ULTRAVIOLET B-INDUCED T CELL APOPTOSIS: IMPLICATIONS FOR PHOTOTHERAPY

Ph.D. thesis

Zoltán Novák M.D.

2004

Supervisor: Lajos Kemény M.D., Ph.D., D.Sc.

Department of Dermatology and Allergology
University of Szeged, Szeged, Hungary

List of original papers related to the subject of the thesis

I. Novak Z, Berces A, Ronto G, Pallinger E, Dobozy A, Kemeny L.
Efficacy of different UV emitting light sources in the induction of T cell apoptosis.
Photochem Photobiol 2004 May, 79(5):434-9. IF(2002): 2,241

II. Novak Z, Bonis B, Baltas E, Ocsovszki I, Ignacz F, Dobozy A, Kemeny L.
Xenon chloride ultraviolet B laser is more effective in treating psoriasis and in inducing T cell apoptosis than narrow-band ultraviolet B.
J Photochem Photobiol B. 2002 May; 67(1):32-8. IF: 1,573

III. Baltas E, Nagy P, Bonis B, **Novak Z,** Ignacz F, Szabo G, Bor Z, Dobozy A, Kemeny L.
Repigmentation of localized vitiligo with the xenon chloride laser.
Br J Dermatol. 2001 Jun; 144(6):1266-7. IF: 2,405

IV. Jakab K, **Novak Z,** Engelhardt JI, Kemeny L, Kalman J, Vecsei L, Rasko I.
UVB irradiation-induced apoptosis increased in lymphocytes of Huntington's disease patients.
Neuroreport. 2001 Jun 13; 12(8):1653-6. IF: 2,374

CONTENTS

1. INTRODUCTION

1.1. Ultraviolet light

Ultraviolet (UV) light is a form of electromagnetic radiation situated in the wavelength spectrum 100-400 nm. The shortest wavelengths of UV radiation are called vacuum UV. The UV region above 200 nm has been artificially subdivided on the basis of the responses of human skin and the wavelengths contained in sunlight. Three regions are recognized. UVC radiation (200-280 nm) is not found in sunlight at the surface of the earth, as it is filtered out by ozone and water vapor in the atmosphere. UVB radiation (280-320 nm) is the most biologically active waveband of UV radiation in sunlight. UVA radiation (320-400 nm) is biologically less active than UVB, but it is partially responsible for a sun-induced erythema [Morison, 1992].

UV light is a widely used therapeutic modality for different skin diseases. UVA phototherapy is effective in the treatment of inflammatory skin diseases such as acutely exacerbated atopic dermatitis, localized scleroderma, urticaria pigmentosa and disseminated granuloma annulare. Delivery of 8-methoxypsoralen and subsequent UVA irradiation (PUVA therapy) is an effective alternative for the treatment of psoriasis, mycosis fungoides, localized scleroderma, urticaria pigmentosa or lichen planus. UVB light can be administered as total body or localized therapy for psoriasis, atopic dermatitis, pruritus, vitiligo [Njoo, 2000], lichenoid graft versus host reaction (Simon, JC, 2000).

1.2. Conventional UVB light sources

The first treatment of psoriasis with UV light was introduced by Goeckerman more than 75 years ago [Goeckerman, 1925]. This highly effective regimen consisted of daily exposure to erythemogenic levels of ultraviolet light, delivered from high pressure mercury vapor lamps, in conjunction with application of coal-tar containing ointment. Initially, broad-band (BB)-UVB light sources were applied in UVB phototherapy, these emit wavelengths throughout the whole spectrum of UVB light [Menter, 1983]. Nowadays, they are less and less frequently used, mainly for the treatment of psoriasis, atopic dermatitis and pruritus. In 1980, an action spectrum study in patients with psoriasis was carried out. With the use of a monochromator, the action spectrum for ultraviolet phototherapy of psoriasis was determined for radiation between 254 and 313 nm, and compared with the action spectrum for erythema of the uninvolved adjacent skin. Daily exposures of different doses of 254, 280, 290, 296, 300, 304 and 313 nm radiation were observed. Wavelengths of 254, 280 and 290 nm were erythemogenic, but not therapeutic even at 10 to 50 times the minimal erythema dose (MED).

At the other wavelengths studied, the 2 action spectra were similar, at wavelengths of 300 and 304 nm, complete clearing occurred on daily exposure to doses equal to or less than MED. In every subject, suberythemogenic exposure doses of 313 nm resulted in complete clearance of the plaques [Parrish, 1981]. Therefore, radiation of wavelengths less than 296 nm is very phototoxic to normal skin, as manifest by a low threshold for induction of delayed erythema. This radiation is, however, not effective in phototherapy of psoriasis. The small number of suberythemogenic exposure doses required suggested that monochromatic radiation might have advantages over broad-band sources.

These findings led to the introduction of the selective UVB phototherapy (SUP) and narrow-band (NB)-UVB phototherapy. SUP has peaks at 305 and 325 nm. While it has not proven as effective in treating psoriasis as originally hoped, SUP appears superior to BB-UVB for the treatment of atopic dermatitis [Paul, 1983]. NB-UVB also emits polychromatic light, but the 311-313 nm wavelength range predominates in its emission spectrum (fig 1). In a bilateral comparative study, the ability of suberythemogenic doses of NB-UVB versus conventional BB-UVB to remit psoriasis was compared. The NB-UVB treatment (50% of the MED) produced virtual clearing of psoriatic plaques in 9 of 11 patients within 6 weeks. In contrast, the response to BB-UVB, delivered at somewhat higher levels (75% of the MED) was quite poor, with only 1 of 11 patients attaining resolution after 6 weeks. Another study also compared the effectiveness of the two different UVB sources: clinical and histopatological resolution was achieved in 86% of sites treated with NB-UVB versus 59% treated with BB-UVB [Coven, 1997]. Therefore, NB-UVB proved to be superior to BB-UVB for the treatment of psoriasis.

1.3. The xenon chloride UVB laser

Earlier, we observed that supraerythemogenic fluences of UVB result in faster clearing of psoriasis, however, the limiting factor for the use of such high fluences lies with the intolerance of the uninvolved surrounding skin, since psoriatic lesions can often withstand much higher UV exposures. Because the laser light can be selectively directed towards lesional skin, and all of the energy of a 308 nm excimer laser is emitted within the action spectrum for the phototherapy of psoriasis (Fig. 1), our group investigated the therapeutic effect of the 308 nm Xenon chloride (XeCl) excimer laser for psoriasis. This laser emits its total energy at 308 nm and may therefore be regarded as a"super narrow band" UVB light source. The laser has been used to treat skin tumors and tattoos. In six patients with chronic plaque type psoriasis, we compared the efficacy of NB-UVB with 308 nm UVB laser. The

number of treatments up to complete clearance with the NB-UVB was 29-33, while that with the XeCl laser was 8-10. The cumulative doses were 26-32 J/cm^2, and 2.5-8.1 J/cm^2 for the NB-UVB and XeCl laser, respectively, so the cumulative dose required for the complete clearance of psoriatic plaques was 6 times less with the XeCl laser than with NB-UVB phototherapy [Bónis, 1997]. The high clinical efficacy of the XeCl laser for psoriasis was later confirmed by other studies. Asawanonda et al determined the dose-response relationship of XeCl laser for psoriasis. They used 8 different fluences from 0.5 to 16 MED. The use of such high fluences resulted in a prolonged remission of psoriasis, even after a single treatment. They could demonstrate that fluence was the single most important determinant in the clinical clearing of psoriasis [Asawanonda, 2000]. Trehan et al also investigated the antipsoriatic efficacy of one single high fluence XeCl laser treatment. 11 of 14 patients showed significant improvement within 1 month, and 5 still demonstrated persistent areas of clearing at 4 months [Trehan, 2002]. Another studies established the high efficacy of XeCl laser in chronic inverse psoriasis [Mafong, 2002], and vitiligo [Baltás, 2002]. According to a multicenter open trial from 5 dermatology practices including 124 patients, 84% of the patients achieved at least 75% clearing in after 10 or fewer treatments. No serious side effects were observed, just erythema, blisters, hyperpigmentation, but they were well tolerated [Feldman, 2002]. In summary, XeCl laser might therefore be regarded as a new and promising form of UVB phototherapy, which seems to be superior to conventional UVB sources in the treatment of psoriasis and vitiligo [Spann, 2oo1, Kemény, 2001]. The clinical efficacy of the XeCl laser in psoriasis is therefore well documented, but the mechanism of its high efficacy has not been investigated so far.

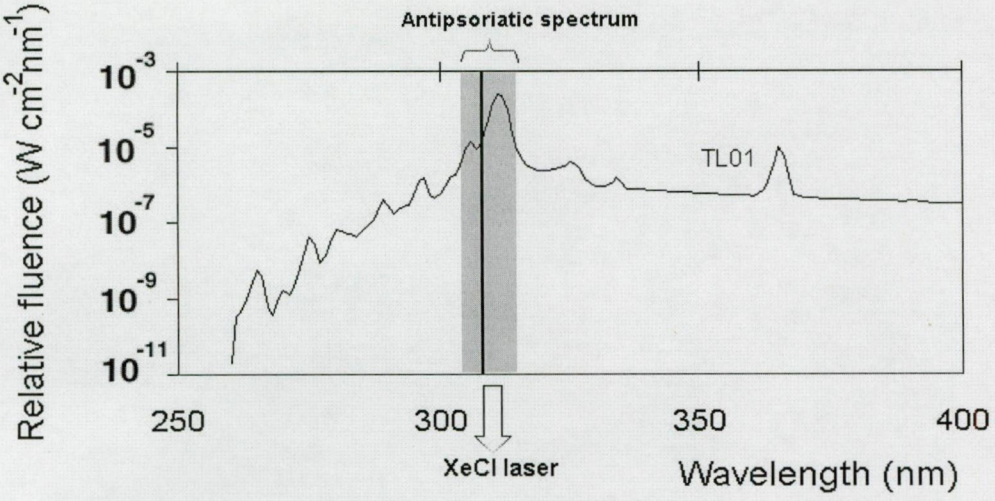

Figure 1. Emission spectra of NB-UVB (TL01) and 308 nm XeCl laser. The spectrum of Philips TL01 UVB source was obtained from Modos et al. [Modos, 1999]. The grey area indicates the most effective wavelengths for the phototherapy of psoriasis as measured by Parrish et al [Parrish, 1981].

1.4. Psoriasis vulgaris

Psoriasis vulgaris is a chronic inflammatory skin disease that affects 2-3% of the population. In Western Europe the disease is about as common as diabetes mellitus. Clinically, psoriasis is considered a disease of the entire skin, with the most common presentation being well circumscribed erythematous scaling plaques that may be symmetrically distributed (Fig.2.). Histologically, the disease is characterized by hyperplasia and incomplete differentiation of epidermal keratinocytes, tissue inflammation with neutrophils in the stratum corneum and influx of immunocytes including dendritic antigen presenting cells and both CD4 and CD8 positive lymphocytes (Fig. 3.). In an experimental system, full thickness human psoriatic, non lesional skin was transplanted onto severe combined immunodeficient mice. Psoriasis could be induced by injecting autologous immunocytes into the dermis [Wrone-Smith, 1996]. In the xenotransplant model, an epidermal hyperplasia response appears to arise from cytokines derived from T cells infiltrating into skin. Aberrant keratinocyte proliferation, regenerative epidermal differentiation and infiltration of CD8+ T cells in psoriatic epidermis were recently shown to be sensitive to a new therapeutic modality, the IL-2-diphteria toxin conjugate [Gottlieb, 1995]. Since IL-2 directs the toxin to activated immune cells, a pathogenic role for T cells is indicated [Wrone-Smith, 1996]. The pathogenesis of the disease is not yet known, the search for the etiology has concentrated on epidermal proliferation and differentiation,

inflammatory changes and the dermal vasculature. Although each of these broad areas might hold the answer, now it seems that skin infiltration by activated cutaneous lymphocyte-associated antigen-positive T cells appears to cause a complex inflammatory tissue phenotype, leading to the presence of activated leukocytes in skin lesions, a diverse array of cytokines produced by leukocytes and keratinocytes, proliferation of small blood vessels and epidermal keratinocytes and increased expression of leukocyte-trafficking adhesion molecules. Therefore, there is considerable evidence, that psoriasis vulgaris is mediated by activated T lymphocytes infiltrating the epidermis and the dermo-epidermal interface [Wrone-Smith, 1996].

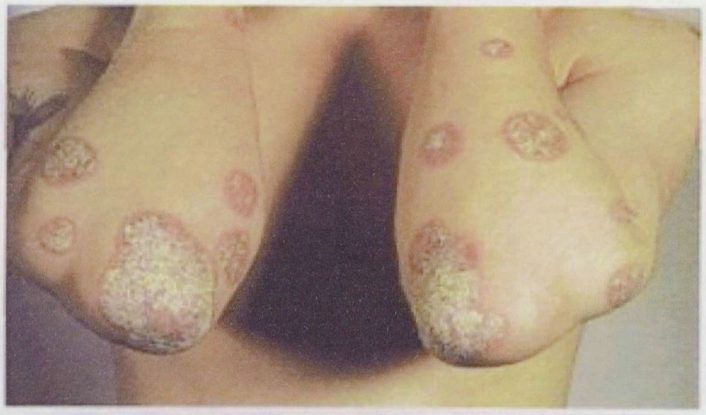

Figure 2. Chronic plaque type psoriasis on the elbows of a patient.

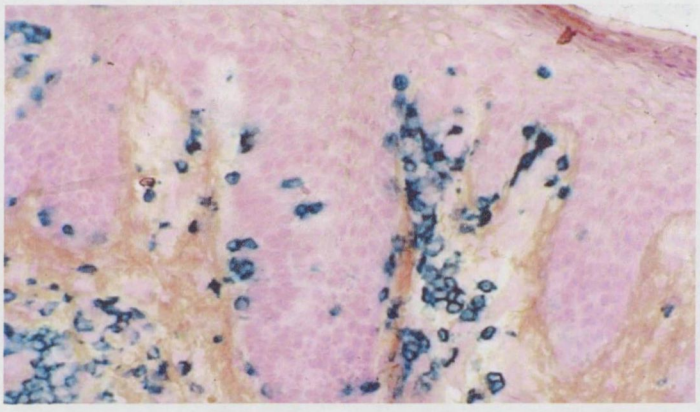

Figure 3. CD3+ T cells in the dermo-epidermal junction and in the epidermis of psoriatic skin.

