property strongly enhanced by an amino-terminal alanine-methionine swap. *J Immunol* 164:1488–97

Nold MF, Nold-Petry CA, Zepp JA *et al.* (2010) IL-37 is a fundamental inhibitor of innate immunity. *Nat Immunol* 11:1014–22

Nomura I, Gao B, Boguniewicz M *et al.* (2003) Distinct patterns of gene expression in the skin lesions of atopic dermatitis and psoriasis: a gene microarray analysis. *J Allergy Clin Immunol* 112:1195–202

O'Reilly N, Menezes N, Kavanagh K (2012) Positive correlation between serum immunoreactivity to Demodex-associated Bacillus proteins and erythematotelangiectatic rosacea. *Br J Dermatol* 167:1032–6

Oestreich KJ, Weinmann AS (2012) Transcriptional mechanisms that regulate T helper 1 cell differentiation. *Curr Opin Immunol* 24:191–5

Palau N, Julia A, Ferrandiz C *et al.* (2013) Genome-wide transcriptional analysis of T cell activation reveals differential gene expression associated with psoriasis. *BMC Genomics* 14:825

Powell FC (2005) Clinical practice. Rosacea. *N Engl J Med* 352:793–803

Rathcke CN, Vestergaard H (2006) YKL-40, a new inflammatory marker with relation to insulin resistance and with a role in endothelial dysfunction and atherosclerosis. *Inflamm Res* 55:221–7

Riis JL, Johansen C, Vestergaard C *et al.* (2011) Kinetics and differential expression of the skin-related chemokines CCL27 and CCL17 in psoriasis, atopic dermatitis and allergic contact dermatitis. *Exp Dermatol* 20: 789–94

Rufli T, Buchner SA (1984) T-cell subsets in acne rosacea lesions and the possible role of Demodex folliculorum. *Dermatologica* 169:1–5

Rutz S, Eidenschenk C, Ouyang W (2013) IL-22, not simply a Th17 cytokine. *Immunol Rev* 252:116–32

Sakabe J, Umayahara T, Hiroike M *et al.* (2014) Calcipotriol increases hCAP18 mRNA expression but inhibits extracellular LL37 peptide production in IL-17/IL-22-stimulated normal human epidermal keratinocytes. *Acta Derm Venereol* 94:512–6

Salmond RJ, Filby A, Qureshi I *et al.* (2009) T-cell receptor proximal signaling via the Src-family kinases, Lck and Fyn, influences T-cell activation, differentiation, and tolerance. *Immunol Rev* 228:9–22

Scapini P, Lapinet-Vera JA, Gasperini S *et al.* (2000) The neutrophil as a cellular source of chemokines. *Immunol Rev* 177:195–203

Schwab VD, Sulk M, Seeliger S *et al.* (2011) Neurovascular and neuroimmune aspects in the pathophysiology of rosacea. *J Investig Dermatol Symp Proc* 15:53–62

Singh SP, Zhang HH, Foley JF *et al.* (2008) Human T cells that are able to produce IL-17 express the chemokine receptor CCR6. *J Immunol* 180: 214–21

Smith JR, Lanier VB, Braziel RM *et al.* (2007) Expression of vascular endothelial growth factor and its receptors in rosacea. *Br J Ophthalmol* 91: 226–9

Steinhoff M, Buddenkotte J, Aubert J *et al.* (2011) Clinical, cellular, and molecular aspects in the pathophysiology of rosacea. *J Investig Dermatol Symp Proc* 15:2–11

Steinhoff M, Schauber J, Leyden JJ (2013) New insights into rosacea pathophysiology: a review of recent findings. *J Am Acad Dermatol* 69: S15–26

Suarez-Farinas M, Dhingra N, Gittler J *et al.* (2013) Intrinsic atopic dermatitis shows similar TH2 and higher TH17 immune activation compared with extrinsic atopic dermatitis. *J Allergy Clin Immunol* 132:361–70

Sulk M, Seeliger S, Aubert J *et al.* (2012) Distribution and expression of non-neuronal transient receptor potential (TRPV) ion channels in rosacea. *J Invest Dermatol* 132:1253–62

Turner JE, Steinmetz OM, Stahl RA *et al.* (2007) Targeting of Th1-associated chemokine receptors CXCR3 and CCR5 as therapeutic strategy for inflammatory diseases. *Mini Rev Med Chem* 7:1089–96

Two AM, Hata TR, Nakatsuji T *et al.* (2014) Reduction in serine protease activity correlates with improved rosacea severity in a small, randomized pilot study of a topical serine protease inhibitor. *J Invest Dermatol* 134:1143–5

Wang JH, Reinherz EL (2012) The structural basis of alphabeta T-lineage immune recognition: TCR docking topologies, mechanotransduction, and co-receptor function. *Immunol Rev* 250:102–19

Wilkin J, Dahl M, Detmar M *et al.* (2002) Standard classification of rosacea: Report of the National Rosacea Society Expert Committee on the Classification and Staging of Rosacea. *J Am Acad Dermatol* 46:584–7

Wollina U (2014) Recent advances in the understanding and management of rosacea. *F1000Prime Rep* 6:50

Yamasaki K, Di Nardo A, Bardan A *et al.* (2007) Increased serine protease activity and cathelicidin promotes skin inflammation in rosacea. *Nat Med* 13:975–80

Yamasaki K, Gallo RL (2011) Rosacea as a disease of cathelicidins and skin innate immunity. *J Investig Dermatol Symp Proc* 15:12–5

Yamasaki K, Kanada K, Macleod DT *et al.* (2011) TLR2 expression is increased in rosacea and stimulates enhanced serine protease production by keratinocytes. *J Invest Dermatol* 131:688–97

Zlotnik A, Yoshie O (2012) The chemokine superfamily revisited. *Immunity* 36: 705–16