DERMATO-ENDOCRINOLOGY
2018, VOL. 9, NO. 1, e1356518 (13 pages)
https://doi.org/10.1080/19381980.2017.1356518

Taylor & Francis
Taylor & Francis Group

RESEARCH PAPER

OPEN ACCESS Check for updates

Insulin and Insulin-like growth factor-1 can activate the phosphoinositide-3-kinase /Akt/FoxO1 pathway in T cells *in vitro*

Yasaman Mirdamadi[a], Ursula Bommhardt[b], Alexander Goihl[b], Karina Guttek[b], Christos C. Zouboulis [c], Sven Quist[a], and Harald Gollnick[a]

[a]Department of Dermatology and Venereology, Otto-von-Guericke-University Magdeburg, Magdeburg, Germany; [b]Institute of Molecular and Clinical Immunology, Otto-von-Guericke-University Magdeburg, Magdeburg, Germany; [c]Departments of Dermatology, Venereology, Allergology and Immunology, Dessau Medical Center, Dessau, Germany

ABSTRACT

Hyper-glycemic food increases insulin-like growth factor 1 (IGF-1) and insulin signaling and regulates endocrine responses and thereby may modulate the course of acne. Inflammation and adaptive immune responses have a pivotal role in all stages of acne. Recent hypothesis suggests that hyperglycemic food reduces nuclear forkhead box-O1 (FoxO1) transcription factor and may eventually induces acne. The aim of our study was to investigate the role of IGF-1 and insulin on the phosphoinositide-3-kinase (PI3K)/Akt/FoxO1 pathway in human primary T cells and on the molecular functions of T cells *in vitro*. T cells were stimulated with 0.001 μM IGF-1 or 1 μM insulin +/− 20 μM PI3K inhibitor LY294002. T cells were also exposed to SZ95 sebocyte supernatants which were pre-stimulated with IGF-1 or insulin. We found that 0.001 μM IGF-1 and 1 μM insulin activate the PI3K pathway in T cells leading to up-regulation of p-Akt and p-FoxO1 at 15 and 30 minutes. Nuclear FoxO1 was decreased and FoxO transcriptional activity was reduced. 0.001 μM IGF-1 and 1 μM insulin increased T cell proliferation but have no significant effect on Toll-like receptor2/4 (TLR) expression. Interestingly, supernatants from IGF-1- or insulin-stimulated sebocytes activated the PI3K pathway in T cells but reduced T cell proliferation. Taken together, this study helps to support that high glycemic load diet may contribute to induce activation of the PI3K pathway and increase of proliferation in human primary T cells. Factors secreted by IGF-1- and insulin-stimulated sebocytes induce the PI3K pathway in T cells and reduce T cell proliferation, which probably can reflect a protective mechanism of the sebaceous gland basal cells.

ARTICLE HISTORY
Received 25 April 2017
Revised 9 July 2017
Accepted 11 July 2017

KEYWORDS
Acne; FoxO1; High glycaemic load diet; IGF-1; PI3K; sebocytes; T cells

Introduction

Acne is presenting as a chronic and relapsing inflammatory disorder.[1] Inflammatory events have been found at all stages of acne including the early microcomedone development.[2-4] However, the whole pile of mediators of inflammation and the ranking of events has been not elucidated in detail yet. Although numerous investigations demonstrated *Propionibacterium acnes* (P. acnes) as a key factor in the development of inflammation in acne, other reports showed that this microorganism is not always required, regardless of the acne lesion type.[5-8] Instead, several investigations indicated that sebaceous glands by themselves play a role in the development of inflammatory acne.[9,10] Regular T cell trafficking has been detected around normal infundibula of the follicle in biopsies of the sebaceous unit from acne patients; however, around clinically non inflamed follicles the number was already significantly increased. Accordingly, T cells contribute to the initiation of inflammation in acne.[1,11] Recently, it has been hypothesized that hyper-glycemic load diet and skim milk consumption which increase insulin-like growth factor 1 (IGF-1) and insulin signaling may modulate the course of acne via activation of the phosphoinositide 3-kinase (PI3K)/Akt pathway and reduction of nuclear forkhead box-O1 (FoxO1) transcription factor. Our previous study showed that 1 and 0.1 μM IGF-1 and

CONTACT Yasaman Mirdamadi y_mirdamadi@web.de Department of Dermatology and Venereology, Otto-von Guericke University, Leipziger Str. 44, 39120 Magdeburg, Germany.

This paper is part of the special section based on the 3rd ICSGAD conference held in September 2016.

insulin activate the PI3K/Akt/FoxO1 pathway and can induce expression of toll-like receptor (TLR2/4) in human SZ95 sebocytes as a *P. acnes* independent and possibly be an explanation of the very early event in microcomedogenesis.[12] The aim of our present study was to investigate the role of IGF-1 and insulin on the PI3K/Akt/FoxO1 pathway in human primary T cells and on the molecular functions of T cells *in vitro*. Furthermore, the effect of supernatants from IGF-1- or insulin-stimulated sebocytes on the PI3K/Akt/FoxO1 pathway in human primary T cells was investigated.

Results

The PI3K/Akt/FoxO1 pathway is activated by IGF-1 and insulin in human primary T cells

To investigate the effects of IGF-1 and insulin on PI3K pathway activation in human T cells isolated from peripheral blood of healthy donors, CD3-activated T cells were stimulated with 0.001 μM IGF-1 and 1 μM insulin for different time points between 15 to 120 minutes. Western blot results from whole cell protein fractions showed that p-Akt expression was significantly up-regulated at 15 minutes which declined again at 60 minutes upon IGF-1 stimulation compared to levels of CD3-activated control cells (Fig. 1a and e). Up-regulation of p-FoxO1 was detected first already at 15 minutes (Fig. 1c and g) when T cells were stimulated with 0.001 μM IGF-1. Expression of p-Akt and p-FoxO1 was not changed in CD3-activated control cells after different time points (data not shown). Conversely, phosphorylation of Akt and FoxO1 was blocked upon pre-incubation of cells with the PI3K inhibitor LY294002 (20 μM) at time points 15 to 120 minutes (Fig. 1b, d, f and h). Insulin (1 μM) raised p-Akt and p-FoxO1 expression at 15 and 30 minutes in the normal situation without blocking with LY (Fig. 2a, c, e and g) and expression of p-Akt and p-FoxO1 was reduced after 60, 90, and 120 minutes. Up-regulation of p-Akt and p-FoxO1 was blocked upon pre-treatment with LY (Fig. 2b, d, f and h). These results propose that both, IGF-1 and insulin activate the PI3K/Akt/FoxO1 pathway in activated human T cells.

IGF-1 and insulin promote accumulation of nuclear p-Akt and reduce FoxO1 expression in human primary T cells

To investigate the effect of IGF-1 and insulin on cytoplasmic and nuclear expression of p-Akt, p-FoxO1, and FoxO1, immunofluorescence imaging of T cells was done. CD3-activated T cells were stimulated with 0.001 μM IGF-1 and 1 μM insulin for 15 and 30 minutes which were the best time points according to western blot results. The amount of cytoplasmic p-Akt was increased at 15 minutes, whereas nuclear accumulation of p-Akt was detected later after 30 minutes upon

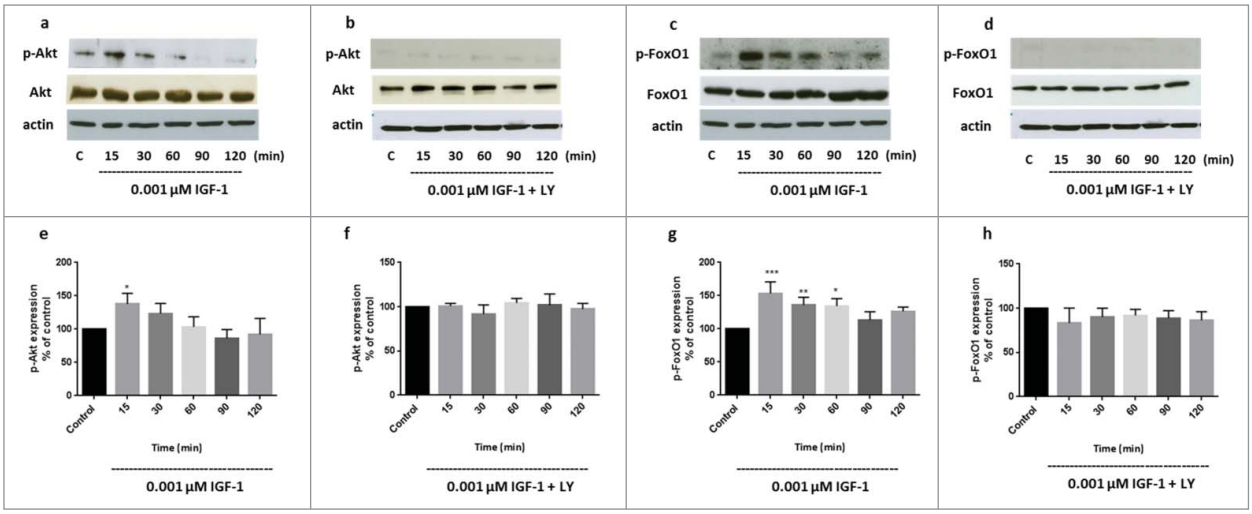

Figure 1. The expression of p-Akt and p-FoxO1 is induced in human CD3-activated (T)cells after stimulation with 0.001 μM IGF-1. Activated T cells were treated with 0.001 μM IGF-1 in the presence or absence of the PI3K inhibitor LY294002 (20 μM) for 15 to 120 minutes. Whole cell protein extracts were analyzed by western blot. (a and b) Representative blots were probed for p-Akt and (c and d) for p-FoxO1. (e-h) Densitometric quantification of western blots and normalization to β-actin. Densitometric data are given as the percentage of the value in untreated cells. Data represent the means + SEMs (n = 3); * $p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$, one-way ANOVA.

