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Stimulation of T cells by autologous mononuclear leukocytes and epidermal cells in psoriasis

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Summary. Based on reports suggesting aberrant cell-mediated immunity and altered infiltration of immunocompetent cells into the skin in psoriasis, we studied the stimulation of T cells by autologous non-T mononuclear leukocytes (autologous mixed lymphocyte reaction, AMLR) and by epidermal cells isolated from lesional and clinically uninvolved skin in psoriasis (autologous mixed epidermal cell lymphocyte reaction, AMECLR). Age- and sex-matched individuals served as controls. We found that the AMLR in psoriasis ($n = 11$) was similar to that in healthy controls ($n = 16$); furthermore, cell proliferation was alike in the presence of either 5% AB-serum or autologous serum. By contrast, while the AMECLR in healthy controls ($n = 9$) resembled that in psoriatics employing epidermal cells from univolved skin, epidermal cells from lesional sites ($n = 10$) induced a significantly higher proliferation of autologous T cells in the AMECLR ($P < 0.01$). We conclude that the in vitro stimulation of T cells by non-T mononuclear leukocytes is normal in psoriasis and is not regulated by autologous serum. Lesional psoriatic epidermal cells, however, are more active in stimulating autologous T cell proliferation than cells from univolved psoriatic or normal epidermis.

Key words: Autologous T cells – Epidermis cells – Proliferation – Psoriasis

Introduction

Several reports have indicated abnormal lymphocyte subpopulations and aberrant immune reactions in patients with psoriasis [3, 14–16, 23, 27, 28, 34]. On the other hand, normal distribution of T helper and T suppressor cells as well as unimpaired immune functions have also been found in psoriatics [2, 8, 11]. Based on these conflicting data, we employed the autologous mixed lymphocyte reaction (AMLR) to compare patients with psoriasis and healthy control individuals. The AMLR comprises the in vitro stimulation of T cells by irradiated non-T mononuclear leukocytes, resulting in a unique self-regulated system of intracellular communication [42]. As serum abnormalities have been described in psoriasis [19, 38], we measured the AMLR in the presence of both AB- and autologous serum.

Furthermore, as recent findings have exhibited the importance of T cell-epidermal cell interaction in normal skin [summarized in 25, 32], as well as in pathogenesis of psoriasis utilizing mostly phenotypic characterization of lymphoid cell infiltrates in psoriatic skin [3, 7, 20], we also set out to study epidermal cell–T cell interaction on the functional level by using the autologous mixed epidermal cell lymphocyte reaction (AMECLR) involving the in vitro stimulation of T cells by isolated epidermal cells. For this purpose, we obtained viable epidermal cells from clinically uninvolved and involved psoriatic skin. Moreover, as metabolic abnormalities have been shown in lesion-free psoriatic skin [9, 13, 17, 29, 33], we also included healthy individuals as a control for the AMECLR.

Our results disclose the ability of T cells in psoriasis to proliferate in a normal fashion upon stimulation by irradiated non-T mononuclear cells both in the presence of AB- and autologous serum. By contrast, while the AMECLR utilizing epidermal cells isolated from skin clinically not involved with psoriasis was similar to that with epidermal cells from healthy controls, we found that epidermal cells from involved skin led to a significantly higher stimulation of autologous T cells in psoriasis.

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Patients and methods

Patients

Patients with active psoriasis were studied. They were 20–40 years of age and had no other metabolic disorders. The AMLR was measured in 11 patients (five females, six males), the AMECLR in 10 patients (five females, five males). Heparinized blood (5 U/ml) was drawn before treatment was started, other than topical nonirritating lubricants or salicylic acid ointments to remove scales; thereafter epidermis was isolated.

Controls

Sixteen individuals aged 20–40 years (eight females, eight males) served as controls for the AMLR. Nine similar persons were included for the AMECLR (four females, five males). None had a personal or family history of psoriasis or metabolic disorders, none had other dermatoses, and none received any medication. All blood was drawn and all epidermal cells were isolated between 8:00 and 10:00 a.m.

Isolation of mononuclear leukocytes

Cell isolation was performed according to the standard density gradient method [6]. Minimum essential medium (MEM, Gibco) supplemented with 0.1 mg/ml penicillin/streptomycin was used. In common with previous findings [37], the number of monocytes among the mononuclear leukocytes (MNL) was in the range of 9%–21% as judged by esterase staining [40], and did not differ between patients and controls. Cells were counted in a Neubauer chamber.

