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INVESTIGATIVE REPORT

Topical Application of Imiquimod Induces Alterations in Peripheral Blood Lymphocytes in Healthy Individuals*

Efi PASMATZI[1], Antigoni CHAIDAROGLOU[2], Theophilos SAKKIS[1], Alexandra MONASTIRLI[1], Sophia GEORGIOU[1], Alexandros SAGRIOTIS[1], George BADAVANIS[1], Eleftheria VRYZAKI[1], Panagiota MATSOUKA[3], Dimitrios DEGIANNIS[2] and Dionysios TSAMBAOS[1]

Departments of [1]Dermatology and [3]Haematology, School of Medicine, University of Patras, and [2]Molecular Immunopathology Laboratory, Onassis, Cardiac Surgery Center, Athens, Greece

The aim of this study was to determine whether imiquimod, a Toll-like receptor-7/8 agonist, in addition to its well-known topical action on the cutaneous immune response, might also induce alterations in the peripheral blood lymphocytes. A 62.5 mg quantity of imiquimod (5% cream) was applied topically under occlusion once daily every second day for 3 weeks to the skin of 10 healthy volunteers, age range 30–57 years. Ten sex- and age-matched healthy controls applied corresponding quantities of the vehicle under occlusion. Before, and one and 3 weeks after the start of treatment, peripheral blood lymphocyte subpopulations were measured by flow cytometry. Statistically significant alterations in the percentage or absolute numbers of peripheral blood lymphocyte subpopulations were found in the imiquimod-treated group compared with the control group. These alterations indicate for the first time that topical application of imiquimod induces alterations in peripheral blood lymphocyte subsets in healthy individuals, which may be of importance in the immunotherapy of neoplastic and infectious disorders and should be taken into careful consideration in patients who are treated with imiquimod. ***Key words: imiquimod; immunophenotyping; lymphocyte subsets; flow cytometry; immunomodulation.***

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Efi Pasmatzi, Department of Dermatology, School of Medicine, University of Patras, PO Box 1413, GR-26504 Rio-Patras, Greece. E-mail: pasmatzi@med.upatras.gr

Imidazoquinolines represent a recently developed class of synthetic low molecular weight immune response modifiers shown to be Toll-like receptor (TLR) agonists, which have been shown to have distinct anti-viral and anti-neoplastic effects in various animal models (1). Imiquimod [1-(2-methylpropyl)-1*H*-imidazo(4,5-*c*) quinolin-4-amine], is a topically applicable TLR-7/8 agonist capable of stimulating the cutaneous innate immunity and the cellular arm of the adaptive immune response and of exerting potent anti-viral, anti-tumour and immunoregulatory effects (1, 2). Clinical trials have clearly shown that this compound is an efficient topical agent for the immunotherapy of some types of cutaneous infections, neoplasms and autoimmune disorders, most of which previously represented frustrating therapeutic problems (3–6).

The biological effects of imiquimod at the site of its application are predominantly due to the activation of nuclear factor-kappa B (NF-κB) subsequent to stimulation of TLR7/8-mediated signalling pathways, leading to enhanced transcription and local release of pro-inflammatory cytokines, chemokines and other mediators (7). Antigen-presenting cells, including epidermal Langerhans' cells (LCs), respond to imiquimod with a rapid increase in the production of interferon-alpha (INF-α) and other pro-inflammatory cytokines; in addition epidermal LCs show a functional maturation and an increase in their migration to the regional lymph nodes, where they induce a distinct T-helper type 1 (Th1) immune response (7–10). The pro-inflammatory activity of imiquimod may be augmented through interference with adenosine receptor signalling pathways and TLR-independent reduction of adenyl cyclase activity, whereas the pro-apoptotic activity of this compound may be related to TLR-dependent regulation of Bcl-2 family proteins (7). The aim of this study was to test the hypothesis that topically applied imiquimod, apart from its well-known local action on the cutaneous immune response, may be capable of inducing alterations in the peripheral blood lymphocytes.

MATERIALS AND METHODS

A 62.5 mg quantity of imiquimod (Aldara; 5% cream, Lavipharm S.A., Athens, Greece) was applied topically under occlusion once daily every second day for 3 weeks to an apparently lesion-free abdominal skin area (10 × 10 cm) of 10 healthy male volunteers, age range 30–57 years. Abdominal skin was chosen as the site of drug application because it fulfils the following criteria: (*i*) no exposure to sunlight and other environmental factors that might interfere with the immunomodulating effects of imiquimod; (*ii*) easier self-application by the study participants; and (*iii*) stable occlusion.

