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Tissue Expression of Nuclear Factor Kappa Beta in Psoriasis Vulgaris Before and After Psoralen Ultraviolet-A Therapy: A Prospective Study

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Abstract

Background: **Nuclear factor kappa beta is an inducible nuclear transcription factor regulating a range of cellular processes. Nuclear factor kappa beta is considered to impact many cellular phenomena such as inflammation, immune responses, proliferation, differentiation, apoptosis, and tumor progression, therefore considered to have a major role in many inflammatory skin diseases including the pathogenesis of psoriasis.** ***Aim:*** **This study aims to investigate nuclear factor kappa beta tissue expression in patients with psoriasis vulgaris before and after Psoralen Ultraviolet A therapy.** ***Subjects and Methods:*** **Thirty patients with generalized chronic plaque psoriasis were assigned as a test group (Group I) and 10 age-and sex-matched healthy individuals assigned as a control group (Group II) were enrolled in this study. Skin biopsy specimens were subjected to histopathological study and immunohistochemical determination of nuclear factor kappa beta before and after 30 sessions of Psoralen Ultraviolet A therapy.** ***Results:*** **This study revealed highly constitutive tissue expression of nuclear factor kappa beta in all psoriatic lesions with variable degrees in comparison with control specimens ($p< 0.001$). Also, there was a significant decrease in nuclear factor kappa beta expression in psoriatic skin after Psoralen Ultraviolet A therapy ($p< 0.001$).** ***Conclusion:*** **Psoralen Ultraviolet A therapy among psoriatic patients demonstrated a decrease in PASI score, epidermal thickness; mononuclear cellular infiltration, as well as nucleocytoplasmic positivity (active nuclear factor kappa beta form) expression, accordingly selective blockade of nuclear factor kappa beta could be an effective treatment for psoriasis.**

Keywords: Nuclear factor kappa beta, Psoriasis, Psoralen Ultraviolet A.

Introduction

Psoriasis vulgaris is a common skin disorder characterized by the focal formation of inflamed, raised plaques that constantly shed scales derived from the excessive growth of skin epithelial cells. The disease is defined as a series of linked multiple cellular changes in the skin: hyperplasia of epidermal keratinocytes, infiltration of T lymphocytes, neutrophils, and other types of leukocytes with vascular hyperplasia in affected skin[(1)]. Nuclear factor kappa beta (NF-κβ) is a transcription factor that is considered to impact many cellular phenomena such as inflammation, immune responses, proliferation, apoptosis, tumor progression, and differentiation, NF-κβ is

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known to serve as an important transcription factor for many cytokine genes, inducible nitric oxide synthesis (iNOS), cyclo-oxgenase 2 (COX-2), growth factors, inhibitors of apoptosis and effector enzymes in response to ligation of many receptors involved in immunity including B-cell receptors (BCRs), T-cell receptors (TCRs) and members of the Toll-like receptor/IL-1 receptor superfamily[2]. Nuclear factor kappa beta activates transcriptions of downstream genes of at least 27 cytokines, chemokines, receptors involved in immune recognition, proteins involved in antigen presentation, and receptors required for leukocyte adhesion and migration[3,4]. In psoriasis, keratinocytes differentiation and proliferation would be regulated and modulated by many cytokines transcription factors and inflammatory mediators released from chronic inflammatory cells which accompany this lesion. NF-κβ is one of the transcriptional factors that play a critically important role in the regulation of cell cycle as well as influencing cell death pathways. In the skin, NF-κβ is proposed to protect the epidermis against apoptosis by enhancing the expression of anti-apoptotic factors[5,6]. When not activated, NF-κβ is found in the cytoplasm bound to its inhibitory protein I kappa β (Iκβ). Upon Iκβ degradation, NF-κβ is activated and translocated to the nucleus. Its activated form consists of a heterodimer containing a p65 (also known as relA) and a p50 subunit. It then stimulates the transcription of cytokines including TNFα, IL-1, IL-2, IL-6, and IL-8, as well as inducible nitric oxide synthesis and the adhesion molecules ICAM-1, VCAM-1, and E-selectin. In addition to increasing the production of NF-κβ, TNF-α can activate NF-κβ by degrading Iκβ, its inhibitory protein. NF-κβ activation may contribute to the pathogenesis of psoriasis through inhibiting TNF-α induced apoptosis[7,8]. Moreover, TGF-β expression is also reported to be upregulated by NF-κβ[9]. The combination of psoralen and UVA radiation (PUVA photochemotherapy) utilizing oral 8-methoxypsoralen (8-MOP) is an established treatment for many skin disorders such as psoriasis vulgaris. PUVA's mechanism of action in psoriasis is a result of its direct lymphotoxic effects and UVA-induced psoralen-DNA interactions are assumed to contribute to the cutaneous anti-inflammatory and anti-proliferative effects of PUVA. PUVA-induced DNA modifications might interfere not only with DNA replication, but also with gene transcription of proinflammatory genes[10]. The aim of this study was to investigate the constitutive expression of NF-κβ in psoriasis and to determine if treatment of psoriatic patients with PUVA would result in downregulation of NF-κβ and in turn if this would correlate with disease resolution and restoration which is the first work to be done up to the best of our knowledge.

