Egyptian Dermatology Online Journal
Volume 1 Number 1

Evaluation of the role of humoral immunity through assessment of monocyte-FC gamma receptors in pathogenesis of acne vulgaris

Egyptian Dermatology Online Journal 1 (1): 6, June 2005

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Abstract

Inflammatory acne has been elucidated by many researchers as a sort of hypersensitivity reaction to bacterial components of Propionibacterium acnes (P. acne), but which one of the immunological armies dominates in the pathogenesis of this type of acne remains unclear. For this purpose we chose 20 acne patients (10 males and 10 females) matched with 5 normal controls. Assessment of Fc receptors percentage on monocytes before and after vaccination by P .acne had been done which revealed that Fc receptor percentage on monocytes were significantly lower in patients with acne than in control and increased after vaccine injection but still were significantly lowered. So we concluded that cellular immunity plays a major role in the pathogenesis of inflammatory acne than humoral immunity.

Introduction

Acne vulgaris is a common, self-limited skin disease which involves the face and upper trunk, manifested clinically by comedones, papules, pustules and nodules. It usually affects individuals aged 20-40 years old.

The pathogenesis of inflammatory acne is not fully understood. While there is increasing information about it, many questions remain to be answered. For example, what causes the variety of distribution of the lesions in patients? What factors are involved in the initiation of inflammatory lesion? What causes follicular rupture, and what mechanisms are involved in the natural regression of the disease in the majority of patients in mid twenties.[3]

Propionibacterium acnes contribute to the inflammatory nature of acne by inducing monocytes to secrete proinflammatory cytokines including TNF-alpha, IL-beta, and IL-8. In particular, IL-8 along with chemotactic factors may play an important role in attracting neutrophils to the pilosebaceous unit.[5]

The mechanism by which P. acnes activate monocyte cytokine release is unknown but is thought to involve pattern recognition receptors (PRRs) of the innate immune system. Recently identified Toll-like receptors (TLRs) are one example of PRR. Toll receptors can discriminate between Gram+ve and Gram-ve organisms, bacterial ligands from Gram+ve bacteria can activate monocytes by TLR2 or TLR4.[10,12]Activation of monocytes by P. acnes or its products may play a role in the establishment of chronically inflamed acne lesions.[14]

Fc receptors are cell-surface receptors on monocytes for Fc domain of IgG and defined by their ability to bind IgG-antigen complexes. This binding couples the humoral and cellular immune responses and in the human, these receptors have been divided into three classes based on differences in apparent molecular mass, affinity for IgG, cellular distribution and reactivity with mAbs.[1, 2] Fc receptors represent one of the important links between the antibody defense mechanism and the cellular immunity in the form of phagocytosis.[7]

The aim of this work is to measure Fc gamma receptors of monocytes in patients with varying degrees of acne vulgaris and normal controls in order to assess the nature of humoral immune response to P. acnes. This of course will help to concentrate more light on how mediation of inflammation in acne vulgaris occurs.

Patients and methods

The study was carried out on ten age-matched healthy white male rabbits, weighing approximately 2 Kg each. Animals were housed under the same controlled environmental conditions at the animal house of the pharmacology department, Alexandria faculty of medicine, fed normal laboratory diet and they had free access to tap water.

Animals were anesthetized with thiopental sodium (2.5mg/kg I.V.). Full thickness four circular excisional wounds were performed down to bare cartilage on the ventral surface of each ear by using a 4-mm punch biopsy. A magnifying binocular loupe C 2.3x340mm (Heine, USA) was used. Hemostasis was then obtained by applying pressure. All wounds were covered using an occlusive polyurethane dressing (Tegaderm 3M, Minneapolis, Minn.) until the entire wound appeared re-epithelialized on gross examination.

Photographs were obtained and treatment of one of four wounds per ear was begun immediately with quercetin cream three times daily for four weeks. The second wound was treated with placebo cream at the same time to serve as control for the preventive group (n=10). The remaining two wounds per ear remained untreated during this period till a hypertrophic scar was established. After four weeks, treatment of the third wound that developed elevated scar was begun three times per day for eight more weeks. The fourth wound was treated with placebo cream to serve as control group for the curative group (n=10).

