The role of Staphylococcus superantigens in chronic plaque type psoriasis

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Background: T cell activation is discussed as an important factor in the pathogenesis of psoriasis. Recently, a lot of attention has been paid to the role of superantigens in T cell activation in the pathogenesis of psoriasis. In this study, the role of staphylococcal superantigens in the pathogenesis of psoriasis with identification of staphylococcal toxin levels in the skin lesions of patients with chronic plaque psoriasis has been studied.

Method: In this case-control study, biopsies were taken from the skin of 40 patients with chronic plaque type psoriasis and 40 controls. Staphylococcal superantigens such as staphylococcus enterotoxin A, staphylococcus enterotoxin C, and toxic shock syndrome toxin 1 were investigated using polymerase chain reaction.

Result: Staphylococcus aureus was isolated from 6.5% of the psoriasis patients and 2.5% of the individuals in the control group and all of them were toxin producer. There was a significant difference between controls and patients.

Conclusion: Bacterial superantigens probably play an important role in the pathogenesis of chronic plaque type psoriasis.

Keywords: polymerase chain reaction, psoriasis, Staphylococcus aureus, superantigen

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INTRODUCTION

Psoriasis is a skin disease with unknown genetic and immunological pathogenesis. Activation of T cells is considered as an important factor in the pathogenesis of this disease, as laboratory studies have shown that populations of T cells isolated from the skin of psoriasis patients are capable of stimulating proliferation in keratinocytes[1]. Superantigens, including a group of viral or bacterial proteins, can directly bind to MHC class II molecules and the Vβ component of T cell receptors and cause activation of T cells [2-3].

Recently, much attention has been paid to the role of superantigens as a triggering factor in the pathogenesis of psoriasis. Exacerbation of psoriatic lesions subsequent to staphylococcal and streptococcal infections is a clue over this subject [4].

Some examples of staphylococcal superantigens are staphylococcus enterotoxin A, B, and C (SEA, SEB, SEC), toxic shock syndrome toxin 1 (TSST-I) and exfoliative toxin (ET). In various studies, evidence for their cellular effects in the pathogenesis of psoriasis is available [5-6]. In this study, we assessed the role of staphylococcus antigens in psoriasis patients with identification of TSST-I, SEB, SEC produced by Staphylococcus aureus in skin lesions by polymerase chain reaction (PCR).

PATIENTS AND METHODS

Patients

The study population included all psoriatic patients that were referred to the dermatology clinic of Hazrat Rasoul Hospital during 2006-2009. Inclusion criterion of this study was clinical diagnosis of plaque type psoriasis that was confirmed pathologically. Patients with history of treatment with oral or topical antibiotics or PUVA therapy in the two past weeks were excluded. In all patients informed consent was obtained before enrollment. The control group included non-psoriatic individuals who were referred due to cutaneous nevus removal. Their samples were used as control experiments.

The sampling method was simple and according to previous studies, 40 patients (26 males and 14 females) and 40 controls were selected. Patients and controls were age and sex matched. The mean age of the participants was 43 (86-9 years) (Table 1).

The collected data were analyzed with SPSS software. This study was approved by the ethics committee of our university and was performed according to the Declaration of Helsinki Principles. All of the participants were instructed about the study and they signed the informed consent forms.

Bacterial culture

Through a cotton applicator stained with 0.15 mole NaCl, specimens were prepared from lesions to culture in blood agar for staphylococcus aureus. Scaly lesions, pustules and infected ulcers were avoided. In the control group, tissue samples from removed nevi were used for culture.

Production of superantigens

Since production of superantigens requires a new culture medium, staphylococcus aureus was cultured overnight in a TY environment saturated with CO2 containing 1% yeast extract, petpon triptycase 1.7%, NaCl 0.5% and K2 HPo4 0.25%. Then, its floating part was deposited by aluminum sulfate 80% at 4°C. After centrifugation for 15 minutes at 12000rpm, the deposited liquid was solved with 100 µl buffer SDS-PAGE.

Table 1. Demographic data of patient and control groups

	number	male	female	mean age
Patient	40	26	14	43
Control	40	26	14	39.2

PCR

After washing cultured Staphylococcus aureus, incubation was done with 50µl Lysostaphyn (from a preparation of 500 mg/ml) and RNOSEI (610 mg/ml) at 37°C for 30 minutes.

