

## Expression of Toll-like Receptor 2 on Peripheral Blood Monocytes of Patients with Inflammatory and Non-Inflammatory Acne Vulgaris

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The pathogenesis of acne vulgaris is multifactorial and entails the interplay of hormonal, microbial and immunological events. The bacterium *Propionibacterium acnes* is involved in the induction of comedogenesis and maintenance of the inflammatory phase of acne. Toll-like Receptor 2 (TLR2) expressed on mononuclear inflammatory cells and possibly on keratinocytes and sebocytes is thought to be of vital importance in mediating *P. acnes*-induced inflammatory response in acne vulgaris. This work aimed to study the degree of expression of TLR2 on peripheral blood monocytes (PBM) from patients with non-inflammatory and inflammatory acne and to investigate the influence of systemic isotretinoin therapy on TLR2 expression. Sixteen patients with predominantly non-inflammatory acne, 16 patients with predominantly inflammatory acne and 16 age and sex matched healthy subjects were involved in this study. Cell surface expression of CD14 and TLR2 were determined by cell surface staining and flowcytometry. TLR2 expression was analyzed for 12 patients with severe and/or scarring inflammatory acne after oral isotretinoin therapy for two months. The mean fluorescence intensity (MFI) of TLR2 on PBM reported a statistically significant difference between patients with non-inflammatory acne, patients with inflammatory acne and control subjects. MFI of TLR was significantly lower for patients with inflammatory acne after systemic isotretinoin therapy. Data obtained suggest that TLR2 expression on PBM is an important event in acne pathogenesis and targeting this molecule might be a useful therapeutic goal in the future.

**A**cne vulgaris is the most prevalent skin condition encountered by dermatologists, as it affects nearly 85% of the population between the ages of 12 to 24 years (White, 1998). It affects the pilosebaceous units and its pathogenesis is multifactorial, as it entails the interplay of hormonal, microbial and immunological events. Previous reports have implicated anaerobic gram-positive organism *Propionibacterium acnes*, a harmless member of the skin flora, to play a significant role in acne pathogenesis (Strauss & Kligman, 1960). *P. acnes* was suggested to stimulate pilosebaceous ductal hypercornification and to initiate a cytokine-mediated inflammatory response with complement activation and neutrophil chemotaxis that ends in follicular

wall disruption (Scott *et al.*, 1979; Webster & Leyden, 1980)

Mammalian Toll-like receptors (TLR) comprise a family of germ line-encoded transmembrane pattern recognition receptors that recognize conserved microbial structures known as pathogen associated molecular patterns (PAMP), such as peptidoglycan (PG) and lipopolysaccharides (LP) (Janeway & Medzhitov, 2002). Activation of TLR leads to the induction of inflammatory responses and the development of antigen-specific adaptive immunity (Akira *et al.*, 2001). The engagement of TLR by pathogen-derived ligands rapidly initiates a signaling pathway that activates the transcription nuclear factor  $\kappa$ B (NF- $\kappa$ B) and the mitogen-activated protein kinase (MAPK) cascade leading to the

synthesis and to the release of pro-inflammatory cytokines and co-stimulatory molecules (Guha & Mackman, 2001). CD14 is a 55-KD GPI-linked glycoprotein that participates in pathogen recognition by using TLRs as co-receptors in signal transduction (Yang, 1999, da Silva *et al.*, 2001; Van Amersfoort *et al.*, 2003).

Pro-inflammatory cytokines are released from *in vitro* stimulated monocytes, obtained from persons with and without acne, in response to *P. acnes* immunogenic components (Vowels *et al.*, 1995). PG is the most important PAMP of *P. acnes* that stimulate monocytes by binding to its receptor TLR2 (Schwandner *et al.*, 1999). The role of TLR2 in acne pathogenesis was further investigated. TLR2-receptors were found to be abundantly expressed on perifollicular and peribulbar macrophages, and that the expression of TLR 2-bearing cells increased with the evolution of acne lesions (Kim *et al.*, 2002)

In this study, we investigated the role of TLR2 in the pathogenesis of acne vulgaris by comparing the intensity of TLR2-receptor expression on peripheral blood monocytes obtained from patients with or without inflammatory acne and in healthy control subjects. In addition, we investigated the impact of oral isotretinoin on monocytes expression of TLR-2, as a drug regimen for patients with inflammatory acne.

