Psoriasis is a chronic inflammatory skin disease characterized by abnormal epidermal proliferation. Several studies have shown that skin-infiltrating activated T cells and cytokines play a pivotal role during the initiation and maintenance of the disease. Interferon (IFN)-α plays an important role in host defense against infections, but recent data have also implicated IFN-α in psoriasis. Thus, IFN-α induces or aggravates psoriasis in some patients, and mice lacking a transcriptional attenuator of IFN-α/β signaling spontaneously develop a psoriasis-like inflammatory skin disease characterized by CD8⁺-infiltrating T cells. In this study, we therefore investigate IFN-α signaling in T cells isolated from involved skin of psoriatic patients. We show that psoriatic T cells have increased and prolonged responses to IFN-α, on the level of signal transducers and activators of transcription (STAT) activation, compared with infiltrating T cells from skin of non-psoriatic donors. Functionally, the increased IFN-α signaling leads to an increased binding of STAT4 to the IFN-γ promotor, IFN-γ production, and inhibition of T cell growth. In contrast, STAT responses to other cytokines were not changed in psoriasis. In conclusion, we provide evidence that psoriatic T cells have an increased sensitivity to IFN-α. Thus, our data suggest that increased IFN-α signaling is involved in the pathogenesis of psoriasis.

Key words: cytokines/inflammation/signal transduction/skin/T lymphocytes

Psoriasis is a chronic inflammatory skin disease affecting around 2% of the global population. It is characterized by T-lymphocyte infiltration and hyperproliferation of the epidermis in involved areas of the skin (Lew et al, 2004). Of the infiltrating lymphocytes, both CD4⁺ Th1 cells and CD8⁺ cytotoxic T cells have been reported to play a pathogenic role (Austin et al, 1999; Friedrich et al, 2000), but also cells expressing NK and NK-T cell markers have recently been found in psoriatic plaques (Nickoloff et al, 2000; Cameron et al, 2002). By their production of Th1 and pro-inflammatory cytokines, especially the type II interferon (IFN), IFN-γ, and growth factors, the T cells contribute to the activation and hyperproliferation of keratinocytes (Nickoloff, 1987; Badadsgaard et al, 1990; Fierlbeck et al, 1990; Strange et al, 1993; Uyemura et al, 1993; Schlaak et al, 1994; Szabo et al, 1998; Bonifati and Ameglio, 1999). Indeed, it is now well established that skin-infiltrating activated T cells and the local overexpression of pro-inflammatory cytokines play a pivotal role during initiation and maintenance of the disease (White et al, 1972; Baker et al, 1984; Gottlieb et al, 1995; Wrone-Smith and Nickoloff, 1996; Lew et al, 2004).

The type I IFNs, IFN-α/β, are believed to play an important role in host defense against viral and bacterial infections and are involved in many immunoregulatory processes (Brassard et al, 2002). Because of its antiviral and antiproliferative activity on many cell types, IFN-α has proved to be useful for treating several clinical conditions, including chronic viral hepatitis and chronic myeloproliferative and lymphoproliferative disorders (Brassard et al, 2002). IFN-α transduces signals directly to the nucleus through rapid activation of the Janus kinase (JAK)/signal transducers and activators of transcription (STAT) signaling pathway. By binding of IFN-α to its high-affinity receptor, receptor-associated JAKs become activated by tyrosine phosphorylation. Once activated, the JAK phosphorylate key tyrosine residues in the cytoplasmic receptor tails, creating docking sites for STAT transcription factors. Upon tyrosine phosphorylation by JAKs, the recruited STATs dimerize and translocate to the nucleus where they induce transcription of cytokine-inducible genes (Kisseleva et al, 2002). IFN-α induces activation of Tyrosine kinase 2/JAK1 bound to the IFN-α receptor (IFNAR)-1/IFNAR-2, respectively (Kotenko and Pestka, 2000). IFN-α stimulation thereby leads to activation of several STAT proteins, including STAT1, STAT2, and STAT4. Once activated, STAT1/STAT2 heterodimers together with interferon regulatory factor (IRF)-9, thereby constituting the IFN-stimulated gene factor 3 complex, bind to the IFN-stimulated response element in the promotor region.
of IFN-α-inducible genes, thereby assisting in their transcriptional activation (Kotenko and Pestka, 2000). STAT4 has been demonstrated to bind to the promoter of IFN-γ gene, and assists in the regulation of its transcription (Xu et al, 1996).