1.5. The mechanism of action of UVB light

Initially it was thought that UVB phototherapy acts through the induction of antiproliferative effects resulting from UVB-induced DNA damage [Epstein, 1968]. Later, UVB has been shown to modify cutaneous immune responses, a phenomenon that is called photo-

immunosuppression [Kripke, 1984, Bataille, 2000]. UVB radiation effects the soluble mediators, e.g. it increases the IL-10 protein expression in human keratinocytes [Rivas, 1999] therefore suppresses the production of interferon-γ by T lymphocytes [Grewe, 1995]. UVB is capable of modulating the expression and function of adhesion molecules, e.g. ICAM-1 expression can be efficiently prevented by exposing human keratinocytes to UVB radiation [Krutmann, 1990]. UVB induced also down-regulation of growth factor production and abrogation of growth factor receptor expression [Takashima, 1995]. The mixed lymphocyte reaction (MLR) and mixed epidermal cell lymphocyte reaction (MECLR) showed that the alloactivating capacity of cells was decreased by UVB exposure [Vermeer, 1994]. UVB can also be immunosuppressive possibly at the level of antigen-presenting dendritic cells [Hart, 2000]. The results of some new investigations provide evidence that UVB irradiation can induce regulatory/suppressor T cells [Aubin, 2004]. According to the observations of Krueger et al, UVB treatment produced consistent and profound depletion of T lymhocytes from psoriatic epidermis. T cell activation appeared to be decreased even further, as judged by expression of the IL-2 receptor. Dermal lymphocytes were much less affected [Krueger, 1995].

UVB treatment caused the induction of Fas ligand on keratinocytes in human epidermis [Gutierrez-Steil, 1998]. As apoptosis is induced by in vitro UVB irradiation of T cells [Yaron, 1996, Hill, 1999], or by incubation of T cells with keratinocytes expessing UVB-induced Fas ligand, it has been proposed that UVB may have immunosuppressive effects in psoriasis through the induction of apoptosis in disease-mediating T cells. Apoptosis is one of two modes of cell death, necrosis is the other. Apoptosis, or programmed cell death is a physiological suicide mechanism that preserves homeostasis, in which cell death naturally occurs during normal tissue turnover [Wyllie, 1994, Scaffidi, 1999]. During apoptosis, the cell draws inward shrinking dramatically, the plasma membrane blebs, the endoplasmic reticulum forms vacuoles, the mitochondrium membrane potential drops, and the chromatin is digested and condenses along the nuclear membrane. The nuclear collapse is associated with extensive damage to chromatin and DNA-cleavage into oligonucleosomal length DNA fragments after activation of a calcium-dependent endogenous endonuclease [Duvall, 1986]. In the final stages, the nucleus and the cell fragment form smaller compact units called apoptotic bodies. These apoptotic cells and bodies are primarily removed by professional or nonprofessional phagocytes [Godar, 1999]. Apoptosis is essential in many physiological processes, including maturation and effector mechanism of the immune system, embryonic development of the nervous system and hormone-dependent tissue remodeling. To characterize the mechanism of

the extent of apoptosis. 100 mJ/cm^2 NB-UVB light resulted in measurable T cell apoptosis by flow cytometry 20 hours later [Ozawa, 1999]. Breuckmann et al. demonstrated delayed apoptosis (within 24-48 hours) on human T cells after BB-UVB irradiation as well [Breuchmann, 2003].

In summary, the major mechanism of action of UVB light in the treatment of inflammatory dermatoses is a cytotoxic effect on the infiltrating T cells, where the mechanism of cell death is most probably apoptosis. Earlier, psoriatic plaques in 23 patients were treated daily with NB-UVB or BB-UVB in a bilateral comparison study. NB-UVB cleared the psoriatic plaques more effectively than did BB-UVB light. Both forms of UVB reduced intraepidermal T cells from lesional skin, but quantitative reductions were greater with NB-UVB. On the other hand, NB-UVB light has been found to be a more potent inductor of T cell apoptosis *ex vivo* than BB-UVB light, therefore, the T cell apoptosis-inducing capacity of a UVB light source can be paralleled by its clinical efficacy [Coven, 1997].

1.6. Biological important physical parameters of UVB

UVB radiation can be characterized by several physical parameters, the most important ones are wavelength spectrum, energy density, irradiance, or light intensity and frequency of impulses if the UVB light is emitted in pulse-mode. Although much scientific work has been carried out on the biological effects of UVB light, little is known about the role of optical parameters of radiation in the effect of UVB radiation on biological processes.

The DNA-damaging effect of UVB is mediated through cyclobutane pyrimidine dimer and (6-4) photoproduct formation. Matsunaga et al. determined the action spectra for the induction of thymine dimers and (6-4) photoproducts in DNA by nearly monochromatic UV light ranging from 150 nm to 365 nm. The most efficient wavelength for the formation of UV light-induced DNA damage proved to be 260 nm, corresponding to the absorption spectrum of DNA [Matsunaga, 1991].

Urocanic acid is a major UV chromophore in the upper layers of the skin where it is found predominantly as the *trans* isomer. UVB irradiation induces photoisomerisation of *trans* urocanic acid to *cis* isomer. *Cis* urocanic acid is suggested to be a mediator of UVB light-induced immunosuppression, because its administration can mimic many of the effects of UVB light on the skin [Webber, 1997]. Gibbs et al. investigated the wavelength dependence for *trans*-to-*cis* photoisomerisation *in vitro* over the spectral range 270-340 nm, and found that the resulting action spectra had maximal effectiveness at 300-315 nm [Gibbs, 1993, Macve, 2002].

The mixed epidermal cell lymphocyte reaction (MECLR) and the mixed lymphocyte reaction (MLR) have been commonly used to study the immunosuppressive effects of UVB radiation. The alloactivating capacity in MLR and MECLR experiments is decreased by exposure to UVB light. Using monochromators, Hurks et al. irradiated PBMC with nearly monochromatic UVB light at 254, 297, 302 and 312 nm, measured the decreases in the MLR and MECLR responses, and hence determined the action spectra for the MLR and MECLR from 254 nm to 312 nm. Both the MLR and MECLR action spectra displayed a maximum at 254 nm and a relative sensitivity at 312 nm. The action spectra were strikingly similar to those for the induction of pyrimidine dimers and 6-4 photoproducts [Hurks, 1995].

The role of the impulse frequency of UVB light in biological processes has not been investigated so far, because none of the experiments were performed with pulse-mode UVB light source.

It has become clear that wavelength spectrum and dosimetry is very important in UVB experiments. However, little emphasis was taken to the importance of light intensity. In 1980, DeFabo et al found, that UV radiation-induced immunologic unresponsiveness of mice to a UV induced fibrosarcoma was independent of the intensity of the UV light [DeFabo, 1980]. Kelfkens showed that intensity did not influence UV tumorigenesis [Kelfkens, 1991]. Hurks et al performed in vitro experiments about the biological importance of light intensity. Using FS40 lamps with variable UV intensities (7 W/m2 and 1.6 W/m2) without alteration of the spectral distribution, epidermal cells were in vitro irradiated, and then the MECLR responses were measured. They found that the irradiation of epidermal cells with high irradiance impaired the alloactivating capacity more than did irradiation with low irradiance. They concluded that UVB radiation-induced suppression of MECLR was critically dependent on irradiance [Hurks, 1995]. In the literature, no data can be found about the role of light intensity, impulse frequency on the therapeutical efficacy of UVB. The only study on the importance of wavelengths of UVB in the therapy of psoriasis was performed by Parrish et al [Parrish, 1981] and has been described above.

1.7. Transmission of human epidermis in the ultraviolet wavelengths

In absorbing radiation, the outermost layers of human skin, stratum corneum and epidermis, act as an optical barrier and protect the viable cells. The extent of the protection provided by this barrier depends partly on its thickness. Thickness of stratum corneum and epidermis varies from one individual to another and in the same individual it varies in different regions of the body [Anderson and Cassidy, 1973]. The average thickness of healthy human stratum

varies from one individual to another and in the same individual it varies in different regions of the body [Anderson and Cassidy, 1973]. The average thickness of healthy human stratum corneum and the epidermis from the lower back is 25 μm and 70 μm, respectively [Bruls, 1984]. The psoriatic skin is characterized by acanthosis, therefore the epidermal thickness increases. Welzel et al used optical coherence tomography to measure the thickness of psoriatic human epidermis from the lower forearm: it was 180-360 μm according to the extent of acanthosis [Welzel, 2003]. In 1988, a detailed analysis of the thickness of psoriatic skin from the forearm was carried out by an Image Analysis Computer System. The suprapapillar part of the stratum malphigii seemed to be constantly 43 μm thick, while the thickness of the rete pegs the stratum malphigii increased as the degree of acanthosis increased (160-420 μm) [Cahpman, 1988].

In 1984, Bruls and al. examined the transmission of human epidermis and stratum corneum as a function of thickness in the ultraviolet wavelengths. The skin samples were taken from the lower back and upper leg of healthy volunteers. They found that the way in which the transmission of samples of stratum corneum and epidermis depends on thickness can be satisfactorily described by an exponential relation, which is in accordance with the Lambert-Beer law. Therefore the transmission of the skin as a function of thickness can be written as:

$$t(d) = 2^{-d/dhalf}$$

where t(d) is the transmission of a layer of thickness d, and dhalf is a constant representing the thickness of the layer which halves the transmission of the penetrating radiation. These authors computed the dhalf values of the stratum corneum and the full thickness epidermis as a function of wavelength [Bruls, 1984]. By using this formula, it is possible to determine the in vivo transmission at several depths in epidermis as a function of wavelength.

2. AIMS OF THE STUDY

The aims of our study were:

1. To determine the mechanism of action of XeCl UVB laser.
2. To find the reason of its higher clinical efficacy compared to NB-UVB light.
3. As different UVB sources were used in UVB phototherapy and there are great differences in their clinical efficiency, to examine the apoptosis-inducing capacity of these UVB lamps.

5. To determine the effect of different physical parameters of UVB light on its apoptosis inducing capacity and antipsoriatic efficacy and to examine the therapeutic efficacy of XeCl laser in the treatment of vitiligo.
6. Although much work has examined the action spectra of UVB for different biological processes, no scientific work focused on the action spectrum of UVB for induction of T cell apoptosis. Our aim was to establish such an action spectrum by using different polychromatic UVB light sources.
7. To compute the theoretical action spectrum of UVB for the induction of intraepidermal T cell apoptosis.

3. PATIENTS AND METHODS

3.1. Patients and UVB treatment

48 plaques of 21 patients with chronic plaque type psoriasis and a 24-year-old female patient with vitiligous patches on the elbows were treated with XeCl laser phototherapy. Informed consent was obtained before the start of the study. Upon entry to the study, the patients had not been treated with systemic antipsoriatic medication for a minimum of 4 weeks. For each patient, a MED dose was established in uninvolved, unexposed gluteal skin. XeCl laser therapy was given 3 times weekly until the treated plaques had cleared completely. The initial dose was 0.6 MED, which was increased by 20% on each subsequent treatment. A 308-nm XeCl excimer laser (Lambda Physik LPX 105 E, Göttingen, Germany) was used: its output consisted of a train of short pulses (15 nanoseconds) at 5.5 mJ/cm^2 per pulse (the size of the light spot is 3 cm x 3 cm). In two groups of patients, XeCl laser phototherapy was performed with different impulse intensities (0.06 mJ/cm^2 and 20 mJ/cm^2) or impulse frequencies (1 Hz and 20 Hz), and the cumulative doses and the number of treatments up to complete clearance were determined. In five patients, symmetrical psoriatic plaques were irradiated with the same energy density with XeCl laser, but the frequency of laser impulses was 1 Hz or 20 Hz, and local psoriasis severity index (LPSI) [Ashcroft, 1999] scores were determined for each plaque following each treatment.

Eight HD patients, two asymptomatic carriers and ten healthy controls were enrolled to the second part of the study.

3.2. UVB light sources

Seven different artificial UVB sources were used in the *in vitro* irradiation experiments.

- FS20 (Westinghouse, Pittsburgh, PA) lamp without any filter (abbreviation: FS20);
- FS20 lamp with tissue culture plate top filter (Corning, London, England) (abbreviation: FS20+Plastic)*;
- NB-UVB (Philips TL01, Philips, Eindhoven, the Netherlands) lamp without any filter (abbreviation: TL01);
- NB-UVB lamp with 0.055%, 17 mm thick phtalic acid filter in pyrex tube (abbreviation: TL01+phtalic acid)**;
- NB-UVB lamp with tissue culture plate top filter (Corning, London, England) (abbreviation: TL01+Plastic)*;
- Solar simulator (Oriel ozone free Xe arc lamp, 2000W, Oriel, Stamford, CT) with WG305 filter;
- 308-nm XeCl excimer laser (Lambda Physik LPX 105 E, Göttingen, Germany) without any filter (abbreviation: XeCl).

The power of the XeCl laser was metered by an energy detector (Gentec ED-200, Quebec, Canada) and a 100 MHz oscilloscope (WATSU Electronic Co. Ltd., Tokyo, Japan). The output of the non-laser UVB light sources was metered by a calibrated UVB detector (Laser Precision Corp. RT-10l, Utica, CA). The irradiance values and incident doses used for irradiation with the different UVB light sources are shown in Table 1. The spectral irradiances were determined in 1 nm steps with a spectroradiometer (Optronic 742). The spectral distribution of the 7 different UVB light sources is represented in Figure 4. The absolute spectral irradiance values, indicated on the vertical axis, only show values above the noise level of the instrument.

---

*The top of the 24-hole tissue culture plate (Corning, England) was used as a UVB filter.

**The 35 mm diameter original TL01 tube was placed into a 52 mm diameter pyrex tube and the space between the two was filled with 0.055% potassium phthalate which is a UVB filter. The phtalic acid filter has to be refilled after 4 hours of use.