## Subjects and Methods

This study was conducted in the Suez Canal University Hospital and included 32 patients with facial acne vulgaris and 16 age and sex matched healthy volunteers without acne vulgaris. After obtaining written consents, patients and control subjects were subjected to clinical history taking and general medical examination. Patients who received systemic or topical anti-acne treatments for less than 2 month at enrollment were not included in this study. In addition, patients suffering from any acute or chronic systemic illness were also excluded. A dermatologic examination was done for

patients to confirm the diagnosis and to determine the clinical type of acne vulgaris.

Acne patients were categorized into two groups; patients with predominantly non-inflammatory acne (n=16) and patients with predominantly inflammatory acne (n=16). Non-inflammatory acne patients were 9 females and 7 males, their ages ranged between 18 and 20 (18.7±2.0) years and the duration of their skin condition ranged between 6 and 36 (23±19) months. Inflammatory acne patients were 11 females and 5 males, their ages ranged between 17 and 22 (19.3±3.4) years and the duration of their skin condition ranged between 6 and 72 (22±18) months. Control subjects were 10 females and 6 males and their ages ranged between 17 and 22 (19.2±3.0) years.

Fresh venous blood (3-ml) added in vacutainers with EDTA was obtained from patients and controls. Cell surface expression of CD14 and TLR2 were analyzed by cell surface staining and flowcytometry. From each sample of whole blood, 100 µl was incubated with 10 µl FITC-conjugated anti-TLR2 monoclonal antibodies (mAb) (TL2.1 Biocarta, Diego CA) and 10 µl PE-conjugated anti-CD14 mAb (IQ Products, Groningen, Netherlands) for 20 min at room temperature, in the dark. This was followed by red cell lysis and washing. Cells were then re-suspended in 300 µl of PBS. Analysis was done by FACS Calibur flowcytometry (Becton Dickinson) and monocytes were specifically analyzed by a selective gating based on parameters of forward and side light scatter. Cell Quest soft ware was used for data analysis. Monocytes were gated according to light scatter properties and gate was confirmed by CD14 expression. CD14+ cells in patients and control samples were determined and the mean fluorescence intensity (MFI) of TLR2 expression was estimated on gated CD14+ cells.

Patients with severe and/or scarring inflammatory acne (n= 12) were then treated with a daily oral dose of 30 mg isotretinoin (Netlook, El-Andalos Pharmaceutical Co.) for two months. Safety criteria for isotretinoin therapy were considered before starting this line of therapy. Before and one month after starting isotretinoin therapy, liver function tests and serum cholesterol were done for each patient. Thereafter, another flowcytometric assessment of TLR2 expression on peripheral mononuclear cells was done, as previously mentioned.

## Statistical Analysis

Statistical analysis was done using SPSS computer software. Data were expressed as mean ± SD. Independent sample (Student's) t-test and paired

sample t-test were used when appropriate and the *P*-value was considered significant at the 5% level of probability.

## Results

In control subjects, the percentage of gating monocytes ranged between 3-11% while in patients with non-inflammatory or with inflammatory acne was 4-13% and 4-13%,

respectively (Table 1). The MFI of TLR2 range was from 118 to 208 in healthy individuals while in patients with non-inflammatory acne and inflammatory acne MFI of TLR2 was 124-360 and 155-650, respectively (Table 1).

Table 1. Summary of CD14+ monocyte percentages and TLR2 mean fluorescence intensity for acne patients and control subjects

Variable	Control subjects	Non-inflammatory acne patients		Inflammatory acne patients	
	Range (mean± SD)	Range (mean± SD)	<i>P</i> - value	Range (mean± SD)	<i>P</i> - value
% Total monocytes	3-11 % (6.4 ± 2.2)	4-13 % (7.4 ± 2.5)	NS	4-13 % (7.6 ± 2.3)	NS
MFI of TLR2	118-208 (149 ± 27)	124-360 (203±79)	0.018	155-650 (287 ± 133)	0.001

*P*<0.05 is significant. NS= not significant.

When we compared the difference between the means of TLR2 MFI for both groups of non-inflammatory and untreated inflammatory acne patients, it revealed a statistically significant difference (*P*=0.04). Monocyte gating is depicted in Figure 1.

The flowcytometric analysis of TLR2 expression on gating monocytes for a control subject and a patient of each group is shown in Figure 2. Scatter plot for different values of TLR2 expression for patients and control subjects are seen in Figure 3.

