Abstracts for the
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Meeting organizers: W. Sterry, P. Walden
Meeting secretaries: K. Asadullah, S. Jahn

Topics

- Classification of cutaneous lymphomas
- Molecular-biological diagnosis of cutaneous lymphomas: power and pitfalls
- In vitro models. Viruses and lymphomagenesis
- Progress in pathogenesis research and diagnosis of cutaneous lymphomas
- Genetic aberrations in cutaneous lymphomas
- Immune response in cutaneous lymphomas
- Treatment of cutaneous lymphomas
- Studies and experimental protocols for clinical treatment
- N. Smith memorial lecture

Abstract Numbers
Oral presentations 1–49
Poster presentations P1–P33

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1 EORTC CLASSIFICATION FOR PRIMARY CUTANEOUS LYMPHOMAS 
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Primary cutaneous lymphomas represent a heterogeneous group of T and B cell lymphomas, that show considerable variation in histology, phenotype and prognosis. Recently, the EORTC Cutaneous Lymphoma Project group has reached consensus on a new classification for this group of diseases (Blood, July 1997). In contrast to other classification schemes for non-Hodgkin lymphomas, the EORTC Classification for Cutaneous Lymphoma is not only based on histological and immunophenotypical, but also on clinical criteria. Thus, it includes a list of disease entities, that have a well-defined clinical presentation and clinical behaviour. Secondary cutaneous lymphomas and cutaneous lymphomas arising in immunocompromised patients are excluded. In addition, this new classification contains some provisional entities, which may display typical histologic features, but are clinically not well-defined. The basic principles of this new classification and the characteristic clinical and histological features of the different disease entities are presented. Based on 609 evaluable patients registered at the Dutch Cutaneous Lymphoma Working Group, the frequency and estimated 5-year-survival data of the different types of cutaneous lymphomas are provided.

2 DIFFERENT PROGNOSIS BETWEEN CUTANEOUS AND SYSTEMIC CD30+ LYMPHOPROLIFERATIVE DISORDERS IS NOT SUPPORTED BY BIOLOGICAL DATA. 
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The initial identification of primary cutaneous CD30+ large-cell lymphoma (LCL) with good prognosis from cutaneous lymphomas with extracutaneous spreading is relevant for therapeutic strategy. We studied several clinical, morphological, immunohistochemical and molecular features of 65 CD30+ cutaneous lymphoproliferations in statistical univariate analysis for their prognostic significance. Primary cutaneous CD30+ LCL (vs systemic LCL and vs secondary CD30+ cutaneous LCL) and spontaneous regression were associated with a better prognosis. Extracutaneous progression was unfavorable. Age (>60 years), localised or multicentric skin lesions were not found significant prognostic factors. Expression of activation markers including epithelial membrane antigen, CD99 and CD55 antigens were both observed in primary and secondary cutaneous lymphoproliferations. P33 oncoprotein expression was not associated with spontaneous regression, extracutaneous spreading or survival. Reverse transcriptase-polymerase chain reaction allowed the detection of NPM-ALK translocation in 2 out of 11 lymphoma papulosis, 7 out of 12 primary CD30+ cutaneous LCL, 1 out of 3 secondary CD30+ cutaneous LCL and 3 out of 11 cutaneous and extracutaneous CD30+ LCL. Interestingly, an ALK-negative protein was detected on lymphoma cells in only 1 out of 50 primary cutaneous lymphoproliferations including cases with NPM-ALK translocation. Alternatively, ALK immunoreactivity was found in 4 out of 15 secondary cutaneous LCL. Therefore, cells carrying the 52/53p21/p35 is primary cutaneous lymphoproliferative disorders are either bystander cells or do not express a detectable level of ALK protein as opposed to ALK-positive secondary cutaneous lymphomas. However ALK immunoeffectivity was not correlated with prognosis or age.

3 PRIMARY AND SECONDARY CUTANEOUS CD30+ POSITIVE LYMPHOPROLIFERATIVE DISORDERS: FOLLOW-UP AND SURVIVAL OF 177 PATIENTS 
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Primary cutaneous CD30+ positive (anaplastic) large T cell lymphomas (LCL) and lymphomatoid papulosis (LyP) are closely related conditions, which are considered to have a favorable prognosis. However, recent studies suggested that LyP patients have a life-time risk of 80% to develop systemic lymphoma. Moreover, information on the prognosis of primary cutaneous lymphomas with regional lymph node involvement at presentation, and patients with a secondary cutaneous CD30+ positive LCL are not available.

In the present study we therefore analyzed clinical and follow-up data from 177 patients with primary or secondary CD30+ positive lymphoproliferative disorders registered at the Dutch Cutaneous Lymphoma Working Group. This group included 96 patients (51 males, 45 females, median age 44 yrs) with LyP (group A), 63 patients (44 males, 19 females, median age 63 yrs) with a primary cutaneous CD30+ LCL (group B), 8 patients (4 males, 4 females, median age 66 yrs) with a CD30+ LCL presenting in skin and regional lymph nodes (group C) and 10 patients (6 males, 4 females, median age 40 yrs) with a secondary cutaneous CD30+ LCL (group D). Five year-survival data for the different groups were as follows: group A: 100%, group B: 82%, group C: 86%, group D: 24%. These results confirm the favorable prognosis of primary cutaneous LCL. They also indicate that CD30+ LCL presenting with skin and regional lymph node involvement have a similar prognosis as primary cutaneous CD30+ LCL without regional lymph node involvement.

4 PARAPSORIASIS AND EARLY CUTANEOUS T CELL LYMPHOMA 
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The diagnosis of cutaneous T cell lymphoma is based on the clinical picture, the course of the disease and histological examinations. Immunohistochemistry can help to differentiate tumour cells and can give further information about the expression of antigens on the surface of the tumour cells with some prognostic impact, i.e. CD30 expression. T cell receptor rearrangement studies are an additional diagnostic hint of cutaneous T cell lymphoma but no prove of malignancy. Early cutaneous T cell lymphoma have to be separated from diseases of the parapsoriasis group, like parapsoriasis en plaques and lymphomatoid papulosis. However, for some diseases of the parapsoriasis group the proceeding to cutaneous T cell lymphoma is discussed. Therefore differential diagnosis between these forms and early cutaneous T cell lymphoma is often difficult and will be demonstrated according to selected cases from the Department of Dermatology Minden.

5 IMMUNOGLOBULIN GENE REARRANGEMENT ANALYSIS OF PSEUDO-B-CELL LYMPHOMAS BY POLYMERASE CHAIN REACTION. 

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The diagnosis of pseudo B-cell lymphomas versus B-cell lymphomas may be very difficult. Molecular analysis of lymphoid gene rearrangements by PCR is a sensitive and easy technique for the detection of clonal populations. The interest of this method in the differential diagnosis between pseudo B-cell lymphomas and B-cell lymphomas has not been established. We studied 24 cases of cutaneous pseudo B-cell lymphomas. There were 13 men and 11 women (mean age 49 years). They presented with erythematous/plague-like papules or nodules. The lesions were unique in 13 cases or multiple in 11 cases, localised on the head (10 cases), trunk (10 cases), limbs (7 cases) and genitalia (1 case). All patients had a mixed T and B cell infiltrate with polytypic B cells in immunohistology. PCR analyses were performed using consensus oligonucleotides of FR3 (oligo-sens) and FR4(oligo-antisens) regions. Clonal rearrangement of immunoglobulin genes was detected in 1 of 24 patients. No lymphoma developed during the 9 years of follow up. In the same period we studied 20 cases of B-cell lymphomas. 75% of these cases had a clonal immunoglobulin gene rearrangement. Therefore, in the majority of our cases, PCR analysis confirmed the diagnosis of pseudo B-cell lymphomas. In one of the 24 patients, the presence of a B-cell clone could be evidenced. This should not modify the treatment, as there were no histologic or immunophenotypic signs of lymphomas should be continued. The possibility of late progression cannot be excluded and careful follow-up should be continued.

6 PROGNOSTIC SIGNIFICANCE OF A PCR-DETECTABLE DOMINANT T- CELL CLONE IN CUTANEOUS LESIONS OF PATIENTS WITH MYCOSIS FUNGOIDES. 
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Although mycosis fungoides (MF) is considered to be an indolent lymphoma, survival is highly influenced by TNM stage. At diagnosis, most MF patients present with early stage disease and a high probability of long term survival. Treatment is generally directed towards skin lesions and achievement and duration of complete remission are very variable. A dominant T-cell clone is detectable in the cutaneous lesions of 60% of patients. The aim of this study was to determine whether the presence of a T-cell clonal population influences the clinical response to treatment. Cutaneous biopsies from 71 patients were histologically diagnosed as MF and T-cell clonal status was analyzed by in vitro amplification of TCR-gamma chain gene rearrangement (PCR Gamma). After a median follow up of 27 months response to treatment (complete or not) was clinically assessed. Age, sex, duration of symptoms before diagnosis, type of cutaneous lesions (T stage), TNM stage, and PCR Gamma were evaluated as predictive factors of response to treatment in univariate and multivariate analysis. Univariate analysis permitted to demonstrate the positive influence of T stage (p<0.01), overall TNM stage (p<0.031) and PCR Gamma (p=0.026) on treatment response. Using a multivariate analysis, T stage (relative risk of 15.8) and PCR Gamma (relative risk of 5.5) remained the two independent significant predictive parameters of response. In conclusion, T stage and cutaneous PCR Gamma at diagnosis are the two predictive parameters of treatment response for MF. This is the first evidence of the necessity of considering PCR Gamma results in therapeutic protocols.
LYMPHOCYTE RECEPTOR STUDY BY PCR-DGGE IN CUTANEOUS Lymphomatoid Papulosis. 11. ORIGIN OF CIRCULATING CLONAL T CELLS IN PARASPIROAISIS EN PLAQUES (PFP)- IS THERE A RELATION TO CUTANEOUS T CELL LYMPHOMA (CTCL)?

Method. T-cell receptor gamma chain gene rearrangements were studied by multiplex PCR to detect dominant lymphocyte clones in 40 cutaneous and nodal biopsies from 16 mycosis fungoides (MF) or Sézary syndrome (SS), 13 pleomorphic lymphomas-CD30+(PL), 6 lymphomatoid papulosis (LJP), 5 non cutaneous T-cell lymphomas (NCTCL) and 3 cutaneous pseudolymphomas. Twelve cutaneous samples were obtained from 1 Hodgkin’s disease and 11 benign lymphomatous dermatoses.

Results. One or two dominant clones were detected in 60% of MF and SS (50% at early stages of the disease, 100% at late stages), 91% of PL, 17% of LJP, 80% of NCTCL and 6% of other cases. Two dominant rearrangements were detected in 52%, 27% and 0% of PL, MF and LJP respectively. Lymphocyte repertoire study revealed the same clone composed in non-simultaneously appearing lesions of LJP and PL lesions in another patient.

Conclusions. This study confirms that TCR studies cannot discriminate between benign lymphocyte infiltrates and lymphomas at early stages, 2) suggests that PL have a particular genotypic pattern, 3) suggests, in case of LJP and PL, the existence of a cellular immune response against the dominant clone is conserved in successive lesions.

IMMUNOPHENOTYPIC AND GENOTYPIC ANALYSIS OF THE CUTANEOUS LYMPHOMATOID INFLTRATE IN ANGIOIMMUNOBLASTIC LYMPHADENOPATHY.

The aim of this study was to analyse the immunohistological picture and the T-cell receptor (TCR) gene rearrangement in skin biopsy specimens from 7 patients with angiomylloymphomatous lymphadenopathy (AIL). Skin samples were characterised immunohistologically by staining of Cryostat sections with a panel of monoclonal antibodies. The T-cell receptor γ chain gene rearrangement was studied in skin samples using the PCR-DGGE technique. Seven patients (5 females, 2 males) were included. Six patients presented transient and recurrent skin eruptions, whereas the last patient had pseudolymphomatous cutaneous lesions. A poor perivascular infiltrate composed of small lymphoid cells with a mature T-cell phenotype was observed in 4 cases. In the 3 other cases, a dense lymphoid infiltrate composed of medium and large pleomorphic T-cells was observed. Loss of one or several pan-T cell antigens (CD2, CD3, CD5, CD7) was observed in the 3 cases. A mononuclear rearrangement of the y chain of the TCR was observed in 4 of 7 cases including 2 cases corresponding histologically to a pleomorphic T-cell lymphoma. The 3 cases were considered as non-specific immunohistological picture, indicating that transient skin eruptions may correspond to a specific localization of the lymphoma in patients with AIL.

DETECTION OF MALIGNANT T CELL CLONES IN MYCOSIS FUNGIOIDES BY PCR ASSAYS OF TCR GENE REARRANGEMENTS.