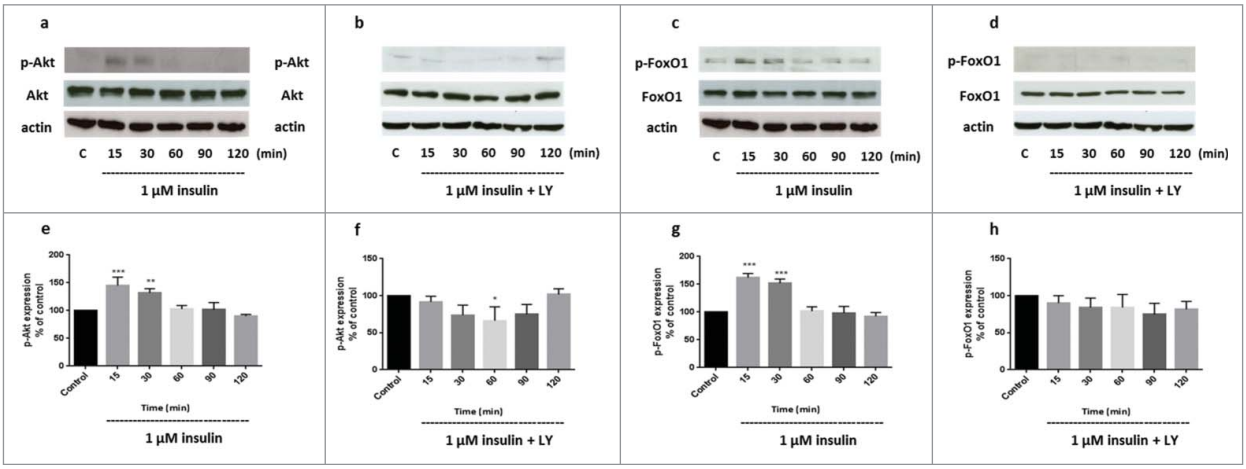

Figure 2. The expression of p-Akt and p-FoxO1 is induced in human CD3-activated (T)cells after stimulation with 1 μM insulin. CD3-activated T cells were treated with 1 μM insulin in the presence or absence of the PI3K inhibitor LY294002 (20 μM) for 15 to 120 minutes. Whole cell protein extracts were analyzed by western blot. (a and b) Representative blots were probed for p-Akt and (c and d) for p-FoxO1. (e-h) Densitometric quantification of western blots and normalization to β-actin. Densitometric data are given as the percentage of the value in untreated cells. Data represent the means + SEMs (n = 3); * $p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$, one-way ANOVA.

0.001 μM IGF-1 stimulation. IGF-1 induced upregulation of cytoplasmic and nuclear p-FoxO1 after 15 minutes; however, nuclear p-FoxO1 expression declined at 30 minutes. The expression of nuclear FoxO1 was decreased at 15 and 30 minutes after IGF-1 stimulation compared to CD3-activated control cells. Furthermore, pre-incubation of the cells with the LY inhibitor (20 μM) decreased expression of p-Akt and p-FoxO1 and increased expression of nuclear FoxO1 (Fig. 3).

1 μM insulin increased cytoplasmic p-Akt after 15 and 30 minutes. Furthermore, cytoplasmic p-FoxO1 was increased after 15 and 30 minutes. Expression of nuclear FoxO1 was reduced after insulin stimulation compared to CD3-activated control cells. Pre-incubation with LY blocked both Akt and FoxO1 phosphorylation, and hence cytoplasmic translocation of FoxO1 proteins was blocked (Fig. 4). These results further demonstrate that IGF-1 and insulin activate the PI3K/Akt pathway leading to FoxO1 phosphorylation and its cytoplasmic accumulation.

IGF-1 and insulin reduce FoxO transcriptional activity in human primary T cells

Firefly luciferase FoxO reporter assays were performed to analyze the transcriptional activity of FoxO in response to PI3K/Akt pathway activation by IGF-1 and insulin. CD3-activated T cells were transiently transfected with a FoxO reporter construct and were stimulated with 0.001 μM IGF-1 or 1 μM insulin for 15 minutes. The luciferase assays showed that FoxO transcriptional activity is significantly decreased 15 minutes after exposure to IGF-1 and insulin with a maximum suppression of 38% and 28%, respectively, compared to CD3-activated control cells. Furthermore, pre-treatment of transfected T cells with the PI3K inhibitor prevented the decrease of FoxO transcriptional activity in the presence of IGF-1 or insulin (Fig. 5). These results confirm that IGF-1 and insulin regulate FoxO transcriptional activity in activated T cells via the PI3K/Akt pathway.

IGF-1 and insulin increase T cell proliferation via the PI3K/Akt pathway

To investigate the role of IGF-1 and insulin on human T cell proliferation, CD3-activated T cells were stimulated with IGF-1 and insulin for 24 to 72 hours. [^{3}H]-thymidine incorporation assays showed that 0.001 μM IGF-1 and 1 μM insulin enhance DNA synthesis in viable T cells at 24, 48, and 72 hours with a maximum increase of 29% at 72 hours compared towith CD3-activated control cells (Fig. 6a and b). LY decreased T cell proliferation; however, proliferation of T cells was even more reduced after treatment of cells with a combination of LY and IGF-1 or insulin (Fig. 6a and b). These data suggest that IGF-1 and insulin significantly increase proliferation of human T cell proliferation via PI3K/Akt activation.

