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CELL-MEDIATED AUTOHYPERSENSITIVITY IN PSORIASIS

In vitro Tests with Extracts from Psoriatic Skin and Scales

V. K. Hopsu-Havu and I. Helander

From the Departments of Dermatology and Medical Microbiology, University of Turku, Turku, Finland

Abstract. Extracts were prepared from psoriatic scales and plaque epidermis as well as from healthy epidermis of psoriatics and normal controls, and their effect on the lymphocyte transformation and migration of autologous leukocytes was tested. Lymphocyte transformation, as indicated by increased thymidine uptake, was significantly stimulated by plaque epidermis in one case of 11 psoriatics. Extracts from normal epidermis, or healthy epidermis of psoriatics, did not affect the transformation of autologous lymphocytes. The phytohemagglutinin response of lymphocytes from psoriatics was the same as that of the normal controls. The ranges of variation and mean values of the autologous leukocyte migration indices obtained in the presence of plaque extract or sediment from 21 psoriatics did not differ from those recorded in the presence of similar extracts prepared from the epidermis of 18 normal control patients. The results of this investigation do not indicate that cell-mediated hypersensitivity toward epidermal antigens plays any significant role in the ethio-pathogenesis of psoriasis.

The possible involvement of immunological mechanisms in the pathogenesis of psoriasis remains to be elucidated. Immunologic mechanisms might be activated by the appearance of abnormal antigens in psoriatic skin, by changes in the physiological barriers normally protecting epidermal antigens from immunologic attack, or by changes in the effector mechanisms which become reactive towards normal epidermal antigens. In principle, either humoral or cell-mediated mechanisms might be involved. Investigations into the role of immunologic factors in psoriasis have been reviewed by Marghescu & Braun-Falco (19) and more recently by Epstein (10). In this paper we limit our discussion to the cell-mediated immunological mechanisms.

Studies on cellular immune reactions have revealed that the psoriatic patient reacts normally in previously acquired delayed hypersensitivity. Landau et al. (16) measured the inflammatory response in skin windows, injecting intracutaneously eight different antigens representing bacteria, viruses, and protozoa (but no autoantigen) and found no difference in the reactions of the controls and psoriatics. Depaoli (9) found a delay in onset of sensitization to DNCB and Meneghini et al. (21) observed a decreased frequency of sensitization to DNCB in psoriatic patients. Skog (24), on the other hand, observed no significant decrease in frequency of sensitization to PDC (pentadecacathecol) in Sweden. Epstein & Maibach (11) found that patients with psoriasis are less prone to be sensitized by simple chemicals (DNCB, NDMA – paranitrosodimethylaniline) than healthy persons. These findings could be summarized to suggest a slight tendency to depressed sensitization to external agents in psoriasis.

Skog & Linder (25) obtained uniformly negative results in efforts to demonstrate reactions to autologous skin extracts and leukocytes in patients with psoriasis. Linder & Skog (17) found no signs of delayed-type immunologic reaction in a large number of autologous skin grafts in psoriatics, irrespective of whether the graft consisted of apparently healthy skin or of plaque skin. The psoriatic and normal autografts of Clendenning & Van Scott (8) were also considered to reveal no signs of immunologic hypersensitivity.

After measles, psoriasis is often observed to clear temporarily for long periods of time (5, 27). Infection with measles virus is also known to depress the cellular immune system (4, 6). Thus, the pathogenesis of psoriasis might be connected with increased rather than with decreased activity of the cell-mediated hypersensitivity.

The purpose of the present study was to investigate the cellular autoimmunity of psoriatic patients by

using the techniques of lymphocyte transformation (LT) and the leukocyte migration inhibition tests (MIF). Psoriatic scale, psoriatic epidermis and normal epidermis of psoriatic patients and normal control persons were used as antigen.

MATERIALS AND METHODS

Patients

Twenty-one patients with psoriasis of medium severity and 18 healthy control persons, in the age range 19 to 75 years were selected from our in-patient department. They received no systemic or topical medications during 2 weeks prior to the experiments.