UVB source	Fluence rate (mW/cm^2)	Energy densities (mJ/cm^2)
TL01	0.89	50-800
TL01+phtalic acid	0.5	200-800
TL01+plastic	0.51	200-800
FS20	0.51	50-400
FS20+plastic	0.18	117-300
Solar simulator	1.35	800-2400
XeCl laser	$3.6*10^8$	66-300

Table 1. The irradiance values and the energy densities used in the irradiation experiments determining the dose effect curves with 7 different spectral distributions of UVB light

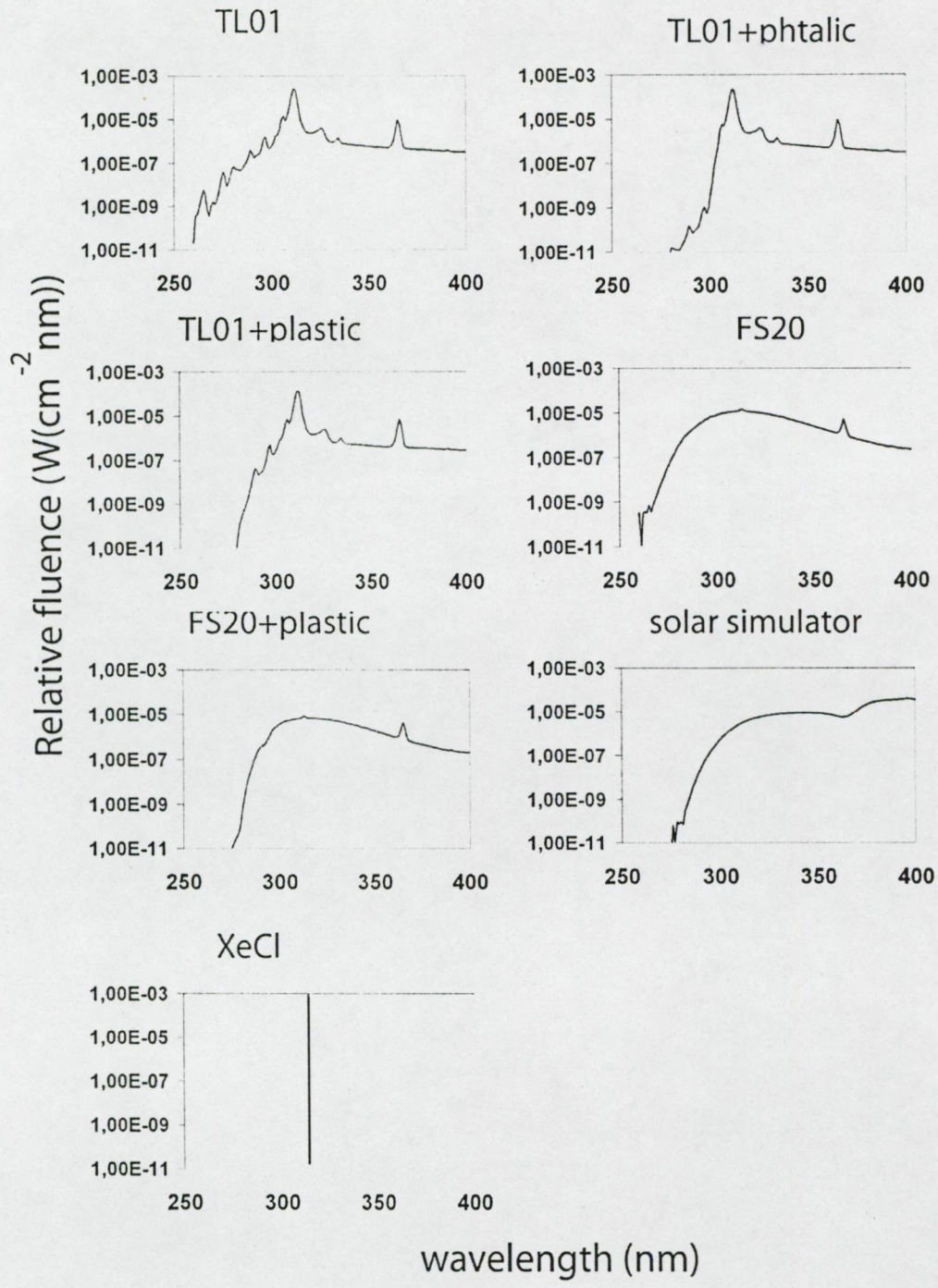

Figure 4. The spectral distribution of the 7 different UVB light sources determined at 1 nm steps with a spectroradiometer. The absolute irradiance values, indicated on the vertical axis, show values above the noise level of the instrument.

UVB irradiation *in vitro*

PBMC were prepared from heparinized venous blood of healthy volunteers by centrifugation over Ficoll-Isopaque gradient. The PBMC were washed twice in phosphate-buffered saline (PBS), and then the irradiation was performed in uncovered tissue culture plates (Corning, London, England) at a density of 10^6 cells/well in 0.3 ml PBS. The preparation of the cells was performed in dark to prevent them from accidental photodamage. In order to prevent a warming side-effect, the PBMC were placed into a 25 °C water bath during irradiation with a solar simulator. For studying the role of impulse frequency, 66 and 200 mJ/cm2 XeCl laser radiation was delivered at 1 to 40 impulses/s. In the other experiments, the laser light was emitted at 20 impulses/s.

To determine the effect of temperature at the time of the irradiation to T cell apoptosis induction, tissue culture plates were placed into 25°C or 37 °C wather bath for the duration of XeCl laser treatment. In order to prevent the warming side effect, PBMC were also placed into a 25°C water bath during irradiation with sun simulator.

After irradiation, the PBMC were washed once in PBS and resuspended in RPMI 1640 (Gibco, Scotland) supplemented with 10% AB+ heat-inactivated human serum, 2 mM L-glutamine and antibiotics. The cells were cultured in a humified 5% CO_2 atmosphere at 37 °C for 24 hours. To establish the time-dependency of T cell apoptosis induction, PBMC were incubated for 6, 12, 24 and 48 hours at the atmosphere described above.

3.3. Detection of apoptotic cells

3.3.1. TUNEL labeling:

The TUNEL (TdT-mediated dUTP-FITC nick end labeling) reaction was performed according to the manufacturer's instruction (Boehringer Mannheim, Switzerland). Briefly: PBMC were fixed in 4% paraformaldehyde solution for 30 min, washed twice in PBS, permeabilised in 0.1% Triton X-100 in 0.1 % sodium citrate for 2 min on ice, washed twice in PBS containing 1% BSA, the cells were then labeled with the TUNEL reaction mixture at 37°C for 60 min and anti-CD3-PE monoclonal antibody (mAb) (Dako, Denmark) on ice for 15 min. After washing in PBS, the cells were resuspended in 0.5 ml PBS.

3.3.2. Apo2.7 labeling

PBMC were fixed in 2% paraformaldehyde solution for 30 min, washed in PBS and permeabilised in 0.1% saponin in PBS supplemented with 1% fetal bovine serum and 0.02% NaN_3 for 15 min on ice. The cells were then washed in PBS containing 1% BSA, the supernate was discarded, and each sample was stained with 20 µl Apo2.7-PE mAb (Immunotech, Paris,

France) and 5 µl anti-human CD3-FITC mAb (Dako, Copenhagen, Denmark) in 75 µl PBS for 25 min at room temperature. To exclude false positive reactions, control samples were stained with isotype-matched anti-human IgG1-PE mAb. After washing, cells were resuspended in 0.5 ml PBS.

3.3.3. Flow cytometry

Sample data were acquired on FACStar and FACSCalibur (Becton Dickinson, Franklin Lakes, NJ) flow cytometers equipped with a 488 nm argon ion laser. 10^3 events were collected per samples. Mononuclear cells were discriminated by their forward and side scatter properties. FITC fluorescence was collected through a 525 nm bandpass filter, PE fluuorescence was collected through a 625 nm bandpass filter. The samples were analyzed using CellQuest software (Becton Dickinson, Franklin Lakes, NJ). T cells were selected via anti-CD3 mAb staining, and then Apo2.7 mAb binding, or TUNEL activity of these cells was quantified by histogram analysis.

3.4. Statistical analysis

All of the comparisons were performed by the Student paired t-test, except data on figure 5. were compared using Wilcoxon signed ranks test. A probability level of $p<0.05$ was considered statistically significant.

3.5. Determination of the DNA-weighted efficiency spectra and the median wavelength values

Using the result of the experiments described above, dose-effect curves were created for the induction of T cell apoptosis in the case of each light source. The formula for the biologically effective dose rate (DNA-weighted efficiency spectra) was calculated applying the recommendation of the Commission Internationale d'Éclairage, briefly:

$$BED / t = \Sigma E(\Delta\lambda) * S^{DNA}(\lambda) * \Delta\lambda,$$

where BED/t is the biologically effective dose rate (DNA-weighted efficiency spectra of a UV source), $E(\lambda)$ is the spectral irradiance of the UV source, $S^{DNA}(\lambda)$ is the action spectrum for UV radiation-induced DNA-damage as published by Setlow [Setlow, 1974] and $\Delta\lambda$ is the wavelength step from spectroradiometric measurements for the determination of irradiance spectra of the light sources.

The areas under these DNA-weighted efficiency spectra were calculated with the use of the rectangle method, briefly: an area under the curve was distributed to many rectangles of 1 nm

of width, and the area could has been calculated as the total of the areas of rectangles. The median wavelength was determined as the wavelength value which divides the area under the curve into two equal parts in the case of each light source.

4. RESULTS

4.1. The therapeutic effect of the XeCl laser

48 plaques of 21 psoriatic patients were treated with the XeCl laser. In order to optimize certain parameters of the UVB phototherapy, we examined whether the therapeutical effect of the XeCl laser depends on the intensity and frequency of the laser impulses.

The mean cumulative doses up to complete clearance of the psoriatic plaques were 4.062 and 4.050 mJ/cm^2 for impulse intensities of 20 and 0.06 mJ/cm^2, respectively (data not shown); the mean number of treatments was 9.2 in both cases. When the XeCl treatment was carried out with an impulse frequency of 1 or 20 Hz, the mean cumulative doses were 4.635 and 4.308 mJ/cm^2, the mean number of treatments was 8 (data not shown). None of these differences proved statistically significant. When the irradiation was performed with 1 or 20 impulses/s, there was also no significant difference in the decrease in local psoriasis severity index (LPSI) scores (Fig. 5.). LPSI is a widely used scoring system for assessment of severity of psoriasis. For each plaque, erythema, induration and desquamation are rated according to a five-point scale. The LPSI score then can vary from 0 to 15, with higher scores representing greater degree of psoriatic severity.

The patient with vitiligous pathes was showed continuous repigmentation during the XeCl laser therapy. The repigmentation seemed to be stable and no side-effects were observed [data not shown].

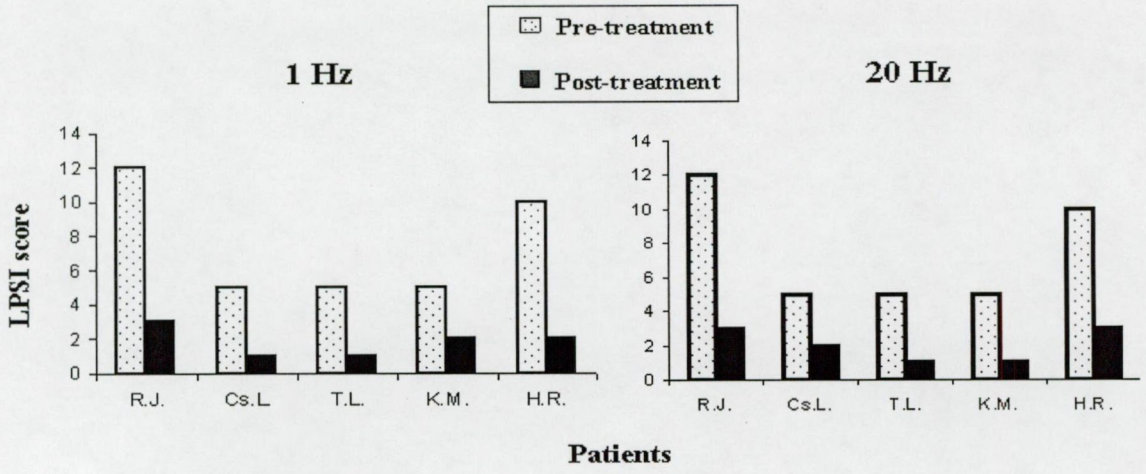

Figure 5A. The local psoriasis severity index scores measured before and after treatments with XeCl laser with 1 Hz or 20 Hz impulse frequencies. Symmetrical psoriatic plaques of 5 patients were compared by a bilateral comparison study. The initials of the patients are represented under the bars.

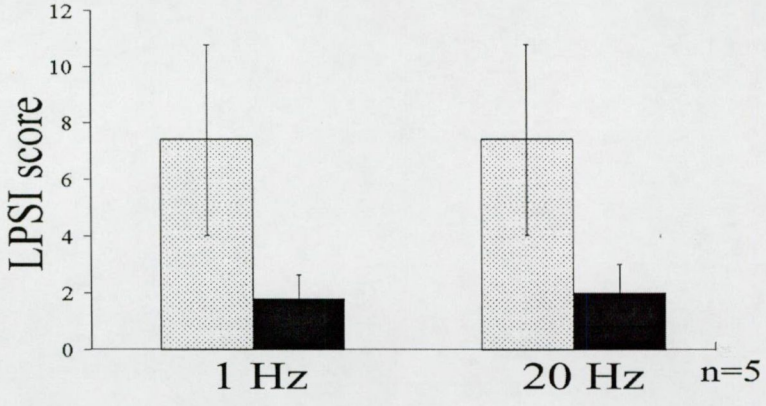

Figure 5B. The average±standard deviation of local psoriasis severity index scores measured before and after treatments with XeCl laser with 1 Hz or 20 Hz impulse frequencies. Symmetrical psoriatic plaques of 5 patients were compared. The differences between the decreases of LPSI scores proved to be statistically not significant measured by Wilcoxon Signed Ranks Test.

4.2. XeCl laser induces T cell apoptosis *in vitro*

The therapeutic efficacy of XeCl laser in dermatology is now well documented, but nothing is known about its mechanism of action. It was shown that psoriasis is mediated by activated epidermal T cells and NB-UVB depletes these T cells from the skin. The mechanism of the cytotoxicity seems to be apoptosis. To establish the possible mechanism how XeCl laser treats

psoriatic plaques, the ability of XeCl laser to induce T cell apoptosis was studied *in vitro*. Freshly separated PBMC were irradiated with 308 nm XeCl laser with 66, 100, 200 and 300 mJ/cm^2 at 20 Hz impulse frequency at room temperature. Following 6, 12, 24 or 48 hours of incubation, the apoptotic cells were labeled with TUNEL reagent, or Apo2.7 mAb, the T cells were stained with anti-CD3 mAb.

In the TUNEL reaction, deoxynucleotidyl transferase, which catalyzes polimerization of nucleotides to free 3'-OH DNA ends in a template-independent manner, is used to label DNA strand breaks. These DNA stand breaks are produced by the cleavage of genomic DNA to oligonucleosome-sized fragments during apoptosis. Fluorescein labels incorporated in nucleotide polymers are detected and quantitated by flow cytometry [Gavrieli, 1992; Sgonc, 1994].

Apo2.7 is a mitochondrial membrane protein-specific monoclonal antibody conjugated with phycoerythrin (PE) fluorescent dye [Zhang, 1996, Koester, 1997]. Recently, the involvement of mitochondria in apoptotic signaling has elicited considerable interest [Koester, 1999]. Cells having lost their mitochondrial transmembrane potential, through the opening of mitochondrial permeability transition pores appear to be irreversibly programmed to die [Marchetti, 1996].

After XeCl laser irradiation the light scatter changes were characteristic to apoptosis: decreased forward scatter (FSC: reflects to the size of the cells) and increased side scatter (SSC: reflects to the granularity of the cell surface) characterized the cells [Darzynkiewicz, 1992] (Fig. 6.). Dual measurements of CD3 binding and Apo2.7 or TUNEL positivity made it possible to distinguish apoptotic T cells (CD3+/TUNEL+ or CD3+/Apo2.7+) from non-apoptotic T cells (CD3+/TUNEL- or CD3+/Apo2.7-). Hence a flow cytometer with three-color detection was used to simultaneously assess anti-CD3-PE or anti-CD3-FITC and TUNEL or Apo2.7-PE stainings on individual cells. T cells were selected by anti-CD3 mAb staining, and then Apo2.7 mAb binding, or TUNEL activity of these cells was quantified by histogram analysis.