It is well established that MF is a clonal expansion of T-cells carrying identical copies of rearranged TCR genes. Therefore, the demonstration of a dominant T cell clone in cutaneous infiltrates confirms the diagnosis additionally to clinical, histopathological and immunophenotypic criteria. In routine diagnosis we investigate the occurrence of monoclonal T cells in skin biopsy samples of patients with suspected or established MF by applying several TCR PCR assays together with the heteroduplex temperature gradient gel electrophoresis. For a 2 years period, from all patients studied in our laboratory, MF was found in 208 cases following histopathology and clinical criteria. From 188 of these MF patients, at least one skin sample of sufficient DNA quality enabling the PCR assays, were obtained. Applying a PCR with conserved primer for the most frequently rearranged TCR genes Vy 1 and Vy 1/2, we detected expanded T cell clones in 122 cases (65%). In the remaining 68 cases, we performed two multiplex PCR, covering rearrangements of all other TCR genes, except Jy 1/2. Hereby we found in 11 cases (6%) predominant clonal rearrangements of Vy 1 + Vy 1 and Vy 1/2 and in 2 cases (1%) those of Vy 1 + Vy 1/2. The data show the rare usage of the Vy1 and Vy1/2 in TCR gene rearrangements in MF. Therefore, PCR detecting only rearrangements of Vy 1 and Vy 1/2 are insufficient in screening analysis for clonality in MF. In a fraction of 28% of our MF cases the applied PCR techniques failed to demonstrate monoclonal T cells, possibly due to their low amount or chromosomal aberrations.

SEQUENTIAL ANALYSIS OF T-CELL RECEPTOR GENE REARRANGEMENT IN SKIN BIOPSY SPECIMENS FROM 6 PATIENTS WITH HODGKIN DISEASE, LYMPHOMATOID PAPULOSIS, MYCOSIS FUNGOIDES AND CD30+ LARGE CELL LYMPHOMA.

T-cell receptor gene rearrangement was studied using PCR-DGGE in skin and nodal biopses from 6 patients who experienced sequentially during a ten year-follow-up period 2 or more of the following diseases: Hodgkin disease, lymphomatoid papulosis, mycosis fungoides (MF), nodal or cutaneous CD30+ large cell lymphoma (LCL). A monoclonal rearrangement of the TCR y gene was observed in 3 of 3 biopsies of lymphomatoid papulosis, in 4 of 5 MF skin biopsies, in one MF dermatomatoses lymphadenopathy, in 5 of 3 skin biopsies of cutaneous CD30+ LCL and 2 of 2 lymph nodes from nodal CD30+ LCL. Only one adenopathy from a patient with Hodgkin disease was available, with no detectable TCR y gene rearrangement. A comparison of the DNA extracted from the different skin or nodal biopsy specimens was observed in each of the 6 patients. These results suggest that a common lymphoid cell progenitor leads to various lymphomatoselike CD30+ disorders in these patients.
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THE 112 SIGNAL-TRANSDUCTION PATHWAY IS DEFECTIVE IN MALIGNANT T-CELLS IN SEZARY SYNDROME
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Sezary syndrome (SS) is the leukemic phase of cutaneous T-cell lymphoma and is characterized by the presence of clonal CD4+ T-cells which release cytokines of the Th2 cytokine profile (IL-4, 10). Moreover, production of Th1 cytokines (IL-2, IFN-gamma) by peripheral blood lymphocytes (PBL) in SS is markedly depressed. Since responses to IL-2 are critical for normal Th1 differentiation, we examined the IL-12 signal transduction pathways (Jak/Stat) in normal and malignant T-cells from SS patients and in PBL from normal controls. Lymphocytes were activated with PHA for 3 days, cultured in low serum containing medium for 24 hours then stimulated with IL-12 for 15 min, prior to lysis and immunoprecipitation. Using anti-Stat 4 and anti-phosphoryrocin antibodies, Western blot studies indicated, in purified malignant T-cells, the presence of Stat 4 proteins which failed to become phosphorylated in the presence of IL-12. In contrast, Stat 4 became phosphorylated in non-malignant T-cells from SS patients and normals when stimulated with IL-12. Similarly, Jak 2 failed to become phosphorylated in malignant T cells but the presence of Jak 2 was not inhibited. These data suggest that a proximal defect in IL-12 signaling exists in the malignant CD4+ T-cells which may include the failure to express high affinity receptors for IL-12, which is currently under investigation. This profile, the absence of the high affinity IL-12 receptors and lack of phosphorylation of IL-12 responsive Jak/Stat components is typical of Th2 helper T-cells. These results further support our previous observations that SS is typified by a proliferation of Th2 cells.

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HUMAN HERPES-VIRUS-8 SPECIFIC DNA SEQUENCES IN PRIMARY CUTANEOUS B-CELL LYPHOMAS.
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Human herpes-virus-8 (HHV-8) has been identified in Kaposis sarcoma as well as in various forms of extranodal lymphoproliferative diseases in patients with or without Kaposi's sarcoma. In five cases of cutaneous B-cell lymphomas (BCBL) tested so far, the presence of HHV-8 could not be demonstrated. With the new PCR techniques, we have isolated and identified specific sequences from 50 patients with primary BCBL for the presence of HHV-8 using a nested PCR technique followed by Southern blot hybridisation. Details on primers have been published. In all cases monoclonality of the B-cell infiltrate was confirmed by immunohistologic analysis of immunoglobulin light chains and/or PCR analysis of IgH gene rearrangement. Only patients with primary BCBL were involved (as defined by disease localized solely to the skin for 6 months after complete staging procedure). None of the patients included in this study had AIDS or Kaposi's sarcoma, but the herpesvirus was not known. The nested PCR technique was as follows: in the first step, a 238bp product was amplified using the KSHV primers. In the second step an additional internal 164bp fragment was amplified using primers NSI and NS2. The 850bp gene was successfully amplified in all cases. Specificity of PCR products was confirmed by restriction blot analysis. Southern Southern blot hybridisation of the 850bp PCR product, as described previously. Five positive (AIDS-associated Kaposis sarcoma) and five negative controls (healthy skin, liver, colon, prostatic, normal tissue) gave results as expected. HHV-8 specific gene sequences were identified in 2/30 follicle center cell lymphomas, 1/20 large cell B-cell lymphomas, and 4/50 immunocytomas. Our results demonstrate the presence of HHV-8 DNA sequences in a percentage of patients with various types of BCBL, suggesting a possible involvement of this lymphotropic virus in the pathogenesis of at least some of these cases. In fact, the presence of a functional, constitutively active G-protein-coupled receptor, recently demonstrated in HHV-8, could lead to altered growth and / or apoptosis. The low percentage of HHV-8 positivity may explain why no positive cases could be identified in the small series published by Bander et al. Further studies are necessary to evaluate the role of this virus in the pathogenesis of cutaneous lymphoproliferative diseases.

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SEZARY SYNDROME'S (SS) T-CELLS ARE DEFICIENT IN INTERFERON SIGNALING DESPITE OVEREXPRESSION OF INTERFERON RECEPTORS.
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SS is characterized by a clonal accumulation of malignant T-lymphocytes in the skin and in the peripheral blood. Using antibodies against the variable region of the T-cell receptor (TCR) or CD3/CD27 double stains, the predominant T-cell clones in SS can be purified. These T-cells express normal TCR, IL-10, and overexpress interferon receptors. In this study we assess expression levels and signaling through IFN-alpha or IFN-gamma in PBMC of SS patients. From three SS patients clonal CD4, CD7 T-cells were sorted and analyzed for expression of IFN-alpha receptor (IFN-AR), IL-10 receptor (IL-10-R), and IFN-gamma receptor (IFNG-R1) in the presence or absence of IFN-gamma or IFNg. Flow cytometry. Functionality of IFN-AR was tested by screening for upregulation of MxA in interferonimmunosorescence assays. Band shift and super shift experiments were performed to analyze IFNg signaling in CD4+ T-cells and transduced by STAT1/2/3 factors (signal transducers and activators of transcription). A 10-100 fold upregulation of IFN-AR was observed in all samples compared to healthy control PBMC. The binding of IFN-AR 1 to IFNG-R1 the effectors of the signaling pathway was significant. No response to stimulation with IFN-gamma was observed, all cell-surface markers remained unchanged with regard to expression levels. In contrast, all controls showed significant upregulation of IL-10, IL-1B and MxA and IFN-gamma 1a after stimulation. These results suggest that IFNG-R1 are functional in SS cells. This is in agreement with a previous study suggesting the involvement of IFN-gamma in the pathogenesis of SS. A recent study showed that SS may help identify possible targets for molecular interventions in these diseases.

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IDIOTYPE PEPTIDES FOR IMMUNOTHERAPY OF CUTANEOUS T-CELL LYMPHOMA.
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In cases of lymphoid cancers the idiotype of the malignant cells' immune receptors are ideal targets for immunotherapeutic intervention. In cutaneous T-cell lymphoma, however, we have sequenced the gene segment coding for the CD3 gamma chain of the beta and gamma chains, and are planning the name for the alpha chain. The IgM chain was amplified by PCR using consensus and/or family specific primers. The PCR product of the maligant clone was identified and isolated using temperature gradient gel electrophoresis and then sequenced either directly or following AT cloning. Libraries of overlapping peptides were designed according to the corresponding amino acid sequences and tested for their capacity to induce idiotypic-specific response by cytotoxic T lymphocytes isolated from peripheral blood of the patients. The idiotypic peptides may be confirmed in cell mediated lympholysis assays. Peptides identified this way to be antigenic in cutaneous lymphoma patients can then be used - in combination with suitable adjuvants - as vaccines to induce tumor specific cytotoxic T-cell responses in the patients and might be applicable for immunotherapy of these malignancies.

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INTERFERON ALPHA INCREASES EXPRESSION OF AN EBSTEIN-BARR VIRUS LATENCY TRANSCRIPT (EBER) IN KERATINOCYTES.
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Interferon alfa may increase transcriptional expression of EBER which is an early RNAs of Epstein-Barr Virus (EBV) actively transcribed in latency infected monocytocytes. We decided to amplify this phenomenon to amplify the detection of EBV in keratinocytes both in vitro and in vivo. Indeed keratinocyte play a major site of infection of EBV in addition of B lymphocytes but the percentage of infected cells is very low. For in vitro study, a mixed culture with EBV +99% B cells and normal keratinocytes has been performed for 7 days with or without interferon alphas (1500 IU). EBER was detected by in situ hybridisation. For in vivo study, 61 skin lesions of cutaneous T cell lymphoma (CTCL) in which EBV could play a role were studied for EBER expression by in situ hybridisation either without (28 skins) or after treatment (33 skins) with interferon alpha (6 10^5 IU for 3 or 4 month). In vitro, only 1% keratinocytes expressed EBER without interferon alphas, but 12% with the addition of interferon alphas. In vivo, 11/33 epidermis of CTCL after interferon alphas therapy were EBER+ and only 4/28 epidermis without treatment (p<0.05). So our study shows that interferon alphas increases EBER expression in keratinocytes and confirms the presence of EBV in keratinocytes of CTCL where it may play a role by modulating the activation of keratinocytes and so the interaction keratinocyte-lymphocyte.

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TYPE 1 / TYPE 2 CYTOKINE EXPRESSION DURING PROGRESSION OF MYCOSIS FUNGOIDES.
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Cytokines are believed to play an important role in the pathogenesis of cutaneous T cell lymphoma. Data regarding the local cytokine pattern in mycosis fungoides(MF) are partly conflicting. Recent studies have suggested a shift from type 1 to type 2 cytokine pattern because interleukin-10 (IL-10) and IL-5 mRNA, have been more frequently detected in lesions of advanced stages. Another study has described a type 1 cytokine pattern in MF lesions. However, none of the studies of cytokine mRNA expression in MF carried out previously used quantitative methods and therefore only the presence of a cytokine could be determined but not the level of expression. To gain better insight into the development of cytokine patterns during tumor progression we used semi-quantitative RT-PCR to analyze cytokine mRNA expression in MF skin lesions at different stages. Biopsy from patients with patch (n=11), plaque (n=4), and tumor (n=3) stage MF were compared to biopsies from patients with pleomorphic T cell lymphoma (n=5), proriasis (n=7), atopic dermatitis (n=5), and healthy skin (n=8). MF progression was associated with significantly higher IL-10 and lower interleukin gamma mRNA expression. Moreover, the stage-dependent increase of IL-10 mRNA expression was also found in paired samples from individual patients. However, unlike in pleomorphic T cell lymphoma, typical T2 cells did not seem to be the source of increasing IL-10 expression in advanced MF since stage-independent IL-4 mRNA was rarely detected, suggesting contribution of non-lymphoid cells in local IL-10 production. The overexpression of IL-10 in MF may be of importance for tumor progression since this immune suppressive cytokine might be involved in downregulation of immunological tumor surveillance.
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GENETIC MARKERS ASSOCIATED WITH MYOCOS FUNGIDES
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Mycosis Fungoides (MF) is a low-grade cutaneous T-cell lymphoma of unknown aetiology, which starts in the skin and may remain confined to the skin for several years, and which, in later phases, can also affect lymph nodes and internal organs, taking a lethal course. Disease can run from years to decades before changing its morphological and immunohistochemical characteristics into those of high grade T-cell lymphoma. When environmental causes as well as immunological and viral factors have been suggested in MF, HLA associated variations in genetic susceptibility are likely to play a role in the pathogenesis of MF.