Compared with the well-studied role of IFN-γ, little is known about the eventual role of IFN-α in the pathogenesis of psoriasis. Nevertheless, several observations suggest that IFN-α might be involved. The use of IFN-α as a therapeutic agent in some susceptible individuals has led to the development or aggravation of psoriasis (Conlon et al, 1990; Funk et al, 1991; Pauluzzi et al, 1993; Brenard, 1997; Calvino et al, 1999; Erkek et al, 2000), and the association between psoriasis and chronic viral infections has been reported (Yamamoto et al, 1995; Erkek et al, 2000). Furthermore, the immunoregulatory actions of IFN-α include upregulation of type 1 immune responses through the direct or indirect enhancement of differentiation and activation of both CD4+ Th1 cells and CD8+ Tc1 cells, and NK cell activation (Ortaldo et al, 1980; Brinkmann et al, 1993; Tough et al, 1996; Brassard et al, 2002). Finally, mice lacking a transcriptional attenuator of IFN-α/β signaling, the IRF-2 protein (IRF-2−/− mice), have recently been shown to develop psoriasis-like inflammatory skin disease, which was dependent on CD8+ -infiltrating T cells, and was caused by uncontrolled IFN-α/β signaling (Hida et al, 2000). Given the crucial role of T cells in the onset and maintenance of the disease, this study was therefore undertaken to investigate IFN-α signaling in T cells isolated from involved skin of psoriatic patients. We show that psoriatic T cells have increased and prolonged responses to IFN-α, on the level of STAT activation, compared with infiltrating T cells from skin of non-psoriatic donors. Functionally, the increased IFN-α signaling leads to an increased binding of STAT4 to the IFN-γ promoter, IFN-γ production, and inhibition of T cell growth. In contrast to the abnormal IFN-α response, STAT responses to other cytokines were not changed in psoriasis. We hereby provide evidence that psoriatic T cells have an increased sensitivity to IFN-α. Our data thus suggest that increased IFN-α signaling is involved in the pathogenesis of psoriasis.

**Results**

Psoriatic T cells show increased IFN-α-induced signaling In order to study IFN-α signaling in psoriatic T cells, we first examined the IFN-α-induced STAT activation in a CD8+ T cell line, Psor-2, isolated from involved skin of a psoriatic patient, and compared it with the activation in a skin-infiltrating CD8+ control T cell line, Control-1, from a non-psoriatic donor. As shown in Fig 1A, IFN-α induced a strong tyrosine phosphorylation of STAT1, STAT2, and STAT3 in Psor-2 cells in a concentration-dependent manner. Interestingly, the IFN-α-induced activation of STAT1 and STAT2, and to a lesser extent STAT3, was significantly stronger in Psor-2 cells than in Control-1 cells, even though a comparable amount of STAT and extracellular-signal-regulated kinase (ERK) protein was present in each lane. Essentially, similar responses were obtained when comparing psoriatic T cells with CD8+ -infiltrating skin cells from other donors without psoriasis (Fig 1B, data not shown). Interestingly, the activation of STAT4 was also increased in psoriatic T cells (Fig 1B). In contrast to IFN-α, interleukin (IL)-2-induced tyrosine phosphorylation of STAT5 was not significantly increased (Fig 1C), suggesting that psoriatic T cells did not have an increased response to cytokines in general. In human psoriatic keratinocytes, STAT proteins were found to be constitutively active (van der Fits et al, 2004; Sano et al, 2005). In contrast, STAT proteins were not constitutively activated in psoriatic T cells (Figs 1 and 2, data not shown).

Prolonged response to IFN-α in psoriatic T cells To investigate the IFN-α response over time, a kinetic study was performed, where Psor-2 cells were stimulated with IFN-α for different periods of time and compared with the response in Control-2 cells. As shown in Fig 2A, Psor-2 cells responded to IFN-α for longer periods of time, suggesting that psoriatic T cells from this patient also have prolonged

---

**Figure 1** Increased interferon (IFN)-α-induced signaling in psoriatic T cells. (A–C) CD8+ psoriatic T cells, Psor-2, and CD8+ skin-infiltrating control T cells from two different non-psoriatic donors, Control-1 and Control-2, were stimulated with IFN-α or interleukin (IL)-2 as indicated for 10 min and lysed. Total cell lysates were subjected to western blotting using an Ab recognizing tyrosine-phosphorylated signal transducers and activators of transcription (PY-STAT1) or PY-STAT5. The blots were subsequently stripped and reprobed with the indicated Abs (P2, Psor-2; C2, Control-2).
responses to IFN-α. Essentially identical results were obtained in seven independent experiments (Fig 2B, data not shown).