*This paper is dedicated to the memory of Dr K. Koniavitou.

Ten sex- and age-matched healthy individuals who applied corresponding quantities of the vehicle topically under occlusion served as controls. All subjects in the two groups had no evidence or history of dermatological, hepatic, renal, neurological, endocrine, gastrointestinal, cardiovascular, haematological, infectious, neoplastic or immunological disorders, had not undergone radiological examination and had not received any topical or systemic medication during a period of 6 months prior to their enrolment. Subjects with alcohol and/or drug dependency and those who had been systematically exposed to sunlight or ultraviolet (UV) light sources in the last 3 months were excluded. All individuals gave their informed consent subsequent to a detailed description of the possible side-effects of imiquimod and of the purposes of the study, the protocol of which had been approved by the local ethics committee. The drug under investigation and the vehicle (supplied by Lavipharm SA, Athens, Greece) were applied at bedtime and left in place for 8 h. The subjects in both groups were advised to avoid any exposure to sunlight or UV light during the 3-week treatment period.

Venous blood samples, obtained from all subjects of both groups before treatment, and one and 3 weeks after the onset of treatment were processed for flow cytometry (Becton Dickinson Facscan, San Jose, California, USA) and stained for CD3, CD20, CD4, CD8, CD5, CD56, CD69, HLA-DR, CD45RO and CD45RA cell surface marker expression with anti-CD3 (conjugated with fluorescein isothiocyanate), anti-CD20 (conjugated with fluorescein isothiocyanate), anti-CD4 (conjugated with fluorescein isothiocyanate), anti-CD8 (conjugated with fluorescein isothiocyanate), anti-CD5 (conjugated with phycoerythrin), anti-CD56 (conjugated with phycoerythrin), anti-CD69 (conjugated with phycoerythrin), anti-HLA-DR (conjugated with phycoerythrin), anti-CD45RO (conjugated with phycoerythrin) and anti-CD45RA (conjugated with phycoerythrin) monoclonal antibodies, respectively (Becton Dickinson Biosciences, San Jose, CA, USA). Staining was performed by incubating 100 µl of whole blood with the relevant monoclonal antibodies or an isotype monoclonal control antibody (to test for non-specific antibody binding) for 30 min at 4ºC. Stained cell suspension was washed with CellWash (Becton Dickinson Biosciences) and subsequently suspended in 2.0 ml BD FACS Lysing Solution (Becton Dickinson Biosciences), vortexed, incubated at room temperature for 10 min, washed again and finally suspended in 500 µl CellWash buffer. Lymphocytes were distinguished from other leukocyte populations using a combination of CD45/CD14 staining (anti-CD45 conjugated with phycoerythrin; anti-CD14 conjugated with fluorescein isothiocyanate; Becton Dickinson Biosciences) and forward and orthogonal light scatter. Lymphocyte gates were set in regions where resting and activated cells are normally found (Fig. 1). Thus, in this contest and in the present study in the region of resting cells, lymphocytes with small size and low granularity (SSLG) were found, whereas in the region of activated cells, lymphocytes with large size and high granularity (LSHG) were observed. This approach has been implemented in clinical and experimental protocols for differentiation of lymphocyte subpopulations (11, 12). Statistical analysis was performed using the analysis of covariance (ANCOVA) model with SAS® software (SAS Institute, Cary, NC, USA). The level of significance was fixed at $\alpha = 5\%$. A p-value of 0.05 or less was considered statistically significant.

RESULTS

Topical application of the drug or the vehicle was well tolerated by all subjects in the two groups. No application site or systemic adverse events were observed in the subjects treated either with the drug or the vehicle and none of them discontinued the treatment. The results of flow-cytometry of peripheral lymphocyte subpopulations are summarized in Tables I and II.