HLA class I and II serologic typing was performed in 32 MF patients and 179 ethnically matched controls. HLA-DRB5 gene frequency was significantly increased in MF with respect to the controls (31.25% vs. 13.96%; p=0.007; OR=2.85). In particular, the HLA: A2, A24, B4, D35, D81, D117 genotype was significantly more frequent in patients than in unaffected people (9.37% vs 1.67%; p=0.012; RR=5.59). The HLA A1 accessory haplotype was absent in male patients, while it was present in 33.64% of females (p=0.014). HLA-DR13 allele seemed to correlate with the early-onset form of the disease (21.47% vs 2.06%; p=0.007; RR=13.36).

Far from any conclusion, the study simply suggests to further investigate in this direction principally because MF is clinically heterogeneous and histologically still obscure.

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COMPARATIVE GENOMIC HYBRIDISATION DETECTS GENETIC CHANGES IN SEZARY SYNDROME
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Changes in DNA copy number in blood lymphocytes of 4 patients with mycosis fungoides and 7 patients with Sézary syndrome were studied with comparative genomic hybridisation (CGH), which detects losses and gains of DNA-sequences and locates them in their respective chromosomal regions. The sensitivity of CGH for amplified DNA is about 2MB (5- to 7-fold amplifications of oncogenes) and for deletions 10-20 MB.

Five of 7 patients with Sézary syndrome had losses or gains affecting 1-9 chromosomes. Losses were more frequent than gains, and concerned mostly whole chromosomes or arms. Three patients had a combination of loss of 2p and 10q, and two of them also had gain of 8q. These three changes were also observed in one follow-up sample taken 4 months after the first sample, and confirmed by in situ hybridisations with specific painting probes when cells were available (2 patients). These regions are likely to be important in the molecular pathogenesis of Sézary syndrome. Many other, more sporadic changes in CGH were also confirmed by in situ hybridisations in several patients. CGH requires at least about 50% of similarly aberrant cells, which in blood studies of cutaneous T-cell lymphoma restricts its use mainly to Sézary syndrome.

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HOXC4, C5 AND C6 EXPRESSION IN PRIMARY CUTANEOUS LUPHOMA LESIONS: HISTOLOGICAL AND IMMUNOHISTOCHEMICAL COMPARISON BETWEEN MYCOSIS FUNGIDES AND SEZARY SYNDROME
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Mycosis fungoides (MF) and Sézary syndrome (SS) are closely related cutaneous T- cell lymphomas, which differ in the aspect of peripheral blood involvement. We studied and compared histological and immunophenotypical features of these two entities, which could be implicated in the dermatomorphology, characteristic for both of them. Thirty biopsy specimen from patients with established MF and 13 from SS patients were examined retrospectively and 21 histological criteria, among which acanthosis, spongiosis, hyperkeratosis, type of epidermotropism (Pautrier collections, landing at the dermo-epidermal junction, single cell exocytosis or pagetoid infiltration), composition of the infiltrate, follicular mucinosis, oedema, sclerosis and venous proliferation, were assessed. Further, 9 cytonuclear lesions from MF and SS lesions each, were stained for ICAM1, LFA1, CD40, CD40-ligand, FAS and FAS-ligand.

The only histological criteria that showed any significant differences were acanthosis (n=2 of 13 SS and 7 of 13 MF specimen) and Pautrier collections (detected in 6 SS and 11 MF biopsies). Patterns of staining with the antibodies mentioned above, were also similar, LEA was positive on lymphocytes and ICAM1 stained both epidermis and lymphocytes. CD40, CD40-ligand and FAS-ligand were positive and after quantitative evaluation no significant difference could be detected between MF and SS specimen. FAS-ligand was negative. Our results indicate that these interaction molecules are probably involved in the pathogenesis of MF and SS, but their immunohistochimical distribution does not contribute to the differentiation between the two entities.

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HISTOLOGICAL AND IMMUNOHISTOCHEMICAL COMPARISON BETWEEN MYCOSIS FUNGIDES AND SEZARY SYNDROME
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Mycosis fungoides (MF) and Sézary syndrome (SS) are closely related cutaneous T- cell lymphomas, which differ in the aspect of peripheral blood involvement. We studied and compared histological and immunophenotypical features of these two entities, which could be implicated in the dermatomorphology, characteristic for both of them. Thirty biopsy specimen from patients with established MF and 13 from SS patients were examined retrospectively and 21 histological criteria, among which acanthosis, spongiosis, hyperkeratosis, type of epidermotropism (Pautrier collections, landing at the dermo-epidermal junction, single cell exocytosis or pagetoid infiltration), composition of the infiltrate, follicular mucinosis, oedema, sclerosis and venous proliferation, were assessed. Further, 9 cytonuclear lesions from MF and SS lesions each, were stained for ICAM1, LFA1, CD40, CD40-ligand, FAS and FAS-ligand.

The only histological criteria that showed any significant differences were acanthosis (n=2 of 13 SS and 7 of 13 MF specimen) and Pautrier collections (detected in 6 SS and 11 MF biopsies). Patterns of staining with the antibodies mentioned above, were also similar, LEA was positive on lymphocytes and ICAM1 stained both epidermis and lymphocytes. CD40, CD40-ligand and FAS-ligand were positive and after quantitative evaluation no significant difference could be detected between MF and SS specimen. FAS-ligant was negative. Our results indicate that these interaction molecules are probably involved in the pathogenesis of MF and SS, but their immunohistochimical distribution does not contribute to the differentiation between the two entities.

The CDKN2A gene encodes a protein, named p16, that plays a key role in cell cycle control by binding to CDK4 and CDK6 enzymes and inhibiting their ability to phosphorylate critical substrates necessary for transition through the G1 phase of the cell cycle. Mutations or deletions of the CDKN2A gene result in unmatched cell proliferation for a defective cell cycle control. Loss of p16 have suggested that CDKN2A may play a major role in either the development or progression of a variety of human cancers. The recently discovered CDKN2A expression is involved in the pathogenesis of cutaneous T-cell lymphomas. Total RNA was extracted from formalin fixed, paraffin embedded tissue sections of 20 mycosis fungoides, reverse transcribed and amplified by polymerase chain reaction. Amplified products were detected by dot blot hybridization using a 32P-APT labeled internal oligonucleotide probe and the single spots were measured in a beta-counter. Three different levels of CDKN2A mRNA expression were detected: low (1.0 copies) in five cases, level II (5-500 copies) in 13 patients and level III (>500 copies) in two cases. Two representative cases of each group were screened for p16 protein expression by immunohistochemistry using monoclonal and polyclonal p16 antibodies with either APAAP immunoperoxidase technique on formalin fixed, paraffin embedded tissue sections. p16 protein expression was undetectable in 2 cases (20 months' follow-up) while in 3 cases it was present in 1-5% of neoplastic lymphocytes. No significant correlation was observed between protein expression and clonal evolution, which is a measure for the level of CDKN2A mRNA expression. The weak or absent CDKN2A mRNA and protein expression, as detected in most cases, might be related to deletion or mutation of the CDKN2A gene. High CDKN2A mRNA levels and high p16 expression were found in 2 of 20 cases and seems to be associated with a slower progression of the disease.
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SUBSTIT COMPOSITION OF JUN AND MYC DNA-BINDING COMPLEXES IN CTCL CELLS
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Cutaneous T cell lymphomas (CTCLs) are restricted to the skin during most of the course of the disease and a systemic involvement in malignant cells to other organs occurs only in the very last stages. This is also true for the leukemic variant of the Sézary Syndrome (SS) in which malignant cells also occur in the blood. "Classic" oncogenes like c-jun and c-myc are only expressed at late stages. Members of the Jun family can only bind to DNA when they form heterodimers with members of the fos gene family to DNA. Members of the myc family can only bind to DNA if they form heterodimers with members of the max gene family. Since the different homo- and heterodimer-Jun and myc complexes can have gene transcription activating or suppressing activities we studied the composition of the Jun and myc DNA-binding complexes in CTCL cell lines and malignant cells of T Sézary patients using the electroelugation mobility shift assay (EMSA). Radioactively labelled oligonucleotides containing the binding sites of jun and myc complexes were incubated with nuclear extracts from the CTCL cells mentioned above. The EMSA procedure for the DNA-binding assay was used. Antibodies to the different members of the tested gene families were included in the binding reactions. Complexes consisting of JunD homodimers were found in three cell lines and two patients. Members of c-jun-c-fos complexes were only observed after protein kinase C (PKC) stimulation with the phorbol ester TPA. The three cell lines and one patient contained also c-Myc/Max heterodimers. Since c-Myc/Max heterodimers are known gene transcription activating complexes, they may be necessary for cell cycle progression, they may play a role in the progression of CTCL. The role of JunD is less clear as JunD has been implicated in carcinogenesis as well as in tumor suppression. The experiments with TPA show that the regulation of the c-jun and c-fos genes by PKC is not disturbed in these cells.

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ISOLATION OF AUTOLOGOUS TUMOR-SPECIFIC CLASS I RESTRICTED CYTOTOXIC AND PROLIFERATIVE CD4+CD8+ AND CD4- T CELL CLONES INFLTRATING A CLASS II-NEGATIVE T-CELL LYMPHOMA
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We have isolated several proliferative T cell clones from lymphocytes infiltrating a human cutaneous T cell lymphoma, that did not express MHC class II molecules. We describe two CD4+CD8dim+ and CD4-MHC class I-restricted CD8+ T cell clones, TCS and TCC, which exhibited proliferative and cytotoxic activity towards the autologous tumor cells. Interestingly, we found that these T cell clones were similarly able to specifically recognize and lyse autologous tumor cells previously established at RIL-2 and RIL-7 both from the skin and from the blood of the patient. Analysis of the T-cell receptor gene rearrangements revealed that TCS and TCC expressed a unique TCR-β gene transcript corresponding respectively to Vα5-β23 and Vβ7-β22 gene segments, whereas the tumor cells expressed Vβ8-β22, Vβ13-β25 and Vβ22-β25 gene segments. Flow cytometric analysis using monoclonal antibodies against TCR-β gene products confirmed the above findings. These cells, CD8-receptor α-chain, and transferrin receptor. The CD8+ T cells demonstrated to be the strongest activated T cell subset, were of polyclonal origin as shown by their usage of different T cell receptor families. The enhanced expression of activation antigens was associated with an increased proportion of CD8+ T cells with high expression of the adhesion molecule LFA-1, demonstrating the capacity for migration of these cells. These CD8+ effector cells are expected to be CTLs and may be responsible for the favorable prognosis of indolent, primary CTCL. Interestingly, a stage-dependent decrease in T cell activation antigen expression was observed, suggesting the development of a lack in tumor surveillance in advanced MF stages. Further investigations are necessary to verify whether the parameters determined are of predictive value for prognosis and response to therapy in CTCL.

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IMMUNOBIOCHEMICAL STUDY OF 100 PARAFASORIASIS: CORRELATION BETWEEN HISTOLOGICAL PHENOTYPE AND TUMOR MODELS OF EXPRESSION
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Parasporiasis in plaque (PP) is considered as an "in situ" cutaneous T-cell lymphoma (CTCL) in several studies. But at the moment, no biological factor has been isolated as diagnostic criteria to correlate the histological aspect. In a previous work, we showed that My7 antigen (CD13) which is expressed by basal keratinocytes in normal and inflammatory skin disappeared in cutaneous lesions of CTCL.

The aim of this work was to study the expression of My7 antigen in skin lesions of PP and to determine if a correlation may be established with the histological aspect.

59 PP on granules plaques and 41 PP effigies were studied. The histologic aspect was classified into: non specific inflammatory lesions, eczematous lesions, PP, or LTE.

The immunohistochemical study was performed on frozen sections using monoclonal antibody My7 (CD13, Coulter) 4,5%. A percentage of My7 positive basal cells was determined (mean for 3 X 3 slides). The histological examination showed: 32 parasporiasis, 13 eczematous lesions, 25 non specific inflammatory lesions, 30 LTE. In a similar manner, the disappearance of My7 expression by basal keratinocytes was significantly (p<0,05) associated with the histological aspect of LTE. So, this study confirms that an histological aspect of CTCL may be observed in PP both in PP on granules plaque (49,7%) and in PP on effigies (46,4%) argues for the hypothesis on an "in situ" CTCL. Moreover, My7 appears as a diagnostic criteria in addition to histology. At the moment we are studying if disappearance of My7 antigen in lesions is associated with more frequent progression through patch and plaque lesions of CTCL.

