Ethanol Enhances the Mitogen-driven Lymphocyte Proliferation in Patients with Psoriasis

RUDOLF E. SCHOPF, HANS M. OCKENFELS and BERND MORSCHES

Department of Dermatology, Johannes Gutenberg University, Mainz, Germany

Ethanol has been reported to exacerbate psoriasis. Since immunological mechanisms are considered to be important for the pathogenesis of psoriasis, we compared the effects of ethanol on lymphocyte proliferation in 15 healthy control individuals and 15 patients with psoriasis. We employed the spontaneous and phytohemagglutin in (PHA)-induced uptake of ^{3}H-TdR to measure lymphocyte proliferation. Ethanol was added to cultures at concentrations ranging from 0.5 to 0.0005% (vol./vol.). We found that both spontaneous and PHA-driven lymphocyte proliferations were significantly lower in patients with psoriasis ($p < 0.002$). Spontaneous blastogenesis in both controls and patients remained stable under ethanol. In controls, ethanol suppressed the PHA-driven lymphocyte proliferation in a dose-dependent fashion. By contrast, in patients with psoriasis ethanol significantly increased lymphocyte proliferation by 2–3 times ($p < 0.002$). Our data indicate that in psoriasis the lower lymphocyte transformation is abnormally enhanced by minimal doses of ethanol.

It is a well-known clinical phenomenon that alcohol intake by patients with psoriasis may induce exacerbation of disease (1–10). Investigations carried out on large numbers of psoriatic patients have disclosed an aggravation of psoriasis after alcohol uptake in 10–18% of patients (5). Chaput et al. described a significant correlation between alcohol abuse and psoriasis, independent of alcoholic liver damage (1). Vincenti & Blunden reported that withdrawal of alcohol led to an improvement of the psoriatic skin lesions, whereas psoriasis was exacerbated again after renewed uptake of alcohol (2).

It has been demonstrated that ethanol affects the immune system by decreasing the number of peripheral blood T-lymphocytes, impairing the formation of T-lymphocyte rosettes as well as the proliferation of lymphocytes that is not caused by a lack of interleukin-2 (IL-2) production by T-cells (11–13). Moreover, phagocyte functions were demonstrated to be suppressed by ethanol (14). It is still unknown, however, in which way ethanol affects the immune cell function in psoriasis.

As immunological mechanisms are considered to be important in the pathogenesis of psoriasis (15, 16), we attempted to explain the ethanol-induced exacerbation of psoriasis by testing the effect of the agent on lymphocyte proliferation induced by phytohemagglutinin (PHA). Our results show an abnormal enhancement of T-lymphocyte stimulation under the influence of ethanol in patients with psoriasis.

MATERIAL AND METHODS

Patients

Seven females and 8 males aged 52 ± 4.5 years (range 23 to 83 years) with active psoriasis were included in our study. Thirteen patients showed extensive lesions; the extent of surface area involved varied from 20 to 50%. Only 2 patients had fewer lesions. Patients with psoriatic arthropathy were excluded. Only 2 patients had had no psoriatic eruptions before. None of the patients had received systemic treatment including PUVA or immunosuppressive therapy during the previous 3 months before lymphocytes were isolated. Topical treatment during the week before venipunctures was allowed to consist only of emollients and 10% salicylic acid emollients to remove scales. HLA phenotypes B27, B37, and Cw6 were found in 6 patients.

Controls consisted of 15 healthy volunteers (10 females, 5 males) aged 34 ± 4.5 years (range 19 to 75 years).

Isolation of lymphocytes

Twenty-five ml peripheral blood was drawn into syringes preloaded with 250 U heparin to prevent coagulation. The blood was mixed 1:4 with minimum essential medium (MEM, Gibco). Peripheral blood mononuclear leukocytes (MNL) were purified by density gradient centrifugation on Ficoll Hypaque® according to Böyum (17). The cells were then washed twice in RPMI 1640 medium (Seromed-Biochrom, Berlin) and adjusted to 1×10^6/ml. Cell viability was gauged by exclusion of 0.4% trypan blue (Merck, Darmstadt, Germany) and found to exceed 98% in all fresh cell preparations.

Stimulation of lymphocytes

1×10^5 lymphocytes/well were cultured at 37°C, in 5% CO_2 atmosphere in 96-well microculture plates (Greiner). The cell culture medium consisted of RPMI 1640 supplemented with 2 mM glutamine, 100 U penicillin, 100 µg streptomycin/ml as well as 10% heat-inactivated (56°C, 30 min) autologous serum to rule out possible lymphocyte stimulation by allogeneic or xenogenic serum. Ten µg/ml purified PHA (Biochrom, Berlin, Germany) that had been found to yield optimum stimulation with lymphocytes from controls (data not shown) served as mitogen. Twelve-fold replicate cell cultures of each individual were used to determine spontaneous and mitogen-induced blastogenesis. To assess the effect of ethanol on the proliferation rate, dilutions were prepared from 99.8% ethanol (Fluka, Buchs, Switzerland) (ethanol diluted in 154 mM NaCl) and added to fourfold replicates of cultures to determine both the spontaneous and PHA-driven blastogenesis (final concentration: 0.5–0.0005%). The PHA experiments without and with alcohol were performed from the same lymphocyte samples. Cells were cultured for 4 days; 0.5 µCi ^{3}H-TdR (5 µCi/mM specific activity) was added to each well for the final 24 h to label newly synthesized DNA as a measure for lymphocyte proliferation. After incubation, the cells were collected with a semiautomatic cell harvester (Skatron AS), and radioactivity was determined employing a liquid scintillation spectrometer (SL 30, Intertechnique).

