The histiocytoses represent a heterogeneous group of disorders that are characterized by the proliferation and accumulation of reactive or neoplastic histiocytes within various tissues. Langerhans cell histiocytosis (LCH) is the commonest of these disorders and it is associated with high morbidity and mortality, especially in children. LCH is a poorly understood disease with features suggestive of a neoplastic, reactive, or immune dysregulation process. The clinical spectrum of LCH is considered to be broad and includes from self-resolving involvement of a single organ to a potentially fatal multisystem disease. The purpose of this review is to undertake an update of LCH with emphasis on the current recommendations regarding the classification, evaluation and treatment of this enigmatic disease.

**Keywords:** Dendritic cells - Histiocytosis, Langerhans cell - Review.

The histiocytoses represent a heterogeneous group of disorders that are characterized by the proliferation and accumulation of reactive or neoplastic histiocytes within various tissues. The generic term “histiocyte” encompasses cells of both the monocyte/macrophage series and dendritic cells. Dendritic cells can be further subdivided into Langerhans cells (LC) and dermal dendrocytes.

While the exact ontogeny is not completely understood, histiocytes are hypothesized to arise from a common CD-34 (+) progenitor cell within bone marrow and, depending upon the cytokine environment, they differentiate along two major pathways, namely into CD-14 (+) cells or CD-14 (-) cells. CD14 (-) cells further differentiate into LC, whereas CD 14 (+) cells differentiate into either dermal dendrocytes or monocyte/macrophages. LC occupy a central role in Langerhans cell histiocytosis (LCH), dermal dendrocytes in juvenile xanthogranuloma (JXG) and related disorders, and macrophages in hemophagocytic syndromes and Rosai-Dorman disease (Figure 1).

In 1987, the Histiocyte Society proposed a classification of histiocytic disorders based on three different classes: LCH (class I), non-LCH (class II), and malignant histiocytosis (class III). More recently, a revision of this classification has been suggested in the light of new developments in the characterization of the central cell type involved in each disorder. This contemporary classification included 1) dendritic cell disorders, of which LCH is by far the most common; 2) macrophage-related disorders; and 3) malignant histiocytic disorders. Correlations between the two classifications are summarized in Table I.

The purpose of this review is to undertake an update of LCH with emphasis on the current recommendations regarding the management of this enigmatic disease.
Definition

LCH is the term currently used to describe a disease characterised by pathological accumulation of a monoclonal population of LC in one or more organs causing tissue damage mediated in part by the production of inflammatory cytokines.

At present the clinical spectrum of LCH is considered to be broad and includes from self-resolving, asymptomatic involvement of a single organ to potentially fatal multisystem involvement. For years various terms have been used to describe the different clinical presentations of this disease. Names such as eosinophilic granuloma, Letterer-Siwe disease, Hand-Schuller-Christian syndrome or Hashimoto-Pritzker syndrome are still widely used by the medical community. However, the use of these terms implies an attempt to rigidly classify an extremely heterogeneous clinical picture. Nowadays all these eponyms are considered obsolete and the current classification of LCH is based on Histioocyte Society recommendations.¹

Etiology

The etiology of LCH remains unclear. Although it has been suggested that viral etiologic factors may play a...
role in LCH pathogenesis, studies have only rarely reported Epstein Barr virus or human herpes virus-6 as possible triggering factors.5-7 The possible role of viruses has not been completely ruled out, but no viral genomes have been consistently detected in LCH lesions.8 A study of 56 LCH samples using in situ hybridization and polymerase chain reaction (PCR) analysis failed to show any consistent evidence of Adenovirus, Cytomegalovirus, Herpesvirus, Parvovirus, human T-cell virus or human immunodeficiency virus infection.9 In addition, the paucity of epidemiological reports of any clustering of cases by time or place argues against it being due to a conventional virus. For these reasons, the relationship between virus and LCH is, at best, inconclusive at this time. No other environmental pathogenetic factors have been identified, except for the fact that an elevated incidence of smoking has been found in patients with pulmonary LCH (PLCH).10

The hypothesis that a defective immune response is the underlying cause in the etiopathogenesis of LCH has been put forward.11 Various associated immunological disorders such as anomalous circulating immunoglobulin counts,12 reduction of the CD8+ T-cell subpopulation,13 presence of cutaneous anergy,14 or an in vitro decrease in the response of T-cells to mitogens 14 or in the suppressive activity of T CD8 lymphocytes have been reported.13 Only one post-mortem study has shown deficiencies in T-cell alloanti-gen presentation by LC in a patient with multisystem LCH.15 However, since it has not been possible to consistently demonstrate any of these findings, most authors consider them to be an epiphenomena. Cases of vertical transmission or familial grouping have been reported.16, 17 and some HLA-DR phenotypes have been shown to appear more frequently in localized LCH than in multisystem LCH.18 All these data may suggest the existence of an increased genetic susceptibility to immune dysregulation against certain stimuli that are currently unknown.

According to another hypothesis there may be a non-neoplastic proliferation of LC secondary to an erratic and uncontrolled activation of cytokines ("cytokine storm"), secreted by both the LC themselves and the accompanying population of T lymphocytes. The existence of raised levels of tumour necrosis factor alpha (TNFα), interferon gamma (IFNγ), granulocyte-monocyte colony stimulating factor (GM-CSF), IL-1, IL-2, IL-4, IL-10 and IL-11 has been shown in lesions of patients with LCH.19-21 Although it is not yet known whether they are responsible for the disease developing, these cytokines and interleukins are thought to be the cause of various symptoms related to LCH and are involved in the development of tissue damage in multiple organs. Thus, it is believed that all these inflammatory mediators contributed to the osteoclastic activity or hepatic and pulmonary fibrosis that appear as late sequelae of LCH.21 Based on this hypothesis, anti-TNFα drugs such as etanercept or thalidomide have been successfully used to treat LCH.22, 23

Using X-linked polymorphic DNA probes, such as human androgen receptor (HUMARA), it has been shown that LCH lesions are clonal, regardless of the extent or severity of the disease.24, 25 One exception to these findings exists, however: adult PLCH is a polyclonal disorder.26 In contrast to other forms of LCH, PLCH in adults is often an isolated disease which is closely associated with smoking and frequently regresses after cessation of the smoking habit.
However, the significance of this monoclonality and its implications remain controversial. There are arguments both for and against LCH being a neoplastic process. Apart from the monoclonality, other findings that support the existence of a neoplastic process include an elevated expression of p53, H-ras and c-myc oncoproteins, the detection of molecular cytogenetic aberrations, and the increased incidence of second malignancies. In contrast, factors favoring a reactive process include the following: LCH cells histologically resemble normal LC rather than showing maturation arrest typical of malignancy; LCH lesions can develop spontaneous regression, which is only rarely seen in true malignant neoplasms; many reactive diseases, i.e. lymphomatoid papulosis, have been shown to be clonal, showing that monoclonality is not a definitive proof of malignancy. In conclusion, LCH pathogenesis does not appear to fit either a truly neoplastic or purely reactive pattern.

### Incidence

The exact incidence of LCH is difficult to determine due to its great clinical heterogeneity and the lack of national registers. Different epidemiological studies carried out in France, the United Kingdom, Denmark and Germany report an annual incidence in children under the age of 15 years of 4-6 cases per million. Although LCH may appear at any age, there is a clear predominance in children between the ages of 1 and 3 years. The annual incidence in children under 1 year has been estimated to be approximately 15 cases per million. In children from 10 to 14 years the annual incidence drops drastically to 2 cases per million. The annual incidence in adults is clearly lower, and is calculated to be around 1-2 cases per million. The mean age at onset of LCH in adults is 35±14 years. The disease does not appear to have any preference for a particular race and the distribution by sex is similar, with just a slight predominance in males, both in children and adults.

### Clinical presentation

The disease can involve almost any organ system; thus, the clinical presentation of patients with LCH differs on a case-to-case basis, mostly because of the variety of organ systems that may be involved and the level of disease activity present in each case.

As mentioned above, LCH is currently considered as a clinical spectrum. At one end of the spectrum are patients with skin or bone single-system disease with a 100% survival. At the other end are patients, usual-
ly very young children, with a multisystem disease with life-threatening organ dysfunction and a high risk of mortality (Table II). Between the two extremes are patients whose disease runs a chronic fluctuating course that eventually ‘burns out’, but often leaves serious residual disabilities.27

**Bone involvement**

Bone is the commonest single organ involved in childhood and the majority of cases presents with a single lesion and an excellent outcome. All bones may be involved, however, except for the hands and feet.27 The commonest involved sites are the bones of the skull, followed by the long bones of the extremities and the flat bones such as pelvis, vertebrae, ribs, and scapula.27, 40 Lesions may be asymptomatic, appearing as an incidental radiological finding during the evaluation of unrelated disorders, or may present with painful swelling over the affected bone or pain that may initially be present only at night. Involvement of the long bones of the extremities may result in pathological fractures. Vertebral body involvement is the commonest cause of vertebra plana in children and an associated soft tissue mass may result in significant neurologic impairment.27 Vertebral collapse with subsequent spinal cord compression has occasionally been described with LCH. Mastoid and temporal bone disease may cause chronic otitis media and middle ear extension may result in deafness or vertigo.40 Involvement of the external auditory canals may result in chronic otitis externa with high risk of superinfection by *P Aeruginosa*. Proptosis may result from orbital wall involvement, and swelling and redness of the eyelid secondary to orbital masses has also been described (Figure 2A).41, 42 The leading clinical symptom of LCH within mandibular and maxillary bones is pain, which is sometimes misdiagnosed as a marginal parodontal infection. There may be severe alveolar bone resorption producing the radiological appearance of teeth “floating in space”.43 Loosening of the teeth may occur as a result of severe gingivitis, especially when there is concomitant bony involvement of the alveolar ridge and jaw.44

The classical radiologic finding is a punched-out lytic lesion in bone, but some LCH lesions may mimic other benign or malignant conditions such as an aggressive bone sarcoma with destruction of bone and periosteal elevation.45 For the evaluation of bone involvement, magnetic resonance imaging (MRI) has no advantage over conventional X-ray. Radiographic appearance of osseous LCH depends on site of involvement and phase of the disease.46 In children the most frequent radiological pattern seems to be a purely osteolytic lesion followed by permeative lesions, vertebra plana, periosteal reactions, and soft tissue masses.47

**Skin involvement**

Cutaneous involvement is very common in LCH, appearing in almost 80% of patients with multisystem LCH and is often the presenting complaint. However, single-system skin disease is observed in only 10%.48, 49 The spectrum of skin findings in LCH is very heterogeneous, but the commonest presentation...
that resolve spontaneously without involvement of other organs. While multiple lesions are commonly seen, solitary lesions have been reported. Although Hashimoto-Pritzker disease invariably regresses over a period of weeks, postinflammatory pigmented changes, atrophic scars, or secondary milia may be observed as permanent sequelae. Currently, there are no criteria other than clinical that can reliably distinguish CSHR from cutaneous involvement by disseminated LCH. A recent study failed to show a significant difference in the histologic features and the expression of E-cadherin, Ki-67, and phosphorylated histone-3 between these disorders, supporting the theory that CSHR and LCH represent different ends of a spectrum of the same condition. Exceptionally, patients diagnosed with CSHR may have late relapse or progression to systemic involvement after the initial autoinvolution.

Oral involvement may occur in patients with LCH, and is often associated with a pattern of multifocal or multisystem disease. Oral involvement may result in gingival erythema, erosions, ulceronecrotic lesions and hemorrhage; these manifestations can be the presenting features of the disease. The most common dental manifestation of LCH is a destructive periodontitis which results from osseous infiltration by proliferative cells. This can lead to total destruction of mandibular and maxillary periodontal support and loosened teeth. Vulvar involvement is rare. Symptoms and signs at the time of presentation can include pruritus, pain, dyspareunia, burning, discharge and presence of erosions or ulcerations. Exceptionally, pure genital LCH, in which the disease is localized to the genital tract, has also been described. Several neoplastic processes can mimic vulvar involvement in LCH, including squamous cell carcinoma, malignant melanoma, and Paget’s disease. A few cases of LCH on the penis have been reported in the literature, either as a localized disease or in the context of disseminated LCH.

Nail changes in patients with LCH have rarely been reported and they may manifest as subungual hyperkeratosis, purpuric striae, subungual pustules, onycholysis, longitudinal grooving, and paronychia of the proximal or lateral nail folds. Permanent nail dystrophy may occur. Various fingernails and toenails are usually affected. Exceptionally, nail changes can be the first clinical manifestation of LCH, appearing some months before characteristic symptoms of the disease. Although some authors consider it an unfavourable prognostic sign, there are insuffi-

Figure 4.—Multiple yellow-brown, scaly papules in a child with multisystem LCH.
enough data to draw definite conclusions as to whether nail involvement in LCH is an independent prognostic factor of poor outcome.65

Central nervous system and endocrine involvement

Infiltration of the hypothalamic-pituitary regions can result in diabetes insipidus (DI) which is present in over 50% of cases, frequently in patients with bony involvement of the skull and extensive disease. Established DI is generally permanent and does not respond to any available treatment, except symptomatically. Gadolinium-enhanced MRI examination, which is considered to be the method of choice for the evaluation of hypothalamic-pituitary axis, shows an absence of a normally bright signal in the posterior pituitary gland or thickening of the pituitary stalk. Anterior pituitary deficits may follow DI and can include growth hormone deficiency, precocious or delayed puberty, thyroid-stimulating hormone deficiency, adrenocorticotropic deficiency, amenorrhea, and hyperprolactinemia.27, 68 It is important for patients with isolated or partial pituitary hormone deficiency to be monitored at regular intervals using established endocrine investigational protocols to detect further pituitary hormone deficiencies.69 Hypothalamic involvement can also occur in patients with LCH, leading to pituitary dysfunction, neuropsychiatric and behavioral disorders, and autonomic and metabolic abnormalities. Disturbances of appetite, thermoregulation, sleeping pattern and behavioral skills have been reported.69, 70

Cerebral LCH is not common (1-4%) and is exceptional as single-organ LCH.71 Clinical manifestations are progressive and most frequent in patients with

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<th>TABLE III. – Long-term sequelae of LCH.</th>
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CNS: central nervous system; HPA: hypothalamo-pituitary axis
bony involvement of the skull and DI. Cerebellar symptoms are the most common clinical symptoms.\(^72\) Other manifestations of central nervous system (CNS) involvement include hyperreflexia, cranial nerve deficits, dysarthria, cognitive defects, and rarely seizures. It is believed that brain involvement in the course of LCH might arise from different disease mechanisms: primary histiocyte proliferation and secondary atrophy or demyelination and gliosis of unknown origin. The former acts as a cerebral mass with a good response to LCH treatment protocols, whereas the latter probably results from cytokine/chemokine-mediated neural damage \(^73\) or an immune reaction to the preceding LCH.\(^74\) There is no known effective therapy for patients with this late progressive CNS disease and it usually results in long-term sequelae (Table III).

### Lung involvement

PLCH is rare in childhood but occurs most commonly in children with multisystem LCH. In adults, by contrast, the lung is the most common and usually the sole organ affected.\(^75\) Lung involvement is seen in less than 10% of childhood LCH, but it has been reported in 20-50% of children with multisystem LCH.\(^76\),\(^77\) About half of children with PLCH may be asymptomatic, and the prognosis appears to depend on the presence or absence of other risk-organ involvement.\(^76\) The association of adult PLCH with smoking,\(^10\) the clinical course of the disease and the observed differences in clonality suggest that PLCH in childhood may be a different disease. Patients with pulmonary involvement may present nonproductive cough, chest pain, hemoptysis, dyspnea, and pneumothorax. With disease progression, clubbing and cor pulmonale may be observed. An abnormal chest radiographic finding may be the only clue to the disease in the 25% of asymptomatic patients with PLCH. A spectrum of chest radiographic abnormalities is seen in patients with PLCH but the most common image is a reticulomicronodular pattern which predominate in the mid-lung zones. Significant lower-lobe involvement has been reported as well, but is less common.\(^78\) High resolution CT (HRCT) has proved to be a superior modality in diagnosing PLCH as compared to plain chest radiography. HRCT defines the numerous nodular and cystic lesions characteristic of PLCH and provides a high diagnostic specificity often avoiding the need for an invasive diagnostic procedure.\(^79\) To diagnose LCH by bronchoalveolar lavage, more than 5% LC must be detected by electron microscopy or immunostaining. One percent may be found in normal lungs and are not indicative of disease.\(^80\)

### Gastrointestinal involvement

Gastrointestinal (GI) tract involvement may present as abdominal pain, anorexia, vomiting, diarrhoea or bloody stools. Failure to thrive may result from malabsorption. However, GI involvement may be asymptomatic and thus is, at times, overlooked. Diarrhoea may be the result of infiltration of the lamina propria by LCH cells or may be caused by abnormal bile acid metabolism. Liver involvement is very common as part of multisystem LCH, producing hepatomegaly or ascites. Cholestatic jaundice may be a late feature due to fibrotic obstruction of the biliary tree (sclerosing cholangitis).\(^81\),\(^82\)

### Miscellaneous findings

Nonspecific constitutional symptoms such as fever, weight loss, and malaise are common in multisystem LCH. Peripheral lymph nodes can be affected in isolation as a single organ disease or as part of multisystem disease. Common sites include the cervical area and the groin, but lymphadenopathy can occur throughout the mediastinum and throughout the abdominal cavity.\(^51\) Enlarged mediastinal nodes can mimic lymphoma or an infectious etiology and cause asthma-like symptoms. Bone marrow involvement is rare and occurs late in the course of the disease. It may result in pancytopenia, and with concomitant hypersplenism may contribute to life-threatening sepsis and hemorrhage.\(^44\) Thyroid tissue is a rarely affected site, and without histopathological evaluation it may be difficult to distinguish from other thyroid disorders because of the similar physical examination, laboratory and imaging findings.\(^83\) Infiltration of the thymus is not common and is usually accompanied by skin, bone or lung disease.\(^84\)

### Pathology and standardized diagnostic criteria

Diagnosis of LCH is based on the presence of compatible clinical findings and their confirmation on
immunohistochemical and histological study. Since 50-80% of LCH present cutaneous involvement, skin biopsy is a quick, accessible method to confirm this diagnosis.

The histological appearance of skin LCH is as variable as its clinical expression. Histological findings depend on the location and on the stage of the biopsied lesion, and it is not unusual to find an overlapping of various histological patterns. So far no histological finding has been shown to have prognostic value.85

In the earliest lesions the infiltrate is mainly located in the upper dermis and consists of clusters of rather uniform cells with a characteristic “coffee-bean” or reniform nuclei and fairly abundant pale-staining or eosinophilic cytoplasm (Figures 5, 6). Within the epidermis, LCH cells are often observed either singly or forming Pautrier-like microabscesses. Mitoses may be evident, but typically they are not numerous. Scattered lymphocytes, eosinophils, and occasional neutrophils are often admixed with the LCH cells. Eosinophils are sometimes conspicuous (Figure 6B). As mentioned above, the morphology of LCH varies as lesions mature or regress. The most chronic lesions may have a more granulomatous or xanthomatous pattern with few LCH cells.

In most cases, the histology of CHSR is indistinguishable from other forms of LCH.54, 86 Sometimes, however, it may present a characteristic pathology consisting of an infiltrate located deep within the dermis and the presence of several multinucleate giant cells and large histiocytic cells with copious glassy eosinophilic cytoplasm.

A presumptive diagnosis of LCH may be made based upon light microscopic findings and a compatible clinical picture, but a definitive diagnosis requires that lesional cells exhibit positive staining with S-100 and CD1a, and the sine qua non is the identification of Birbeck granules upon electron microscopy.1, 87 The

Figure 5.—A) Papule on the forehead of a child with low-risk multisystem LCH. B-D) Histological images showing an infiltrate located within the upper dermis consisting of histiocytes with scattered eosinophils and lymphocytes. Epidermotropism is marked (hematoxylin & eosin x20, x40 and x100, respectively).
number of LCH cells with identifiable Birbeck granules can vary in different lesions, with limited numbers seen in tissue taken from the liver, spleen or CNS. Currently, the presence of Birbeck granules is assumed by immunohistochemical demonstration of langerin (CD207), a mannose-specific lectin whose intracellular component is found in association with these cytoplasmic organelles. It appears to be more sensitive and specific for LCH cells than CD1a, and, in the future it may be a key component of an immunohistochemical panel to diagnose LCH.\(^1\)\(^{,88,89}\)

**Evaluation**

The current classification of LCH follows the guidelines of the Histiocyte Society incorporating study results of multicentre (DAL HX-83 and DAL HX-90)\(^{90}\) and randomized trials (LCH-I, LCH-II and LCH-III) in children. The results of these large clinical trials have added to the evidence-based knowledge of LCH and are being used extensively by physicians and treatment centres throughout the world (available on the Histiocyte Society Web site, www.histiocytesociety.org).