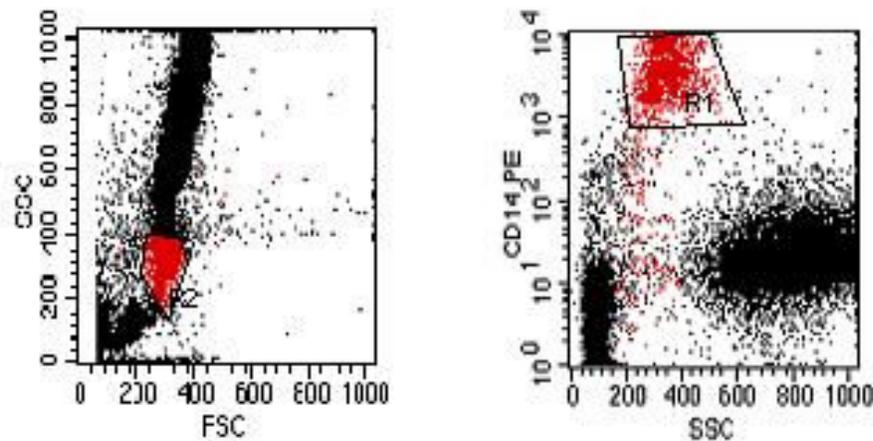
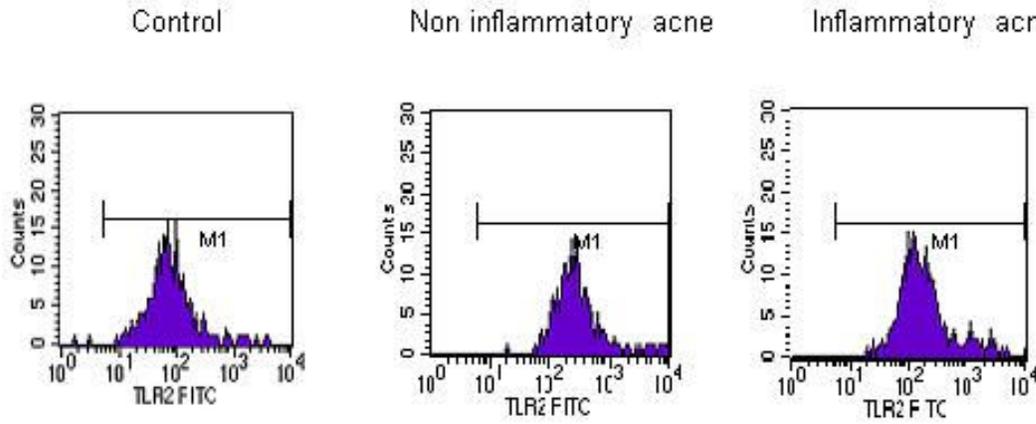
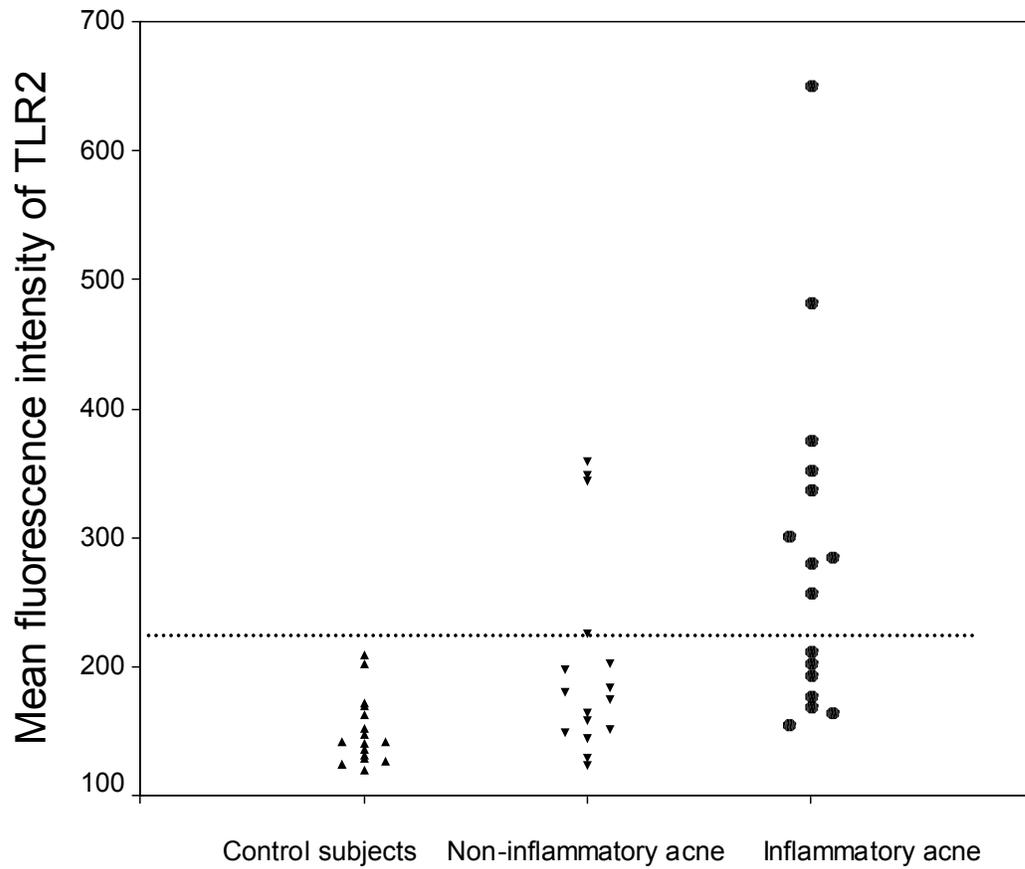


