The Interferon Inhibiting Cytokine IK is Overexpressed in Cutaneous T Cell Lymphoma Derived Tumor Cells That Fail to Upregulate Major Histocompatibility Complex Class II Upon Interferon-γ Stimulation

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Cutaneous T cell lymphomas are characterized by an accumulation of malignant clonal lymphocytes in the skin and occasionally in the blood. We compared gene transcription profiles from cultured clonal lymphocytes with autologous healthy blood lymphocytes by microarray hybridization. Cutaneous T cell lymphoma derived cells transcribed high amounts of an interferon inhibiting cytokine factor. The presence of an interferon inhibiting cytokine factor was confirmed in 12 skin biopsies of mycosis fungoides and Sézary syndrome derived blood lymphocytes by reverse transcriptase–polymerase chain reaction. The presence of interferon inhibiting cytokine factor mRNA in Sézary syndrome derived lymphocytes was associated with a lack of HLA class II upregulation after stimulation with interferon-α and interferon-γ. This was not due to a loss of the interferon signaling cascade as the presence of interferon-signaling components was confirmed by reverse transcriptase–polymerase chain reaction on the transcriptional level. The elevated constitutive interferon inhibiting cytokine factor expression observed in cutaneous T cell lymphoma derived cells was insensitive to interferon-γ stimulation, but was enhanced in normal peripheral blood mononuclear cells. We suggest that interferon inhibiting cytokine factor contributes to the lack of HLA class II upregulation in lymphoma cells. Interferon inhibiting cytokine factor may participate in providing a microenvironment at the tumor site insensitive to interferon-γ stimulation and thus prevents an efficient local immune response. ***Key words: immune escape/microarray hybridization/Sézary syndrome. J Invest Dermatol 116:874–879, 2001***

Cutaneous T cell lymphomas (CTCL) comprise a heterogeneous group of neoplasms characterized by an accumulation of malignant T lymphocytes in skin and occasionally, as in Sézary syndrome (SS), in skin and blood.

Based on clinical, histologic, and immunologic features, we suppose that the tumor cells in SS and probably also in mycosis fungoides (MF) are well differentiated skin homing (Kamarashev *et al*, 1998) T helper 2 lymphocytes (Sterry and Mielke, 1989; Vowels *et al*, 1994; Asadullah *et al*, 1996; Dummer *et al*, 1996), often lacking CD7 expression (Wood *et al*, 1993) with a low proliferation rate, but an increased expression of anti-apoptotic proteins, such as bcl-2 in early stages and enhanced proliferation in advanced stages (Dummer *et al*, 1995; Dummer *et al*, 2000). Recently, it was demonstrated that clonal T cells derived from SS fail to respond to interferon (IFN) -α and IFN-γ (Dummer *et al*, 1998, 2001) and to interleukin-12 (Showe *et al*, 1999). As tumor-specific molecular alteration might present targets for future therapeutic strategies (Rook *et al*, 1999; Dummer *et al*, 2001), we aim to identify genes preferentially expressed in the malignant T cells.

The microarray technique is a powerful tool to identify transcriptional differences between benign and malignant cells for large numbers of genes (Sikic, 1999; Lockhart and Winzeler, 2000). We perform microarray hybridization on isolated CTCL-derived cells and compared the resulting profile with autologous healthy peripheral blood derived T helper cells, otherwise displaying widespread similarities. The IFN-γ inhibiting cytokine (IK) first described by Krief *et al* (1994) appeared overexpressed in CTCL-derived cells. This factor IK was shown to inhibit IFN-γ induced expression of HLA class II antigen.

MATERIALS AND METHODS

Patients MF and SS was diagnosed using standard clinical criteria (Burg *et al*, 1995) (erythroderma, lymphadenopathy), histologic (subepidermal lymphoid infiltrate showing strong epidermotropism and expressing a T helper cell phenotype), and for SS hematologic criteria (more than 1000 SS cells per ml), and an elevated CD4/CD8 ratio (van Willemze *et al*, 1983; Heald *et al*, 1994). All patients were seronegative for human T cell lymphocytic virus I antibodies by enzyme-linked immunosorbent assay. Patients did not receive any systemic therapy in the last 2 wk before blood was taken; however, topical steroids had to be applied to control itching. The biopsy site was spared at least 5 d before tumor sampling. For detailed patient information refer to **Table I**.