These collaborative studies have established guidelines to assist in the diagnosis and evaluation of LCH.\(^{91}\) All patients with suspected LCH should undergo a through physical examination, inclusive of height and weight measurements. Laboratory evaluation should include a complete differential blood count, coagulation studies, erythrocyte sedimentation rate (ESR), liver function tests, C-reactive protein (CPR), and urine osmolality after overnight water deprivation. In addition, the patient should have a chest radiograph, and radiographic skeletal survey, which is more sensitive than a radionuclide bone scan for detecting bone lesions. Some authorities advocate that a bone marrow examination should be mandatory in every baseline examination. Further evaluation should be tailored to patients based on specific presenting signs and symptoms (Table IV).\(^1\)

LCH are currently grouped according to the sites and types of tissues/organs involved and the presence or absence of organ failure. The Histiocyte Society’s Treatment Protocol for LCH categorizes patients as those with single-system disease involving a single site (unifocal), those with single-system disease affecting multiple sites (multifocal), and those with multisystem disease. Patients determined to have multisystem disease should be further stratified according to the specific organs involved and the presence or absence of organ dysfunction. Specific criteria now exist for the classification of organ dysfunction (Table V). High-risk LCH includes all patients with multisystem disease with involvement of one or more key organs (liver, spleen, lung and hematopoietic system). The low-risk multisystem group does not include any of the above organs. Recently, several studies have concluded that lung involvement as the only risk organ does not confer an increased risk of death and it will not be considered a key organ in future Histiocyte Society studies.\(^{27}\)

Disease activity and response criteria may be assessed based upon the Histiocyte Society protocols. Alternatively, a new clinical staging system using a

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**Figure 6.** A) Eosinophils are conspicuous in this field (hematoxylin & eosin x100). B) This field shows typical coffee bean, vesicular nuclei (hematoxylin & eosin x200).
risk-assessment score has been developed. This methodology is based upon a score derived from three primary parameters; clinical examination, laboratory evaluation, and radiological findings. It provides an objective tool for assessing disease severity, both at diagnosis and during follow-up and treatment.92
Treatment

Treatment of LCH depends on the extent of the disease. Although some patients with LCH may undergo spontaneous remission, the disease is unpredictable and many patients progress from single-system disease to multisystem disease. Consequently, previous suggestions that LCH could be untreated are now debatable.

Patients with limited disease have an excellent prognosis, usually without the need for systemic therapy. Single-system skin disease may respond to local measures with topical corticosteroids, topical nitrogen mustard, or psoralen with UVA (PUVA). Topical nitrogen mustard, an alkylating cytostatic agent, is the best studied and most effective topical therapy for cutaneous disease but contact allergy and the concern about potential cutaneous carcinogenicity limit its use. Several reports have documented that thalidomide is an effective treatment for LCH, particularly in cutaneous disease. Thalidomide has been shown to decrease the production of TNFα in vitro and in vivo and is a logical candidate for the treatment of LCH. The response is often temporary and there is a risk of neuropathy with prolonged use. An adult patient with skin limited disease achieved a complete response to topical imiquimod, lending support to the idea that a local release of interferon-alpha and other cytokines by imiquimod can induce clinical resolution of cutaneous LCH. Excimer laser as adjuvant therapy for adult cutaneous LCH has also been proposed. As mentioned above, close monitoring for disease progression is mandatory if isolated cutaneous LCH is managed by the "wait and see" approach.

Treatment for disease limited to bone is dictated by the extent of bone involvement and symptomatology. In single bone lesions, curettage to establish the diagnosis may be curative. In weight-bearing bones that are symptomatic, intralesional steroid injections may lead to resolution. If critical structures are compromised, such as the optic nerve or spinal cord, low-dose radiation or chemotherapy can be employed.

Currently, the LCHIII treatment protocol is probably the most common therapeutic strategy used for patients with multiorgan involvement (Table II). Treatment of multisystem LCH is given to improve survival and to prevent late sequelae. Combination therapy with vinblastine and prednisone has been evaluated and proposed as first-line treatment for pediatric low-risk multisystem LCH as well as, more recently, for adult low-risk multisystem LCH. Similarly, patients with multifocal disease of bone, or recurrent lesions of bone are also candidates for this chemotherapy regimen. On the other hand, combination therapy with vinblastine/prednisone/6-mercaptopurine with or without methotrexate has proven useful in high risk multisystem LCH. As demonstrated by large clinical trials in children (DAL-HX 83 and 90, LCH I, and LCH II), the standard etoposide regimen has not shown any additional benefit in terms of response, survival, or reactivation frequency as monotherapy or in combination with vinblastine and prednisolone. Therefore, etoposide was not included in the treatment schedule of LCH III because of its potential leukemogenicity.

Treatment of PLCH remains to be defined. Smoking cessation is recommended because of a potential pathogenetic association, the occasionally documented resolution of disease following cessation of smoking and the increased risk of bronchogenic cancer in PLCH. Corticosteroids and other immunosuppressive therapies have not been evaluated in well-designed clinical trials, largely because of the relative rarity of the disorder and the confusion in segregating the adult PLCH from multisystem LCH in the pediatric age group.

Several studies have shown that a lack of response to chemotherapy at 6 weeks is the single most important predictor of poor survival. Patients who respond to chemotherapy have a 88% to 91% survival rate, but for patients who do not demonstrate an early response the survival rate drops to 17% to 34%. Recent data from a French pilot study using 2-chlorodeoxyadenosine and high-dose cytosine arabinoside suggest that early switch of poor responders to intensive salvage regimens improves survival. The majority of patients who fail these intensive salvage regimens die and hematopoietic stem cell transplantation should be considered early on in this group. Innovative treatment approaches include the development of monoclonal antibodies directed against CD1a and the use of immunomodulatory agents such as the kinase inhibitor imatinib mesylate, the monoclonal anti-CD52 alemtuzumab, and antiTNF blockers.

Prognosis

Based upon the results of several large multi-centre therapeutic trials, four important, independent, outcome predictors in LCH have been proposed. It has
been shown that prognosis is dependent upon the number of organs involved, the evidence of key organ involvement, the age of the patient at the onset of the disease and, finally, the patient’s response to chemotherapy during the 6-week induction phase, as mentioned above. Patients with disease that is localized (skin, bone, and lymph node) have a good prognosis and need minimal or no treatment. In contrast, multisystem involvement, which is particularly common in children younger than 2 years but also occurs in adults, carries the risk of a poor outcome (Table II).

Traditionally, an age of 2 years has been considered the cut-off point below which the probability of survival decreases considerably. However, newborns with localized skin disease usually have an excellent prognosis, and there is even the possibility of spontaneous resolution. Therefore, the patient’s age should only be considered as a prognostic factor in patients with multisystemic disease.

Long-term sequelae

Reactivation is a frequent and early event in multisystem LCH, but involvement of risk organs at reactivation is rare and mortality is minimal (Table II). Incidence of sequelae correlates with the occurrence of these reactivations. In a retrospective analysis of 335 patients with multisystem LCH and documented complete disease resolution, the probability of a reactivation within the first 5 years was 46%. These reactivations increased the risk of permanent consequences approximately 2-fold.

Despite adequate treatment, at least 71% of multisystem and 24% of single-system patients have at least one permanent consequence, some of which may not become apparent until many years later (Table III). A pilot study from the Histiocyte Society-Late Effects Study Group has shown that the most frequent long-term sequelae are DI (24%), orthopedic abnormalities (20%), hearing loss (13%), and neurological consequences (11.0%). In addition, this study confirmed previous findings by Grois et al. that LCH localization to the skull is significantly associated with the risk of DI.

However, not only patients with multisystem disease are at risk of permanent consequences. Even those patients diagnosed with congenital self-healing LCH may have late relapse or progression to systemic involvement up to 4 years after the initial disappearance of the congenital lesions. Consequently, all patients with LCH require long term follow-up to identify disease recurrence or late-term sequelae.

On the other hand, second malignancies occur in LCH patients with a much higher frequency than expected. Malignant diseases may precede, co-occur, or follow LCH. Overall, the majority of cases develop malignancy following LCH treatment. Acute leukaemia is the most common associated malignancy in children with LCH but solid tumors and other hematopoietic malignancies have also been reported. Two different explanations for this have been proposed. First, it is possible that therapy for LCH promotes a secondary malignancy. Second, it is possible that a genetic predisposition, with or without the associated immunosuppression therapy for the malignancy, plays a role in the development and expression of disseminated LCH.

Riassunto

Istiocitosi a cellule di Langherans: un aggiornamento

L’istiocitosi rappresenta un gruppo eterogeneo di disturbi che sono caratterizzati dalla proliferazione e dall’accumulo di istiociti reattivi o neoplastici nell’ambito di diversi tessuti. L’istiocitosi a cellule di Langherans (Langherans cell histiocytosis: LCH) è la più comune tra questi disordini ed è associata ad un’elevata morbidità e mortalità, specialmente nei bambini. La LCH è una patologia scarsamente compresa, con aspetti suggestivi di un processo neoplastico, reattivo o derivante da immuno-disregolazione. Lo spettro clinico della LCH è ampio e può variare dal coinvolgimento di un singolo organo, che si autorisolva, alla malattia multisistematica potenzialmente fatale. L’obiettivo di questa review è quello di fornire un aggiornamento sulla LCH, con particolare attenzione alle attuali raccomandazioni circa la classificazione, la valutazione e il trattamento di questa patologia enigmatica.

Parole chiave: Cellule dendritiche - Iстiocitosi - Cellule di Langherans.

References


LANGERHANS CELL HISTIOCYTOSIS

MATAIX


Lupus erythematosus is a chronic and inflammatory multiorgan disease with variable clinical appearance and variable course. Typically, it affects the skin, and in addition to a different extent also the joints, lungs, kidneys, nervous system, serous membranes and other organs. Most patients with systemic lupus erythematosus (SLE) show cutaneous manifestations. Conversely, all forms of cutaneous LE may change into a systemic involvement but to different extent. About 50% patients with subacute cutaneous lupus erythematosus fulfill four to five criteria defined by the American College of Rheumatology (ACR) for SLE but do not develop fully blown disease.

Lupus erythematosus is a chronic and inflammatory multiorgan disease with variable clinical appearance and variable course. Most patients with systemic lupus erythematosus show cutaneous manifested and conversely, all forms of cutaneous LE may change into a systemic involvement. Specific lesions of cutaneous LE are classified in different subtypes of acute cutaneous lupus erythematosus (ACLE), subacute cutaneous lupus erythematosus (SCLE), chronic cutaneous lupus erythematosus (CLE) and intermittent cutaneous lupus (ICLE) according to clinical, histological and immunoserological parameters. Regular laboratory tests are important to monitor the activity and course of the disease or side effects of the therapy. In case of clinical or laboratory dysfunctions of internal organs, additional technical investigations are necessary. Histology is needed to support clinical diagnosis. A large number of drugs are able to induce SCLE, e.g. hydrochlorothiazide, terbinafine, or angiotensin-converting enzyme inhibitors. Drug-induced SCLE can be differentiat-ed by possible complementary immunoserological parameters. Neonatal lupus can be induced by transplacental transmission of maternal anti-Ro(SS-A) and anti-La(SS-B)-antibodies. Children with neonatal lupus might suffer from congenital atrioventricular block. Their mothers may suffer from active LE, but can be clinically healthy as well. As a consequence, pregnancies at risk should be monitored in short intervals by serial echocardiographic interventions. Protection against UV light is recommended for all types of CLE. There are some topical and many systemic treatment options, e.g. topical and systemic glucocorticosteroids, antimalarial drugs, dapsone, azathioprine, or mycophenolat mofetil with different response to skin or organ involvement.

KEY WORDS: Lupus erythematosus, cutaneous, diagnosis - Lupus erythematosus, cutaneous, drug therapy - Autoimmunity.

Classification

Gilliam in 1977 defined specific and non-specific skin lesions. Non-specific lesions are more common in SLE, but can also occur in association with typical...
cutaneous lesions. Non-specific lesions are mostly vascular lesions like periungual teleangiectasias, thrombophlebitis, and the syndrome of Raynaud. In addition vasculitic lesions are typical for these non-specific features like livedo racemosa, leukocytoclastic vasculitis or hypocomplementemic urticarial vasculitis. Other non-specific manifestations are alopecia, erythema multiforme or calcinosis cutis. Alopecia with telogen effluvium is typically found during disease flares-ups.

Specific lesions of cutaneous LE (CLE) are classified in different subtypes (Figure 1). They are specified according to clinical, histological and immunoserological parameters into acute cutaneous lupus erythematosus (ACLE), subacute cutaneous lupus erythematosus (SCLE), chronic cutaneous lupus erythematosus (CCLE) and intermittent cutaneous lupus erythematosus (ICLE).

In 2005, a new disease index score called CLASI (cutaneous lupus erythematosus disease area and severity index) was described for the evaluation of cutaneous lesions. In this score, “activity” is separated from “damage”. Each skin lesion is assigned a point score between 0 and 3. Scarring and non-scarring forms of alopecia are scored separately. This CLASI score decreases in activity after successful therapy, whereas the damage score may increase in scarring forms of CLE. Therefore, this score correlates with the improvement of global skin health, pain, and itch and is a useful tool to measure clinical response to different treatments. Alternatively, activity scores like the SLAM (systemic lupus activity measure) or the SLEDAI (systemic lupus erythematosus disease activity index) are available; however, they evaluate laboratory parameters or organ involvements which are not that representative for CLE.

Acute cutaneous LE

Acute cutaneous LE (ACLE) may present in a localised and generalised form. It is mostly associated with active SLE. The acute form is characterized by the typical centrofacial erythema, well known as the butterfly blush with spared nasolabial folds. The generalised form shows as maculopapulous exanthema, particularly with erythema and periungual teleangiectasias. Both forms heal without scarring but sometimes with hyperpigmentation. Some patients also show oral ulcerous lesions.

Both forms are associated with the exposure to sun light. Often, the clinical activity of ACLE runs parallel to the activity of SLE. About 40-90% of the patients have autoantibodies to dsDNA.

Histological features are often not as significant as in other forms of CLE, but interface dermatitis might be seen as well as dermal edema and sparse dermal cellular infiltrate.

Subacute cutaneous LE

Subacute cutaneous LE (SCLE) presents with two clinical forms, the polycyclic annular and the erythematous-papulosquamous variant (Figure 2). Some patients only develop one single type of lesions, oth-
er may present both simultaneously. As SCLE is highly UV-sensitive, skin lesions are found predominantly in the UV-exposed areas like the upper body, neck and the dorsal sides of the upper extremities in a symmetrical distribution. Interestingly, the scalp and face are rarely involved. Lesions often resolve with hypopigmentation but without scarring.

SCLE is sometimes interpreted as a borderline form between CLE and SLE. Patients might fulfill up to 4 criteria defined by the ACR. Most of the patients do not suffer only from skin lesions but also from mild systemic disease like arthralgia or myalgia.

Normally, patients show characteristic immunological parameters like anti-Ro (SS-A) and anti-La (SS-B)-antibodies. SS-A-Ro-autoantibodies are tightly associated with UV-sensitivity in SCLE. In drug induced SCLE patients might develop additional anti-histone-antibodies. A large number of drugs are able to induce SCLE, e.g. diuretics like hydrochlorothiazide, terbinafine, calcium-channel blockers or angiotensin-converting enzyme inhibitors (Table I).

**TABLE I.—Associated drugs in drug-induced lupus erythematosus*.**

<table>
<thead>
<tr>
<th>Definitely</th>
<th>Likely</th>
<th>Possibly</th>
</tr>
</thead>
<tbody>
<tr>
<td>Procainamide</td>
<td>anti-convulsants (e.g. Phenytoin)</td>
<td>Lipid-lowering agents</td>
</tr>
<tr>
<td>Hydralazine</td>
<td>Quinidine</td>
<td>Penicillin</td>
</tr>
<tr>
<td>D-Penicillamine</td>
<td>Sulfonamide</td>
<td>Tetracycline</td>
</tr>
<tr>
<td>Diltiazem</td>
<td>Rifampicine</td>
<td>Reserpine</td>
</tr>
<tr>
<td>Minocycline</td>
<td>β-blockers</td>
<td>Griseofulvin</td>
</tr>
<tr>
<td>Isoniazide</td>
<td>Lithium</td>
<td>TNF-α-inhibitors</td>
</tr>
<tr>
<td>ACE-Inhibitors (Captopril)</td>
<td>Anti-RNP</td>
<td>Anti-Sm</td>
</tr>
<tr>
<td>Diuretics (e.g. HCT, Spironolactone)</td>
<td>Anti-dsDNA</td>
<td>Anti-Histone</td>
</tr>
<tr>
<td>Terbinafine</td>
<td>Complement</td>
<td></td>
</tr>
<tr>
<td>Amiodarone</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>Nonsteroidal anti-inflammatory agents</td>
<td>→</td>
<td></td>
</tr>
</tbody>
</table>


**TABLE II.—Differences in additional markers apart from the skin drug-induced in LE and idiopathic LE.**

<table>
<thead>
<tr>
<th></th>
<th>Idiopathic LE</th>
<th>Drug-induced LE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>20-40</td>
<td>~50</td>
</tr>
<tr>
<td>F:M</td>
<td>9:1</td>
<td>1:1</td>
</tr>
<tr>
<td>Occurrence of symptoms</td>
<td>Gradually, relapsing</td>
<td>Immediately, remits with drug cessation</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Skin lesions</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Renal involvement</td>
<td>++</td>
<td>(+)</td>
</tr>
<tr>
<td>CNS involvement</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>ANA</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Anti-RNP</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Anti-Sm</td>
<td>+</td>
<td>(+)</td>
</tr>
<tr>
<td>Anti-dsDNA</td>
<td>+++</td>
<td>(+)</td>
</tr>
<tr>
<td>Anti-Histone</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Complement</td>
<td>?</td>
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</table>
Drug-induced SCLE cannot be differentiated by histopathology alone but possible complementary signs may be blood or tissue eosinophilia (Table II).

Characteristic histopathological changes are hyperkeratosis, degeneration of the basal cell layer and a mononuclear infiltration of the dermoepidermal junction in addition to dermal edema, necrosis of epidermis or vesicular changes at the active border.11-13 The epidermis may be mildly atrophic.

Rowell’s syndrome is widely considered a clinical subtype of SCLE. Zeitouni et al.14 defined the major and minor diagnostic criteria with presence of LE, erythema multiforme-like lesions, speckled pattern of antinuclear antibody as major and chilblains, positive anti-La (SS-B) or anti-Ro (SS-A) antibody and reactive rheumatoid factor as minor criteria.

SCLE can be associated in special cases with malignancies.15 Until now, it is unclear whether SCLE is a paraneoplastic disease or just coincident as a consequence of chronic inflammation and/or of chronic immunosuppressive therapy.16 In patients who do not respond to therapy or have malignant diseases in their medical history a screening for malignancies should be initiated. Lymphomas and cancer of solid internal organs are most common.17

**Chronic cutaneous LE**

Chronic cutaneous LE can be classified as discoid LE (DLE), LE profundus (LEP) and chilblain LE (CHLE).

**Discoid LE (DLE)**

This is the most common form of chronic CLE and CLE as a whole group. DLE is characterized by erythematousquamous plaques with well-demarcated margins to the normal-appearing skin (Figure 3). DLE may occur in 20% of patients with SLE. It is therefore necessary to exclude organ involvement in any patients with DLE, particularly in those 5% of patients who show distinctly positive antinuclear autoantibodies (ANA).

Skin lesions present as discoid plaques with hyperpigmentation secondary to inflammation. These plaques mostly show scaling and are painful when being touched or when the hyperkeratoses are lifted. This is called the “carpet tack sign”. Most of theses plaques heal with atrophy and distinct scarring, as well as hypopigmentation in the centre of the lesion. As DLE is UV-sensitive, most of the lesions are located on the face and the scalp and limited to this area in 80% (localised form) but can also involve the lips or the oral mucosa. Chronic plaques of the oral mucosa are sharply demarcated with irregular borders and teleangiectasias and might imitate lichen planus lesions.