After one week of topical treatment, statistically significant differences were found between the imiquimod- and the vehicle-treated individuals with regard to the membrane markers of their LSHG peripheral lymphocytes (Table I). Thus, there was a statistically significant increase in the percentage of helper/inducer T lymphocytes (CD4+) ($p = 0.0036$), naive T lymphocytes (CD45RA+) ($p = 0.0064$), activated suppressor/cytotoxic T lymphocytes (CD8+/HLA-DR+) ($p = 0.0249$) and naive helper/inducer T lymphocytes (CD4+/CD45RA+) ($p = 0.0214$) of the imiquimod-treated individuals, compared with the controls.

After 3 weeks of topical treatment statistically significant differences were found between the imiquimod- and the vehicle-treated individuals with regard to the membrane markers of their LSHG peripheral lymphocytes (Table I). Thus, a statistically significant increase in the percentage of helper/inducer T lymphocytes (CD4+) ($p < 0.0001$), activated T lymphocytes (CD69+) ($p = 0.0016$), memory T lymphocytes (CD45RO+) ($p = 0.0358$), memory suppressor/cytotoxic T lympho-

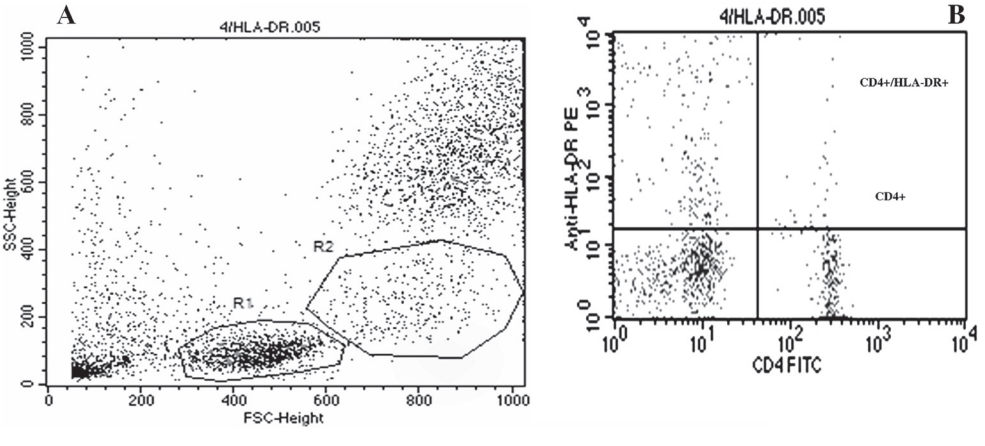

Fig. 1. (A) Forward Scatter/Side Scatter (FSC/SSC) dot blot of peripheral blood cells from healthy individuals labelled with anti-CD4-FITC/anti-HLA-DR monoclonal antibodies. Gate R1 defines the area where "resting" lymphocytes (lymphocytes with small size and low granularity) are located. Gate R2 defines the area where lymphocytes with large size and high granularity are located. (B) Staining of cells in R2 with the two monoclonal antibodies used. The upper right quadrant shows the population of CD4 lymphocytes bearing the HLA-DR activation marker.

Table I. *Membrane markers of lymphocytes with large size and high granularity (LSHG). Results are expressed as means ± SD and least square means (LSMs) of lymphocyte percentage after one and 3 weeks of treatment with imiquimod and the vehicle.*