Enrichment of T lymphocytes

Mononuclear leukocytes in MEM supplemented with 5% AB-serum (for the AMLR) or in 5% fetal calf serum (FCS) as reported for the interaction of T cells with epidermal cells (AMECLR) [1,5] were passed over a nylon-wool column exactly as described previously [24]. Of the cells eluting, 75%–91% could be identified as T cells by rosetting with neuraminidase-treated sheep erythrocytes or by OKT11 (Ortho) monoclonal antibody. All cell fractions enriched for T cells were free of monocytes as checked by esterase staining. Before further processing, cells were washed in cell culture medium (RPMI 1640, Gibco), supplemented with 2 m*M* L-glutamine, 0.1 mg/ml penicillin/streptomycin, 5×10^{-5} *M* mecaptoethanol (Merck) and 5% AB-serum or autologous serum as indicated. Only heat-inactivated (56°C, 30 min) sera were used.

Isolation of non-T MNL

Non-T NML were also isolated as described previously [24] by rinsing the nylon wool in MEM supplemented with 5% AB-serum in a plastic Petri dish at 37°C. Cell suspensions thus obtained consisted of 78%–89% non-T MNL, consistent with values reported in the literature [24].

Isolation of epidermal cells

Up to five, in some cases up to ten separate 7-mm-diameter vaccuum-induced blisters were raised over normal, uninvolved and involved psoriatic skin employing the method of Kiistala and Mustakallio [26]. Involved sites in psoriasis were at least 5 cm away from clinically uninvolved psoriatic skin. Each blister top, consisting of pure epidermis [26], was removed with scissors and forceps and washed in phosphate buffered saline at pH 7.2. The epidermis was then floated on 0.5% trypsin (Serva, Heidelberg, FRG, 35 U/mg) for 30 min at 37°C. It was then transferred into tissue culture medium containing 20% FCS to stop the trypsin action, and epidermal cell suspensions were prepared by teasing and pipetting the epidermis gently. Epidermal cell suspensions were pipetted and washed three times with tissue culture medium, the final wash being with 5% AB-serum. By this method we obtained $2-6 \times 10^6$ viable cells. The percentage of DR-positive cells in this suspension was 4%–7% as determined by immunofluorescence using OKIa1 (Ortho) monoclonal antibody.

Viability testing

Cell viablility, gauged by exclusion of 0.4% tryptan blue (Merck, Darmstadt, FRG), was found to be in excess of 98% in leukocyte suspensions and in the range 85%–95% in epidermal cell suspensions.

Autologous mixed lymphocyte reaction

In order to explicit a proliferative response of T cells in the AMLR, non-T stimulator cells were irradiated with an X-ray dose of 3000 R. We used the cell culture period of 144 h in an incubator (37°C, 5% CO_2) in order to obtain optimum proliferation as previously shown [35]. Cultures were set up in culture medium with either 5% AB-serum or autologous serum in triplicate in 96-well round-bottom microtiter plates (Nunc) consisting of 1×10^5 irradiated stimulator cells and 2×10^5 responder cells at a final volume of 200 µl. To rule out xenogeneic protein as a source of stimulation in the AMLR as has been claimed [21], we performed the AMLR in cells exposed to homologous or autologous serum only. Four hours before harvesting, each culture was pulsed with 1 µCi ^{3}H-thymidine (specific activity 5.0 µCi/m*M*, Amersham Buchler). Cells were collected with an automated cell harvester (Skatron) onto glass fiber filters and the amount of incorporated radioactivity was determined by liquid scintillation spectroscopy. Data were expressed either as cpm (mean counts per minute of triplicate cultures with a coefficient of variance < 20%) incorporated ^{3}H-thymidine, or Δ cpm [(cpm responder + cpm stimulator cells) – cpm responder cells].

Autologous mixed epidermal cell lymphocyte reaction

The incubation mixture consisted of 1×10^5 viable epidermal cells and 2×10^5 enriched T cells in tissue culture medium (PRMI 1640) containing 5% AB-serum. As control experiments have shown an about twofold lower proliferative response in the AMECLR using irradiated (3000 R) stimulator cells, epidermal cells were not irradiated, allowing sufficient time for restoration of membrane antigens that may have been destroyed by salicylic acid and trypsin treatment. The culture period of 6 days, previously shown [36] to result in optimum proliferation, was chosen. Cell processing and data expression were as in the AMLR.

Statistical analyses

The Wilcoxon matched pairs signed rank test served to compare the results of the AMECLR in psoriasis. Comparisons of cell cultures between controls and patients with psoriasis were made using the *U*-test according to Wilcoxon, Mann, and Whitney.