To address whether the difference in IFN sensitivity was because of different levels of IFNAR expression, we compared IFNAR levels in total lysates and on the surface of psoriatic T cells and control T cells. Differences in IFN sensitivity, however, did not seem to be mediated by an increased amount of the IFNAR, as a comparable amount of the IFNAR-1 was found in psoriatic and control T cells (Fig 2C). Similar results were obtained when measuring the surface expression of IFNAR-1 by fluorescence-activated cell sorting (FACS) analysis (Fig 2D). Having found that Psor-2 cells were both more sensitive and had prolonged responses to IFN-α, we wished to address whether this was also true for psoriatic T cells from other patients. We therefore analyzed the IFN-α response in psoriatic T cells isolated from involved skin of three additional patients: Psor-3, from one patient, Psor-19 and -18 from another patient, and Psor-17 and -16 from a third patient. All psoriatic T cells clearly showed increased IFN-α-mediated activation of STAT1, STAT2, STAT3, and STAT4 (Fig 3A–C, data not shown). Again, this correlated with a comparable surface expression of the IFNAR measured by FACS analysis (data not shown). The apparent differences in STAT activation as judged from band intensities between individual experiments are caused by differences in exposure time during enhanced chemiluminescence (ECL) development. This is illustrated in Fig 3B and C where different exposures of STAT1 and STAT2 are shown. Although an increase in exposure time manifests itself as an increase in band intensities, it has no influence on the relative difference in IFN-α responses between patients and controls. Thus, the increased sensitivity to IFN-α in psoriasis is seen at all exposure times (Fig 3B and C). Similar results were also obtained in identical, but independent experiments (Fig 4A, data not shown). As shown recently by Kohlmann et al (2004), expansion of T cell lines in culture might change the characteristics of the cells. Yet, an increased sensitivity to IFN-α is seen in psoriatic T cells from the same patient cultured for a short (<2 wk) and longer (>1 mo) period of time (Fig 4A vs B). In conclusion, these findings support the above observations, that psoriatic T cells have increased sensitivity to IFN-α.

Peripheral blood lymphocytes (PBL) from psoriasis patients show increased IFN-α-induced signaling In order to address whether an increased sensitivity to IFN-α was a unique feature of lesional T cells or a general phenomenon of psoriatic T cells, we compared IFN-α responses in PBL obtained from psoriasis patients and healthy donors (see “Materials and Methods”). As shown in Fig 5, an enhanced STAT1, STAT2, and STAT4 (and to some extent STAT3) activation was observed in PBL from psoriasis patients when compared with healthy donors. Similar amounts of proteins had been loaded in all lanes as illustrated by the band intensities for ERK1/2. The amount of total STAT proteins, however, was also elevated in PBL when compared with healthy donors, indicating that peripheral T cells from psoriasis patients also differ from healthy controls.

Increased STAT–DNA binding to the IFN-γ promoter To address whether the increased IFN-α- induced STAT activation had a functional effect on downstream events, we examined the STAT–DNA binding and cytokine production. Activation of STAT4 triggers binding of STAT4 to the
promotor of the IFN-\(\gamma\) gene (Xu et al., 1996) and STAT4-dependent expression of IFN-\(\gamma\) (Rogge et al., 1998). Accordingly, Psor-2 and Control-2 cells were stimulated with IFN-\(\alpha\) prior to affinity purification with a biotinylated oligonucleotide probe representing a STAT4-binding sequence for the IFN-\(\gamma\) promotor. As shown in Fig 6A and B, IFN-\(\alpha\) induced an increased binding of STAT4 to the IFN-\(\gamma\) promotor in Psor-2 cells when compared with non-psoriatic skin T cells. Likewise, IFN-\(\alpha\) induced an enhanced IFN-\(\gamma\) production in Psor-2 cells when compared with the non-psoriatic skin T cells (Fig 6C).