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Abbreviation: IK, interferon inhibiting cytokine factor.

Table I. Patient characteristics[a]

Patient	Sex	age	EORTC classification	TNM classification (stage)	Biopsy: IK mRNA	Cell culture: IK mRNA	Microarray hybridization
ZK	F	35	PBMC, healthy donor		ND	–	ND
MD	M	63	Psoriasis vulgaris		–	ND	ND
MB	F	42	Psoriasis vulgaris		–	ND	ND
BR	F	36	Psoriasis vulgaris		–	ND	ND
MT	M	45	Eczema		–	ND	ND
HT	M	30	Eczema		–	ND	ND
AE	M	61	Atopic dermatitis		(+)	ND	ND
VM	M	74	Parapsoriasis en grande plaques	Ia	ND	+	+
DM	F	50	Mycosis fungoides	IIb	+	ND	ND
TR	M	47	Mycosis fungoides	IVa	+	ND	+
RM	F	47	Mycosis fungoides	IVa	+ (NS)	+	+
SW	M	65	Mycosis fungoides	IVa	+ (NS)	+	+
BB	F	53	Mycosis fungoides	IVb	+	ND	ND

Patient	Sex	Age	EORTC classification	Ratio CD4/ CD8	TNM classificaiton (stage)	$CD7^-$ cells: IK mRNA	$CD4^+CD7^-$ (%)	$V\beta^+$ (%)[b]
SM	F	62	SS	8.1	III	+	ND	
CT	F	53	SS	12.0	III	+	50	80.0
ZA	F	69	SS	6.3	III	+	33	
WR	F	62	SS	16.1	III	+	67	
XB	M	64	SS	15.1	III	+	80	73.8
KH	F	67	SS	6.0	IVa	+	60	
PL	M	56	SS	12.0	IVa	ND	74	81.3

[a]ND, not determined; NS, not shown; EORTC, European Organization for Research and Treatment of Cancer.
[b]Clonal dominance of T cell receptor Vβ chain of all $CD4^+$ cells

Purification and culture of malignant clones and normal $CD4^+$ cells All human material was obtained with the patient's informed consent. $CD4^+$ cells were separated by an immunomagnetic procedure using paramagnetic polystyrene beads (Dynal, Lake Success, NY) and a magnetic separation device (Dynal). An anti-human CD4-specific murine IgM antibody (Dako, Glostrup, Denmark) was conjugated to Dynabeads M-450 (Dynal) by overnight incubation at pH 9.5, washed twice in phosphate-buffered saline containing 10% fetal bovine serum and incubated under constant slow agitation with peripheral blood mononuclear cells (PBMC) generated by Ficoll gradient enrichment from the patient's blood at 4°C. Purified $CD4^+$ T cells were stimulated by exposure to 10μg per ml PHA (Gibco, Grand Island, NY) and harvested on day 3.

Lymphocytic cells from a diagnostic MF tumor sample were cultured for 8 wk in RPMI 1640 medium (500 ml), supplemented with 100 ml fetal bovine serum (≈ 20%), 10 ml fungizone/streptomycin, 10 ml glutamine, 5 ml sodium pyruvate (all Gibco BRL), 100 μl human epidermal growth factor (= 1 μg), 200 ml interleukin-2 (10^6 u per ml), 2 ml interleukin-4 (50,000 u per ml), 2 ml granulocyte-monocyte colony-stimulating factor (80,000 u per ml), and 400 μl basic fibroblast growth factor (250,000 u per ml) (all Becton Dickinson, San Jose, CA). Clonality of the morphologically homogenous tumor cell population was determined by PCR/denaturing gradient gel electrophoresis as described previously and compared with original cryopreserved biopsy material (Wood *et al*, 1994). Autologous peripheral $CD4^+$ T cells were cultured for 3 d under identical conditions in order to normalize for tissue culture artifacts.