Membrane markers	Imiquimod Mean ± SD (LSMs)	Vehicle Mean ± SD (LSMs)	*p*-value
HLA-DR			
Baseline	68.5 ± 10.3	65.6 ± 6.8	
Week 1	66.2 ± 7.2 (65.6)	68.1 ± 4.4 (68.7)	0.2072
Week 3	67.5 ± 8.2 (66.5)	63.1 ± 8.6 (64.1)	0.4037
CD4			
Baseline	11.0 ± 4.2	11.2 ± 4.0	
Week 1	13.8 ± 7.2 (13.9)	8.5 ± 2.8 (8.4)	0.0036
Week 3	26.6 ± 6.6 (26.7)	8.2 ± 2.6 (8.1)	<0.0001
CD8			
Baseline	10.5 ± 3.8	8.6 ± 1.0	
Week 1	10.9 ± 4.4 (10.1)	7.9 ± 1.6 (8.7)	0.2298
Week 3	10.2 ± 3.1 (9.6)	9.1 ± 2.3 (9.7)	0.8867
CD69			
Baseline	21.7 ± 7.1	42.1 ± 5.3	
Week 1	17.2 ± 6.8 (26.7)	40.8 ± 9.8 (31.3)	0.4202
Week 3	15.4 ± 6.7 (19.9)	51.3 ± 9.7 (46.8)	0.0016
CD45RO			
Baseline	69.5 ± 4.7	60.6 ± 5.0	
Week 1	69.7 ± 6.7 (67.1)	65.9 ± 7.1 (68.5)	0.7448
Week 3	76.6 ± 5.9 (74.7)	64.7 ± 5.9 (66.6)	0.0358
CD45RA			
Baseline	46.3 ± 5.3	43.3 ± 4.8	
Week 1	52.8 ± 8.1 (51.7)	42.2 ± 4.7 (43.2)	0.0064
Week 3	51.2 ± 7.8 (49.9)	44.9 ± 4.0 (46.2)	0.0975
CD8/HLA-DR			
Baseline	3.2 ± 1.5	3.1 ± 1.5	
Week 1	3.6 ± 1.5 (3.6)	2.2 ± 1.0 (2.2)	0.0249
Week 3	3.4 ± 1.9 (3.3)	1.9 ± 0.9 (1.9)	0.0509
CD8/CD45RO			
Baseline	5.4 ± 2.9	3.3 ± 1.2	
Week 1	7.4 ± 5.6 (6.9)	2.2 ± 0.9 (2.7)	0.0542
Week 3	6.2 ± 1.7 (5.8)	2.9 ± 1.6 (3.2)	0.0042
CD8/CD45RA			
Baseline	11.0 ± 3.4	12.2 ± 3.0	
Week 1	12.5 ± 4.3 (12.8)	11.3 ± 2.0 (11.0)	0.2180
Week 3	9.6 ± 4.4 (9.9)	12.5 ± 3.6 (12.2)	0.1941
CD4/HLA-DR			
Baseline	4.2 ± 2.5	4.7 ± 2.0	
Week 1	13.8 ± 13.9 (13.7)	4.0 ± 1.7 (4.1)	0.0524
Week 3	37.5 ± 14.5 (37.9)	3.3 ± 1.7 (2.9)	<0.0001
CD4/CD69			
Baseline	3.5 ± 2.0	4.8 ± 1.7	
Week 1	2.5 ± 2.3 (2.7)	3.6 ± 1.6 (3.4)	0.4692
Week 3	2.7 ± 1.7 (2.8)	3.6 ± 1.3 (3.5)	0.3677
CD8/CD69			
Baseline	3.6 ± 2.5	3.5 ± 1.1	
Week 1	2.8 ± 1.4 (2.8)	3.5 ± 1.6 (3.5)	0.3272
Week 3	2.2 ± 1.6 (2.2)	4.1 ± 2.9 (4.1)	0.0667
CD4/CD45RA			
Baseline	11.8 ± 5.7	11.2 ± 4.5	
Week 1	16.5 ± 10.7 (16.3)	7.9 ± 2.5 (8.0)	0.0214
Week 3	22.4 ± 5.8 (22.2)	8.8 ± 4.6 (9.0)	<0.0001
CD4/CD45RO			
Baseline	4.0 ± 1.2	6.5 ± 2.6	
Week 1	5.9 ± 3.4 (6.6)	4.5 ± 2.2 (3.8)	0.0685
Week 3	15.1 ± 6.5 (15.6)	4.6 ± 1.7 (4.1)	0.0004

cytes (CD8+/CD45RO+) ($p = 0.0042$), activated helper/inducer T lymphocytes (CD4+/HLA-DR+) ($p < 0.0001$), naive helper/inducer T lymphocytes (CD4+/CD45RA+) ($p < 0.0001$) and memory helper/inducer T lymphocytes (CD4+/CD45RO+) ($p = 0.0004$), was found in the imiquimod group after 3 weeks of treatment, compared with the control group.

In the SSLG lymphocytes after one week of topical treatment there was a statistically significant decrease in the absolute numbers of activated T lymphocytes (CD69+) ($p = 0.0056$), natural killer cells (CD56+) ($p = 0.0212$) and memory T lymphocytes (CD45RO+) ($p = 0.0216$) of the imiquimod-treated individuals, compared with the vehicle-treated controls (Table II). In the SSLG lymphocytes after 3 weeks of topical treatment there was a statistically significant increase in the absolute number of activated helper/inducer T lymphocytes (CD4+/HLA-DR+) ($p = 0.0016$) of the imiquimod-treated individuals, compared with the controls (Table II).

