

## INVESTIGATIVE REPORT

# Cytokine Profile of Patients with Mycosis Fungoides and the Immunomodulatory Effect of AS101

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Cytokines are known to play a major role in the pathogenesis of mycosis fungoides, a cutaneous malignant neoplasm of CD 4 T cells. In the present study, we investigated the effect of AS101, a tellurium-based compound with immunomodulating properties, on the pattern of lymphokine production by peripheral blood mononuclear cells (PBMCs) from patients with mycosis fungoides. PBMCs were isolated from 35 patients with mycosis fungoides stage IA and IB before initiation of treatment and from 20 healthy sex and age-matched controls. Unstimulated and phytohaemagglutinin-stimulated PBMCs were tested with and without the addition of AS101. The production of interferon- $\gamma$ , interleukin 2 (IL-2), IL-2 receptor (IL-2R), interleukin 5 (IL-5) and interleukin 10 (IL-10) was determined by enzyme-linked immunosorbent assays. The effects of AS-101 on mycosis fungoides PBMCs were compared to those of healthy donor PBMCs. Significantly higher levels of IL-2R, IL-5 and IL-10 and significantly lower levels of interferon- $\gamma$  were found in the patients compared to the controls. There was no significant difference between the groups in the production of IL-2. AS101 inhibited the production of IL-2R, IL-5 and IL-10 and induced a significant increase in IL-2 levels in the mycosis fungoides PBMCs. These findings may have important clinical implications for the possible therapeutic benefit of AS101 in mycosis fungoides. **Key words:** mycosis fungoides; immunomodulation; T-helper 2 profile; T-helper 1 profile; AS101.

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Mycosis fungoides (MF) is a lymphoproliferative disorder characterized by infiltration of the skin with clone-derived malignant CD4+ lymphocytes which phenotypically resemble mature T helper (Th) cells (1–3). Cytokines are considered to play a major role in the pathogenesis of MF (2, 4, 5). The skin manifestations are divided into clinicopathologic stages of increasing aggression: patch, plaque and tumour (4, 6, 7). Saed et al. (8) reported that the cutaneous lesions of plaque stage MF are characterized by an epidermal Th1-type cytokine profile, whereas Vowels et al. (5) detected a Th2 cytokine profile in some plaque biopsies and in all tumour-stage biopsies. MF progression has been associated with increasing cutaneous overexpression of IL-10 mRNA (9).

AS101, a tellurium-based compound with known immunomodulating properties, has been shown to inhibit the release of IL-10 by lipopolysaccharide-stimulated mouse peritoneal macrophages and human monocytes and to block the

transcription of IL-10 mRNA (10). It also enhances the production of interleukin-2 (IL-2), tumour necrosis factor and interferon-gamma (IFN- $\gamma$ ) by human mononuclear cells (11–15). The effects of AS101 on interleukin 5 (IL-5) production in general and on peripheral blood mononuclear cells (PBMC) in MF have not been studied previously.

In the present study, we investigated the cytokine profile of resting and activated PBMC in patients with MF and the effect of AS-101 on this profile compared with healthy donor PBMCs.

## MATERIALS AND METHODS

### Subjects

The study population, comprising 35 patients (25 males and 10 females) aged between 40 and 75 years, were diagnosed based on clinical, histopathological and immunohistologic criteria (16). All patients were in either stage IA or IB before initiation of treatment and had no atopic diseases. Twenty healthy age- and sex-matched subjects served as the control group.

### Methods

AS-101 was prepared by diluting a sterile solution of this compound, which was supplied by Wyeth-Ayerst, Randor, PA, and licensed by IVAX, Miami, FL, USA.

Blood samples were drawn from all participants, and PBMCs were isolated on Ficoll-Paque gradients and cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100  $\mu$ /ml penicillin and 100  $\mu$ /ml streptomycin. The cells were incubated in 24-well flat-bottomed culture plates at 37°C in a humidified atmosphere with 5% CO<sub>2</sub> for 48 h. Phytohaemagglutinin (PHA) (Ta'asiot Biologiot, Beit Haemek, Israel) 0.5% was used as a stimulus. Supernatants were harvested at 48 h and stored at –70°C until tested. IL-10, IFN- $\gamma$ , IL-2, IL-2 receptor (IL-2R) and IL-5 were measured with enzyme-linked immunosorbent assay (ELISA) kits (Endogen, Inc., Woburn, MA, USA) in accordance with the manufacturer's instructions. All samples were run in duplicate. AS101 was added at a concentration of 0.5  $\mu$ g/ml, as this concentration was found to yield optimal effects. Statistical analysis was performed with Student's *t*-test. The results are presented as means  $\pm$  SD. The detection limits for IL-10 were 3 pg/ml for IFN $\gamma$ , 2 pg/ml for IL-2, 6 pg/ml for IL-2R 24 u/ml and 2 pg/ml for IL-5 $\gamma$ .