As shown on Figure 7, dose-dependent increases in Apo2.7 or TUNEL binding occurred after exposure of cells to 66 or 200 mJ/cm^2 XeCl laser. Flow cytometric analysis revealed time-dependent increases in the percentage of apoptotic T-cells, as measured with Apo2.7 and TUNEL stainings (Fig. 8.). The first Apo2.7-positive T-cells appeared 12 hours after irradiation. Following 48 hours of incubation, Apo2.7 positivity could not be well detected, because the light scatter properties of the cells were characteristic to late apoptosis/necrosis (data not shown). In contrast, DNA stand break assay showed a steady increase starting at 24

hours, continuing until 48 hours post-irradiation. The two different methods of observing apoptosis gave corresponding results. Irradiation with 200 mJ/cm^2 induced apoptosis in 59% and 65% of the T cells measured with the TUNEL and Apo2.7 methods, respectively, 24 hours later. At least 20 mJ/cm^2 XeCl laser irradiation needed to induce T cell apoptosis detectable with both methods (Fig. 8.).

HD is an autosomal dominant neurodegenerative disorder caused by CAG repeat expansion in the huntingtin gene [Reiner, 1988]. The mechanism of neuronal degeneration seems to be apoptosis [Saudou F, 1998]. Huntingtin is widely expressed in cells, so abnormalities can be expected also in non-neuronal tissues. Therefore, the susceptibility of lymphocytes from HD patients and healthy controls to XeCl UVB laser-induced apoptosis was compared. Lymphocytes from 8 HD patents and 2 asymptomatic carriers showed increased apoptotic cell death following *in vitro* XeCl laser irradiation [data not shown].

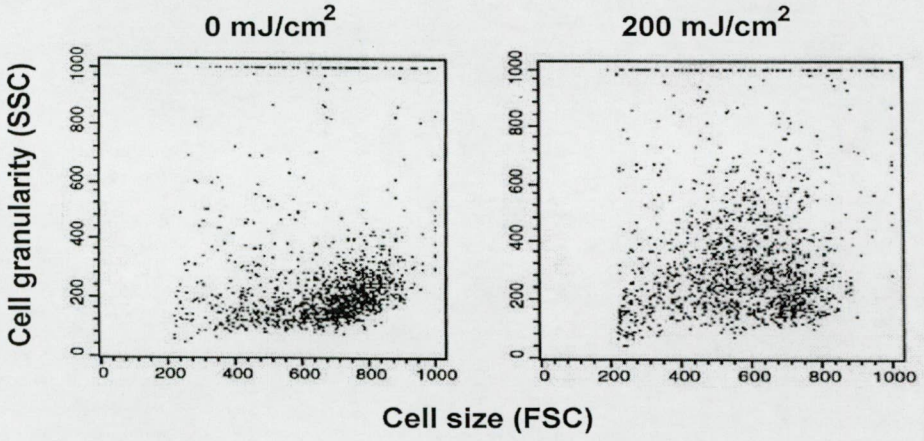

Figure 6. Light scatter properties of PBMC before and after XeCl laser irradiation measured by flow cytometry. These changes are characteristic to apoptosis: decreased forward scatter (FSC: reflects to the size of the cells) and increased side scatter (SSC: reflects to the granularity of the cell surface) can be observed following 200 mJ/cm^2 XeCl laser light.

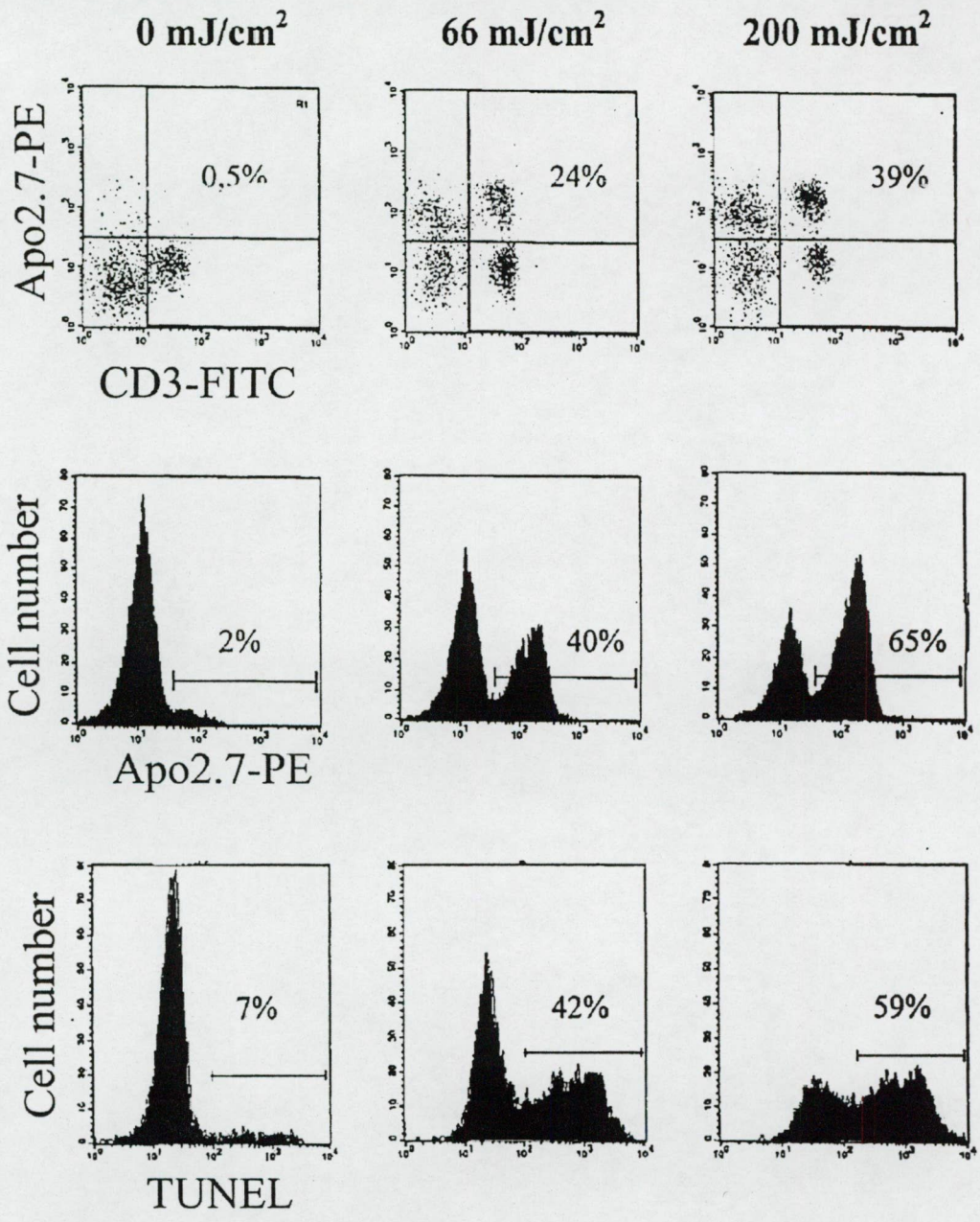

Figure 7. Flow cytometric analysis of T cell apoptosis. PBMC were irradiated with increasing amounts of energy from the XeCl laser, and apoptosis was assessed 24 hours after irradiation. The left panels are untreated controls. The top panels show simultaneous CD3 and Apo2.7 stainings, the upper right quadrant indicating apoptotic T-cells. The middle and bottom histograms show only CD3+ T cells, as identified by FITC-CD3 or PE-CD3 staining. The abscissa represents the extent of apoptosis, measured with the Apo2.7 or TUNEL method.

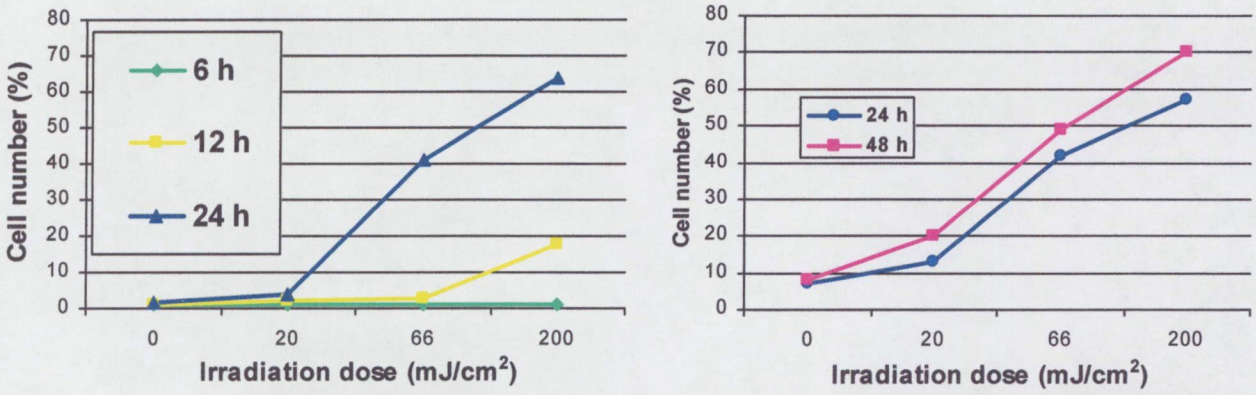

Figure 8. XeCl laser induces T cell apoptosis in a time-dependent manner. PBMC were irradiated with increasing doses of XeCl laser and then T cell apoptosis was detected 6, 12. 24 and 48 hours later with Apo2.7 (left graph) or TUNEL (right graph) method.

4.3. XeCl laser is more effective in inducing T cell apoptosis than NB-UVB

According to our earlier clinical study, the XeCl laser more efficiently cleared psoriatic plaques, than the widespread used NB-UVB. As the T-cell apoptosis-inducing capacity of a UVB light source can be paralleled by its clinical efficacy, we compared the T cell apoptosis inducing capacities of the NB-UVB and XeCl laser. PBMC were irradiated *in vitro* with the XeCl laser or with NB-UVB light with the same energy densities (0, 66, 100, 200, 300 and 600 mJ/cm2) at room temperature at 20 Hz impulse frequency. After 24 hours of incubation T cell apoptosis was studied with Apo2.7 and CD3 antibodies using flow cytometry. Both UVB light sources induced T cell apoptosis, but the induction was quantitatively greater with the 308 nm XeCl laser (Fig. 9A). The difference was significant at each energy density used as measured by Student's paired T test, but greater differences were observed when the cells were irradiated with smaller amounts of energy (53% vs. 5% induction at 100 mJ/cm2). The energy sufficient to induce apoptosis in 50% of the T cells (apoptotic dose 50: AD_{50}) was calculated. The AD_{50} proved to be 95 mJ/cm^2 with XeCl laser and 310 mJ/cm^2 with NB-UVB. The T cell apoptosis was induced in the highest degree (76% of the T cells) when irradiation was performed with 300 and 600 mJ/cm^2, with the XeCl laser and NB-UVB, respectively. Irradiation with 600 mJ/cm^2 XeCl laser induced necrosis of T cells, therefore no apoptotic cells were detected (fig 9B).

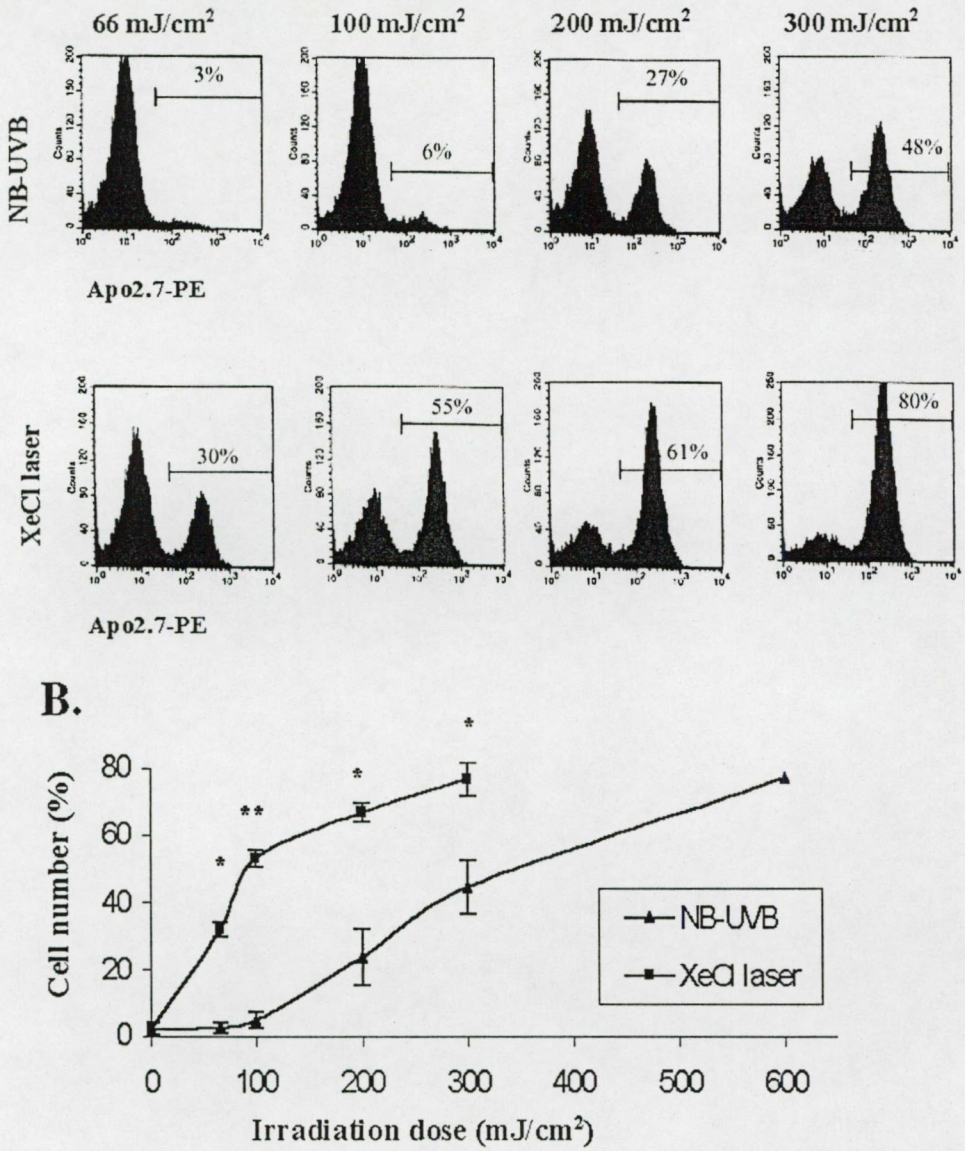

Figure 9. Comparison of the T cell apoptosis-inducing capacities of NB-UVB light and the XeCl laser. PBMC were irradiated with increasing amounts of NB-UVB light and XeCl laser. Apoptosis was measured with Apo2.7 staining 24 hours after irradiation. All of the histograms show only CD3+ T cells.

(A) The extent of apoptotic T cells are represented on the abscissa following NB-UVB (upper panels) or XeCl laser (lower panels) irradiation with the same energy densities.

