Treatment of Peripheral Blood Mononuclear Cells with 8-Methoxypsoralen plus Ultraviolet A Radiation Induces a Shift in Cytokine Expression from a Th1 to a Th2 Response

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Treatment with 8-methoxypsoralen plus ultraviolet A radiation and extracorporeal photochemotherapy (photopheresis) are widely used for the treatment of psoriasis and other inflammatory skin diseases, graft-versus-host disease, and mycosis fungoides. As the ratio of Th1 and Th2 cells appears to be critical for pathogenesis and progression of these disorders the effect of psoralen plus ultraviolet A on Th1 and Th2 cytokine production by CD4+ lymphocytes was investigated. Human peripheral blood lymphocytes were incubated in the presence of anti-CD3, rh-IL2, and rh-IL4 for 48 h. After subsequent stimulation with rh-IL2 and rh-IL4 for 72 h cells were treated with 8-methoxypsoralen (100, 500, 1000 ng per ml) plus ultraviolet A (2 J per cm²) and incubated for a further period of 5 h in the presence of ionomycin, phorbol-12-myristate acetate and monensin.

Fluorescence-activated cell sorter analysis revealed a significant reduction of interleukin-2- and interferon-γ-producing CD4+ cells upon psoralen plus ultraviolet A treatment depending on the concentration of 8-methoxypsoralen. In contrast, interleukin-4-producing CD4+ cells were increased, indicating a shift from Th1 to a Th2 cell cytokine profile upon psoralen plus ultraviolet A treatment. These results indicate that 8-methoxypsoralen photochemotherapy of lymphocytes is able to modulate their Th1/Th2 distribution. Inhibition of Th1 cytokine expression by psoralen plus ultraviolet A might help to explain its beneficial effects in the treatment of Th1 dominated skin diseases. Key words: flow cytometry/interferon-γ/interleukin-2/interleukin-4. J Invest Dermatol 116:459–462, 2001
Biochemical, NJ) to allow cell disaggregation. Cell viability was checked flow cytometrically by propidium iodide (PI) exclusion stain. Briefly, PI incubated for 5 min at 37°C. Cells were washed and resuspended in PBS (Bio Whittaker) for FACS analysis.

Cell culture and prestimulation Isolated cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 100 U per ml streptomycin and penicillin, and 2 mM glutamine (all Bio Whittaker, Vervier, Belgium) at a density of 2 × 10^6 cells per ml. They were activated with 25 μg per ml anti-CD3 antibody (Anellon, Bayport), 10 ng per ml rh-IL-2, and 10 ng per ml rh-IL-4 (R&D Systems, Abingdon, U.K.) for 2 d.

RESULTS AND DISCUSSION

Viability of PBMC after 8-MOP/UVA is independent of psoralen concentrations Viability of prestimulated PBMC was determined by dye exclusion and FACS analysis between 0 h and 48 h after exposure to 8-MOP/UVA (Fig 1). Neither 8-MOP alone nor UVA alone had an effect on cell survival. In accordance with what has been described earlier for lymphocytes after photopheresis, a time-dependent increase in the percentage of dye-uptaking cells was observed after 8-MOP/UVA treatment (Edelson et al., 1987). This effect was independent of 8-MOP concentration at a range between 100 ng per ml and 1000 ng per ml, indicating that, at least in our system, photopheresis-induced cell death and immunomodulation (see below) are not necessarily linked and that it is possible to increase 8-MOP levels without a concomitant increase in cytotoxicity.

At 5 h after UVA exposure, the time point of cytokine determination, cytotoxicity was the same as in the control samples, namely 4%–5%.

