Serum-Mediated Inhibition of the Interferon-Gamma-Induced HLA-DR Expression on Monocytes in Patients with Psoriasis

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Psoriatic patients have a decreased proportion of monocytes expressing class II major histocompatibility complex antigens (DR⁺ monocytes) in their peripheral blood. The expression of the DR antigen on monocytes after culture in the presence of either autologous lymphocytes (endogenous interferon-gamma, IFN-γ) or exogenous IFN-γ was investigated. In normal AB serum the lymphocytes of the majority of the patients showed a spontaneous IFN-γ production which was sufficient for DR antigen induction, while the monocytes displayed approximate normal susceptibility to exogenous IFN-γ as judged by DR antigen expression. However, the sera of psoriatic patients contained one or more factors that interfered with the IFN-γ-mediated DR antigen expression on cultured monocytes of the same patients. Restoration of IFN-γ-induced DR antigen expression on monocytes in the presence of the patient's serum was achieved by the addition of superoxide dismutase, 2-mercaptoethanol, or indomethacin. The clinical significance of these observations is discussed. J Invest Dermatol 87:524-527, 1986

Recently, much attention has been focused on the fact that interferon (IFN) regulates the expression of histocompatibility antigens (MHC antigens) in several cell types [1,2]. It has been shown that the expression of class II MHC antigens on monocytes/macrophages, a feature critical to the antigen-presenting function of these cells, is regulated by IFN-γ [2,3].

In psoriasis, several abnormal functions of immune cells similar to those found in patients suffering from the classical autoimmune diseases, have been described [4-6]. Recently, IFN-like activity has been detected in suction blister fluid from lesions [7] and in the peripheral blood [8] of patients suffering from psoriasis. Similar findings have been published for systemic lupus erythematosus [9]. Furthermore, we have found that the mitogen-induced production of IFN-γ by peripheral blood cells of the patients, was significantly reduced, and some of these patients failed completely to produce IFN-γ in vitro, as did patients suffering from several autoimmune diseases [5]. This prompted us to look for the class II MHC (DR) antigen expression in the peripheral blood monocytes both in vivo and in vitro after cultivation in the presence of added IFN-γ.

Our findings, described in this paper, show that patients suffering from psoriasis have a decreased proportion of DR⁺ monocytes in their peripheral blood. Three explanations are proposed for our observation: (1) diminished IFN-γ-production by patients' lymphocytes; (2) impaired susceptibility to IFN-γ; and (3) the existence of soluble factors that inhibit the action of IFN-γ on monocytes. The results suggest that the third possibility is most likely to be true.

MATERIALS AND METHODS

Patients The heparinized blood was obtained from hospital patients with exacerbating psoriatic lesions. Blood samples were drawn before the patients had undergone any internal therapy or PUVA treatment. Healthy volunteers matched for age and sex were used as controls.

Preparation of Monocytes The mononuclear cells were isolated from heparinized blood samples by density gradient centrifugation as described [10]. The cells were resuspended in culture medium RPMI-1640 (Sifin, Berlin, G.D.R.), supplemented with t-glutamine 2 mM, penicillin 100 U/ml, streptomycin 100 µg/ml, and 10% heat-inactivated AB serum or fetal calf serum (FCS). FCS-coated glass cover slips were placed in Petri dishes and overlaid with the cell suspension (5 × 10⁶/ml). After incubation at 37°C for 2 h, the nonadherent cells were removed by repeated washing with prewarmed medium (6-8 times). The cover slips with the adherent cells were either assayed immediately or incubated further in 2 ml of fresh culture medium in Petri dishes; the adherent cells consisted of more than 93% monocytes characterized by nonspecific esterase and fluorescence staining using a monoclonal antimonocyte antibody (BL-M/G). Less than 3% of T lymphocytes, as assayed by staining with a monoclonal pan-T cell antibody (BL-T2), were usually observed among the adherent cells. One to four days later, the number of adherent cells dropped by an average of only 10-15%. During cultivation, a part of the adherent cells acquired the morphologic appearance of mature large "histioocyte-like" cells. After 3 days in culture,
the adherent cells displayed only a minimal contamination by cells other than monocytes/macrophages (they were more than 98% BL-M/G), and no T lymphocytes could be detected.