IFN-\(\alpha\) signaling correlates with increased inhibition of T cell growth As another functional study of increased IFN-\(\alpha\)

Figure 3
Prolonged response to interferon (IFN)-\(\alpha\) in psoriatic T cells from other patients. Psoriatic T cells, Psor-3 (A), Psor-19 (B), Psor-17 (C), and Control-2 cells were stimulated with IFN-\(\alpha\) (10,000 U per mL) for the indicated periods of time. Total cell lysates were subjected to western blotting using the indicated Abs.

Figure 4
Prolonged response to interferon (IFN)-\(\alpha\) in psoriatic T cells cultured for short and longer periods of time. Psoriatic T cell lines from the same patient, Psor-19 and -18, were subjected to short-term culture (A) or long-term culture (B). Psoriatic T cells and Control-2 cells were subsequently stimulated with IFN-\(\alpha\) (10,000 U per mL) for the indicated periods of time. Total cell lysates were subjected to western blotting using the indicated Abs.

Figure 5
Peripheral blood lymphocytes (PBL) from psoriasis patients show increased interferon (IFN)-\(\alpha\)-induced signaling. PBL from psoriasis patients and healthy control donors were stimulated with IFN-\(\alpha\) as indicated for 10 min and lysed. Total cell lysates were subjected to western blotting using the indicated Abs.
response in psoriatic T cells, we looked at the ability of IFN-α to induce growth arrest in human T cells (Balkwill and Oliver, 1977; Brassard et al, 2002). Psor-2 cells and Control-2 cells were incubated with IFN-α in culture medium with IL-2 (1000 U per mL) for 96 h. Cell cultures were subsequently subjected to [3H]thymidine uptake measurement. The median counts per minute from triplicate cultures are depicted as percentage of the [3H]thymidine uptake detected in cells incubated without IFN-α. (B) Psor-2 and Control-2 cells were cultured with IFN-α (10,000 U per mL) for 24 h. Cells were then subjected to quantification of apoptotic cells using the fluorescent DNA-binding dye, 7-aminoactinomycin D. Mean fluorescence intensity (MFI) values from IFN-α-stimulated cells are depicted as change in percent of MFI values detected in cells incubated without IFN-α.

**Discussion**

In this study, we demonstrate that T cells isolated from involved skin and blood of psoriatic patients have increased responsiveness to IFN-α. Thus, IFN-α-induced signaling is increased and prolonged on the level of STAT activation, compared with infiltrating T cells from skin of non-psoriatic donors. The increased IFN-α signaling functionally leads to
an increased STAT–DNA binding, IFN-γ production, and inhibition of T cell growth. Our finding that increased IFN-α signaling is associated with psoriasis is in accordance with a number of observations of psoriasis or exacerbated psoriasis in patients treated with IFN-α, i.e. IFN-α treatment of viral infections (Yamamoto et al., 1995; Erkek et al., 2000), viral hepatitis and myeloproliferative and lymphoproliferative disorders (Conlon et al., 1990; Funk et al., 1991; Pauluzzi et al., 1993; Brenard, 1997; Calvino et al., 1999; Erkek et al., 2000). Moreover, Hida et al. (2000) reported that the development of a psoriasis-like inflammatory skin disease, because of lack of IRF-2 in IRF-2-/- mice, was associated with excessive IFN-α/β signaling, and that this signaling was responsible for the development of the skin disease. By investigating human psoriatic skin, van der Fits et al. (2004) recently demonstrated that the IFN-α/β signaling pathway was indeed constitutively activated in psoriatic keratinocytes. The sensitivity to IFN-α in these keratinocytes was, however, not increased, and in contrast to IFN-γ, IFN-α did not directly induce a psoriatic phenotype on keratinocytes (van der Fits et al., 2004). This suggests that the effect of IFN-α in the pathogenesis of psoriasis might be indirect, and thus might include other cell types involved in the stimulation of keratinocyte hyperproliferation, such as T cells or NK cells. Here, we demonstrate that psoriatic T cells have increased responsiveness to IFN-α, suggesting that T cells mediate the disease-promoting effect of IFN-α. In support of this, CD8+ T cells were directly involved in the pathogenetic process of the psoriasis-like skin disease in IRF-2-/- mice (Hida et al., 2000).