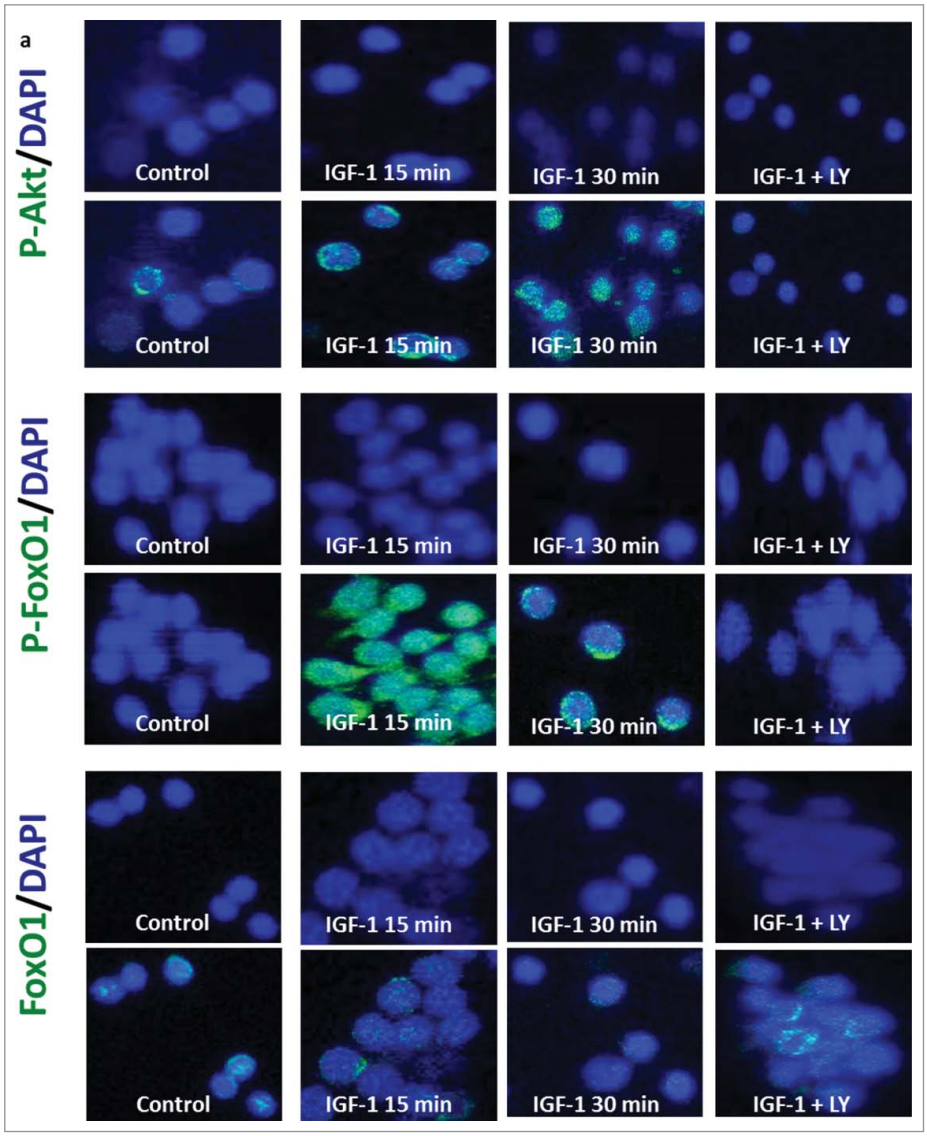

Figure 3. IGF-1 induces p-Akt and p-FoxO1 expression in human CD3-activated T cells. Activated T cells were treated with IGF-1 (0.001 μM) in the absence or presence of the PI3K inhibitor LY294002 for different time points and analyzed by immunofluorescence staining. Merged confocal microscopic images show p-Akt, p-FoxO1, FoxO1 (FITC) (pseudo-colored in green) and DAPI (pseudo-colored in blue) upon stimulation with IGF-1 (0.001 μM) for 15 and 30 minutes.

Expression of TLR2 and TLR4 is not significantly changed upon IGF-1 and insulin stimulation in T cells

Next, we explored the effect of IGF-1 and insulin on TLR2/4 expression in CD3-activated T cells. Activated T cells were stimulated with 0.001 μM IGF-1 or 1 μM insulin for 48 hours and TLR2/4 expression was measured by flow cytometry. TLR2/4 expression was slightly up-regulated at 48 hours following IGF-1 and insulin incubation, but this was not significant (Fig. 7a and b). Pre-treatment with the PI3K inhibitor, which blocks T cell proliferation, significantly decreased TLR2 expression in the presence of IGF-1 or insulin (Fig. 7a). Expression of TLR4 was hardly affected by IGF-1, insulin or LY treatment (Fig. 7b).

The PI3K/Akt/FoxO1 pathway is activated by supernatants from IGF-1- or insulin-stimulated sebocytes in human primary T cells

To investigate whether stimulated sebocytes could affect T cells, CD3-activated T cells were co-cultured for 15 minutes (the best time point according to previous T cell western blot results (Fig. 1)) with supernatants from IGF-1- or insulin-stimulated sebocytes and western blots were performed. The results showed that p-Akt and p-FoxO1 expression in T cells is significantly up-regulated at 15 minutes upon co-culture with supernatants of IGF-1- and insulin-stimulated sebocytes. Phosphorylation of Akt and FoxO1 was

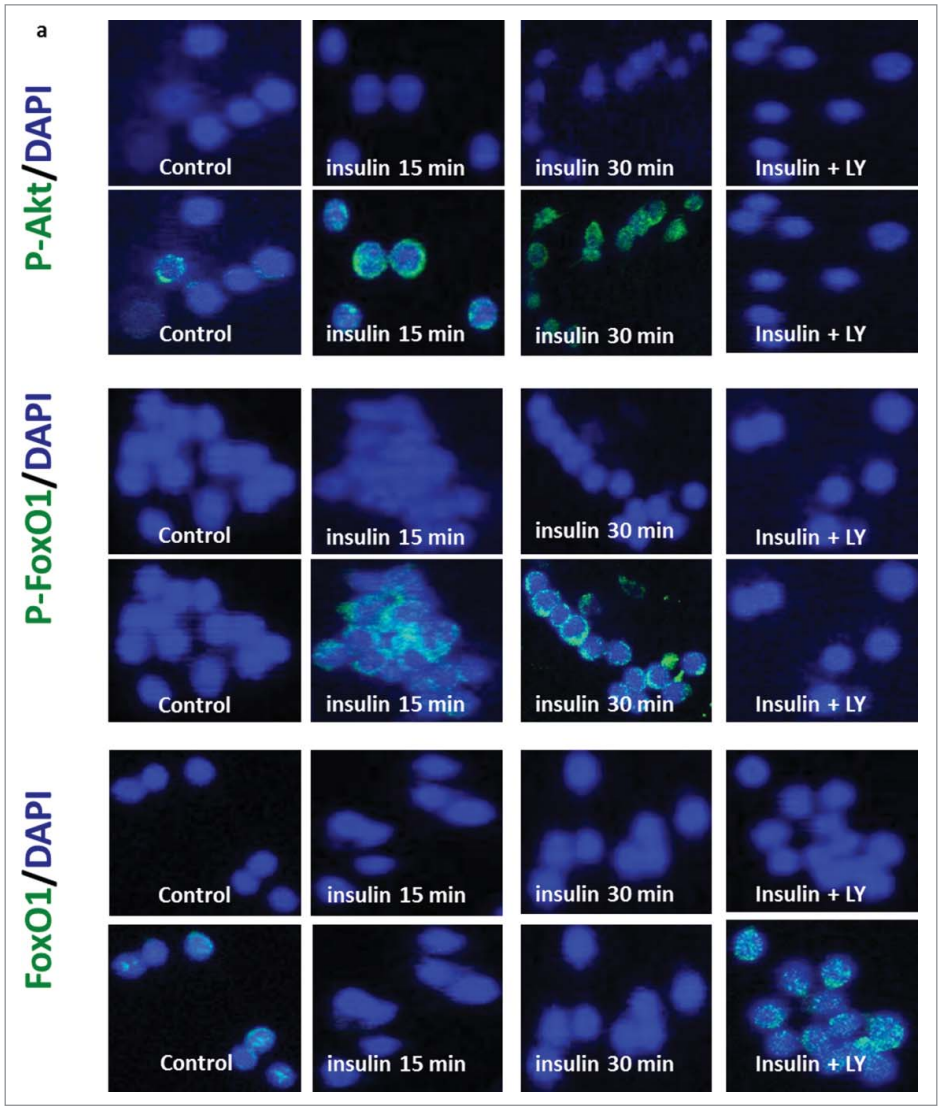

Figure 4. Insulin induces p-Akt and p-FoxO1 expression in human CD3-activated T cells. Activated T cells were treated with insulin (1 μM) in the absence or presence of the PI3K inhibitor LY294002 for different time points and analyzed by immunofluorescence staining. Merged confocal microscopic images show p-Akt, p-FoxO1, FoxO1 (FITC) (pseudo-colored in green) and DAPI (pseudo-colored in blue) upon stimulation with insulin (1 μM) for 15 and 30 minutes.

blocked upon pre-incubation with the PI3K inhibitor LY (20 μM) (Fig. 8a-d). These results give some evidence that culture supernatants from growth factor-stimulated sebocytes have an ability to activate the PI3K/Akt/FoxO1 pathway in human primary T cells.

Supernatants from cultures of IGF-1- or insulin-stimulated SZ95 sebocytes reduce human primary T cell proliferation

To investigate the effect of IGF-1- or insulin-stimulated SZ95 sebocytes on T cell proliferation, CD3-activated T cells were treated with supernatants from IGF-1- or insulin-stimulated SZ95 sebocyte for 24 to 72 hours. [^{3}H]-thymidine incorporation assays showed that supernatants from IGF-1- or insulin-stimulated SZ95 sebocytes suppress DNA synthesis in viable T cells after 24, 48, and 72 hours, with a maximum suppression of 50% after treatment with supernatants of IGF-1-stimulated sebocytes at 72 hours (Fig. 9). Pre-treatment with the PI3K inhibitor decreased T cell proliferation; however, LY together with supernatants from IGF-1- or insulin-stimulated SZ95 sebocytes reduced T cell proliferation more effectively (Fig. 9). These results demonstrate that IGF- or insulin-stimulated sebocytes secret factors into the supernatant which have an ability to decrease T cell proliferation.