The disseminated form (20%) of DLE is spreading below the neck to the trunk and the upper extremities but can also occur anywhere on the body surface, including areas that are not regularly exposed to the sun.8

Hypertrophic or verrucous DLE is a variant with predominant hyperkeratotic lesions.

**Lupus erythematosus profundus (LEP)**

LEP is also referred to as LE panniculitis and is a very rare form of chronic CLE. It is reported in about 1-3% in all CLE patients.18, 19 LEP shows characteristic tender subcutaneous nodular infiltrations in the deep dermis and subcutaneous fat at the proximal extremities, the buttock, the head and trunk sometimes with overlying discoid lesions. LEP resolves with typical deep lipatrophia and scarring, and older lesions may calcificate (Figure 4). LEP can be induced by trauma or irritative stimuli as a Koebner phenomenon and may be associated with DLE as well as with SLE.10

Histopathological findings are sometimes different from other forms of CLE with lobular panniculitis, dense inflammatory infiltrations of lymphocytes and plasma cells as well as deposition of mucin between the adipocytes.

About 75% of the patients show elevated ANA titers but no specific autoantibodies. Though the clinical manifestation of additional SLE is rare, about 30-50% of the patients fulfill up to four ACR-criteria.8

**Chilblain lupus erythematosus (CHLE)**

Chilblain lupus erythematosus (CHLE) is normally located at the distal extremities like fingers, toes, as well as nose or ears. It presents periodically with livid red to purple nodules and plaques in response to cold temperatures and humidity which may occasionally ulcerate. Clinically and histologically, it is often difficult to differentiate CHLE from perniones.

Some of the patients show elevated ANA, anti-SS-Ro or positive rheumatoid factor but to a variable
Cutaneous lupus erythematosus

Specific autoantibodies are rarely found and SLE may occur in about 20%.

Recently, familiar chilblain lupus has been described in association to specific gene loci on chromosome 3. Accordingly, other variants of LE may be genetically determined as well.

Intermittent cutaneous LE

Lupus erythematosus tumidus

This is a very rare and more urticaria-like variant of LE without significant epidermal manifestations. Recent work related intermittent cutaneous LE (ICLE) to the group of cutaneous LE. Consequently in 2004 it was suggested as a separate identity in a modified classification. The skin lesions are erythematous papules and plaques in an annular or centrifugal presentation. As LET is highly photosensitive in 72% of cases lesions are typically localised in the UV-exposed areas like face, upper chest or trunk as well as the upper proximal extremities. Skin lesions heal without scarring or pigmentation abnormalities.

Histologically, skin lesions show an increased dermal deposition of mucin and inflammation of the superficial periadnexal and perivascular areas. Elevated ANA are detectable in about 10-30% of all patients, with anti-Ro or anti-La-autoantibodies in about 5%. Spontaneous healing is possible. The course and prognosis of ICLE is very good, especially an association with SLE is not common.

Special variants

Bullous LE

This form is a very rare and severe form of LE with subepidermal blistering, mostly associated with active variants of SLE. Blistering can occur with small vesicles or bullae on erythematous or on normal appearing skin.

Most patients show autoantibodies to collagen type VII. (Immuno)histologically, BLE shows neutrophilic infiltration and granular or linear deposits of IgG, IgM, IgA and complement at the basement membrane zone. The so called “acute syndrome of apoptotic pan-epidermolysis (ASAP)” which is clinically similar to forms of toxic epidermal necrolysis is discussed to be a maximal variant of this entity.

Neonatal LE

Infants with neonatal LE (NLE) show skin lesions which are similar to SCLE with erythematous macules, papules and annular plaques, mainly on the typically UV-exposed areas like face, trunk and extremities. Skin lesions may be present at birth or appear during the first weeks of life while the involvement of other organs with resulting hematopoetic, hepatic or renal abnormalities are less frequent. NLE is caused by transplacental transmission of maternal anti-Ro(SS-A) and anti-La(SS-B)-antibodies. About 2% of infants of mothers with anti-Ro(SS-A) and anti-La(SS-B)-antibodies develop NLE, but the risk is increased up to 25% for following pregnancies. The mothers may suffer from active LE, but can be clinically healthy as well. As a consequence, pregnancies at risk should be monitored in short intervals by serial echocardiographic interventions.

The skin lesions disappear in infants within 6 months parallel to the disappearance of the transmitted autoantibodies. Unfortunately, anti-Ro(SS-A)-antibodies have a distinct affinity to the conductive system of the fetal heart and may directly damage it, resulting in a congenital and persisting atrioventricular block. This complication can appear during pregnancy as well as within the first few weeks of life. The only effective treatment is a pace maker implantation but about 14% all affected children will die. Children who survive do not have other specific health related problems, but might in their later life develop autoimmune diseases and should therefore be clinically controlled in regular intervals. The development of CLE is rare with clinical variants similar to those of adults.

Pathogenesis

The most extensively studied pathogenic factor in CLE is UV-light. Clinically, CLE often deteriorates during spring and summer. Accordingly, photosensitivity is one of the ACR-criteria. Whereas in the epidermis of patients with CLE apoptotic cells accumulate after 72 h of UVB exposure, healthy persons normally show a decrease of these cells. Furthermore patients with CLE show increased expression levels of different cytokines (interferons, TNF-alpha etc.) in non-irradiated skin. This pro-inflammatory environment as well as the presence and persistence of apoptotic cells may result in the development of inflammatory...
lesions. This is further aggravated by the binding of autoantibodies to apoptotic cells or released RNA/DNA and the consecutive activation of phagocytes.

Additionally, CD4+CD25+ regulatory cells (T_{reg}) play a role in modulating the immune response in LE patients. These patients seem to suffer from a deficient control of this specific T_{reg} subpopulation. Patients with CLE do not show a general defect of the T_{reg}, but they are not able to suppress other T-cell-populations and their proliferation in cutaneous lesions.28

CHLE was found to be associated with different genetic mutations 20, 21 and may apply to other forms of CLE as well. However, an immunogenetic background as described for SLE with associations to different HLA factors as well as to polymorphisms of complement factors coded within the HLA region seems less likely.

Certain drugs can exacerbate an underlying SLE or induce lupus-like clinical manifestations.29 Most of these drug-induced forms of LE are SLE or SCLE,30-34 but in case reports other CLE lesions like LET, CHLE or CDLE have been described after exposure to squaric acid dibutylester for therapy of alopecia areata,35 and the TNF-alpha-inhibitor infliximab.36, 37

Other possible organ involvement besides skin involvement

Most patients with CLE suffer from additional clinical symptoms of rather nonspecific character. A systemic involvement is rare and may indicate incipient SLE. The most common features are musculoskeletal manifestations with arthralgia, arthritis or periarticular swelling in 95% of all these patients. Most commonly the proximal interphalangeal joints are symmetrically affected followed by the knees and wrists. Additionally, most patients with SLE suffer from fatigue and sometimes slightly elevated or subfebrile body temperatures.

Other organ involvements like cardiovascular manifestations, pulmonary or renal disease and neurological or psychological problems are less frequent.

Diagnostic approach in CLE

The case history and clinical features are essential for further diagnostic procedures as well as histopathologic and laboratory tests.

Laboratory tests are important to monitor the activity and course of the disease or side effects of the therapy. Basic screening should include a complete blood cell count, liver and renal function tests, acute phase proteins as well as urinalysis. The blood count can indicate SLE with anemia, leukopenia or thrombocytopenia. Leukopenia can be drug-induced, whereas qualitative and quantitative shifts among leukocyte subsets can indicate bacterial or viral infections which might complicate the therapy of LE. With the help of the erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), inflammatory and infectious processes can be differentiated as well as CLE from SLE. In CLE, both are only slightly elevated whereas in SLE, the ESR is highly elevated in contrast to an only slightly elevated CRP. Elevated CRP would on the other side would indicate complication acute infections. In addition serum electrophoresis can reflect both inflammatory activity and liver function.

Additional liver and renal function tests are necessary to detect possible organ involvement in LE or differentiate side effects of immunosuppressive or immunomodulatory drugs. For liver functional tests, transaminases (e.g. ASAT, GGT), and alkaline phosphatase are sufficient for routine screening. Elevated liver enzymes are tolerable to the two-fold of the normal limit. In case of distinctly elevated transaminases, infectious or autoimmune hepatitis should be excluded. If patients complain about weakness and pain of the muscles, elevated LDH and CK can indicate myositis.

Renal function parameters include serum creatinine, serum urea, uric acid, sediment of morning urine as well as 24-hour urine collection to determine creatinine clearance and quantitative proteinuria. Alternately, the creatinine clearance may be determined using the MDRD formula.38, 39

Specific laboratory tests include inflammatory, autoimmune and infectious disease parameters. Depending on the CLE variant, typical autoantibodies can be found (Table III) but the absence of autoantibodies might be diagnostically helpful. Antibodies against nuclear antigens (ANA) identify antibodies against nucleic acid and cytoplasmic or nuclear proteins within the nucleus. If ANA are present, a specification is mandatory by testing for autoantibodies against extractable nuclear antigens (ENA) and native double-stranded DNA (dsDNA). ANA should be monitored in yearly intervals or more closely if SLE is clin-
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ically highly suspicious. Anti-dsDNA-antibodies can be identified by ELISA with high sensitivity, but should be confirmed by indirect immunofluorescence using Crithidia lucilia. They indicate the presence or future the development of SLE and correlate with disease activity, especially with renal involvement.

Other specific autoantibodies are anti-histone-antibodies. They are indicative of drug-induced lupus (e.g. SLE or SCLE) when anti-dsDNA antibodies are absent, but can also occur in patients with idiopathic lupus (24-95%) in conjunction to anti-dsDNA antibodies.

Positive rheumatoid factors are of only limited diagnostic value because they can be detected to a variable extent in patients with SLE and CLE; particularly an association to SCLE is observed. For differentiation of arthralgias or arthritis in CLE / SLE from rheumatoid arthritis (RA), antibodies to cyclic citrullinated peptide are highly specific for RA and found in SLE in only <5%.

To evaluate the inflammatory activity of the disease, consumption of complement factors e.g. C2 and C4 is monitored. Their levels are usually normal in CLE in contrast to acute SLE. In association with hypocomplementemic urticarial vasculitis, C1q and anti-C1q antibodies should be determined.

If there is evidence of an abnormal coagulation in the medical history of the patients like thromboembolic events, miscarriages and/or Raynauds syndrome, it makes sense to screen for anti-phospholipid antibodies. Lupus anticoagulans should be tested in addition to cryoglobulines or cold agglutinines. Their presence is most likely associated with systemic manifestations of LE.

In case of clinical or laboratory dysfunctions of internal organs, additional technical investigations are necessary. Because of the rare organ involvement in CLE they should be performed based on individual parameters.

If lung involvement is supposed, high-resolution computer tomography in addition to tests of pulmonary function and diffusion capacity should be performed. Cardiac involvement with inflammation of the valves, endocardium or other parts of the heart as well as pul monary hypertension can be monitored by echocardiography, whereas ECG might not be sensitive enough.

A renal involvement can be estimated best by the above mentioned laboratory parameters like e.g. serum creatinine and proteinuria. Ultrasonography can only demonstrate severe renal changes.

Other technical investigations like neurological examination, cerebrospinal fluid examination, MRT/CT, ophthalmological examination or further gastrointestinal investigations can be necessary and should be adapted individually.

The direct immunofluorescence test (DIF) has been used for a long time to support the clinical diagnosis of LE due to the lack of alternative diagnostic methods. It may be performed on skin lesion which should preferably be older than 4-6 weeks, on non-involved UV-exposed and on non-involved non-UV exposed skin. Band-like deposits of immunoglobulins (IgG, IgM and IgA) as well as complement factors (e.g. C3) can be detected along the basement membrane zone. The positive “lupus band test” in a narrow sense is defined as linear deposits of IgG in non-involved UV-exposed skin and is indicative of SLE. In sun exposed skin, false positive results are possible. Whereas DIF on involved skin is still of clinical value, the diagnosis of SLE resides on the more sensitive anti-dsDNA tests available today.

UV-sensitivity and provocation tests following a standardized protocol should be carried out in addition to laboratory tests to verify the individual impact of photosensitivity and to try to provoke skin lesions for further diagnostic evaluations by histological and immunohistological examinations. Further it will increase the patient’s awareness of a correlation between UV exposure and skin lesion as well as to

TABLE III.—Different autoantibody profiles in different CLE variants.

<table>
<thead>
<tr>
<th>Table III.</th>
<th>Different autoantibody profiles in different CLE variants.</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>ACLE</td>
</tr>
<tr>
<td>ANA</td>
<td>+++</td>
</tr>
<tr>
<td>anti-dsDNA</td>
<td>+++</td>
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<tr>
<td>anti-Sm</td>
<td>++</td>
</tr>
<tr>
<td>anti-Ro</td>
<td>(+)</td>
</tr>
<tr>
<td>anti-La</td>
<td>(+)</td>
</tr>
<tr>
<td>anti-histone</td>
<td>++</td>
</tr>
</tbody>
</table>
comply with specific recommendations for adequate sun protection according to the causative UV spectrum.

Optional and mandatory investigations for the different subsets of CLE are listed in Table IV.

**Treatment**

**Topical treatment options**

Protection against UV light is recommended for all types of CLE. UV-protection can be achieved by textiles (sun hat, long-sleeved clothes) and additional physical and chemical sunscreens with high sun protection filters for UVA and UVB with factors well above 25. Patients with CLE should not work in professions related to high UV-exposure and should not travel to Mediterranean and tropical countries without ample protection.

Additional covering of skin lesions with make-up (camouflage) is psychologically helpful, e.g. in patients with scarring CLE or acute inflammatory lesions.42

All subsets of CLE can be improved by the topical application of glucocorticosteroids. Treatment should be initiated once daily with medium-strength topical glucocorticosteroids like prednicarbate (e.g. for the face), for body application more potent glucocorticosteroids like mometasonfuroat or betametasone 0.1% can be tried. An intralesional injection or occlusive application of potent glucocorticosteroids might be indicated to prevent disfigurating scarring or in distinctly hyperkeratotic lesions. In the latter cases the topical application of retinoids might be useful.

Topical immunomodulators like the calcineurin

<table>
<thead>
<tr>
<th>Table IV.—Different autoantibody profiles in different CLE variants.</th>
<th>Necessary investigations</th>
<th>Additional investigations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Routine</strong></td>
<td>• Complete blood count</td>
<td>• X-ray chest</td>
</tr>
<tr>
<td></td>
<td>• Serum creatinine</td>
<td>• Abdominal sonography</td>
</tr>
<tr>
<td></td>
<td>• Serum urea</td>
<td>• anti-ENA</td>
</tr>
<tr>
<td></td>
<td>• Liver enzymes (ASAT, ALAT, GGT, AP)</td>
<td>• Anti-nucleosome-antibodies</td>
</tr>
<tr>
<td></td>
<td>• ESR, CRP</td>
<td>• Anti-histon-antibodies</td>
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<td></td>
<td>• Serum electrophoresis</td>
<td>•</td>
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<tr>
<td></td>
<td>• Urinalysis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• C3, C4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• ANA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Anti-dsDNA</td>
<td></td>
</tr>
<tr>
<td><strong>Skin lesions</strong></td>
<td>• Skin biopsy incl. DIF of skin lesion</td>
<td>• Skin biopsy with DIF of UV-protected skin</td>
</tr>
<tr>
<td></td>
<td>• UV testing</td>
<td>•</td>
</tr>
<tr>
<td></td>
<td>• Microscopic evaluation of pertungual teleangiectasias</td>
<td>•</td>
</tr>
<tr>
<td><strong>Cardiac involvement</strong></td>
<td>• Echocardiography</td>
<td>•</td>
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<td></td>
<td>• ECG</td>
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<tr>
<td><strong>Lung involvement</strong></td>
<td>• HR-CT</td>
<td>•</td>
</tr>
<tr>
<td></td>
<td>• Pulmonary function tests</td>
<td>•</td>
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<tr>
<td></td>
<td>• Pulmonary diffusion capacity</td>
<td>•</td>
</tr>
<tr>
<td><strong>Renal involvement</strong></td>
<td>• Determination of glomerular filtration rate</td>
<td>•</td>
</tr>
<tr>
<td></td>
<td>• Creatinine-clearance</td>
<td>• Sm-Ag-antibodies</td>
</tr>
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<td></td>
<td>• Protein in 24-hour urine collection</td>
<td>• U1-RNP-antibodies</td>
</tr>
<tr>
<td></td>
<td>• Uric acid</td>
<td>• SS-A/SS-B-antibodies</td>
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<td></td>
<td>• Abdominal sonography</td>
<td>•</td>
</tr>
<tr>
<td><strong>Raynaud syndrome/thrombosis</strong></td>
<td>• Anti-phospholipid antibodies</td>
<td>• Thrombophilia-screening (e.g. protein C/S, AT-III)</td>
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<tr>
<td></td>
<td>• Cryoglobulines</td>
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<td>• Cold agglutinines</td>
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<tr>
<td><strong>Myositis/arthralgia</strong></td>
<td>• Rheumatoid factor</td>
<td>• LDH, CK, CK-MB</td>
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<td>• Anti-CCP-antibodies</td>
<td>• X-ray of the bones / joints</td>
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<tr>
<td><strong>Central nervous system</strong></td>
<td>• Neurological examination</td>
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<tr>
<td></td>
<td>• Cranial MR/CT</td>
<td>• Anti-rRNP-antibodies</td>
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inhibitors tacrolimus or pimecrolimus can improve skin lesions but with divergent response. The primary target seems to be the decrease or blocking of cytokine production by activated T lymphocytes.43 Calcineurin inhibitors may to be effective in acute and subacute cutaneous LE manifestations in addition to systemic treatments and cause only minor local side-effects. Chronic discoid LE and hypertrophic plaques do not well respond because of limited penetration.43

All surgical or invasive procedures like tunable dye laser, dermabrasio or excision should only be performed in individual cases of stable or preferably extinct disease because of the possible Koeber phenomenon.44

Systemic treatment options

Systemic therapy is indicated in patients non-responding to topical therapeutic options and presenting with scarring lesions, widespread skin manifestations or internal organ involvement. However, CLE often responds only moderately well to systemic immunosuppressive drugs even at dosages that can control systemic involvement in SLE. This is especially true for glucocorticoids which have to be administered in high dosages to improve inflammatory skin lesions. Therefore in most cases, a combination of topical and systemic therapy is necessary.

Antimalarial drugs are the treatment of choice for all forms of CLE. Their mechanism of action is still unknown. Recent date suggest that the acidification of lysosomal compartments may be modulated which results in an inhibition of signalling through Toll-like receptor 7 and Toll-like receptor 9.45 Activation of Toll-like receptors 7/8 and 9 was shown to increase the IFN-alpha secretion and subsequently the autoantibody production. Hydroxychloroquine is usually better tolerated than chloroquine. The dosage should be adapted to the optimal body weight (max <6.5 mg/kg of hydroxychloroquine, <4 mg/kg of chloroquine), but most patients are sufficiently treated with e.g. hydroxychloroquine 200 mg/d. The most important rare, but typical side effect is a retinopathy which is rather related to the maximal daily dose, than the maximal cumulative dosage. An ophthalmologic control should be performed at the start of the treatment and every 6-12 months thereafter according to guidelines.46 In addition to an increase of liver enzymes, alopecia and whitening of the hair 47 antimalarial myopathy may rarely occur.48 Combination of both drugs should be avoided due to the risk of renal toxicity.

Mepacrine (quinacrine) at a dose of 100 mg/d (max 200 mg/d) can be combined with antimalarials.49 In addition, other immunosuppressive drugs like mycophenolate mofetil can be combined to improve therapeutic response.50 Smoking should be abandoned as it will reduce the efficacy of the antimalarial drugs, possibly through the inhibition of the P450 enzyme system.51, 52

Dapsone (diaminodiphenylsulfone: DADPS) has an anti-inflammatory effect via inhibition of lysosomes and inhibition of neutrophile chemotaxis.53 It is normally administered in a dosage between 50-150 mg/d and may result in mild hemolysis and met-hemoglobinemia. To avoid severe side effects, the activity of the enzyme glucose-6-phosphate-dehydrogenase should be monitored before therapy. At a critical value of >5% met-hemoglobin, clinically relevant cyanosis of the lips or the tongue, shortness of breath, dizziness and/or significant hemolysis, dapsone should be decreased or stopped. Vitamin C or vitamin E can mitigate these side effects; cimetidine (1.6 g/dL) can be used as an alternative but is more expensive and may result in additional side effects.

