

## ORIGINAL ARTICLE

## Trail to Induce Psoriatic Skin Lesion in the Skin of BALB/C Mice by Injection of *Staphylococcus Aureus* Exotoxin

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### ABSTRACT

An In Vivo attempt to induce psoriatic lesions in the skin of BALB/C mice by injection of bacterial superantigen. *Staphylococcus aureus* that was isolated from skin lesions of psoriatic patients was subjected to gel filtration chromatography for extraction and purification of their exoproteins. The skin of BALB/C mice was injected by 0.2 ml of the extracted purified exotoxin. The induced lesions were markedly similar to that of human psoriatic lesions, although the histopathological changes were not completely mirrored to that of human psoriatic skin lesions. There is an important role of *Staph. aureus* exotoxine (superantigen) in induction, triggering and maintenance of psoriatic lesions

Keywords: *Staphylococcus aureus*, psoriasis, superantigens, animal model.

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### INTRODUCTION

Psoriasis is a chronic inflammatory and proliferative disorder of the skin clinically manifested as well circumscribed, erythematous papules and plaques covered with silvery white scales typically located over the extensor surfaces of the limbs and scalp [1]. Psoriasis is a very common disease affecting one to two percent of the population in all geographic regions [2,3]. The cause of psoriasis is still unknown, but experts agree that the skin lesions are the result of inflammation in the dermis and hyperproliferation with abnormal differentiation of the epidermis [4].

Recent studies have showed a potential role for bacterial superantigens in the induction of the localized inflammatory response that leads to the clinical lesions of psoriasis especially the guttate and chronic plaque psoriasis [5,6,7].

Yokote *et al.* [8] showed that the toxins of *Staph. aureus* strains behave as superantigens, and if present in patients, might play a role in the exacerbation of psoriatic lesions by activating certain V-beta ( $V\beta$ ) T-lymphocyte subsets. The finding of persistent T-cell clones bearing  $V\beta$  3 or  $V\beta$  13 in skin biopsy specimens from patients with chronic plaque psoriasis suggest the assumption of a superantigen – induced inflammatory response [9]. In this regard, *Staph. aureus* has been found on the skin of more than half of patients with chronic plaque psoriasis [10].

Many studies showed that psoriasis might be a T-cell mediated disease triggered by bacterial superantigen [11,12]. A number of important factors have been identified as relevant in the pathogenesis of psoriatic skin lesions: keratinocyte proliferation, vascular alterations [13,14] and activation of T lymphocytes, dermal macrophages and dendritic cells [15,16]. The close association in psoriatic lesions of the inflammatory infiltration with cytokine and chemokine production, and the basal keratinocyte hyperplasia has suggested a role of immunological processes in the pathogenesis of the disease [17-19]. In order to study the role of superantigen in the pathogenesis of psoriasis we try, in this work, to find if *Staph. aureus* superantigen can induce psoriatic lesion when injected in mice skin.

### MATERIALS AND METHODS

This is a conventional clinically – based (experimental) study which was carried out on 122 patients with plaque psoriasis attending the out patient dermatology department of Al-Sadder Teaching Hospital during the period from September 2004 and continued till December 2006. All patients were diagnosed clinically by the same dermatologist and they had no previous administration of antibiotics, at least two weeks prior to their inclusion in this study.

Skin swabs were taken from psoriatic lesions, and then put in nutrient broth and transported to the laboratory for isolation and identification of bacteria. Skin swabs were then subcultured on agar plate media. The following media were used for isolation and identification of isolates [20-22]: Blood Agar Base Medium (HIMEDIA M 089); Mannitol Salt Agar Medium (HIMEDIA); Nutrient Agar Medium (SCOTT); DNase Agar (BECTON DICKINSON); Brain Heart Infusion Broth (DIFCO).

It must be mentioned that all previous media were prepared and sterilized by autoclave according to the manufacturer's recommendations. Inoculated media were incubated aerobically at 37°C for 24-48 hours, while anaerobic cultivation was excluded in this study.

For rapid and accurate identification, API staph system, besides all necessary and valid tests were done by using particular diagnostic and biochemical tests [23,24].