Microarray hybridization Four different pairs of samples have been analyzed. Total mRNA (3 mg) obtained from CTCL patient's derived clonal T cell cultures and autologous normal $CD4^+$ PBMC, respectively, was reverse transcribed using the cDNA Synthesis (CDC) Primer Mix including (α-^{32}P)deoxyadenosine triphosphate into radioactively labeled cDNA (Atlas TM Pure Total Labeling System, Clontech, Palo Alto, CA). The probes were then hybridized to separate Atlas Arrays (''Human Cancer'' comprising 588 different tumor-related genes) following the manufacturer's instructions (Atlas TM cDNA Expression Array, Clontech) and expression profiles visualized by phosphoimaging scans.

Flow cytometry Surface receptor status of PBMC and CTCL cells was analyzed using anti-HLA-ABC monoclonal antibody (MoAb) W6.32 (Dako Diagnostics, Zug, Switzerland), anti-HLA-DR MoAb L243 (BD/Pharmingen, Heidelberg, Germany), anti-IFN-α receptor 1 MoAb (kindly provided by Dr. Novick), anti-IFNAR2 MoAb (Transduction Laboratories, Maechler AG, Basel, Switzerland), anti-IFN-γ receptor (anti-IFNGR) 1 MoAb A6-C5 (kindly provided by Dr Hemmi, Institute for Molecular Biology, Zürich), anti-IFNGR2 MoAb Hub159 (Genzyme Diagnostics, Kings Hill, U.K.). As isotype control mouse IgG1, IgG2a antibodies, and hamster IgG antibodies (all BD/ Pharmingen) have been used, respectively. As secondary reagents fluorescence conjugated rabbit anti-mouse (RAM) polyclonal antibodies (Dako), biotin-labeled goat anti-hamster polyclonal MoAb (CALTAG, Burlingame, CA) and fluorescence conjugated streptavidin (Dako) have been used.

Reverse transcriptase–polymerase chain reaction (RT–PCR) For IK, mRNA detection total mRNA was extracted from CTCL cell cultures, tissue samples and PBMC culture using RNAeasy Mini Kit (Qiagen AG, Basel, Switzerland), following the manufacturer's instructions (**Table I**). In addition, from two SS patients (PL and XB) and normal donor blood, PBMC were isolated with Ficoll-Paque (Amersham, Uppsala, Sweden), pelleted, and then resuspended in buffer A (10 mM HEPES, 10 mM KCl, 1 mM ethylenediamine tetraacetic acid, 1 mM ethyleneglycol-bis-(β-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid) and lyzed by vortexing with the addition of 1/16 vol. of 10% nonidet P40. The supernatant was added to an equal volume of buffer B (7 M urea, 1% sodium dodecyl sulfate, 0.35 M NaCl). After one phenol/chloroform/ethanol extraction the RNA was dissolved in RNAse-free water. From all isolated RNA samples mounts between 2 and 4 μg RNA were used to synthesize cDNA (M-MuLV Reverse Transcriptase from New England Biolabs, Beverly, MA). PCR was performed with the incubation buffer supplied with the Taq DNA polymerase (Boehringer Mannheim, Mannheim, D), with PCR digoxigenin labeling nucleotide mix (Boehringer Mannheim), and with 2.0 μM oligonucleotide primers. The cDNA probes were first amplified with primers for β-actin to test the quality and quantity of the cDNA. Only satisfactory cDNA was then subjected to PCR with primers for

Table II. Primer sequences[a]

Gene	5′-primer (5′-3′)	3′-primer (5′-3′)	amplicon size (bp)
IFNAR1	TTG CTC TCC CGT TTG TCA TTT A	GAC CTC AGG CTC CCA GTG TAA C	395
IFNAR2	CCA CCT CTA CAG AAT CCC AGT T	GGA GAA CAC TTG CAG ACG ATA A	591
IFNGR1	TTG GAT TCC AGT TGT TGC TGC TT	TGT CCA GGA AAA TCA GAC TTC AA	775
IFNGR2	CCT CCA CTG AGC TTC AGC AAG TC	CAA AAG GGA ATA CTG GTC TCT GG	394
STAT-1	CAA GGT GGC AGG ATG TCT CAG TG	TGG TCT CGT GTT CTC TGT TCT GC	565
JAK-1	GGA CCC CAC ACA TTT TGA GAA GC	TGC AGA GTG ACT CCA AAA GAC CA	645
IK	GCA GCT GGG AGA TTT CTT TG	GGG CAA AGC TTC TTT GTT GT	148
β-actin	CGA CAA CGG CTC CGG CAT GTG C	CGT CAC CGG AGT CCA TCA CGA TGC	419

[a]All primers are intron-spanning.

