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In vitro T Cell Response to Staphylococcal Enterotoxin B Superantigen in Chronic Plaque Type Psoriasis

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Recent studies have demonstrated the important role of CD4+ T cells in the pathophysiology of psoriasis. One of the current hypotheses is that triggering of the psoriatic inflammatory process could be secondary to CD4+ T cell activation by bacterial superantigens in the skin. In this study, IL-2-derived T cell lines were recovered from the blood and the skin of 4 patients with chronic plaque type psoriasis and of 2 patients with allergic contact dermatitis (ACD). Blood and skin T cell lines were tested for their ability to proliferate in vitro to staphylococcal enterotoxin B (SEB) presented by MHC class II expressing antigen-presenting cells. The results showed a significantly higher SEB-induced T cell proliferation in skin T cell lines as compared to blood T cell lines in 3 out of 4 psoriatic patients and in one of the 2 ACD patients. No difference between the skin and blood T cells for their response to phytohemagglutinin was observed. Furthermore the blood T cell lines from both patients and control individuals responded equally well to SEB. Thus psoriatic skin T cell lines were characterized by an enrichment in SEB-responding T cells. Since similar enhancement of SEB-responsive T cells was occasionally found in ACD patients, we propose that SEB could be an environmental factor associated with rather than responsible for psoriatic inflammation. ***Key word: T lymphocyte activation.***

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Psoriasis is a chronic inflammatory disease of unknown etiology, in which genetic factors play a role in the onset and course of the disease (1). Indirect evidence suggests that activation of $CD4^+$ T cells could be the primary cellular event in the pathogenesis of psoriasis, leading to the inflammatory and the hyperproliferative epidermal process (2–4). In this respect, cyclosporin A, anti-CD3 and anti-CD4 monoclonal antibodies, which are all known to downregulate T cell activation, have been shown to be effective in psoriasis (5–7).

Superantigens (SAgs), including molecules derived from bacteria, viruses or mycoplasma (8, 9), activate T cells by interacting with specific variable regions of the T cell receptor (TcR) β chains and MHC (major histocompatibility complex) class II molecules on antigen-presenting cells (10–12). SAgs have recently been proposed as potential triggering factors in psoriasis, and staphylococcal enterotoxin B (SEB) seems to be one of the SAgs involved in T cell activation in the disease (13).

In this study, we show that T cell lines recovered from lesional skin are enriched for SEB-responding T cells, as compared to blood-derived T cell lines in 3 out of 4 cases of chronic plaque psoriasis. These data support the hypothesis that SEB may contribute to local T cell activation in psoriasis.

MATERIALS AND METHODS

Patients

This study involved 4 patients with active chronic plaque-type psoriasis. The patients were all informed of the aim of the study and consented to take part in it. They had not used topical treatment or PUVA therapy for the preceding 2 weeks, before entering the study. Peripheral venous blood was collected in heparinized tubes and immediately processed for peripheral blood mononuclear cells (PBMC) isolation. A 6-mm punch biopsy was taken from the edges of the inflammatory skin; selected areas were washed with alcohol, biopsies were removed, and the specimens were placed in HBSS with penicillin (200 U/ml) and streptomycin (200 μg/ml). As controls, blood was obtained from voluntary healthy donors by the same procedures. Furthermore, 2 patients with allergic contact dermatitis (ACD) to nickel were included in the study; lesional skin and peripheral blood were obtained 48 h after a patch test to nickel and processed as indicated above.

Preparation of peripheral blood mononuclear cells

PBMC were prepared from patients' blood by Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) density gradient centrifugation. Cells from the interface were washed 3 times in HBSS and resuspended at $1x10^6$ cells/ml in culture medium: RPMI 1640 medium (Gibco, Grand Island, NY) containing 10% heat-inactivated human AB serum (Centre de Transfusion sanguine, Lyon, France), penicillin (100 U/ml), streptomycin (100 μg/ml), gentamicin (100 μg/ml), and L-glutamine (2 mM) (all from Vie 3000, Balan, France), hereafter referred to as complete RPMI medium.

In order for us to prepare blood-derived antigen presenting cells (APC), PBMC obtained after separation on Ficoll were γ-irradiated at 4,000 rads, washed 3 times in HBSS and used as presenting cells in proliferation assays. The same procedure was used to obtain feeder cells from blood, for maintenance of the T cell lines.