### **Extraction and Purification of *Staphylococcus aureus* Exoprotein**

#### **1- Primary Screening Test**

*In vitro* primary screening test was carried out for selection of the potent isolates [25,26] of *Staph. aureus* which have the ability to produce exoproteins.

Twenty isolates of *Staph. aureus* were tested against three types of reference strains which were supplied from Microbiology Department of Basrah Medical College. These strains are: *Staph. aureus* ATCC 25923, *E.coli* ATCC 25922 and *Ps. aeruginosa* ATCC 27853.

Brain Heart Infusion broth (100 ml) was inoculated with bacteria and incubated for 48 hours for obtaining heavy growth. Standard strains were inoculated on Muller Hinton agar and left for 10 min to dry.

Sterile filter papers (Whatman no.4) were used for preparation of discs (6 mm in diameter). These filter discs were saturated with bacterial growth on Brain Heart Infusion to evaluate its activity against reference strains [27]. Filter papers were placed on the agar surface Plates of Mueller Hinton agar and then were incubated for 24 hours at 37°C. After incubation, the diameter of inhibition zone was measured for each isolate.

Primary screening test was done again with the most active isolates for testing their filtrates activity against the same reference strain.

#### **2- Extraction of Exotoxin**

The potent isolates of *Staph. aureus* were inoculated in Brain Heart Infusion for production of exoproteins (enterotoxin), depending on Orwin *et al.* and Schlievert [28,29].

#### **3- Gel Filtration Chromatography for Purification of Exotoxin**

Depending on Orwin *et al.* [28]; Chang & Bergdoll [30]; Leslie & Frank [31]; Whitaker & Granum [32].

#### **Induction of Psoriatic Skin Lesion in Laboratory Animals (*In Vivo* Study)**

To investigate the role of *Staph. aureus* exotoxine (superantigen) as a possible additional causative agent in initiation and triggering of psoriasis in laboratory animals, BALB/C mice were selected as an animal model for this study. The age of mice that was used ranged between 14-16 days. All possible typical conditions for animal growth were performed.

#### **Mice were infected by three methods:**

1- Intradermal injection (0.2 ml of exotoxin) 3 mice.

2- Spot method (0.2 ml of exotoxin) 3 mice.

3- Prick method (0.2 ml of exotoxin) 3 mice.

4- Control group (0.2 ml of normal saline) 3 mice.

After injection, mice were monitored for observing any change on the injected skin, any appearance of skin lesion and other different behaviors. Skin biopsy was taken from the lesion appeared at site of injection and kept in formalin (10%) for sectioning and then processed for examination (All of histological sectioning and staining procedures were done by Pathology Department of Basrah Medical College).

#### **Statistical analysis**

Agreement of the local ethical committee was obtained prior to starting this work. Statistical Program for Social Science (SPSS) was used to analyze the data. Chi - square ( $\chi^2$ ) test, differences between two proportions (Z- test) and t- test were used to assess the significance of differences between groups. P- value less than 0.05 was considered as statistically significant and P- value less than 0.01 considered as highly significant.

## **RESULTS**

### ***Staphylococcus aureus* among Psoriatic Patients**

Out of a total 122 psoriatic skin lesions, 72(59%) showed positive culture of *Staph. aureus*. The results were also showed that patients with plaque type harbored *Staph. aureus* (62.7%) more than those with guttate type (35.3%) (Table-1).

**Table-1: Frequency of *Staphylococcus aureus* among psoriatic types**

<i>Staph. aureus</i> growth	Psoriasis types No. (%)			Total
	Plaque	Guttate	Others	
Positive	64 (62.7)	6 (35.3)	2 (66.7)	72 (59)
Negative	38 (37.3)	11 (64.7)	1 (33.3)	50 (41)
Total	102 (83.6)	17 (13.9)	3 (2.5)	122

P= 0.0332

X<sup>2</sup> (plaque and guttate)= 4.5; (Mantel-Haenszel corrected)

#### Extraction and Purification of *Staphylococcus aureus* Exoprotein

Out of a total 72 isolates of *Staph. aureus*, twenty potent isolates were chosen for testing their ability to produce exotoxin.