The observation by Hida et al. (2000), that the development of psoriasis-like inflammatory skin disease was dependent on the signaling components IFNAR-1 and IFR-9, suggests a selective involvement of the IFNAR/IFN-stimulated gene factor 3 pathway. In accordance with this, our findings on psoriatic T cells also included an increased IFN-α-mediated STAT1 and STAT2 activation, which, together with IFR-9, constitute the IFN-stimulated gene factor 3 complex. IFN-α-induced STAT3 activation has been linked to T-Bet expression (Hibbert et al., 2003), and recent findings on a constitutive activation of STAT3 in keratinocytes implicated STAT3 in the pathogenesis of psoriasis (Sano et al., 2005). In agreement with these findings, we observed an enhanced activation of STAT3 in psoriatic T cells. Interestingly, we also found an increased IFN-α-mediated activation of STAT4, suggesting that the involvement of IFN-α signaling in human psoriasis includes other IFN-α-mediated signaling pathways. STAT4 is critically involved in the generation of Th1 responses (Kaplan et al., 1996; Magram et al., 1996), and has been shown to bind to the promoter of and thereby regulate the IFN-γ gene (Kaplan et al., 1996; Xu et al., 1996). At the same time, IFN-α is known to upregulate type 1 immune responses by enhancing differentiation and activation of CD8+ T cells (Parronchi et al., 1996; Tougou et al., 1996; Sareneva et al., 1998, 2000), and to induce the development of IFN-γ-producing Th1 cells (Rogge et al., 1998), the latter IFN-α-induced STAT4 of which is believed to play an important role. It is therefore conceivable to speculate that increased sensitivity to IFN-α would induce increased IFN-γ production via STAT4. In this study, we show that increased IFN-α-induced STAT4 activation in psoriatic T cells does indeed correlate with an increased DNA binding to the IFN-γ promoter, and an increased IFN-γ production.

The IFN-α-induced signaling was shown to be increased in psoriatic T cells even though a comparable amount of the IFNAR was found on the surface of psoriatic and control T cells, and comparable amounts of intracellular STAT proteins were present in both cell types. At present, we are unable to explain this increase in IFN-α sensitivity. The increased IFN-α responsiveness in psoriatic T cells might result from a deficient expression or function of proteins regulating the IFN-α signaling pathway. In a recent report (Hida et al., 2000), excessive IFN-α/β signaling and the spontaneous development of psoriasis-like inflammatory skin disease were indeed found in mice lacking a transcriptional attenuator of IFN-α/β signaling. In our study, the IFN-α-induced STAT activation was increased in psoriatic T cells already after 15 min. Our preliminary data suggest that TYK2 activation is also increased in psoriasis. Studies are in progress to address whether the enhanced STAT activation is because of deficiencies in negative regulators, such as tyrosine phosphatases like SH2 domain-containing protein tyrosine phosphatase-1 (David et al., 1995) and suppressors of cytokine signaling proteins (Alexander and Hilton, 2004; Brender et al., 2005).

As a functional study of increased IFN-α response in psoriatic T cells, we looked at the antiproliferative capacity of IFN-α and measured the IFN-α-induced inhibition of IL-2-mediated growth. Indeed, the inhibition of IL-2-mediated growth by IFN-α was significantly stronger in psoriatic T cells (Psor-2 cells) than in corresponding non-psoriatic T cells (Control-2 cells) or T cells isolated from the blood of healthy donors (alloreactive T cells) (Fig 7A, data not shown). These results supported the conclusion above that psoriatic T cells are more sensitive to IFN-α. IFN-α-induced growth-arrested psoriatic T cells, however, did not correlate with an increase in apoptosis (Fig 7B and data not shown). In view of the actual in vivo situation in the local lesions of psoriasis, one could therefore imagine that psoriatic T cells, even under the influence of IFN-α, remain in the skin as viable cells, able to perform their pathogenic function in psoriatic plaques for a long period of time. Indeed, psoriasis-plaque xenografts on severe combined immunodeficiency mice can be preserved for weeks to months and in most cases continue to show the presence of viable T cells, without the need for a continuing supply of fresh T cells from the peripheral circulation (Wron-Smithe and Nickoloff, 1996; Gilhar et al., 1997).