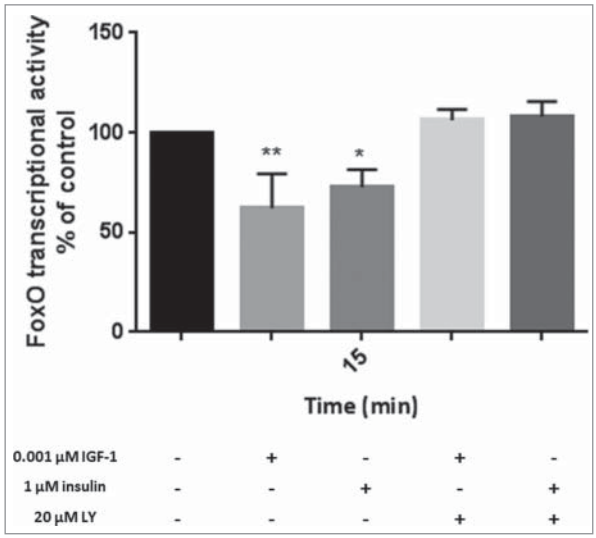

Figure 5. Stimulation with IGF-1 and insulin reduce FoxO transcriptional activity in human CD3-activated T cells. Activated T cells were transfected with a FoxO Reporter construct and FoxO transcriptional activity was measured by luciferase assay after stimulation of T cells with 0.001 μM IGF-1 or 1 μM insulin in the absence or presence of PI3K inhibitor LY294002 (20 μM) for 15 minutes. Data are shown as a percentage of the values in untreated cells. Data represent the means + SEMs of 3 independent experiments; ($^{*}p < 0.05$ and $^{**}p < 0.01$, Student's *t-test*).

Discussion

Acne is a chronic and relapsing inflammatory disorder. Inflammation and adaptive immune responses play an important role in all stages of this disorder.[1,13-15] The skin of normal adults contains around 20 billion T cells, which have a role in inflammatory skin disorders.[16,17] In addition, increased amounts of $CD4^+$ T cells have been detected around clinically uninvolved follicles and $CD8^+$ T cells are detected at later time points.[1,18,19] The number is dramatically increasing in papules not older than 6hrs of clinical visibility. $CD4^+$ T cells and macrophages (CD209) have been detected around normal follicles in biopsies from acne patients.[20] T cells contribute therefore to the initiation of inflammation in acne. Recently, the role of diet in acne is newly discussed.[21,22] It has been reported that hyper-glycemic food and skim milk consumption increase insulin and IGF-1 signaling and regulates endocrine responses and thereby may modulate the course of acne.[23-26] Additionally, a new hypothesis in the pathogenesis of acne suggests that the acnegenic stimuli may activate the PI3K/Akt pathway and therefore, reduce nuclear FoxO1 expression and activity.[27] The PI3K pathway has an important role in cell growth, cell cycle and differentiation. FoxO transcription factors are targets of the PI3K pathway and play a pivotal role in T cell proliferation and apoptosis.[28,29]

Our results in SZ95 sebocytes showed that IGF-1 and insulin reduce nuclear FoxO1 and FoxO transcriptional activity via PI3K/Akt pathway activation.[12] Insulin-like growth factor 1 receptor (IGF-1R) and insulin receptor (IR) are tyrosine kinase receptors which are expressed on many cell types including T cells.[30,31] IGF-1 and insulin regulate T cell development, proliferation, differentiation, and function.[32-36] To investigate the role of dietary factors on PI3K pathway activation and T cell function, one aim of this study was to explore the effect of IGF-1 and insulin as putative acnegenic stimuli on the PI3K pathway activation and expression of FoxO1 in human primary

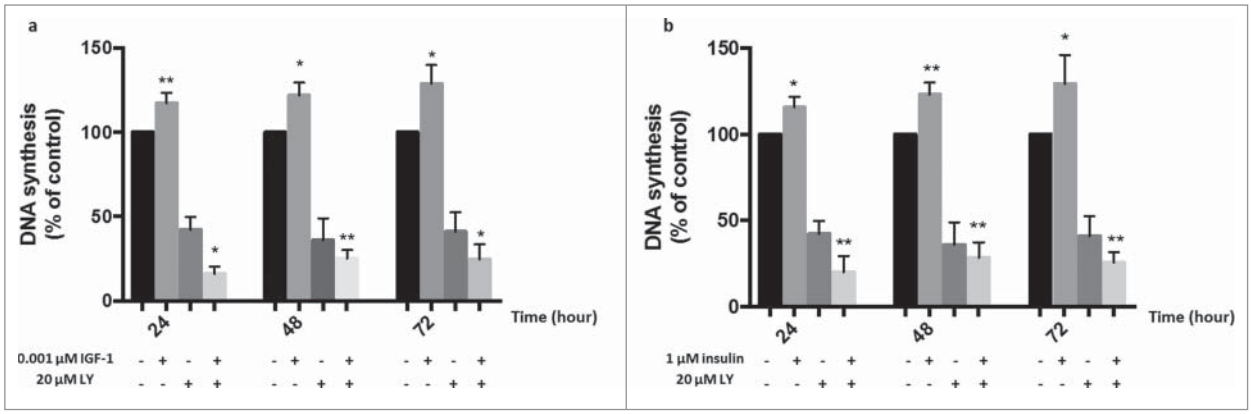

Figure 6. IGF-1 and insulin increase proliferation of CD3-activated human T cells. T cell proliferation was measured by [^{3}H]-thymidine incorporation following stimulation with (a) 0.001 μM IGF-1 and (b) 1 μM insulin in the presence or absence of LY294002 (20 μM) after 24, 48, and 72 hours. DNA synthesis values were expressed as a percentage of untreated cells and represent means + SEMs (n = 4); ($^{*}p < 0.05$ and $^{**}p < 0.01$, Student's *t-test*).

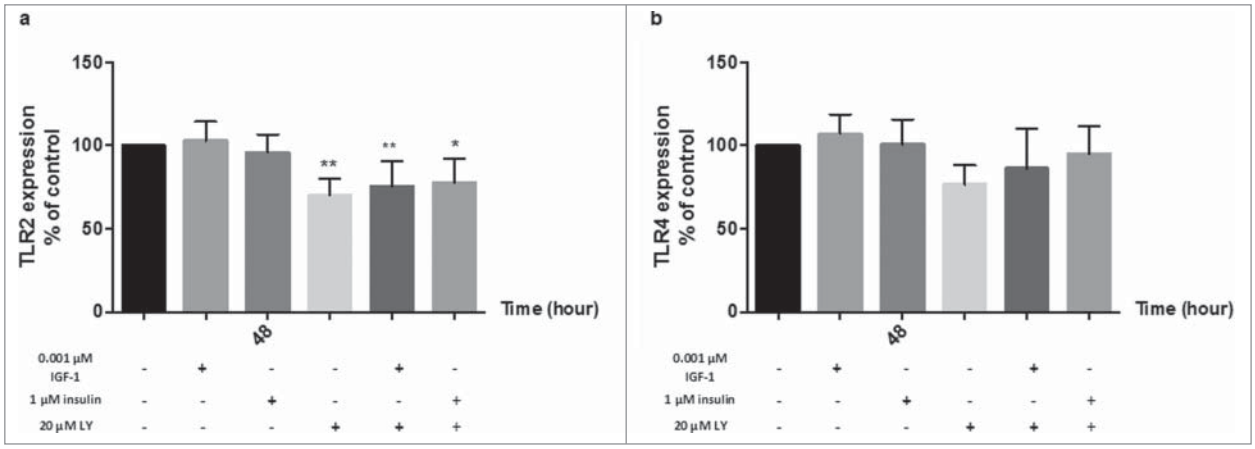

Figure 7. Expression of TLR2 and TLR4 in CD3-activated human T cells upon stimulation with IGF-1 and insulin. Activated T cells were stimulated for 48 hours and subsequently analyzed for (a) TLR2 and (b) TLR4 expression by flow cytometry. Data represent the means + SEMs of 4 independent experiments; (* $p < 0.05$, $^{**}p < 0.01$, Student's *t-test*).