Out of a total 20 selected isolates ten was shown to be more active in inhibiting growth of the reference strains: *Staph. aureus* ATCC 25923, *E. coli* ATCC 25922, and *Ps. aeruginosa* ATCC 27853 according to the diameter of inhibition zone.

The second screening test was also done and the result showed the ability of *Staph. aureus* filtrates of the last 10 isolates from table-2 of inhibiting growth of reference strain and to ensure the entity of their potential ability in the filtrates. Three typical local isolates (A, B, and C) of *Staph. aureus* were chosen to test their ability to produce exoproteins.

**Table-2: Diameter of inhibition zone (cm) of *Staph. aureus* isolated from psoriatic lesions against reference strains**

No.	Presence of inhibition zone	Diameter of inhibition zone against reference strains (cm)		
		<i>E. coli</i> ATCC 25922	<i>Staph. aureus</i> ATCC 25923	<i>Ps. aeruginosa</i> ATCC 27853
3	+ve	1	1	1
4	-ve	0	0	0
6	-ve	0	0	0
7	+ve	1.2	1	1
8	+ve	1	1	1
10	+ve	1.3	1.2	1
11	+ve	1	1	0
12	+ve	0.9	1	0
13	+ve	1.1	1.2	1
16	+ve	2	2.1	1

#### Purification of Protein by Gel Filtration Chromatography

The purification of exoprotein was achieved by gel filtration chromatography. The protein fractionation and purification were performed by using sephadex G-75.

One protein peak was obtained with gel filtration from fraction number 6 – 13 for protein A and B and 13-22 for protein C. These protein peaks were estimated spectrophotometrically at 280 nm. The elution peak was also determined for dextran blue at 600 nm with fraction number 7 (Figure 4-1). Refiltration with gel chromatography was also done to achieve the purity of the same protein peaks.