## RESULTS

The mean levels and SD of IL-10, IFN- $\gamma$ , IL-2, IL-2R and IL-5 production by PBMC obtained from the MF patients and healthy controls are shown in Fig. 1A–E. As no significant difference in cytokine release was noted between the PBMCs obtained from the MF patients, stage IA and IB, we analysed all the patients as a single group.

For IL-2, IL-2R and IL-5, the effect of AS101 was tested only on PHA-stimulated PBMCs, as unstimulated PBMCs showed undetectable levels of these cytokines. The levels of

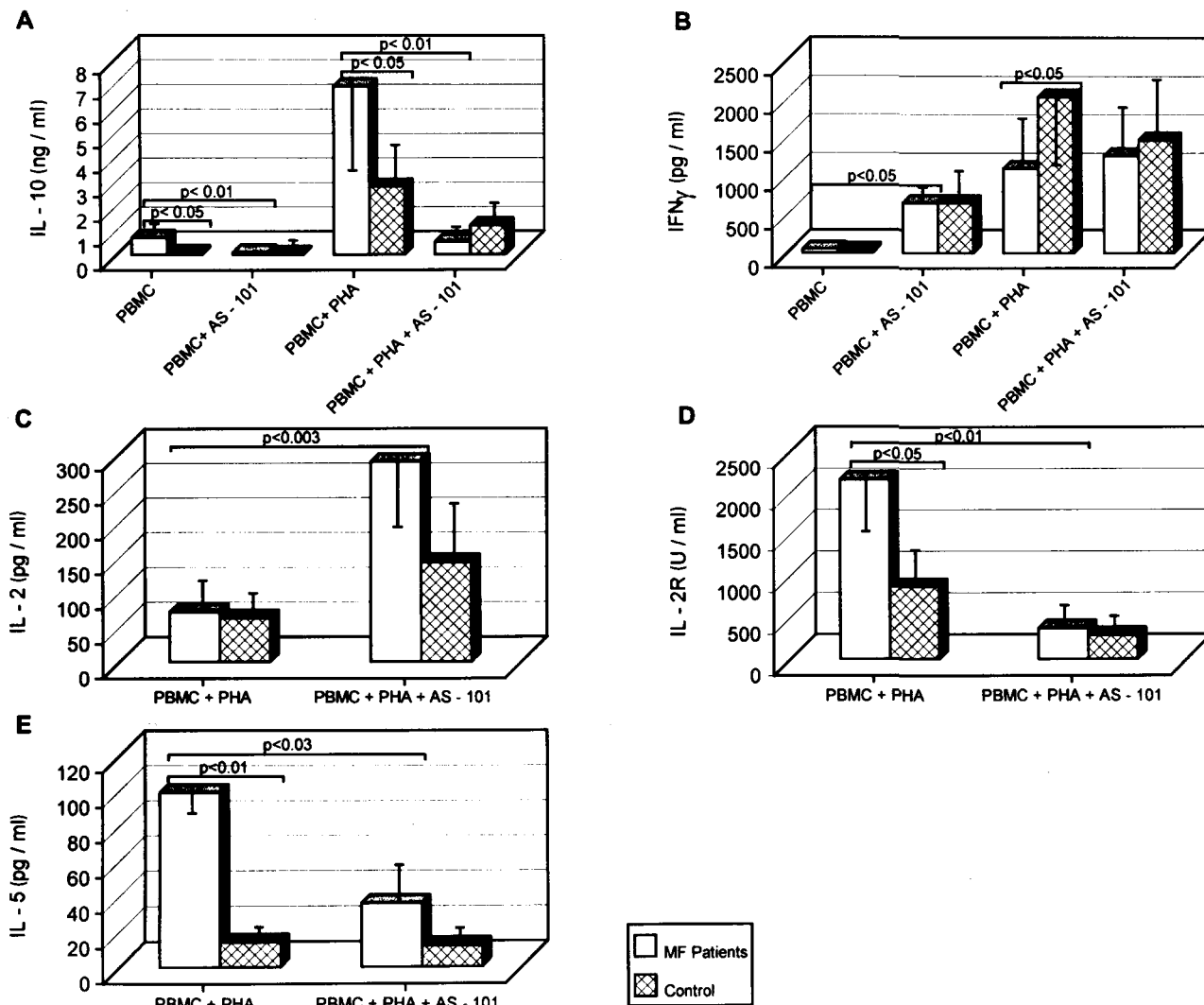


Fig. 1. Cytokine levels (mean ± SD) in resting and activated peripheral blood mononuclear cells (PBMCs) of patients with mycosis fungoides (MF) and healthy donors and the effect of AS-101. (A) IL-10 production is significantly higher in resting and activated PBMCs of MF patients and is inhibited by AS-101; (B) IFN-γ production is lower in activated cells of patients with MF, and significantly induced by AS101 in resting PBMCs of MF patients; (C) IL-2 production is similar in MF patients and controls but significant enhancement by AS101 is seen in PBMCs of MF patients; (D) Release of soluble IL-2R is significantly higher in PBMCs of MF patients and significantly inhibited by AS101; and (E) IL-5 production is significantly higher in PBMCs of MF patients and significantly inhibited by AS-101.