Subjects and Methods

This study included 30 psoriatic patients recruited from the phototherapy unit at Suez Canal University Hospital, Dermatology Outpatient Clinic, Ismailia, Egypt, during the period from March 2015 to June 2016. Permission from the ethics committee and institutional review board was taken and informed written consent was obtained from each patient and control before starting the study. Patients who have a past or family history of skin cancers, pemphigus, uremia, hepatic diseases, severe myocardial diseases, cataracts, aphakia, and photosensitive dermatoses, and also pregnant or lactating females were excluded from the study[11]. Before starting treatment, all individuals were subjected to complete history

taking, dermatological and ophthalmological assessment, in addition to some routine laboratory investigations including complete blood picture, liver, and kidney function tests. The severity of psoriasis was assessed by Psoriasis Area Severity Index (PASI) score[12]. For computing the PASI score, the body was divided into four sections, each section had a coefficient depending on its extension (namely, 0.1 head, 0.2 upper limbs, 0.3 trunks, 0.4 lower limbs). Each area was scored separately, taking into consideration the characteristics of the plaque (scaling, induration/thickness, and erythema) then, the scores were calculated to give the final score. A lesional skin punch biopsy around 5 mm was taken from each patient after infiltration anesthesia with xylocaine 2% before and after PUVA therapy and from healthy controls. Each skin biopsy specimen was fixed in neutral buffered formalin and processed for paraffin-embedded blocks. Sections (5µm) were stained with hematoxylin and eosin (H&E) to detect the different histopathological changes. Other sections were cut onto poly-L-lysine coated slides to IHC determination of NF-κβ [p65/Rel A] (Santa Cruz Biotechnology Inc., California, USA; catalog Sc-7154) using streptavidin/horseradish peroxidase detection kit [DAKO LSAB system] without antigen retrieval. The results of IHC staining were given in positivity manner, grading from no staining to very strong staining as follows: 0= no staining, 1= faint or weak staining, 2 = moderate staining, 3= strong staining, and 4= very strong staining[13].

PUVA Therapy

Patients received treatment with the irradiation system PUVA 7001-PUVA spectrum-27 F85/100W PUVA-wave length 315-400 nm maximum 365nm (Herbert Waldmann GmbH & Co., Villingen-Schwenningen city, Baden Württemberg, Germany) on the whole body 3 times/week. The patient ingested 8-methoxypsoralen tablets (dose of 0.6 mg/kg body weight). After two hours, the whole body has been exposed to UVA. Patients wore protective goggles in the machine and thereafter they used sunglasses during the rest of the day. The male genitalia was shielded during exposure to UVA therapy. The starting PUVA dose was 1-2 J/cm^2 according to the skin type and increments of ½ J/cm^2 were increased every other session. Treatment was terminated and evaluated at the end of 30 sessions[11].

Quantitative Analysis

By the aid of Leica Q 500 image analysis system (Leica Qwin 500 Microsystem Corporation, Milton Keynes, UK) at the Faculty of Medicine, Cairo University. Quantitative measurement of mononuclear cellular infiltrate in H&E sections and optical density of NF-κβ [p65/Rel A] expression in the immunostained sections were done [10 fields per section].