Preparation of antigens

Psoriatic scales, taken by tweezers, and involved and uninvolved epidermis from psoriatics and normal controls, caught by keratotome, were collected. The tissue samples were weighed and homogenized in Tenbrock-type tissue grinders in 1 or 2 ml isotonic phosphate buffer ($Na_2HPO_4 \times 2H_2O$ 0.8 g, KH_2PO_4 0.3 g, NaCl 0.44 g, Aq.ster. 100.0). After centrifugation the supernatant was used as one antigen. The precipitate was homogenized in the original volume of phosphate buffer and was used as another antigen. In some experiments, dilutions 1:1, 1:10, 1:100 and 1:1 000 of the antigen preparation was used in phosphate buffer. Such a wide range was tested because the optimal concentration was unknown. Subsequently, antigen solutions were used undiluted (1:1). The amounts of the psoriatic epidermis used are given in the tables.

Preparation of leukocytes

Twenty to thirty ml of blood (from the cubital vein) was drawn into a heparinized syringe (200 IE/10 ml blood) and allowed to settle in upright position at 37°C for 60 min. In most cases the blood sedimentated spontaneously but in a few cases when the sedimentation rate was low, the blood was mixed with 5% dextran (Dextran 150, mol.wt. 150 000 from Pharmacia, Uppsala, Sweden) in RPMI 1640 tissue culture medium (Grand Island Biological Company, Berkeley, California, USA) in the proportion 1:20. The supernatant leukocyte-rich plasma was centrifuged at 150 *g* for 15 min and plasma was withdrawn. The remaining cell pellet was resuspended in 5 ml of RPMI 1640 containing Na-Penicillin (Leiras, Turku, Finland) 100 IE/ml and streptomycin (Hoechst, Germany) 100 μg/ml. The cells were counted and diluted to the final concentration 1×10^6 lymphocytes per ml. The final mixture contained 80% RPMI 1640, the antibiotics, and 20% of autologous plasma. Triplicate 1 ml cultures gassed with 7% CO_2 in air were set up in 17 × 100 mm Falcon sterile disposable plastic tubes (No. 2001) for lymphocyte transformation tests. For migration inhibition test the cell pellet was washed three times in Hanks balanced salt solution. The washed leukocytes were then resuspended in RPMI 1640 medium containing 10% fetal calf serum to give a final concentration of 4×10^7 leukocytes per 0.2 ml.

Lymphocyte transformation test

One-tenth ml of the antigen solution was added to lymphocyte cultures and 0.1 ml of (isotonic) phosphate buffer into the control cultures. Twenty-five μl of phytohemagglutinin-M (PHA, Difco) reconstituted with 5 ml aqua was added to the positive control cultures, but nothing to the negative cultures for PHA. Cultures containing PHA and the corresponding control cultures were incubated for 65–66 hours and those containing antigens and the corresponding control cultures for 136–138 hours. Four hours before the harvesting, 1 μCi of H^3T, specific activity 2 000 μCi/mM (New England Nuclear) was added to each culture. Cultures were terminated by adding 2 drops of cold, non-radioactive thymidine and 1 ml of cold NaCl. After centrifugation at 150 *g* for 15 min the supernatant was removed. DNA was precipitated with 5 ml of 10% TCA, washed in 10% TCA and once in absolute alcohol. The dried residue was dissolved in 5.0 ml of Hydroxyde of Hyamine (Packard) which was then transferred into 10 ml of scintillation fluid (PPO 19.6 g, PoPoP 0.4 g, toluene 3.5 g, alcohol 0.5 l) for counting.