Table 1. The autologous mixed lymphocyte reaction in healthy control individuals and patients with psoriasis

	n	R		R + S		(R + S) − R	
		AB-serum	Autologous	AB-serum	Autologous	AB-serum	Autologous
Healthy controls	16	2029 ± 517	1420 ± 351	8135 ± 2465	6845 ± 2114	6106 ± 2121	5424 ± 1991
Patients with psoriasis	11	2374 ± 809	2242 ± 641	20111 ± 6449	18575 ± 6428	14348 ± 5041	14129 ± 5604

Values are expressed as cpm ^{3}H-thymidine incorporation (mean ± SEM)
R, responder T cells; S, stimulator non-T mononuclear cells. S controls were all < 200 cpm. Time of incubation: 144 h. Cells were separated in 5% AB-serum only, and cultured in either 5% AB-serum or 5% autologous serum

Results

The AMLR in psoriasis

Table 1 summarizes the results of the AMLR in 11 patients with psoriasis and 16 healthy controls. Although the mean stimulation of the psoriasis group was higher in the control individuals, the values failed to reach statistical significance ($P < 0.2 > 0.1$). Furthermore, the presence of autologous serum did not lead to statistically different values either in controls or in psoriasis patients. From these results it was concluded that T cells reactivity stimulated by antigens presented on non-T leukocytes was normal; moreover, serum factors were also normal in psoriasis regarding T cell proliferation in the AMLR.

The AMECLR in psoriasis

Figure 1 compares the results of 9 healthy controls and 10 patients with psoriasis. Cells from clinically uninvolved epidermis induced a mean stimulation of autologous T cells quite similar to that of epidermal cells in healthy controls. By contrast, epidermal cells from involved epidermis brought about a markedly higher proliferative response of autologous T cells. In addition, stimulator epidermal cells from clinically involved psoriatic skin also exhibited a higher spontaneous proliferation than from uninvolved and normal skin.

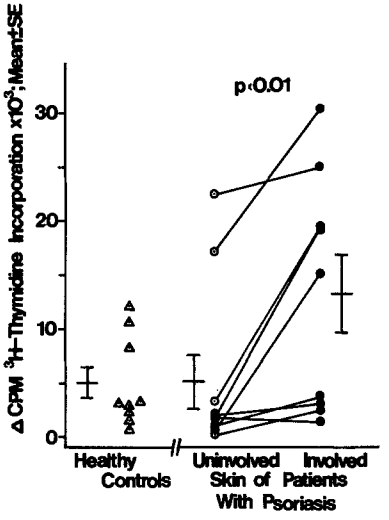

Fig. 1. Stimulation of 2×10^5 autologous enriched T cell by 1×10^5 isolated epidermal cells in 144-h cell cultures. *Δ cpm* (cpm R + cpm S) − cpm R; R, responder T cells; S, stimulator epidermal cells. Control values for healthy controls: R = 473 ± 93 cpm; S = 288 ± 42 cpm. Control values for psoriatics: R = 577 ± 64 cpm; S (from uninvolved skin) = 358 ± 67 cpm; S (from involved skin) = 472 ± 92 cpm. The difference between stimulator controls in lesional and uninvolved skin in psoriasis also reached statistical significance ($P < 0.01$)

Discussion

Our findings in the AMLR show that there is no difference between psoriatics and controls. In contrast, others reported cellular abnormalities in psoriasis suggesting a suppressor cell defect [14, 34]. Some of our patients also exhibited a higher proliferative response in the AMLR compatible with diminished suppression, but taken together, the values we obtained with the 11 psoriatics failed to reach statistical significance. Furthermore, it can also be ruled out that serum factors exert differential influences on the proliferative response in the AMLR, since the incorporation of ^{3}H-thymidine into DNA of enriched T cells was similar to that of cell cultures employing AB-serum. Glinski et al. [16] suggested that serum factors in psoriasis may coat the lymphocyte surface membrane and thus be responsible for interfering with T lymphocyte function. The techniques used by these authors were different from the ones we employed; thus, no direct comparison is possible. In particular, others used only mixed mononuclear cell suspensions, whereas we utilized defined numbers of stimulating and responding cells. Our results do not exclude the possibility that under certain circumstances in vitro (e.g., different concentrations of mitogens), psoriatic lymphocytes may respond in an altered manner. The AMLR, which we used as a self-regulated system of cooperation between stimulating non-T cells and re-

sponding helper and suppressor cells in the absence of any foreign material, was normal in psoriasis.

In contrast to MNL, autologous epidermal cells from clinically involved skin in psoriasis exerted significantly greater stimulation than those from controls. Remarkably, epidermal cells from clinically uninvolved psoriatic skin, stimulated to a similar extent as those from healthy controls. Although abnormalities in uninvolved psoriatic epidermis have been reported, including cell cycle time [17], sterol metabolism [9], and arylhydrocarbon hydroxylase [33], ornithine decarboxylase [29], and phospholipase A_2 activities [13], our results suggest that these findings are of no critical importance in T cell–epidermal cell interaction.