Generation of T cell lines

To prepare blood-derived T cell lines, we cultured $1x10^5$ PBMC in 96-well round-bottom microtiter plates (Costar, Cambride, MA), in 200 μl complete RPMI medium with 20 U/ml interleukin-2 (recombinant human IL-2 (rIL-2), Biogen, Geneva, Switzerland) for 7 days, and 20 U/ml rIL-2 was added to the culture medium for the last 3 days. T cell lines were established from lesional skin by culturing skin biopsies, cut in small fragments, in 48-well culture plates (Costar) in complete RPMI supplemented with 20 U/ml rIL-2 and $1x10^6$ autologous feeder cells (4,000 rads γ-irradiated autologous PBMC) for 7 days. 20 U/ml rIL-2 was added to the cultures for the last 3 days.

All T cell lines were expanded by weekly cycles alternating addition of 0.2 μg/ml phytohemagglutinin (PHA-P, Difco, Detroit, MI), 20 U/ml rIL-2 and $1x10^6$/ml irradiated PBMC as feeder cells (4,000 rads γ-irradiated allogeneic PBMC, from unrelated healthy donors) during 4 days (day 1 to day 4), and addition of 20 U/ml rIL-2 alone during the

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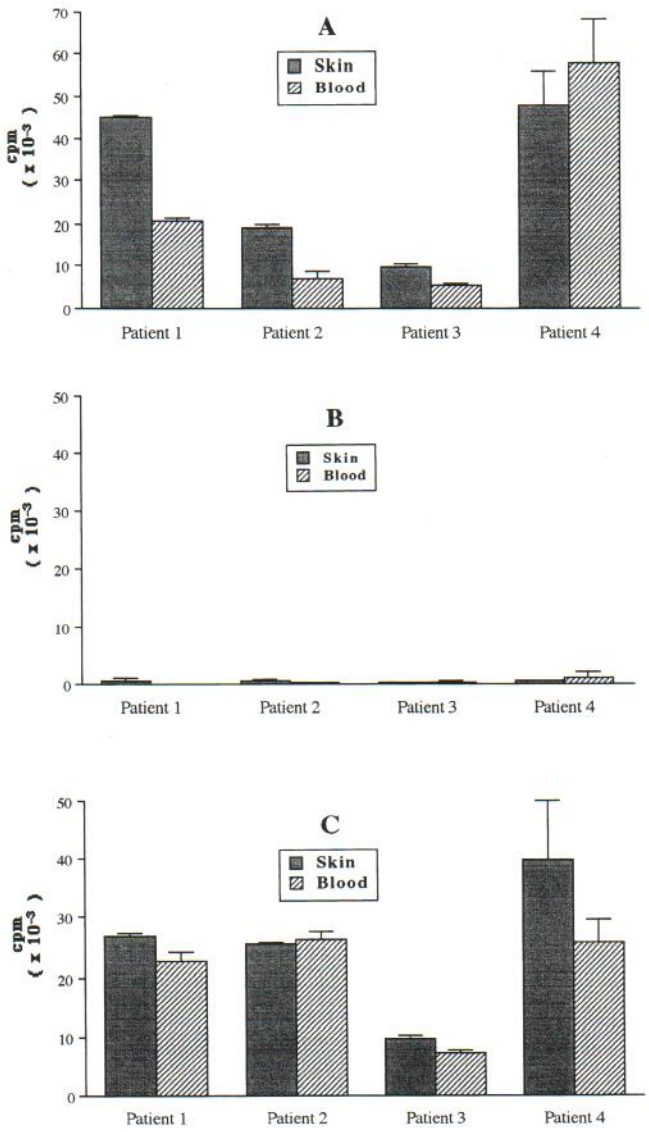

Fig. 1. Comparative response to different stimuli of T cell lines derived from peripheral blood or lesional skin of 4 psoriatic patients. $1x10^4$ T cells were incubated with $1x10^5$ allogeneic 4,000-rads irradiated antigen-presenting cells in the presence of 100 ng/ml SEB (A), with antigen-presenting cells alone (B), or with antigen-presenting cells, 0.2 μg/ml PHA and 20 U/ml rIL-2 (C).

last 3 days (day 4 to day 7). The T cell lines were incubated at 37°C in a humidified atmosphere with 5% CO_2.