**Surface Antigens** Prior to the staining of cells, the medium was aspirated and replaced by 1% paraformaldehyde solution for 15 min at room temperature. The cells were then washed twice and overlaid with isotonic buffer solution (PBS) containing 2% bovine serum albumin. After 15 min the solution was removed and the cells were incubated with the appropriate monoclonal antibodies. After washing, the coverslips were incubated with a fluorescein-conjugated goat antimouse antiserum diluted 1:40 in PBS. The cells were then studied under a fluorescent microscope (Jenularum, Carl-Zeiss Jena, G.D.R.). At least 200 cells were counted each time.

**Monoclonal Antibodies (Mab)** The data of the utilized Mab have been described in detail elsewhere [11,12]. Briefly, BL-T2 is a pan-T cell Mab (CD-5 cluster), and the Mab BL-M/G is directed against all monocytes, granulocytes, and a proportion of the null cells. Three different Mab recognizing nonpolymorphic determinants of class II MHC antigens (here called DR) were used: BL-1a/1 [12], VID-1 (kindly provided by Prof. Knapp, Vienna), and anti-HLA-DR (Becton-Dickinson).

**Interferon** Semipurified IFN-γ (Pascal, Frankfurt, F.R.G.) having 10⁴ IU/mg protein and semipurified IFN-α, 10⁴ IU/mg protein (a gift by Prof. Cantell, Helsinki), were used. In some experiments purified recombinant human IFN-γ (Genentech), 3 × 10⁴ IU/mg protein, was used with the same results.

**Supplements** Superoxide dismutase (SOD) was prepared from human erythrocytes (kindly provided by Dr. Hörming, Dessau). β-Carotene was obtained from Hoffmann-La Roche as Phenoro capsules containing 10 mg β-carotene and 15 mg canthaxantheme.

**RESULTS**

**DR Antigen Expression on Monocytes In Vivo** Peripheral blood monocytes were isolated with the adherence technique and immediately tested for their DR antigen expression by several Mab directed against class II MHC. As shown in Fig 1, the proportion of DR⁺ monocytes in the group of psoriatic patients was significantly lower than in the normal controls (p < 0.01). The results from the tests with all 3 Mab were comparable (only the results with VID-1 are shown). The mean percentage of DR⁺ cells from 31 normal volunteers was 59%, ranging from 35–81%. Only 6 of 21 patients showed a DR expression within this normal range. The mean value of the 21 patients was 30%, ranging from 12–55%.

**Interferon-Gamma Production by Lymphocytes In Vitro** We have shown that peripheral blood mononuclear cells of psoriatic patients have a reduced in vitro response to concanavalin A and phytohemagglutinin, as measured by IFN-γ production [7]. However, very small doses of IFN-γ are necessary for the induction of DR antigens on monocytes (in vitro) as low as 1 IU/ml [2,3]. Thus the antiviral assay is not sensitive enough to determine whether lymphocytes are able to produce sufficient amounts of IFN-γ for DR antigen induction on monocytes. Recently, we [3] and others [2] were able to show that highly purified monocytes expressed progressively fewer DR antigens after 2–3 days of culture. The addition of either a low dose of IFN-γ (exogenous IFN-γ) or autologous lymphocytes (endogenous IFN-γ) to the monocytes prevented this loss of DR antigens. The reinduction of DR antigen expression in the presence of autologous lymphocytes was abrogated by anti-IFN-γ antibodies or cyclosporine A, indicating that IFN-γ produced spontaneously by the lymphocytes is the active mediator in this very sensitive assay. In the antiviral assay usually only marginal amounts of IFN activity (<10 IU/ml) in these supernatants are detectable. We have cultured 10⁴ monocytes of healthy donors or psoriatic patients in the presence or absence of 5 × 10⁴ autologous lymphocytes in culture medium containing 10% AB serum for 3 days. Thereafter, the DR antigen expression on monocytes was determined. As shown in Table I, the lymphocytes of the majority of the patients produced sufficient amounts of IFN-γ for conservation of DR antigen expression on cultured monocytes. Only 3 of 8 patients showed a decreased spontaneous IFN-γ production in this assay. Therefore, it seems unlikely that a diminished IFN-γ production is the sole reason for the decreased DR antigen expression on monocytes of psoriatic patients in vivo.