Statistical Analysis

Statistical package SPSS version 11 was used for data analysis. Mean and standard deviation was used for continuous variables and percentage for discrete variables. Differences between pre and post-treatment were assessed using paired *t-tests*. For correlation between continuous variables, Pearson's correlation (r) was used[14].

Results

Clinical results

The study included 30 psoriatic patients; 16 males (53.3%) and 14 females (46.7%) aged between 18-70 years with a mean of 37.6±13.3 years. The duration of their disease ranged between 1 to 30 years with a mean of 9.90±2.84 years. 15 patients (50%) were skin type III, 11 patients (36.7%) of skin

type IV, and 4 patients (13.3%) of skin type V. Ten healthy individuals were taken as controls; 4 males (40%) and 6 females (60%) aged between 18-65 years with a mean age of 32.87±12.7 years. The extent of the disease in psoriatic patients ranged from mild to very severe; PASI score before the first session of PUVA therapy ranged between 5.6-56.4 with a mean of 29.1±16. Most of the patients achieved either almost complete clearing or moderate improvement at the end of the study as PASI score was 6.75± 8.28 (Table 1 and Fig. 1).

Histological and IHC results

Hematoxylin and eosin (H&E) stained sections of pre-treatment specimens showed hyperkeratosis, parakeratosis, papillary edema, dilatation, and tortuosity of capillaries and mononuclear cellular infiltration in the dermis (Fig. 2a, 2b) Sections of post-PUVA therapy showed marked decreased in both epidermal thickness and mononuclear cellular infiltration (Fig. 2c, 2d) and Table 2.

Table 1: Clinical data of the patients and controls

Variables		**Psoriasis patients N=30** no. (%)	**Control N=10** no. (%)
Sex	*Males*	16 (55.3%)	4 (40%)
	Females	14 (46.7%)	6 (60%)
Skin types	*III*	15 (30%)	4 (40%)
	IV	11 (36.7%)	4 (40%)
	V	4 (13.3%)	2 (20%)
Age (Yrs.)	*Range*	18-70	18-65
	Mean ± SD	37.6 ± 13.3	32.87 ± 12.7
Disease duration (yrs.)	*Range*	1-30	
	Mean ± SD	9.90±2.84	
PASI score	Before treatment		
	Range	5.6-56.4	
	Mean ± SD	29.1 ± 16	
	After treatment		
	Range	2.3-18.7	
	Mean ± SD	9.75 ± 7.28	

Table 2: Histopathological changes in different studied groups

Variables	Epidermal thickness (µm)	Mononuclear cellular infiltrate (cell/HPF)
	Mean ± SD	Mean ± SD
Control	4.44±2.08	18.42±9.11
Before PUVA	24.53±2.13	98.6±17.35
After PUVA	6.37±2.45	33.16±12.14

Immunohistochemical (IHC) detection of NF-κβ expression in the controls specimens showed only epidermal diffuse cytoplasmic staining (a non-active form of NF-κβ) in all of them (100%). On the other hand, nucleo-cytoplasmic positivity (active NF-κβ form) expression in non-treated psoriatic specimens showed; 6.7% of very strong staining, 36.7% showed strong staining, 33.3% of moderate staining and 23.3% showed weak or faint NF-κβ staining. In patient's specimens after treatment, no very strong staining of NF-κβ was detected, only 6.7% showed strong staining, 13.3% showed

moderate staining and 43.3% showed weak staining; while 36.7% showed negative active NF-κβ form staining (Table 3 and Figure 3). Comparing psoriatic and control skin, it was found that epidermal nucleocyto-plasmic positivity (active NF-κβ form) expression in psoriatic patients was significantly higher than in controls that lacked any nuclear staining (p< 0.001). Also, there was a highly significant decrease in NF-κβ expression in psoriatic skin after PUVA therapy (p< 0.001). It was found that there was no significant correlation between the variable degree of NF-κβ expression and age, sex of patients, or duration of psoriasis (p>0.05).

