Decreased Production of Interferon in Whole Blood Cultures Derived from Patients With Psoriasis

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Patients suffering from psoriasis show many alterations with respect to their immune system as documented by in vitro test systems. In the present study we investigated the in vitro production of interferons (IFN) of leukocytes from psoriatic patients to stimulation with a variety of IFN inducers. Furthermore, the lymphoproliferative responses were tested. Whole blood cultures of 30 psoriatic patients showing moderate to severe disease activity and 21 cultures from healthy controls were stimulated with the mitogens PHA, ConA, and PWM, with PPD and Tetanus Antigen as IFN γ inducers and with C. parvum, PolyI-PolyC, and Herpes simplex virus as inducers of IFN α. Interferon activity was tested in the supernatant of 48-h cultures by using an antiviral assay. Lymphoproliferation was assayed in 5-d cultures in parallel. Psoriatic patients showed a significantly decreased IFN production to all the stimuli tested. There were no significant differences in the lymphoproliferative responses; only the response to PWM was slightly decreased. The decreased IFN production by leukocytes from psoriatic patients seems to be very remarkable since increased susceptibility to infections is not generally known in these patients. *J Invest Dermatol 90:511–514, 1988*

Increasing evidence suggests that abnormalities of the immune system may be of major importance in the pathogenesis of psoriasis. Several authors report alterations of monocytic and lymphocytic functions [1–6] as well as abnormalities of humoral immunity [7,8]. Recently the presence of interferon in suction blister fluid from psoriatic lesions was described [9]. Furthermore, PUVA treatment [10] as well as Goeckerman therapy [11] resulted in a significant increase of interferon in serum and suction blister fluid from patients with psoriasis. Interferon usually is induced by viruses. Therefore, it is of interest that at least one group has presented evidence that retroviruses may play a role in psoriasis [12,13]. Interferon represents a potent modulator of several cellular functions [14]. Any alteration in the production of these mediators, particularly any regarding the cellular components of the immune system, might result in a disturbance of the immune response [15]. We, therefore, investigated the production of interferons (IFN) in leukocyte cultures upon stimulation with a variety of IFN α as well as IFN γ inducers. The lymphoproliferative responses were tested in parallel.

MATERIALS AND METHODS

Patients and Controls The psoriasis group consisted of 30 patients (18 male and 12 female), exhibiting symptoms of plaque-type psoriasis with moderate to severe disease activity. Neither control nor psoriasis subjects had received any medication (particularly local and systemic steroid therapy, Goeckerman treatment, and therapy with ultraviolet light) for at least 3 weeks prior to our studies. The age and sex distribution for control and test subjects was not statistically different; further, there were no correlations between results obtained and age or sex. The control group consisted of 21 (10 male and 11 female) healthy blood donors.

Whole-blood Technique Measurements were performed as described previously [16]. In brief, 10 ml of heparinized (50 units/ml) blood was collected and immediately mixed at a ratio of 1:15 with culture medium (RPMI 1640 supplemented with 2 mM glutamine, 100 units/ml penicilline, and 100 μg/ml streptomycine but without additional serum). The white cell count was routinely determined in an automated particle counter. Furthermore, a differential lymphocyte count yielded normal results in all instances. Because of these findings the blood/medium ratio remained unchanged in all experiments. For both the lymphoproliferative and the IFN tests, the blood suspension was distributed in 0.9 ml aliquots into tubes to which mitogens or antigens were added at the appropriate concentrations (see below) in aliquots of 0.1 ml. The contents of the tubes were mixed and distributed into the wells of round-bottom 96-well plates (Nunc, Wiesbaden, FRG) at aliquots of 0.2 ml for the lymphoproliferative test and 0.1 ml for the IFN test.

Lymphoproliferative Test After 5 days of incubation at 37°C cells were labeled with the addition of ^{3}H-thymidine (1 μCI/well; specific activity 25 Ci/mmol, Amersham Buchler, Braunschweig, FRG) to the wells and incubated for an additional 4 h. The nonsoluble radioactivity, precipitated on filter paper by using a harvesting device (Skatron, Norderstedt, FRG), was determined in a liquid scintillation counter (LKB, Bromma, Sweden).