Sometimes induction of a hypersensitivity syndrome/DRESS is possible. It presents with fever, generalised exanthema, lymphadenopathy and elevated liver enzymes.45 In such cases, dapsone therapy should be stopped immediately. Dapsone works best on inflammatory lesions of CLE including lupus tumidus, urticarial vasculitis, oral ulcerations and SCLE, not however on hyperkeratotic forms of CLE variants.

Systemic glucocorticosteroids are differentially effective in the treatment in CLE. For monotherapy two dosage regimes are established: First an oral dosage regime with 1 mg/kg BW prednisolone equivalent for 6-8 weeks with dosage reduction by 10% per week to the minimal dosage needed or secondly a pulse therapy with methylprednisolone 1 g/day intravenously on three consecutive days in monthly intervals.55 They are indicated in acute inflammatory skin rashes or in very severe forms of LE in combination with other drugs. If glucocorticosteroids are necessary for a longer period of time, the daily dosage should preferably be below the Cushing dose of 7.5 mg prednisolone equivalent. As the risk of osteoporosis is increased initial and regular annular osteodensitometry should be performed and vitamin D (400-800 IE/d) and calcium (1-1.5 g/dL)
substituted. In addition, gastric protection might be necessary as well as prophylaxis of oral and intestinal fungal infection.

Azathioprine is an antimetabolite in the purine metabolism resulting in an inhibition of lymphocyte proliferation. Normally, azathioprine is well tolerated but can result in severe side effects like bone marrow depression or severe infections. During therapy, amply contraception must be carried out in women as well as in men during therapy and for at least 6 months after stopping. Normally, 1-2 mg/kg BW have to be administered with an initial dose of 50-100 mg/d. Azathioprine should not be combined with allopurinol or furosemide because of their interactions with the enzyme thiomyethyltransferase and an increased bone marrow toxicity. If a combination is necessary, the azathioprine dosage should be reduced pre-therapeutically to 75% of the regularly administered dosage.

Mycophenolate mofetil (MMF) is an inhibitor of the inosin-monophosphate-dehydrogenase which is a pivotal enzyme in the synthesis of purines. Hence it inhibits T- and B-lymphocytes as well as their migration into inflammatory tissues. The clinical effects on various autoimmune diseases seem similar to azathioprine, but side effects are less frequent. The dosage is administered between 1-3 g/dL. MMF should be combined with e.g. glucocorticosteroids because its effects on cutaneous lesions as monotherapy are limited and delayed. However, in single cases the successful therapy of SCLE, DLE or chilblain lupus has been reported.60, 61

Retinoids are second-line choice for CLE and are favoured in hyperkeratotic-verrucous forms. Acitretin or isotretinoine are administered at a dosage between 0.2 mg and 1 mg/kg BW. Contraception is necessary.

**TABLE V.** — Possible systemic therapeutic options and dosages for treatment of CLE.

| Antimalarials | Hydroxychloroquine 2000/d (max. <6.5 mg/kg optimal BW) | Retinopathy | 2-4 months | Smoking |
| Dapsone 50-150 mg /d (max. 2 mg/kg BW) | Hemolytic anemia | 3 months | Glucose-6-phosphate-dehydro-genase |
| Azathioprine 50-100 mg/d (max. 1-2 mg/kg BW) | Gastrointestinal side effects | 6-12 weeks | Thiomethyltransferase |
| Mycophenolate mofetil 1-3 g/d | Blood cell count | 4-8 weeks | Expensive |
| Acitretin 0.2 mg/kg BW | Dryness of skin | 3-8 weeks | Contraception |
| Isotretinoine 1 mg/kg BW | Lipid metabolism | | Hyperkeratotic forms of CLE |
| MTX 5-25 mg/week | Gastrointestinal side effects | Interindividual variability in oral application |

Notes: Dapsone: 1 mg/kg BW in decreasing dosage or pulses 1 g/d for 3 days. Corticosteroids: 50-150 mg/d (max. 1-2 mg/kg BW). Azathioprine: 1 mg/kg BW in decreasing dosage or pulses 1 g/d for 3 days. Mycophenolate mofetil: 1-3 g/d. Acitretin: 0.2 mg/kg BW. Isotretinoine: 1 mg/kg BW. MTX: 5-25 mg/week.
mandatory during therapy and at least for 1 month after stopping isotretinoin and 2 years after therapy with acitretin. Typical side effects are dryness of the lips and mucous membranes in addition to an elevation of liver enzymes and serum lipids, temporary effluvium as well as muscle and bone pain.

Methotrexate is a folic acid antagonist and inhibitor of purine synthesis. The clinical effects seem related to an inhibition of the migration of neutrophils into the tissue and a suppression of cellular immunity. Methotrexate is administered once weekly between 5-25 mg. The oral administration is most widely used but subcutaneous or intravenous application is better tolerated and improves resorption. Side effects are gastrointestinal pain, vomiting, diarrhea or stomatitis. Methotrexate is preferably metabolised by the liver and eliminated by the kidney. As a consequence elevation of liver and renal function parameters can occur. Dosage should be reduced if transaminase levels are elevated >3 times the normal level. A rare side effect is a hypersensitive pneumonitis with cough and dyspnea. In this case therapy should be stopped immediately. Strict contraception should be followed during therapy. Therapy is preferred in patients with recalcitrant SCLE and DLE.

Leflunomide is a novel anti-inflammatory and immunomodulatory agent which through inhibition of the pyrimidine synthesis is able to mainly affect the proliferation of B and T lymphocytes as well as to block the CD43-mediated T-lymphocyte aggregation and the proliferation of B and T lymphocytes as well as to block the CD43-mediated T-lymphocyte aggregation. There is evidence that leflunomide is effective and safe in the treatment of patients with SLE especially if standard therapies are contraindicated or of limited clinical effect. It should be kept in mind, however, that the induction of SCLE has been published in single cases. Methotrexate is preferably metabolised by the liver and eliminated by the kidney. As a consequence elevation of liver and renal function parameters can occur. Dosage should be reduced if transaminase levels are elevated >3 times the normal level. A rare side effect is a hypersensitive pneumonitis with cough and dyspnea. In this case therapy should be stopped immediately. Strict contraception should be followed during therapy. Therapy is preferred in patients with recalcitrant SCLE and DLE.

A novel anti-inflammatory and immunomodulatory agent which through inhibition of the pyrimidine synthesis is able to mainly affect the proliferation of B and T lymphocytes as well as to block the CD43-mediated T-lymphocyte aggregation.

Novel therapeutics like TNF-alpha-inhibitors, have not been evaluated in CLE yet. On the contrary the induction of CLE has been published in single cases.

An overview of therapeutic options is provided in Table V.

Riassunto

I differenti aspetti del lupus eritematoso cutaneo

Il lupus eritematoso (LE) è una patologia cronica infiammatoria ch'è coinvolta numerosi organi con variabili manifestazioni e decorso clinico. La maggior parte dei pazienti affetti da LE sistemico presenta manifestazioni cutanee, viceversa tutte le forme di LE cutaneo possono modificarsi determinando un coinvolgimento sistemico. Le lesioni specifiche del LE cutaneo sono classificate nei differenti sottotipi di lupus eritematoso cutaneo acuto (ACLE), lupus eritematoso cutaneo subacuto (SCLE), lupus eritematoso cutaneo cronico (CDLE), e lupus eritematoso cutaneo intermitente (ICLE), in accordo a parametri clinici, istologici e immunoserologici. Test di laboratorio seriati sono fondamentali per monitorizzare l'attività e il decorso della patologia o gli effetti collaterali della terapia. In caso di disfunzioni di organi interni rilevate clinicamente o con gli esami di labaratorio, si rendono necessarie ulteriori indagini strumentali. L'esame istologico è necessario per supportare la diagnosi clinica. Un vasto numero di farmaci è in grado di indurre il SCLE, per esempio l'idroclorotiazide, la terbinafina o gli inhibitione dell’enzima di conversione dell’angiotensina. Il SCLE indotto da farmaci può essere differenziato tramite parametri immunoserologici complementari. Il lupus neonatale può essere indotto dalla trasmissione trans-placentare di anticorpi materni anti-Ro (SS-A) e anti-La (SSB). I bambini affetti da lupus neonatale spesso soffrono di blocco atrio-ventricolare congenito. Le loro madri possono essere affette da LE in forma attiva, anche se possono essere clinicamente sane. Di conseguenza, le gravidanze a rischio devono essere monitorizzate a breve termine tramite controlli ecocardiografici seriati. La protezione contro i raggi UV è raccomandata per tutti i tipi di CLE. Vi sono alcune opzioni terapeutiche topiche e molte sistemiche, per esempio i glucocorticosteroidi sistemic, i farmaci antimalarici, il dapsonico, l’azatioprina o il micofenolato mofetile con differenti risposte a livello cutaneo e degli organi coinvolti.


References


Lentigo maligna: current concepts in diagnosis and management

H. W. WALLING

Lentigo maligna (LM) is a cutaneous malignancy with increasing incidence and significant risk of morbidity. Early diagnosis of LM requires a high index of suspicion for the often subtle atypical signs of pigmented lesions on sun-damaged skin. A variety of treatment options are available to the physician. Surgical options include simple excision and margin-control techniques such as staged excision and Mohs micrographic surgery. Tissue may be embedded vertically or en face, and processed as frozen or paraffin-embedded sections. Destructive options include radiotherapy, cryotherapy, curettage, and electrodestruction. Topical therapy may be appropriate in selected cases; immunotherapy with topical imiquimod may have advantages over other topical agents. The opportunity for curative therapy is maximized by surgical treatment. Margin-control surgery offers the highest cure rate while minimizing loss of normal tissue.

KEY WORDS: Lentigo - Skin, neoplasms, diagnosis - Skin, neoplasms, therapy.

Lentigo maligna (LM) is an in situ melanocytic neoplasm typically arising on the head and neck of older persons with actinically-damaged fair skin. A significant number of LM cases will progress to invasive LM melanoma (LMM). LM represents up to 15% of all cases of malignant melanoma and up to 26% of head and neck melanomas. The risk of developing LM/LMM increases with age and with increasing sun-exposure. The prognosis for LMM correlates with depth of invasion and is similar to that of other types of melanoma. Some authors have preferred the term “lentiginous melanoma” as the appropriate designation for this disease. This term encompasses LM and LMM, as well as melanomas on sun-damaged skin with other histologic features. Recent data suggests that lentiginous melanoma is now the most common form of melanoma.

This review will discuss current concepts in the diagnosis and treatment of LM.

Clinical presentation

LM typically develops on chronically sun-exposed skin of the head and neck. In a recent series of 59 cases of LM/LMM, 43 (73%) occurred on the head and neck, with a predilection for the cheek (21; 36%). The mean age at diagnosis in this study was 69 years, which is similar to other published studies. The clinical appearance is an ill-defined tan to brown patch with irregular pigment and border which gradually increases in size. Early cases may be clinically subtle and resemble background photodamage (Figure 1). The clinical differential diagnosis includes solar lenti-
go, pigmented actinic keratosis, actinic damage, and seborrheic keratosis.8

**Diagnosis**

Diagnosis is accomplished with skin biopsy. While an excisional biopsy is preferred to allow analysis of the entire lesion,8 this may not be possible for large, ill-defined lesions on cosmetically sensitive skin. As such, incisional or punch biopsies may be performed for initial diagnosis; shave biopsies may be adequate for diagnosis of LM but risk transecting unsuspected invasive LMM.

**Histopathology**

There are currently no uniform histologic criteria for diagnosing lentigious melanoma.9 Features associated with LM include confluent atypical melanocytes, nesting of atypical melanocytes, and presence of melanocytes above the dermal-epidermal junction (pagetoid spread). LM is commonly defined as melanoma in situ arising in actinically damaged skin, with continuous proliferation of atypical melanocytes, as single confluent cells or in nests, along the basal layer of the epidermis and adnexae, without the requirement for pagetoid spread.7 Single scattered atypical melanocytes is generally not considered sufficient for diagnosis of LM.9 Increased melanocyte density, confluence of up to six melanocytes, and follicular penetration are noted in “normal” sun-damaged skin.10

**Therapeutic approaches**

As randomized controlled trials are lacking with regard to treatment of LM, little consensus exists regarding the most appropriate treatment of this disease.11 While close clinical monitoring has been proposed as an option for LM in light of its slow growth pattern,12 treatment of LM is advisable for several reasons. First, what appears to represent in situ melanoma upon biopsy is not infrequently (10-32% in most studies) found to be invasive melanoma when the entire lesion is excised.1, 13, 14 Second, an estimated 5% of melanomas-in situ will progress to invasive disease,1, 2 which may have life-threatening implications.15, 16 It is currently not possible to predict which cases will progress to invasive disease. It is similarly not possible to ascertain with certainty whether invasive disease is concealed within a given tumor without histologic examination.11

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**TABLE I.—Treatment options for lentigo maligna.**

<table>
<thead>
<tr>
<th>Surgical, histologic margin control</th>
<th>Surgical, destructive without histologic analysis or margin control</th>
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<tbody>
<tr>
<td>Staged-excision</td>
<td>Electrosurgery (electrodesiccation or electrotulgaration)</td>
</tr>
<tr>
<td>En face or vertically-oriented, paraffin-embedded</td>
<td>Electro surgery with curettage</td>
</tr>
<tr>
<td>Mohs micrographic surgery</td>
<td>Curettage</td>
</tr>
<tr>
<td>En face oriented, frozen sections</td>
<td>Cryosurgery</td>
</tr>
<tr>
<td>“Slow Mohs”</td>
<td>Laser destruction</td>
</tr>
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</table>

**Non surgical**

Radiotherapy
Topical immunotherapy
Imiquimod
Other topical agents
Azelaic acid
5-flurouracil
Retinoids
Cidofovir

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Figure 1.—Clinical presentation.
Treatment for LM can be classified into surgical and nonsurgical treatment (Table I). Surgical treatments include excision with margin analysis and destructive treatments without margin analysis. Non-surgical treatments include topical therapies, radiotherapy, and cryotherapy. Surgical treatments with margin analysis generally have a lower rate of recurrence compared to non-surgical or destructive treatment. In a large retrospective study, the recurrence rate of melanoma in situ managed with destructive therapies was 31%, compared with a recurrence rate of 7% for surgical excision. However, due to tumor and patient characteristics, not every case can be managed surgically.

Destructive therapies

Superficial destructive techniques include cryosurgery, curettage and electrodesication, radiotherapy, and laser therapy. In a study of 101 patients with LM/LMM treated with Grenz rays or soft X-rays with at least 2 years of follow-up, 7% developed recurrence after a mean interval of 45 months. In a study of 64 patients with LM/LMM followed a mean 23 months after treatment with fractionated radiotherapy, none of 42 patients with LM developed clinical recurrence, but 2 of 22 patients (9%) with LMM developed local recurrence and one (4.5%) developed metastasis. In a study of 18 patients with LM treated with cryosurgery (two freeze-thaw cycles), no patient developed clinical recurrence after a mean follow-up interval of 75 months. In a study of 30 patients with LM treated with cryosurgery, 2 (6.6%) developed recurrence after a mean follow-up of 3 years. Disadvantages of destructive approaches include uncertainty of margin control, the possibility of not diagnosing unsuspected foci of invasive LMM, risk of progression to invasive LMM in incompletely treated LM, and risk of metastasis of LMM.

Topical therapies

Several options for topical therapy of LM have been reported. Azelaic acid and imiquimod are the best studied. Based on a study of 50 patients with LM followed 5-10 years, azelaic acid (applied twice daily for duration according to clinical response) may clinically control LM in up to half of cases. However, in another case series, 2 of 9 patients so treated developed invasive LMM. Immunotherapy with imiquimod has recently generated significant interest. Six of 7 patients (86%) with LM treated with imiquimod showed histological and clinical resolution after a mean 19 months of follow-up. In a study of 34 cases of facial LM, treatment with imiquimod (applied once to twice daily for 2-20 weeks until the endpoint of weeping erosion) was associated clinical clearance in 33 cases (6 confirmed histologically), with one recurrence (3%) after a mean follow-up interval of 17 months. In a recent review of 4 clinical trials and 11 case reports of patients treated with topical imiquimod for LM, 59/67 (88%) responded at least partially to this immunotherapy over short follow-up intervals. However, the development of invasive LMM in two patients is concerning. Comparison between these trials is difficult, and the medication usage is non standardized, with the application instructions, dosing schedules, treatment periods, and tumor characteristics variable or not mentioned. The concept of treatment with imiquimod prior to staged excision may prove to be a valuable approach to managing anatomically challenging cases. In a study of 40 patients with LM treated with imiquimod 5 times per week for 3 months, 30/40 (75%) had a complete clinical and histologic response in 33 cases (6 confirmed histologically), with one recurrence (3%) after a mean follow-up interval of 17 months. In a recent review of 4 clinical trials and 11 case reports of patients treated with topical imiquimod for LM, 59/67 (88%) responded at least partially to this immunotherapy over short follow-up intervals.

Surgical therapy

The ideal surgical treatment is the one which most reliably removes the entire tumor, minimizes the chance of recurrence, and minimizes compromise of normal tissue. To date, wider margins have not shown a significant survival benefit compared to narrow margins for invasive melanoma. Thus, many experts have argued that the appropriate margin is that which simply removes the entire tumor. However, melanoma is likely to display discontinuous spread with skip areas of tumor advancement. There is little data to support the necessary depth of excision for in situ melanoma. About two-thirds of dermatologists in a recent survey report carrying the excision to within subcutaneous fat without extending to fascia. Planning surgical excision requires accurate assessment of the clinical margins of the lesion. As the clinically visible lesion of LM may often underestimate the extent of the tumor, use of a dermatoscope or Woods
A recent review of MMS for 116 cases of LM found that frozen sections deemed clear which were subsequently thawed and paraffin-embedded were verified as clear in 95.7% of cases. This may represent the upper-limit of accuracy achievable with frozen sections when performed by an experienced Mohs surgeon. Practitioners will need to take this into consideration in deciding whether the advantages of MMS (primarily same-day completion of the procedure and repair) outweigh the chance that perhaps 1 in 25 cases will be falsely clear on frozen sections.

### Orientation of excised tissue

Excisions processed by conventional serial cross-section (“bread-loaf”) techniques are embedded vertically, with the section containing superficial and deep tissue in the same cut. In conventional MMS, the excised tissue is embedded horizontally, or en-face, such that the entire peripheral margin is analyzed in the same cut. A beveled peripheral margin allows the peripheral and deep margin to be visualized in the same histologic plane. En face embedding allows examination of 100% of the margin. Vertical embedding allows only sampling of the margin.

### Frozen vs paraffin sections

Frozen sections (as per standard MMS) allow rapid determination of margin status, but the technical quality of the slides is inferior. Paraffin sections are the gold standard for histologically diagnosing cutaneous melanoma. Accurate visualization of atypical melanocytes on frozen sections is difficult, particularly on sun-damaged skin where prominent single melanocytes may reside in the absence of disease. Frozen processing artifically alters the appearance of melanocytes and also obscures the nuclear detail required for diagnosis.

### Table II. Recommended margins for wide local excision of primary cutaneous melanoma.

<table>
<thead>
<tr>
<th>Maximal tumor depth</th>
<th>Clinical excision margin</th>
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<tr>
<td>In situ</td>
<td>0.5 cm</td>
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<tr>
<td>≤ 1 mm</td>
<td>1 cm</td>
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<tr>
<td>1.01-2 mm</td>
<td>1-2 cm</td>
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<tr>
<td>&gt; 2 mm</td>
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</table>

to judge atypia in single cells. Frozen section interpretation of LM and LMM has been associated with a sensitivity of 59-73% and a specificity of 68-81%. This reflects interobserver variability when interpreting sections even when identical histologic criteria are recognized. In a study in which 15 dermatopathologists independently assessed en face frozen and standard paraffin-embedded sections, diagnostic discrepancies occurred in over a third of cases. Discrepancies most often involved a false-negative margin on frozen sections. This may contribute to increased recurrence of LM in cases involving frozen section histology.

Immunohistochemical stains

A number of melanocyte-highlighting immunohistochemical (IHC) are available, including S-100, MART-1/MelanA, HMB-45, and Mel-5. These stains have been used in both paraffin and frozen sections to allow better differentiation between melanocytes and other cells. In a study of 56 tissue redundancies excised from sun-damaged skin, Davis et al preferred anti-MART-1/MelanA to anti-HMB-45 or anti-S-100, and found the azure B counterstain to allow differentiation between melanocytes and melanophages.