IL-10 and IFN-γ were detectable in both stimulated and unstimulated PBMCs of MF patients.

IL-10 production was significantly higher in both unstimulated and PHA-stimulated PBMCs from the patients with MF ( $p < 0.05$ ), and was significantly decreased by AS101 from a mean of  $6.89 \pm 3.24$  ng/ml in PHA-stimulated PBMC to only  $0.52 \pm 0.14$  ng/ml ( $p < 0.01$ ) (Fig. 1A), and from a level of  $0.70 \pm 0.361$  in unstimulated PBMCs of MF to a level of only  $0.09 \pm 0.004$  ( $p < 0.01$ ).

IFN-γ production was significantly lower in PHA-stimulated PBMCs from patients with MF (mean  $1099 \pm 552$  pg/ml) compared to controls (mean  $2032 \pm 860$  pg/ml) ( $p < 0.05$ ). AS101 significantly increased the spontaneous release of IFN-γ by unstimulated PBMCs of patients with MF ( $p < 0.05$ ), but not of PHA-stimulated PBMCs (Fig. 1B). IL-2 production by PHA-stimulated PBMCs was similar in the two groups, but the addition of AS101 induced a fourfold increase in the MF PBMCs, from a mean of  $72 \pm 34$  pg/ml to  $288 \pm 109$  pg/ml

(Fig. 1C). This difference was highly significant ( $p < 0.003$ ). No significant difference was found in the control group after addition of AS-101. Soluble IL-2R and IL-5 production by the PHA-stimulated PBMCs was higher compared to controls ( $p < 0.05$  and  $p < 0.01$ , respectively). AS101 significantly inhibited the L-2R and IL-5 release by the MF PBMCs ( $p < 0.01$  and  $p < 0.03$ ), respectively (Fig. 1D, E), but had no effect on IL-5 of the healthy control cells (Fig. 1E). The cytokine values were normally distributed in each single group of both MF patients and controls.

DISCUSSION

The progression of MF has been found to be associated with increasing overexpression of mRNA IL-10 in cutaneous lesions (9). As IL-10 suppresses the production of IFN-γ, it may be assumed that the stage-dependent decrease in IFN-γ mRNA expression observed in tumour stage MF compared to the

patch and plaque stage (5, 9) may be the result of IL-10 enhancement. Previous studies have shown that the transcription of IL-10 mRNA can be blocked by AS101 (8, 10). Saed et al. have tested the blood cytokine mRNA levels in patients with MF and reported no difference in cytokine pattern between MF and healthy controls (8). However, our study differs from theirs in that we tested the production of five different cytokines by PHA-stimulated PBMCs. We found a significantly lower release of IFN- $\gamma$  and a significantly higher production of IL-10, IL-5 and IL-2R, while no difference was found in the IL-2 production in PHA-stimulated PBMC from patients with MF compared to controls. The decrease shown in IFN- $\gamma$  is in agreement with the findings of Lee et al. (17) and of Vowels et al. (5) in stimulated MF and Sézary cells; however, they did not test IL-10 production.

We did not find any defect in the ability of PBMCs of patients with MF to synthesize IL-2. In a study by Rook et al. (18), the poor IL-12 production by PBMCs from patients with Sézary syndrome increased five- to sixfold with the addition of neutralizing monoclonal anti-IL-10 antibodies. These authors also detected a significant increase in IFN- $\gamma$  production by the Sézary syndrome PBMCs when the IL-10 was neutralized (18). Our finding of a more than twofold increase in soluble IL-2R release by stimulated PBMCs of MF patients compared to controls is in accordance with that of Wasik et al. (19), who showed an increased serum concentration of soluble IL-2R in cutaneous T-cell lymphoma.

The different effects of AS101 on PBMCs from patients with MF and normal healthy controls can be explained by its immunomodulator properties, which enable it to act in opposite ways (10).

We conclude that there is a Th2-type cytokine production in MF stage IA and IB PBMCs in response to PHA stimulation similar to that found in Sézary syndrome. AS101, by inhibiting IL-10 production, may induce an increase in IFN- $\gamma$  production and a shift from Th2 to a Th1-type cytokine profile. This finding has important implications for the possible therapeutic role of AS101 in MF.

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