Interferon assay The Interferon assay was performed as described previously [16,17]. In brief, the Interferon standard (human recombinant IFN α2, diluted to 10^3 IU/ml, Hoffmann La Roche, Bale, Switzerland) and three-fold dilutions (1/3, 1/9, 1/27, etc.) of the test samples were prepared in medium RPMI 1640, supplemented

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Abbreviations:
Con A: concanavalin A
HSV: herpes simplex virus
IFN: interferon
PHA: phaseolus vulgaris phytohemaglutinin
PPD: tuberculin derivative
PWM: pokeweed mitogen

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with 2% to 5% fetal calf serum and added to confluent monolayers of human HEp-2 cells in 96-well plates (4×10^4 cells/well). The plates were incubated for 24 h and the cells were washed twice before they were infected with 200 μl versicular stomatits virus at an m.o.i. of 1.0 (diluted in basal medium Eagle, Gibco Biocult, Karlsruhe, FRG, supplemented with 2% FCS) and incubated for another 48 h. Then they were fixed, stained, and evaluated for the presence of the cytopathic effect. The antiviral activity, expressed in IFN units, was calculated as the reciprocal of the highest dilution of the sample, reducing the number of the viral plaques by 50%. One laboratory unit of IFN is equivalent to 1 international unit of IFN.

In selected patients we have typed the interferon induced by the different stimuli. The mitogens and the antigens exclusively induced IFN γ, and the IFN induced by C. parvum, Poly I-Poly C, and HSV represented IFN α to the largest extent.

Mitogens and antigens The following mitogens were used: Phytohemaglutinin, from Phaseolus vulgaris (PHA/Wellcome, Burgwedel, FRG; 2,5 μg/ml); Concanavalin A (Con A/Pharmacia, Freiburg, FRG; 5 μg/ml); and pokeweed mitogen (PWM/Gibco, Wiesbaden, FRG; 1:500). The following additional stimulants were used: Tuberculin derivative (PPD/Statens Serum institut, Copenhagen, Denmark; 10 μg/ml); C. parvum, a killed suspension of bacteria (strain CN 6134, Wellcome, Burgwedel, FRG; 100 μg/ml); Tetanus antigen (TET/Institute Merieux, Lyon, France; 10 μg/ml); and poly I-poly C (Serva, Heidelberg, FRG, 100 μg/ml). Herpes simplex virus type I (HSV strain WAL, 10^7 phys. particles/ml) was prepared as described previously [18].

Serum IFN titers Sera from patients and controls were tested for IFN activity as described above.

Statistical analysis Statistical evaluations were performed by using the Mann-Whitney U-test.

RESULTS

The results of the in vitro IFN responses of psoriasis patients and controls to three different mitogens (PHA, Con A, and PWM) and to the antigens PPD and tetanus inducers of IFN γ are shown in Fig 1. The IFN response upon stimulation with C. parvum, poly I-poly C, and HSV as IFN α inducers are presented in parallel. Psoriatic patients showed a significantly decreased IFN production to all the inducers of IFN γ and IFN α tested.

The results of the in vitro lymphoproliferative responses of normal blood donors to the three mitogens (PHA, Con A, and PWM) and to the antigens PPD, tetanus, and HSV are shown in Fig 2. The same type of data is summarized for patients with psoriasis in parallel. Only the response to PWM was found to be slightly reduced ($p < 0.05$). No significant difference could be detected upon stimulation with the other stimuli tested. In addition, no significant serum IFN levels were detected in the sera of patients and controls (data not shown).

DISCUSSION

Our results clearly demonstrate a significantly decreased production of IFN to all stimuli tested. The defect is not a result of decreased proliferative capacity of lymphocytes, because the lymphoproliferative responses generally were found to be normal and in agreement with previous reports [1,3]. A significantly decreased response could only be detected after stimulation with PWM, which could be the cause of the decreased immunoglobulin synthesis by PWM-stimulated mononuclear cells from psoriatic patients [2]. However, there are reports describing a decreased mitogenic response of peripheral blood lymphocytes in psoriasis to stimulation with Con A [1,4]. Conflicting results may be caused by stimulation with a different concentration of the mitogen or by testing patients in different states of the disease and could be the effect of preceeding antipsoriatic therapy [19], which was excluded in our study.