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POLETIC EFFECTS OF CD3-CD38 INTERACTION ON PRIMARY CUTANEOUS T LYMHPHOMA (CTCL) NURDBREPHORANT EXPRESSION
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CD30 antigen is a member of Tumor Necrosis Factor superfamily originally identified on Reed-Sternberg cells in Hodgkin lymphoma (HL). The biologic significance of CD30 molecule is strictly related to the existence of a natural ligand (CD30L), recently identified in marine activated cells and in human peripheral blood cells. Recently, it has been demonstrated that CD30+ cells enhance proliferation of HL-derived T cells and, on the contrary, they induce cytotoxic cell death (apoptosis) of CD30+ aggressive large cells from nodal lymphomas; this pleiotropic activity suggests the possibility that the CD30-CD30L interaction might play a determinant pathophysiologic role in both HL and non-HL. We analyzed the phenotypical and genotypical expression of CD30 and CD38 in T cells of CD30+ cutaneous lymphomas, non Mycosis Fungoides (non NF) and mycosis fungoides (NF) patients. Our results confirm that CD30+ cutaneous lymphomas display a similar expression pattern both in mycosis fungoides and non NF patients, only the molecular study (RT-PCR and Southern blot) showed CD30 and CD38 mRNA expression in all examined lesions. The results of our study, notwithstanding the differences in the results obtained by immunohistochemistry and molecular analysis (possibly related to a quantitatively different expression of CD30), suggest the possibility that the CD30-CD30L interaction may have a role in the induction of self regression (apoptosis?).
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IP-10 mRNA EXPRESSION IN CUTANEOUS T CELL LYMPHOMAS

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IP-10, a CXC chemokine, is considered to play an important role in attracting T cells via its chemotactic and cell adhesion promoting properties. Recently, also tumor growth inhibitory activities have been described. Elevated levels of IP-10 protein have been found in the epidermis of cutaneous T cell lymphomas (CTCL). The notion that infiltration of the epidermis by neoplastic T cells (epidermotropism) is a characteristic feature of early stage CTCL, but often disappears with progression to tumor stage CTCL, prompted us to study IP-10 mRNA expression in different stages of CTCL in order to assess its potential role in epidermotropism and tumor cell growth regulation.

A quantitative RT-PCR method was developed to determine absolute IP-10 mRNA levels in skin biopsies of early stage (0-6) and advanced tumor stage (6+) CTCL. In addition, RNA situ hybridization (ISH) was employed to visualize the site of IP-10 mRNA expression. IP-10 mRNA expression levels were correlated with clinical and histological parameters. In the early stages of CTCL, up to 1800 copies of IP-10 encoding mRNA per total RNA could be recovered, while this amount decreased to 10 or less copies/ng total RNA within tumor stages. Extremely high levels of IP-10 mRNA were found in lymphomatoid papulosis, a spontaneously regressing type of CTCL. RISH studies showed a strong association between expression of IP-10 mRNA in the basal layer keratinocytes and the presence of epidermotropic tumor cells. The decline in IP-10 mRNA levels during progression to late tumor stage CTCL suggests that IP-10 plays an important role in the host immune response and may prevent tumor progression, either by attracting tumor infiltrating lymphocytes or via a direct effect on the neoplastic T cells.

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CYTOTOXIC RELATED PROTEINS EXPRESSION IN A GROUP OF CD8+
PRIMARY CTCL

E. Berth 
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CD8+ primary cutaneous T cell lymphomas (CTCL) are a group of CTCL characterized by high clinical aggressiveness. Agnarsson, analysing a cohort of CD8+ CTCL patients, has identified an aggressive-acute and a chronic form, the former with phenotypical profile typical of CD2-, CD4+, CD3+, CD7+, CD8+ and the latter by CD2-, CD3+, CD4+, CD8+. To understand the relation of specific cytokine and chemokine gene expression among these patients and to late tumor stage CTCL, we studied 18 patients (2 series of 10 patients at different stages) and found that in early stage CTCL, the expression of IP-10 plays an important role in the host immune response and may prevent tumor progression, either by attracting tumor infiltrating lymphocytes or via a direct effect on the neoplastic T cells.

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CYTOXIC PROTEIN EXPRESSION IN CUTANEOUS T-CELL LYMPHOMAS

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Cytotoxic keratins are known to play a major role in the defense against neoplastic processes and viral infections. They include keratins, cytotoxic CT8+ T cells and normal y8+ T cells. We postulated that the presence of cytotoxic keratins within the infiltrates of cutaneous T cell lymphomas could influence the prognosis of these tumors. Recently, the development of monoclonal antibodies against human cytotoxic keratins have made the immunohistochemical identification of keratins expressing these proteins possible. Our objective was to see whether the expression of these proteins displays different patterns in cutaneous T cell lymphomas. Using monoclonal antibodies on paraffin-embedded sections, we studied 54 cases of cutaneous T cell lymphomas for the expression of TIA-1 and granzyme B proteins. Cytotoxic keratins were variable. In 10% of the cases, we found an aberrant expression of granzyme A and CDS45R0, a marker of TIA-1 and granzyme B in the tumor cells, whereas only scattered reactive lymphocytes expressed cytotoxic proteins in CD4+ mycosis fungoides (13 cases). Our results further support an overlapping between two distinct clinical entities such as mycosis fungoides and CD8+ lymphomas.

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INDUCTION OF INTERFERON-INDUCIBLE PROTEIN-10 BY TUMOR NECROSIS FACTOR-ALPHA INTERFERON-5: BUT NOT BY HLT-V1 IS LIKELY TO EXPLAIN THE EPIDERMOTROPISM OF CUTANEOUS T CELL LYMPHOMA


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Interferon-induced Proteins-10 (IP-10) is a cytokine chemotactic for CD4+ positive lymphocytes that is expressed by keratinocytes of normal epidermis. TNF-α is specific for IFN-γ for the induction of IP-10 by keratinocytes. The tax gene of human T-lymphotropic virus (HTLV)-1 immobilizes CD4+ positive lymphocytes, induces IFN-γ, and has been detected in variable number of patients with cutaneous T cell lymphomas (CTCL) that are seronegative for HTLV-I. We therefore decided to determine the presence of IP-10, IFN-γ, TNF-α, and tax in lesions of adults with unstratified classic CTCL who were seronegative for HTLV-I. IP-10, IFN-γ and TNF-α were detected and localized with immunohistochemistry of frozen sections. In addition, immunostaining for IFN-γ, TNF-α was detected by reverse-transcriptase polymerase chain reaction (RT-PCR) amplification. IP-10 was overexpressed in lesional keratinocytes of 30/31 patients where it was detected throughout the epidermis. By RT-PCR, mRNA for TNF-α was detected in lesions of 81%, and for TNF-α in lesions of 13.19 patients. By immunocytochemistry, TNF-α was expressed by lesional keratinocytes in 10/13 tested patients, whereas IFN-γ was focally expressed by lesional lymphocytes in 9/12 tested patients. Tax mRNA was detected in lesions of 81% patients, but was easily detectable in cutaneous lesions of peripheral blood of control patients with T cell malignancies who were seronegative for HTLV-I. We conclude that IFN-γ secretion by CTCL may induce IP-10 secretion by epidermal keratinocytes and thus form a cytokine loop responsible for the epidermotropism of CTCL. TNF-α may contribute to epidermotropism by synergizing with IFN-γ in the induction of IP-10. The tax gene of HTLV-I does not appear to be involved in classic CTCL. These cytokine loops may be exploited in the design of novel therapies for CTCL.
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**CYTOTOXIC T LYMPHOCYTE ACTIVITY SPECIFIC FOR MALIGNANT T CELLS IN A PATIENT WITH SEZARY SYNDROME: IMPLICATIONS FOR THE MECHANISM OF INTERFERON-Gamma THERAPY**

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It has been suggested that CD8+ cytotoxic T lymphocytes (CTL) serve as a tumor-killer against malignant cutaneous T cell lymphoma (CTCL) cells. When malignant T cells are of the T8-type, the activities of Th1 cells, CTL, and natural killer cells are expected to be depressed. We investigated the tumor-specific cytotoxicity of CD8+ T cells residing in peripheral blood of a patient with advanced Sezary syndrome of the T8-type, focusing on the effect of interferon-2 (IL-2), interferon-2 (IFN-gamma), and IL-12 on their cytotoxic activity, and the relationship between their lytic capacity and the patient’s clinical course. At four different time points during a two-month clinical period, CD4+CD8+ Sezary tumor cells and CD8+ T cells were separated from patient’s circulating cells by negative and positive selections with immuno-magnetic beads. CD8+ T cells were cultured with chemically attenuated Sezary cells in the presence or absence of cytokines. When cultured with IL-2, CD8+ cells exhibited specific lytic activity. IFN-gamma or IL-12 exerted a synergistic cytolytic effect with IL-2 on the tumor cells. Cloned CD8+ T cells from the patient proliferated in response to IL-2 and IFN-gamma in vitro. We assessed the serum level of LDH. This suggests that CTL down-regulate the growth of malignant T cells in this long-standing disease. Our study also implies the mechanism by which IFN-gamma exert the therapeutic effectiveness in the treatment of CTCL.

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**GRANULOMATOUS SLACK SKIN**

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Granulomatous slack skin (GSS) is a rare cutaneous disorder characterized clinically by the evolution of circumscribed erythematous lax skin masses especially in the body folds, and histologically by granulomatous T cell infiltration and loss of elastic fibres. GSS is often associated with preceding or following lymphoproliferative malignancies especially mycosis fungoides (MF) and Hodgkin’s disease (HD). No effective treatment is available. Whether this entity is a benign disorder, a peculiar host reaction to a malignant lymphoma, a precursor of malignant lymphoma, or an indolent cutaneous T cell lymphoma (CTCL) in itself, is still a matter of debate.

The results of three patients with GSS from the Netherlands are compared with the cases reported in the world literature. A female patient had GSS since 8 years without developing a secondary malignancy. In a second female patient with a histologically confirmed diagnosis of MF, GSS developed 18 years later in the lower extremities. A third patient, who suffered from lymphadenopathy for 4 years, was noted to have a 6-year history of erythematous nodular skin disease diagnosed as CTCL. Developed GSS. Granuloma formation was also found in a facial basal cell carcinoma, in a lymph node in the neck, and in a lymph node. Clinical examination of the T cell receptor genes were found in both female patients (I and II). The development of GSS within plaque MF lesions has not been reported before. As compared to follow up of earlier reported patients, the last case described by us developed very extensive skin lesions and showed a strong propensity to develop granulomas.

We hypothesize that GSS is a very rare and rather indolent variant of CTCL. Long term follow up is needed because in about 50% a second malignant lymphoproliferative disease occurs.

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**THE IMMUNOPATHOGENESIS OF CUTANEOUS T-CELL LYMPHOMA: THE CASE FOR BIOLOGICAL RESPONSE MODIFICATION**

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Cutaneous T-cell lymphoma (CTCL) is a clonally-derived, skin-invasive malignancy of CD4+ T lymphocytes which shares the phenotype of mature helper T-cells. Previous work from our laboratory has demonstrated that the Sezary form, or typically leukemic form of CTCL, is characterized by prominent immunologic defects including depressed cell-mediated immunity. We have also demonstrated increased production of T-selectin type 2 (Tg2) cytokines (IL-2, IL-3) by the malignant T-cells and deficient Th1 cytokines (IL-2 and interferon gamma (IFN-gamma)) by their peripheral blood cells (PBMC). We have also noted a marked defect in IL-12 production in CTCL, which may also play a role in depressed cell-mediated immunity. Overall, our data suggest that immune abnormalities can be attributed to the cytokine abnormalities triggered by the malignant T-cell proliferation. Since CTCL is a disease that is responsive to biological response modifiers, we have focused on strategies for reversing the cytokine and immune defects by in vitro testing of available, and novel biological response modifiers including IFN-gamma, IL-12, IL-15 and retinoids. Our results have indicated that IFN-gamma potently suppresses the abnormal IL-2 and IL-5 production and that IL-12 can correct the deficient IFN-gamma production and that retinoids induce IL-12 production. We have also commenced studies of the in vivo growth characteristics of the malignant CD4+ cells and have determined that IL-12 and IFN-gamma significantly suppress growth of the malignant cells. These studies have led to a phase I trial of IL-12 to treat CTCL. This work has defined mechanisms of action of biological response modifiers, targeted potential future combinations of therapeutic biologic agents for CTCL.

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**FLUDARABINE PHOSPHATE IN ADVANCED MYCOSIS FUNGOIDES/SEZARY SYNDROME: A PHASE II TRIAL**

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Fludarabine is an adenosine nucleoside analogue with promising activity in low-grade non- Hodgkin lymphomas. Preliminary reports have suggested its potential clinical activity in the treatment of advanced Cutaneous T-Cell-Lymphomas (CTCL). The aim of this study was to assess the efficacy and toxicity of fludarabine in patients with advanced or refractory Mycosis fungoides (MF) or Sezary Syndrome (SS).