Figure 1. Dot plots Show gating of Monocytes according to their light scattering properties



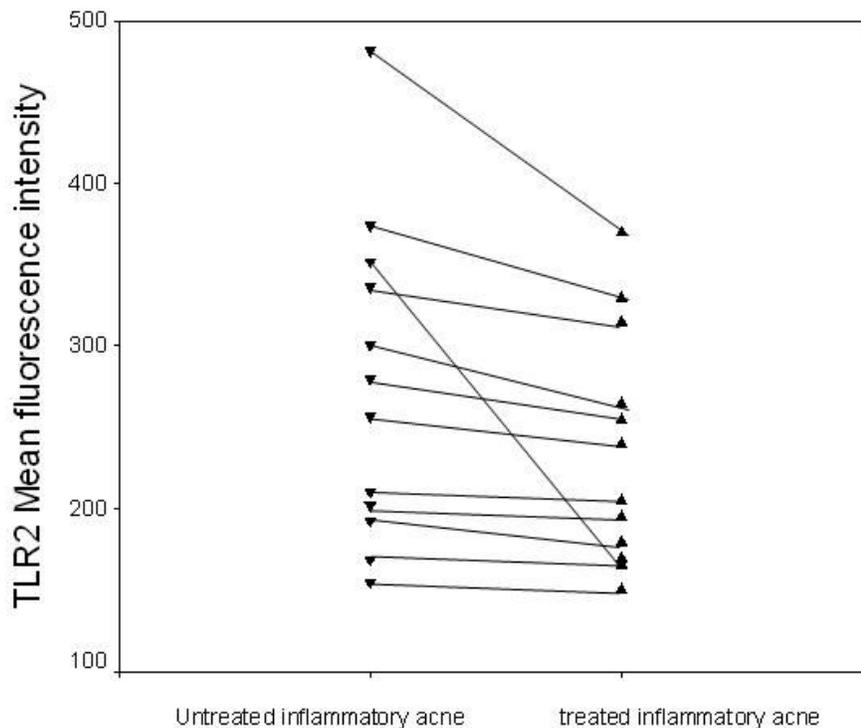
**Figure 2.** Histograms show the expression of TLR 2 on the gated Monocytes.



**Figure 3.** A scatter plot shows MFI of TLR2 on monocytes from patients and control subjects. Dashed line represents control mean value ± 2SD

After two month treatment with isotretinoin, 9 patients with inflammatory acne showed marked improvement and 3 patients showed mild improvement with reduction of inflammatory papules and pustules, none of them revealed any significant elevation of liver enzymes or serum cholesterol levels. MFI of TLR2 for untreated patients ranged between 155 and 482 (mean  $276 \pm 28$ ), while

range of MFI of TLR2 detected after treatment was 150-370 (mean  $237 \pm 21$ ). Comparing the difference of the mean values for TLR2 expression for patients with inflammatory acne before and after treatment by paired sample t-test, a significant difference ( $P= 0.028$ ) was observed between both groups. Reduced MFI of TLR2 was observed after treatment (Figure4).