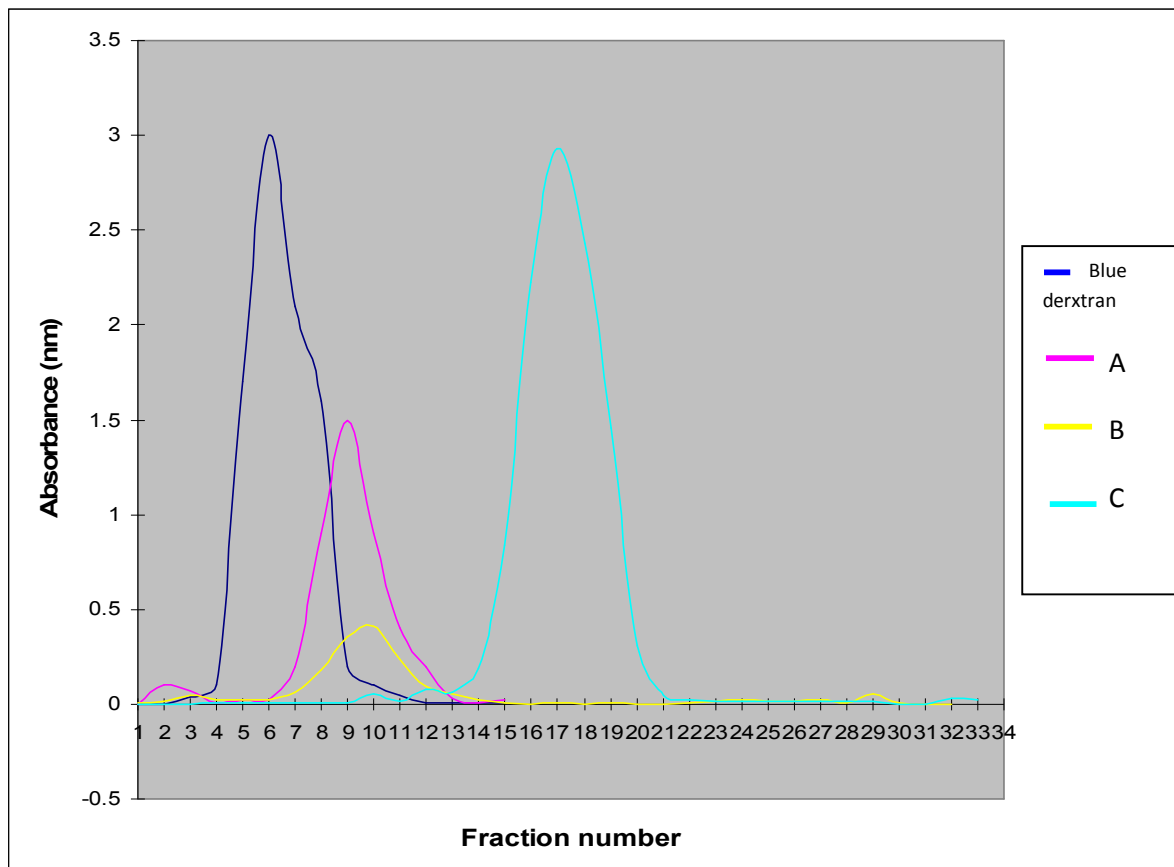


Figure-1 Gel filtration chromatography with Sephadex G75 for elution purified protein (exotoxin) with blue dextran

### Protein Concentration

The result showed that protein concentrations of purified proteins A, B and C were (0.054, 0.07 and 0.4) mg/ml respectively. The protein content of C type (0.4 mg/ml) was more than those of other proteins.

### Induction of Psoriatic Skin Lesion in Laboratory Animals

The impact of exoprotein (superantigen) that was produced by skin-colonizing *Staph. aureus* of psoriatic patients was studied. This was performed by topical application and intracutaneous injection of small amount of *Staph. aureus* purified exotoxin by using three different techniques. Intradermal injection was appeared to be the best method to induce psoriatic skin lesion.

The skin lesion that was induced in BALB/C mice appeared nearly similar to that of psoriatic lesions in human. The fully developed of *in Vivo* induced lesions were erythematous, scaly that was remarkably elevated from the adjacent normal skin, occurring mostly after 2-4 days of injection.

The scales were easily removed by gentle scraping leaving a pinpoint bleeding which simulate the characteristic feature of human psoriasis (Ausptiz's sign). It must be mentioned that the induced lesions disappeared after 4-6 days of appearance (picture 1-8). The histopathological changes of *InVivo* induced skin lesions were as following: Focal parakeratosis, acanthosis, club-shaped elongated rete ridges and dilated blood vessels, but neither monomicroabscess nor permeation of the epidermis were seen in the submitted histopathological slides (picture 9-14).



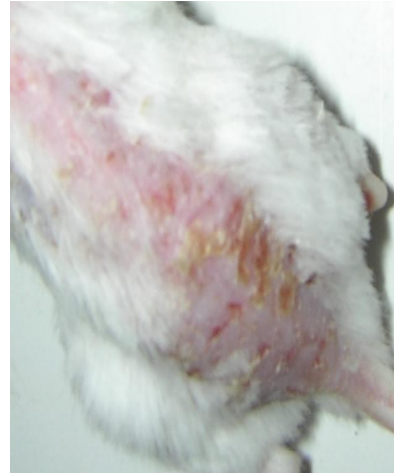
**Picture-1** Skin lesion after 4 days of intradermal injection of exotoxin