Altogether, these findings on an increased responsiveness of psoriatic T cells to IFN-α, which correlates with an increased STAT–DNA binding to the IFN-γ promoter and IFN-γ production, support and add to the proposed events by Hida et al. (2000) for an involvement of IFN-α in the pathogenesis of psoriasis (Fig 8): (i) recruitment of CD8+ T cells to the skin; (ii) hyper-responsiveness of these T cells to IFN-α, because of a decreased control of the IFN-α signaling; (iii) increased IFN-α-mediated responses, including the production of IFN-γ; and (iv) activation and proliferation of other cell types, such as keratinocytes, which in turn would further activate the T cells.

In conclusion, we provide evidence that T cells from involved skin of psoriatic patients have an increased

941
sensitivity to IFN-α. Thus, our data suggest that increased IFN-α signaling is involved in the pathogenesis of psoriasis.

Materials and Methods

Antibodies and other reagents Recombinant human IFN-α (Introna) was from Schering-Plough (Kenilworth, New Jersey). Recombinant human IL-2 (Prol leukin) was from Chiron (Emeryville, California). Recombinant human IL-4 was from Leinco Technologies (St Louis, Missouri). Rabbit anti-human IFNAR-2 mAb was from Research Diagnostics Inc. (Flanders, New Jersey). FITC-conjugated mouse anti-human IFNAR-2 mAb was from Santa Cruz Biotechnology (Santa Cruz, California). Phospho-specific STAT3 (Tyr704) mAb was from Zymed Laboratories (San Francisco, California). Phospho-specific STAT4 (Tyr693) pAb was from Becton Dickinson (Franklin Lakes, New Jersey). Phospho-specific STAT2 (Tyr689) pAb was from Upstate Biotechnology (Lake Placid, New York). Phospho-specific STAT3 (Tyr704) mAb was from Dendreon Pharmaceuticals (San Diego, California). (The relevant isotype control Ab for the above Abs was used.) Phospho-specific pAb against STAT1 (Tyr701) and STAT5 (Tyr694) was from Cell Signaling Technology (Beverly, Massachusetts). Phospho-specific STAT2 (Tyr689) pAb was from Upstate Biotechnology (Lake Placid, New York). Phospho-specific STAT3 (Tyr704) mAb was from nanoTools (Denzlingen, Germany). Phospho-specific STAT4 (Tyr693) pAb and mAb against STAT4 was from Zymed Laboratories (San Francisco, California). STAT1 (E23), STAT2 (C-20), IFNAR-1 (R-100), and ERK1/2 (K23) pAb were from Santa Cruz Biotechnology (Santa Cruz, California). The ECL kit was from Amersham (Buckinghamshire, UK). DNA-binding dye 7-amino-actinomycin D (7-AAD) was from Sigma (St Louis, Missouri). The biotinylated double-stranded oligonucleotide probe used for affinity purification was: Bio-IFN-γ (5′-Bio-TCTGGTCATTCTCTTTTCCAAAGAAAAGTTT-3′), representing the STAT4-binding sequence derived from the IFN-γ-responsive promoter of the IFN-γ transcript (Xu et al, 1996). (The core STAT4-recognition sequence is underlined.)

Subjects All subjects enrolled in this study had no anti-psoriatic treatment for 2 wk, and no systemic antipsoriatic treatment for at least 4 wk prior to the obtaining of biopsies or blood samples. Nor did they have any concurrent infections or medications. The enrollment of subjects and all experimental procedures were carried out under protocols approved by the medical ethical committees of each participating institution, and informed written consent was obtained from all subjects. All experimental procedures were conducted according to the Declaration of Helsinki Principles.

T cell lines Biopsies taken from psoriatic patients were all taken from the center of lesions with stable chronic plaques. The psoriatic cell lines, Psor-2 and -3, were continuous CD8+^/CD4^/IFNAR-/-/CD25+ T cell lines established from punch biopsy specimens of two different patients with psoriasis vulgaris as previously described (Kaltoft et al, 1995). The human CD8+^/CD4^-2^/IFNAR-/-/CD25+ T cell lines, Control-1, -2, and -3, are continuous T cell lines derived from uninfected skin of three different patients with diseases other than psoriasis as described in detail elsewhere (Kaltoft et al, 1992, 1994). Allo-reactive human CD8+^/CD4^-/IFNAR-/-/CD25+ T cell lines were obtained from healthy individuals and have previously been described (Nielsen et al, 1994). The human T cell lines, CD8+^/CD4^-/IFNAR-/-/CD25+ Psor-16 and -17, and Psor-18 and -19, from two different psoriasis patients were established from T cells migrated from biopsy fragments grown in culture for a few weeks as described previously (Skov et al, 1997). The different psoriasis T cell lines do primarily have a Th1-cytokine profile (Nielsen et al, 1998, data not shown), and all cells from both control and psoriasis patients respond equally well to growth factors such as IL-2, IL-4, IL-7, and IL-15. Cells were cultured in RPMI 1640 (Sigma) supplemented with 2 mM L-glutamine, 100 μg per mL penicillin, 100 μg per mL streptomycin (Sigma), 10% pooled human serum, 1000 U per mL IL-2, and 10 ng per mL IL-4. Cells were washed twice and starved for IL-2 and IL-4 for 16–18 h before initiation of the experiments described below.