Proliferation assays

For SEB-specific proliferation assay of the T cell lines, $1x10^4$ T lymphocytes were cultured in the presence of $1x10^5$ allogeneic PBMC used as APC (4,000 rads γ-irradiated allogeneic PBMC, from unrelated healthy donors) and 100 ng/ml SEB (Sigma, Saint-Louis, MO). Negative controls included responder T cells incubated alone or incubated with APC in the absence of SEB. Positive controls included T cells cultured with APC in the presence of 0.2 μg/m1 PHA-P and 20 U/ml rIL-2. Triplicate cultures were maintained in 200 μl complete RPMI medium in 96-well round-bottom microtiter plates (Costar) for 6 days at 37°C in a humidified atmosphere with 5% CO_2. One μCi of ^{3}H-thymidine (Amersham, Arlington Heights, IL) was added to each well for the last 16 h. Cells were then harvested on glass fiber strips, and ^{3}H-thymidine incorporation was measured by using a liquid scintillation counter (Packard Instrument Co, Downers Grove, IL). Results from triplicate wells were expressed as mean cpm ± standard deviation (SD).

RESULTS

Phenotypic analysis of skin and blood T cell lines

T cell lines were established by IL-2 stimulation of T lymphocytes recovered from PBMC of normal individuals, and from PBMC and lesional skin of patients with psoriasis and ACD. FACS analysis demonstrated that all the T cell lines comprised a majority of TcR $\alpha\beta$+ T cells with variable percentages of CD4+ and CD8+ T cells.

SEB-specific response of T cell lines from normal individuals and psoriatic patients

The response of the T cell lines to the staphylococcal SAg SEB was tested in a proliferation assay in the presence of allogeneic PBMC as antigen-presenting cells. All T cell lines proliferated to SEB (Fig. IA) but did not respond to allogeneic PBMC alone (Fig. IB). Comparison of SEB-specific responses of skin T cells, as compared to blood T cells, showed that the magnitude of the T cell response, for the T cell lines of patients 1 to 3, was significantly higher (2.5-fold) for skin T cells than for blood T cells from the same patient.

The proliferative responses to SEB of blood T cell lines derived from the 4 psoriatic patients were compared to 5 blood T cell lines from normal individuals (Table I). No significant difference between SEB-induced stimulation of blood T cell lines was observed between normal and psoriatic patients. Fur-

Table I. *SEB-induced proliferation of T cell lines derived from normal or psoriatic blood*

T cell lines were established by culturing PBMC of psoriatic or control individuals with 20 U/ml rIL-2. $1x10^4$ T lymphocytes from T cell lines were incubated with $1x10^5$ APC (allogeneic 4,000-rads irradiated PBMC) alone, or in the presence of 100 ng/ml SEB or of 0.2 μg/ml PHA and 20 U/ml rIL-2. Culture was performed during 6 days at 37°C, the last 16 h with 1 μCi ^{3}H-thymidine. Results are expressed as mean cpm±SD (x 10^{-3}).

APC: antigen-presenting cells; SEB: staphylococcal enterotoxin B; PHa: phytohemagglutinin.

Exp[1]	Patients	T + APC	T + APC + SEB	T + APC + PHA-IL-2
1	Patient 1	0,10±0,01	20,58± 0,69	22,74±1,60
	Control 1	0,16±0,02	26,56± 0,55	19,52±2,62
2	Patient 4	0,32±0,10	20,54± 0,72	46,15±7, 73
	Control 2	0,31±0,13	15,56± 1,55	10,80±1,65
3	Patient 1	4,23±0,43	42,46± 3,14	52,12±4,62
	Patient 3	0,15±0,01	22,00± 1,74	10,88±1,34
	Patient 4	1,06±0,92	57,88±10,26	25,75±3,67
	Control 3	12,13±1,26	83,87±13,76	75,54±4,02
4	Patient 2	0,05±0,01	6,82± 2,89	6,90±0,78
	Patient 4	0,20±0,06	8,75± 0,64	59,65±4,18
	Control 4	4,27±1,80	11,00± 2,31	38,07±1,23
5	Patient 4	0,44±0,09	4,48± 0,22	14,37±1,23
	Control 5	0,30±0,11	18,84± 1,36	9,91±0,47

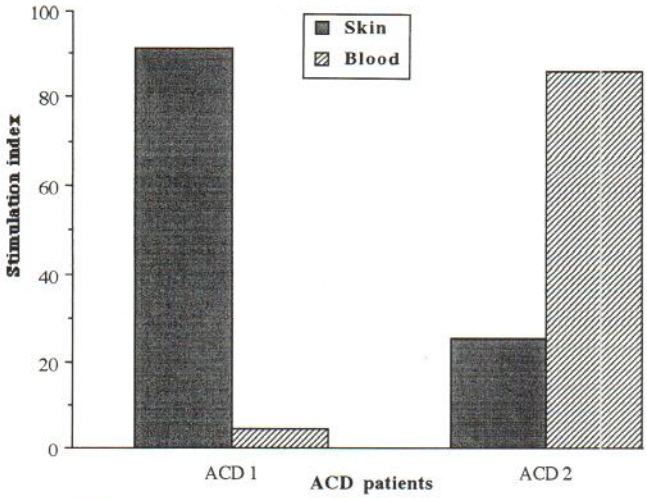

Fig. 2. SEB-induced proliferation of T cell lines derived from lesional skin or peripheral blood of 2 patients with allergic contact dermatitis. $1x10^4$ T cells were incubated with $1x10^5$ allogeneic antigen-presenting cells alone or in the presence of 100 ng/ml SEB.