DISCUSSION

Prior to the development and clinical use of its topical form, imiquimod has been orally administered to patients with HIV infection and cancer. A systemic immune activation in terms of elevation of serum levels of INF-α, beta$_2$-microglobulin, neopterin, and of peripheral blood mononuclear cell 2-5A synthetase was observed in the treated immunocompromised patients, but it was mostly associated with dose-limiting imiquimod toxicity (13–15). In guinea pigs with experimental genital herpes simplex virus (HSV) infection, monotherapy with topical imiquimod was found to exhibit a potent anti-HSV activity and to suppress post-therapy recurrences apparently due to the significant augmentation of systemic immune response caused by this compound in the treated animals (16, 17). Moreover, in other experimental studies it was found that adjunctive topical administration of imiquimod enhanced the systemic immunity attained by local cryosurgery destruction of cutaneous melanoma lesions in mice (18).

Recently, exacerbation of autoimmune or inflammatory disorders was reportedly observed in patients topically treated with imiquimod. Topical application of this compound to the warts of an 18-year-old woman with a well-controlled HLA B27 spondyloarthropathy led to a severe flare of her arthritis requiring more than 3 months to improve, despite marked immunosuppressive therapy (19). Topical imiquimod treatment of superficial basal cell carcinoma and of actinic keratoses in a 64-year-old woman, and a 77-year-old man, respectively, both with a clinical history of psoriasis, and of a psoriatic plaque in a 58-year-old man, induced the development of widespread psoriatic lesions even

Table II. *Membrane markers of lymphocytes with small size and low granularity (SSLG). Results are expressed as means ± SD and least square means (LSMs) of the absolute numbers of lymphocytes after one and 3 weeks of treatment with imiquimod and the vehicle.*