8-MOP/UVA leads to an increase in IL-4-producing Th2 cells and a concomitant decrease of IL-2- and IFN-γ-producing Th1 cells PBMC were pretreated as described in Materials and Methods and exposed to increasing doses of 8-MOP and UVA (2 J per cm^2). After blocking of cytokine secretion with antibody cocktails (all Pharmingen); mouse antihuman anti-CD4, mouse-IgG1, mouse-anti-human IL-4, rat anti-human IL-2R, mouse anti-human IFN-γ (all Pharmingen); mouse antihuman CD4, mouse-IgG1 fluorescence controls (Becton Dickinson). Multicolor staining for cell surface antigens and intracellular cytokines Cells from the different incubations were divided into FACS staining vials (Falcon, Becton Dickinson) and further treated with 8-MOP concentrations (100, 500, and 1000 ng per ml) and UVA (2 J per cm^2). After blocking of cytokine secretion with antibody cocktails (all Pharmingen); mouse antihuman anti-CD4, mouse-IgG1, mouse-anti-human IL-4, rat anti-human IL-2R, mouse anti-human IFN-γ (all Pharmingen); mouse antihuman CD4, mouse-IgG1 fluorescence controls (Becton Dickinson). Multicolor staining for cell surface antigens and intracellular cytokines Cells from the different incubations were divided into FACS staining vials (Falcon, Becton Dickinson) and further treated with 8-MOP concentrations (100, 500, and 1000 ng per ml) and UVA (2 J per cm^2). After blocking of cytokine secretion with antibody cocktails (all Pharmingen); mouse antihuman anti-CD4, mouse-IgG1, mouse-anti-human IL-4, rat anti-human IL-2R, mouse anti-human IFN-γ (all Pharmingen); mouse antihuman CD4, mouse-IgG1 fluorescence controls (Becton Dickinson). Multicolor staining for cell surface antigens and intracellular cytokines Cells from the different incubations were divided into FACS staining vials (Falcon, Becton Dickinson) and further treated with 8-MOP concentrations (100, 500, and 1000 ng per ml) and UVA (2 J per cm^2). After blocking of cytokine secretion with antibody cocktails (all Pharmingen); mouse antihuman anti-CD4, mouse-IgG1, mouse-anti-human IL-4, rat anti-human IL-2R, mouse anti-human IFN-γ (all Pharmingen); mouse antihuman CD4, mouse-IgG1 fluorescence controls (Becton Dickinson). Multicolor staining for cell surface antigens and intracellular cytokines Cells from the different incubations were divided into FACS staining vials (Falcon, Becton Dickinson) and further treated with 8-MOP concentrations (100, 500, and 1000 ng per ml) and UVA (2 J per cm^2). After blocking of cytokine secretion with antibody cocktails (all Pharmingen); mouse antihuman anti-CD4, mouse-IgG1, mouse-anti-human IL-4, rat anti-human IL-2R, mouse anti-human IFN-γ (all Pharmingen); mouse antihuman CD4, mouse-IgG1 fluorescence controls (Becton Dickinson).
dependent on the 8-MOP concentration. At 1000 ng per ml a maximal induction of 338% and 240% of control levels was observed.

The percentage of IL-2+CD4+ and IFN-γ/CD4+ cells was determined after stimulation of the 8-MOP/UVA-treated cells with PMA and ionomycin in the presence of monensin. In two independent experiments we observed a decrease in the number of IL-2+ as well as IFN-γ/CD4+ cells that was inversely related to the 8-MOP concentration (Fig 2b, c). At 1000 ng per ml 8-MOP the number of IL-2+ producing Th cells was reduced to 5% and 15% of control levels. IFN-γ-producing cells were reduced to 19% and 62% of controls.

8-MOP alone (see below, Fig 3) had no effect on the production of IL-4, IL-2 and IFN-γ by CD4+ T-cells.

8-MOP/UVA-induced Th1/Th2 skewing occurs at therapeutically relevant 8-MOP concentrations. As described above, the effect of 8-MOP/UVA on the distribution of Th1/Th2-cytokine-producing CD4+ T-cells is more pronounced at 8-MOP levels that are usually not achieved in the clinical setting. Serum levels in photochemotherapy are usually in the range of 50 ng per ml to 200 ng per ml. To investigate the influence of therapeutically relevant 8-MOP concentrations on Th1/Th2 distribution, PBMC from six healthy volunteers were exposed to UVA (2 J per cm²) and 8-MOP (100 ng per ml). Each individual sample served as its own control. We could demonstrate a small but significant increase of IL-4+/CD4+ cells from 4.4% ± 2.6% to 5.9% ± 3.0% (mean ± SD, Fig 3a). Neither 8-MOP nor UVA alone had a significant effect. For IL-2+/CD4+ cells we observed a significant reduction from 11.0% ± 4.8% to 9.02% ± 4.2% and to 9.2% ± 4.4% (mean ± SD) for UVA alone and 8-MOP/UVA, respectively (Fig 3b). 8-MOP alone had no effect. Although with our relatively small sample of donors the downregulation of IL-2 by UVA alone might be a chance finding a specific effect of UVA on IL-2-producing CD4+ T-cells cannot be ruled out, particularly as effects of low doses of UVA on lymphocytes have been described earlier (Yoo et al, 1996).