(B) Summary of three independent experiments. The mean percentage of Apo2.7-positive cells is represented after irradiation with increasing amounts of NB-UVB light (triangles) or XeCl UVB laser light (squares). Irradiation with 600 mJ/cm^2 XeCl laser induced necrosis of T cells, therefore apoptotic changes could not be detected. The paired t-test was performed. P values: *P<0.005, **P<0.0005

4.4. The apoptosis-inducing effect does not depend either on the frequency of impulses, nor on the temperature during irradiation

One of our main goals was to characterize the efficacy of UVB phototherapy by different optical parameters. As the main mechanism of UVB immunosuppression seems to be T cell apoptosis, we examined whether the T cell apoptosis-inducing effect of the XeCl laser depends on important physical parameters, like the frequency of laser impulses and the temperature during irradiation. The PBMC were irradiated with the pulse-mode XeCl laser using the same energy density (66 and 200 mJ/cm^2), but the frequency of the impulses varied between 1 and 40 Hz. The intensity of each laser impulse remained the same: $3,6*10^8$ mW/cm^2. Following 24 hours of incubation, apoptotic T cells were stained with Apo2.7 and CD3 mAbs. Although slight differences could be observed, there was no significant change in the extent of T cell apoptosis using different impulse frequencies at neither of the irradiation doses used. Figure 10. demonstrates the extent of T cell apoptosis in 3 independent experiments, irradiated with 66 mJ/cm^2 energy density. The use of 200 mJ/cm^2 energy density resulted in even decreased difference (data not shown).

There is considerable evidence that the extent of some biological processes strongly depend on the temperature of the cell. Usually UVB irradiation experiments are carried out at room temperature. We investigated whether an increase in the temperature of the cells during irradiation results in increased T cell apoptosis. PBMC were irradiated with 66, 100, 200 and 300 mJ/cm^2 XeCl laser light at 20 Hz impulse frequency. During treatment, cells were divided into two tissue culture plates and one-one plate was placed into a 25°C, and a 37 °C water bath. After 24 hours of incubation on 37 °C, following Apo2.7 and CD3 mAb labelings, flow cytometry revealed, that there was not difference in the extent of T cell apoptosis between the two groups (data not shown). In summary, between the parameters investigated, neither the temperature of irradiation, nor the frequency of impulses influences the T cell apoptosis inducing capacity of XeCl laser.

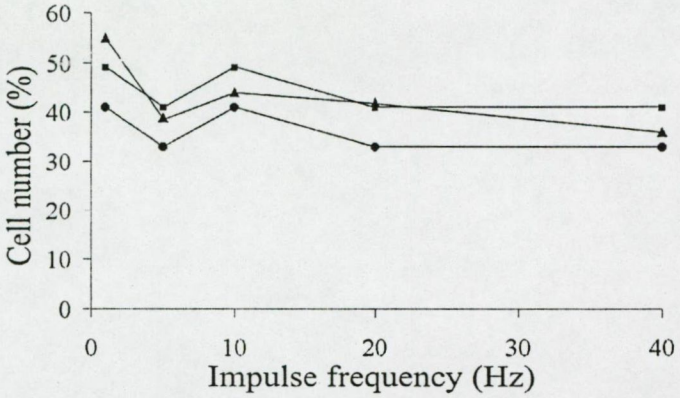

Figure 10. The percentage of Apo2.7 positive T cells 24 hours after XeCl laser irradiation. The PBMC were irradiated with 66 mJ/cm^2 energy density, but the frequency of impulses varied between 1 and 40 Hz. The three curves represent the results of three independent experiments. The differences proved to be statistically not significant.

4.5. The T cell apoptosis inducing capacity of 7 different UVB sources

In order to establish an action spectrum of UVB for T cell apoptosis-induction, we used different spectral distributions of UVB light. The BB-UVB or FS20 lamp, the NB-UVB or TL01 lamp, the XeCl laser and the Xe arc solar simulator are well known from the UVB phototherapy of different skin diseases. By using a phtalic acid filter with NB-UVB light and by using the plastic tissue culture plate top as a filter with FS20 and NB-UVB light, we could create 3 more spectral distributions of UVB light. The emission spectra of these 7 UV sources were then determined with a spectroradiometer (Fig. 4.). PBMC were irradiated *in vitro* with increasing doses, and T cell apoptosis was measured by means of simultaneous Apo2.7 and anti-CD3 stainings with flow cytometry. Dose-effect curves were then created. All 7 UVB sources induced T cell apoptosis in a dose-dependent manner. Figure 11. represents these dose-effect curves. The dosis necessary to induce apoptosis in 50% of the T cells (AD50) was calculated and listed in Table 2.

TL01 induced apoptosis in 50% of the T cells at a dosis of 300 mJ/cm^2; this result is in good agreement with our previous experimental data (Chapter 4.3.). The use of a plastic plate top as a filter eliminated most of the short-wavelength UVB radiation. This modification of the spectrum led to a decrease in the T cell apoptosis-induction capacity of NB-UVB and FS20. The filtering effect of phtalic acid was well observable too: a decrease of about 2 orders of magnitude was found at the wavelength 300 nm and phtalic acid eliminated UV radiation almost completely below 300 nm, which resulted in a severe decrease in the apoptosis-

inducing capacity of NB-UVB light (AD50: 301 mJ/cm^2 and 553 mJ/cm^2 without and with phtalic acid filter, respectively).

FS20 was the most effective of the 6 non-laser UV sources in inducing T cell apoptosis. It contains considerable amount of UVC light, and shorter UVB wavelengths predominate in its emission spectrum. The elimination of most of this short-wavelength radiation resulted in a decrease in its apoptosis-inducing capacity (AD50: 168 mJ/cm^2 and 210 mJ/cm^2 without and with plastic filter, respectively).

The solar simulator proved to be the least effective in inducing T cell apoptosis. Although it contains some UVC light and a considerable amount of UVB radiation, UVA predominates in its emission spectrum. The energy density required for the induction of apoptosis in 50% of the T cells was 1912 mJ/cm^2, which is more than 6 times more than the AD50 for NB-UVB light.

The 308 nm XeCl laser is a coherent monochromatic pulse-mode UVB excimer laser with a very high irradiance (approx. 10^9 times more than those of the above mentioned non-laser UVB sources). The XeCl laser induced T cell apoptosis in a dose-dependent manner too, but quantitative induction was much higher than with non-laser UVB sources. The AD50 for the XeCl laser was 95 mJ/cm^2, this laser light therefore induced apoptosis to a higher degree than did any of the non-laser UV sources.

4.6. Determination of the wavelength dependence of T cell apoptosis induction

One of the major mechanisms of action of UVB light is the induction of T cell apoptosis. It is well known that the biological effects of UV light are strongly dependent on the wavelength spectrum, though few scientific data exist about the wavelength dependence of T cell apoptosis induction. We decided therefore to construct an action spectrum for T cell apoptosis induction in the UVB range. The above mentioned 6 different polychromatic light sources were used and dose-effect curves were created by performing the irradiation with increasing energies. Data are expressed as the percentages of Apo2.7 and CD3 double-positive cells in relation to all the CD3-positive cells. The energy densities required for the induction of apoptosis in 50% of the T cells were then determined. The apoptosis-inducing capacities of each UV light source were calculated by reciprocating the AD50 values (Table 2.). DNA damage seems to be the major mechanism in the background of UVB radiation-induced T cell apoptosis (Breuckmann, 2003), thus the emission spectra of the polychromatic UV sources were weighted by the action spectrum of UV radiation-induced DNA damage as described in Patients and Methods. These DNA-weighted efficiency spectra of the UV sources used are

presented in Figure 12. The median wavelength values of the areas under the curves were calculated and presented in Table 2. For constructing the action spectrum for T cell apoptosis induction at polychromatic UVB sources the modification of the method applied in [Modos, 1999] was used as follows. The median wavelength value of each UV source has been associated with its apoptosis-induction capacity (1/AD50 value) obtained from the dose-effect curves and a linear regression curve was calculated. Figure 13. depicts the wavelength dependence of T cell apoptosis induction in the range 290 to 311 nm. This regression curve revealed a consistent decrease from shorter to longer wavelengths, e.g. irradiation at 290 nm is 3 times more effective in T cell apoptosis induction than that of 311 nm. The real apoptosis inducing capacity of the XeCl laser is almost 4 times higher than that calculated on the basis of this spectrum.

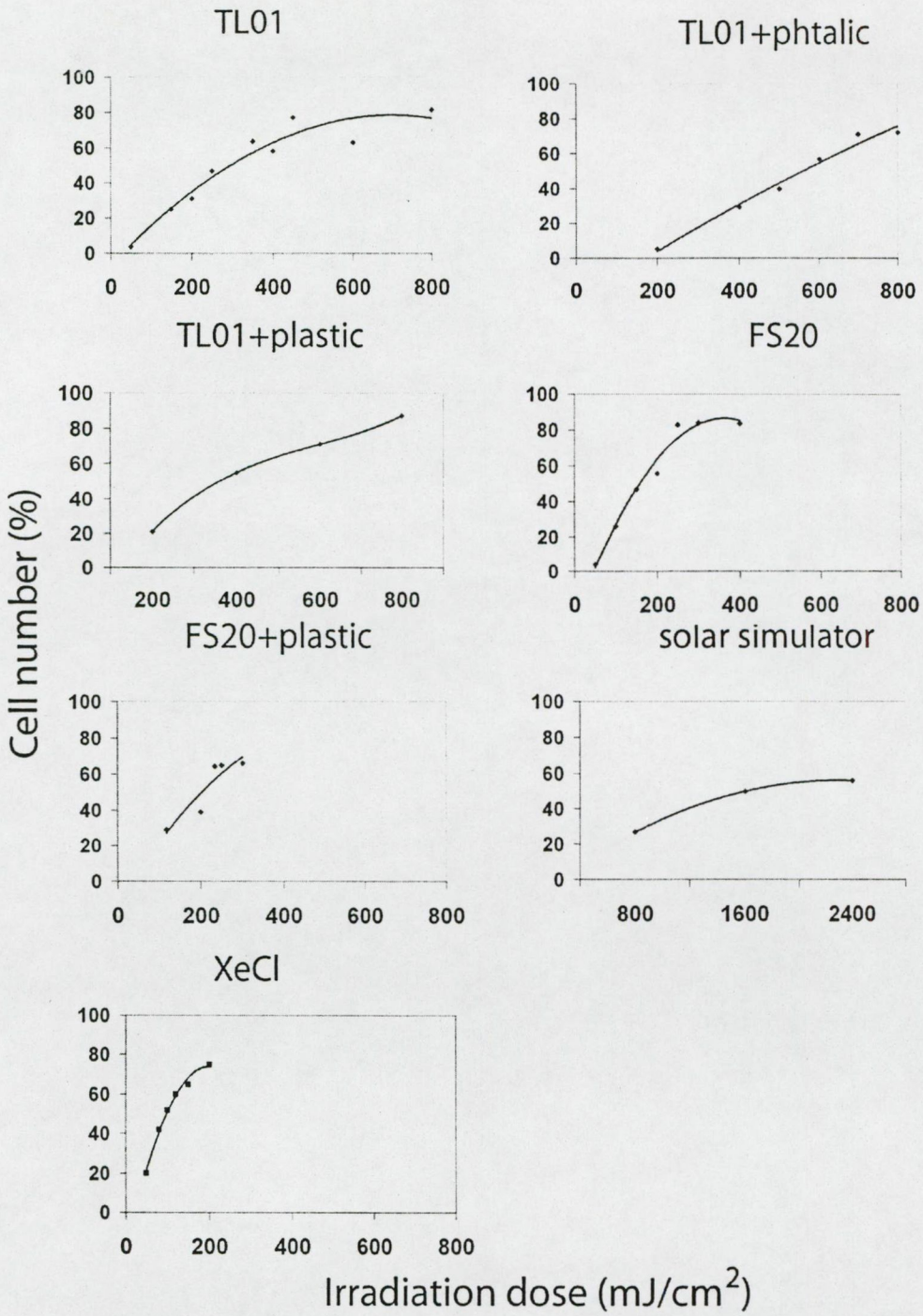

Figure 11. Dose-dependent induction of T cell apoptosis after irradiation with various polychromatic UVB light sources. Data are expressed as the percentage of Apo2.7 and CD3 double positive cells in relation to the all CD3 positive cells. Third order polynomial regression line was fitted to the individual data.

UVB source	wavelength median (nm)	AD50 (mJ/cm2)	1/AD50 (cm2/mJ)
TL01	299	301	0,0033
TL01+phtalic acid	311	553	0,0018
TL01+plastic	309	420	0,0024
FS20	290	168	0,0060
FS20+plastic	297	210	0,0048
Solar simulator	299	1912	0,0005
XeCl laser	308	95	0,0105

Table 2. The median values of the spectral distribution of UVB sources corrected by the action spectrum of UV induced DNA damage (calculated as described in Materials and Methods). The AD50 values or the energy densities necessary to induce apoptosis in 50% of the T cells were calculated from the dose-effect curves of each lamp. The apoptosis inducing capacities (1/AD50) of each UV light sources were calculated by reciprocating the AD50 Values.

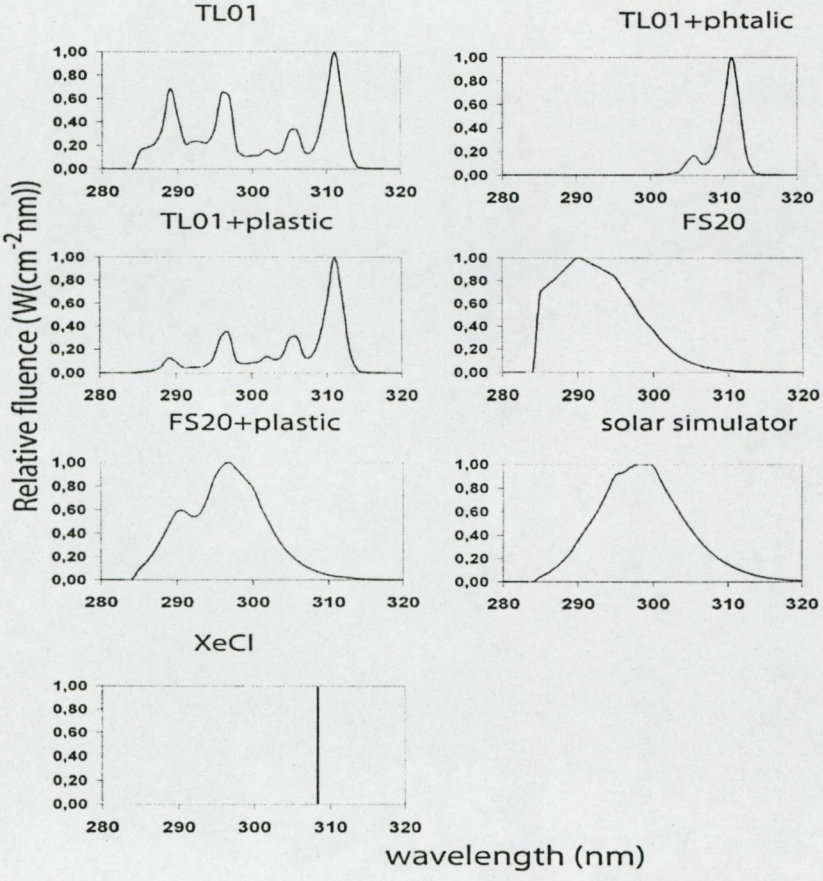

Figure 12. The emission spectra of the polychromatic UV sources were weighted by the action spectrum of UV induced DNA damage resulting in efficiency spectrum.