**Picture-2** Scaly, erythematous skin lesion after 4 days of intradermal injection



**Picture-3** Easily removed scales leave pinpoint bleeding on scraping



**Picture-4** Skin lesion after 5 days of intradermal injection of exotoxin



**Picture-5** Skin lesion with erythematous scales after exotoxin application by brick test. After 5 days



**Picture-6** Skin lesion after 4 days of scales after intracutaneous injection

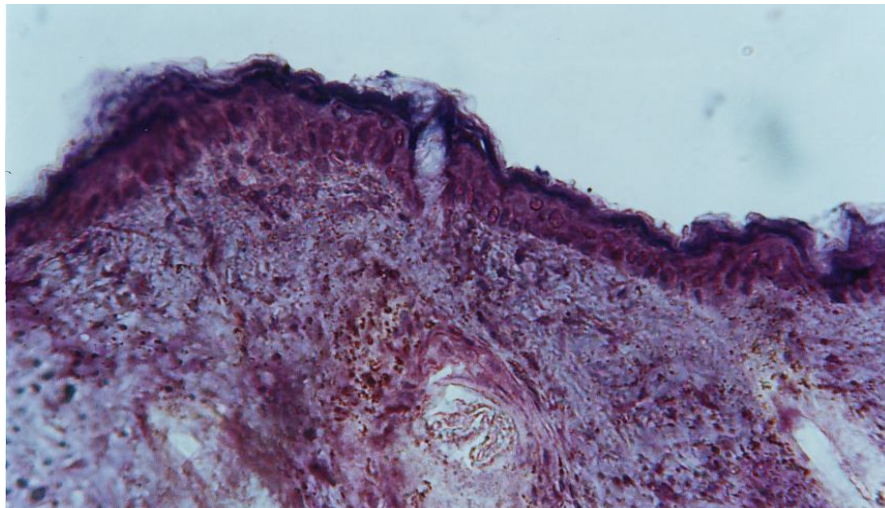




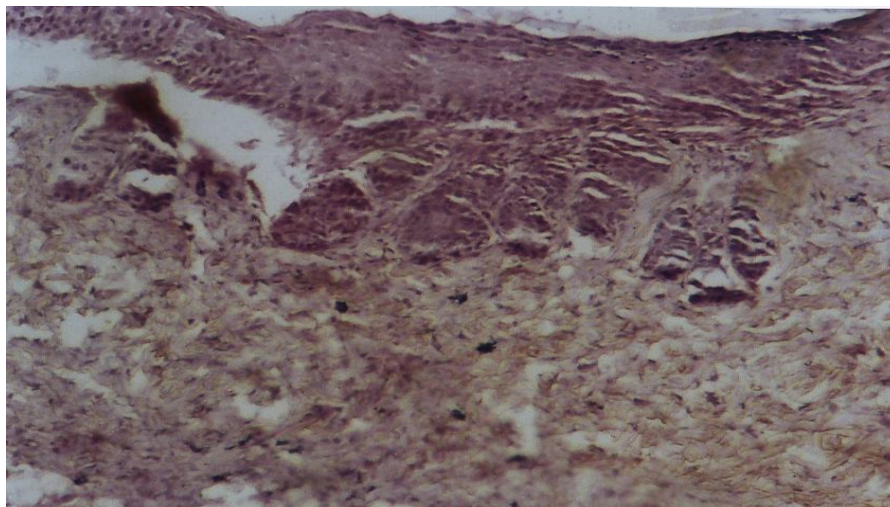
Picture-7 typical skin lesion



Picture-8 Normal skin of control that was injected with normal saline



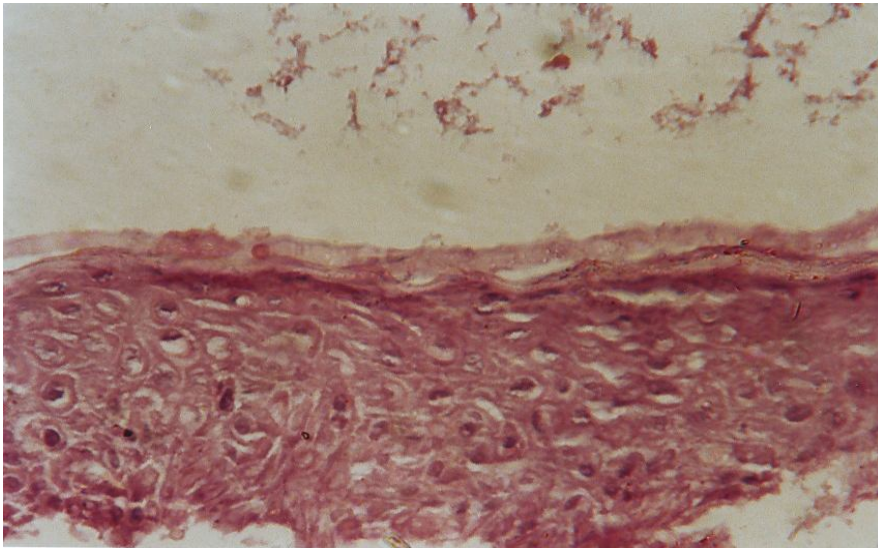
Picture-9: Normal structure of mice skin; control.(25x, HE)