In parallel with IL-2 also IFN-γ/CD4+ T-cells were significantly reduced from 7.2% ± 3.7% to 5.3% ± 3.0% (Fig 3c). UVA and 8-MOP alone had no effect on IFN-γ production.

The observed shift towards a Th2 expression pattern is weak at 100 ng per ml 8-MOP, the currently recommended therapeutic serum level in PUVA and photopheresis. Thus, at least in clinical conditions where the enhancement of Th2 responses might be therapeutically desirable, higher 8-MOP levels might improve the efficacy of photochemotherapy. This might be particularly true for photopheresis where the ex vivo exposure conditions are similar to our in vitro model. In photopheresis 8-MOP can be applied extracorporeally to the treatment bag without dose-limiting side-effects, making this treatment variant particularly suitable for dose escalation studies (Knobler et al, 1993).

Inhibition of IL-2 by photochemotherapy has been described earlier in murine spleen cells and in lymphocytes from psoriatic patients (Okamoto et al, 1985, 1987; Vonderheid et al, 1990). In contrast, the production of IL-2 and IFN-γ was transiently increased after in vitro PUVA of PBMC from healthy donors (Tokura et al, 1999). In this study a concomitant decrease of IL-4 and IL-10 was observed. This study provides only indirect evidence for the effect of photochemotherapy on Th1/Th2 distribution, however, as the methods used do not allow for the phenotypical characterization of the cytokine-secreting cells. Similarly, a reversal of the Th2 to a Th1 cytokine expression pattern was observed in
patients with cutaneous T cell lymphomas under photopheresis (Di Renzo et al, 1997). It is unclear whether this change in cytokine secretion is a direct effect of photochemotherapy, however, or simply reflects the disappearance of the malignant Th2 clone from the circulation. Another study showed by competitive polymerase chain reaction that in a T cell line (HUT-87) derived from a patient with Sezary syndrome photochemotherapy can increase IFN-γ mRNA (Saed and Fivenson, 1994). Clearly this type of model is useful for the investigation of the molecular mechanisms of PUVA-induced gene regulation but cannot be applied to the clinical setting. Thus, to generalize that 8-MOP/PUVA induces a shift towards a Th1 response in diseases other than mycosis fungoides and Sezary syndrome is not possible. Our results provide evidence that under different experimental conditions 8-MOP/PUVA can have an opposite effect, namely the suppression of Th1 and induction of Th2 cytokines from CD4+ lymphocytes. The clinical efficacy of photopheresis and PUVA in the treatment of graft-versus-host disease is within the context of these observations (Greinix et al, 1998). In particular the acute form of the disease is characterized by a Th1 response of graft-T cells against host tissues (Ferrara and Krenger, 1998) and according to our data photochemotherapy might help restore the protective function of Th2 cells. Another disease where a Th1 predominance has been recently described in lesional skin as well as in circulating T cells is psoriasis (Austin et al, 1999). Here the effect of PUVA might be at least in part mediated through exposure of skin-resident lymphocytes and their reversal from Th1 to Th2. Modification of the activity of other cytokine-producing cells in the skin and the peripheral blood such as keratinocytes, dendritic cells, other lymphocytes (B cells, CD8+ T cells), and macrophages might further influence the immunomodulatory effect of photochemotherapy and may thus help explain the varied clinical effects ascribed to photochemotherapy.

Future experimental studies should identify the conditions that determine the effect resulting from 8-MOP photochemotherapy and elucidate the molecular basis of these differing cellular responses. Clinical research should integrate the results obtained in order to design the optimal conditions for photochemotherapy-induced T cell modulation.

REFERENCES

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Figure 3. 8-MOP/PUVA-induced Th1/Th2 skewing occurs at therapeutically relevant 8-MOP concentrations. PBMC from six healthy donors were treated as indicated in Materials and Methods prior to exposure to 8-MOP (100 ng/ml) and UVA (2 J/cm2). Cytokine-expressing CD4+ cells were determined by FACS analysis 5 h after 8-MOP/UVA. Bars represent mean ± SD; asterisks indicate statistical significance compared with untreated controls at a significance level of p < 0.05 (paired t test). (a) IL-4; (b) IL-2; (c) IFN-γ.