The ingredients in the cream were modified from the formula according to Katsarou et al.[9] Each 100 gm cream contain: quercetin (Carl Roth, GmbH co. 76185 Karlsruhe, Germany) 7.5 gm, white soft paraffin 9.5 gm, liquid paraffin 4.75 gm, acetyl alcohol 3.5 gm, glyceryl monostearate 2.5 gm, cremophor RH40 4.00gm, methyl paraben 0.25gm, propyl paraben 0.10gm, propylene glycol 10.00gm and water to 100gm. The placebo control was identical in composition except for quercetin.

With the animals under anesthesia, serial photographs were taken. The scars on the left ear were carefully excised and stored at -80°C for use in the biochemical measurements of: 1-Hydroxyproline concentration which is considered a reflection of collagen content as it comprises approximately ten percent of collagen.[10] 2-Histamine concentration was carried out according to the method of Shore et al.[11]

Statistical analysis[12]: All data were expressed as mean $\pm$ standard deviation (SD). One-way analysis of variance (ANOVA) techniques were used to examine the studied parameters. For pairwise comparisons among groups, the least significance difference test (LSD) was used. P value was calculated and statistical significance was set at ($P \leq 0.05$)

Results

The mean values of Fc receptor percentages in acne patients (males 35.2 and females 37.6) were highly significantly decreased ($P < 0.001$), compared with normal controls (males 89 and females

87,5) before P. acnes vaccination., as illustrated in Figures (3, 4). After P. acnes vaccine injections, the mean values of Fc receptor percentages on monocytes appeared highly significantly increased (P<0.001) in both male patients (62.4) and female patients (60.4), as shown in Figures (1,2). However, they still appeared highly significantly lower (P<0.001) than those of normal controls, as in Figures (5,6). On the other hand, there was no significant difference (P>0.60) in the mean values of Fc receptor percentages in male acne patients versus female patients, either before the vaccination or after, as shown in Figures(7,8).

Figure 1: Table & graph show the results of Fc receptor percentages in male acne patients before and after vaccination

Patient's Age (Years)	Before vaccination	After vaccination
20	15	55
25	25	54
22	28	45
19	30	52
17	35	60
16	35	68
22	40	65
22	40	78
20	50	77
25	54	70

X = 27.2 **Paired T = 10.681**
SD =8.052 **P<0.001(highly significant)**
SE =2.564

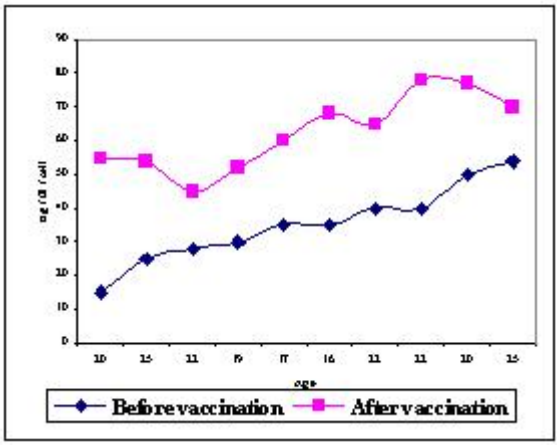


Figure 2: Table & graph show the results of Fc receptor percentages in female acne patients before and after vaccination

Patient's Age (Years)	Before vaccination	After vaccination
19	22	52
18	29	65
20	30	50
18	30	62
24	35	48
17	35	55
25	40	60
22	45	64
21	50	66
20	60	82

X =22.8 Paired T =9.755
SD =7.39 P< 0.001(highly significant)
SE =2.337

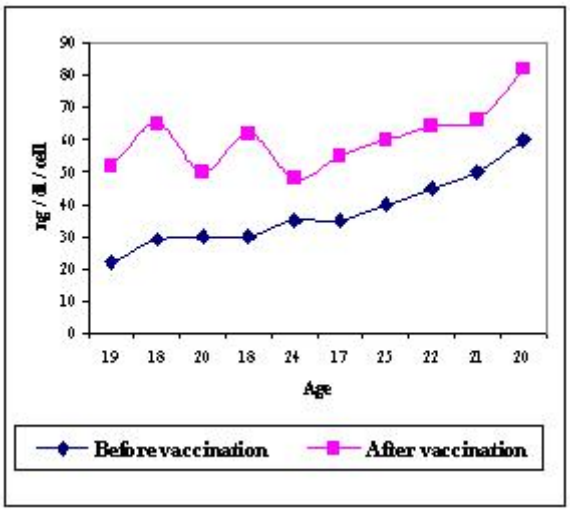


Figure 3:Table & graph show the results of Fc receptor percentages in male acne patients before vaccination compared to male controls

	male patients (before)	Male controls
N	10	3
$\bar{x}$	35.2	89
SD	11.593	7.937
SE	3.666	4.58