Membrane markers		Imiquimod Mean ± SD (LSMs)	Vehicle Mean ± SD (LSMs)	*p*-value
HLA-DR	Baseline	684.4 ± 232.8	531.9 ± 139.1	
	Week 1	663.7 ± 266.5 (596.8)	556.7 ± 171.0 (623.6)	0.7208
	Week 3	757.7 ± 287.7 (696.9)	576.5 ± 176.6 (637.3)	0.5234
CD4	Baseline	1179.8 ± 483.2	890.5 ± 272.5	
	Week 1	1030.3 ± 397.4 (907.1)	971.5 ± 365.4 (1094.7)	0.0553
	Week 3	1253.9 ± 464.5 (1136.8)	928.4 ± 340.8 (1045.4)	0.4785
CD8	Baseline	760.4 ± 315.5	550.4 ± 175.7	
	Week 1	683.6 ± 266.6 (597.6)	567.4 ± 185.1 (653.4)	0.2526
	Week 3	726.3 ± 269.6 (646.9)	592.2 ± 195.1 (671.6)	0.7187
CD69	Baseline	199.3 ± 55.8	307.1 ± 124.5	
	Week 1	170.7 ± 71.9 (192.6)	333.3 ± 88.0 (311.4)	0.0056
	Week 3	223.7 ± 95.6 (233.6)	301.2 ± 97.3 (291.2)	0.2709
CD45RO	Baseline	962.1 ± 238.0	810.7 ± 185.9	
	Week 1	898.9 ± 208.6 (830.2)	893.5 ± 232.4 (962.1)	0.0216
	Week 3	1003.6 ± 171.3 (950.4)	858.3 ± 236.7 (911.5)	0.5845
CD45RA	Baseline	1735.1 ± 611.4	1186.2 ± 409.1	
	Week 1	1543.3 ± 561.0 (1305.5)	1278.6 ± 431.6 (1516.3)	0.0834
	Week 3	1793.2 ± 495.6 (1605.6)	1326.5 ± 353.0 (1514.2)	0.4830
CD8/HLA-DR	Baseline	221.5 ± 115.6	192.6 ± 126.5	
	Week 1	194.9 ± 103.5(181.5)	206.1 ± 138.3 (219.5)	0.1043
	Week 3	214.6 ± 150.7 (198.2)	203.5 ± 166.6 (219.9)	0.5618
CD8/CD45RO	Baseline	322.2 ± 174.1	293.3 ± 142.2	
	Week 1	372.2 ± 212.6 (361.2)	301.7 ± 134.9 (312.6)	0.4339
	Week 3	328.0 ± 150.5 (315.7)	315.3 ± 164.9 (327.6)	0.7512
CD8/CD45RA	Baseline	664.8 ± 284.3	763.7 ± 272.6	
	Week 1	610.2 ± 247.2 (653.4)	839.0 ± 329.8 (795.8)	0.0748
	Week 3	688.7 ± 268.4 (732.3)	860.6 ± 282.6 (817.0)	0.1671
CD4/HLA-DR	Baseline	159.9 ± 53.6	136.0 ± 47.3	
	Week 1	197.7 ± 134.3 (183.5)	135.8 ± 57.6 (150.0)	0.4126
	Week 3	239.3 ± 68.4 (234.7)	140.5 ± 34.3 (145.1)	0.0016
CD4/CD69	Baseline	59.1 ± 44.3	132.8 ± 47.0	
	Week 1	64.9 ± 53.0 (104.2)	168.8 ± 78.3 (129.5)	0.3743
	Week 3	69.4 ± 45.9 (81.8)	155.2 ± 67.3 (142.9)	0.0862
CD8/CD69	Baseline	129.7 ± 49.8	156.0 ± 116.6	
	Week 1	79.5 ± 61.1(90.8)	149.2 ± 119.3 (137.9)	0.0831
	Week 3	114.3 ± 45.5 (123.2)	159.1 ± 99.4 (150.2)	0.2412
CD4/CD45RA	Baseline	511.5 ± 311.9	738.2 ± 288.6	
	Week 1	490.0 ± 306.1 (593.1)	743.3 ± 279.5 (640.3)	0.3827
	Week 3	672.5 ± 332.3 (758.2)	822.0 ± 216.8 (736.3)	0.7925
CD4/CD45RO	Baseline	525.6 ± 87.2	483.5 ± 115.9	
	Week 1	496.5 ± 81.6 (474.4)	519.4 ± 169.2 (541.5)	0.0857
	Week 3	541.2 ± 105.7 (527.1)	492.0 ± 138.4 (506.0)	0.6677
CD3	Baseline	1725.0 ± 510.9	1357.9 ± 340.7	
	Week 1	1845.4 ± 399.8 (1703.4)	1479.6 ± 421.9 (1621.5)	0.5019
	Week 3	1899.0 ± 576.4 (1741.6)	1273.7 ± 378.7 (1431.1)	0.0669
CD5	Baseline	1842.6 ± 497.6	1445.9 ± 290.8	
	Week 1	1897.2 ± 379.4 (1741.3)	1541.3 ± 444.0 (1697.1)	0.7471
	Week 3	1988.0 ± 586.0 (1790.2)	1414.1 ± 449.6 (1611.9)	0.3079
CD20	Baseline	183.3 ± 56.4	170.5 ± 44.0	
	Week 1	165.8 ± 71.0 (159.6)	164.0 ± 51.0 (170.2)	0.5551
	Week 3	165.0 ± 56.9 (160.6)	183.6 ± 68.3 (187.9)	0.2804
CD56	Baseline	1017.7 ± 366.5	362.3 ± 183.8	
	Week 1	594.2 ± 237.3 (393.0)	360.4 ± 155.6 (561.6)	0.0212
	Week 3	639.3 ± 194.1 (578.0)	402.3 ± 131.3 (464.2)	0.3237
CD20/CD5	Baseline	73.6 ± 23.4	79.9 ± 21.7	
	Week 1	58.8 ± 23.4 (61.2)	63.2 ± 21.0 (60.9)	0.9680
	Week 3	67.4 ± 24.5 (68.4)	64.6 ± 25.8 (63.6)	0.6709
CD3/CD56	Baseline	627.3 ± 277.0	108.6 ± 46.9	
	Week 1	267.4 ± 129.4 (162.2)	93.5 ± 62.9 (198.7)	0.4631
	Week 3	295.1 ± 134.7 (281.4)	116.2 ± 67.3 (129.9)	0.0855

at sites distant to the application, followed by a generalized psoriatic eruption (20–22). Another 80-year-old woman, with multiple squamous cell carcinomas on her extremities, experienced exacerbation of her pre-existing and well-controlled myasthenia gravis one week after onset of topical imiquimod therapy for her carcinomas and recovered after cessation of imiquimod administration (23). These clinical data, taken together with the systemic adverse reactions (flu-like symptoms, fatigue, headache, diarrhoea and myalgias) seen in patients treated with topical imiquimod, may be regarded as evidence suggesting that in humans this compound, apart from its well-known local action on the cutaneous immune response, may also be capable of exerting systemic immunomodulatory effects. This hypothesis is supported by the results of our paper, which, to our knowledge, show for the first time that topical application of imiquimod causes alterations in the peripheral blood lymphocytes in healthy human subjects.