Then, 350 µl of the lytic solution containing SDS 1% and NaOH 0.2 normal along with 250ml potassium acetate with PH = 4.8 were added. After 10 minutes, putting on ice, lysed bacteria precipitated at 12000 rpm for 10 minutes and the deposited DNA was washed with ethanol 70%. PCR was done on 50ml of the mixture. In this step, 50 mM KCl, 10 mM TRIJ - Hcl (PH 8.3), 15 mM $MgCl_2$, 0.01% gelatin, 200 mM dntps, 0.2 mM Oligonucleotide primer and 1.25 unit of *Taq* polymerase were centrifuged at 94°C for 1.5 minutes, at 50°C for 1.5 minutes and at 73°C for 1.5 minutes at 40 cycles; then, this product was added to agarose gel 1.5% containing ethidium bromide and electrophoresis was done.

RESULTS

Of 40 individuals in the case and control groups, S. aureus was isolated from four participants, 3 (6.5%) patients and one (2.5%) control. All of these four cases were toxin producers (Table 2,3). A significant difference was observed in the production of TSST-1, enterotoxin A and enterotoxin C between patients and controls (PV < 0.05).

DISCUSSION

Various studies have shown that bacterial toxins can cause stimulation and activation of cutaneous T cells as superantigens which stimulates the release of cytokines from keratinocytes and other inflammatory cells; therefore, they should play a

Table 2. Positive result in patient group

Code	Age (year)	Sex	SEA	SEC	TSST1
7	45	male	+	+	-
17	23	male	+	+	-
24	26	male	-	+	+

Table 3. Positive result in control group

Code	age(year)	Sex	SEA	SEC	TSST1
50	50	male	-	+	+

major role in the process of induction or exacerbation of psoriasis lesions.

Superantigens include a group of bacterial products that are presented to T Cell Receptor (TCR), particularly the Vβ area, after binding to molecules of MHC class II. These molecules are significantly different from common peptidic antigens [7].In contrast to common antigens identification that is restricted to molecules, this limitation does not exist about superantigens. Finally, in order to bind to MHC, superantigens do not require intracellular processing and bind to different sites as compared to common antigens binding sites.

Staphylococcus enterotoxin (SE) [A, B, C], TSST-I, exfoliative toxin (ET) and streptococcal exotoxin (SPE) are considered as leader bacterial superantigens. Superantigens are capable of stimulating T cells thousands of times more than common antigens [8].

In one study, biopsy specimens from the non-lesional skin of psoriasis patients and healthy individuals were cultured in vivo and bacterial superantigens and lipopolysaccharides were added as a stimulus to both environments. Finally, it was noted that in the two environments, different T cell proliferations, cytokines and growth factors were produced [9]. In another study, Staphylococcus toxins were compared between psoriasis patients and healthy individuals. Staphylococcus was isolated from 60% of the psoriasis patients out of whom 36% were toxin producers, and 12% of healthy controls who all lacked S. aureus toxin [6].

In another study, to evaluate the role of bacterial superantigens in psoriasis, the purified toxins TSST-I, SPES, SPEA, and SEB were used topically on the non-lesional skin of psoriasis patients and healthy individuals. Evidence showed more dramatic reactions in psoriatic patients than the control group [10].

On toxins measurement, various methods including immunodiffusion, ELISA and agglutination are available but amplification methods such as PCR that provide the detection of responsible genes for TSST-I, SEC, SEB are highly sensitive and specific. Although a number of studies, such as a study conducted by Sayama et al, from Japan, were not able to identify any significant difference in the rate of colonization of S. aureus in skin lesions of psoriasis patients and normal individuals [12], but most studies have shown the role of bacterial superantigens in the development and especially aggravation of psoriasis lesions. The results of our study showed isolation of toxin producing staphylococcus aureus in 6.5% of plaque type psoriasis patients and 2.5% of the controls. This significant difference suggests the possible role of bacterial superantigens in the pathogenesis of psoriasis. Design and implementation of broader studies with larger sample size with cellular and molecular study to achieve greater certainty in this area is necessary.

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