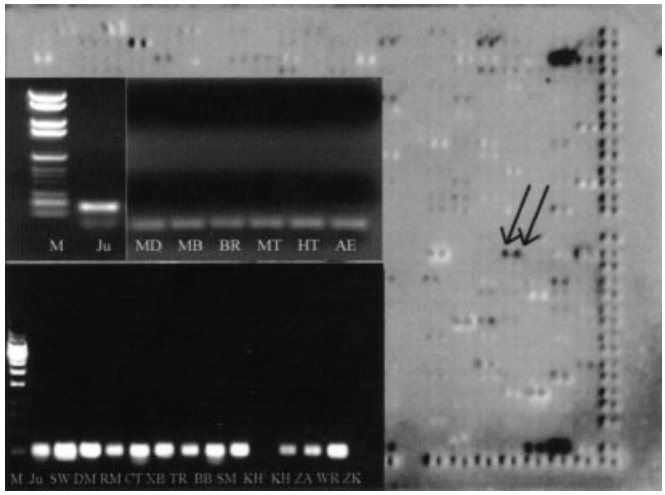

Figure 1. Differential expression of 588 genes in autologous PBMC and cultured CTCL cells analyzed by microarray hybridization. mRNA has been isolated from CTCL cells and healthy PBMC (both derived from patient SM) and reverse transcribed into cDNA. Then, samples have been subjected for hybridization with an array comprising 588 different genes related to human tumors. Results are presented as an overlay of two blots. Black dots indicate mRNA that is present in tumor cells only, the arrows indicate specific hybridization of CTCL mRNA with a probe coding for IFN-γ antagonist cytokine (GenBank: A25270; cytokine IK). Insert: Expression of IK mRNA in psoriasis vulgaris biopsies (MD, MB, BR), eczema biopsies (MT, HT), atopic dermatitis biopsies (AE), cell cultures or tissue samples from mycosis fungoides lesions (SW, DM, RM, TR, BB), PBMC from patients with Sézary syndrome (CT, XB, SM, KH, ZA, WR), Jurkat cells (Ju) and healthy donor PBMC (ZK). KH^1 = as a control, RNA has been isolated from sorted $CD7^+$ PBMC only.

IFNAR1 and 2, IFNGR1 and 2, JAK-1 and STAT-1, respectively. **Table II** shows the primer sequences. Intron-spanning primers were selected using the OLIGO Primer Analysis Software program version 4.0 (National Biosciences, Cascade, CO). Primer sequences were sent to GenBank to exclude cross-binding to other published sequences (Altschul *et al*, 1990). PCR was performed with Perkin Elmer 9600 GenAmp cyclers (Wellesley, MA). DNA-fragments were amplified with 30 cycles and an annealing temperature of 55°C. An aliquot of PCR product was electrophoresed on a 1.6% agarose gel and visualized by ethidium bromide staining. In all PCR reactions positive controls from various cell lines and water as negative control were included.

Real-time quantitative PCR A Hot-Start PCR was performed using 2μl ready-to-use mastermix (LightCycler – Faststart DNA Master SYBR Green I, Roche Molecular Biochemicals, Mannheim, Germany), containing thermostable recombinant Taq polymerase, reaction buffer, deoxyadenosine triphosphate, deoxycytidine triphosphate, deoxyguanosine triphosphate, deoxyuridine triphosphate), 0.5 μM of each oligonucleotide primers (Microsynth, Balgach, Switzerland), variable free $MgCl_2$ concentrations, 2 μl cDNA and water to a final volume of 20 μl. Amplification occured as a three-step cycling procedure: denaturation 95°C, 15 s; annealing 66°C, 10 s; and elongation 72°C, 18 s for 40 cycles. External standards for the β-actin quantitation consisted of four serial 1 : 10 dilutions (5.4×10^4 to 5.4×10^7 copies per reaction) of a pCR2.1 β-actin plasmid. External standards were run concomitantly with patients' samples under identical conditions. The fluorescence signal was plotted against the cycle number for all samples and external standards. The Fit Points method option was used in the course of analyzing quantitation data, allowing the definition of a noise band and subsequent background fluorescence subtraction, and resulting in the display of only log-linear and plateau amplification phases. A standard curve was then generated for each run, plotting the crossing point against the log-concentration of the standards. Normalization of estimated IK amounts was achieved by calculating the ratios between IK and β-actin, in order to compensate the variations in quantity and quality of starting mRNA. Ten microliters of each amplification product were checked by electrophoresis on a 2% agarose gel stained with ethidium bromide. The estimated size of the amplified fragments matched the calculated size. Product identity was in parallel checked by melting curve analysis, which is another application in the LightCycler analysis program.