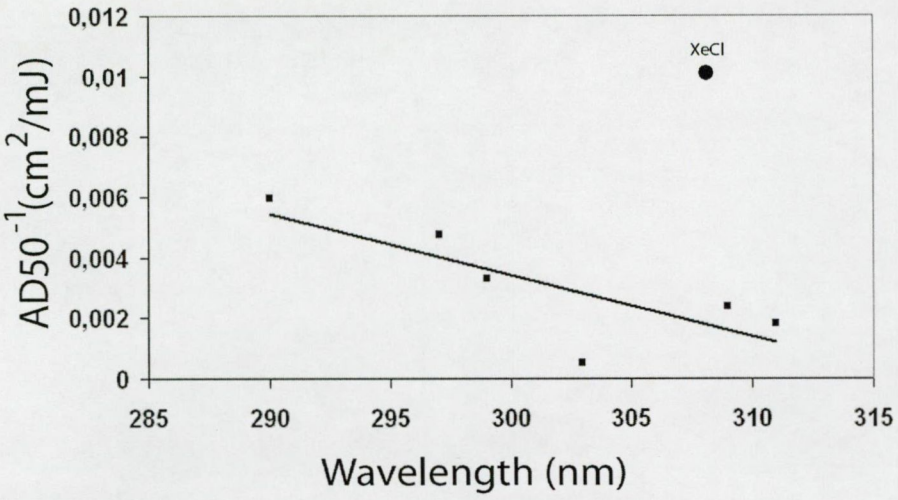

Figure 13. Wavelength dependence of UVB light for the induction of T cell apoptosis in the spectral region 290- 311 nm. The round shaped plot represents the 1/AD50 of the XeCl laser.

4.7. Determination of the theoretical action spectrum of intraepidermal T cell apoptosis induction as a function of epidermal thickness

As the extent of T cell apoptosis induction and the penetration of UVB light into the dermis is wavelength-dependent, the wavelength spectrum of optimal dermato-phototherapy might depend on the thickness of the skin lesion. Therefore, the formula created by Bruls et al [Bruls, 1984] was modified to compute the theoretical wavelength dependence of intraepidermal T cell apoptosis as a function of epidermal thickness. As the pathognomic T cells in psoriasis are localised along the dermo-epidermal junction and in the epidermis, we computed how thick layer of epidermis has to be transmitted by the UVB radiation to reach these intraepidermal T cells. As the average thickness of stratum corneum and suprapapillary part of the stratum malpihigii is 20 μm and 43 μm, respectively, and the thickness of the living layer of epidermis varies from the extent of acanthosis, these T cells are localised at 65-460 μm from the surface of the skin. The extent of intraepidermal T cell apoptosis induction was determined by multiplying the extent of *in vitro* T cell apoptosis induction and the percentage of transmitted UVB light at each wavelength studied.

$$AI_{ie} = 1/AD50 * 2^{-d/dhalf}$$

Where AI_{ie} is the theoretical extent of intraepidermal T cell apoptosis induction, AD50 is the energy density to induce apoptosis in 50 % of the T cells *in vitro*, d is the distance of the T cells from the surface of the skin, and dhalf is a constant representing the thickness of the layer which halves the transmission of the penetrating radiation.

By using this formula, one can determine the theoretical extent of intraepidermal T cell apoptosis induction in the range 290-313 nm depending on the epidermal thickness of the irradiated skin. For T cells situated at 65 μm and 250 μm from the surface, 302 nm and 313 nm seems to be the most efficient wavelengths, respectively. For T cells localised from appr. 140 μm from the skin surface, the wavelengths 302-313 nm are equally efficient in inducing apoptosis. The Figures 14-16 depict these 3 theoretical action spectra.

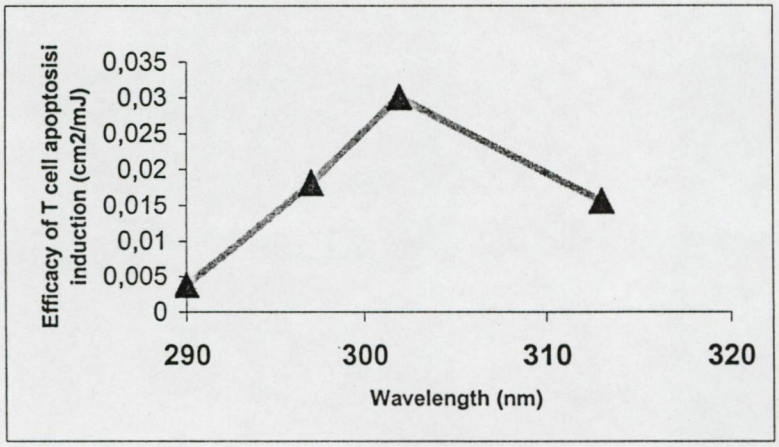

Figure 14. Theoretical action spectrum of UVB for the induction of intraepidermal T cell apoptosis in case of thin epidermis (65 μm from the surface of the skin).

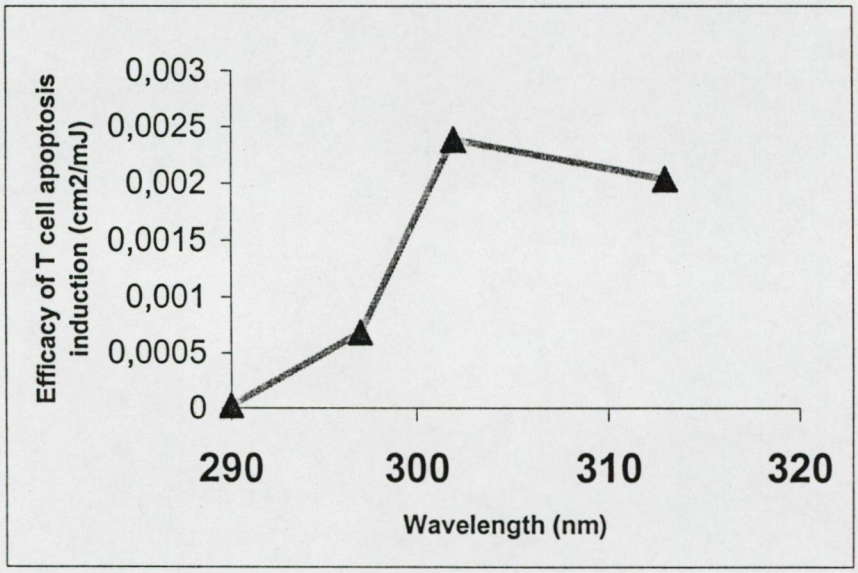

Figure 15. Theoretical action spectrum of UVB for the induction of intraepidermal T cell apoptosis in case of moderately acanthotic epidermis (140 μm from the surface of the skin).

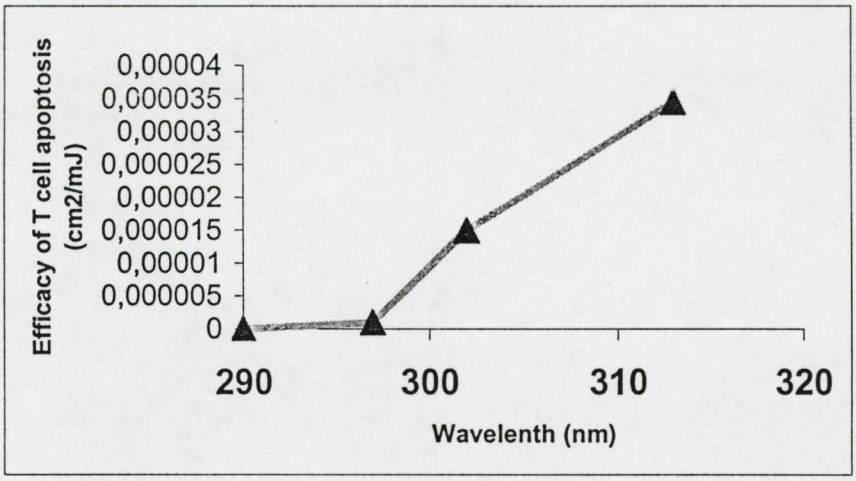

Figure 16. Theoretical action spectrum of UVB for the induction of intraepidermal T cell apoptosis in case of extremely acanthotic epidermis (250 μm from the skin surface).

5. Discussion

UV phototherapy is widely applied to treat different dermatoses. The prototypic skin disease showing a favorable response to UV phototherapy is psoriasis vulgaris [Fischer, 1976]. There is growing evidence that the efficacy of UVA and UVB phototherapy may not simply be attributed to antiproliferative effects, but most likely involves immunomodulatory consequences [Morita, 1997; Gilmour, 1993]. One of the major mechanisms of action of UVB light in the treatment of inflammatory dermatoses seems to be a cytotoxic effect on the infiltrating T cells, where the mechanism of cell death is most probably apoptosis [Ozawa, 1999; Aragane, 1998].

Earlier, we found that XeCl UVB laser clears psoriatic plaques more efficiently, than conventional NB-UVB. This finding was confirmed later by other studies too. In order to expand our earlier pilot study, in the present study we investigated the efficacy of the XeCl laser for the treatment of psoriasis. All 21 patients tolerated the treatment well and no serious side-effects were observed. The XeCl laser phototherapy was highly effective in all of the treated plaques, the LPSI scores decreasing quickly following each visit [Publ. I). The XeCl laser was also effective in inducing repigmentation in a patient with vitiligo without any side-effects [Publ. III]

Conventional UVB sources emit polychromatic continuous incoherent light, whereas the XeCl laser emits coherent, monochromatic UVB light in short impulses, which permits the variation of certain important phototherapeutic parameters, such as impulse frequency and light intensity. Earlier, when psoriatic skin was irradiated with multiple MED doses of XeCl

laser, the application of higher frequency of laser impulses led to the induction of stronger erythema (unpublished data). These observations indicated that the frequency of impulses influences the biological effect of phototherapy. In the present study, we compared the antipsoriatic efficacy of the XeCl laser when the irradiation was performed at different intensities and impulse frequencies. We did not find significant differences in either of the investigated parameters [Publ. I].

For most of the plaques, the 3x3 cm light spot of the XeCl laser is sufficient for treatment, however we are seeking possibilities to extend the area being irradiated at a given time. One potential solution would be to mount the XeCl laser with a real-time scanner. The scanner would perform a total body scan to recognize psoriatic plaques and would automatically direct the laser light toward the involved areas. However, this technique would result in the same plaque receiving irradiation at a reduced impulse frequency. Another possibility for total body treatment is if the laser light is scattered to increase the area of irradiation. In this case, the light intensity of the laser impulses would be reduced. Our present results indicate that the introduction of a scanning system or laser light scattering would not lower the efficacy of XeCl treatment.

As UVB acts through the induction of apoptosis on different cell types [Aragane, 1998, Peter, 1998], we set out to learn more about the cellular mechanism of action of the XeCl laser, and demonstrated that it induces T cell apoptosis *in vitro* measured with Apo2.7 mAb staining and TUNEL reaction. The extent of UVB-induced T cell apoptosis showed dose-and time dependency as measured with both methods [Publ. I].

Parallel with the clinical studies we found that changes in the frequency of impulses did not influence the apoptosis-inducing capacity of the XeCl laser [Publ I].

To check our method in a clinical setting, we determined the susceptibility of lymphocytes from HD patents and healthy individuals to XeCl UVB-induced apoptosis. According to our results, that sensitivity of HD cells to induced apoptosis is not restricted to neurons [Publ. IV].

Earlier, psoriatic plaques in 23 patients were treated daily with NB-UVB or BB-UVB in a bilateral comparison study. NB-UVB cleared the psoriatic plaques more effectively than did BB-UVB. On the other hand, NB-UVB light has been found to be a more potent inductor of T cell apoptosis *ex vivo* than BB-UVB light; therefore, the T cell apoptosis inducing capacity of a UVB light source can be paralleled by its clinical efficacy [Murphy, 1995]. In our present study, the *in vitro* T cell apoptosis-inducing capacities of widely used BB-, NB-UVB sources and the XeCl UVB laser were determined and compared to each other. XeCl laser was the strongest apoptosis inducer, BB-UVB (FS20) induced T cell apoptosis more efficiently than

NB-UVB (TL01) [Publ. II]. In clinical studies, XeCl laser also seems to be the most efficient antipsoriatic UVB source [Bónis, 1997], suggesting that the more effective induction of T cell apoptosis may be responsible for the greater clinical efficacy of the XeCl laser as compared with non-laser UVB light. In fact, given that approximately 5-10% of the UVB energy incident on the skin penetrates the epidermis [Anderson, 1981], there is reasonably good agreement between the doses of XeCl laser required to kill T-cells (200 mJ/cm^2) and the therapeutic amounts of 308 nm laser light delivered to psoriatic lesions (1624-3248 mJ/cm^2 [Bónis, 1997]). Clinically, however, NB-UVB is a more potent antipsoriatic light source than BB-UVB [Walters, 1999]. The main reason for the difference between in vitro and clinical data might be the absorbance of most of the radiation shorter than 300 nm by the epidermis. Thus, that short wavelength UVB range that is emitted by BB-UVB induces apoptosis efficiently *in vitro*, but cannot penetrate into the dermis or the dermo-epidermal junction [Tjioe, 2003].

The apoptosis-inducing efficacy of the XeCl laser proved to be much higher than all of the non-laser sources. The high efficacy of the UVB laser was observed in clinical studies too, therefore this super narrow-band light is more and more widespread used in phototherapy of different skin diseases. The background of its very high efficacy is still unknown, but we hypothesize that its high fluence rate can mostly be responsible for it: e.g. the XeCl laser emits its energy in nanoseconds, while the performance of NB-UVB irradiation requires minutes. On the other hand, though the NB-UVB light source emits most of its energy in the wavelength interval 311-313 nm [Green, 1988], its emission spectrum contains longer wavelengths too, which may exert a less cytotoxic effect on T cells. Additionally, when metered with biological dosimeters, NB-UVB light has been found to include some radiation of wavelength of 280 nm, which has a high DNA-damaging potential [Modos, 1999]; however, this short-wavelength light is fully scattered and absorbed by the upper layer of the epidermis [Bruls, 1984]. This might also contribute to the observed higher clinical efficacy of the XeCl laser as compared with that of NB-UVB light. Hurks et al examined the influence of irradiance on the MECLR *in vitro* and *in vivo* using broad-band UV lamp with variable UV intensities. They found, that irradiation of epidermal cells with high irradiance impaired the alloactivating capacity more than irradiation with low irradiance. They concluded that UVB-induced suppression of MECLR was critically dependent on irradiance [Hurks, 1995]. According to other authors, the role of light intensity in UVB-induced immunosuppression is questionable [DeFabo, 1980, Kelfkens, 1991]. According to our results, fluence rate may

influence the effects of UVB radiation, higher UVB fluence rates can result greater UVB induced T cell apoptosis [Publ. II].