**Figure 4.** Overlay scatter plot shows TLR2 expression values for patients with inflammatory acne before and after treatment

## Discussion

Years ago, many investigators pointed towards the magnificent role played by *P. acnes* in the pathogenesis of acne, regarding not only the evolution of inflammatory lesions, but also the initial development of non-inflammatory comedons (Lavker *et al.*, 1981, De Young *et al.*, 1984). The role of APCs in acne pathogenesis was suggested by Vowels *et al.* (1995) who showed that pro-inflammatory cytokines are released from monocytes in persons with and without acne in response to *in vitro* stimulation with both *P. acnes* and *P. acnes* culture supernatants. This meant that an antigen on *P. acnes* is necessary for monocytes to release its chemical mediators.

This paved the way for the identification of PG as the most important antigen of *P. acnes* that stimulate monocytes by binding to its pattern recognition receptor, TLR2 (Schwandner *et al.*, 1999). Thereafter, Kim *et al.* (2002) investigated the role of TLR2 in acne and found that this receptor was abundantly expressed on perifollicular and peribulbar macrophages, and that the concentrations of TLR 2-bearing cells increased with the evolution of acne lesions. Hence, the rationale of this study was to suspect a role for TLR2-bearing monocytes in the pathogenesis of acne vulgaris.

Despite the fact that peripheral blood TLR2-bearing monocytes may be increased in some other inflammatory disorders, yet we thought that proper selection of patients by exclusion of those who suffered any other medical condition particularly acute illness or chronic infectious diseases may confer specificity of our results. In this context, Pons *et al.* (2006) applied a similar flowcytometric technique to study the degree of expression of TLR2 on peripheral blood monocytes in response to bacterial infections among

patients with chronic obstructive pulmonary diseases (COPD). The authors found that the expression of TLR-2 was up-regulated in peripheral blood monocytes from COPD patients who are either clinically stable or during acute exacerbations.

In this study, we found a non-significant difference in the percentage of CD14+ peripheral blood monocytes among acne patients and healthy controls. These results indicate that acne patients were not suffering from any systemic disease with an infective nature. However, the MFI of TLR2 on PBM was significantly increased in acne patients, and the expression was still higher among those with predominantly inflammatory lesions than those with predominantly non-inflammatory lesions. These results indicated that TLR2 bearing monocytes are involved to some extent in the pathogenesis of non-inflammatory acne lesions and to a higher extent in mediating inflammatory acne lesions.

In addition to the increased expression of TLR2 on PBM observed in this study, Kim *et al.* (2002) reported increased expression of TLR2 on the cell surface of macrophages surrounding pilosebaceous follicles. Furthermore, Jugeau *et al.* (2005) demonstrated increased expression of TLR2 on keratinocytes and sebocytes in acne lesions which suggested that lesional skin keratinocytes participates in the inflammatory response to *P. acnes* through up-regulation of TLR2.

In an *in vitro* study conducted by Liu *et al.* (2005), treatment of primary human monocytes with all-trans retinoic acid (ATRA) led to the down-regulation of cell surface expression of TLR2 as well as its co-receptor CD14. The authors indicated that ATRA exerts an anti-inflammatory effect on monocytes through two pathways, one specifically affecting TLR2 and CD14

expression and the other through down-regulation of monocyte cytokine induction. In another *in vitro* study conducted by Tenaud *et al.* (2007), adapalene, a locally applied retinoid for acne treatment, was found to decrease the expression of TLR-2 in explants of normal skin and explants of acne lesions. The authors suggested that the decreased expression of TLR-2 by the keratinocytes can contribute to explain the anti-inflammatory activity of adapalene observed in clinical practice. In this study, treatment of patients with oral isotretinoin was found to decrease the expression of TLR2 on PBM. The reduced MFI of TLR2 receptors observed on monocytes obtained before and after treatment may explain the anti-inflammatory activity of isotretinoin, hence adding a new possible mechanism by which this drug works in treating acne patients.

In conclusion, this work demonstrated that *P. acnes* induce TLR expression and that this mechanism could play an essential role in acne-linked inflammation. In addition, retinoids exert their anti-inflammatory action as an effective therapeutic tool, in part, through down-regulation of TLR2-induced cytokine response. As such, we suggest that agents that target TLR expression and function may provide a novel strategy for the treatment of this common skin disease.

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