RESULTS

Overexpression of IK mRNA in cutaneous lymphoma cells detected in microarray hybridization Total RNA of patient's skin derived CTCL cell cultures was isolated and subjected for hybridization with 588 tumor-related genes on a microarray. An overlay subtracts the hybridization signal obtained with CTCL cells, from the signal obtained with autologous healthy PBMC (SW, TR) or with allogenic $CD4^+$ cells (VM, RM). A consistent overexpression in all four microarray tested patient samples was found for the IK mRNA. **Figure 1** demonstrates the microarray hybridization results for patient SW. Subsequent reverse transcriptase–PCR analysis with extended patient numbers revealed the presence of the IK mRNA in 11 skin biopsies, blood samples or cell cultures of CTCL patients and the lymphoma cell line Jurkat (**Table I**, **Fig 1** insert). In contrast to that, RNA isolated from healthy donor's PBMC showed no band (ZK). The same was true for RNA isolated from $CD7^+$ cells of the SS patient KH (**Fig 1** insert; KH^1).

Unresponsiveness of patient's PBMC to IFN stimulation As IK was found to be overexpressed in CTCL cells we asked, whether IFN are still effective on CTCL cells and if they can stimulate the HLA class II expression in these cells. A clear upregulation of HLA class II expression with either IFN-α or IFN-γ was only seen in gated lymphocytes from healthy controls but not in gated lymphocytes derived from two SS patients with high clonality (XB, PL), although a basic level of HLA class II protein expression was detected (**Fig 2**). Jurkat cells did not express HLA class II at all.

Differential expression of IFNR chains on patients' PBMC and healthy donor PBMC The patients XB and PL were tested for expression of IFNR chains and associated proteins by