Table 3: Comparison between active NF-κβ form expression in psoriatic patients before and after PUVA therapy

NF-κβ (IHC) Staining	Before treatment (n=30)		After treatment (n=30)	
	No.	%	No.	%
No staining	0	0	11	36.7
Faint staining	7	23.3	13	43.3
Moderate staining	10	33.3	4	13.3
Strong staining	11	36.7	2	6.7
V. strong staining	2	6.7	0	0

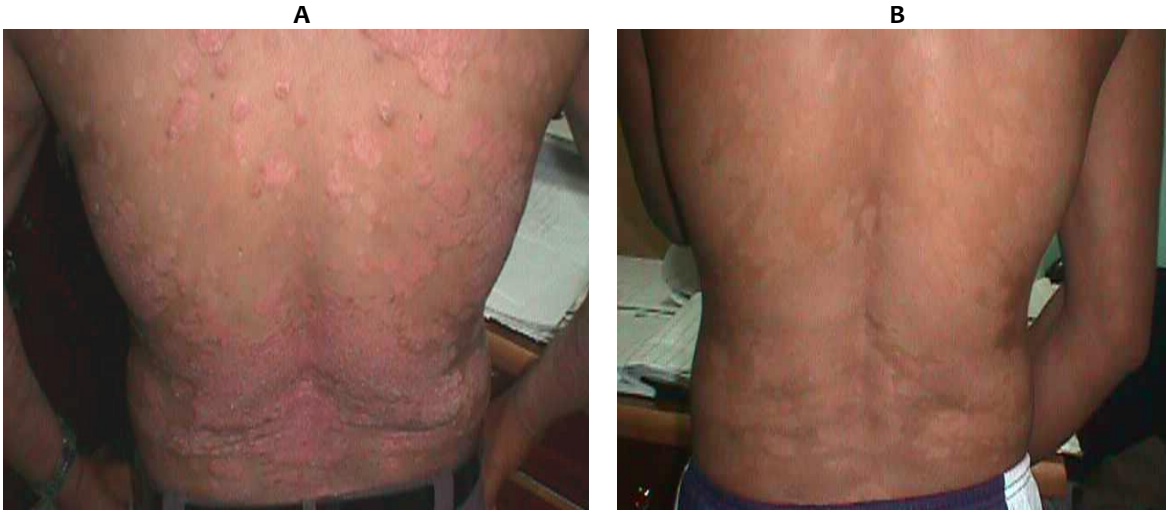

Figure 1: Psoriatic skin lesions before (A) and after (B) PUVA therapy

Discussion

Psoriasis is a chronic, genetically influenced, remitting, and relapsing inflammatory papulosquamous disease of immunologic nature that is mediated by T-helper-1 cytokines characterized by epidermal hyperproliferation with abnormal differentiation, and inflammatory infiltration of the epidermis and dermis[15]. Nuclear Factor Kappa Beta (NF-κβ) is a member of the transcription factor family, which belongs to the Rel family[2]. Many researchers reported that the NF-κβ pathway is involved in psoriasis-related immune responses and the increased epidermal thickness present in psoriatic plaques is the net sum of both rates of proliferation and cell death.

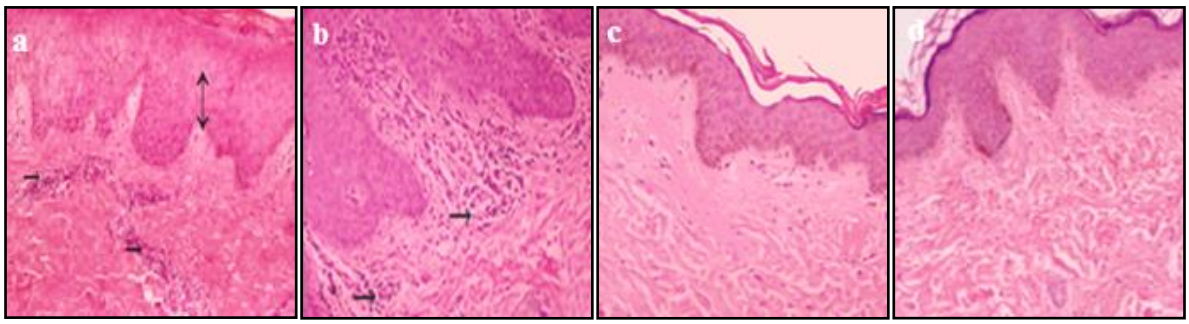

Figure 2: Histopathology of psoriatic skin before and after PUVA therapy.