We isolated epidermal cells by raising suction blisters. Considering the histopathology of psoriasis, it is easily conceivable that the elongated rete ridges with thickening in their lower parts counter blister formation and may not exclude the isolation of different cell compartments. Although in most cases we obtained only two to three smaller blisters in involved sites (vs. five or more 7-mm-diameter blisters in uninvolved sites) we nevertheless succeeded in gaining sufficient numbers of viable cells, i.e., about 2×10^6, for setting up cell cultures. Applying prolonged suction resulted in bleeding. This suggests that we did isolate cells from all epidermal layers clear down to the basement membrane, and not just suprabasal or superficial cells with different stimulating properties. Ethical considerations notwithstanding, we did not perform histologic examinations after raising suction blisters; presumably we acquired epidermis mostly from the suprapapillary parts of involved skin down to the basement membrane.

Immunofluorescent studies have disclosed conflicting results in lesional vs. uninvolved psoriatic skin, indicating both reduced [7, 18] and enhanced [3] numbers of Langerhans cells, as well as a selective preponderance of T suppressor/cytotoxic cells [7, 20] or conversely T helper cells [3] in lesional psoriatic epidermis. Unfortunately, controls performed in normal skin were not reported in these studies. Although several authors report a clustering of Langerhans cells in diseased psoriatic epidermis [7, 18, 30], the cellular interaction on a functional basis cannot be fully understood by immunofluorescent studies alone. Moreover, it has been suggested that the interaction of lymphoid cells with the epidermis may be important in pathogenesis of psoriasis based on immunofluorescent criteria [3, 7, 20]. A well-known clinical correlate may be the exacerbation of psoriasis (Koebner phenomenon) at sites of positive epicutaneous skin tests for contact sensitivity in patients with active psoriasis. Based on these observations, the AMECLR should be of considerable help to define the role of epidermal cell–lymphocyte interaction in psoriasis.

The reasons for the enhanced AMECLR in lesional vs. univolved psoriatic epidermis are not fully understood: however, several possibilities emerge, including the following:

1. Epidermal cells may have functioned as feeder layers, and therefore the stimulation could be of nonimmunologic nature, particularly as stimulator controls also showed enhanced ^{3}H-thymidine incorporation. This seems unlikely, however, as direct cell–cell contact is required for a proliferative response in a mixed skin cell lymphocyte reaction [10].

2. Alternatively, epidermal cells may have produced mitogenic substances for T cells. This is more likely, as we have preliminary evidence for enhanced generation of an interleukin-1-like substance in lesional psoriatic epidermis. In addition, the proliferation was higher when stimulator cells were not irradiated.

3. Protease inhibitors have been shown to inhibit the AMECLR [36]. The increased proteolytic activity expressed in lesional psoriatic epidermis [12] may have contributed to cell proliferation.

4. Although cells were cultured in the presence of AB-serum, the isolation of epidermal cells and T lymphocytes for the AMECLR was performed in FCS, in common with other reports [1,5]. Comparing cell isolation from healthy controls in the presence of AB-serum vs. FCS, the AMECLR was lower with cells isolated in AB-serum (837 ± 235 cpm for AB-serum vs. 4894 ± 2752 cpm for FCS, mean $\pm$ SEM; $n = 4$). More important, contact with FCS alone was not sufficient to induce proliferation in the responder controls (273 ± 68 cpm for AB-serum vs. 357 ± 123 cpm for FCS; $n = 4$). Furthermore, the addition of FCS to the AMECLR resulted in an about twofold increase of cell proliferation (data not shown). Our experimental design therefore amply compares the antigen-presenting capacity of epidermal cells. This would suggest enhanced Langerhans cell density or function in involved psoriatic epidermis, supporting the immunofluorescent findings of Baker et al. [3].

5. Antigen presentation by epidermal cells requires the presence of Ia-positive cells [39]. As cells of other than macrophage or interdigitating lineage may also possess antigen-presenting properties [41], the potentiality of Ia-antigen induction on keratinocytes cannot be excluded, particularly in the light of Ia synthesis under culture conditions [4, 22] or using interferon gamma [31]. Hence, de novo synthesis of Ia antigens may possibly be involved in the AMECLR, as treatment of stimulating epidermal cells by anti-Ia

antibodies plus complement has failed to abrogate the proliferative response [5].

In conclusion, our results indicate on the functional level an enhanced stimulatory capacity of epidermal cells from lesional psoriatic skin for autologous T cells. As irritations, including contact allergy, are known to induce the Koebner phenomenon, our study lends support to the hypothesis that the activity of psoriasis may be dependent upon the stimulation of T cells by antigens presented on epidermal cells.

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