T = 9.167 P < 0.001 (highly significant)

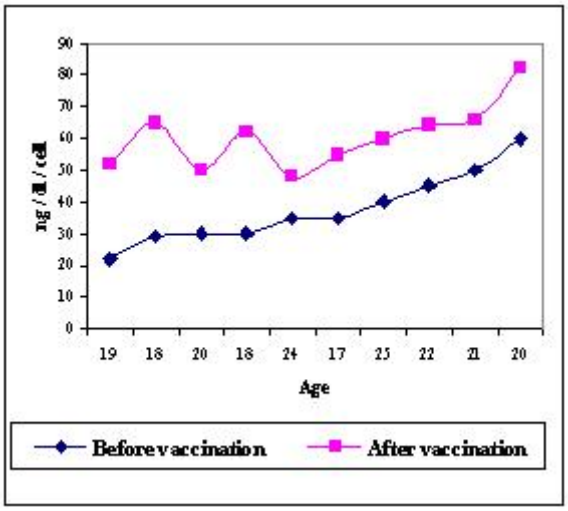


Figure 4: Table & graph show the results of Fc receptor percentages in female acne patients before vaccination compared to female controls

	female patients (before)	Female controls
N	10	2
$\bar{x}$	37.6	87.5
SD	11.364	20.6
SE	3.593	7.495

T = 6 P value < 0.001 (highly significant)

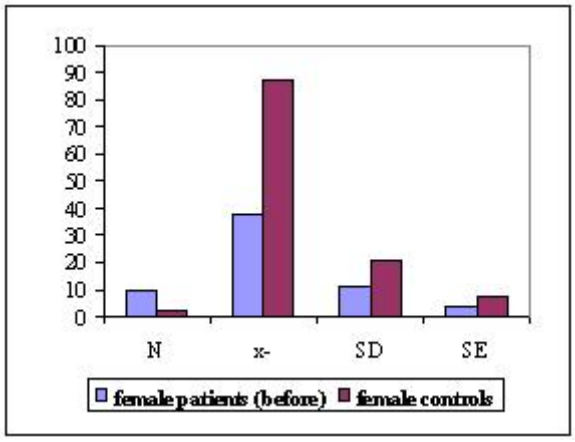


Figure 5: Table & graph show the results of Fc receptor percentages in male acne patients after vaccination compared to male controls.

	male patients (after)	male controls
N	10	3
$\bar{x}$	62.4	89
SD	11.027	7.937
SE	3.487	4.58

T = 4.61941

P value < 0.001 (highly significant)

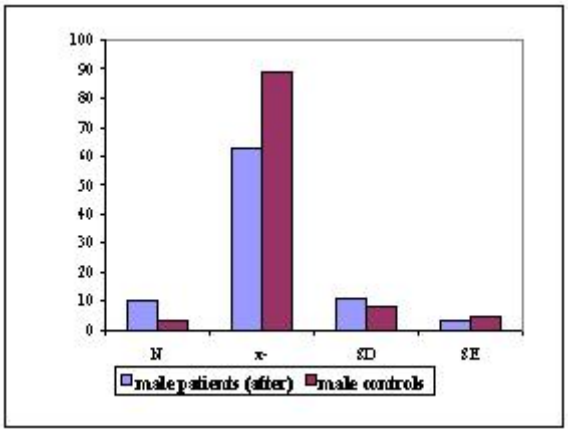


Figure 6 : Table & graph show the results of Fc receptor percentages in female acne patients after vaccination compared to female controls .

	female patients (after)	female controls
N	10	2
$\bar{x}$	59.6	87.5
SD	9.8	10.6
SE	3.099	7.495

T = 3.43989

P value < 0.001 (highly significant)