Picture-10: parakeratosis; absence of granular layer, acanthosis with elongated rete ridges & suprapapillary thinning.(25x, HE)



**Picture-11:** Dilated elongated tortuous blood vessels reaching the tip of dermal papillae (25x, HE)

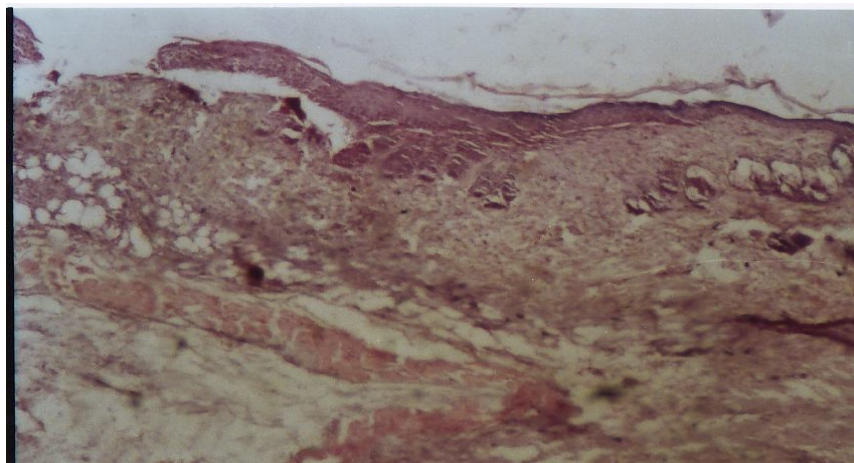


**Picture-12:** Spongiosis associated criteria with induced Psoriatic – like lesion in mice (40x, HE)



**Picture-13:** Parakeratosis: retention of nuclei in stratum corneum and decreased granular layer (100x, HE)





**Picture-14:** Test tube- like rete ridges with irregular acanthosis (10x, HE)

## DISCUSSION

As with many complex diseases, both genetic and environmental factors play a role in the development of psoriasis [33,34]. Regarding the environmental factors, staphylococcal and streptococcal infections are among the most common triggering factors that initiate and exacerbate the disease [35]. Epidemiological evidence implicates bacterial infection as a common triggering stimulus for psoriasis. The triggers that exacerbate psoriasis are important to identify, as they could become as an important therapeutic targets [36].

This study reported that, *Staphylococcus aureus* inhabited plaque type more than other psoriasis forms. This finding might be explained by the fact that psoriatic plaque is a good habitat for the growth of *Staph. aureus* or it may assume that psoriatic skin is unable to inhibit the growth of *Staph. aureus* or other bacteria [37] or the psoriatic lesion is the result of the heavy abnormal bacterial growth.

### Extraction and Purification of *Staphylococcus aureus* exotoxin

*Staphylococcus aureus* that were selected as mentioned previously, were subjected to gel filtration chromatography for extraction of exoprotein. One purified protein peak was revealed from one isolate. In addition, two other protein peaks were also arising from other *Staph. aureus* isolates.

Many studies hypothesized that *Staph. aureus* enterotoxin play a role in triggering and aggravating psoriasis by acting as superantigen [38,39,40,41]. The possibility of this hypothesis might be true since the idea about enterotoxin came from many supporting studies [42,43,44] about the properties of *Staph. aureus* enterotoxin which include: an ability to cause emesis and gastroenteritis in a primate model, superantigenicity, intermediate resistance to heat and pepsin digestion, and tertiary structural similarity.

### Induction of Psoriatic Lesions by Injecting *Staphylococcus aureus* exotoxin in BALB/C mice

The exact cause of psoriasis in human is not known although it is generally accepted as an immunologically mediated disease toward unrecognized antigen in genetically predisposed individual. Whether a person usually develops psoriasis is hypothesized to depend on a triggering factor. Examples of triggering factors include systemic infection, skin injury, vaccination, certain medication and intramuscular injections or oral steroid medication [45].