However, these stains may not allow accurate differentiation between normal and abnormal melanocytes. Any of the above stains may highlight melanocyte hyperplasia in the absence of melanoma. In a study of frozen sections obtained from chronically sun-exposed normal skin, MART-1 staining identified moderate-to-severe melanocyte confluence in 35% of cases, with nonspecifically-stained dermal cells in half of cases. MART-1/Melan-A has been found also to stain keratinocytes in pigmented actinic keratoses.

Another drawback to IHC is the increased time required to process the tissue. While some protocols may add an hour to processing time, a recent study estimated only an additional twenty minutes required for Mel-5 staining with an automated immunostainer. The additional laboratory complexity may also increase the potential for technical errors. Moreover, few Mohs labs (<15% according to a recent survey) currently utilize immunostains.

Patient follow-up

In light of the propensity for recurrence of LM, ongoing clinical follow-up is essential. In a single-institution review of 1996 cases of LM/LMM, 2.2% of cases developed recurrence, and nearly 20% of recurrences occurred over 5 years after initial treatment. This concurs with two studies reporting a mean time to recurrence of 45–46 months. As such, therapy-specific recurrence rates in studies with short follow-up intervals must be interpreted cautiously. In addition to the risk of recurrent disease, patients with prior LM/LMM are at increased risk of a subsequent primary melanoma. In an Australian study of 52,997 patients with prior melanoma followed over a 20 year period, the rate of development of a second melanoma was 6 per 1 000 person-years. This highlights the importance of continued dermatologic examination.

Conclusions

LM is a cutaneous malignancy with increasing incidence and significant risk of morbidity. Early diagnosis of LM requires a high index of suspicion for the often subtle atypical signs of pigmented lesions on sun-damaged skin. A variety of treatment options are available to the physician. The opportunity for curative therapy is maximized by surgical treatment. Margin-control surgery offers the highest cure rate while minimizing loss of normal tissue.

Riassunto

Lentigo maligna: concetti attuali nella diagnosi e nella terapia

La lentigo maligna (LM) è un tumore cutaneo che presenta un'incidenza in aumento e che ha un rischio significativo di morbidità. La diagnosi precoce di LM richiede un alto indice di sospetto a causa dei segni spesso sottilmente atipici delle lesioni pigmentate che compaiono su una cute danneggiata dal sole. Il medico ha a disposizione una varieta di trattamenti. Le opzioni chirurgiche comprendono l'asportazione semplice e le tecniche con margini controllati, la chirurgia micrografica di Mohs e la chirurgia tastata. Il tessuto asportato può essere asportato verticalmente o orizzontalmente e le sezioni processate dopo congelamento e immersione in paraffina. Le opzioni distruttive comprendono la radiotherapia, la crioterapia, la curatage e la distruzione. La terapia topica può essere appropriata in casi selezionati; l'imminoterapia con imiquimod topico può essere vantaggiosa rispetto ad altri agenti topici. La possibilità di una terapia risolutiva è massimizzata dal trattamento chirurgico. La chirurgia del margine controllato offre il mag-
gior taso di guarigione, minimizzando la perdita di tessuto normale. Parole chiave: Lentigo maligna - Cute, neoplasie, diagnosi - Cute, neoplasie, terapia.

References


The expanding spectrum of cutaneous borreliosis

K. EISENDEL, B. ZELGER

The known spectrum of skin manifestations in cutaneous Lyme disease is continuously expanding and can not be regarded as completed. Besides the classical manifestations of cutaneous borreliosis like erythema (chronicum) migrans, borrelial lymphocytoma and acrodermatitis chronica atrophicans evidence is growing that at least in part also other skin manifestations, especially morphea, lichen sclerosus and cases of cutaneous B-cell lymphoma are causally related to infections with Borrelia. Also granuloma annulare and interstitial granulomatous dermatitis might be partly caused by Borrelia burgdorferi or similar strains. There are also single reports of other skin manifestations to be associated with borreliac infections like cutaneous sarcoidosis, necrobiosis lipoidica and necrobiotic xanthogranuloma. In addition, as the modern chameleon of dermatology, cutaneous borreliosis, especially borrelial lymphocytoma, mimics other skin conditions, as has been shown for erythema anulare centrifugum or lymphocytic infiltration (Jessner Kanof) of the skin.

KEY WORDS: Borrelia infections - Lymphoma, B-cell - Granuloma annulare - Immunohistochemistry - Lyme disease - Necrobiosis lipoidica.

The germ Borrelia (B.) burgdorferi is a slowly growing microaerophil gram negative spirochete. The generation time is about 7-12 hours. At the time 13 different species are included in the B. burgdorferi sensu latu (s.l.) complex. They show different geographic distributions and are associated with different vectors and hosts. For example B. garinii and B. afzelii are frequently found in Europe but not in the United States. The different species of Borrelia also show different patterns of pathogenicity. Only B. burgdorferi sensu strictu, B. garinii, B. afzelii and B. spielmanii are clearly known to cause disease in the human host. Different Borrelia species also show a different tissue preference in humans (tissue tropism).

Overview about the diseases caused by B. burgdorferi

Lyme disease is the most frequent tick born disease in the northern hemisphere. Borrelia are mainly transmitted in Europe by Ixodes (I.) ricinus, in Asia by I. persulcatus and in the United States by I. scapularis,

Abbreviation list.—ACA: acrodermatitis chronica atrophicans; BL: borrelial lymphocytoma; EM: erythema migrans; FFM: focus floating microscopy; LS: lichen sclerosus

Fundings.—None.

Conflict of interests.—None.

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I. pacificus or Amblyomma americanum, also named “lone star tick” for the prominent white dot on the back of the adult female.6, 8 Borrelia cause characteristic diseases and, similar to syphilis, borreliosis has been separated into three stages. Stage I (stage of first manifestation) comprises the erythema migrans (EM, Figure 1A) and the early borrelial lymphocytoma (B, Figure 2A), which develop weeks to months after the tick bite and are accompanied by mild influenza like symptoms. The second stage (stage of dissemination) includes the involvement of the musculoskeletal system (acute Lyme arthritis with additional painful muscles, tendons, bursae and bones), the nervous system (meningitis, lymphocytic meningoradiculoneuritis, also called Bannwarth syndrome, mild encephalitis and myelitis), the heart (atrioventricular block, myopericarditis, pancarditis), the skin with late borrelial lymphocytoma, acute inflammatory acrodermatitis chronica atrophicans (ACA Figure 3A) and multiple erythema migrans, as well as the involvement of all other organs (lymphadenopathy, splenomegaly, hepatitis, mild haematuria, conjunctivitis, iritis and ophthalmmitis). The third stage (“stage of chronicity”) is limited to organ diseases with irreversible organic or functional damage in joints, nervous system or in the skin. This are in the case of skin diseases chronic-atrophic stages of ACA and in part morphea (Figure 4A) and lichen sclerosus. These chronic changes develop after months to years.1, 9 Single stages might overlap or be completely missing.10, 11

Figure 1.—A) Characteristic clinical manifestation of erythema migrans; B) histological examination (H&E, x20) reveals superficial and middermal dense perivascular infiltrate of (C) lymphocytes, some plasma cells, and an increase of fibroblasts and mucin between collagen bundles (H&E, x200).

Figure 2.—A) Borrelial lymphocytoma with concomitating erythema migrans with characteristic histological features (B) of dense infiltrates of lymphocytes (H&E, x10) with (C) follicle formation (H&E, x200).

Figure 3.—A) Acrodermatitis chronica atrophicans of the left leg characterized by ill-defined, hyperpigmented, and atrophic patch (note prominent veins!); B) histology (H&E) reveals a dense lichenoid and middermal perivascular infiltrate with (C) hints of follicle formation composed of (D) lymphocytes, some plasma cells, and an increase of fibroblasts between fibrosclerotic collagen bundles (H&E, x200).
History of cutaneous borreliosis

In the newer literature infections with *B. burgdorferi sensu lato* are named after the city Lyme in Connecticut although first descriptions have occurred long before in Europe. Already Afzelius wrote in 1909 in the *Archiv für Dermatologie und Syphilis* about an EM caused by the tick *Ixodes reduvius* (*Ixodes scapularis* in the new taxonomy) and recognized already the tick as vector for this disease. The first description and nomination of ACA occurred even earlier in the year 1902 by Herxheimer and Hartmann. The BL was first described as *lymphadenosis cutis benigna* by Brävferstedt in 1943, Brävferstedt also suspected insect bites, mainly tick bites, as triggers of the disease. Finally, Willy Burdorfer discovered the spirochetal etiology of borreliosis (Lyme disease) in 1982 and the pathogenic agent was named *B. burgdorferi* in his honor.

Histopathologic patterns in classical cutaneous borreliosis

The dermatohistopathological changes in cutaneous borreliosis are a consequence of the continuous antigenic stimulus by persisting *Borrelia* in the tissue. The consequence are histopathologic similarities in between the different cutaneous manifestations, like structural changes in the collagen texture and the frequent presence of B lymphocytes, especially plasma cells. B lymphocytes can be easily shown by staining with anti-CD20 antibodies. The different clinical and histological manifestations can be explained by the location and duration of the infection and by the different known borrelial strains, on the other hand also by the dominating immune response involving B and T cells, as well as the number of inoculated bacteria and the genetic predisposition of the infected host (HLA type, disposition to autoimmune reactions). The damage in the connective tissue collagen is on one hand the result of the inflammatory infiltrate ("cytokine storm", collateral damage), on the other hand *Borrelia* show collagenotropism and might directly influence and damage the collagen structure.

Histopathology of classical cutaneous borrelioses is characteristic, but unfortunately non specific, meaning that the diagnosis is first of all a clinical one. In the case of EM histopathology shows perivascular lymphocytic infiltrations not always containing plasma cells in all the dermal layers, sometimes with admixture of eosinophilic granulocytes and macrophages, as well as slight changes in the structure of the collagen texture in the connective tissue (Figure 1 B,C). In the case of ACA (Figure 3 B,C), depending on the duration of the disease, the changes include more or less pronounced atrophy of the epidermis, dermis and subcutaneous tissue with ectatic capillaries in the upper corium. The inflammatory infiltrate is accentuated perivascular or even band-like and contains plasma cells. In addition there is a loss of elastic fibers with the development of fibrosis or incipient sclerosis of the papillary and reticular dermis. Some authors consider BL as special form of an EM. Histopathology shows an unremarkable epidermis with sharp defined partially confluent lymphocytic infiltrates (Figure 2 B,C). There are two histopathologic types of BL with (follicular type) or without (diffuse/nodular type) follicular structures, resembling the germinal centers of lymph nodes. Combinations between the two types can be observed. Plasma cells and sometimes eosinophils and multinucleated giant cells can additionally be found at the border of the infiltrate. An unaltered grenz zone between the epidermis and the lymphocytic infiltrate can regularly be observed. Table I shows the expression of leukocyte differentiation antigens based on a score of 0-3+ in lesional skin.
Diagnostic aids for cutaneous borreliosis

After initial enthusiasm, the detection of microorganisms has turned out to be difficult, frequently unreliable, and almost always extremely time-consuming by different procedures, including histochemical stains (Gram, Wright, Wright-Giemsa, and polychromes), fluorochromes (thioflavine-T, acridine orange, and rhodamine), silver impregnation techniques (Warthin-Starry, modified Dieterle, modified microwave-Dieterle, and Bosma-Steiner) in the 1980s, and immuno-histochemical analysis in the 1990s. Sero-logic techniques (immunofluorescence, enzyme linked immuno sorbent assay [ELISA], and immunoblot) are similarly unsatisfying, with false-negative (20-80%) and false positive results occasionally due to cross-reactions with Treponema pallidum or, more commonly, to a positive endemic background of 20% to 30% in many parts of Europe. Cultures with specified media such as modified Pettenkofer-Kelly or Barbour-Stoenner-Kelly can detect Borrelia in all clinical forms, but these techniques are limited to special laboratories and are unreliable, with less than 50% sensitivity. Moreover the time delay to get a positive culture can be up to four weeks. Molecular techniques initially seemed to solve the riddle, but in due course, it became clear that sensitivity varies (30-90%) according to the Borrelial strains, the material (fresh frozen tissue or paraffin material), and the applied primers. There is further a risk of contamination leading to false positive results. So, cutaneous borreliosis remains a diagnosis based on circumstantial evidence combining clinicopathologic and laboratory information and clinical response to therapy.

Immunohistochemistry and focus floating microscopy

The histopathologic diagnosis was recently made easier by the direct detection of the pathogen by immunohistochemistry and focus floating microscopy (FFM). The method is an advancement to older immunohistochemistry techniques employing a polyclonal anti-borrelial antibody, which recognizes all different borrelial strains. FFM combines several strategies to detect minuscule organisms in tissue sections. The key point to this technique is an almost holoscopic approach to the slide by tuning the focus of the microscope through the thickness of the slide (3-4 µm). So with FFM the section is scanned through in two planes: horizontally in serpentines as in routine cytology, and, simultaneously, vertically at a magnification of 200 to 400 times. This approach allows detection of B. burgdorferi (diameter 0.2 µm compared to 2 µm of collagen bundles) which pass through the section at various angles and accordingly may appear as undulated, comma-like to dot forms (Figure 5). In addition omission of counter stain as well as bright illumination of the scanning field proves to be helpful as the bright red color of the 3-amino-9-ethylcarbazole-stained microorganisms best contrasts with the faint yellow color of unstained collagen bundles as well as other tissue structures. The technique can be applied successfully on fresh material, nitrogen-frozen material and paraf-
Fin-embedded material and it is an easy, quick and inexpensive method to reliably detect *Borrelia* in cutaneous tissue sections. FFM proved to be more sensitive than PCR and ELISA-PCR in cases of classical borreliosis. Cases of prominent *Borrelia* detection by FFM are usually positive by PCR, which becomes negative in later stages as the number of microorganisms drop. So, it was possible to detect *Borrelia* by FFM in 47 of 71 ticks, in 34 of 66 tick bites, in 30 of 32 cases of EM, in 41 of 43 cases of BL (Figure 5) and in 50 of 51 cases of ACA. With a sensitivity of over 90% FFM was more sensitive than PCR with a sensitivity of 45% (P<0.001) and nearly equally specific (99% versus 100%). All control cases, except one case of false-positive secondary syphilis, were negative with FFM (Table II).

**Detection of *B. burgdorferi* in morphea**

Morphea is an inflammatory connective tissue disorder of unknown etiology. The involvement of *B. burgdorferi* as a causative agent was first proposed by Aberer *et al.* in 1985. Since then conflicting results have been obtained by different studies using serological, immunohistochemical, culture and PCR approaches. *Borrelia* has been frequently detected in Europe and Asia patients, but not in cases from the United States or Scotland. Studies reporting a positive association between *B. burgdorferi* infection and morphea found evidence of the organism in 26-100% of cases (Table III); on the other hand there are at least 10 reports where no positive cases could be

| Table II.—Detection of *Borrelia* in classical cutaneous Lyme disease by focus floating microscopy (FFM) only and by direct comparison of FFM with PCR. |
|---------------------------------|------------------|------------------|------------------|
| **Diagnosis**                   | **FFM only**     | **FFM PCR**      | **PCR**          |
| Ticks                           | 47/71 [66.2]     | NA               | NA               |
| Tick bites                      | 34/66 [51.5]     | NA               | NA               |
| Erythema chronicum migrans     | 15/17 [88.2]     | 15/15 [100]      | 7/15 [46.7]      |
| Borrelial lymphocytoma          | 22/24 [91.7]     | 19/19 [100]      | 4/19 [21.1]      |
| Acrodermatitis chronica atrophicans | 22/23 [95.7]   | 28/28 [100]      | 17/28 [60.7]     |
| Controls*                       | 0/109 [0]        | 1/60 [1.7]**     | 0/66 [0]+        |
| **Total number of cases**       | 310              | 122              | 128              |

NA (not available). *including atopic and stasis dermatitis, prurigo, insect bite, scabies, (false positive) secondary syphilis**, drug eruption, psoriasis, pityriasis rubra pilaris, lichen planus, erythema multiforme, urticaria, polymorphous light eruption, lupus erythematosides, systemic scleroderma, pemphigus seborrhoeicus, Wells syndrome, rheumatoid nodule, ganglion, foreign body reaction due to ruptured infundibular cyst, folliculitis, acne, rosacea, lichen nitidus, (hypertrophic) scar, keloid, dermatofibroma, fibroma molle, connective tissue naevus, melanocytic naevi, lentigo senilis, seborrhoeic keratoses, acinic keratoses, squamous and basal cell carcinomas, mycosis fungoides. +in six cases no material was left on paraffin blocks for FFM.

**Table III.—Literature with direct detection of *Borrelia* in morphea.**

<table>
<thead>
<tr>
<th>Author</th>
<th>Country</th>
<th>Year</th>
<th>Method</th>
<th>Results pos./n</th>
</tr>
</thead>
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<tr>
<td>Aberer <em>et al.</em></td>
<td>Austria</td>
<td>1987</td>
<td>Immunoperoxidase</td>
<td>7/21</td>
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<td>Aberer <em>et al.</em></td>
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<td>1987</td>
<td>Culture</td>
<td>1/4</td>
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<tr>
<td>Weber <em>et al.</em></td>
<td>Germany</td>
<td>1988</td>
<td>Culture</td>
<td>1/1</td>
</tr>
<tr>
<td>Aberer <em>et al.</em></td>
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<td>1988</td>
<td>Immunoperoxidase</td>
<td>3/9</td>
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<tr>
<td>Ross <em>et al.</em></td>
<td>Puerto Rico</td>
<td>1990</td>
<td>Silver stain</td>
<td>10/25</td>
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<tr>
<td>Aberer <em>et al.</em></td>
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<td>1991</td>
<td>Culture</td>
<td>1/11</td>
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<tr>
<td>Schempp <em>et al.</em></td>
<td>Germany</td>
<td>1993</td>
<td>PCR</td>
<td>9/9</td>
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<tr>
<td>Schempp <em>et al.</em></td>
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<td>1993</td>
<td>PCR/immunohistochemistry</td>
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<tr>
<td>Weidenthaler <em>et al.</em></td>
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<td>Granter <em>et al.</em></td>
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<td>1994</td>
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<td>1/1</td>
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<td>Trevisan <em>et al.</em></td>
<td>Italy</td>
<td>1996</td>
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<td>6/10</td>
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<td>Fujiwara <em>et al.</em></td>
<td>Japan</td>
<td>1997</td>
<td>PCR</td>
<td>2/5</td>
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<tr>
<td>Breier <em>et al.</em></td>
<td>Austria</td>
<td>1999</td>
<td>Culture</td>
<td>1/1</td>
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<tr>
<td>Oxkan <em>et al.</em></td>
<td>Turkey</td>
<td>2000</td>
<td>PCR</td>
<td>3/10</td>
</tr>
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<td>Hercogova <em>et al.</em></td>
<td>Czech Republic</td>
<td>2002</td>
<td>PCR</td>
<td>1/1</td>
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<tr>
<td>Eisendle <em>et al.</em></td>
<td>Germany/Austria</td>
<td>2007</td>
<td>PCR</td>
<td>1/29</td>
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</table>

Pos. positive; N number
identified. The different studies concerning the detection of *Borrelia* in morphea have been reviewed by Weide *et al.* 2000 and Goodland *et al.* 2002. One major difficulty in assessing the association between morphea and borreliosis is the challenge of reliably detecting *Borrelia* in tissue specimens. These conflicting results at least in part reflect the difficulties of the various techniques used to prove/document the participation of *Borrelia* in the disease process.

Some patients with morphea show clinical similarities to classical borreliosis (Figure 6A). Early stages of morphea with a prominent lilac ring are not always easy to differentiate from EM with its active border. In late stages red-purple-brown macules of burned out morphea may mimic ACA. Remarkably, these clinical entities can occur at the same time in the same patient, and co-existent morphea has been described in patients with EM, BL, ACA and Lyme arthritis. Moreover, there are descriptions of new patterns in borreliosis such as interstitial granulomatous dermatitis, which clinically most closely resembles morphea. There are also similarities in the histopathological findings (variable infiltrates of lymphocytes, macrophages and plasma cells; slight vacuolar degeneration of the basal layer in late stages; an increase of fibrocytes/fibroblasts with variable fibrosis to sclerosis) of EM and ACA on the one hand and morphea on the other. A bacterial etiology is further suggested because some cases of morphea respond well to antibiotic therapy, such as penicillin and ceftriaxone. D-penicillamine, discussed in older textbooks as inhibiting cross connections between collagen fibers, was likely to have been effective because of its metal-chelating activity which deprived the bacteria of essential trace elements such as manganese, zinc and magnesium.