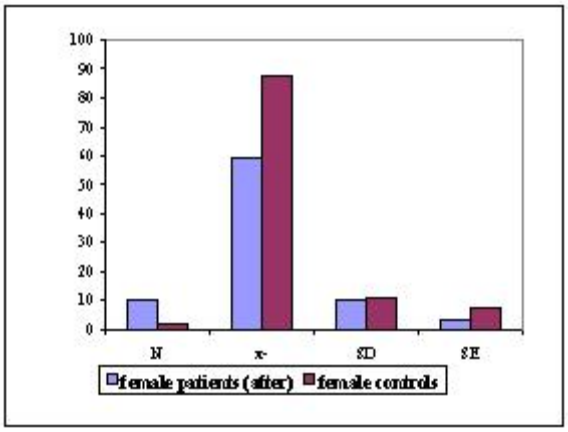


Figure 7: Table & graph show the results of Fc receptor percentages in male acne patients versus female acne patients before vaccination .

	male patients (before)	female patients (before)
N	10	10
$\bar{x}$	35.2	37.6
SD	11.593	11.364
SE	3.666	3.593

T = 0.467

P value > 0.06 (not significant)

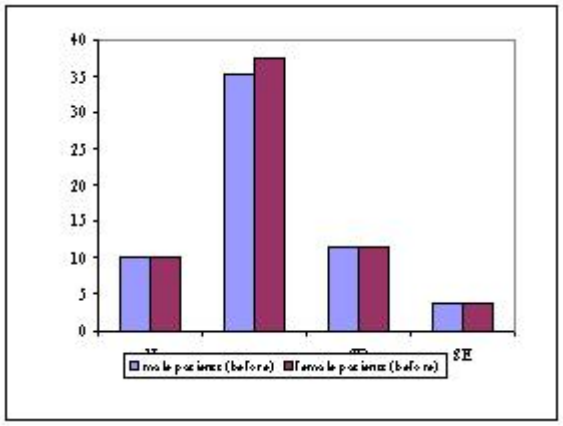
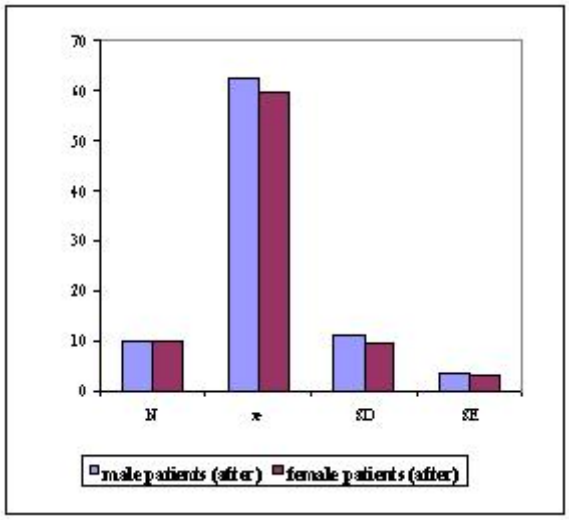


Figure 8: Table & graph show the results of Fc receptor percentages in male acne patients versus female acne patients after vaccination .

	male patients (after)	female patients (after)
N	10	10
$\bar{x}$	62.5	59.6
SD	11.027	9.8
SE	3.487	3.099

T = 0.6002

P value > 0.60 (not significant)



Discussion

Acne vulgaris is considered as one of the most common skin disorders, it can present in a variety of clinical forms depending on the type, number and severity of predominant lesion. Thus, there may be mild, moderate or severe comedonal or inflammatory acne, the latter with many subtypes.