Nineteen patients (13 MF, 6 SS) were treated with fludarabine at a dosage of 25 mg/m² intravenously on days 1 to 5 every 28 days for up to eight cycles; seventeen (12 MF/5 SS) are evaluable for response. MF patients were classified according to TNM staging system (5 stage II, 7 stage III, 3 stage IV, 1 stage Vb). Ten patients (6 MF/4 SS) had received prior chemotherapy. Four out of twelve MF patients and six out of seven SS had a partial remission (PR) response rate (33%). A median response duration of 7 months (range 3-10 months). Currently, 3/5 SS patients achieved a clinical response (2 CR, 1 PR), for an overall response rate of 60%. Another patient showed a cutaneous stable disease, with a more than 50% reduction in the number of circulating Sezary cells. In all patients a remarkable reduction in pruritus was seen. Median response duration time was 3.5 months (range: 1-8). Remission durations were 51, 11 and 24 months. Systemic side effects and toxicities were unremarkable; cutaneous rashes were observed in 8 patients, whereas only 1 case of grade I neutropenia occurred. These results show that fludarabine is an effective and well-tolerated first-line chemotherapy in SS; conversely, in tumor stage MF patients, fludarabine results do not compare favourably with other therapeutic approaches (i.e. VIMOPH).
43 PROGNOSTIC FACTORS IN SÉZARY SYNDROME
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Sézary syndrome prognostic factors are quite unknown. However, clinical histopathological and the most experimental studies have suggested that SS is a heterogeneous group which may comprise forms with a different clinical course. The specific goal of this prospective study was to assess by multivariate analysis the predictive value of a series of clinical, haematological and immunological factors and their influence on survival. A cohort of 62 SS patients diagnosed in our clinic since 1975 was examined; 51 were included in the multivariate analysis model.

Diagnostic criteria were: a) erythroderma and perianal lymphomatous; b) circulating SC greater than or equal to 10% of peripheral blood leukocytes and greater than or equal to 1000/mm3; c) cutaneous and nodal biopsy proven CTCL. Median survival time was 48.2 (range 1 month-15.2 years) for 42 patients included in the analysis. The two prognostic factors were: high number of circulating SC, presence of large SC, PAS positive SC, lack of CD7 expression and previous history of documented MF. On basis of these data, a risk index was calculated using Cox model and two low-risk high risk (risk index < or = 1) groups have been singled out. Survival differences between the two groups are striking (5 years survival: 60.8% vs. 83.3%). Even if our model needs to be confirmed by larger groups of patients, it can lead to be of clinical relevance in improving SS knowledge and management.

44 TREATMENT OF CUTANEOUS T-CELL LYMPHOMA WITH LOW DOSES OF INTERFERON ALFA-2B
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This study was designed to evaluate the optimal dose and route of interferon alpha-2b (INF-a-2b) given to the Chinese Patients with cutaneous T-cell lymphoma (CTCL). 16 patients with CTCL treated with INF-a-2b were divided into groups according to the TNM classification: IB (8 patients), IIA (2), IIB (2) and IV (4). During induction INF-a-2b were given intramuscularly every two days over two weeks, beginning with a dose of 1-3 x 10^6 IU. Depending on the patients tolerance level, the dosage could be increased weekly to a maximum tolerated dose of 15 x 10^6 IU (mean, 6 x 10^6 IU/m2/dose administered two or three times a week. Patients with clinical response were maintained at initial dosage. Thereafter the dosage were gradually decreased after disappearance of skin lesions. 8 patients were treated by combining intramuscular and intradermal injection of INF, 6 patients (B, II, A, and II B, 2) achieved complete response (CR). 7 patients (B, V, II B, and IV B) showed partial response (PR). The overall response rate in this group was 62.5%. The prompt clinical initial response in 3 patients were manifested early at one week after the intramuscular injection of INF and then the skin lesions were complete to the therapy but disappeared rapidly by combining intradermal injection of INF. The conditions of those patients maintaining CR within 2 to 6 weeks were stabilized for 1-36 weeks. Minor side effects have been observed in 4 patients with “flu-like” symptoms and 1 patient with low fever. This study demonstrated that the overall response rate in treating Chinese Patients with CTCL was 81.25% and higher (83.7%-97.6%) in the subgroup at early stages of disease progress. The authors believe that Chinese Patients with CTCL may have a different tolerance dosage for INF treatment. Starting with low doses of INF treatment may help to determine the optimal dosage for different patient and achieve a more cost-effective ratio in the treatment. Intradermal injection of INF could be recommended for achieving longer periods of therapeutically active serum concentration.

45 LONGTERM EFFICACY OF ORAL PHOTOCHIMOTHERAPY IN MYCOSIS FUNGOIDES H. Maier, B. Schubiger, F. Weening, M. Kauder, A. Zuppinger, H. Tschopp
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Since the introduction of oral photochemotherapy (PUVA) as treatment modality for cutaneous mycosis fungoides (MF), further studies in small groups of patients have been carried out. In order to evaluate long-term efficacy we reviewed the fate of 22 MF patients (3 IA, 11 IB, 1 III, 1 IA, 1 IB, 1 III, 1 IV A) in whom PUVA had been initiated before 1981. Six male and 4 female patients (2 IA, 5 IB, 1 III A) of the 12 MF patients who did not appear at follow-up had died. One patient with initial stage IB disease had died of leucaemia which may be related to his progressive nature. In all other deaths no other than tumour progression was noted. No patient had died in the recent examination (1981-1985). At the time of the last examination (1985) 13 patients (1 IB, 2 IA, 2 III, 1 IV A) remained in the same stage and 4 patients (1 IA, 3 IB) showed clear improvement at the last recorded examination which was 10 (0.25-6) months after the diagnosis of MF and 2 (0.9-3) months after the last PUVA session. The survival rate of the 12 patients was 92% (90-100) at the end of the follow-up (1981-1985). One out of 22 patients showed a complete regression of the lesions, 7 out of 22 (31.8%) patients showed a significant regression. Eight patients had a complete remission. The five-year disease-free periods after the last PUVA session were found in 2 stage IB patients, 123 and 195 months respectively. In 2 stage IA patients the involved area was less than 1% of body surface at follow-up and one in patient with stage IB disease the skin was cleared except one single tumor by four consecutive PUVA courses. All follow-up were patients were alive and in good condition 12 to 34 (mean 22.8) years after the first symptoms of their malignant disease emerged.

Summing up, our data indicate that oral photochemotherapy alone or in combination with other treatment modalities may induce longterm remission in MF patients with early disease. Furthermore, it seems to be possible to maintain the same stage of disease for a long period even in some cases of advanced MF.

46 PRELIMINARY RESULTS OF THE TREATMENT OF CUTANEOUS LYMPHOMAS WITH CEMICITABINE
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Gemcitabine, a novel nucleoside analogue, has been shown to be active as single agent in many solid tumors, particularly in advanced NSCL, breast and pancreatic cancer. We tested efficacy and toxicity of gemcitabine in 7 pre-treated patients (6 p) affected by cutaneous lymphomas. Patients’ characteristics: 6 males and 1 female; median age 70 years (range 41-93). Histology: T-cell: 6 p, B-cell: 1 p. Pre-treatment consists of interferon (IFN) 1 p, IFN and chemotherapy 1 p, plenotherapy 6 p, PUIA 6 p. Gemcitabine was administrated: 4 (1p), 2 (1p), 1 (1p). In 2 p the third dose was omitted due to WHO grade 3 threembocytopenia and leukopenia. No clinically relevant toxicity was reported. In the 5 patients evaluable for response we observed: 2 complete responses and 3 partial responses, all appearing after the first dose of gemcitabine. The study is going on.

47 CHRONIC DERMATITIS ENVOLVING INTO SÉZARY SYNDROME IN 2 YOUNG ADULTS
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Sézary syndrome (SS) is a variant of cutaneous T cell lymphoma (CTCL) characterized by erythroderma, lymphadenopathy and the presence of circulating atypical lymphocytes (Sézary cells). SS has a peak incidence in middle aged persons. According to epidemiological studies and case reports, patients with CTCL can have inflammatory dermatitis, including atopic dermatitis before they develop their cutaneous lymphoma. In the few patients who developed SS at a young age the erythroderma was not evolving from a pre-existing dermatitis neither associated with an atopic diathesis. Two young patients are presented who developed SS at an unusual young age after a period of chronic dermatitis. A 23-year-old male patient with a history of severe atopic dermatitis since childhood, developed SS after 9 years of erythroderma. A 29-year-old female patient developed SS after a recurrent eczema with erythroderma of 4 years duration. These cases contribute to the still ongoing discussion whether their exists a relationship between inflammatory dermatitis and subsequent SS or not. In the presented patients with chronic dermatitis a systemic lymphoma developed on a young age, making a coincidence therefore unlikely. We hypothesize that, in individuals with genetic susceptiblely lymphomas as atopic/chronic dermatitis may specifically stimulate these cells and contribute to their escape from immunologic control. This mechanism offers an explanation for an association between pre-existing dermatitis and the development of SS on an unusual young age in these two patients.

48 LEUKAEMIA CUTIS: A CLINICOPATHOLOGICAL ANALYSIS OF 29 PATIENTS
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Leukaemia Cutis (LC) is known to occur in patients during the course of leukaemia. In part of the patients LC is the presenting symptom, whether as first sign of the disease or as sign of a relapse of a treated leukaemia. We analysed 16 male and 13 female patients (French-American-British classification for non-lymphocytic leukaemia M2=4; M3=14; M4=7; M5=2; AML=11; CML=1; EML=3). Fourteen patients had CL in presenting symptom of which 7 showed no detectable bone marrow involvement. The interval between cutaneous diagnosis and appearance of systemic leukaemia varied from 0.9 months. Fifteen patients developed LC during the course of disease after 1.5 months since the diagnosis of leukaemia. 25/29 were treated with standard polychemotherapy. All patients died after 1-34 months since the diagnosis of LC. The skin lesions consisted of macules, papules and nodules. LC may mimic other dermatoses. The cutaneous infiltrate varied from perivascular to diffuse. The infiltrate showed a mononuclear proliferation of atypical undifferentiated monocyted cells with a moderate amount of finely stained cytoplasm and round to oval or slightly indented nuclei. A moderate number of mitosis was present. LC is a manifestation of systemic leukaemia to the skin and is associated with a very poor prognosis.
P2

PCR-BASED DETECTION OF T CELL RECEPTOR-GAMMA REARRANGEMENT IN TUMOR STAGES 1-4: DIAGNOSTIC VALUE AND LIMITATIONS

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Cutaneous T-cell lymphoma (CTCL) is considered to represent a monoclonal T cell proliferative disease. In CTCL, monoclonality has initially been demonstrated by Southern blotting using T-cell receptor (TCR) beta chain probes. Presently, clonality in CTCL is analysed by PCR of TCR-gamma chain genes followed by temperature gradient gel electrophoresis. Using this method, several groups have shown sensitivity for detection of clonality to be in the 60-70% range. However, these studies included initial stages of CTCL as well. To exclude that the low sensitivity was due to minimal T-cell infiltrates in early lesions or to a lack of detectable monoclonality, we designed a study analysing 18 patients with a clear-cut clinical and histological diagnosis of advanced stage CTCL (clinical stages II-V). We employed an improved standard consensus primer-based TCR-gamma PCR method followed by fluorescence-conjugated allele-specific oligonucleotide (ASO) hybridization and sequential agarose and acrylamide gel electrophoresis of the fixed paraffin-embedded tumor tissue. Of 18 samples tested, 14 showed a clonal rearrangement of the TCR-γ chain indicating a sensitivity of only 77%. This low sensitivity in undoubted CTCL tumors invalidates standard consensus primer-based TCR-γ PCR as a reliable tool in the diagnosis of CTCL, especially with regard to early CTCL lesions. Currently, we try to improve the success rate. 

P4

PROLIFERATING CELL NUCLEAR ANTIGEN AND SOLUBLE INTERLEUKIN-2 RECEPTOR LEVELS IN MYCOSIS FUNGOIDES.

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Expression of proliferating cell nuclear antigen (PCNA) in skin infiltrates and serum levels of soluble interleukin-2 receptor (sIL-2R) was studied in a group of 26 patients with mycosis fungoides (MF) in various clinical stages. The aim of the study was to investigate a possible correlation between these parameters and activity of the disease. Skin specimens from patients with advanced stage MF revealed the highest percentage of PCNA positive cells compared to early stages. The elevated concentration of sIL-2R was detected in all 3 patients with erythrodermic variant of MF who also presented the highest proliferation index. The median value of sIL-2R level was higher in patients with tumor phase compared to patients in patch and plaque stage. No difference was found between sIL-2R serum level and expression of membrane-bound IL-2R alpha chain (CD25) on lymphoid cells in skin lesions. The study indicated that PCNA immunostaining and serum sIL-2R level may useful prognostic parameters in MF.

P1

LYMPHOMATOID CONTACT DERMATITIS: 2 CASES

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Lymphomatosid contact dermatitis (LCD) is an allergic contact dermatitis with anatomocellular features close to mycosis fungoides. We report two additional cases: the first in a 58-year-old female with symmetrical infiltrated erythematous plaques of the pre-auricular areas, the second a 54-years-old man with infiltrated plaques of the temples. In both cases, lesions were 3 to 5 cm wide and the rest of the skin was spared. Biopsies showed an aspect of MF with a dense lymphocytic infiltrate of the upper dermis associated with epidermotropism and marked nuclear atypia. These cells were CD3+, CD4+ and CD7+. The PCR for the T-cell receptor gene revealed either a polyclonal or an oligoclonal aspect. The first patient noticed that lesion appeared after she changed her glasses. For the second case, the unusual localisation of <5%Spoon> lesions and the knowledge of the first case's history led us to suspect a LCD, as much as the patient had glasses. Patch tests were positive in both cases with isopropyl-diphenylenediamine (IPPD) after 72 hours. Biopsy of the test showed an epidermotropic lymphocytic infiltrate associated with dysgranulosis. IPPD is present in a rubber part of the glasses: therefore we obtain in the first case a disappearance of the lesion in 2 months with the avoidance of the allergen. The follow-up of the second case is currently insufficient. LCD must be confused with MF, as in our second case that has been treated by electrocoagulation before diagnosis. Indeed, infiltrated plaques, histological aspect are very cloned to MF. However, localised skin lesions, as the presence of spirogotic foci must led to suspect LCD and to investigate patients with pseudo-LCD. In one case of LCD has been studied by PCR in literature and was polyclonal for the TCR gene areas as in our first case. However, this is not a formal argument for the benignity of this proliferation.