T cells *in vitro*. T cell proliferation and expression of TLR2/4 were investigated. FoxO proteins are phosphorylated and inactivated after TCR/CD28 stimulation in T cells.[37] Vav1 is a crucial part in TCR/CD28 induced signaling which leads to phosphorylation. Inhibitions of FoxO1 leads to downregulation of $p27^{kip1}$ cell cycle inhibitor and increases proliferation of murine T cells.[38] It has been shown that IGF-1

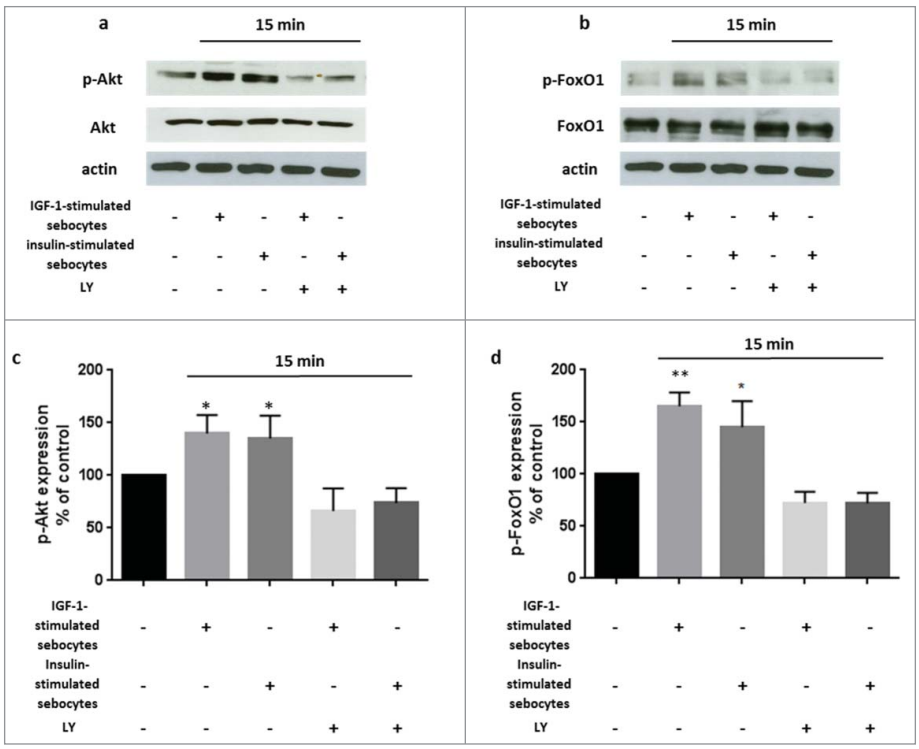

Figure 8. The supernatants of IGF-1- and insulin-stimulated SZ95 sebocytes up-regulate the expression of p-Akt and p-FoxO1 in CD3-activated T cells. SZ95 sebocytes were stimulated with 1 μM IGF-1 or insulin. After 72 hours, the medium was replaced and the cells were incubated for another 72 hours. The supernatants were collected and added to activated T cells in the presence or absence of PI3K inhibitor LY294002 (20 μM) for 15 minutes. Whole protein fractions were analyzed by western blot. (a) Representative blots were probed for p-Akt, (b) Representative blots were probed for p-FoxO1. (c and d) Densitometric quantification of blots and normalization to β-actin. Densitometric data were calculated as a percentage of the values in untreated cells. Data represent the means + SEMs (n = 3); * $p < 0.05$, $^{**}p < 0.01$, one-way ANOVA.

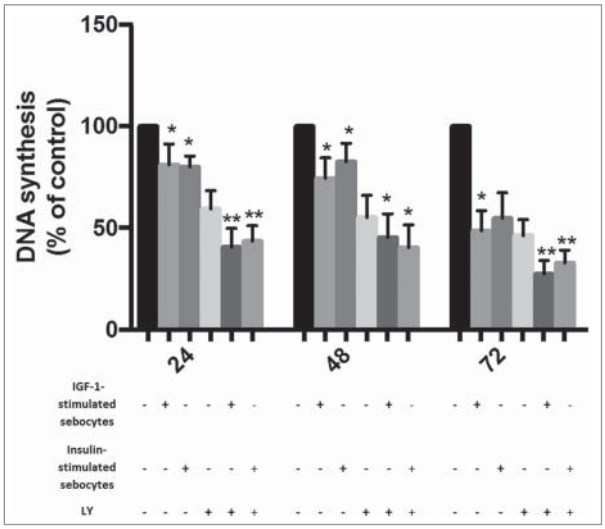

Figure 9. Supernatants from IGF-1- or insulin-stimulated SZ95 sebocytes reduce proliferation of CD3-activated T cells. SZ95 sebocytes were stimulated with 1 μM IGF-1 or insulin. Activated human primary T cells were stimulated with sebocyte supernatants in the presence or absence of PI3K inhibitor LY294002 (20 μM) for 24, 48, and 72 hours. T cell proliferation was measured by [^{3}H]-thymidine incorporation assay. DNA synthesis values are expressed as a percentage of untreated cells and represent means + SEMs (n = 4); (* $p < 0.05$ and ** $p < 0.01$, Student's *t-test*).

induces phosphorylation of Akt at 60 minutes in concanavalin-activated T cells and inhibits apoptosis.[39] Insulin activates the Akt pathway in murine regulatory T cells (Treg cells).[40] Our results showed that 0.001 μM IGF-1 and 1 μM insulin up-regulate p-Akt and p-FoxO1 at 15 and 30 minutes in human T-cells. PI3K pathway inhibitor LY294002 (20 μM) blocked p-Akt and p-FoxO1 upregulation. Cytoplasmic p-Akt was increased after 15 minutes while, nuclear p-Akt was up-regulated after 30 minutes which indicates a translocation of p-Akt from the cytoplasm to the nucleus in human T cells. In addition, up-regulation of p-FoxO1 was first detected in the cytoplasm and nucleolus after 15 minutes. However, expression of nuclear p-FoxO1 was down-regulated after 30 minutes upon IGF-1 and insulin stimulation. Nuclear FoxO1 was decreased after 15 and 30 minutes. These findings indicate that FoxO1 transcription factor is phosphorylated and degraded upon IGF-1 and insulin treatment in human primary T cells.

Furthermore, FoxO transcriptional activity was reduced upon PI3K pathway activation at 15 minutes compared to CD3-activated control cells.

Mammalian target of rapamycin complex 1 (mTORC1) is a nutrient-sensitive kinase complex which regulates transcription, cell proliferation, and lipogenesis.[41-44]We analyzed FoxO1 but of course it has to be considered that Akt is a major activator of mTORC1 signaling. The expression of mTOR is enhanced in acne patients compared to healthy controls.[45] Furthermore, mTORC1 has an important role in T cell differentiation, proliferation and functions.[46-49]

It is well established that proliferation of T cells is induced after TCR stimulation and PI3K pathway activation.[38,50,51] Our results showed that 0.001 μM IGF-1 and 1 μM insulin further increase CD3-activated T cell proliferation after different time points with a maximum increase of 29% at 72 hours compared to CD3-activated control cells. LY inhibitor alone reduced T cell proliferation and LY together with IGF-1 or insulin significantly blocked increase of T cell proliferation confirming that T cell proliferation increases via PI3K pathway activation.

TLR activation leads to antigen-presenting cell (APC) maturation and cytokine production.[52] These receptors play a pivotal role in innate as well as adaptive immunity.[53] TLR2 is expressed on $CD4^+$ and $CD8^+$ T cells.[54,55] TLR4 is expressed on T cells and has a role in $CD4^+$ and $\gamma\delta$ T cell proliferation and survival.[56] The cytosolic domains of TLRs contain a PI3K binding motif [57] and naturally, there is an interaction between PI3K and TLRs.[58-60] It has been reported that PI3K activation reduces pro-inflammatory events and inhibits TLR signaling in immune cells.[61] We showed in a previous study that IGF-1 and insulin up-regulate TLR2/4 expression in SZ95 sebocytes indicating an important link between dietary factors and inflammation in acne without microbial involvement.[12] Next, we investigated the role of IGF-1 and insulin on TLR2/4 expression in human primary T cells. No significant effect on TLR2/4 expression was detected in CD3-activated T cells. LY inhibitor significantly reduced TLR2/4 expression which may result from a general inhibition of T cell activation. These findings suggest that contrary to human sebocytes, dietary factors such as IGF-1 and insulin mimicked in the *in vitro* system do not affect TLR expression via the PI3K pathway in human T cells and therefore, increased activity might be inhibited.

To get more insight in possible interaction of sebocyte factors after stimulation with IGF 1 or insulin and their release affecting T-cells, we investigated the effect of supernatants from IGF-1- or insulin-stimulated sebocytes on T cell PI3K pathway activation.

The results showed the up-regulation of p-Akt and p-FoxO1. Pre-incubation with LY blocked p-Akt and p-FoxO1 up-regulation in human T cells. These data suggest that IGF-1- and insulin-stimulated sebocytes may synthesize some unknown factors and may activate the PI3K pathway in human T cells. We found in previous study that IGF-1 and insulin increase sebocyte lipogenesis and reduce sebocyte proliferation which can be in part a secondary effect of the induction of differentiation and peroxisome proliferator-activated receptor (PPAR) activation in sebocytes.[12] Interestingly, in the T cell study, [^{3}H]-thymidine incorporation assays indicated that supernatants from IGF-1- or insulin-stimulated sebocytes significantly reduce T cell proliferation with a maximum suppression of 50% after 72 hours. Pre-incubation with LY reduced T cell proliferation more effectively than supernatants from IGF-1- and insulin-stimulated sebocytes alone. PPAR-γ reduces c-Myc protein and prevents cell proliferation. PPAR-α mediates Bcl$^-$2 and Bad and initiates apoptosis.[62] Exportation of FoxO1 from the nucleus to the cytoplasm promotes PPAR target genes,[63, 64] which mediate sebocyte differentiation.[65] The reduction of human T cell proliferation induced by supernatants from IGF-1- and insulin-stimulated sebocytes clearly needs further investigation as it could result from enhanced apoptosis or enhanced differentiation of the treated T cells. Future studies should also investigate the involvement of PPAR and the cytokine profile of IGF-1- and insulin-treated human T cells.