Our findings indicate that topical imiquimod has no significant impact on the absolute numbers of the peripheral mature T lymphocytes (CD3+ and CD5+), B lymphocytes (CD20+) and their activated CD5+ subset (CD20+/CD5+) in the treated healthy individuals. On the contrary, it exerts distinct, differential and time-dependent effects on peripheral T-lymphocytic subpopulations that regulate the innate and the adaptive immune response. Thus, as far as LSHG lymphocytes are concerned, already after one week of treatment there was an induction of helper/inducer (CD4+), naive (CD45RA+), naive helper/inducer (CD4+/CD45RA+) and activated suppressor/cytotoxic T lymphocytes (CD8+/HLA-DR+). After 3 weeks of imiquimod treatment, probably due to cytokines released by the stimulated helper and helper/inducer cells, an induction of memory T lymphocytes (CD45RO+) and memory subpopulations of helper/inducer (CD4+/CD45RO+) and suppressor/cytotoxic T lymphocytes (CD8+/CD45RO+) was observed together with a decline in the expression of the early activation marker CD69. The loss of this marker was also seen in SSLG lymphocytes after one week of imiquimod treatment accompanied by a decrease in natural killer (CD56+) and memory T lymphocytes (CD45RO+), whereas after 3 weeks of treatment an induction of activated helper/inducer T lymphocytes (CD4+/HLA-DR+) could be detected.

It is known that the maximum recommended dose of topical imiquimod is one sachet (12.5 mg) and the use of an occlusive dressing is not recommended. Thus, considering the high imiquimod dose (62.5 mg) applied in our study to a large treatment area (100 cm^2) under occlusion every other day, it might be argued that the clinical relevance of our findings is questionable. It should be noted, however, that identical or higher amounts of this compound (75–83 mg) (under occlusion or not) have been applied by other research groups to identical or even larger skin surface areas 3–7 times per week (4, 6, 24–32). It seems reasonable, therefore, to suggest that our data are at least relevant to regimens similar to that used in our study. Nevertheless, a linear dose proportionality between serum concentrations and the applied imiquimod dose (29) is lacking, whereas there is a tremendous heterogeneity of dose-response among individual patients (33), with cytokine-mediated systemic side-effects of imiquimod occurring even with normal dosing. However, whether alterations in the peripheral blood lymphocytes occur even under conventional treatment conditions warrants further investigation.

The exact molecular and cellular mechanisms underlying these imiquimod-induced alterations in the peripheral lymphocyte subpopulations of healthy individuals observed in our study are presently unknown. However, it seems reasonable to suggest that they are caused by the circulating cytokines and chemokines produced at the site of imiquimod application and by the stimulated immunocompetent, mainly dendritic, cells. The modulatory effects of topical imiquimod on the peripheral blood lymphocytes found in the present study may be of importance in the immunotherapy of neoplastic and infectious disorders and should be considered carefully in patients with autoimmune diseases.

In a recent multicentre study on the efficacy and safety of topical imiquimod in the management of actinic keratoses in organ transplant recipients, no graft rejection or trend for deterioration of graft function could be detected after 16 weeks of treatment (34). Thus, it has been concluded that this compound appears to be a safe alternative for the treatment of actinic keratoses in patients with solid organ transplants. Nevertheless, since the prevalence of HPV-associated cutaneous infections and malignancies in organ transplant recipients increases with the duration of immunosuppression (35, 36), multiple courses of topical imiquimod application will be necessary to arrest the ever-increasing occurrence of these disorders during the lifetime of the immunocompromised patients. However, no study data are available on retreating HPV-associated cutaneous infections that have cleared after initial treatment and subsequently recur. Those studies are warranted to provide further insights into the efficacy and safety of imiquimod.

The authors declare no conflicts of interest.

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