P3

CONTRIBUTION OF HISTOPATHOLOGICAL AND MOLECULAR ANALYSES TO THE DIAGNOSIS OF CUTANEOUS B-CELL INFLTRATATES.

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To evaluate the morphology, immunohistochemical and molecular analyses, we have studied 21 skin biopsies from 19 patients with primary cutaneous B-cell infiltrates. Morphological review by two independent dermatopathologists allowed the consensus diagnosis of lymphoma (n=6) or benign lymphoid hyperplasia (n=15). A discordant diagnosis was made for the other samples (n=9) thereafter considered as unclassifiable lymphoid infiltrates. Immunohistochemistry allowed either monoexpression of immunoglobulin light chain or a positive staining with anti-CD20 antibodies in a respective 3 and 4 samples of lymphomas. Polymerase chain reaction (PCR) was used to analyze immunoglobulin heavy chain and T-cell receptor chain gene rearrangement and to amplify (t[4;11]; (11;14) breakpoints. A clonal morphology was detected in 12 out 19 patients. Among these 12 patients, final diagnosis of lymphoma was confirmed in 8 patients including the 6 patients with morphological diagnosis of lymphoma. Two patients with chronic benign lymphoid hyperplasia and 2 patients with clonal unclassified lymphoid infiltrate presented a benign clinical outcome and one was last. Alternately, no clonal molecular marker was found in two cases of lymphoma. When two pathologists agree for the diagnosis of either malignant or benign lesion, molecular analyses are of limited interest for the diagnosis. When the lesion is unclassified or when histopathological review is discordant, the detection of a clonal lymphoid population should lead to the follow-up of patients at risk for lymphoma.

P5

LACK OF IL-7 mRNA OVEREXPRESSSION IN MYCOSIS FUNGOIDES.

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Interleukin 7 (IL-7) is thought to be a growth factor for cutaneous T-cell lymphomas (CTCL) since it has been shown that IL-7 transgenic mice developed a cutaneous disease characterized by enhanced T-cell proliferation with progression to malignancy and b) in vitro growth of Sezary cells line was IL-7 dependent. However, no direct in vivo evidence exist for the involvement of IL-7 in the pathogenesis of CTCL. Therefore, we examined IL-7 mRNA expression in skin biopsies from patients with CTCL such as mycosis fungoides (MF) (n=20) or polymorphic T-cell lymphoma (n=5). By competitive RT-PCR IL-7 mRNA was neither detectable in any of the CTCL nor in normal skin samples (n=8) or skin from patients with psoriasis (n=7) or atopic dermatitis (n=5). In contrast, IL-7 mRNA could be detected in a biopsy from a kidney allograft transplant, normal keratinocytes under various culture conditions and several cell lines. Interestingly, using a highly sensitive nested PCR, IL-7 mRNA was detectable in all specimens tested, but there was no indications of IL-7 overexpression in CTCL when analysing patch, plaque, and tumor stage B. In contrast, increasing CD3 expression was found which is most likely due to the enhanced density of malignant T-cells in advanced tumor stages. In summary, by using competitive RT-PCR we could not detect IL-7 overexpression in CTCL. This indicates that IL-7 is not an autoocrine growth factor in MF.
P6

EXPRESSION OF EGFR, EGFR RECEPTOR, AND TGFR IN CUTANEOUS T CELL LYMPHOMAS WITH PSEUDOMONAL EPIDERMAL HYPERPLASIA P. Couteau-Ji, J. Wehler, E. Thonnion, Y. Fontas, B. Vergez, M. Bayle-Jeay, M. Bazot, P. Souteyrand, P. Joly. French Study Group on Cutaneous Lymphomas Cytokine Genomics: Hôpital Charles Nicolle in Rouen and Dijon, Hôpital Henri Mondor Creteil, France

The clinical and histological features of 11 cases of cutaneous T-cell lymphomas (CTCL) with pseudomonal epidermal hyperplasia (PEH) are reported. The expression of EGFR, EGFR and TGFR was evaluated by immunohistochemistry and compared to 10 cases of CTCL without PEH. The cases of CTCL with PEH consisted of 8 of the 28 CD30+ CTCL (29%) and 3 of the 148 Mycosis Fungoides (2%) registered by the group from 1999 to 1995. Epidermal expression of EGFR, EGFR and TGFR was stronger in CTCL than that observed in normal human skin. Surprisingly, lymphomatous T-cells from CTCL with and without PEH expressed EGFR and TGFR, whereas no expression of these cytokines could be detected in 5 control cases of B-cell lymphoma. No difference in the epidermal expression of EGFR and TGFR could be evidenced between CTCL with and without PEH. In the contrary, the expression of the EGFR by the keratinocytes from CTCL with PEH was stronger than that of CTCL without PEH. Our results suggest that lymphomatous cells of CTCL produce EGFR and TGFR. An EGFR hyperexpression by the overlying epidermis may explain the PEH seen in some cases of CTCL.

P7

MYCOSES FUNGOIDES WITH EPIDERMAL NECROSIS P. Couteau-Ji, P. Joly, S. Dalcé, E. Thonnion, T. Periloz, J. Wechler1 Departments of Dermatology, Charles Nicolle Hospital Rouen1, Henri Mondor Hospital Creteil2, Dijon Hospital3, French study group on cutaneous lymphomas in Charles Nicolle Hospital Rouen and Dijon Hospital3

The clinical and histological features of 3 cases of Mycosis Fungoides (MF) with epidermal necrosis are reported. After a several year follow-up period of plaque stage MF, an erythroderma with numerous and disseminated superficial skin erosions occurred in the 3 cases. Histological examination of these lesions showed a dermal infiltrate of Sézary cells, numerous necrotic keratinocytes and epidermotropic atypical lymphocytes. Lymphomatous cells were CD4+ CD8- in all cases. The expression of TNFα and apoptosis marker was evaluated by immunohistochemistry using anti-TNFα MoAb and cell death in situ labeling and detection system ApoptoDECT™. In the 3 cases, no expression of TNFα nor apoptosis marker could be evidenced. One to 3 months after the onset of erosive lesions, the 3 patients died, of an extracutaneous dissemination of the MF in one patient, or of infectious complications in the two others. In conclusion, this rare and particular form of MF, 1 seems to be associated with a poor prognosis and 2) raises the question about the mechanism of the epidermal necrosis.

P8

ANTIGEN-DRIVEN CLONAL EXPANSION OF B-CELLS INFILTRATING SALIVARY GLANDS AND LYMPH NODE TISSUE IN PATIENTS WITH JOEGRÉEN’S SYNDROME S. Görgen, A. Becker, S. Golembowski, S. Rutz, E. Georgieva-Blä, W. Stüry, S. Jahn Department of Dermatology, Medical Faculty (Charité), Humboldt-University Berlin and *Re Benniers-Klinik Berlin-Buch, Berlin, FRG

In Joergens’ patients, B cells were found to infiltrate salivary glands resulting in sialadenitis and keratoconjunctivitis (Isica syndrome). Early in the disease course a tiboclonial gammopathy is detectable in sera of those patients and the frequent occurrence of B-cell malignancies is reported.

To analyze in more detail the V_{H} gene repertoire expressed in B cells infiltrating salivary glands and lymph nodes in two Joergens’ patients biopsies were taken, mRNA and cDNA were amplified using specific primers for the FR1 region (V_{H}) and the J_{H} or the CH1 domain gene segments. A total of 137 V_{H}DNA transcripts were cloned and sequenced. Suggesting from sequence analyses the B-cell infiltrates in all tissue specimen were of polyclonal origin. Nevertheless in both labial salivary gland and lymph node biopsies the expansion of a number of B-cell clones became evident.

The V_{H}DNA-ChIY transcripts obtained by PCR suggest that these clones produce IgG antibodies. The analysis of the mutational pattern indicated the maturation and expansion of the B-cells in the result of antigen stimulation. For one individual clone, an ongoing mutational process was documented. Members of this clone have been found both in the labial lymph node material suggesting an emigration and circulation of these B cells, without histological evidence of a developing lymphoma.

Our data provides further evidence that a clonal B-cell expansion under antigen stimulation takes place in salivary glands of Joergens’ patients. It may be speculated that those B-cell clones during disease progression may expand resulting in lymphomagenesis.

P9

PILOTROPIC SKIN INVOLVEMENT IN LOW-GRADE B-CELL LYMPHOMA F. Granget1, M.C. Testa1, C. Le Cluyt1, M.F. Apps, J.J. Thonnion, O. Castejón, B. Lemerand1, J. Wechler1, M.H. Delattre2, P. Feyma3, J.C. Guillame1, P. Joly3 and the French Study Group On Cutaneous Lymphomas Calmar, St Aubin, Villejuif, *Rouen, Creteil, France

Papular or papulo-vesicular eruptions of uncertain nature have been reported during the course of B-cell chronic lymphocytic leukaemia (B-CLL) or low-grade B-cell lymphoma (LGCL). We describe five patients with a distinctive eruption consistent with pilotropic skin involvement in LGCL.

Case 1: A 64-year-old male had a progressive annular plaque on his right calf for 1 year. A biopsy showed a papulapapulo-nodular eruption with epidermotropic T cells.

Case 2: A 57-year-old male with a history of cutaneous T-cell lymphoma (CTCL) presented with papular and papulonodular lesions of the head.

Case 3: A 66-year-old male with a history of CTCL presented with a disseminated papulo-pustular eruption involving the trunk and extremities.

Case 4: A 69-year-old female with a history of CTCL presented with a disseminated papulo-pustular eruption involving the trunk and extremities.

Case 5: A 72-year-old female with a history of CTCL presented with a disseminated papulo-pustular eruption involving the trunk and extremities.

Our cases share a common histological feature, a pustular eruption with epidermotropic T cells. The association of these eruptions with CTCL and LGCL suggests a possible relationship between these diseases.

P10

PRIMARY CUTANEOUS T-CELL-RICH B-CELL LYMPHOMA EVOLVING INTO HODGKIN’S DISEASE IN A PATIENT WITH GARDNER’S SYNDROME L. Kaatsch1, K. Homburg1, K. Jäger1, M. Konig2, G. Lang3, H. Schäfer4, B. Vergez5, M. Bayle-Jeay6, M. Kopp7, C. Cazau1, J. Homburg1, C. Schmitt1, A. Dörries4, R. Bramer5, M. Ziller8, M. Bazot9, H. TonnB, P. Souteyrand10, J. Souteyrand4, M. Bazot4, J. Souteyrand10

A 50-year-old patient was referred to the Department of Dermatology in Zirich for the evaluation of several nodules of slow evolution, situated on the outer aspect of his left arm. Histological examination revealed a dermal infiltrate consisting predominantly of small lymphocytes in a small number of large pleomorphic cells. Immunohistochemical examination revealed a prominent small lymphocytes to be T-cells, while the fewer large cells expressed B-cell markers (CD20+ CD30-). Routine staging procedures failed to detect any systemic involvement. The diagnosis – primary cutaneous T-cell-rich B-cell lymphoma was established. The lesions were excised and the patient was followed up regularly. Three years later enlarged lymph node in his right axilla and a significant sphenopalatine ganglion were detected. Histological and immunohistochemical examination revealed a lymphocytic predominant infiltrate with large cells of typical Reed-Sternberg cell morphology, which were CD20+ CD30-, resulting in a diagnosis of Hodgkins’ disease. Primary cutaneous T-cell-rich B-cell lymphoma is an extremely rare entity. The two previously reported cases with follow-up suggest benign course of this variant. Our case however suggest that systematization with shift to Hodgkin’s disease is another possible outcome.

P11

EXPRESSION OF ADHESION MOLECULES ON ENDOTHELIA IN VIVO IN PATIENTS WITH ERYTHRODERMA P. Granget1, J.M. de Vries1, J. Tomesen1, I.C. Bhui2, C.A.E.M. Brugnagel-Koosten3, T. Thepen1, W.A. van Vliet2

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Erythroderma may result from different causes. At present it’s unclear whether the pathomechanisms of different types of erythroderma are identical or different. In order to explore the differences between the pathomechanisms of erythroderma, we investigated the expression of adhesion molecules on endothelial cells in vivo.

A total of 42 snap-frozen skin specimens were used in this study. Two patients from two different cases of erythroderma (1 idiopathic erythroderma, 1 erythrodermic atopic dermatitis, 3 Sézary syndrome and 6 erythroderma from miscellaneous causes), 10 patients with mycosis fungoides, 5 atopic dermatitis, and 5 healthy volunteers.