Migration inhibition test

Migration inhibition of the leukocytes in agarose medium was studied using the method introduced by Clausen (7). Agarose medium was prepared by mixing agarose (Indubiose®, supplied by l'Industrie Biologique Française), Auto-Pow (Basal medium Auto-Pow cat. no. 1 A-010, Flow Laboratories, Rockville) and aqua dest. under heating. The solution was left to cool to ~45°C. Glutamine, fetal calf serum (Difco) and sodium bicarbonate were added in such proportions that the final solution contained 0.75% agarose, 10% fetal calf serum and 0.8% Auto-Pow basal medium. Penicillin (100 IU/ml) and streptomycin (100 μg/ml) were added and 7 ml agarose medium was poured into disposable plastic Petri dishes, diameter 9 cm (Nunc, Denmark). After a gel had formed, holes of 2 mm diameter were cut out in each agarose plate, using a stainless steel punch. Antigen was added to one-half of the leukocyte suspensions and the same amount of phosphate buffer was added to the other half, which served as control. The leukocyte suspensions were incubated with antigen at 37°C for 30 min before being placed in holes in the agarose plates. Five μl of the suspension was placed in each hole. The agarose was incubated at 37°C in 7% CO_2 in air for 18 hours. The cells were fixed on the bottom of Petri dishes by pouring formalin solution into the dishes. Agarose medium was removed and the cells were stained with 0.1% cresyl violet. The migration areas were measured by planimetry under a projection microscope. Three parallel cultures with antigen and 3 control cultures were prepared from each sample. The degree of migration was expressed as a migration index (MI), i.e. the ratio of the average areas obtained in the cultures containing antigen and the controls.

RESULTS

Lymphocyte transformation test

The results of our initial studies with a series of dilutions of antigen preparations from scales and living epidermis obtained from plaques and healthy skin areas of 5 psoriatic patients are given in Table I. It appears that the presence of none of the dilutions of any of the different antigen preparations consistently

Table I. *Effect of different amounts of healthy and plaque epidermis and psoriatic scales on H^3T-thymidine uptake by patients own lymphocytes in vitro*

The amount of tissue subjected to extraction is given in parentheses

Antigen	Antigen dilution	Patient number and counts per 10 min				
		No. 1	No. 2	No. 3	No. 4	No. 5
Normal epidermis	—	(54.0 mg)	—	(37.0 mg)	(78.9 mg)	(88.0 mg)
supernatant	1:1	74	—	55	140	35
	1:10	54	—	48	51	16
	1:100	83	—	40	68	14
	1:1 000	51	—	58	53	17
sediment	1:1	87	—	59	137	29
	1:10	93	—	66	88	58
	1:100	95	—	59	44	17
	1:1 000	87	—	70	67	31
Psoriatic epidermis	—	(144.1 mg)	(34.2 mg)	(51.4 mg)	(116.8 mg)	(91.2 mg)
supernatant	1:1	295	216	47	93	72
	1:10	83	89	86	86	30
	1:100	58	89	71	80	20
	1:1 000	48	78	119	159	41
sediment	1:1	93	652	94	130	34
	1:10	55	555	124	73	52
	1:100	57	74	85	107	36
	1:1 000	57	52	62	90	63
Psoriatic scale	—	(76.7 mg)	(25.2 mg)	—	—	—
supernatant	1:1	55	58	—	—	—
	1:10	96	70	—	—	—
	1:100	58	82	—	—	—
	1:1 000	44	87	—	—	—
sediment	1:1	37	485	—	—	—
	1:10	40	225	—	—	—
	1:100	40	324	—	—	—
	1:1 000	45	137	—	—	—
Control (phosph. buf.)		86	76	127	108	31
Phytohemagglutinin		31 791	93 397	74 161	54 807	—
Control (3 days)		49	116	59	193	30

stimulated the thymidine uptake. Only in one preparation (No. 2), taken from a patient suffering from a widespread psoriasis of several years duration, were the counts recorded in the presence of antigen (sediment) more than twice that of the control level. Further data from studies with 6 psoriatic patients using a single antigen concentration, shown in Table II, revealed no stimulation of lymphocyte transformation. Supernatant and sediment preparations from epidermis of normal healthy individuals did not affect transformation of autologous lymphocytes in the 10 subjects tested (data not given in the tables).

Furthermore, the PHA response of the lymphocytes from normal individuals did not differ from that obtained with the cells from psoriatics. Control cultures revealed an uniformly low uptake of thymidine by the lymphocytes from normal subjects as well as in those from psoriatics.

Migration inhibition test

The data on the effect of the sediment and supernatant antigen preparations of normal control skin and of psoriatic plaque epidermis on leukocyte migration are given in Fig. 1. It appears that the scatter in all groups is similar and there is no significant difference in the mean. The amount of epidermis in the assay varied from 20 to 70 mg and no correlation was revealed when the amount of epidermis was plotted against the migration index. Neither was any correlation observed when the migration index was plotted against the age of the patients (varying between 19 and 75 years) or against the duration of psoriasis (varying between 0.5 and 32 years).