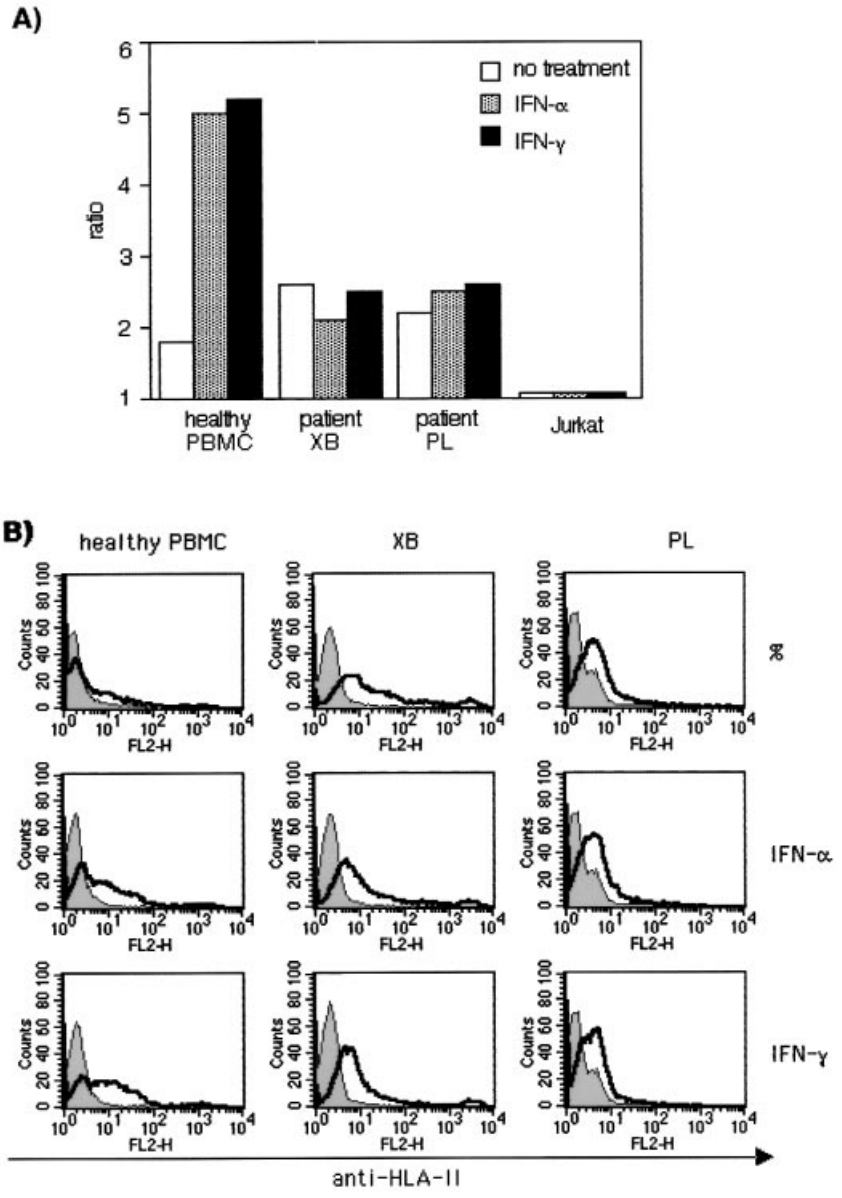

Figure 2. Lack of HLA class II upregulation in SS-derived cells upon IFN stimulation. Freshly isolated PBMC from two SS patients containing a clonal T cell population of 73.8% (XB) and 81.3% (PL) of all $CD4^+$ cells in the blood were cultured 70 h with or without 500 U per ml IFN-α, or IFN-γ, respectively. Then, cells were washed and stained with fluorescence conjugated antibodies against HLA class II. Stained cells were analyzed in a flow cytometer and gated on lymphocytes. Flow cytometric data are presented as average shift (ratio of mean fluorescence of specific antibody *vs* appropriate isotype-matched control antibody). Jurkat cells showed no HLA class II expression at all (*A*). Additionally, (*B*) compares histograms of isotype controls (*filled gray curves*) vs. specific antibody stainings (*open black curves*).

fluorescence-activated cell sorter (FACS) analysis and reverse transcriptase–PCR in order to evaluate a functional signal transduction pathway. There were prominent differences in basal levels of protein expression and, however, after IFN stimulation no consistent regulation of IFNR chains expression was detectable (**Table III**). The same was true for healthy control PBMC and Jurkat cells. Patient XB revealed strong expression of IFNGR1 and 2 chains, whereas patient PL expressed high amounts of IFNGR1, which disappeared after stimulation and almost no expression of IFNGR2. Reverse transcriptase–PCR analysis revealed the presence of mRNA for all tested IFN signaling components in both patients. The results for PBMC and Jurkat cells, however, appeared heterogeneous.

IFN-γ upregulated IK transcription in healthy donor PBMC but not in CTCL patient derived $CD4^+$ cells In order to evaluate the functional properties of IK we incubated sorted patient derived $CD4^+$ cells (SM, CT, ZA, WR) and outgrown clonal lymphoma cells (BB) with IFN-γ and then isolated RNA for reverse transcriptase–PCR analysis. With real-time PCR we have been able to measure IK mRNA levels and β-actin mRNA levels as external standards. **Figure 3** shows the ratios of IK mRNA compared with β-actin mRNA. Four of five CTCL patients showed almost no change in IK mRNA transcription after IFN-γ stimulation. One patient, however, responded with an increase of IK mRNA. It is noteworthy that this particular patient showed an extremely low level of IK mRNA in unstimulated $CD4^+$ cells. In comparison with that, we found an increase of IK mRNA levels after IFN-γ stimulation in $CD4^+$ cells from healthy donors as well.

DISCUSSION

The study of differential gene expression patterns in tumor cells *vs* their benign autologous counterpart by array technology opens new dimensions in illuminating the molecular biology of neoplasms. Expression levels of large amounts of genes can be compared simultaneously and may help to identify characteristic profiles for tumor cells (Bittner *et al*, 2000). First approaches in diffuse large B cell lymphomas demonstrate a correlation of tumor-specific properties and prognosis (Alizadeh *et al*, 2000).