The expression patterns of adhesion molecules in erythroderma were: VCAM-1 49.5%, ICAM-1 68.9%, E-selectin 59.2% and P-selectin 50.9%. No significant difference was found between different groups of erythroderma.

The expression of adhesion molecules on skin vascular endothelium may contribute to the pathomechanisms of erythroderma.
P12
LYMPHEDEMA-AREA RESTRICTED LYMPHOMATOID PAPULOSIS
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We report a case of lymphomatoid papulosis strictly localized on an arm with lymphedema. Case report:

A 60-year-old woman presented with a 3-year history of asymptomatic, non-pruritic papules on her right arm. The lesions were firm, red, and slightly elevated. The skin surrounding the lesions was normal. The patient was initially diagnosed with lymphedema of the arm and treated with compression therapy. Despite this treatment, the lesions continued to grow and spread to other areas of the body. Biopsy of the lesions revealed features consistent with lymphomatoid papulosis.

The clinical presentation and histological findings of the lesions were consistent with lymphomatoid papulosis. The patient was treated with systemic chemotherapy, which resulted in partial remission of the lesions. The patient is currently under close surveillance and regular follow-up for any signs of recurrence or progression.

P13
THE BIOLOGICAL EFFECTS OF VIT. E IN PUVA TREATED CELLS.
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PUVA therapy and extracorporeal photochemotherapy (photopheresis = EoP) are well known therapeutic modalities for the treatment of a series of cutaneous disorders. Although the immune modulating effect of PUVA is thought to mainly arise from DNA damage and induction of transcription factors, its release has been postulated to be co-initiated by photo induced cellular membrane damage. As previously shown, the impact of PUVA treatment induces a reduction of intracellular levels of Vitamin E, inhibition of superoxide anion generation and peroxidation of polyunsaturated fatty acids to a variable degree, in a time and 8-MOP/PUVA dosage dependent fashion. In this study, we investigated on Vitamin E and its possible role in the prevention of the oxidative damage induced in a lymphomasurine cell line (BMA) after 0, 10, 100 ng/ml 8-MOP/125 cm EVA treatment. The addition of Vitamin E to the cell cultures restores a 6 hours prior to PUVA, led to significant enhancement of intracellular levels of Vitamin E in treated as well as in untreated BMA cells. Addition of 8-MOP in the peroxidation pattern of unsaturated fatty acids as well as reduction of 13% of catalase activity and 15% of GSH-Px activity at time 0. Under the same culture conditions, at 24 hrs, an increase of the peroxidative damage could be seen. In contrast, the addition of Vitamin E immediately after PUVA treatment was able to completely reverse intracellular Vitamin E levels and catalase activity after 24 hours, when no peroxidation of polyunsaturated fatty acids was evident at the time of PUVA treatment and remained peroxidized also after 24 hours. In conclusion, Vitamin E pre- and post treatment is capable of reversing the oxidative damage induced by the photooxidation of PUVA and should be considered when PUVA/EoP is therapeutically associated with antioxidants.

P14
B-CELL LYMPHOMA MALIGNUM IN STADIO IV.
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A 40-year-old male patient developed a year ago infiltrated plaques on his arm and on extensor side of thigh. 7 months later tumor lesions in the area of the infiltrated plaques appeared. Immunocytochemical examination revealed the lymphoma malignant of large B-cell lymphocytes origin (CD20+, CD38, UCHL-1+). Fraction of the tumor growth determined by applying MIB1 antibody was 50%. In the blood smear a numerous lymphoid cells were found. Patient was qualified for cytostatic treatment.

P15
CUTANEOUS INTOLERANCE TO MECLORETAMINE IN CUTANEOUS LYMPHOMAS: A PROSPECTIVE STUDY OF 43 PATIENTS.
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Methloretamine hydrochloride is a cost-effective and convenient treatment of cutaneous T-cell lymphomas. We carried a prospective study from 1 January 1996 to 31 May 1996 to precise the exact frequency and the histological features of cutaneous intolerance to mecloretamine. This series included 35 men and 17 women, aged from 18 to 87, with Mycosis Fungoides (35/52), non-epidermotropic cutaneous lymphoma (8/52), Lymphomatoid Papulosis (7/52), Sezary Syndrome (1/52) and Langerhans Histiocytosis (1/52). 43 patients were evaluable at the end of the study. Patients with cutaneous reaction to mecloretamine were patch-tested and cutaneous biopsies of the positive tests were performed. Cutaneous intolerance occurred in 23/43 (53%) patients, from 4 days to 9 months after the onset of treatment (4 was observed), on the contrary 1 patient was at time 0. Under the same culture conditions, at 24 hrs, an increase of the peroxidative damage could be seen. In contrast, the addition of Vitamin E immediately after PUVA treatment was able to completely reverse intracellular Vitamin E levels and catalase activity after 24 hours, when no peroxidation of polyunsaturated fatty acids was evident at the time of PUVA treatment and remained peroxidized also after 24 hours. In conclusion, Vitamin E pre- and post treatment is capable of reversing the oxidative damage induced by the photooxidation of PUVA and should be considered when PUVA/EoP is therapeutically associated with antioxidants.

P16
SEZARY SYNDROME (8%): A DESCRIPTIVE STUDY OF 29 CASES.
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Clinical, histopathological, immunohistological and immunogmolecular features of 29 cases of SS from 4 Dermatology Departments of Barcelona were reviewed. 18 patients were males and 11 females with a mean age of 66 years (range: 37-90 years). A medical history (from 2 month to 23 years), of "eczematous dermatitis" was recorded in all cases. 7 patients had a previous diagnosis of parapsoriasis/mycosis fungoides. One patient was under ciclosporine therapy because of a heart transplantation. All, but 6 patients, presented with erythroderma at SS diagnosis. Aderrophy and papulosis were constant symptoms. Severe lymph node involvement (LNM/14) was detected in only 1 cases and visceral infiltration in 20%. Chlorambucil + prednisone was the first treatment in about 50% of patients. Most patients received more than 2 different consecutive therapeutic regimens which included polychemotherapy, alfa-interferon, extracorporeal phoopheresis, methotrexate, etretinate, etc. Partial or complete response were the main treatment results. Only 3 patients are in complete remission at the end of the study. Median follow-up was 2 years (range: 1 month - 9 years). 17 patients died, most of them because of septic complications. Most were characterized by epidermotropic dermal incontinence lymphoid infiltrates. Whole hemato-cardiologic investigations were seen in only 50% of skin specimens. Predominance of blastic-large cells, high proliferation rate, granulomatous infiltrates and follicular mucinosis were rare phenomena. Presence of eosinophilic and plasma cells was variable. Neoplastic cells showed a helper T-cell phenotype with occasional loss of mature T cell antigens. Secondary CD30+ lymphomas were not detected. Immunogmolecular studies, done on peripheral blood and/or skin specimens of 9 patients, demonstrated monoclonality.

P17
PARAPSORIASIS IN PLACITUS PROBIBILIERT TOXICA IN T CELL LYMPHOMA CUTES VIRIDIS.
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We present three cases of cutaneous T cell lymphoma in the workers employed for about 20 years in the manufacturing textile industry. Case 1. — A 45 years old female, has been suffering from the skin itching for ten years. In 1985 desquamative, erythematous, homogenous foci appeared within the skin of armpit and groin, corresponding to large plaque parapsoriasis. The patch tests to furfuryldehyde, benzidine and sodium sulphate were positive. Case 2. — A 42 years old female. In 1986 a skin itching had started, then in 1987 the rash of poikilodermic character appeared on the skin of the face and the neck. The rash sprayed to her armpits, upper extremities, groins and lower extremities. In 1996 the hair falling out was stated. The allergenic patch test was negative, the photo patch test was positive. Case 3. — A 64 years old male. In 1976 the dryness and itching of the skin had appeared. Ten years later hair falling out was also found. In 1996 the erythematous-desquamative foci were recorded in the region of clavicle on the left side and on lower extremities. The patch tests were negative. The diagnose of CTCL in cases 1, 2 and 3 was proved histopathologically — standard histochmical methods as well as immunohistochmical techniques employing monoclonal antibodies were used.
P18
THERAPEUTIC ALGORITHM IN MYOCOS FUNGIDOS
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The treatment of mycosis fungoides is a therapeutic challenge. The aim of this study was to evaluate the effectiveness of different therapeutic methods used according to the clinical stage of the disease. Treatment results were analyzed in a group of 52 patients aged from 21 to 81 years, treated during 1980-1997. Early-stage disease was treated with topical chemotherapy and photochemotherapy. Local application of nitrogen mustard solution produced the complete clearing in 5 of 7 patients, lasting for more than 4 years in 3 of them. Photocemotherapy was used in 31 subjects and resulted in complete and partial remission in 71% and 19% respectively. In the case of relapse, PUVA was administered with administration of etretinate or psoralene and radiotherapy. Patients with advanced disease or therapeutic failure were treated with systemic chemotherapy. Combination chemotherapy was applied to 15 patients and the complete or partial response was achieved in 80% with median duration of remission lasted for 3.5 months. Summarizing the management in presented group of patients, we found median survival rate of 8.2 years (range 3.5 to 14).

P20
CUTANEOUS T-CELL LYMPHOMA WITH HISTIOCYTIC CYTOPHAGIC PARANICLITIS (HCPC) IN 2 CASES
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‡Department of Anatomopathology, Hôpital Cochin, Paris, France

Patient 1: A 27-year-old woman had ulcerated nodules of legs and trunk. Biopsy showed a lymphohistiocytic parainflammatory infiltrate. Two years later, biopsy revealed a HCPC (3 months later). It presented with fever, diffuse nodules, splenomegaly, panlymphocytosis, raised liver enzyme and skin. Cutaneous biopsy showed a perivascular infiltrate of plump cells atypical lymphocytes with eosinophils. A mediastinal lymphohistiocytic infiltrate without lymphomatous involvement was observed. Remission was achieved with chemotherapy. She was then radiologically clear of disease.

Patient 2: A 66-year-old woman had nodules of legs. Biopsy showed a lymphoid infiltrate of normal histiocytes. Six months later, she presented with diffuse nodules, raised liver enzymes and lymphocytosis. Biopsy showed a HCPC and a dense subcutaneous infiltrate of large atypical lymphocytes CD3+ and CD8+, with evidence of EBV expression. An isolated increased number of histiocytes was observed in bone marrow and liver. She died of sepsis. Autopsy revealed no histiocytic and lymphomatous infiltrate.

Twenty-two cutaneous T-cell lymphomas with HCPC have been reported. The subcutaneous and dermal infiltrate is more often composed by large size CD4+ lymphocytes. No extranodal involvement has been observed. Histiocytic nodular infiltrate is often present. Sixteen patients died (72%) from a hematopoietic syndrome, sepsis and intravascular disseminated coagulation. Repeated biopsies are often needed because of the initial non specific histologic pattern of these paraproteins. HCPC may precede the appearance of lymphoma. Patient 2 raises the value of an isolated increased number of histiocytes as a first sign of HCPC and/or hematopoietic syndrome.

P21
INTRALESIONAL CISPLATIN-EPINUPHRINE-COLLAGEGEL FOR THE TREATMENT OF CUTANEOUS B-CELL LYMPHOMA

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Because CBCCL is a rare disease, there are no generally accepted standards for the treatment of primary cutaneous B-cell lymphomas. Experimental and clinical trials indicate that intratumoral injected cisplatin in a collagen-epinephrine gel is a new potent local chemotherapy for various tumors, especially in head and neck cancers. Cisplatin gel is a three component gel system consisting of a collagen gel containing cisplatin (4 mg/ml) to avoid rapid diffusion of cisplatin in tumor environment and circulation. We report on a 56-year-old white woman presenting with a primary cutaneous follicular center B-cell lymphoma on the scalp. An intratumoral injection of a 1% cisplatin gel in a collagen gel was performed. A complete remission was observed after 6 months. One year later, 15% of the tumor mass was visible. No recurrences have been observed during the past 5 years. Cisplatin gel has been used for cutaneous tumors, with a partial response in 5 cases and no change in 1 case. Treatment of cutaneous lymphomas with cisplatin gel is an effective method for the treatment of cutaneous lymphomas.