**Susceptibility to IFN-γ In Vitro** The percentage of monocytes expressing DR antigens decreased within 3 days of culture both in healthy donors and psoriatic patients. When 10 IU/ml

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**Table I.** Spontaneous IFN-γ Production by Lymphocytes As Judged by DR Antigen Expression on Autologous Monocytes After Co-culture

<table>
<thead>
<tr>
<th>Supplement</th>
<th>Percent DR⁺ Monocytes of Healthy Donors</th>
<th>Psoriatic Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>18 (5)</td>
<td>16 (5)</td>
</tr>
<tr>
<td>IFN-γ (5 IU/ml)</td>
<td>63 (7)</td>
<td>58 (8)</td>
</tr>
<tr>
<td>Autologous lymphocytes</td>
<td>60 (8)</td>
<td>48 (15)</td>
</tr>
<tr>
<td>Anti-IFN-γ and autologous lymphocytes</td>
<td>25 (7)</td>
<td>ND (N)</td>
</tr>
</tbody>
</table>

Highly purified monocytes (10⁴/well) were cultured in medium with 10% AB serum and various supplements for 3 days; thereafter, the DR antigen expression on the monocytes was determined. In the absence of any IFN-γ a slope in DR antigen expression was observed. In the presence of either 5 IU/ml IFN-γ (exogenous IFN-γ) or 5 × 10⁴ autologous lymphocytes per well (endogenous IFN-γ) the loss of DR antigens was prevented. The DR antigen-preserving effect of added lymphocytes is caused by spontaneous IFN-γ synthesis, because a Mab against IFN-γ was able to abolish this effect.

*Mean (SD); n = 8.*

*Not done.*

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**Figure 1.** Proportion of DR⁺ monocytes recorded immediately after separation from the peripheral blood. Bars, mean ± SD.
IFN-γ was added to cultures containing AB serum, the values remained significantly higher in both groups (Fig 2a), whereas IFN-α (1-500 IU/ml) addition had no effect (not shown). The marginal difference between the healthy group (68 ± 8%) and the patient group (56 ± 11%) was not significant (p > 0.05). Thus, the susceptibility of patients’ monocytes to IFN-γ with regard to DR antigen induction seems to be normal. However, as shown in Fig 2b, the action of IFN-γ was prevented by autologous serum (10%) in 10 of 12 patients (24 ± 13%). This inhibitory activity in the patients’ sera was not genetically restricted since sera of psoriatic patients also reduced the DR antigen expression on several allogeneic monocytes (not shown). However, the IFN-γ effect on monocytes of healthy donors was rarely influenced by the sera of patients; in only 3 of 12 healthy donors the proportion of DR + monocytes was significantly decreased by sera of patients in the presence of IFN-γ. The mean value of DR + monocytes of healthy donors in the presence of both IFN-γ and sera of patients was 58 ± 12%, that is, 15% inhibition vs 57% inhibition in the group of psoriatic patients (p < 0.01).

Characteristics of the Inhibitory Activity in the Sera of Psoriatic Patients. It has been shown that prostaglandins (PG) of the E series inhibit the IFN-γ effect on class II MHC antigen expression on monocytes/macrophages [1,13]. Furthermore, we have demonstrated that reactive oxygen intermediates induced by phagocytosis also reduced the DR antigen expression on monocytes [13]. We tested whether the inhibitory activity in the sera of patients could be abolished by the addition of reducing agents such as 2-mercaptoethanol, scavengers of reactive oxygen intermediates such as SOD and β-carotene, or the inhibitor of PGE synthesis, indomethacin.