Viability testing by trypan blue exclusion ruled out cytotoxic effects of the ethanol concentrations employed.

Statistical methods

The statistical significance of differences between psoriatics and controls was determined by the two-tailed Wilcoxon-Mann-Whitney test. The Wilcoxon rank sum test was used for PHA values measured with and without ethanol in psoriatics.

RESULTS

Lymphocyte proliferation

The spontaneous blastogenesis of lymphocytes was almost twice as high in healthy controls compared to patients with psoriasis ($400:44$ vs. 216 ± 38 ($p < 0.002$)). Likewise, the PHA-driven stimulation of lymphocytes was 2.6 times higher in healthy controls than in patients with psoriasis (13226 ± 1346 vs. 5019 ± 1867 ($p < 0.002$)).

There was no correlation between routine blood parameters for liver and kidney function, antistreptolysin titer or HLA phenotype as well as extent of skin disease, either in spontaneous or PHA-driven lymphocyte blastogenesis.

Effects of ethanol on lymphocyte proliferation

The spontaneous blastogenesis remained stable under the influence of ethanol both in healthy controls and patients with psoriasis, and there was no significant difference of cell proliferation in the absence or presence of ethanol (data not shown).

By contrast, under the influence of ethanol the PHA-driven lymphocyte proliferation differed markedly between healthy controls and patients with psoriasis. While lymphocytes from controls were suppressed in a dose-dependent fashion by increasing ethanol concentrations, psoriatic patients responded with a two- to threefold enhanced PHA-induced lymphocyte proliferation, as displayed in Fig. 1. Individual PHA values without and with ethanol (0.005%) for each of the 15 psoriatics are shown in Fig. 2. From these data it was concluded that in psoriasis PHA-driven lymphocyte proliferation is abnormally stimulated by minimal doses of ethanol.
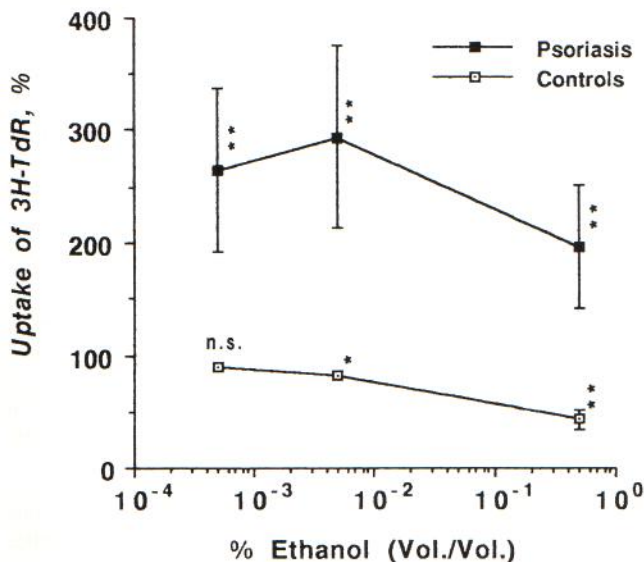

Fig. 1. Effects of 0.0005 to 0.5% ethanol on PHA-induced lymphocyte proliferation of 1×10^5 peripheral blood mononuclear cells (means ± SEM). Values are based on the mean cpm of 4-fold cell cultures, standardized to the PHA-induced proliferation without ethanol (= 100%). The 100% values refer to the absolute cpm $*p < 0.05$, $**p < 0.002$, n.s.: not significant. The p values derive from comparisons between the ethanol and non-ethanol value in psoriatics, and also between the ethanol and non-ethanol value in controls.
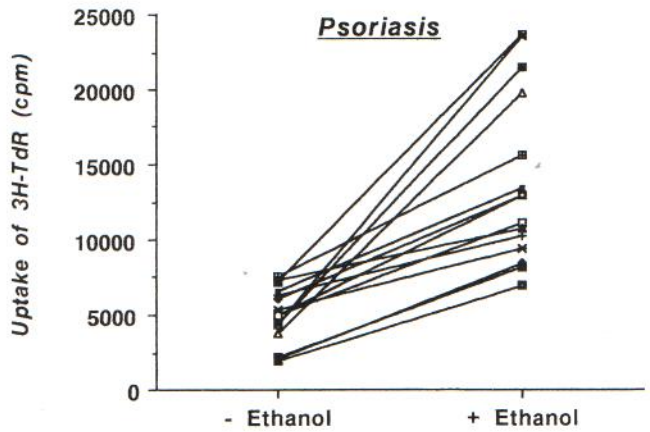

Fig. 2. Individual PHA values without and with ethanol (0.005%) for each of the 15 patients with psoriasis. When the Wilcoxon rank sum test was employed, the differences reached statistical significance at the $p = 0.0007$ level.