In the current study, an *In Vivo* attempt to induce psoriatic skin lesion with BALB/C mice was performed by injecting a particular dose of purified *Staph. aureus* exotoxin (superantigen).

Clearly the lesion that was induced is markedly similar to that of human psoriatic lesion. However, the histopathological examination was not completely similar to that of human lesions.

The main histopathological features are focal parakeratosis, a markedly thickened stratum corneum (acanthosis), mild spongiosis in addition to somehow broadening and elongation of rete projection to a uniform depth in the dermis with vasodilatation of dermal blood vessels.

**The incomplete similarity between the induced lesion and that for human psoriasis could be attributed to some reasons:**

- 1- Original differences between human and mouse skin structure were responsible of some discrepancies. The minimal or focal elongation of rete ridge elongation in mice, a major hall mark in human disease, was attributed to the relatively flat dermoepidermal junction in normal skin mice [45].



- 2- The response to *Staph. aureus* enterotoxin appeared to be dose dependent and requiring prolonged exposure for typical psoriatic lesion to develop otherwise signs of inflammation slowly disappeared over 5 to 7 days [46]. So that the exposing of the mice skin to short or transient small dose of SAg may not allow the resulting lesion to be completely similar to that of human psoriasis.
- 3- Psoriasis is a multifactorial disease occurs in the genetically predisposed persons. Accordingly typical psoriasis might only be induced in those who are genetically predisposed and the mice may need to be prepared in certain way to be susceptible to have the typical psoriatic changes. However the ability of superantigen to stimulate immunocytes to induce psoriasis lesions was proposed by many studies [47, 48, 49]. On the other hand studies concerned with the pathogenesis of psoriasis have been hampered by the lack of an animal disease just resembling this common human skin disorder. Over the past few years, however, various rodent models that mirrored aspects of psoriatic phenotype and pathogenesis have become available [50,51]. Several spontaneous mutations, transgenic animals, xenotransplantation models or T-cell transfer models have been utilized to study aspects of psoriasis. The mutation features moderate epidermal acanthosis, increased dermal vascularity [52] and a dermal infiltrate composed of macrophages and mast cells [53] although these skin alteration do not mirror psoriatic lesions, but they provide a strong handle on many specific phenotypic and pathogenic aspects of this common disorder [50].
- 4- The absence of microabscess and lack of epidermal permeation by neutrophil, which are a characteristic histopathological feature of human psoriasis, probably indicating that these feature occur early in psoriatic lesions and they need time to appear, meanwhile, it signifies that the induced mice skin lesions are neither a result of skin infection nor a skin reaction to the irritation caused by intradermal injection of SAg, as one might thought.

The attempt for induction of psoriatic lesion was done to demonstrate the immunological and intracellular pathway that mediate these phenotypes and assess the utility of the animal models for better understanding of this disease. However, that was done because it is still debated which the earliest event leading to psoriatic lesion [36, 51].

Capuano *et al.* [54] investigated the early histopathologic events in both uninvolved psoriatic skin and non psoriatic control induced by traumatic injury as changes occurring in Koebner's reaction. The result indicated that histopathological alterations were detected in more than 50% of psoriatic patients and in a single positive control with a family history of psoriasis.

Travers *et al.* [36] put the accumulating evidence for the role of *Staph. aureus* superantigen in the initiation and propagation of psoriasis by topically applied *Staphylococcus* and *Streptococcus* exotoxin to induce cutaneous reaction in volunteers.

The development of improved animal models having the clinical features simulating that of human psoriasis would be of great benefit for screening potential therapies for this chronic disease [45,55]. Notably, from our present findings, we certainly assume that there is an important role of *Staph. aureus* exotoxins (superantigen) in induction, triggering and maintenance of psoriatic lesions, although another trial on a well genetically prepared mice, is advisable.

Furthermore, a therapeutic trial with antistaphylococcal antibiotics is also recommended to evaluate their effect in treating psoriasis.

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