We cultured tumor cells from four patients with MF and compared their gene expression profile with autologous or allogenic $CD4^+$ cells, respectively. In repeated experiments CTCL biopsies and outgrown clonal T cells derived from 12 MF and SS patients demonstrated consistent overexpression of factor IK (**Figs 1** and **3**), an IFN-γ inhibiting cytokine first described by

Table III. Differential expression of IFNR chains[a]

		IFNAR1		IFNAR2		IFNGR1		IFNGR2			
	Treatment	FACS	RT–PCR	FACS	RT–PCR	FACS	RT–PCR	FACS	RT–PCR	STAT-1 RT–PCR	JAK-1 RT–PCR
PBMC	–	1.2	–	1.0	+	1.7	–	1.8	–	–	±
	IFN-α	1.0		1.0		1.6		0.7			
	IFN-γ	1.0		1.0		1.3		1.8			
Patient	–	1.2	+	1.2	+	5.2	+	8.4	+	+	+
XB	IFN-α	1.2		1.0		5.2		9.9			
	IFN-γ	1.7		1.0		3.7		9.6			
Patient	–	1.2	+	1.0	+	9.6	+	1.0	+	+	+
PL	IFN-α	1.3		1.0		1.3		1.0			
	IFN-γ	1.4		1.0		1.1		3.0			
Jurkat	–	1.0	+	4.0	+	2.2	–	0.7	±	–	±
	IFN-α	1.0		1.0		5.3		1.0			
	IFN-γ	ND		1.0		1.3		1.0			

[a]Flow cytometric (FACS) data are presented as average shift (ratio of mean log fluorescence of specific antibody vs. appropriate isotype-matched control antibody). Because of limited patient material only single experiments could be performed. RT–PCR analysis: + = strong band; ± = faint band; – = no band detectable; ND = not determined. RT, reverse transcriptase.

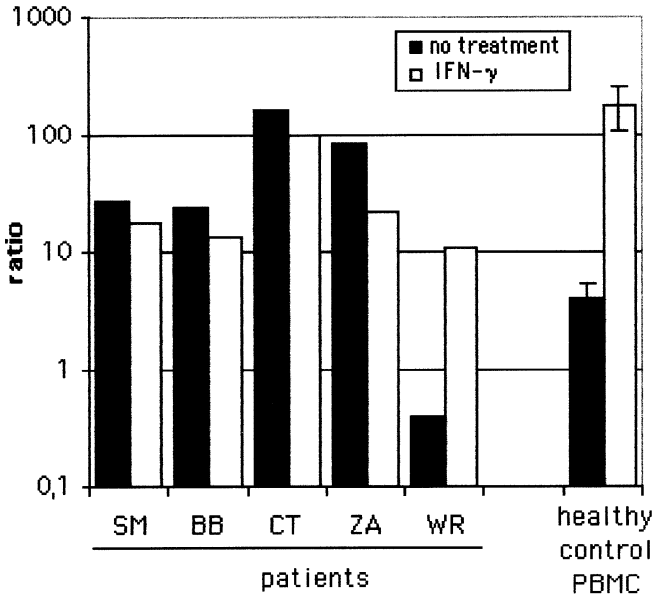

Figure 3. IFN-γ fails to enhance IK mRNA expression in CTCL derived lymphocytes as measured by real-time reverse transcriptase–PCR (LightCycler). From five CTCL patients malignant cells have been isolated and stimulated for 24 h with IFN-γ (500 U per ml). Then, RNA has been isolated and transcribed into cDNA. The quality and quantity of cDNA has been checked with primers specific for β-actin, followed by a PCR with primers specific for IK. Data are expressed as ratios of IK transcripts *vs* β-actin transcripts. Because of limited patient material only one experiment per sample could be performed. In case of healthy control PBMC the mean of three individual experiments including standard error of the mean (SEM) are presented. Each single control sample showed higher IK level after IFN-γ stimulation.