NB-UVB contains considerable amount of short UVB. By using phtalic acid filter almost all of the radiation shorter than 300 nm could be eliminated, however, this short-wavelength spectrum was very effective in our *in vitro* study [Publ. II]. Earlier measurements disclosed that this short-wavelength UV light is almost completely absorbed in the upper part of the epidermis, therefore it cannot induce T cell apoptosis *in vivo* [Anderson, 1981]. Thus, the use of phtalic acid-filtered UVB light in skin phototherapy might have advantages over traditional UVB phototherapy by eliminating the erythema-inducing, but therapeutically not efficient short-wavelength UV radiation. However, this hypothesis has to be proven by *in vivo* clinical studies.

The present study also describes the determination of the action spectrum of UVB radiation-induced T cell apoptosis. As 95% of the UVC radiation is scattered, reflected and/or absorbed in the upper thin (about 25-30 μm) part of the epidermis [Environmental Health Criteria, 1994], and UVB was found to be superior in biological efficacy than UVA [Nishigaki, 1999, Peak, 1991], we restricted the range of our interest to the UVB spectrum. The spectral sensitivity for various biological UV effects, like killing efficiency of simple organisms, production of erythema, DNA damage, skin cancer induction, immunosuppression has been determined mostly with monochromatic light [Matsunaga, 1991; Webber, 1997; Hurks, 1995]. However, in this case, the determination of the precise sensitivity value with a monochromatic source is somewhat obscure due to the difficulty in determining the accurate light dose (e.g. as monochromatic light created by monochromators is of variable and low intensity, the irradiation procedure would take hours to induce T cell apoptosis). This fact appears in the literature as the discrepancy between sensitivity curves measured with a monochromatic source and with polychromatic radiation observed in normal human skin fibroblasts [Rosenstein, 1987]. A precise and comprehensive action spectrum determination has been performed earlier [de Gruilj, 1993; de Gruilj, 1995] for Utrecht-Philadelphia skin cancer induction on hairless mice using 14 different polychromatic UV sources. In the 250-400 nm wavelength range the approximation by a Langrange polynomial fourth order resulted in a satisfactory fitting of the calculated and the measured data.

By using different filters with the above-mentioned UV light sources, we could create more spectral distribution UV light with higher intensity. Then the efficacy of different spectral regions of UVB light to induce T cell apoptosis was determined by the use of these polychromatic UV light sources. This experimental method is accepted and proved to be

successful in photodermatology completed by a polynomial approximation [de Gruilj, 1994]. The emission spectra of sources used were weighted by the action spectrum of UV radiation-induced DNA damage as published by Setlow. The T cell apoptosis-induction capacities of all these lamps were determined by the method described earlier. Dose-effect curves were created and the apoptosis-induction efficacy (1/AD50) of the UVB sources were calculated by reciprocating the dose values which induced apoptosis in 50% of the T cells in the case of each lamp. The wavelength dependence of UVB light to induce T cell apoptosis was determined by associating the 1/AD50 value with the median wavelength of each light source. The regression curve of this action spectrum demonstrates a continuous decrease from 290 nm to 311 nm [Publ. II]. The decreasing trend of this spectrum is similar to those observed earlier, e.g. the erythema action spectrum, the action spectrum for thymine dimer and 6-4 photoproduct formation and the action spectra for suppression of the MLR and MECLR responses. However, these action spectra (erythema, thymine dimer) show several orders of magnitude difference in efficiency between 290-311 nm while the action spectrum reported here show differences far less than this between these wavelengths. The explanation for the similarities might be that all of these processes are predominantly mediated by UV light-induced DNA damage [Godar, 1995].

In summary, our results suggest that the XeCl laser is a new and promising form of UVB phototherapy. Carcinogenicity of different UV therapies increase in parallel with the cumulative UV dose during life [Lavker, 1995]. We found earlier, that the cumulative dose needed for healing was more than 6 times less with the XeCl laser than with NB-UVB therapy. We presumed, that the lower therapeutic cumulative dose therefore involves a lower risk of carcinogenesis. Additionally, as the majority of the psoriatic patients suffer from mild to moderate psoriasis, affecting only 10-20% of the total body surface, and the XeCl laser is selectively directed toward lesional skin, the laser treatment results in sparing the surrounding normal skin from unnecessary carcinogenic UV radiation exposure.

We have also shown that XeCl laser is more effective than BB-UVB and NB-UVB light in inducing T-cell apoptosis. As the induction of T-cell apoptosis seems to be one of the main mechanisms of action of UVB light in the treatment of many inflammatory (psoriasis, atopic dermatitis, lichen ruber, etc.) and hyperproliferative (cutaneous T-cell lymphomas) diseases, the XeCl UVB laser might well be the treatment of choice for these disorders. Additionally, the XeCl laser was found to induce repigmentation in vitiligo [Publ.III], suggesting that this new "super narrow band" UVB light source might well be used in different treatment indications in dermatological practice [Njoo, 1999].

There are further possible fields of application in research for the XeCl laser. The results of UV photobiology research are not always comparable, as UVB lamps often emit shorter or longer wavelengths too, and because of the incoherency of the UVB sources, which makes precise dosimetry difficult [Gasparro, 2000]. The optical properties of the XeCl laser (a monochromatic, coherent, pulse-mode laser; easier precise dosimetry, there are no "contaminating" wavelengths) can make this laser light an ideal tool for studies of the mode of action of UVB light.

Although T cell apoptosis induction seems to be a major mechanism of UVB induced immunosuppression [Moodclyffe, 2000; Tomimori, 2000], the results of this study cannot be directly adapted to clinical application. In our opinion, the spectral distribution of a UVB light source influences the clinical results by having effect on the apoptosis induction capacity and having influence on the absorbance and transmittance of the light in the epidermis. On one hand, shorter wavelengths seem to induce T cell apoptosis more efficiently; on the other hand, longer wavelengths penetrate better into the dermis. Therefore, the formula created by Bruls et al [Bruls, 1984] was modified to compute the theoretical wavelength dependence of intraepidermal UVB induced T cell apoptosis as a function of epidermal thickness [manuscript in preparation]. By using this formula, one can determine the theoretical extent of intraepidermal T cell apoptosis induction in the UVB range 290-313 nm depending on the epidermal thickness of the irradiated skin. Altough UVB induced T cell apoptosis is not the only one mechanism in the background of UV phototherapy [Nghiem,2002; Ullrich, 1996, Schwarz, 2000], we consider that longer wavelength UVB phototherapy would be ideal for the treatment of thick psoriatic plaques, while short-wavelength UVB radiation would be optimal for the treatment of thin skin lesions. We hope that the results of this study will partly help the phototherapists to individualize the UV phototherapy according to the properties of the lesion to be treated.

6. SUMMARY

6.1. We showed that XeCl laser is well tolerated by the patients and highly effective for the treatment of psoriatic plaques. The clinical efficacy does not depend on light intensity nor on laser impulse frequency. The XeCl laser proved to be effective in the treatment of vitiligo also.

6.2. We have shown for the first time that XeCl UVB laser induces T cell apoptosis *in vitro*.

6.3. Lymphocytes from HD patiens were more susceptible to XeCl UVB-induced apoptosis than those from healthy controls.

6.4. XeCl laser is more effective in inducing T cell apoptosis than NB-UVB.

6.5. The apoptosis-inducing effect does not depend either on the frequency of impulses nor on the temperature during irradiation.

6.6. We determined the T cell apoptosis-inducing capacity of 7 different UV light sources.

6.7. We constructed the action spectrum of UVB light for T cell apoptosis induction.

6.8. We calculated the theoretical action spectrum of intraepidermal T cell apoptosis induction as a function of epidermal thickness.

7. ABBREVIATIONS

BB-UVB:	Broad-band ultraviolet B
MED:	Minimum erythema dose
SUP:	Selective UVB phototherapy
NB-UVB:	Narrow-band ultraviolet B
XeCl:	Xenon chloride
MLR:	Mixed lymphocyte reaction
MECLR:	Mixed epidermal cell lymphocyte reaction
PBMC:	Peripheral blood mononuclear cells
TUNEL:	TdT-mediated dUTP-FITC nick end labeling
mAb:	Monoclonal antibody
PBS:	Phosphate buffered saline
LPSI:	Local psoriasis severity index
PE:	Phycoerythrin
HD:	Huntington disease
AD50:	The energy density necessary to induce apoptosis in 50% of the T cells

dhalf(λ): Constant representing the thickness of the layer which halves the transmission of the penetrating radiation

AI_{ie}: Theoretical extent of intraepidermal T cell apoptosis induction

8. ACKNOWLEDGEMENT

I thank Prof. Dr. Attila Dobozy, Member of The Hungarian Academy of Sciences, for providing me the opportunity to work in a highly inspiring scientific environment at his Department.

I am grateful to my supervisor, Prof. Dr. Lajos Kemény for giving me continuous support and for guiding me with his invaluable advices regarding my scientific and clinical work.

I express my gratitude to my collegues, Dr. Zsuzsanna Bata-Csörgő, Dr. Anna Kenderessy Szabó, Dr. Eszter Baltás, Dr. Győző Szolnoky, Dr. Béla Bónis for always being ready to help me.

I was fortunate to work with Dr. Katalin Jakab, from the Department of Neurology, University of Szeged, Hungary; Dr Attila Bérces, Prof. Dr. Györgyi Rontó from the MTA-SE Research Group for Biophysics, Hungarian Academy of Sciences, Budapest, Hungary; Dr Imre Ocsovszki from the Department of Biochemistry, University of Szeged, Hungary; Ferenc Ignácz from the Department of Optics and Quantum Electronics, University of Szeged, Hungary; Dr. Éva Nagy, dr. Andrea Surányi from the Department of Clinical Chemistry, University of Szeged, Hungary, and Dr. Éva Pállinger from the Molecular Immunological Research Group, Hungarian Academy of Sciences, Budapest.

I wish to acknowledge the technical help of Andrea Gyimesi and Edit Márta Gordos.

And last but not least I would like to thank my family the continuous support..

9. REFERENCES

Anderson RR, Parrish JA: The optics of human skin. *J. Invest. Dermatol.* 1981;77:13-19.

Aragane Y, Kulms D, Metze D, Wilkes G, Poppelmann B, Luger TA, Schwarz T.: Ultraviolet light induces apoptosis via direct activation of CD95 (Fas/APO-1) independently of its ligand CD95L. *J Cell Biol.* 1998;140(1):171-82.

Asawanonda P, Anderson RR, Chang Y, Taylor CR: 308-nm excimer laser for the treatment of psoriasis: a dose-response study. *Arch. Dermatol.* 2000;136:619-624.

Ashcroft DM, Wan Po AL, Williams HC, Griffiths CE: Clinical measures of disease severity and outcome in psoriasis: a critical appraisal of their quality. *Br. J. Dermatol.* 1999;141:185-191.

Aubin F.: Mechanisms involved in ultraviolet light-induced immunosuppression. *Eur J Dermatol.* 2003;13(6):515-23.

Aubin F, Mousson C.: Ultraviolet light-induced regulatory (suppressor) T cells: an approach for promoting induction of operational allograft tolerance? *Transplantation.* 2004;77(1 Suppl):S29-31.

Austad J, Braathen LR: Effect of UVB on alloactivating and antigen-presenting capacity of human epidermal Langerhans cells. *Scand J Immunol.* 1985;21(5):417-23.

Baltás E, Nagy P, Bónis B, Novák Z, Ignácz F, Szabó G, Bor Zs, Dobozy A, Kemény L.: Repigmentation of localized vitiligo with the xenon chloride laser. *Br. J. Dermatol.* 2001; 144:1266-1267.

Bataille V, Bykov VJ, Sasieni P, Harulow S, Cuzick J, Hemminki K.: Photoadaptation to ultraviolet (UV) radiation in vivo: photoproducts in epidermal cells following UVB therapy for psoriasis. *Br J Dermatol.* 2000;143(3):477-83.

Bianchi B, Campolmi P, Mavilia L, Danesi A, Rossi R, Cappugi P.: Monochromatic excimer light (308 nm): an immunohistochemical study of cutaneous T cells and apoptosis-related molecules in psoriasis. *J Eur Acad Dermatol Venereol.* 2003;17(4):408-13.

Bónis B, Kemény L, Dobozy A, Bor Zs, Szabó G, Ignácz F: 308 nm excimer laser for psoriasis. *Lancet* 1997;350:1522.

Breuckmann F, von Kobyletzki G, Avermaete A, Radenhausen M, Hoxtermann S, Pieck C, Schoneborn P, Gambichler T, Freitag M, Hoffmann K, Altmeyer P.: Mechanisms of apoptosis: UVA1-induced immediate and UVB-induced delayed apoptosis in human T cells in vitro. *J Eur Acad Dermatol Venereol.* 2003;17(4):418-29.

Bruls WAG, Slaper H, van der Leun JC, Berrens L. Transmission of human epidermis and stratum corneum as a function of thickness in the ultraviolet and visible wavelengths. *Photochem Photobiol.* 1984;40(4):485-94.

Caricchio R, Reap EA, Cohen PL.: Fas/Fas ligand interactions are involved in ultraviolet-B-induced human lymphocyte apoptosis. *J Immunol.* 1998;161(1):241-51.

Chapman DM, Ross JB.: Objective measurement of three epidermal parameters in psoriasis vulgaris and in dermatopathology in general. *Br J Dermatol.* 1988;119(3):333-43.

Compton MM: A biochemical hallmark of apoptosis: internucleosomal degradation of the genome. Cancer Metastasis Rev. 1992;11(2):105-19.

Coven TR, Burack LH, Gilleaudeau T, Keogh M, Ozawa M, Krueger JG: Narrowband UV-B produces superior clinical and histopathological resolution of moderate-to-severe psoriasis in patients compared with broadband UV-B. *Arch. Dermatol.* 1997;133:1514-1522.

Darzynkiewicz Z, Bruno S, Del Bino G, Gorczyca W, Hotz MA, Lassota P, Traganos F.: Features of apoptotic cells measured by flow cytometry. *Cytometry.* 1992;13(8):795-808

de Gruijl FR, Sterenborg HJ, Forbes PD, Davies RE, Cole C, Kelfkens G, van Weelden H, Slaper H, van der Leun JC.: Wavelength dependence of skin cancer induction by ultraviolet irradiation of albino hairless mice. *Cancer Res.* 1993;53(1):53-60.

de Gruijl FR.: Action spectrum for photocarcinogenesis. *Recent Results Cancer Res.* 1995;139:21-30.

Environmental Health Criteria 160 (1994). Ultraviolet Radiation, WHO, Geneva

Epstein JH, Fukuyama K, Epstein WL: UVL induced stimulation of DNA synthesis in hairless mouse epidermis. *J Invest Dermatol.* 1968;51(6):445-53.