Taken together, this study helps to support the hypothesis from *in vitro* results that *in vivo* high glycemic load diet which increases IGF-1 and insulin may contribute to induce activation of the PI3K pathway, reduction of FoxO transcriptional activity, and increase of proliferation in human primary T cells. However, they do not influence TLR expression in T cells. In addition, factors secreted by IGF-1- and insulin-stimulated sebocytes have an ability to induce the PI3K pathway in T cells and they reduce T cell proliferation.

Material and methods

Cell culture

Peripheral blood was obtained from healthy donors. Approval for the studies with human T cells was obtained from the local ethics committee of the Medical Faculty of the Otto-von-Guericke University Magdeburg with the permission number [107/09]. Blood donors gave written informed consent. Mononuclear cells were isolated by Ficoll gradient (Biochrom) centrifugation of heparinized blood. Human T cells were purified by negative selection with the Pan T-cell Isolation Kit according to manufactures instructions and AutoMacs magnetic separation system (Miltenyi Biotec). The purity of T cells was analyzed by flow cytometry and was usually more than 96%. T cells were activated with CD3 antibody (clone OKT3). Plate-bound antibodies were provided as follows.

T cell activation

For cell cultivation, 96-well plates (Nunc) and 24-well plates (Corning®, USA) were coated with the antibodies. Goat anti-mouse IgG + IgM (H$^+$L) (Jackson ImmunoResearch, USA) was diluted 1:100 in phosphate buffered saline (PBS) (Biochrom, Berlin, Germany) and was added to the wells. After overnight incubation at 4°C or 4 hours at 37°C, wells were washed 3 times with PBS. Thereafter, CD3 antibody was diluted 1:100 in PBS and was added. Plates were incubated for 4 hours at 37°C. Wells were washed again 3 times with PBS.

After isolation, T cells were cultured in serum-free AIM V® medium (Invitrogen) at a density of 1×10^5 cells/ml in 96-well plates or at a density of 1×10^6 cells/ml in 24-well plates for overnight.

Sebocyte treatment

To investigate the effects of IGF-1- or insulin-stimulated sebocytes on human T cells, sebocytes were stimulated with 1 μM IGF-1 (BioVision, California, USA) or insulin (Sigma-Aldrich, Steinheim, Germany) for 72 hours. Thereafter, the medium was replaced with the medium without growth factor and cells were cultured for another 72 hours. Supernatants were collected and activated human primary T cells were cultured with sebocyte supernatants in the presence or absence of PI3K inhibitor LY294002 (20 μM) (Cell Signaling Technology, USA).

Western blot

T cell activation was done as described above. Human primary T cells were plated at a density of 1×10^6 cells in 24-well plates and cultivated in serum-free AIM V®

medium. After overnight, cells were stimulated with 0.001 μM IGF-1 or 1 μM insulin for 15 to 120 minutes or with supernatants of IGF-1- or insulin-stimulated sebocytes. For pre-treatment with LY294002, T cells were incubated for 30 minutes with 20 μM LY294002 and then growth factors were added. Cells were lysed with RIPA buffer (Sigma-Aldrich, Missouri, USA). A protease and phosphatase inhibitor cocktails (Sigma, Missouri, USA) were added. 25 μg of protein was run on a 10% SDS-polyacrylamide gel. Protein was transferred to a nitrocellulose membrane and incubated with the following primary antibodies: p-Akt (Cell Signaling #9271, 1:1000), p-FoxO1 (Cell Signaling #9464, 1:1000), Akt (Cell Signaling #9272, 1:1000), FoxO1 (Cell Signaling #2880, 1:1000), or β-actin (SIGMA #A5441, 1:5000) (Sigma, USA) as a loading control. Membranes were incubated with peroxidase-conjugated secondary antibodies: Peroxidase-conjugated AffiniPure Anti-Rabbit IgG (Jackson ImmunoResearch, USA #74425, 1:10000) or Goat anti mouse IgG (H/L): HRP (A Bio-Rad, USA #0300–0108P, 1:10000). Blots were developed with SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific, USA) and exposed to film. Films of blots were analyzed and quantified by densitometry with Kodak 1D Image Analysis Software, Version 3.6 after background reduction.

[^{3}H]-thymidine incorporation assay

T cell activation was done as described above. T cells (1×10^5 cells/ml) were seeded in triplicates in 96-well plates in serum-free AIM V® medium. After overnight, 0.001 μM IGF-1 or 1 μM insulin or supernatants of IGF-1- or insulin-stimulated sebocytes with or without 20 μM LY294002 were added and cells were grown for 24–72 hours. Before cell harvest, cells were labeled with [^{3}H]-thymidine (0.2 μCi/well; ICN, Meckenheim, Germany) for 16 hours. Inc. radioactivity was harvested on glass fiber filters and determined by liquid scintillation counting.

Transfection and luciferase assay

T cells were activated as described above. After overnight, activated T cells were cultured in 25 cm^2 cell culture flasks and transfected at 80% confluence with Cignal FoxO Reporter (QIAGEN, USA) using Lipofectamine 2000 (Invitrogen) transfection reagent. Six hours later, cells were cultivated in 96-well plates. After overnight, cells were stimulated with 0.001 μM IGF-1 or 1 μM insulin in the presence or absence of 20 μM LY294002 for 15 minutes. Luciferase assays were evaluated on a luminescence plate reader using the Dual-Luciferase reporter assay system (Promega, USA) according to the manufacturer's instructions. The ratio of *Firefly* to *Renilla* luciferase was calculated.

Immunofluorescence cell staining

Cell activation was done as mentioned above. 13 mm coverslips (Mariefeld, Germany) were coated with poly-L-lysine (0.01% solution, Sigma, cat.# P4707) at room temperature. After 10 minutes, poly-L-lysine solution was removed and the cells were added and allowed to settle for 30–60 minutes at room temperature. Subsequently, 0.001 μM IGF-1 or 1 μM insulin in the presence or absence of 20 μM LY294002 were added for 15 and 30 minutes and then cells were fixed with 4% paraformaldehyde (PFA) and permeabilized with 0.2% Triton X-100. Then T cells were blocked with PBS containing 4% fetal bovine serum (FBS) (Biochrom) and incubated with FoxO1 (1:50), p-FoxO1 (1:50), or p-Akt (1:25) antibody for 1 hour. After that, cells were incubated with FITC-conjugated anti-rabbit IgG antibody (1:1000, Invitrogen, USA). To stain nuclei, 4, 6-diamidino-2-phenylindole-dihydro-chloride (DAPI, 1:1000, Invitrogen) was used. T cells were analyzed with confocal fluorescence microscopy (Leica TCS SP2, Germany) and images were captured and analyzed with Metaview software.

Flow cytometry

Cell activation was done as mentioned above. Human primary T cells were plated at a density of 1×10^6 cells in 24-well plates and cultivated in serum-free AIM V® medium. After overnight, cells were treated with 0.001 μM IGF-1 or 1 μM insulin in the presence or absence of 20 μM LY294002 for 48 hours. After treatment, cells were washed with PBS and resuspended in PBS. T cells were stained with fluorophore-conjugated TLR2 (PE) and TLR4 (FITC) (Abcam) antibodies for 30 minutes in the dark. After washing with PBS, fluorescence was determined by flow cytometry (BD Bioscience, USA).

Statistics

All experiments were repeated at least 3 times. Statistical significance was evaluated by Student's *t-test* and one-way ANOVA. Significance was set at $p^* < 0.05$.

Abbreviations

ANOVA	analysis of variance
APC	antigen-presenting cell
DAPI	4; 6-diamidino-2-phenylindole; dihydrochloride
FoxO1	forkhead box-O1
IGF-1	insulin-like growth factor-1
IGF-1R	IGF-1 receptor
IR	insulin receptor
mTORC1	mammalian target of rapamycin complex 1
P. acnes	*propionibacterium acnes*
PFA	paraformaldehyde
PI3K	phosphoinositide-3-kinase
PPAR	peroxisome proliferator-activated receptor
Th	T helper
TLR	Toll-like receptor
Treg cells	regulatory T cells

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

This work was supported by department Dermatology & Venereology Otto-von-Guericke University Magdeburg/ Germany.