In a recent large study of 122 morphea cases *Borrelia* could be detected in more than 68% of all cases with a significantly higher percentage (P=0.018) in active (75.0%) than inactive morphea (52.9%). This might reflect intentional or coincidental antibiotic exposure in longer existing cases and/or the natural course of disease with repression of the microorganisms by the immune system. The presence of B lymphocytes as determined by positive staining with CD20 proved to be a good diagnostic predictor for the presence of *Borrelia*, with a positive correlation of 0.85 (P<0.001). This is not surprising as the presence of B lymphocytes reflects an immune response against bacterial infections like borreliosis. The low percentage of detection of borrellial DNA with PCR in this study with one positive case in 30 (3.3%) indicates the problematic role of this technique to reliably detect *Borrelia* in tissue specimens. The reason for the inconsistent results in PCR studies (positive, Table III, negative see 50, 52, 53, 55, 57, 78) could be the low number of microorganisms found in the tissue with the detection threshold being beyond for this technique. Other explanations include previous antibiotic treatment, old stage of disease, wrong biopsy site (e.g. from negative sclerotic area), or wrong fixation of tissue specimens leading to DNA cross-linking e.g. with inadequately buffered formalin. Further — except for the studies done by Ranki 1994, Dillon 1995 and Wienecke 1995 — most other negative PCR studies are lacking appropriate positive controls in terms of detection of borrellial DNA in tissue specimens from classical borreliosis such as EM, BL and ACA. Thus, the reliability of the DNA extraction method for small DNA amounts or the PCR technique used in these studies remains somewhat debatable.

Another explanation for negative PCR results is that *B. burgdorferi* sensu latu includes *B. burgdorferi* sensu strictu, *B. garinii* and *B. afzelii* VS461, but also
newer *Borrelia* species have been identified. The pathogenic significance of these species, such as *B. valaisiana*, *B. hermsii*, *B. turicatae*, *B. parkeri* and most recently *B. spielmani* is not yet fully answered. While *B. burgdorferi* sensu strictu is the only well-established cause of Lyme disease in the United States, *B. afzelii*, *B. garinii* and probably *B. valaisiana* additionally cause “Lyme disease” in Europe and Asia. Relapsing fever borreliosis by *B. hermsii*, *B. turicatae* and *duttonii* and EM by *B. spielmani* have been described. The study by van Dam suggests that different *B. burgdorferi* genotypes have different pathogenic potentials. This is well documented for the classical borrelial manifestations, so ACA rarely occurs in the United States but is commonly seen in Europe where *B. afzelii* and *B. garinii* are more prevalent. Maybe, subspecies variations dictate the clinical manifestations that follow infections, with only certain strains possessing the characteristics required to initiate the development of morphea. This is well documented for the classical borrelial manifestations, so ACA rarely occurs in the United States but is commonly seen in Europe where *B. afzelii* and *B. garinii* are more prevalent. Maybe, subspecies variations dictate the clinical manifestations that follow infections, with only certain strains possessing the characteristics required to initiate the development of morphea.

So, borreliosis is a vector transmitted disease whose causative agents, *B. burgdorferi* and variants, share collagenotropism, whereas other spirochaetes or spirochaetal bacteria are epithelio- and endotheliotropic (*e.g.* T. pallidum) or mucotropic (*e.g.* H. pylori). Fibronectin binding proteins of *B. burgdorferi* promote bacterial attachment to glycosaminoglycans which are most prominent in connective tissue between collagen bundles. While the immune system can cope with the organisms in EM and BL comparatively quickly, in ACA and morphea the situation seems more complicated. The low level of microorganisms probably indicates that the disease is not only due to the effect of the infectious agent, but also reflects the challenge for the immune system due to the location of the microorganisms and or even a compromised immune reaction in morphea patients themselves, where *Borrelia* might trigger a subsequent autoimmune reaction. This could also explain why not all patients benefit from antibiotic therapy. Indeed, a recent study published in 2009 by Prinz JC found evidence for the induction of autoimmunity by *Borrelia* infection. The authors examined the relationship between *Borrelia* exposure, serologic autoimmune phenomena and age at disease onset in morphea patients. In 90 morphea patients the presence of *Borrelia*-specific serum antibodies was correlated to the age at disease onset and the presence and titers of antinuclear antibodies. A statistically highly significant association between morphea, serologic evidence of *Borrelia* infection, and high-titer antinuclear antibodies was observed, when disease onset was in childhood or adolescence. The conclusion was that *B. burgdorferi* infection may be relevant for the induction of a distinct autoimmune type of scleroderma, which the authors suggested to be called “*Borrelia*-associated early onset morphea”. This condition is characterized by the combination of disease onset at younger age, infection with *B. burgdorferi*, and evident autoimmune phenomena as reflected by high-titer antinuclear antibodies.

Detection of *Borrelia* in patients with *lichen sclerosus*

*Lichen sclerosus* (LS), frequently reported in the dermatologic literature as *lichen sclerosus et atrophicus*, is a chronic inflammatory skin disease of unknown etiology leading to substantial discomfort and morbidity. It commonly affects adult woman in the genito-anal region (Figure 7A) but also occurs elsewhere (Figure 7B). LS has clinical and histological similarities with morphea and some investigators consider this entity a superficial variant of morphea, an opinion supported by its frequent coincidence with morphea. LS further shares similarities and common features with ACA, a chronic form of borreliosis, in particular including histological findings such as an infiltrate of lymphocytes admixed with some plasma cells, an increase of fibrocytes and fibroblasts and a diffuse dermal fibrosis to sclerosis (Figures 3B, C, 7C, D). These observations have led several investigators to consider the possibility of *B. burgdorferi* as a common etiologic factor for both diseases. The involvement of *B. burgdorferi* as a causative agent for LS was first proposed by Aberer et al. in 1987 and subsequently further supported at least in part by several other studies (Table IV). A bacterial etiology is further suggested because several cases of LS respond well to antibiotic therapy, such as dirithromycin, penicillin and ceftriaxone. *Borrelia* have frequently been detected in Europe, but not...
Figure 7.—Clinical photograph of lichen sclerosus in the female genital area (A) and on the upper back of a young female (B). Histopathology at different magnifications (C,D) shows infiltrates of lymphocytes admixed with some plasma cells, an increase of fibrocytes and a diffuse dermal sclerosis (H&E, x100 b, x200 c).

### Table IV

Results of studies investigating *B. burgdorferi* in lichen sclerosus patients. For the PCR involving studies the amplified gene is shown in parenthesis.

<table>
<thead>
<tr>
<th>Author</th>
<th>Country</th>
<th>Year</th>
<th>Method</th>
<th>Results pos./N</th>
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<tr>
<td>Aberer et al.</td>
<td>Austria</td>
<td>1987</td>
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<td>6/13</td>
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<td>1988</td>
<td>Immunoperoxidase</td>
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<td>1990</td>
<td>silver stain</td>
<td>10/21</td>
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<td>Schempp et al.</td>
<td>Germany</td>
<td>1993</td>
<td>PCR (Flagellin)</td>
<td>6/6</td>
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<td>Ranki et al.</td>
<td>Finland</td>
<td>1994</td>
<td>PCR (OspA)</td>
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<tr>
<td>Dillon et al.</td>
<td>USA</td>
<td>1995</td>
<td>PCR (Flagellin)</td>
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<tr>
<td>De Vito et al.</td>
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<td>1996</td>
<td>PCR (Clone 2H1)</td>
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<td>1997</td>
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<td>1997</td>
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<tr>
<td>Eisendle et al.</td>
<td>Austria</td>
<td>2006</td>
<td>PCR (23s-RNA) FFM</td>
<td>38/60</td>
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Pos. positive, N number
in cases from the United States. Studies reporting a positive association between B. burgdorferi infection and LS found evidence of the organism in 10-68% of cases; on the other hand there are reports where no positive cases could be identified (Table IV).

In a recent large study on 61 cases of LS Borrelia could be detected in more than 60% of all LS cases, with a significantly higher percentage (P=0.001) in early (79.5%) than late LS (33.3%), while it made no difference if LS was associated with morphea or not (Table V). This might reflect intentional or coincidental antibiotic exposure in longer existing cases or the natural course of disease with repression of the microorganisms by the immune system. The negative detection of borrelial DNA with PCR in this study again indicates the problematic role of PCR to reliably detect Borrelia in tissue specimens. The low number of microorganisms beyond the detection threshold could be one explanation for the inconsistent results in PCR studies. Other explanations again include old stage of disease, wrong biopsy site (e.g. from negative fibrosclerotic parts), or wrong fixation of tissue specimens leading to DNA cross linking e.g. with inadequately buffered formalin. Further as explained above negative PCR studies are frequently lacking appropriate positive controls in terms of detection of borrelial DNA in tissue specimens from classical borreliosis.

As in the case for morphea there is another explanation for negative PCR results as beside B. burgdorferi sensu latu newer Borrelia species have been identified, as mentioned above with unclear pathogenic significance. So, subspecies variations might dictate the clinical manifestations that follow infections, with only certain strains possessing the characteristics required to initiate the development of LS. Thus, like in the case for morphea, an other explanation for the moderate results by PCR is the specificity of the primers used working only for known human pathogenic strains, while immunohistochemistry with a less specific polyclonal antibody detects most different borrelial species.

In any case, detection of spirochetes in pure LS and LS associated with morphea seems to be a common denominator which indicates the nosologic relationship of these skin disorders. Moreover, the infectious hypothesis with spirochetes helps to explain the most common stereotypical presentation of LS, namely in the genitoanal area. Subclinical dissemination with spread of Borrelia to kidneys and urine occurs in early borrelial infection. Favorable by the moist and frequently traumatized conditions of genitalia, this might allow a superficial Borrelia infection in the perigenital region, i.e. LS. This also explains the frequent occurrence of the disease in the perigenital area and why other mucous membranes such the oral or endonasal mucosa and conjunctiva are practically never affected. The lower level of microorganisms in late LS indicates that the disease is not only the consequence of the infectious agent, but also reflects the challenge for the immune system and or a compromised immune reaction in LS patients themselves, where Borrelia antigens might trigger a subsequent autoimmune reaction in genetically predisposed individuals via molecular mimicry. Favorable by the moist and frequently traumatized conditions of genitalia, this might allow a superficial Borrelia infection in the perigenital region, i.e. LS. This also explains the frequent occurrence of the disease in the perigenital area and why other mucous membranes such the oral or endonasal mucosa and conjunctiva are practically never affected. The lower level of microorganisms in late LS indicates that the disease is not only the consequence of the infectious agent, but also reflects the challenge for the immune system and or a compromised immune reaction in LS patients themselves, where Borrelia antigens might trigger a subsequent autoimmune reaction in genetically predisposed individuals via molecular mimicry. Favorable by the moist and frequently traumatized conditions of genitalia, this might allow a superficial Borrelia infection in the perigenital region, i.e. LS. This also explains the frequent occurrence of the disease in the perigenital area and why other mucous membranes such the oral or endonasal mucosa and conjunctiva are practically never affected. The lower level of microorganisms in late LS indicates that the disease is not only the consequence of the infectious agent, but also reflects the challenge for the immune system and or a compromised immune reaction in LS patients themselves, where Borrelia antigens might trigger a subsequent autoimmune reaction in genetically predisposed individuals via molecular mimicry. Favorable by the moist and frequently traumatized conditions of genitalia, this might allow a superficial Borrelia infection in the perigenital region, i.e. LS. This also explains the frequent occurrence of the disease in the perigenital area and why other mucous membranes such the oral or endonasal mucosa and conjunctiva are practically never affected. The lower level of microorganisms in late LS indicates that the disease is not only the consequence of the infectious agent, but also reflects the challenge for the immune system and or a compromised immune reaction in LS patients themselves, where Borrelia antigens might trigger a subsequent autoimmune reaction in genetically predisposed individuals via molecular mimicry. Favorable by the moist and frequently traumatized conditions of genitalia, this might allow a superficial Borrelia infection in the perigenital region, i.e. LS. This also explains the frequent occurrence of the disease in the perigenital area and why other mucous membranes such the oral or endonasal mucosa and conjunctiva are practically never affected. The lower level of microorganisms in late LS indicates that the disease is not only the consequence of the infectious agent, but also reflects the challenge for the immune system and or a compromised immune reaction in LS patients themselves, where Borrelia antigens might trigger a subsequent autoimmune reaction in genetically predisposed individuals via molecular mimicry.

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For these reasons and the reliable detection of spirochetal microorganisms in morphea and LS, one must conclude that at least some cases of morphea and LS should be integrated in the spectrum of cutaneous borrelioses.

Biofilms of *B. burgdorferi sensu latu* in chronic or recurrent cutaneous borreliosis?

The hypothesis that *B. burgdorferi* might form biofilm structures in BL and ACA was recently proposed based on the finding of large colonies of *Borrelia* in classical cutaneous borrelioses shown by immunohistochemistry and FFM. So, *Borrelia* can grow in a "medusa colony" or in a "granular colony with a reddish veil."

It is a fascinating hypothesis to compare large borrelial aggregations in the tissues with biofilms and to speculate that such biofilms of *B. burgdorferi* might be responsible for a partial resistance to antibiotic therapy in some patients with Lyme disease. Subsequently, the potential that *Borrelia* may shed from these biofilms, might thus provide a possible explanation for chronic relapsing courses of some borrelial infections. Of note, biofilm formation in the human host has already been described for other spirochetes like *Treponema denticula*, and biofilm formation has been associated with antibiotic resistance in *Helicobacter pylori* infections. Bacterial biofilms are responsible for several chronic diseases (*e.g.* periodontitis and chronic lung infection in cystic fibrosis patients) that are very difficult to treat because they show much greater resistance to antibiotics than their free-living counterparts. The biofilm resistance is very unique in a sense that it requires multiple mechanisms such as incomplete penetration of the antibiotics into the matrix, inactivation of antibiotics by altered chemical microenvironment within the biofilm and an altered, protected phenotypic "spore like" state of the resistant bacteria population.

If *B. burgdorferi* is indeed capable forming biofilms, it will change the way, how we think about Lyme disease especially in patients,

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**Table: Known and possible skin manifestations in cutaneous borreliosis**

- **Classical patterns**
  - Known association
    - 1) Erythema (chronicum) migrans
    - 2) Borreli-alymphocytoma/lymphadenosis benigna cutis
    - 3) Acrodematitis chronica atrophicans
    - 4) Juxta articular fibrotic nodules
- **Partly Borrelia associated**
  - Probable association
    - 1) Morphea/localized scleroderma
    - 2) Lichen sclerosus (et atrophicus)
    - 3) Granuloma annulare
    - 4) Interstitial granulomatous dermatitis
    - 5) Cases of cutaneous B-cell-lymphoma
- **Single reports**
  - Potential association
    - 1) Cutaneous sarcoidosis
    - 2) Necrobiosis lipoidica
    - 3) Lymphocytic infiltration Jessner-Kanof
    - 4) Necrobiosis xanthogranuloma
    - 5) Cases of cutaneous T cell-lymphoma (mycosis fungoides)

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[Figure 8](#) — A) Focus-floating microscopy and immunohistochemical staining (Acris BP 1002, no counterstain, x1000) for various borrelial colony forms. Medusa-like cluster of 'planktonic microorganisms' in a case of acrodematitis chronica atrophicans. B) Colony of degenerating fragmented/small granular "dying" spirochetes in a case of morphea. C) Putative biofilm formation of a borrelial colony with a mixture of medusa-like and granular spirochetal aggregations with cystic rounded forms, tubular elements or swollen granules covered by a reddish veil in a case of lichen sclerosus.

[Figure 9](#) — Summary of the various known and possible skin manifestations in cutaneous borreliosis as described in the literature. The skin diseases are subdivided for known, probable and possible borrelial etiology.

173 voci biblio in editing da Catia.
where it seems to be persistent despite antibiotics treatment.\textsuperscript{113} It would be interesting to see if patients with big borrelial colonies that might resemble biofilms are the ones that are more resistant to antibiotic treatment. More research on this topic will answer these questions.

**Conclusions**

The classical manifestations of cutaneous borreliosis are EM, BL — a cutaneous B-cell-pseudolymphoma (or lymphadenosis cutis benigna) — ACA and iuxtaarticular fibrinoid nodules which are related to ACA.\textsuperscript{114, 115} At least in part also other skin manifestations — as described above — especially morphea,\textsuperscript{44, 49, 77, 87, 116, 117} lichen sclerosus\textsuperscript{44, 49, 58, 75, 96, 99, 118} and cases of cutaneous B-cell lymphoma\textsuperscript{119, 126} are causally related to infections with *Borrelia*. In the case of cutaneous B-cell lymphoma the pathogenic mechanism seems to be analogous to the one discussed for MALT-lymphomas which are induced by chronic *Helicobacter pylori* infection.\textsuperscript{127-138} The evidence is also growing that granuloma annulare\textsuperscript{139-147} and interstitial granulomatous dermatitis\textsuperscript{148-150} might be partly caused by *B. burgdorferi* or similar strains. There are single reports which connect other skin diseases to *Borrelia*, for example cutaneous sarcoidosis, especially in the Chinese literature,\textsuperscript{151-164} then necrobiosis lipoidica,\textsuperscript{165} necrobiotic xanthogranuloma\textsuperscript{166} and cases of mycotic fungoides,\textsuperscript{167} but the evidence for the latter skin diseases is not unambiguous. In addition, as the modern chameleon of dermatology, cutaneous borrelioses, especially BL, mimic other skin conditions, as has been shown for erythema anulare centrifugum or lymphocytic infiltration of the skin (Jessner-Kanof).\textsuperscript{168-173}

In summary, it can be concluded that the known spectrum of skin manifestations in cutaneous borreliosis is continuously expanding and can not be regarded as completed (Figure 7).

**Riassunto**

*Lo spettro in espansione della borreliosi cutanea*

Lo spettro conosciuto delle manifestazioni cutanee della malattia di Lyme è in continua espansione e non può essere considerato come completato. Accanto alle classiche manifestazioni della borreliosi cutanea, quali l’eritema (cronico) migrante, il linfocitoma borrelioso e l’acrodermatite cronica atrofizzante, sta crescendo l’evidenza che almeno in parte anche altre manifestazioni cutanee siano correlate all’infezione da *Borrelia*, specialmente la morfea, il lichen sclerosus ed alcuni casi di linfoma cutaneo a cellule B. Anche il granuloma annulare e la dermatite granulomatosa interstiziale potrebbero in parte essere provocati da *Borrelia burgdorferi* o da ceppi simili. Vi sono anche segnalazioni isolate di altre manifestazioni cutanee associate alle infezioni da *Borrelia* quali la sarcoidosi cutanea, la necrobiosi lipoidica e lo xantogranuloma necrobiotico. Inoltre, come un camaleonte dermatologico, la borreliosi cutanea, specialmente nel caso del linfocitoma borrelioso, mima altre patologie cutanee, come è stato dimostrato per l’eritema annulare centrifugo o l’infiltrazione linfocitica (Jessner Kanof) della cute.


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THE EXPANDING SPECTRUM OF CUTANEOUS BORRELIOSIS

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Innate and adaptive immune responses in contact dermatitis: analogy with infections

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Allergic contact dermatitis (ACD) is an inflammatory skin disease of great and steadily increasing importance as an occupational health problem. The disease is induced by chemicals and metal ions which penetrate the skin and form complexes with host proteins. This process is accompanied by a strong, allergen-induced inflammatory reaction and leads to the migration of allergen-carrying dendritic cells (DC) from the skin to regional lymph nodes, where they promote generation of allergen-specific T cells. The latter are the ultimate effector cells of the disease. Re-exposure to the causative agent leads to the recruitment of the T effector cells, which then elicit the typical skin inflammatory reaction at the site of contact. Although DC and effector T cells play a protagonistic role in the sensitization and elicitation phase of ACD, respectively, other cell types including keratinocytes, NK cells, mast cells and B cells contribute to the pathogenesis of the disease. In this review the authors summarize recent findings that identify stress responses and innate immune pathways triggered by contact allergens and review recent data regarding the adaptive T cell response. The new data were collected mainly from studies on contact hypersensitivity (CHS), the corresponding experimental mouse model of human ACD. The elucidation of the molecular events involved in contact allergen-induced innate responses will help to design new treatment strategies and may allow to develop predictive in vitro assays for the identification of contact allergens.

KEY WORDS: Dermatitis, allergic contact - Immunity, innate - Toll-like receptors - NOD-like receptors - Infection - T-Lymphocytes - Dendritic cells - Haptens.