As shown in Table II, the inhibitory effect of the sera of psoriatic patients on the IFN-γ-mediated DR antigen expression can be prevented by SOD, 2-mercaptoethanol, and indomethacin.

Studies on the kinetics of the DR antigen suppression by these sera revealed that when sera of psoriatic patients were added to the monocyte cultures 1 day later than IFN-γ, no suppressive effect was observed (data not shown).

DISCUSSION

Peripheral blood monocytes from patients suffering from psoriasis expressed fewer DR antigens on their surfaces than monocytes from healthy donors (Fig 1). In order to explain this, we tried to detect disturbances in the production or function of IFN-γ on psoriatic patients.

The results show that an insufficient IFN-γ synthesis by lymphocytes (Table I) and/or a diminished IFN-γ response by monocytes (Fig 2a) could not be the reason for the in vivo reduced DR antigen expression on monocytes of psoriatic patients. However, Fig 2b shows that the patients’ sera contain a factor—or maybe more—which neutralizes the effect of IFN-γ. Probably, this serum factor induces a state of diminished susceptibility to IFN-γ, by raising the intracellular level of cAMP directly or indirectly as described for lipopolysaccharides, zymosan, or PGE [1,2,13].

Indeed, the cyclooxygenase inhibitor indomethacin prevented the inhibitory effect of these sera (Table II), and this could indicate that the induction of PG synthesis in the monocytes of psoriatic patients by their sera could be the cause of the inhibition of the DR antigen expression. Evidently, the IFN-γ effect on monocytes of healthy donors was only rarely affected by the sera of psoriatic patients. Obviously, the monocytes of healthy volunteers are more resistant to the inhibitory signal mediated by the patients’ sera and that can imply that monocytes of psoriatic patients differ from those of normal persons in their functional state.

The nature of the inhibitory factor(s) is not clear. The serum-mediated DR antigen inhibition can be prevented by addition of 2-mercaptoethanol or SOD (Table II). This effect is of special interest as it implicates a mechanism analogous to the concanavalin A-derived soluble immune response suppressor (SIRS), a 55,000-dalton glycoprotein produced by concanavalin A-activated T cells which blocks antibody response and mitogen-induced proliferation in vitro and in vivo [14,15]. It is released by suppressor T cells in an inert precursor form and is converted into the active moiety upon oxidation by H2O2 derived from stimulated monocytes [14]. The inhibitory effect of SIRS on various immune functions can be inhibited by reagents which suppress the formation of the monocyte-modified soluble factor (catalase, cyanide) or inactivate it (reducing agents like 2-mercaptoethanol) [14,15]. The SIRS can also induce PGE synthesis [14]. Remarkably, SIRS can be produced by T cells incubated with IFN-α instead of concanavalin A [14].

Recently, IFN-like activity has been detected in both the skin and the blood of psoriatic patients [7,8]. The presence of IFN in the circulation is possibly responsible for the production of a SIRS-like lymphokine by the lymphocytes of patients; activated further by monocytes, this active moiety is probably able to induce PGE synthesis in monocytes, and that could provide us with an explanation for the decreased proportion of DR + monocytes in psoriatic patients described above. Since similar defects in DR antigen expression on monocytes are observed in quite disparate conditions like systemic lupus erythematosus, infections with Mycobacterium leprae, sepsis, and psoriasis [1,16-18], it is quite
unlikely that they are the actual cause of the disease. The possibility that a chronic inflammatory process somehow leads to these effects is at least as likely. These observed phenomena may be the expression of an attempted contraregulation against the autoimmune and/or chronic inflammatory process in the organism. An effective therapy of psoriasis could be an enhancement of the natural regulatory processes, if we know more about them. At present, we are studying the mediators of biologic immunosuppression and the mechanisms of their induction.

Experiments are in progress to investigate whether the effects described in this paper are also detectable in the skin (epidermal cell culture, suction blister fluid).

REFERENCES


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