DISCUSSION

Our results show that lymphocyte proliferation differs in patients with psoriasis compared with healthy controls. Our findings confirm those of others reporting a diminished mitogen-induced lymphocyte proliferation in vitro as well as a suppressed reactivity to recall antigens in vivo (18–20). These abnormalities may be explained by a shift toward T-suppressor cells of the T-helper/T-suppressor cell ratio in the peripheral blood, as reported (21). There are, however, also reports suggesting a normal lymphocyte proliferation in patients with psoriasis (22–24). Furthermore, it has recently been found that soluble IL-2 receptors, indicating lymphocyte activation, are elevated in psoriatic serum even after clinical remission (25–27). Therefore, the issue of T-cell activation in psoriasis is complex and appears to be critically involved with disease activity (15, 16). In our hands, there was a clear distinction between healthy controls and patients with psoriasis, resulting in a decreased spontaneous and mitogen-driven lymphocyte proliferation in patients.

The addition of ethanol to the cell cultures resulted in a different pattern between controls and patients. While PHA-driven lymphocyte proliferation in controls was inhibited by the ethanol concentrations employed, confirming previous reports in rodents as well as non-psoriatic humans where 0.4% ethanol in vitro suppressed the proliferative response by approximately 50% (12, 13, 28), our findings in patients with psoriasis demonstrate a markedly increased mitogen-induced lymphocyte proliferation. In psoriasis the effects of ethanol on the immune system appear not to be restricted to mononuclears, as it has been reported that alcohol also increases in vitro the chemotactic activity of polymorphonuclear leukocytes from patients with psoriasis but not those from controls (T Ternowitz, U Søderberg, presented at the 25th Nordic dermatological congress, Copenhagen, June 1989).

The reasons why concentrations of ethanol that can be measured in humans after uptake of alcoholic beverages suppress the PHA-driven lymphocyte blastogenesis in healthy controls on one hand and enhance the response in patients with psoriasis on the other are not fully understood. However, several possibilities exist:

(1) Ethanol may be cytotoxic to mononuclear leukocytes. In the concentrations tested, this possibility can be ruled out with certainty, since we found no loss of viability of MNL by the trypan exclusion method in controls. Moreover, in patients there was an enhanced function ruling out cytotoxicity by ethanol.

(2) Controls may, in theory, be able to oxidize ethanol slower to acetaldehyde and acetic acid than patients. However, this is unlikely, since the oxidation products of ethanol also directly inhibit lymphocyte proliferation (28), which could not explain the enhanced proliferation in psoriasis.

(3) The possibility cannot be excluded that ethanol may have interfered with the calcium influx due to possible alteration of cell-membrane fluidity, as has been suggested (29). In psoriasis, alterations in cell membrane permeability including steroid penetration have been documented (30).

(4) Ethanol may have increased the intracellular concentration of the cyclic adenosine 3′,5′-monophosphate (cAMP). This seems likely because ethanol has been shown to increase cAMP in leukocytes (31). Increased cAMP concentrations have long been known to be associated with diminished leukocyte functions (32). The biological effects of cAMP are mediated by protein kinases involved in the phosphorylation of proteins. A deficiency of regulatory subunits of cAMP-dependent protein kinase-A has been documented in psoriasis, correlating with the severity of psoriasis (33, 34). Abnormalities in the cAMP – protein kinase metabolism also seem to be responsible for the well-known exacerbation of psoriasis by β-adrenoceptor antagonists or lithium carbonate, both of which act on the cAMP system. It remains to be defined whether a defective cAMP protein kinase-A metabolism is crucially involved in the pathogenesis of psoriasis.

(5) The possibility that ethanol induces an altered utilization of IL-2 by psoriatic MNL has to be considered, in particular since elevations of IL-2 receptor-positive T-cells were found in psoriasis (35). In normal individuals the effects seem comparable with those found in MNL cultures from ethanol-treated rats, showing a decreased ability to proliferate in response to IL-2 despite an unimpaired IL-2 production by T-cells (12).

We presume that ethanol uptake in patients with psoriasis may also stimulate the activation process of lymphocytes in vivo. As defined immunological stimuli, such as interferon-α or IL-2 (36, 37), are known to exacerbate psoriasis and immunosuppressants like cyclosporin A inhibiting IL-2 synthesis are used to successfully treat psoriasis (38), the enhanced lymphocyte stimulation we found in vitro may lead to a Koebner phenomenon in vivo. Our study suggests that ethanol may exacerbate psoriasis by affecting cell-mediated immunity.

ACKNOWLEDGEMENT

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