Krief *et al* (1994). This factor IK was shown to inhibit IFN-γ-induced expression of HLA class II antigen. Moreover, IK is expressed in $CD34^+$ hematopoietic progenitors, and its expression can be upregulated during growth factor-induced differentiation (Cao *et al*, 1997). Studies, that were done in IK transfected Raji B cell lines led to a model in which IK inhibits the constitutive HLA class II expression through repression of the class II transactivator (CIITA) transcription. Differential expression of CIITA plays an important part for the stabilization and activity of the transcription complex. Several, yet unknown intermediate proteins may be involved between IK and CIITA promotors (Vedrenne *et al*, 1997). As CIITA is hardly expressed in IK transfected Raji B cells, it appears that IK acts through a negative regulation of CIITA expression. That means IK inhibits both, the CIITA promoter III and IV of which the first is responsible for a constitutive gene expression and the latter renders responsible for an IFN-γ induced expression (Morris *et al*, 2000).

In order to investigate, whether the overexpressed IK is functional we studied PBMC from advanced SS containing at least 75% clonal T cells as assessed by FACS (Dummer *et al*, 1996, 2001). In contrast to normal PBMC, SS derived PBMC failed to upregulate HLA class II expression upon IFN stimulation.

Stimulation with IFN-γ does not influence the IK levels in most of the CTCL cells; however, normal PBMC respond with an upregulation of IK mRNA transcription. This is especially interesting as these cells are sensitive to IFN-γ and increase the HLA class II expression upon stimulation.

IFN-γ binds to a specific ubiquitous cell surface receptor, consisting of two transmembrane proteins, namely IFNGR1 chain, IFNGR2 chain (Bach *et al*, 1996). The signal transduces through a cascade of phosphorylations and then leads to the activation of CIITA, which in turn induces HLA class II expression (Steimle *et al*, 1994) and to some extend HLA class I expression as well (Martin *et al*, 1997).

A natural role of IFN in tumor surveillance is demonstrated in mouse tumor models where treatment with antibodies directed against IFN leads to the stimulation of growth and proliferation of tumor cells. The $IFNGR^{-/-}$ mouse model was used to address the question whether IFN-γ plays a crucial part in promoting host surveillance that is capable of controlling the growth of primary tumors. After treatment with chemical carcinogens, IFN-γ-resistant animals developed tumors more frequently than wild-type controls (Kaplan *et al*, 1998).

Bach *et al* (1995) used an *in vitro* developmental system showing that IFN-γ stops the expression of the IFNGR2 chain, resulting in Th1 cells that are unresponsive to IFN-γ. This receptor chain loss also occurred in IFN-γ-treated Th2 cells (Bach *et al*, 1995). Their data suggest that IFNGR2 loss is a specific response of $CD4^+$ T cells to IFN-γ treatment rather than a Th1 specific differentiation event. According to Bach *et al* (1995), it is also possible that IFNGR2 overexpression is a sign of IFN-γ depletion in SS blood. We found similar heterogeneous results in our FACS analysis (**Table III**). Although we showed the presence of most IFNR chains in our patients, this does not prove a functional integrity of

the IFN signaling pathway (Dummer *et al*, 2001). Further analysis on mRNA levels revealed the presence of IFNR chain mRNA in both investigated patients.

According to Vedrenne *et al* (1997) the overexpression of the IK protein maintains CIITA expression under a threshold, inducing a latent state of HLA-DR transcription repression. They suggest that the IK/CIITA ratio represents a crucial controlling element for HLA class II membrane expression. We believe that the threshold for HLA class II expression can be easily overcome after IFN-γ stimulation in normal cells, by producing sufficient amounts of CIITA, which is then feedback regulated by rising IK levels. In malignant cells, however, frequently deficient in IFN-signaling this regulatory loop is impaired, thus leading to constant high IK levels and inhibition of IFN-γ induced HLA class II protein expression.

Based on our data, we speculate that the clonal T cells create a microenvironment where high levels of IK inhibit the beneficial effects of IFN-γ in the surrounding tissue. A constant overexpression of IK limits the CIITA activation and, therefore, the induction of HLA class II protein. We are aware of the fact that this regulatory pathway might be only one of many switches in a complex microenvironmental network. The characterization of factors involved in the control of HLA class II expression in healthy and malignant cells, however, will be important to understand the underlying molecular mechanisms. Successful immunointervention in MF and SS might aim to restore reduced HLA class II facilitating a better recognition by immunosurveillance (Martin *et al*, 1999).

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