Feldman SR, Mellen BG, Salam TN, Fitzpatrick RE, Geronemus RG, Vasily DB, Morison WL: Efficacy of 308 nm excimer laser for treatment of psoriasis: results of a multicentric study. *J. Am. Acad. Dermatol.* 2002;46(6):900-6.

Gasparro FP, Brown DB: Photobiology 102: UV sources and dosimetry - the proper use and measurement of "photons as a reagent". *J. Invest. Dermatol.* 2000;114:613-615.

Gavrieli Y, Sherman Y, Ben-Sasson SA.: Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. *J Cell Biol.* 1992;119(3):493-501.

Gibbs NK, Norval M, Traynor NJ, Wolf M, Johnson BE, Crosby J: Action spectra for the trans to cis photoisomerisation of urocanic acid in vitro and in mouse skin. *Photochem Photobiol.* 1993;57(3):584-90.

Gilmour JW, Vestey JP, George S, Norval M.: Effect of phototherapy and urocanic acid isomers on natural killer cell function. *J Invest Dermatol.* 1993;101(2):169-74.

Godar DE, Lucas AD.: Spectral dependence of UV-induced immediate and delayed apoptosis: the role of membrane and DNA damage. *Photochem Photobiol.* 1995;62(1):108-13.

Godar DE.: Light and death: photons and apoptosis. *J Investig Dermatol Symp Proc.* 1999;4(1):17-23.

Goeckerman, W.H.: The treatment of psoriasis. *Northwest Med.* 1925;24:229

Goettsch W, Hurks HM, Garssen J, Mommaas AM, Slob W, Hoekman J, Pierik F, Roholl PJ, Van Loveren H.: Comparative immunotoxicology of ultraviolet B exposure I. Effects of in vitro and in situ ultraviolet B exposure on the functional activity and morphology of Langerhans cells in the skin of different species. *Br J Dermatol.* 1998;139(2):230-8.

Gottlieb SL, Gilleaudeau P, Johnson R, Estes L, Woodworth TG, Gottlieb AB, Krueger JG.: Response of psoriasis to a lymphocyte-selective toxin (DAB389IL-2) suggests a primary immune, but not keratinocyte, pathogenic basis. *Nat Med.* 1995;1(5):442-7.

Grewe M, Gyufko K, Krutmann J.: Interleukin-10 production by cultured human keratinocytes: regulation by ultraviolet B and ultraviolet A1 radiation. *J Invest Dermatol.* 1995;104(1):3-6.

Gutierrez-Steil C, Wrone-Smith T, Sun X, Krueger JG, Coven T, Nickoloff BJ.: Sunlight-induced basal cell carcinoma tumor cells and ultraviolet-B-irradiated psoriatic plaques express Fas ligand (CD95L). *J Clin Invest.* 1998;101(1):33-9.

Hart PH, Grimbaldeston MA, Finlay-Jones JJ.: Mast cells in UV-B-induced immunosuppression. *J Photochem Photobiol B.* 2000;55(2-3):81-7.

Hill LL, Shreedhar VK, Kripke ML, Owen-Schaub LB.: A critical role for Fas ligand in the active suppression of systemic immune responses by ultraviolet radiation. *J Exp Med.* 1999;189(8):1285-94.

Hoffmann K, Kaspar K, Altmeyer P, Gambichler T.: UV transmission measurements of small skin specimens with special quartz cuvettes. *Dermatology.* 2000;201(4):307-11.

Hurks HM, Out-Luiting C, Vermeer BJ, Claas FH, Mommaas AM.: Dose-response characteristics of immunologic unresponsiveness to UV-induced tumors produced by UV irradiation of mice. *Photochem Photobiol.* 1979;30(3):385-90.

Hurks HM, Out-Luiting C, Vermeer BJ, Claas FH, Mommaas AM.: The action spectra for UV-induced suppression of MLR and MECLR show that immunosuppression is mediated by DNA damage. *Photochem Photobiol.* 1995;62(3):449-53.

Hurks HM, Out-Luiting C, Vermeer BJ, Claas FH, Mommaas AM: UVB-induced suppression of the mixed epidermal cell lymphocyte reaction is critically dependent on irradiance. *Photochem Photobiol.* 1995;62(3):485-9.

Hurks HM, Out-Luiting C, Vermeer BJ, Claas FH, Mommaas AM.: In situ action spectra suggest that DNA damage is involved in ultraviolet radiation-induced immunosuppression in humans. *Photochem Photobiol.* 1997;66(1):76-81.

Kelfkens G, van Weelden H, de Gruijl FR, van der Leun JC.: The influence of dose rate on ultraviolet tumorigenesis. *J Photochem Photobiol B.* 1991;10(1-2):41-50

Kemeny L, Bonis B, Dobozy A, Bor Z, Szabo G, Ignacz F: 308-nm excimer laser therapy for psoriasis. *Arch Dermatol.* 2001;137(1):95-6.

Koester SK, Roth P, Mikulka WR, Schlossman SF, Zhang C, Bolton WE: Monitoring early cellular responses in apoptosis is aided by the mitochondrial membrane protein-specific monoclonal antibody APO2.7. *Cytometry.* 1997;29(4):306-12.

Koester SK, Bolton WE.: Differentiation and assessment of cell death. *Clin Chem Lab Med.* 1999;37(3):311-7.

Kripke ML: Immunological unresponsiveness induced by ultraviolet radiation. *Immunol Rev.* 1984;80:87-102.

Krutmann J, Kock A, Schauer E, Parlow F, Moller A, Kapp A, Forster E, Schopf E, Luger TA: Tumor necrosis factor beta and ultraviolet radiation are potent regulators of human keratinocyte ICAM-1 expression. *J Invest Dermatol.* 1990;95(2):127-31.

Lavker RM, Gerberick GF, Veres D, Irwin ChJ, Kaidbey KI: Cumulative effects from repeated exposures with suberythemal doses of UVB and UVA in human skin. *J. Am. Acad. Dermatol.* 1995;32:53-65.

Macve JC, Norval M.: The effects of UV waveband and cis-urocanic acid on tumour outgrowth in mice. *Photochem Photobiol Sci.* 2002;1(12):1006-11.

Mafong EA, Friedman PM, Kauvar AN, Bernstein LJ, Alexiades-Armenakas M, Geronemus RG: Treatment of inverse psoriasis with the 308 nm excimer laser. *Dermatol Surg.* 2002;28(6):530-2.

Marchetti P, Castedo M, Susin SA, Zamzami N, Hirsch T, Macho A, Haeffner A, Hirsch F, Geuskens M, Kroemer G.: Mitochondrial permeability transition is a central coordinating event of apoptosis. *J Exp Med.* 1996;184(3):1155-60.

Matsunaga T, Hieda K, Nikaido O: Wavelength dependent formation of thymine dimers and (6-4) photoproducts in DNA by monochromatic ultraviolet light ranging from 150 to 365 nm. *Photochem Photobiol.* 1991;54(3):403-10.

Menter A. and D.L.Cram: The Goeckerman regimen in two psoriasis day care centers. *J Am. Acad. Dermatol.* 1983;9:59-65.

Modos K, Gaspar S, Kirsch P, Gay M, Ronto Gy: Construction of spectral sensitivity function using polychromatic UV sources. J. *Photochem. Photobiol. B: Biol.* 1999,49:171-176.

Moodycliffe AM, Nghiem D, Clydesdale G, Ullrich SE.: Immune suppression and skin cancer development: regulation by NKT cells. *Nat Immunol.* 2000;1(6):521-5.

Morison WL: Phototherapy and photochemotherapy of skin disease. 1992. Raven Press: 10-12

Morita A, Werfel T, Stege H, Ahrens C, Karmann K, Grewe M, Grether-Beck S, Ruzicka T, Kapp A, Klotz LO, Sies H, Krutmann J.: Evidence that singlet oxygen-induced human T

helper cell apoptosis is the basic mechanism of ultraviolet-A radiation phototherapy. *J Exp Med.* 1997;186(10):1763-8.

Murphy FP, Coven TR, Burack LH, Gilleaudeau P, Cardinale I, Auerbach R, Krueger JG Clinical clearing of psoriasis by 6-thioguanine correlates with cutaneous T-cell depletion via apoptosis: evidence for selective effects on activated T lymphocytes. *Arch Dermatol.* 1999 Dec;135(12):1495-502.

Nghiem DX, Kazimi N, Mitchell DL, Vink AA, Ananthaswamy HN, Kripke ML, Ullrich SE.: Mechanisms underlying the suppression of established immune responses by ultraviolet radiation. *J Invest Dermatol.* 2002;119(3):600-8.

Nishigaki R, Mitani H, Tsuchida N, Shima A.: Effect of cyclobutane pyrimidine dimers on apoptosis induced by different wavelengths of UV. *Photochem Photobiol.* 1999;70(2):228-35.

Njoo MD, Westerhof W, Bos JD, Bossuyt PM.: The development of guidelines for the treatment of vitiligo. *Arch Dermatol.* 1999;135(12):1514-21.

Njoo MD, Bos JD, Westerhof W: Treatment of generalized vitiligo in children with narrow-band (TL-01) UVB radiation therapy. *J Am Acad Dermatol.* 2000;42(2 Pt 1):245-53.

Ozawa M, Ferenczi K, Kikuchi T, Cardinale I, Austin LM, Coven TR, Burack LH, Krueger JG: 312-nanometer ultraviolet B light (Narrow-Band UVB) induces apoptosis of T cells within psoriatic lesions. *J. Exp. Med.* 1999;189:711-718.

Parrish JA, Jaenicke KF: Action spectrum for phototherapy of psoriasis. *J. Invest. Dermatol.* 1981;76:359-362.

Paul BS, Stern RS, Parrish JA, Arndt KA: Low-intensity selective UV phototherapy. A clinical trial in outpatient therapy for psoriasis. *Arch Dermatol.* 1983;119(2):122-4.

Peter ME, Krammer PH.:Mechanisms of CD95 (APO-1/Fas)-mediated apoptosis. *Curr Opin Immunol.* 1998;10(5):545-51.

Reiner A, Albin RL, Anderson KD, D'Amato CJ, Penney JB, Young AB.: Differential loss of striatal projection neurons in Huntington disease. *Proc Natl Acad Sci U S A.* 1988;85(15):5733-7.

Rivas JM, Ullrich SE.: The role of IL-4, IL-10, and TNF-alpha in the immune suppression induced by ultraviolet radiation. *J Leukoc Biol.* 1994;56(6):769-75.

Rosenstein BS, Mitchell DL.: Action spectra for the induction of pyrimidine(6-4)pyrimidone photoproducts and cyclobutane pyrimidine dimers in normal human skin fibroblasts. *Photochem Photobiol.* 1987;45(6):775-80.

Ruiz-Ruiz C, Robledo G, Font J, Izquierdo M, Lopez-Rivas A.: Protein kinase C inhibits CD95 (Fas/APO-1)-mediated apoptosis by at least two different mechanisms in Jurkat T cells. *J Immunol.* 1999;163(9):4737-46.

Saudou F, Finkbeiner S, Devys D, Greenberg ME.: Huntingtin acts in the nucleus to induce apoptosis but death does not correlate with the formation of intranuclear inclusions. *Cell.* 1998;95(1):55-66.

Scaffidi C, Kirchhoff S, Krammer PH, Peter ME.: Apoptosis signaling in lymphocytes. Curr *Opin Immunol.* 1999;11(3):277-85.

Schindl A, Klosner G, Honigsmann H, Jori G, Calzavara-Pinton PC, Trautinger F:. Flow cytometric quantification of UV-induced cell death in a human squamous cell carcinoma-derived cell line: dose and kinetic studies. *J Photochem Photobiol B.* 1998;44(2):97-106.

Schwarz A, Beissert S, Grosse-Heitmeyer K, Gunzer M, Bluestone JA, Grabbe S, Schwarz T.: Evidence for functional relevance of CTLA-4 in ultraviolet-radiation-induced tolerance. *J Immuno*l. 2000;165(4):1824-31.

Setlow, R.B.: The wavelengths in sunlight effective in producing skin cancer: a theoretical analysis. *Proc. Natl. Acad Sci U. S. A.* 1974;71:3363-3366.

Sgonc R, Boeck G, Dietrich H, Gruber J, Recheis H, Wick G: Simultaneous determination of cell surface antigens and apoptosis. *Trends Genet.* 1994;10(2):41-2.

Simon JC, Pfieger D, Schopf E: Recent advances in phototherapy. *Eur J Dermatol.* 2000;10(8):642-5.

Spann CT, Barbagallo J, Weinberg J: A review of the 308-nm excimer laser in the treatment of psoriasis. *Cutis.* 2001;68(5):351-2.

Takashima A: UVB-dependent modulation of epidermal cytokine network: roles in UVB-induced depletion of Langerhans cells and dendritic epidermal T cells. *J Dermatol.* 1995;22(11):876-87.

Tjioe M, Smits T, van de Kerkhof PC, Gerritsen MJ.: The differential effect of broad band vs narrow band UVB with respect to photodamage and cutaneous inflammation. *Exp Dermatol.* 2003;12(6):729-33.

Tomimori Y, Ikawa Y, Oyaizu N.: Ultraviolet-irradiated apoptotic lymphocytes produce interleukin-10 by themselves. *Immunol Lett.* 2000;71(1):49-54.

Trehan M, Taylor CR: High-dose 308-nm excimer laser for the treatment of psoriasis. *J Am Acad Dermatol.* 2002;46(5):732-7.

Walters IB, Burack LH, Coven TR, Gilleaudeau P, Krueger JG.
Suberythemogenic narrow-band UVB is markedly more effective than conventional UVB in treatment of psoriasis vulgaris. *J Am Acad Dermatol.* 1999 Jun;40(6 Pt 1):893-900.

Vermeer BJ, Hurks M: The clinical relevance of immunosuppression by UV irradiation. *J Photochem Photobiol B.* 1994;24(3):149-54.

Webber LJ, Whang E, De Fabo EC.: The effects of UVA-I (340-400 nm), UVA-II (320-340 nm) and UVA-I+II on the photoisomerization of urocanic acid in vivo. *Photochem Photobiol.* 1997;66(4):484-92.

Welzel J, Bruhns M, Wolff HH.: Optical coherence tomography in contact dermatitis and psoriasis. *Arch Dermatol Res.* 2003;295(2):50-5.

Wrone-Smith T, Nickoloff BJ: Dermal injection of immunocytes induces psoriasis. *J. Clin. Invest.* 1996;98:1878-1887.

Wyllie AH.: Apoptosis. Death gets a brake. *Nature.* 1994;369(6478):272-3.

Yaron I, Yaron R, Oluwole SF, Hardy MA.: UVB irradiation of human-derived peripheral blood lymphocytes induces apoptosis but not T-cell anergy: additive effects with various immunosuppressive agents. *Cell Immunol.* 1996;168(2):258-66.

Zhang C, Ao Z, Seth A, Schlossmann SF: A mitochondrial membrane protein defined by a novel monoclonal antibody is preferentially detected in apoptotic cells. *J. Immunol.* 1996;157:3980-3987.