P22
PRIMARY CUTANEOUS CD30+ (Ki-1)+ POSITIVE ANAPLASTIC LARGE T CELL LYMPHOMA ASSOCIATED WITH RENAL T CELL CARCINOMA: LITERATURE REVIEW OF 87 PATIENTS
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We report a case of primary cutaneous CD30+ Anaplastic Large T Cell Lymphoma (ALCL) associated with renal T cell carcinoma. The incidence of renal carcinoma is higher in association with lymphoid malignancies than in normal population. A 43-year-old man developed a tumor on the right upper lip over 6 months which was excised. No palpable lymph nodes were detected and systemic examination was normal. Histology confirmed a CD30+ and ALCL of null T cell type. Staging revealed a mass in the left kidney which proved on nephrectomy to be clear cell renal cell carcinoma. There was focal recurrence of the lymphoma at 5 weeks which resolved with chemotherapy (CHOP). Systematic review of 87 patients in the literature was carried out, 58 were male, 29 female. Cell type was T cell in 82, null cell in 5 and none were B cell. Below are treatments and recurrences (56.3% overall) in parentheses: Excision 5 (18), chemotherapy 20 (13), excision and radiotherapy 11 (7), radiotherapy 11 (4), chemotherapy and radiotherapy 3 (5), no treatment 7 (4). 65.5% are alive and well, 11.5% alive with disease, 10.3% died of disease, 1.9% died of other causes, median survival was 38 months but survival was 1 year in 2 patients. Median survival was 3 after excision, 4 after chemotherapy and 2 after chemotherapy and radiotherapy.
P24
DOES A SMALL CELL VARIANT OF MYCOSES FUNGOSIS EXIST?
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From pityriasis and phaeomelanic cutaneous T cell lymphomas, small cell variants have been described. Although the presence of only small lymphocytes without significant atypia has been described in some cases of mycoses fungoides (MF) the size of the cells or nucleus was not objectivated. In a small percentage of the patient population of Utrecht with otherwise clinical classic MF our pathologist had the impression that the infiltrate sometimes was composed of only small lymphocytes with hyperchromasia, deeply indented nuclei; small cell mycoses fungoides (SCMF) as seen with special fixation procedures (acid metreric chloride formalin fixation). We investigated whether this impression could be objectivated using electronmicroscopy (EM). 14 patients with SCMF were investigated. 10 patients with a clinical and histological MF (CL-MF) and 4 patients with various types of eczema (ECZ) served as control groups. We made 40-60 electron micrographs in each patient of the most dense part of the infiltrates including always epidermal MF cells. These were analyzed with a computer equipment calculating the Nuclear Contour Index (NCI). The mean PERIBAS nuclear area (Perimeter ratio before and after smoothing), the mean nuclear area, the p75 of the nuclear area and the percentage of cells larger than 30µm2.

Statistical analysis revealed that the mean area of lymphoid cells from patients with SCMF and CL-MF differed significantly (mean nuclear area: 17.02 vs. 23.15 µm2; p<0.02 and p75: 26.66 vs. 27.86 µm2; p<0.01). The NCI of SCMF and CL-MF were almost equal, 6.34 vs. 7.20; p=0.06. The nuclear area of SC-MF and ECZ did not differ significantly (17.62 vs. 18.56 µm2; p<0.29).

We conclude that a small cell variant of MF indeed does exist and that it can be objectivated by EM. These results might have diagnostic implications in the patients with parapsoriasis on plaques where the pathologist could fail to diagnose mycoses fungoides.

P26
MYCOSES FUNGOIDES IN A CHILD: CASE REPORT
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We report a case of mycoses fungoides presenting in a child, 9 years old, with no pruritic erythematous, scaling dermatitis on the abdominal region, with two greater plaques and several smaller in periumbilical region, without other clinical manifestation (e.g. no lymphadenopathy, hepatosplenomegaly). Her general health was good. Results of laboratory studies showed normal complete blood counts, biochemical, and liver function tests. There were no circulating Sézary cells at time of admission. Skin biopsy was performed on one greater lesion and histology showed infiltration on superficial derma by small lymphoid cells with hyperchromic nuclei, sometimes with convoluted, and indentation of the nuclear profile. These elements in restricted areas infiltrate the basal layer of the epidermis, configuring Pautrier-like microabscesses. Immunohistological studies revealed a T cell phenotype of the majority of the lymphoid cells, with predominance of CD4+ cells in the intraepidermal compartment.

P27
CUTANEOUS CD4+ CD68+ CELL TUMORS: REPORT OF 6 CASES
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The authors report six cases of particular cutaneous tumors selected from the registry of the French Cutaneous Lymphomas Study Group. The patients (2 males, 4 females) were aged 38 to 84 years. They initially presented cutaneous nodules or infiltrative papules. All but two, presented with regional lymph nodes. Staging were negative except one patient with bone marrow involvement. Histological features were relevant with pleomorphic medium T cell lymphoma, but these cells exhibiting a distinguishing phenotype. They were positive for CD4, CD8, CD20, CD3, CD43. All other T cell, and B cell markers were negative. The myelomonocytic markers were negative except CD68 which was weakly positive in 3 cases. Others NK-cells markers and CD54 were negative. PCR studies did not detect any B cell clonal rearrangement. All patients were treated successfully by polymyxobacteriostatic therapy, but 4 of them relapsed with bone marrow involvement (4/6) and leukemia (3/4). They died in 5 to 24 months. The 2 remaining cases are alive, one is in cutaneous progression at 8 months and the other one is in remission at 12 months.

The origin of these cells is unclear. Despite expression of CD4 or CD56, we failed to demonstrate a T cell or NK-cell origin. CD4 and CD56 are not specific of T or NK lineages. These two markers are known to be expressed by monocytic cells. These 6 cases with other similar exceptional single cases reported, seem to represent a new entity developed from lymphoid or more likely myelomonocytic precursor cells.

P28
ADULT T CELL LEUKAEMIALYMPHOMA
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Adult T cell leukaemia/lymphoma (ATLL) is a malignant lymphoproliferative disorder which is caused by human T cell lymphotropic virus type 1 (HTLV-1). This disease commonly involves the skin as well as the peripheral blood and lymph nodes. Cutaneous involvement has been recognized in more than half of ATLL patients. Our university is located in the southwestern region of Japan where HTLV-1 is endemic. Over the past 15 years we have collected 118 cases of ATLL with cutaneous manifestations. All patients examined were positive for HTLV-1 antibodies. The male to female ratio was 1:9:1. The median age at first visit was 65 years. According to the classification of the clinical subtype of ATLL, 21 patients were classified as acute type, 20 patients as chronic, 25 patients as lymphoma and 32 patients as smoldering type. All patients exhibited skin lesions. Most patients showed indurated erythemas (39.4%), papules (16.5%) and nodules and tumors (31.2%) similar to those occurring with CTCL. Some patients displayed unusual skin manifestations resembling those of other skin diseases, including drug eruptions, viral eruptions, erythematosus edema, palmar-plantar pustulosis, psoriasis, palmar-plantar hyperkeratosis, atypical, folliculitis, acrodermatitis, and purpura. Histologically, individual cutaneous lesions displayed various degrees of tumour infiltration from the epidermis (Pautrier's microabscesses) to the subcutaneous adipose tissue. We report here the clinical and histological features of these cases as well as the statistical data.

P29
EFFECTIVENESS OF COP/CHOP REGIMENS IN THE TREATMENT OF PRIMARY CUTANEOUS LYMPHOMAS: A CLINICAL FOLLOW-UP STUDY OF 80 PATIENTS
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The efficacy of systemic polychemotherapy in the treatment of primary cutaneous B-cell lymphomas (CCHL) or T-cell lymphomas (CTCL) is still controversial. In CCHL patients, polychemotherapy is usually given in the presence of multiple cutaneous lesions. Conversely, it is usually administered to CTCL patients in the presence of advanced viscerale disease or progressive cutaneous disease. The aim of this study was to evaluate the effectiveness of COP/CHOP regimens, in a series of 80 patients with cutaneous lymphomas (45 primary CCHL and 35 CTCL).

In primary CCHL, the overall objective response rate (RR) was 98%, with an 89% CR rate. Five-year DFS survival was 70%, with a 35% relapse-rate. All relapses occurred in the COP treated group. No differences in RR or relapse-rate were found according to the histopathologic diagnosis. Patients with CCHL based on their legs or diffuse to trunk and legs showed a higher relapse-rate (47% vs. 65%) than those with cutaneous lesions localized on their trunk or head. Five-year event-free survival was 57%. In CTCL, patients, the overall objective RR was 40%, with a 23% CR rate and median response duration of 5.9 months. Mycosis fungoides patients showed a higher RR (81/7; 47%) than did Sézary syndrome patients (1/15; 36%). All the 5 patients with polymorphous or immunoblastic primary CTCL achieved a short-lived CR (median survival: 10 months). Our results confirm the good prognosis of primary CChL and the efficacy of polychemotherapy according to COP/CHOP regimens. CHOP regimen is to be preferred to COP in as much as it reduces relapse rates. Conversely, there are no indications for the use of COP/CHOP regimens as first-line chemotherapy in CTCL patients.
P30
T CELL RECEPTOR I' PCR AND IMMUNOPHENOTYPING IN THE DIAGNOSIS OF CUTANEOUS T CELL LYMPHOMAS
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The diagnosis of cutaneous T cell lymphomas (CTCL) is performed by clinical examination, histology, immunophenotyping, ultrastructural electron microscopy and, presently, by PCR techniques also.

We investigated parafin embedded and frozen skin biopsy samples of suspected CTCL patients by histology, immunophenotyping and T cell receptor (TCRγδ) PCR. Immunophenotyping was done with monoclonal antibodies for CD1a, CD2, CD4, CD8, MIB1, CD30, CD79a, CD25, and CDS8 using the APAAP technique. For the PCR, isolated DNA was amplified with consensus TCRγδ and J1/2 primer. The PCR products were separated on a polyacrylamide gradient gel electrophoresis including silver staining in order to discriminate monoclonal and polyclonal TCR rearrangements. In 25 cases of malignant lymphomas and one case of pseudolymphoma were preliminary diagnosed according to histology and clinical investigation. 18 samples from 15 patients showed the typical histologic picture of MF or Sézary Syndrome (SS) and were confirmed by PCR as monoclonal T cell proliferations. In 3 patients the histology and immunophenotyping indicated MF whereas the PCR demonstrated polyclonality. The clinical course of the disease in these patients has been mild. In 2 patients with other CTCL monoclonal rearrangements occurred. Because monoclonality was found by TCRγδ PCR continuously during the observed course of disease in all patients with advanced MF, SS or other CTCL, the method is a valuable tool in the diagnosis of CTCL.

P31
DNA STABILITY OF LYMPHOCYTES FROM PATIENTS WITH CTCL USING THE COMET ASSAY
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DNA instability is found in many types of cancer cells. We used the COMET assay to study both DNA stability and DNA repair mechanisms in lymphocytes from blood or skin cell lines in patients with CTCL. The COMET assay looks at the spreading of DNA during electrophoresis after a standardized damage with X-ray or UVB. DNA repair is studied by incubating the lymphocytes for various periods of time following the damage. The degree of damage is measured by image analysis.

We have observed that both blood lymphocytes and T lymphocytes from skin-derived cell lines of patients with CTCL had increased DNA instability and decreased DNA repair following damages induced by X-ray and UVB. Further investigations will look at eventual differences according to disease stage.

P32
EXTRACORPOREAL PHOTOCHEMOTHERAPY ASSOCIATED WITH ADJUVANT TREATMENT IN SEZARY SYNDROME
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For the last 15 years, extracorporeal photopherapy (ECP) has been used to treat various forms of cutaneous T cell lymphomas mostly as monotherapy. The aim of our study was to evaluate the combination of adjuvant therapy with ECP in Sézary syndrome patients. Sequential addition of adjuvant therapy, defined as a modality not known to be consistently effective in SS, was administered to initial ECP alone in a group of 11 SS patients. It consisted of low-dose methotrexate (MTX), low-dose interferon (IFN), topical chemotherapy such as nitrogen mustard (HN2) followed by BCNU, and retinoids. Unlike MTX, IFN and retinoids in combination with ECP which was unremarkable, addition of topical nitrogen mustard led to 54% clinical remission and normalization of hematological and immunological parameters in some patients. Topical BCNU combined with ECP induced a complete remission in 2/2 patients. Whether or not this effect is linked exclusively to HN2 or BCNU and ECP or previous ECP combined therapies remains to be determined. Randomized trials are needed to further define the best combination modality with ECP.

P33
CAPILLARY LEAK SYNDROME REVEALING A T CELL LYMPHOMA
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Capillary leak syndrome (CLS) is a rare and severe disorder with high mortality. We report a case of a 57-year-old man who had generalized edema associated with a 12 kg weight gain, a non pruritic morbilliform rash, diffuse arthralgia, myalgia and fever. No hypotension or peripheral effusion was observed. For 3 years he had been in complete remission for 3 years for an angioimmunoblastic lymphoproliferative type T cell lymphoma. No tumoral, cardiac or kidney etiology was found. Skin biopsy showed a moderate mononuclear perivascular infiltrate consisting of small T helper lymphocytes with irregular nuclei. Loss of 2 pan T antigens was observed and a TCRγ gene rearrangement similar in blood and skin was detected. Bone-marrow involvement was found. Photopherapy induced a transient complete remission.

This case is a typical example of CLS which consists of generalized erythematous edema associated with weight gain, fever, arthralgia and myalgia. This clinical presentation resembles the idiopathic CLS described by Akkinson. Secondary CLS have been reported during growth factor treatment (IL2, GMCSF) and in association with hematological disorders. CLS associated with lymphoma has been described only three times. Skin biopsy was consistent with a T cell lymphoma associated with a major T clone at molecular level. CLS pathophysiology is poorly understood. Various factors modifying capillary permeability such as VGEF and TNFα, IL1β, IL6, possibly produced by cutaneous tumor cells can be proposed.
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