thermore, blood and skin-derived T cell lines presented similar proliferative capacities to PHA (Fig. 1C). These data indicated that differences observed in SEB-induced proliferation between skin and blood could reflect an enrichment of SEB-specific T lymphocytes in psoriatic lesional skin.

SEB-specific response of T cell lines from patients with ACD

To compare results obtained in psoriasis to another chronic inflammatory skin disease, we performed similar experiments with T cell lines derived from lesional skin and blood of 2 patients with ACD (Fig. 2). In one out of these 2 ACD patients, skin T cell lines exhibited an enhanced SEB-specific proliferative response, as compared to blood-derived T cell lines.

DISCUSSION

The hypothesis of a role of SEB in psoriasis is supported by several findings. Psoriatic inflammatory skin is often superinfected by *Staphylococcus aureus*, which may produce several toxins, e.g. SEB. One of the features of psoriatic skin inflammation is the ectopic expression of MHC class-II molecules by keratinocytes (14). In this respect, SEB can be presented to T cells bearing certain Vβ subfamilies, namely Vβ3, Vβ12, Vβ14, Vβ15, Vβ17 and Vβ20, by both MHC class-II expressing Langerhans' cells and MHC class-II positive keratinocytes (15–17). In active psoriasis, T cells expressing SEB-reactive T cell receptor Vβ families infiltrate the skin and are thought to play a physiopathologic role (13). T cells infiltrating psoriatic skin produce IL-2 and IFN-γ and thus correspond to the Th1 subset (18), which is the $CD4^+$ T cell subset known to be activated by SEB (19). Finally, anti-CD4 monoclonal antibodies (mAbs) have been shown to down-regulate both T cell inflammation in psoriasis (6, 20) and SEB-induced T cell activation in vitro. Alternatively, SAgs derived from *Candida albicans* and from *Streptococcus* have been postulated to be associated with psoriatic inflammation. Recently, Leung et al. (13), using TcR Vβ specific mAbs, reported an enrichment of T cells bearing Vβ5.1 and Vβ12 or Vβ5.1 and Vβ8.1 subfamilies in lesional skin from 2 patients (13). Since SAgs derived from *Candida albicans* can bind to Vβ5, it was concluded that the observed bias in the TcR repertoire of the patients with psoriasis could indicate the effect on T cells of SAgs produced on lesional skin superinfected by *Candida albicans*. Lewis et al. (21) reported an increase, in the lesional skin of patients with guttate and chronic plaque psoriasis, of T cells expressing T cell receptor Vβ2 and Vβ5.1, which bind some streptococcal SAgs (21).

Thus, the potential involvement of SAgs in the pathogenesis of psoriasis implies an enrichment for SAg-specific T cells in lesional skin. In the present study, we report that T cell lines from the lesional skin of 3 out of 4 psoriatic patients exhibit a significantly higher proliferative response to the SAg SEB than peripheral blood T cell lines derived simultaneously from the same patients. This suggests an enrichment in psoriatic lesional skin of T cells expressing T cell receptor Vβ families known to bind SEB. However, the finding that similar SEB-enriched T cells can be occasionally found in the skin of patients with ACD, an inflammatory skin disease secondary to hapten-specific T cell activation, argues against the hypothesis that SEB is the causative agent responsible for psoriatic inflammation. Such an observation is not contradictory to the hypothesis of a role of SAgs in the pathogenesis of chronic inflammatory skin disorders. Indeed, it may be postulated that SAg-T cell responsiveness is consecutive to skin T cell activation following bacterial superinfection of pre-existing inflammatory skin lesions.

Taken together, our results and the results of previous studies suggest that molecules derived from microorganisms and endowed with SAg properties may contribute to the cutaneous inflammation in psoriasis. These molecules may be released by bacteria colonizing the skin, and bound to MHC class II positive Langerhans' cells (22), which can migrate to the dermis and activate SAg-specific T cells. MHC class II positive keratinocytes can also present SAgs to T cells (16) and could thus be involved in the persistent activation of T cells in lesional psoriatic skin.

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