Pathogenesis of allergic contact dermatitis

Allergic contact dermatitis (ACD) is a serious problem in occupational health. More than 4,000 chemicals are known to act as contact allergens. These low molecular weight reactive haptens and metal ions penetrate the skin where they are taken up by antigen-presenting cells (APC) like dendritic cells (DC). This process is accompanied by a strong inflammatory reaction, driven by several skin cells, such as keratinocytes and DC. The first allergen contact induces sensitization (sensitization phase). The activated DC (epidermal Langerhans cells (LC) and dermal DC) then migrate to the skin draining lymph nodes and present the contact allergens to naive T cells. The allergen-specific effector and memory T cells thus generated then circulate in the blood. Upon a secondary allergen expo-
sue of the skin they are recruited to the site of contact where they elicit the allergic response (elicitation phase). The disease becomes manifest due to recruitment of inflammatory cells such as mast cells, neutrophils and NK cells, and due to tissue damage caused by the cytotoxic activity of CD4+ and predominantly CD8+ effector T cells on keratinocytes and other skin cells via death receptors such as Fas.¹

Electrophilic chemicals as contact allergens

Many of the known contact allergens are haptens that can directly react with endogenous proteins due to their electrophilicity which allows covalent binding to endogenous proteins. The chemicals are not immunogenic per se but so after protein binding and are therefore called half-antigens or haptens. Non-reactive pre- and pro-haptens are metabolized in the skin or undergo auto-oxidation on exposure to air ² to form reactive haptens. Recently, the cytochrome P450 system that usually serves the metabolic transformation of endogenous substrates such as fatty acids and vitamins but also of exogenous substrates such as drugs, has been implicated in the metabolic activation of pre-haptens.³ Thus, intrinsic or acquired chemical reactivity with proteins and peptides is a hallmark of contact allergens. Two major events for the induction of ACD may require protein coupling of contact allergens: first, the induction of stress and innate inflammatory responses in the skin. These are a prerequisite for the T cell response to antigens presented on APC. The T cell activating antigens are peptides derived from pathogen proteins or contact allergen-modified self-peptides.

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allergens to thiol groups in proteins such as the cellular anti-oxidant glutathione. They also induce anti-oxidant responses and trigger the inflammatory response that is essential for activation of the adaptive immune response. Recently evidence has been presented for an involvement of innate pattern recognition receptors (PRRs) such as the Toll-like receptors (TLR) and NOD-like receptors (NLR) in the induction of CHS. Highly conserved molecules designated pathogen-associated molecular patterns (PAMPs), such as lipopolysaccharide (LPS, endotoxin) from Gram-negative bacteria, bacterial lipopeptides or nucleic acids of microbial origin (DNA, dsRNA or ssRNA), trigger TLR in the plasma membrane and in intracellular compartments. Furthermore, microbial RNA, muramyl dipeptide and toxins trigger the cytosolic inflammasome, a Caspase-1 activating protein complex containing PRR such as the NOD-like receptor NALP3. The property of contact allergens to bind to different cellular proteins seems to endow them with activities similar to those of PAMPs. Thus, contact allergens may directly or indirectly activate PRR. The latter may result from the induction of endogenous TLR and NLR ligands.

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Bona fide irritants can trigger some, but not all of the innate responses provoked by contact allergens and above all, they do not induce specific T cell responses. The identification of specific and unique receptors triggered by contact allergens or irritants are being exploited for the development of predictive in vitro assays for their identification in order to replace animal testing. Such efforts are very important due to the dramatic occupational health problems caused by these agents. Further they would result in a significant reduction of the number of animals used in such tests.

Induction of cellular stress responses by contact allergens

Many endogenous proteins can be modified by reactive haptens in the skin. Unfortunately, very little is known about the functional targets. For example, proteins whose hapten modification has functional consequences. Similar to posttranslational protein modifications, such as phosphorylation or glycosylation, hapten modification may change protein function, localization or interaction with other proteins. One possible mechanism involved is the modification of proteins involved in relevant signaling pathways. It is also known that contact allergens cause oxidative stress, induce redox imbalance and may thereby trigger pathways that are sensitive to changes in the redox potential. These pathways include those leading to NF-κB and MAPK activation and are known to be required for DC maturation. In fact, treatment with the antioxidant ebselen compromises DC and T cell function and suppresses CHS responses to the contact allergen oxazolone but not to the irritant croton oil. A cross-talk between oxidative stress, TLR triggering and anti-oxidant responses has been shown to exist. This seems to be important for the delivery of signal 3 in addition to MHC/peptide recognition by the T cell receptor (signal 1) and co-stimulation, e.g., via CD28/CD80/86 (signal 2) for full T cell differentiation and polarization to effector cells.

As a cytoprotective response against oxidative stress cells activate an anti-oxidant response, the so-called phase 2 response to protect themselves from inflammation and cytotoxicity. Interestingly, one of the few known functional targets of hapten modification is the cytosolic electrophile sensor kelch-like ECH-associated protein 1 (Keap1) that is instrumental in the phase 2 response. Thus, contact allergens induce a redox imbalance, which is needed for the induction of CHS, but at the same time they also induce a protective response to prevent oxidant induced damage. Hapten-modification of thiol residues in the cysteine-rich substrate adaptor Keap1 prevents targeting of the transcription factor nuclear factor-erythroid 2 related factor 2 (Nrf2) for ubiquitination and proteasomal degradation and, instead, results in its translocation to the nucleus. There, Nrf2 activates the transcription of genes containing anti-oxidant response elements (ARE) in their promoter region. Genes involved in the regulation of inflammation, the function of APC and migration of DC are among the Nrf2 targets. Furthermore, genes involved in the Keap1/Nrf2-dependent antioxidant response such as heme oxygenase 1 (HMOX) and NADPH quinone oxidoreductase 1 (NQO1) are regulated by Nrf2. Since the Keap1/Nrf2-dependent anti-oxidant response is absent in cells activated by irritants, cellular in vitro assays for identification of contact allergens are presently being established in order to replace animal testing. In these assays activation of a Nrf-sensitive (ARE-containing) luciferase reporter construct and measurement of NQO1 activity or monitoring of HMOX/NQO1 gene expression help to identify contact allergens among various substances under investigation.
A role for the Keap1/Nrf2 pathway in CHS has been demonstrated using agents that deplete the cellular anti-oxidant glutathione or by using Nrf2-/- mice. In vitro depletion of glutathione before hapten modification of in vitro generated bone marrow-derived DC (BM-DC) with diethyl maleate abrogated their ability to sensitize mice to DNCB. This treatment prevented DC maturation resulting in a lack of costimulatory molecule upregulation and of the production of proinflammatory cytokines. These data show that a fine balance between oxidative stress and anti-oxidant responses determines the activation of dendritic cells.

CHS responses are generally decreased in older mice. In a recent study, CHS was found to be decreased in old but not in young Nrf2-/- mice. In line with these findings oral administration of the Nrf2 antagonist N-acetyl cysteine resulted in the compensation of the age-related decrease in CHS. This was due to an upregulation of type 1 T cell responses. The effect of the age-related decrease in CHS responses determines the activation of dentritic cells.

These data demonstrate a complex role for oxidative stress in shaping immune responses to contact allergens. A redox imbalance via ROS induction or other mechanisms is needed for the inflammatory response in CHS but also provokes a protective anti-oxidant response. Interestingly, the generation of ROS via NADPH oxidase (NOX), a common feature of infectold mice that were treated was replaced by injection of hapten-modified DC from untreated DC from old mice induced a reduced CHS response compared to those from young mice. In a recent study, CHS was found to be decreased in old but not in young Nrf2-/- mice. In line with these findings oral administration of the Nrf2 antagonist N-acetyl cysteine resulted in the compensation of the age-related decrease in CHS. This was due to an upregulation of type 1 T cell responses. The effect of the age-related decrease in CHS response compared to those from young mice.

Inflammation triggered by contact allergens via innate immune receptors

**TLR**

The family of TLR consists of at least 13 germ-line encoded signaling receptors in mice and humans. TLRs are mammalian homologs of the protein Toll, which is required in Drosophila during development and essential for the resistance of the fly to fungal infections. They belong to type I integral membrane glycoproteins with a leucine-rich repeats (LRR)-containing domain which is important for ligand recognition and is also found in NLR. Further they have a transmembrane domain and a Toll/IL-1 receptor homology (TIR) domain required for initiation of signaling. TLR1, 2, 4, 5, 6 and 10 are localized mainly on the cell surface, while TLR3, 7, 8, 9 and 13 are found in the ER membrane. The latter receptors have to translocate into endosomes before they meet and become subsequently activated by their ligands. Physical association between the ER-resident protein UNC93B and the intracellular TLR promotes the endosomal translocation of these receptors and is essential for their proper signaling.

The identification of TLR4 as the signaling receptor for LPS also disclosed the essential role for this protein in the recognition of Gram-negative pathogens and in the activation of the innate and adaptive immune system. This finding paved the way to new fields of interest, which led to the identification of different microbial components, acting as ligands for the different TLRs. TLRs are receptors that recognize a variety of highly conserved microbial PAMPs and thus enable a rapid sensing of microorganisms including bacteria, viruses, parasites and fungi. TLR on the cell surface recognize lipopeptides, glycolipids and proteins mainly of bacterial, but in some cases also of viral, parasitic or fungal origin. TLR2 in collaboration with TLR1 or TLR6 is activated by several ligands, including diacylated lipopeptides from Gram-positive bacteria and triacylated lipopeptides from Gram-negative bacteria. In concert with Dectin-1, TLR2 recognizes the yeast cell wall component zymosan. TLR4 is the sole receptor for LPS, a major component of the outer membrane of Gram-negative bacteria. On the surface of cells, it is present in association with the adaptor protein MD-2. In this form MD-2 represents the binding site for LPS while TLR4 is the signaling part of the LPS receptor. The TLR4/MD-2-mediated LPS recognition is critical for sensing and elimination of Gram-negative bacterial pathogens. LPS release from bacteria and the subsequent interaction with the TLR4/MD-2 on cells of the innate immune system is responsible for a large number of biological activities, including clinical symptoms of infection. These activities are required for anti-bacte-
inal defense, but may also lead to an uncontrolled inflammatory reaction resulting in endotoxin shock. In addition to LPS, the F protein of respiratory syncytial virus and an as yet unidentified component of vesicular stomatitis virus as well as the mouse mammary tumor virus envelope glycoprotein were reported to activate TLR4.53-55

The triggering of TLR leads to the production of chemokines and of a large number of pro-inflammatory and anti-inflammatory cytokines and proceeds through MyD88- or TRIF-dependent signaling pathways. The pathway initiated upon activation of the adaptor protein MyD88 is common to all TLR, except TLR3, and leads to the induction of pro-inflammatory cytokines via NF-κB. The second pathway involves the adaptor protein TRIF and is used by TLR3 and TLR4. It induces type I interferon and enhances the production of the pro-inflammatory cytokines. Recent reviews 17-19 summarize the current knowledge about signaling through the different TLR. Notably, the unique capability of TLR4 to activate two signaling pathways is thought to be responsible for the high inflammatory potency of its ligand LPS.

**TLR IN CHS**

The molecular mechanisms leading to the activation of the innate immune system by contact allergens are less clear. They can activate NF-κB and MAP kinases,1 and the hypoxia inducible factor subunit HIF-1α.20 It is not known whether signaling cascades are activated by direct hapten modification of components in the respective pathways or by the induction of endogenous ligands for signaling receptors as suggested by us recently for the TLR2 and TLR4 pathways in CHS.14 Finally, also skin commensals and pathogens may contribute to TLR triggering in ACD.

The mechanisms of contact allergen-induced DC activation in the skin are largely unclear. DC express several TLR and their triggering results in the activation and migration of these cells. Intriguingly, DC activated during the sensitization phase of CHS resemble those activated by microbial ligands through TLR. They exhibit enhanced expression of various co-stimulatory molecules, such as CD40, CD80 or CD86, of MHC molecules and of the chemokine receptor CCR7. TLR activation results in the production of pro-inflammatory cytokines such as TNF-α, IL-1α, IL-1β, IL-6 and IL-12 and of type I interferon. These cytokines contribute to the development of CHS and are induced by contact allergens such as the strong contact allergen 2,4,6-trinitrochlorobenzene (TNCB) in the skin. We have now shown that in vitro stimulation of DC with TNCB results in the upregulation of co-stimulatory molecules but does not lead to cytokine production.14 Since the skin as the site of exposure to contact allergens is also continuously exposed to microbial flora and thus to TLR ligands, we initially assumed that the induction of cytokines in DC during sensitization to contact allergens might be the result of microbial TLR activation.

Previously it was shown that exogenously administered ligands of TLR4 and TLR7 (LPS and synthetic R848, respectively) during the sensitizing phase enhance the induction of CHS to 2,4-dinitrofluorobenzene (DNFB) or fluoresceinisothiocyanate (FITC).37 Recently, such receptors, TLR2 and TLR4, were recognized by us to participate in the sensitization of mice to contact allergens.14 The authors demonstrated that the activation of DC through TLR triggering during the sensitization phase of CHS is in fact necessary for the generation of allergen-specific effector CD8+ T cells. A single deficiency of TLR4 had no effect on the induction of CHS to TNCB in mice. The concomitant absence of TLR2 and 4 prevented the development of CHS. The importance of the two TLR was also shown when the initial skin painting with TNCB was replaced by an intracutaneous (i.c.) injection of trinitrophenyl (TNP)-modified BM-DC. In this case only DC from wild type, TLR2 or TLR4 single deficient, but not from TLR2/TLR4 double deficient mice induced sensitization and a CHS response after challenge of the ears with TNCB.14 These results made it evident that TLR2 and TLR4 are essential and to a substantial degree interchangeable in the activation of DC during the sensitization phase of CHS. Surprisingly, mice double deficient for TLR4 and IL-12 receptor β2 (IL-12Rβ2) did not develop CHS when sensitized with TNCB, oxazolone or FITC. Furthermore, TNP-modified TLR4/IL-12Rβ2-deficient DC in contrast to TNP-modified DC from WT mice failed to sensitize WT recipients to TNCB challenge. IL-12 is considered to be important in the induction of Th1 immunity and therefore also in the generation of allergen-specific CD8+ effector T cells. However, a loss of IL-12 function in TLR4 competent mice did not impair, but enhanced the CHS response, indicating the existence of an alternative IL-12 independent CHS development. Accordingly, in TLR4/IL-
12Rβ2 double deficient mice the mere introduction of the TLR4 transgene was sufficient to restore the inducibility of CHS. Thus, during the IL-12 independent CHS induction, the function of TLR4 is essential and cannot be replaced by TLR2. Interestingly, the introduction of the TLR4 transgene also enabled the development of allergen-specific T cells producing high amounts of IFN-γ despite a continuing absence of IL-12 function.14 According to a more recent report, IL-23 could in this case be the Th1 polarizing cytokine.58 In accordance, DC activated through TLR4 upregulate IL-23.14

Notably, in the absence of TLR4, triggering with synthetic cytidine-phosphate-guanosine oligodeoxynucleotides (CpG-ODN) through TLR9 provided sufficient help for the activation of DC in the IL-12 independent CHS.14 At this point it should be mentioned that the activities elicited by the different TLR, although mediated exclusively by the MyD88 signaling pathway, are not identical.17 Thus, contrary to TLR2 ligands, those of TLR9 can induce type I interferon in DC. It is tempting to speculate that this property may explain why the induction of CHS in TLR4/IL-12Rβ2 deficient mice the TLR9 ligand CpG-ODN could substitute for the missing TLR4 function in our study.14 It is therefore conceivable that in the sensitization phase of CHS, TLR2 and TLR4 functions can be replaced by other TLR, if ligands for these receptors are available and the correct signaling pathways become activated. On the whole, the above investigations revealed that sensitization to contact allergens can proceed via both, IL-12-dependent and -independent DC-associated pathways that require TLR activation.

The requirement for innate immune activation after cutaneous exposure to the sensitizing agent was demonstrated in nickel-induced CHS. Nickel, one of the most important contact allergens in man, does not efficiently induce CHS in mice. Injection of NiCl₂ with H₂O₂,59 an inducer of ROS, or with the TLR4 ligand LPS, resulted in a successful induction of CHS in mice.60 These findings once more underline the instrumental role of danger signals 61, 62 in the initiation of immune responses to contact allergens. Obviously, these danger signals are provided by contact allergens directly or indirectly and may be missing in some situations as is the case for nickel in mice. However, one cannot exclude a role of PAMPs that may act as microbial adjuvants in CHS responses.

ENDOGENOUS LIGANDS ACTIVATING TLR

In addition to recognizing pathogen-derived components, TLR must also recognize endogenous ligands as supported by their important role in embryogenesis and post-embryonic development in drosophila. In the fruit fly, Spätzle is generated as an endogenous Toll ligand during dorsoventral patterning of the embryo but also in response to infections by bacteria, fungi and yeast.57

In mammals, endogenous danger and stress signals are generated during sterile trauma or inflammation. Some of these are PRR ligands, also called damage-associated molecular patterns (DAMPs) in analogy to PAMPs.63 Some DAMPs can act as endogenous Toll ligands. A number of these have been identified to date,64 and include TLR2- and/or TLR4-activating inflammatory proteins such as heat shock proteins (HSP). A functional role for HSP27 and HSP70 in DNFB-induced CHS has been demonstrated.65 Intra-dermal pretreatment of mice with blocking antibodies to these HSPs, and subsequent sensitization with HSP at the same site, significantly reduced the CHS response and reduced the IFN-γ and IL-17 production by T cells from draining lymph nodes. A concomitant increase of IL-4 and IL-10 was observed and antibody/DNFB pre-treated mice were tolerant to further sensitization and challenge in an antigen-specific manner. Interestingly, the HSP effects were TLR4 dependent suggesting a role for a HSP27- and HSP70-mediated TLR4 triggering in CHS.

Other endogenous TLR ligands are high mobility group box 1 (HMGB1) protein and oligomeric degradation products of HA. HA is a major component of the extracellular matrix (ECM) and its degradation fragments are known to elicit numerous pro-inflammatory responses and maturation of DC.66 HMGB1 is a nuclear protein that is released extracellularly as a mediator of inflammation as well as after necrotic cell death. Its interaction with TLR2 and TLR4 is supposed to generate an inflammatory response similar to those initiated by LPS.67, 68 Complexes of HMGB1 with nucleosomes were shown to act via TLR2.69 Furthermore, the biological activity of the surfactant protein A was attributed to TLR4.70 Also, nucleic acids of mammalian origin, especially when complexed to auto-antibodies, as well as self-DNA complexed to the human cathelicidin LL-37, may act as endogenous ligands for intracellular TLR.71, 72
HYALURONIC ACID AS ENDOGENOUS TLR LIGAND

The identification of endogenous TLR activators is hampered by the potential presence of contaminating microbial agonists in the preparations under test. This is the reason why several endogenous substances, originally claimed to represent a given TLR activator, on re-examination, could not be confirmed as such. Such examples justified skepticism regarding the existence of endogenous ligands for TLR. More recent in vivo studies however, strongly suggested that such endogenous ligands do exist. Using a model of non-infectious lung injury, the interaction of endogenously produced HA fragments with TLR2 and TLR4 was reported to provide signals that initiate inflammatory responses, maintain epithelial cell integrity and promote recovery from acute lung injury.75, 74

HA is abundant in the skin as a high molecular weight polymer and its degradation products promote the migration and maturation of skin DC, and can also cause inflammation. The enzymatic degradation of HA is accomplished by hyaluronidases. Constitutive HA turnover occurs e.g. in skin but pronounced changes in HA metabolism are associated with inflammation.75-74 (authors’ unpublished observations). Thus, inflammatory stimuli must induce hyaluronidases. In line with this view, it has been shown that the HYAL-l promoter contains a NF-κB binding site allowing its regulation in inflammatory responses.75 Moreover, degradation of ECM components such as HA in the skin may proceed via oxidative breakdown involving ROS during the inflammatory response induced by contact allergens.64, 76 Interestingly, it was shown that extracellular superoxide dismutase (EC-SOD) can directly bind HA and prevent oxidant induced fragmentation. This may explain the protective function of EC-SOD that is expressed at high levels in the lung in models of lung injury and fits with the increased lung inflammation observed in EC-SOD deficient mice.77 Anti-oxidants such as N-acetyl cysteine can inhibit HA fragment-induced inflammatory responses whereas oxidants such as H2O2 enhance them.78 The role of EC-SOD in skin during CHS is unclear but mice with transgenic skin specific expression of this enzyme show a suppression of TNBC induced CHS. This effect is due to an impaired LC migration and diminished infiltration of inflammatory cells, and to a failure to express inflammatory cytokines in the skin.79

A role for HA in the induction of CHS is also supported by the finding that mice deficient for the HA receptor CD44 are more resistant to the induction of CHS. In this connection it is of interest that Taylor et al, demonstrated that a full inflammatory response of cultured cells to HA fragments requires a complex formation between CD44, TLR4 and MD-2.80 The importance of HA in the development of CHS is supported further by the finding that the sensitization to contact allergens in mice can be suppressed by the HA inhibitor Pep-1.81 Moreover, the antimicrobial cathelicidins known for their anti-inflammatory activity by interference with TLR function can inhibit sensitization for CHS in a TLR4 dependent fashion.82 It was also shown that cathelicidins are able to inhibit HA-mediated cytokine release and cell adhesion in a partially CD44-dependent manner.83 Interestingly, we have shown that Pep-1 also reduces the TLR-dependent CHS to TNCB in germ-free wild type mice.14 These data provide strong additional support for the existence of endogenous ligands for TLR and identify HA as one of several possible ligands involved in CHS.