Isolation of PBL Blood from three different psoriasis patients and three different healthy control donors was individually subjected to density gradient centrifugation using Lymphoprep (Axis-Shield PoC AS, Oslo, Norway). PBL were subsequently isolated, washed three times in pre-warmed phosphate-buffered saline, and resuspended and left in culture medium (see description above) without cytokines for 4 h, prior to FACS analysis and western blotting as described below. FACS analysis of psoriasis and control PBL revealed a comparable amount of IFNAR+ and CD3+ cells, and that around 70% of the cells were CD3+.

Protein extraction, oligonucleotide affinity purification of STAT proteins, and western blotting After stimulation with or without IFN-α for the indicated times, the cells (1.5 x 10^6) cells/experiment for whole-cell lysates and 20 x 10^6 cells/experiment for oligonucleotide affinity purification) were rapidly pelleted and lysed in ice-cold lysis buffer as described previously (Eriksen et al, 2004). The oligonucleotide affinity purification and the western blotting procedure are described in detail elsewhere (Eriksen et al, 2001). Blots were evaluated using ECL, stripped, and reprobed according to the manufacturer’s manual (Amersham).

FACS analysis of cell surface expression Cells were harvested (5 x 10^6), washed three times in washing buffer (PBS, 5% fetal calf serum, 0.1% sodium azide), and pelleted. The cells were resuspended in 4 μL labeled Ab, vortexed, and incubated for 30 min at 4°C in the dark. The cells were washed three times in washing buffer and stored at 4°C in the dark until they were analyzed on FACScan (Becton Dickinson, Franklin Lakes, New Jersey). 10^4 cells per sample were analyzed.

ELISA Cells were cultured at 2.0 x 10^5 cells per well in 12-well plates for 24 h with or without IFN-α in a final volume of 800 μL. The IFN-γ production was analyzed by sandwich ELISA according to the manufacturer’s protocol (Becton Dickinson Biosciences Pharmingen).
Proliferation assay Assays were performed in culture medium (RPMI 1640) supplemented with 10% human serum, 2 mM l-glutamine, 100 µg per mL penicillin, 100 µg per mL streptomycin, and 1000 U per mL IL-2 in 96-well round-bottomed tissue culture plates (Nunc, Roskilde, Denmark) with a final volume of 200 µL. Psor-2, Control-2, and alloreactive T cells were cultured at 25 × 10^5 cells per well for 24, 48, 72, or 96 h with or without reagents as indicated. Twelve hours before harvest, [3H]thymidine incorporation was measured in a scintillation counter, and the results were expressed as median counts per minute to triplicate cultures.

Quantification of apoptotic cells Apoptotic cells were quantified by the use of the DNA-binding dye 7-AAD as described in detail (Sommer et al, 2004). Cells were harvested (5 × 10⁶), washed three times in washing buffer (PBS, 5% fetal calf serum, 0.1% sodium azide), resuspended in 0.5 mL 0.05% saponin in PBS, and pelleted. The cells were resuspended in 0.5 mL 4 µg per mL 7-AAD in 0.05% saponin in PBS, incubated at room temperature in the dark in 30–45 min, and analyzed on FACSscan. Untreated cells were used to set gates for viable cells with 2–4N DNA, for apoptotic cells in 30–45 min, and analyzed on FACScan. Untreated cells were harvested onto glass fiber filters, [3H]thymidine incorporation was measured in a scintillation counter, and the results were expressed as median counts per minute to triplicate cultures.

References


Xu X, Sun YL, Hoey T: Cooperative DNA binding and sequence-selective recognition conferred by the STAT amino-terminal domain [see comments]. Science 273:794–797, 1996