(a, b): Pre-treatment classical change of psoriasis, shows epidermal proliferation (↕) (hyperkeratosis, parakeratosis, Munro microabscesses, acanthosis with elongation of rete ridges and loss of granular cell layer). Dermis shows superficial perivascular inflammatory infiltrate of mainly lymphocyte (→) and dilated tortuous superficial blood vessels. **(c, d)** Post-treatment specimen shows decrease epidermal thickness with faint superficial perivascular inflammatory infiltrate (H&E x100).

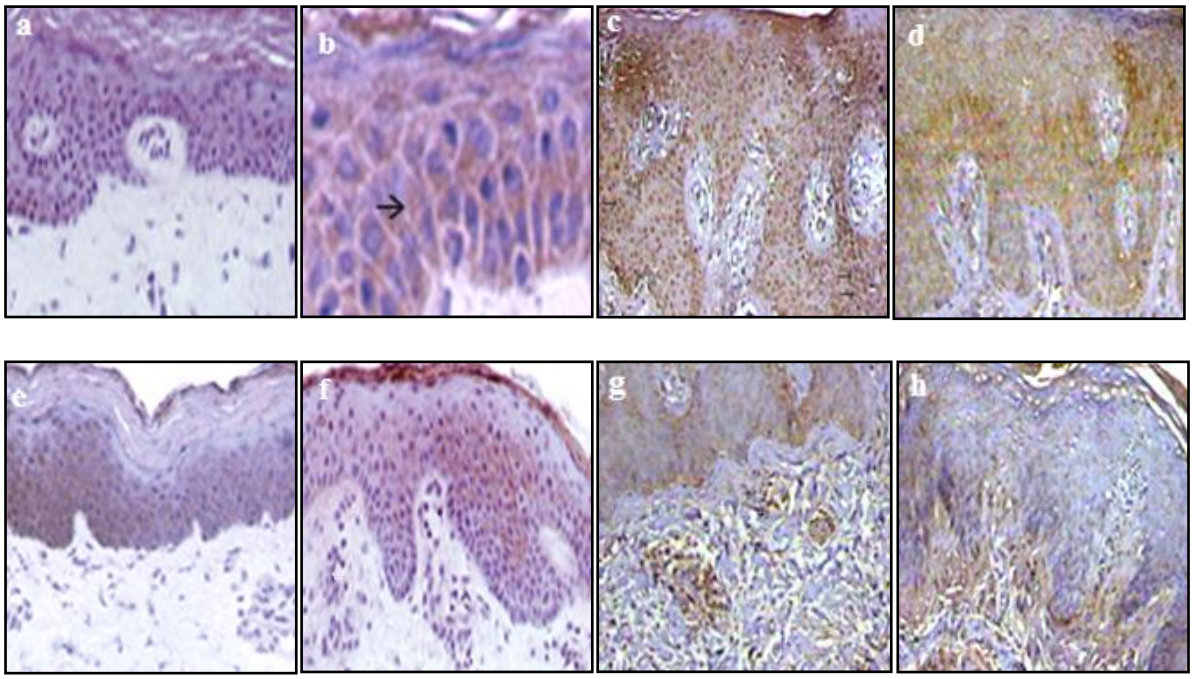

Figure 3: IHC staining of NF-κB immunoreactivity in normal control epidermis and in psoriatic epidermis before and after PUVA therapy

(a, b): Normal control epidermis revealed cytoplasmic staining (non-active form) (IHC, NF-κβ x400). **(c)** Pre-PUVA Diffuse nuclear NF-κB (active form) staining involving the whole layer of psoriatic epidermis (→). **(d)** Pre-PUVA Moderate staining nuclear NF-κB in keratinocyte. **(e, f, g, h)** After PUVA therapy with a marked decrease in nuclear NF-κB in the keratinocyte and dermis (IHC, NF-κβ x100).