ORCID

Christos C. Zouboulis http://orcid.org/0000-0003-1646-2608

References

[1] Taylor M, Gonzalez M, Porter R. Pathways to inflammation: Acne pathophysiology. Eur J Dermatol. 2011;21:323-33. doi:10.1684/ejd.2011.1357. PMID:21609898

[2] Do TT, Zarkhin S, Orringer JS, Nemeth S, Hamilton T, Sachs D, Voorhees JJ, Kang S. Computer-assisted alignment and tracking of acne lesions indicate that most inflammatory lesions arise from comedones and de novo. J Am Acad Dermatol. 2008;58:603-8. doi:10.1016/j.jaad.2007.12.024. PMID:18249468

[3] Tochio T, Tanaka H, Nakata S, Ikeno H. Accumulation of lipid peroxide in the content of comedones may be involved in the progression of comedogenesis and inflammatory changes in comedones. J Cosmet Dermatol. 2009;8:152-8. doi:10.1111/j.1473-2165.2009.00437.x. PMID:19527342

[4] Tanghetti EA. The role of inflammation in the pathology of acne. J Clin Aesthet Dermatol. 2013;6:27. PMID:24062871

[5] Shehadeh N, Kligman AM. The bacteriology of acne. Arch Dermatol. 1963;88:829-31. doi:10.1001/archderm.1963.01590240153025. PMID:14071456

[6] Marples RR, Mcginley KJ, Mills OH. Microbiology of comedones in acne vulgaris. J Invest Dermatol. 1973;60:80-3. doi:10.1111/1523-1747.ep12724149. PMID:4266324

[7] Puhvel S, AMIRIAN DA. Bacterial flora of comedones. Br J Dermatol. 1979;101:543-8. doi:10.1111/j.1365-2133.1979.tb11883.x. PMID:160242

[8] Shaheen B, Gonzalez M. A microbial aetiology of acne: what is the evidence? Br J Dermatol. 2011;165:474-85. doi:10.1111/j.1365-2133.2011.10375.x. PMID:21495996

[9] Boehm KD, Yun JK, StrohI KP, Elmets CA. Messenger RNAs for the multifunctional cytokines interleukin−1α, interleukin−1β and tumor necrosis factor−α are present in adnexal tissues and in dermis of normal human skin. Exp Dermatol. 1995;4:335-41. doi:10.1111/j.1600-0625.1995.tb00057.x. PMID:8608340

[10] Anttila H, Reitamo S, SAURAT JH. Interleukin 1 immunoreactivity in sebaceous glands. Br J Dermatol. 1992;127:585-8. doi:10.1111/j.1365-2133.1992.tb14870.x. PMID:1476918

[11] Das S, Reynolds RV. Recent advances in acne pathogenesis: Implications for therapy. Am J Clin Dermatol. 2014;15:479-88. doi:10.1007/s40257-014-0099-z. PMID:25388823

[12] Mirdamadi Y, Thielitz A, Wiede A, Goihl A, Papakonstantinou E, Hartig R, Zouboulis CC, Reinhold D, Simeoni L, Bommhardt U. Insulin and insulin-like growth factor-1 can modulate the phosphoinositide-3-kinase/Akt/FoxO1 pathway in SZ95 sebocytes in vitro. Mol Cell Endocrinol. 2015;415:32-44. doi:10.1016/j.mce.2015.08.001. PMID:26257240

[13] Kurokawa I, Danby FW, Ju Q, Wang X, Xiang LF, Xia L, Chen W, Nagy I, Picardo M, Suh DH. New developments in our understanding of acne pathogenesis and treatment. Exp Dermatol. 2009;18:821-32. doi:10.1111/j.1600-0625.2009.00890.x. PMID:19555434

[14] Dreno B, Gollnick H, Kang S, Thiboutot D, Bettoli V, Torres V, Leyden J. Understanding innate immunity and inflammation in acne: Implications for management. J Eur Acad Dermatol Venereol. 2015;29:3-11. doi:10.1111/jdv.13190. PMID:26059728

[15] Gollnick H, Dreno B. Pathophysiology and management of acne. Journal of the European J Eur Acad Dermatol Venereol. 2015;29:1-2. doi:10.1111/jdv.13190. PMID:26059728

[16] Clark RA. Skin-resident T cells: The ups and downs of on site immunity. J Invest Dermatol. 2010;130:362-70. doi:10.1038/jid.2009.247. PMID:19675575

[17] Lorenz B, von Stebut E. Isolation of T Cells from the Skin. Methods Mol Biol. 2014:3-13. doi:10.1007/978-1-4939-1212-4_1. PMID:25150991

[18] Norris J, Cunliffe W. A histological and immunocytochemical study of early acne lesions. Br J Dermatol. 1988;118:651-9. doi:10.1111/j.1365-2133.1988.tb02566.x. PMID:2969256

[19] Layton A, Morris C, Cunliffe W, Ingham E. Immunohistochemical investigation of evolving inflammation in lesions of acne vulgaris. Exp Dermatol. 1998;7:191-7. doi:10.1111/j.1600-0625.1998.tb00323.x. PMID:9758417

[20] Jeremy AH, Holland DB, Roberts SG, Thomson KF, Cunliffe WJ. Inflammatory events are involved in acne

lesion initiation. J Invest Dermatol. 2003;121:20-7. doi:10.1046/j.1523-1747.2003.12321.x. PMID:12839559

[21] Cordain L, Lindeberg S, Hurtado M, Hill K, Eaton SB, Brand-Miller J. Acne vulgaris: A disease of Western civilization. Arch Dermatol. 2002;138:1584-90. doi:10.1001/archderm.138.12.1584. PMID:12472346

[22] Cordain L, Eades MR, Eades MD. Hyperinsulinemic diseases of civilization: more than just Syndrome X. Comp Biochem Physiol A Mol Integr Physiol. 2003;136:95-112. doi:10.1016/S1095-6433(03)00011-4. PMID:14527633

[23] Cappel M, Mauger D, Thiboutot D. Correlation between serum levels of insulin-like growth factor 1, dehydroepiandrosterone sulfate, and dihydrotestosterone and acne lesion counts in adult women. Arch Dermatol. 2005;141:333-8. doi:10.1001/archderm.141.3.333. PMID:15781674

[24] Melnik BC, Schmitz G. Role of insulin, insulin−like growth factor−1, hyperglycaemic food and milk consumption in the pathogenesis of acne vulgaris. Exp Dermatol. 2009;18:833-41. doi:10.1111/j.1600-0625.2009.00924.x. PMID:19709092

[25] Vora S, Ovhal A, Jerajani H, Nair N, Chakrabortty A. Correlation of facial sebum to serum insulin-like growth factor-1 in patients with acne. Br J Dermatol. 2008;159:990-1. doi:10.1111/j.1365-2133.2008.08764.x. PMID:18652583

[26] LaRosa CL, Quach KA, Koons K, Kunselman AR, Zhu J, Thiboutot DM, Zaenglein AL. Consumption of dairy in teenagers with and without acne. J Am Acad Dermatol. 2016;75:318-22. doi:10.1016/j.jaad.2016.04.030. PMID:27241803

[27] Melnik BC. FoxO1–the key for the pathogenesis and therapy of acne? J Dtsch Dermatol Ges. 2010;8:105-14. doi:10.1111/j.1610-0387.2010.07344.x. PMID:20151947

[28] Coffer PJ, Burgering BM. Forkhead-box transcription factors and their role in the immune system. Nat Rev Immunol. 2004;4:889-99. doi:10.1038/nri1488. PMID:15516968

[29] Birkenkamp KU, Coffer PJ. FOXO transcription factors as regulators of immune homeostasis: molecules to die for? J Immunol. 2003;171:1623-9. doi:10.4049/jimmunol.171.4.1623. PMID:12902457

[30] Helderman JH, Strom TB. Specific insulin binding site on T and B lymphocytes as a marker of cell activation. Nature. 1978;274:62. doi:10.1038/274062a0. PMID:26882

[31] Kooijman R, Scholtens L, Rijkers G, Zegers B. Type I insulin-like growth factor receptor expression in different developmental stages of human thymocytes. J Endocrinol. 1995;147:203-9. doi:10.1677/joe.0.1470203. PMID:7490549

[32] Kooijman R, Scholtens LE, Rijkers GT, Zegers BJ. Differential expression of type I insulin−like growth factor receptors in different stages of human T cells. Eur J Immunol. 1995;25:931-5. doi:10.1002/eji.1830250411. PMID:7737296

[33] Braciale VL, Gavin J, Braciale TJ. Inducible expression of insulin receptors on T lymphocyte clones. J Exp Med. 1982;156:664-9. doi:10.1084/jem.156.2.664. PMID:7047673

[34] DeBenedette M, Snow E. Insulin modulates the interleukin 2 responsiveness of T lymphocytes. Reg Immunol. 1990;3:82-7. PMID:2150918

[35] Brocardo MG, Schillaci R, Galeano A, Radrizzani M, White V, Guerrico AG, Santa-Coloma TA, Roldán A. Early effects of insulin-like growth factor-1 in activated human T lymphocytes. J Leukoc Biol. 2001;70:297-305. PMID:11493623