Webester (1998), concluded that the variability of acne severity is due to the patient's reactivity to P. acnes. In other words, inflammatory acne is due to hypersensitivity to P. acnes. The oversensitive individual would mount a vigorous response to the organism, while the non-reactive one would form no lesion at all. There is evidence that such reactivity might be present in acne. In vitro complement activation by comedonal material is stimulated by the presence of antibodies to P. acnes and greatly decreased by their removal.[13]

Antibody to P. acnes is also required for neutrophil lysosomal hydrolase release to be triggered.[9] Thus, in the presence of elevated antibody titers, P. acnes is a much more potent and damaging inflammatory stimulus. Patients with severe acne have elevation in immunity. Antibody to P. acnes rises in proportion to the severity of

acne inflammation.[15]

Recently it has been found (Kim et al.,2002) that P. acnes contributes to inflammatory nature of acne by inducing monocyte to secrete proinflammatory cytokines including TNF-alpha, IL-lB and IL8. In particular, IL8 along with other P. acnes induced chemotactic factors that may play an important role in attracting neutrophils to the pilosebaceous unit. The Fc receptors are present on the phagocytic cells but are variable in molecular weight in each group of cells, e.g., the molecular weight of the Fc receptors on monocyte is 80,000 while that on neutrophil is 43,000. At the same time, there are different concentration of Fc receptors on different phage cells.[6]

In the human, leukocyte Fc gamma R are divided into three classes (FcR1, Fc R2, and FcR3), each with their own function and expression pattern. FcR1 binds monomeric IgG with high affinity whereas FcR2&R3 are of low affinity and only capable of binding IgG containing immune complexes.[4]

In the present study, we used a mixture of the three classes, hoping to extend our knowledge to FcR expression on human in vivo situation. However, our results agree against the involvement of an immune disorder in the etiology of inflammatory acne. Before P. acnes vaccine injections, Fc receptor percentage values on monocytes were lower in acne patients (males and females) than controls, and such a decrease was highly significant (Figures 3,4). This may indicate that there were no enough Fc receptors on monocytes to engulf the P. acnes organism, and subsequently, there was no enough processing of the organism which can stimulate lymphocytes. The decrease in Fc receptors could be interpreted by the possible presence of anti Fc receptor like substances that could block Fc binding.[8]

After P. acnes vaccine injection (4 injections with 2 weeks in between), the percentage of Fc receptors increased with high significance (Fig.5,6) this might be the cause of the partial (34%) improvement rate in the patient's acne. A possible explanation is that the injected P. acnes vaccine interacted by some way with the possible anti Fc receptor like substances. Another explanation may be that P. acnes stimulated monocytes to synthesize more Fc receptors. However, although this significant increase, still Fc receptor percentages were highly significantly lower than those of control group (Fig. 5,6) and this means that there is no role of humoral immunity in acne vulgaris. However, the present study was devised to elucidate the mechanism by which P. acnes induces inflammatory cytokines in monocytes by studying Fc receptors on monocytes. It seems that recognition of microbial pathogens by the cells of the immune system triggers host defence mechanisms to contact infection to prevent disease. However, activation of these same pathways can also result in inflammation at the site of disease and subsequent tissue injury. In acne the host response to P. acnes can

result in the production of inflammatory cytokines and contribute to the clinical manifestations of the disease which is disruption of the follicular epithelium and colonization of the follicles with P. acnes with subsequent reactions in the surrounding dermis. The presence of other receptors like TLRs on monocytes and their role in production of cytokines needs a further research which we hope to be done in the future.

Summary & conclusion

Acne vulgaris is a disease of the sebaceous follicles and is characterized by polymorphic skin lesions. These include open and closed comedones, papules, pustules, nodules and cysts. Four factors are considered to be important in the pathogenesis of acne; increased sebum production, comedogenesis, the activity of the follicular microflora and inflammation.

This study attempted to spot light on some of the immunological factors that may play a role in the pathogenesis of acne vulgaris. For this purpose, 20 patients (10 males and 10 females) aged 15-25 years complaining from acne vulgaris and 5 normal controls (3 males and 2 females) were included in this study. Blood samples were taken and submitted to estimation of Fc receptor percentages on monocytes. A P. acnes vaccine was injected intradermally 4 times, with 2 weeks interval with dose of 0.75 ml. The results were tabulated and statistically analyzed.

It was found that, before P. acnes vaccine injections the mean values of Fc receptor percentages in acne patients were highly significantly lower (males 35.2 and females 37.6) than those of normal controls (males 89 and females 87.5). After vaccination, the mean values of Fc receptor percentages were highly increased in male and female acne patients (males 62,4 and females 59,6) but still lower than control group. This mean that there is no role of humoral immunity in acne vulgaris. Thus, we can conclude that cell-mediated immunity is more detrimental than humoral immunity in the pathogenesis of acne vulgaris.

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