Decreased IFN production in the peripheral blood is in contrast to increased levels of IFN γ in suction blister fluid [9] which may be the result of increased local IFN production. IFN γ is produced by activated T-cells [14]. HLA DR^+ dendritic cells, which were present in the plaques from patients with active psoriasis, may directly activate T cells or may present an as yet unknown antigen to T cells [20,21]. Since IFN γ could also be detected in the blister fluids

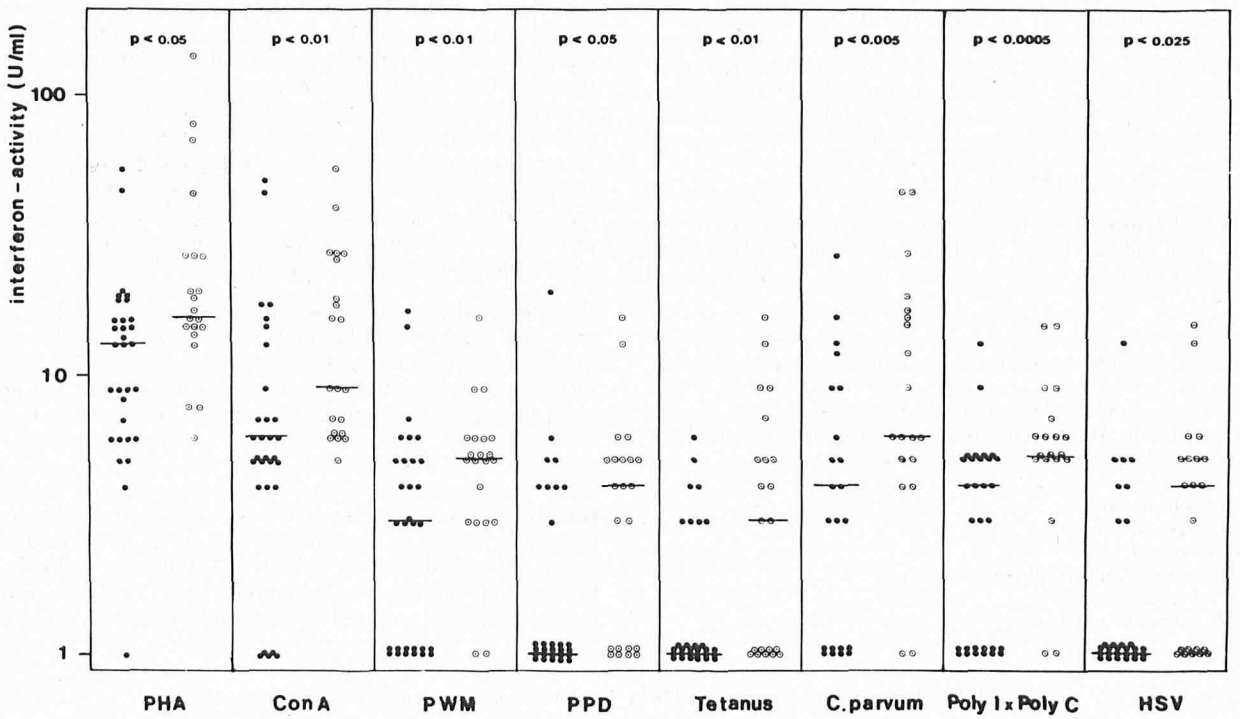

Figure 1. Interferon production of leukocytes from psoriatic patients and normal blood donors to stimulation with inducers of IFN α and γ. The closed symbols represent values obtained from psoriatic patients (n = 30), and the open symbols are those from normal donors (n = 21). Medians are indicated as horizontal lines.