[36] Kecha O, Brilot F, Martens H, Franchimont N, Renard C, Greimers R, Defresne M-P, Winkler R, Geenen V. Involvement of Insulin-Like Growth Factors in Early T Cell Development: A Study Using Fetal Thymic Organ Cultures 1. Endocrinology. 2000;141:1209-17. doi:10.1210/endo.141.3.7360. PMID:10698198

[37] Fabre S, Lang V, Harriague J, Jobart A, Unterman TG, Trautmann A, Bismuth G. Stable activation of phosphatidylinositol 3-kinase in the T cell immunological synapse stimulates Akt signaling to FoxO1 nuclear exclusion and cell growth control. J Immunol. 2005;174:4161-71. doi:10.4049/jimmunol.174.7.4161. PMID:15778376

[38] Charvet C, Canonigo AJ, Bécart S, Maurer U, Miletic AV, Swat W, Deckert M, Altman A. Vav1 promotes T cell cycle progression by linking TCR/CD28 costimulation to FOXO1 and p27kip1 expression. J Immunol. 2006;177:5024-31. doi:10.4049/jimmunol.177.8.5024. PMID:17015685

[39] Walsh PT, Smith LM, O'Connor R. Insulin−like growth factor−1 activates Akt and Jun N−terminal kinases (JNKs) in promoting the survival of T lymphocytes. Immunology. 2002;107:461-71. doi:10.1046/j.1365-2567.2002.01525.x. PMID:12460191

[40] Han JM, Patterson SJ, Speck M, Ehses JA, Levings MK. Insulin Inhibits IL-10–Mediated Regulatory T Cell Function: Implications for Obesity. J Immunol. 2014;192:623-9.doi:10.4049/jimmunol.1302181. PMID:24323581

[41] Inoki K, Ouyang H, Li Y, Guan K-L. Signaling by target of rapamycin proteins in cell growth control. Microbiol Mol Biol Rev. 2005;69:79-100. doi:10.1128/MMBR.69.1.79-100.2005. PMID:15755954

[42] Wang X, Proud CG. Nutrient control of TORC1, a cell-cycle regulator. Trends Cell Biol. 2009;19:260-7. doi:10.1016/j.tcb.2009.03.005. PMID:19419870

[43] Suzuki T, Inoki K. Spatial regulation of the mTORC1 system in amino acids sensing pathway. Acta Biochim Biophys Sin (Shanghai). 2011;43:671-9. doi:10.1093/abbs/gmr066. PMID:21785113

[44] Wang X, Proud CG. mTORC1 signaling: what we still don't know. J Mol Cell Biol. 2010;3(4):206–220. doi:10.1093/jmcb/mjq038. PMID:21138990

[45] Monfrecola G, Lembo S, Caiazzo G, De Vita V, Di Caprio R, Balato A, Fabbrocini G. Mechanistic target of rapamycin (mTOR) expression is increased in acne patients' skin. Exp Dermatol. 2016;25:153-5. doi:10.1111/exd.12885. PMID:26477999

[46] Pierdominici M, Vacirca D, Delunardo F, Ortona E. mTOR signaling and metabolic regulation of T cells: New potential therapeutic targets in autoimmune diseases. Curr Pharm Des. 2011;17:3888-97. doi:10.2174/138161211798357809. PMID:21933144

[47] Powell JD, Pollizzi KN, Heikamp EB, Horton MR. Regulation of immune responses by mTOR. Annu Rev Immunol.

2012;30:39-68. doi:10.1146/annurev-immunol-020711-075024. PMID:22136167

[48] Yang K, Chi H. mTOR and metabolic pathways in T cell quiescence and functional activation. Semin Immunol. 2012:421-8.doi:10.1016/j.smim.2012.12.004. PMID:23375549

[49] Almeida L, Lochner M, Berod L, Sparwasser T. Metabolic pathways in T cell activation and lineage differentiation. Semin Immunol. 2016:514-24. doi:10.1016/j.smim.2016.10.009. PMID:27825556

[50] Reynolds LF, Smyth LA, Norton T, Freshney N, Downward J, Kioussis D, Tybulewicz VL. Vav1 transduces T cell receptor signals to the activation of phospholipase C-γ1 via phosphoinositide 3-kinase-dependent and-independent pathways. J Exp Med. 2002;195:1103-14. doi:10.1084/jem.20011663. PMID:11994416

[51] Na S-Y, Patra A, Scheuring Y, Marx A, Tolaini M, Kioussis D, Hemmings B, Hünig T, Bommhardt U. Constitutively active protein kinase B enhances Lck and Erk activities and influences thymocyte selection and activation. J Immunol. 2003;171:1285-96. doi:10.4049/jimmunol.171.3.1285. PMID:12874217

[52] Janeway Jr CA, Medzhitov R. Innate immune recognition. Annu Rev Immunol. 2002;20:197-216. doi:10.1146/annurev.immunol.20.083001.084359. PMID:11861602

[53] MacLeod H, Wetzler LM. T cell activation by TLRs: a role for TLRs in the adaptive immune response. Sci STKE. 2007;2007:pe48-pe.

[54] Komai-Koma M, Jones L, Ogg GS, Xu D, Liew FY. TLR2 is expressed on activated T cells as a costimulatory receptor. Proc Natl Acad Sci U S A. 2004;101:3029-34. doi:10.1073/pnas.0400171101. PMID:14981245

[55] Mercier BC, Cottalorda A, Coupet C-A, Marvel J, Bonnefoy-Bérard N. TLR2 engagement on CD8 T cells enables generation of functional memory cells in response to a suboptimal TCR signal. J Immunol. 2009;182:1860-7. doi:10.4049/jimmunol.0801167. PMID:19201838

[56] Reynolds JM, Martinez GJ, Chung Y, Dong C. Toll-like receptor 4 signaling in T cells promotes autoimmune inflammation. Proc Natl Acad Sci U S A. 2012;109:13064-9. doi:10.1073/pnas.1120585109. PMID:22826216

[57] Arbibe L, Mira JP, Teusch N, Kline L, Guha M, Mackman N, Godowski PJ, Ulevitch RJ, Knaus UG. Toll-like receptor 2-mediated NF-kappa B activation requires a Rac1-dependent pathway. Nat Immunol. 2000;1:533-40. doi:10.1038/82797. PMID:11101877

[58] Laird MH, Rhee SH, Perkins DJ, Medvedev AE, Piao W, Fenton MJ, Vogel SN. TLR4/MyD88/PI3K interactions regulate TLR4 signaling. J Leukoc Biol. 2009;85:966-77. doi:10.1189/jlb.1208763. PMID:19289601

[59] Li X, Jiang S, Tapping RI. Toll-like receptor signaling in cell proliferation and survival. Cytokine. 2010;49:1-9. doi:10.1016/j.cyto.2009.08.010. PMID:19775907

[60] Ojaniemi M, Glumoff V, Harju K, Liljeroos M, Vuori K, Hallman M. Phosphatidylinositol 3-kinase is involved in Toll-like receptor 4-mediated cytokine expression in mouse macrophages. Eur J Immunol. 2003;33:597-605. doi:10.1002/eji.200323376. PMID:12616480

[61] Hazeki K, Nigorikawa K, Hazeki O. Role of phosphoinositide 3-kinase in innate immunity. Biol Pharm Bull. 2007;30:1617-23. doi:10.1248/bpb.30.1617. PMID:17827709

[62] Martinasso G, Oraldi M, Trombetta A, Maggiora M, Bertetto O, Canuto R, Muzio G. Involvement of PPARs in cell proliferation and apoptosis in human colon cancer specimens and in normal and cancer cell lines. PPAR Res. 2007;2007:93416. doi:10.1155/2007/93416. PMID:17389773

[63] Fan W, Imamura T, Sonoda N, Sears DD, Patsouris D, Kim JJ, Olefsky JM. FOXO1 transrepresses peroxisome proliferator-activated receptor γ transactivation, coordinating an insulin-induced feed-forward response in adipocytes. J Biol Chem. 2009;284:12188-97. doi:10.1074/jbc.M808915200. PMID:19246449

[64] Fan W, Yanase T, Morinaga H, Okabe T, Nomura M, Daitoku H, Fukamizu A, Kato S, Takayanagi R, Nawata H. Insulin-like growth factor 1/insulin signaling activates androgen signaling through direct interactions of Foxo1 with androgen receptor. J Biol Chem. 2007;282:7329-38. doi:10.1074/jbc.M610447200. PMID:17202144

[65] Chen W, Yang CC, Sheu HM, Seltmann H, Zouboulis CC. Expression of peroxisome proliferator-activated receptor and CCAAT/enhancer binding protein transcription factors in cultured human sebocytes. J Invest Dermatol. 2003;121:441-7. doi:10.1046/j.1523-1747.2003.12411.x. PMID:12925198