DISCUSSION

Both lymphocyte transformation and the production of leukocyte migration inhibitory factors have been

Table II. *Effect of healthy skin and plaque (3–5 mg/culture) of six psoriatic patients on H^3T-thymidine uptake by patients own lymphocytes in vitro*

Antigen	Patient no.					
	1	2	3	4	5	6
Psoriatic epidermis						
supernatant	161	112	125	237	316	27
sediment	253	236	147	192	401	34
Control (phosph. buf.)	197	73	451	271	324	47
PHA	46 827	97 936	56 934	33 554	62 773	58 561
Control (3 days)	197	136	55	258	227	383

found to be sensitive though independent detectors of the cell-mediated immunologic reaction. Therefore, a delayed hypersensitivity reaction, if present in psoriasis, should have been detected by the techniques used. The validity of our test methods is not likely to be questioned since both the lymphocyte transformation and migration inhibition tests of ours have given very reliable results in other studies carried out simultaneously with other antigens (to be reported separately). In addition, the controls and the response of PHA in the transformation test behaved as expected.

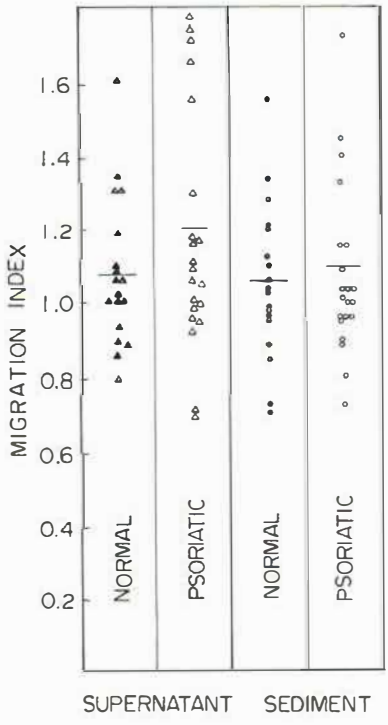

Fig. 1. Effect of the homogenate supernatant and sediment from epidermis of normal control persons and from plaques of psoriatics on the migration of autologous leukocytes.

An unsuitable antigen concentration or false preparation of the antigens could lead to false-negative results. The range of antigen concentration was, however, 1 000-fold and should cover the responsive area. Antigen was prepared from both psoriatic scale and live epidermis of the psoriatic person in order to detect the antigens, wherever they might be located. Furthermore, both the extract prepared in dilute buffer as well as the sediment left after centrifugation were tested in order to check both the water-soluble and insoluble antigens. This was necessary since both soluble epidermal antigens (1, 2, 3, 12, 13, 20) and corneal insoluble antigens (15) have been described.

Psoriasis-specific epidermal antigens inducing sensitization in psoriasis have been investigated by several researchers. Long & Edmonds (18) could find no difference in the rabbit's antigenic response, whether normal or psoriatic skin was injected. Forsey et al. (14) demonstrated in psoriatic scale one soluble antigen not apparent in normal epidermis. Sellei et al. (23), on the other hand, found in immunoelectrophoresis two distinct precipitation lines characteristic of psoriatic epidermis. Moresi et al. (22) and Sönnichsen et al. (26), investigating psoriatic epidermis, could find no antigenic factors specific for psoriasis. Our findings suggest that there exists no cell-mediated hypersensitivity towards psoriasis-specific antigens in psoriasis. This could be due to the lack of specific antigens in psoriatic epidermis or to the fact that they are protected from the immunologic machinery.

Our findings, with both lymphocyte transformation and migration inhibition tests, thus give no indication that consistently detectable delayed-type sensitization, either towards the antigens present in

the psoriatic plaque or in healthy skin areas, had occurred in the psoriatic patients. This would suggest that the cell-mediated hypersensitivity towards epidermal antigens does not play any essential role in the etiopathogenesis of psoriasis. If such a reactivity occurs it may rather be a consequence than a cause of the psoriatic manifestation.

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V. K. Hopsu-Havu, M.D.
Department of Dermatology
University of Turku
SF-20520 Turku 52
Finland