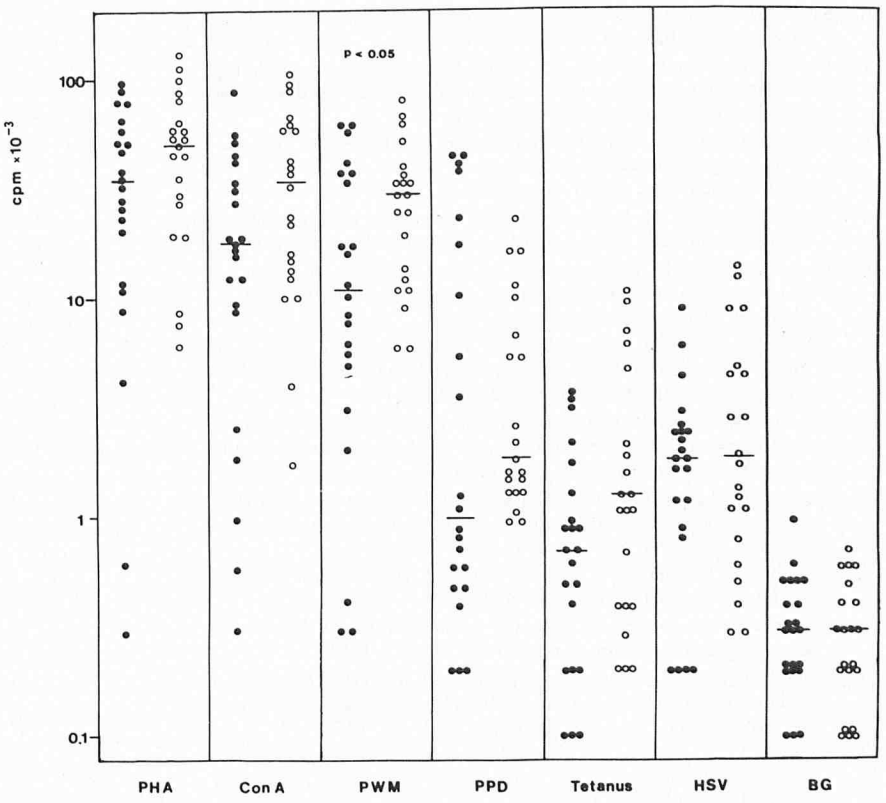

Figure 2. Lymphoproliferative responses of psoriatic patients and of normal blood donors to different stimuli. The closed symbols represent values obtained from psoriatic patients (n = 22), and the open symbols are those from normal blood donors (n = 21). Medians are indicated as horizontal lines.

[11] a viral aetiology of psoriasis could possibly be assumed, particularly when regarding the finding of a retrovirus-like particle in the patients [12,13]. However, IFN α as well as IFN γ exerts an antiproliferative effect on cultured keratinocytes [22]. These in vitro results are in contrast to the significantly increased proliferation of keratinocytes in vivo.

As reported previously [10,11], clearing of the lesions in psoriasis is associated with an increase of IFN in suction blisters and in the serum of the patients. However, we were unable to detect any significant serum IFN levels in psoriatic patients and in the control subjects. Detectable serum IFN levels could be the result of preceeding therapy. Differences could also be due to the methods applied. We have no obvious explanation for this apparent disagreement at present.

Increased levels of IFN in the peripheral blood and skin lesions appear to be in contrast with a well-known hyperproliferation of keratinocytes. However, a variety of different mediators triggering immunologic and proliferative responses, which may be inducers for the proliferation of keratinocytes in the disease exist. Interleukin 1 can stimulate the proliferation of human keratinocytes in vitro [23,24]. However, Takematsu et al. [25] found a significantly diminished presence of IL-1 activity in scale extracts prepared from skin specimens from psoriasis patients in comparison to horny tissue extracts prepared either from normal skin or from noninnflammatory skin. Since interleukin 1 production is regulated by α and β interferons [26], a decreased production of interferon could be the cause of reduced interleukin 1 levels in the skin of the patients. However, there was no correlation between interleukin 1 production and DNA synthesis in keratinocyte cultures from uninvolved psoriatic skin [27].

The finding of a decreased interferon production might suggest that exogenous interferons could be useful in inhibiting skin proliferation in patients with psoriasis [28]. However, neither IFN α nor IFN γ had any effect on a DNA synthesis in psoriatic epidermis following intralesional injection [29]. Furthermore, the in vivo application of IFN α and IFN γ did not support an essential role for these mediators in the improvement of the disease [29–31].

Our results are in agreement with a previous report by Goan et al. [32], who found a decreased production in IFN γ by leukocytes from psoriatic patients upon stimulation with the mitogen Con A. In contrast, normal IFN values were measured with PHA or PWM as IFN inducers. Differences could be due to distinct culture conditions to generate IFN. Because we used whole blood cultures it cannot be excluded at present that the decreased ability of leukocytes to produce IFN in psoriatic patients may be caused by inhibitory factors in the serum of the patients. These factors could inhibit the interferon production of leukocytes at the cellular level. This possibility will be tested in a further study.

Furthermore, it appears that the diminished production of IFN might not be essential for the pathogenesis of psoriasis but rather circumstances blocking the action of IFN. Possible explanations could be the alteration of IFN receptors or the presence of inhibitory factors that were recently found in sera of patients with psoriasis [33].

Therefore, decreased production of interferon in psoriasis does not necessarily have to be a primary event in the pathogenesis of the disease. Rather, it may be the result of a complex regulatory process triggered by the chronic inflammatory process in the skin of the patients.

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