In this study, nuclear NF-κβ (active form) immunoreactivities were significantly higher in allover the psoriatic epidermis, although it was more heavily expressed in the basal keratinocytes with dermal expression in different degrees; whereas all of the controls showed only epidermal diffuse cytoplasmic staining (a non-active form of NF-κβ), these results were in agreement with those of Lizzul et al.[16] who stated that there was a highly significant increase ($p< 0.001$) in nuclear NF-κβ in the epidermis of psoriatic plaques as compared with normal skin. It was also in agreement with what reported

by Abdou and Hanout[17] who found that nuclear expression of NF-κβ was detected in 66% of psoriatic lesions; this active phosphorylated form was significantly overexpressed in psoriasis in comparison with normal skin (p = 0.0004)[17]. On the other hand, Takao et al.[18] reported that, in the absence of stressful stimuli, NF-κβ is present in the cytoplasm in a resting state within normal keratinocytes. Under normal conditions, NF-κβ has also been noted to play an important role in keratinocyte regulation and proliferation. But the function of NF-κβ in keratinocytes has been proposed to be quite paradoxical to its effect in other cell types, such as T or B cells. This is matched with the fact that NF-κβ in unstimulated resting cells is restricted to the cytoplasm bound to I kappa β (Iκβ) that subsequently prevents it from entering the nucleus. When these cells are stimulated, specific kinases phosphorylate Iκβ causing its rapid degradation by proteosomes with the release of NF-κβ and its passage into the nucleus[19]. In the present work, there was a positive correlation between NF-κβ expression and disease severity (p=0.0005), no significant correlation has been detected between the variable degree of NF-κβ expression and age, sex of patients, or duration of psoriasis (p > 0.05). The cellular consequences responsible for the therapeutic benefits of PUVA are not fully understood. However, it is generally assumed that UVA-induced DNA-psoralen photo adducts (type-I reactions) impede replication, causing the inhibition of cell proliferation. PUVA-mediated inhibition of proliferation may be beneficial in psoriasis as it suppresses hyperproliferative epidermal keratinocytes (reduction of acanthosis) and interferes with the expansion of skin infiltrating T cells sustaining inflammation. Higher UVA doses and psoralen concentrations could lead to irreversible cell damage. Lymphocytes are very susceptible to PUVA-induced apoptosis and necrosis, so this lymphocyte death would considerably contribute to the anti-inflammatory effect of PUVA in psoriasis and to the decrease of skin infiltration with tumor cells in cutaneous T cell lymphomas[20]. This study demonstrates a substantial and significant reduction of nuclear NF-κβ expression in psoriatic skin after PUVA therapy (p< 0.001). This result was in accordance with what was reported by Gasparo[21] who stated that PUVA kills diseased cells through the induction of apoptosis which may be through the inhibition of NF-κβ. Song and Tapley[22] reported that PUVA-induced DNA-psoralen photo adducts may not only inhibit DNA replication but may also interfere with gene transcription. Nuclear Factor κβ specific binding activity was not induced 1-24 h after PUVA in extracts from PUVA-treated cells when compared with controls, whereas the pro-oxidant cytokine TNF-α caused a marked increase in NF-κβ binding, and these data suggest that PUVA inhibits cell proliferation, but not transcription, at nonlethal PUVA conditions. Furthermore, the data do not support a major role for PUVA-generated reactive oxygen intermediates in the regulation of gene transcription[23]. Yoo et al.[24] reported that the induction of T-cell apoptosis is thought to be a key mechanism for PUVA therapy. Evidence for the appearance of apoptotic T cells under PUVA therapy was provided for peripheral blood T cells in Sézary syndrome patients undergoing extracorporeal photopheresis. Interestingly, the induction of apoptotic cells is not an immunologically null event, but most likely it has immune-suppressive consequences[25]. Phagocytosis of apoptotic cells has some profound effects on the production of mediators by macrophages. After phagocytosis of apoptotic T cells, macrophage production of proinflammatory cytokines such as TNF-α, IL-1, and IL-12 is down-regulated whereas the production of the anti-inflammatory/immunosuppressive cytokine IL-10 is increased[26].

Conclusion

PUVA in psoriasis demonstrated a decrease in PASI score, epidermal thickness, mononuclear cellular infiltration, as well as nucleocytoplasmic positivity (active NF-κβ form) expression, which indicated a reduction of hyperproliferative epidermal keratinocytes, inflammation and a return to normal keratinocyte proliferation and differentiation. These findings underscore the important role, which NF-κβ plays within the immune system, and the potential of developing therapeutic agents that affect NF-κβ function further work will be needed to expand on these ideas and to elucidate what gene products are stimulated by its activation and how they help to inhibit apoptosis. Selective blockade of NF-κβ could prove to be an efficacious treatment for psoriasis as well as other inflammatory and autoimmune disorders.

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Conflicts of interest: None

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