The identity of the endogenous TLR activator(s) involved in the induction of CHS under germ-free conditions has not been addressed and represents a challenge for further investigations. It is important to stress that the results obtained in germ-free mice by no means rule out an important role for microbial TLR ligands in the induction of CHS under normal environmental conditions. The concomitant presence on the skin of contact allergens and microbes during the sensitization and elicitation phase of CHS makes it likely that microbial components are involved in CHS development.

NOD-like receptors and the NALP3 inflammasome in CHS

NLR are cytosolic PRR that typically contain a C-terminal LRR domain most likely involved in ligand recognition, a centrally located NACHT domain essential for activation of NLR and a N-terminal effector domain reponsible for protein-protein interaction such as the caspase recruitment domain (CARD) or the pyrin domain (PYD). Like TLR, NLR are sensors for microbial ligands such as bacterial peptidoglycan, flagellin, bacterial toxins and nucleic acids. Furthermore, they also recognize endogenous ligands.20-23

Among other functions, NLR are constituents of cytosolic protein complexes designated inflammasomes that contain the adaptor protein ASC and serve to activate caspase-1. Inflammasomes collaborate with TLR, which trigger the production of immature pro-IL-1β
pro-IL-18 and pro-IL-33. The bioactive secreted forms of these cytokines are generated by proteolytic processing of the pro-forms by caspase-1. The NALP3 inflammasome plays an important role in ACD. Previous studies have demonstrated an important role for IL-1β IL-18 and Caspase-1 in the mouse CHS model.14-16

The NALP3 inflammasome is not only triggered by microbial ligands but also by danger signals such as asbestos, silica, uric acid crystals, amyloid beta fibrils and alun and other particulate vaccine adjuvants.14, 57-61 These particles or protein aggregates may act initially via the plasma membrane and induce signaling events.92, 93 Uptake into lysosomes and rupture of these releases the crystals or fibrils into the cytosol, whereby, they may directly activate the inflammasome. Alternatively, the accompanying release of cathepsin B can cause inflammasome activation as shown by the anti-inflammatory effects of cathepsin B inhibitors.14, 94

It is still unclear how contact allergens mediate activation of the inflammasome pathway. Intradermal injection of monosodium urate crystals upon sensitization with TNCB increases the ear swelling response to TNCB challenge indicating a role for the inflammasome in CHS.91 However, it is unknown whether contact allergens induce the release of uric acid from necrotic cells during chemical insult of skin tissue.

A commonality of inflammasome activation by all known ligands is the decrease of intracellular potassium levels. This may be regulated by purinergic receptor signaling e.g. via P2X7, an ATP sensing receptor in the plasma membrane of cells.14, 94 As recently shown, inflammasome activation involves ROS, produced by NOX enzymes. Thus, NOX inhibitors or knockdown of the common subunit p22phox of NOX, prevented inflammasome activation and production of mature IL-1β p17 in the human monocyte cell line THP1.45

The potassium efflux can be induced not only by ROS but also directly, via increase in extracellular ATP, due to cellular stress.21-23, 94 A role for ATP and its metabolization via the ecto-nucleoside triphosphate diphosphohydrolase (NDTPhase) CD39 and CD73 in CHS has been demonstrated. Intradermal administration of non-hydrizable ATP-γS resulted in increased CHS responses.95 Knockout mice lacking CD39 that is expressed on LC exhibited an increased irritant contact dermatitis but, surprisingly, an attenuated CHS.96, 97 The latter result is not easily explained and should be further investigated. The role of ATP in the development of CHS may be explained by its role in the activation of the inflammasome. In addition, its concerted metabolization to adenosine by CD39 and CD73 expressed on regulatory T cells may be important in the regulation of the CHS response. Adenosine can act via the A2A receptor on effector T cells and plays a role in their suppression by Treg.95

T CELL POLARIZATION IN CHS

DC are professional APC and as such play a decisive role in the induction of, not only the normal adaptive immune defense, but also of autoimmune and allergic disorders. In allergic contact dermatitis DC internalize and process contact allergen-coupled proteins in the skin, transport them into the regional lymph nodes and present allergen-coupled MHC associated peptides to naïve T cells.1 In principle, depending on the cytokine milieu during antigen (allergen) uptake and processing, DC mature and promote development of either Th1 helper/cytotoxic(γδ) or Th2 type immune responses. The Th1/Th1c response is characterized by the generation of IFN-γ producing antigen-specific T cells (CD4+ and CD8+) under the influence of IL-12- secreting DC. The Th2 type response proceeds in the presence of IL-4 and leads to the generation of CD4+ T cells producing IL-4, IL-5, IL-10 and IL-13. Human monocyte-derived DC treated with the contact allergens DNCB or oxazolone in vitro were reported to be polarized to a DC1 phenotype.98 In the absence of danger signals however, this process is very inefficient both in murine and human DC, due to the lack of cytokine induction (Esser PR, Martin SF, unpublished data).14 CHS induced by skin allergens is a Th1/Th1c type immune response that leads to the generation of allergen-specific CD8+ effector T cells, which upon re-stimulation with the allergen produce large amounts of IFN-γ.1-24 Interestingly, a Th2-polarized CHS has also been described. Thus, it was reported that in BALB/c and C57BL/6 mice the CHS induced by fluorescein isothiocyanate (FITC),99, 100 but not by TNCB,14, 101, 102 is Th2 dependent. In our study however, the FITC-induced CHS in C57BL/10 mice showed no Th2 polarization.14 A Th2-polarized CHS was also observed in C57BL/6 mice in which IL-12 function had been lost due to a disruption of the Tγk2 gene.103 In the authors’ studies the loss of IL-12 function in IL-12 receptor-deficient C57BL/10 mice did not prevent the Tc1-polar-
oral tolerance. Interestingly, the induction of oral
immunity regulation by Treg cells

An ultimate fate of T cells is their thymic or peripheral differentiation to regulatory T cells (Treg). This immunosuppressive T cell subset does not only prevent autoimmune diseases but is also active in the downregulation of the CHS response and in the establishment of contact allergen-specific low zone or oral tolerance. Interestingly, the induction of oral tolerance to contact allergens requires antigen presentation to T cells by plasmacytoid DC, a process that involves the liver. The latter was shown by an efficient induction of tolerance upon hapten injection into the portal vein seven days prior to sensitization. It is not yet known, whether the cells that downregulate CHS are natural or adaptive Treg and whether they are antigen-specific. Also the crucial site of their action (lymph nodes and/or skin) during immune regulation remains to be determined. Recent data indicate that Treg homing to the inflamed tissue is important in the regulation of the CHS response and in the establishment of contact allergen-modified DC resulted in the generation of effector cells expressing skin homing receptors and sensitization for CHS.

It is very likely that the tissue microenvironment shapes the differentiation of monocyte DC precursors to skin-specific characteristics such as the ability to imprint tissue-specific homing receptors on T cells. In fact, bone marrow-derived DC acquire the potential to imprint skin homing receptors on T cells in the presence of dermal fibroblasts. Similarly, the same DC acquired the potential to induce small intestine-specific homing receptors on T cells in the presence of small intestinal epithelial cells. Both, soluble factors produced by the stromal and epithelial cells, and cell to cell contact were required for imprinting these receptors. These findings imply that the tissue microenvironment of the skin provides specific signals which induce the differentiation of monocyte precursors to skin-specific DC. Moreover, soluble factors that act as tissue specific environmental cues may be produced by stromal and epithelial cells and be transported by DC into the lymph nodes. One of these factors is retinoic acid produced by small intestinal epithelial cells.

Specific signals which allow the DC to imprint homing receptors on T cells are provided also by the stroma of tissue-draining lymph nodes. This was shown by transplantation of skin-draining lymph nodes into the small intestine mesenteries or of mesenteric lymph nodes into the popliteal fossa. Moreover, the lymph nodes supported the induction of the homing receptor profiles corresponding to their previous location. Thus, in this system the stroma provided dominant tissue-specific imprinting signals that override the signals given by immigrating DC. It is conceivable that the lymphoid stroma may not only contribute to the induction of tissue-specific homing receptor profiles on naive T cells but also to their maintenance on central effector and memory T cells.

The importance of tissue-specific T cell subsets also extends to the Treg compartment. This was demonstrated in scurfy mice that lack Treg cells due to a genetic defect in the transcripion factor Foxp3. Such mice develop a multiorgan autoimmune disease. Transfer of wild type Treg cures the disease, while transfer of Treg that lack selectin ligands, adhesion molecules required for skin homing, fails to do so.

**T cell migration to the skin**

The polarization of cytokine profiles is a crucial event in the priming of T cell responses by DC. However, the T cells that are primed in the tissue-draining lymph nodes have to receive the information on the tissue origin of the DC in order to migrate to and exert their effector function in that tissue. This task is accomplished by DC upon T cell priming. It was shown that epidermal LC can in fact induce skin homing receptors on T cells. Moreover, only intracuta-

neous but not intraperitoneal or intravenous injection of contact allergen-modified DC resulted in the generation of effector cells expressing skin homing receptors and sensitization for CHS.
PIECES OF A PUZZLE: A MODERN LOOK AT CONTACT DERMATITIS

If we try to put together the pieces of the puzzle concerning the pathomechanisms of allergic contact dermatitis, we realize that these are reminiscent of the events that lead to the immune response to infections. It becomes clear that similar pathways are activated and operate in concert via feedback mechanisms and cross-talk to generate immunity to infection or the full picture of allergic inflammatory skin disease. The central role of inflammation offers a unique opportunity for therapeutic interference with the innate stress and immune response pathways that lead, not only to allergic, but also to irritant contact dermatitis. Anti-inflammatory therapy would seem to be very promising if the targets are identified and the appropriate drugs become available. Specific differences in the mechanisms by which different allergens and irritants generate the essential danger signals have to be defined. This will help to develop the urgently needed predictive in vitro assays for the identification of putative irritants and contact allergens, as well as risk assessment. Worldwide efforts in this direction are ongoing, e.g. in the EU-funded project Sens-It-iv,122 that aims at the development of alternative testing strategies in order to replace animal testing. In order to achieve that goal we have to identify crucial and specific mechanisms involved in the sensitization and elicitation process. Putting together the increasing number of pieces of the puzzle will help to better explain the pathology of ACD. It is to be expected that certain fundamental mechanisms, such as some aspects of innate responses are common for ACD, infectious and autoimmune diseases or cancer. Thus, drugs that were originally designed for the treatment of one disease may also work in others. One of the main lessons learned is that interference with DAMP- or PAMP-dependent danger signals may be a key to the solution of many problems in inflammation-associated diseases.120,121

It remains extremely fascinating to see how chemicals and metal ions, as non-infectious and non-replicating agents, trigger innate and adaptive immune responses, as well as stress responses very much like pathogens.

Riassunto

Risposte immunitarie innate e adattative nella dermatite da contatto: analogia con le infezioni

La dermatite allergica da contatto (ACD) è una patologia della cute di natura infiammatoria di grande interesse ed in costante aumento essendo un problema di salute occupazionale. Questa malattia è indotta da agenti chimici e ioni metallici che penetrano nella cute e formano complessi con proteine dell’ospe. Questo processo è accompagnato da una forte reazione infiammatoria allergene-indotta e determina la migrazione di cellule dendritiche (DC) portatrici dell’antigene dalla cute ai linfonodi regionali, dove promuovono la generazione di cellule T allergene-specifiche. Queste ultime sono le cellule fondamentali nel generare la patologia. La ri-esposizione all’agente causale determina il reclutamento delle cellule T effettori, che a loro volta provo-cano la tipica reazione infiammatoria cutanea a livello del sito di contatto. Sebbene DC e le cellule T effettori rivestis-cano un ruolo fondamentale nella fase di sensibilizzazione e nella fase di sviluppo della ACD, rispettivamente, altri tipi cellulari tra cui i cheratinociti, le cellule NK, le ma ce-cellule e le cellule B contribuiscono alla patogenesi della patologia. In questa revisione dei dati della Letteratura, gli Autori rias-sumono le recenti acquisizioni riguardanti le risposte allo stress e le risposte immunitarie innate scatenate dagli allergeni di contatto e forniscono una rassegna dei dati recenti che riguardano la risposta adattativa delle cellule T. Questi nuovi dati sono stati raccolti soprattutto da studi condotti sul l’ipersensibilità da contatto (CHS), il corrispondente modello sperimentale murino della ACD umana. La spiegazione degli eventi molecolari coinvolti nelle risposte innate indot- te dagli allergeni di contatto aiuterebbero a disegnare nuove strategie di trattamento e permetterebbero di sviluppare delle analisi in vitro predittive per l’identificazione degli allergeni da contatto.


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63. Heat shock proteins HSP27 and HSP70 are present in the skin and are important mediators of allergic contact hypersensitivity. J Immunol 2006;177:685-72.
Despite the rising incidence of melanoma in the Caucasian population, there has not been a concomitantly dramatic increase in mortality, which is due, in part, to the advent of better tools that have been made available for the early detection of melanoma. This article presents an overview of some of the recent diagnostic developments that are of potential interest to practicing dermatologists. Some of these diagnostic advances include: total body photography; dermoscopy; multispectral imaging; confocal scanning laser microscopy; teledermatology; high-frequency ultrasound; computed tomography; magnetic resonance imaging; immunohistochemical stains; comparative genomic hybridization; microphthalmia transcription factor; and melanoma sniffing dogs. Although not all of these tools are uniformly accepted nor mandatory, a passing familiarity with them will be helpful as additional data regarding their use evolves.

Keywords: Melanoma-diagnosis - Dermoscopy - Microscopy, confocal - Tomography, X-ray computed - Magnetic resonance imaging - Comparative genomic hybridization.

Despite the rising incidence of melanoma in the Caucasian population, there has not been a concomitantly dramatic increase in mortality, which is due, in part, to the advent of better tools that have been made available for the early detection of melanoma. This article presents an overview of some of the recent diagnostic developments that are of potential interest to practicing dermatologists.

Recent diagnostic developments

Magnifying lens, Wood’s light and UV photography

Magnification can detect features to help differentiate a melanoma from other neoplasms. For example, a seborrheic keratosis would demonstrate intact skin tension lines as well as comedo-like openings. The Wood’s lamp emits a wavelength in the UV spectrum (360 nm) that is strongly absorbed by melanin. This feature aids in the detection and delineation of the borders, for example, of lentigo maligna melanoma. It may also have a role in detecting a regressed primary tumor in a patient presenting with metastatic melanoma of unknown primary origin.

UV photography utilizing cameras which emit light between the wavelengths in the 320-390 nm range can
also aid in evaluating pigmented lesions. The UV absorption by epidermal melanin may help detect melanomas.1

**Total body photography (mole mapping)**

Baseline clinical photography is useful to follow patients with numerous pigmented lesions. Those lesions without significant clinical change may continue to be monitored and, thus, may decrease the number of unnecessary biopsies in patients with multiple moles or dysplastic nevus syndrome. To perform this one needs to consider the photographic equipment, patient anatomic poses and the storage of digital images. The patients are given their photographic prints to keep for their personal routine monitoring.

Currently, a topodermatographic sequential image analysis system is being developed to measure subtle clinical changes in the appearance of selected pigmented lesions in a body region over time.1 In addition, automated systems are being developed for three-dimensional skin imaging to automatically detect new or changing lesions.1

**Dermoscopy**

The art of dermoscopy (also called dermatoscopy, epiluminescence microscopy or skin surface microscopy) utilizes a handheld dermatoscope, which requires the application of a liquid (oil, alcohol or water) to the skin surface in order to transilluminate the skin and to minimize light refraction and diffraction. Thus, the epidermis becomes especially translucent and allows the in vivo visualization of any changes in the anatomic structures of the epidermis and papillary dermis not able to seen with the naked eye. There are clinical criteria based on the changes identified which, when interpreted appropriately, can increase the diagnostic accuracy for diagnosing melanoma by an estimated 10% to 20% and can help to distinguish melanoma from other benign cutaneous tumors.1, 2

Dermoscopy can also be performed without fluid immersion using a polarized light microscope. However, polarized dermoscopy is inferior in terms of illumination and resolution as compared to direct contact dermoscopy. Currently, research is ongoing to evaluate the efficacy of dermoscopy in predicting melanoma thickness and in detecting early melanoma foci developing within congenital melanocytic nevi or dysplastic nevi.1, 2

**Multispectral imaging**

Multispectral images are sequences of images taken at different wavelengths of light from the infrared to the near UV range. Currently, two systems — MelaFind and SIAscope (spectrophotometric intracutaneous analysis scope) — use these multispectral dermoscopic images as data for computer analysis. The difference between these two modalities is that MelaFind utilizes a completely automated system, whereas the SIAscope generates graphs which require a physician to translate the data into a diagnosis.1

The SIAscope system performs an in vivo skin examination by capturing images at 4 different filtered wavelengths ranging from 400 to 1,000 nm peaks. These images provide a wealth of information regarding the concentration and distribution of collagen, melanin and hemoglobin.1 The information is displayed in different SIA graphs and topographic images to aid the physician in the diagnosis. With a reported specificity and sensitivity of 80% and 83%, respectively, this instrument not only may help diagnose melanoma, but may also be able to predict the melanoma thickness.1

Similar to the SIA system, the MelaFind also uses multispectral illumination but with 10 different narrow-spectrum wavelengths. The imaging probe creates the digital images and rapidly (within 3 seconds) transmits them for computer processing. The goal of MelaFind is to perfect a completely autonomous, nonoperator-dependent instrument to diagnose melanoma. So far, studies report a sensitivity of 95% to 100% and a specificity of 68% to 85%.1-5

**Confocal scanning laser microscopy**

Confocal scanning laser microscopy (CSLM) allows for noninvasive in vivo imaging of the epidermis and dermis by focusing a laser beam in the visible or near-infrared wavelength on a selected point on the skin. The light beam reflected from that focal point passes through a pinhole-sized spatial filter and is then scanned through a 2-dimensional grid to create horizontal microscopic sections. A series of these horizontal planes are then stacked vertically with an axial thickness of 2 µm to 5 µm, which correlates almost directly with the axial thickness of excised histologic sections.

The standard depth of normal skin which is imaged by CSLM is limited to the level of the papillary dermis,
which averages between 200 \( \mu \text{m} \) to 300 \( \mu \text{m} \). Since the ability to detect depth correlates to the penetrating depth of the wavelength of laser light used,\(^1\, 5\, 6\) longer wavelengths may also be utilized to allow for deeper visualization of deeper dermal tumors.

There are two modes for using CSLM — reflectance and fluorescence mode. The reflectance mode depends on the innate differences in the reflectivity of structures, such as cytoplasmic pigmentated and nonpigmented melanosomes. The fluorescence mode depends on the varying distribution of endogenous or exogenous fluorophores to provide the tissue contrast. The fluorophore is excited by a laser light source and emits a signal that is detected in gray scale. Since only light emitted from the exact focal plane of interest returns to the detector through the pinhole, CSLM is able to target certain specific subcellular structures or proteins of interest. Studies utilizing animal melanoma models have demonstrated increased production of certain pathologic proteins in vivo, which may help detect melanoma in the future.\(^1\)

The usefulness of CSLM for the practicing dermatologist lies in the fact that, since its technology allows for the readily accessible and noninvasive in vivo examination of cellular components of skin approaching histologic accuracy, lesions suspicious for melanoma and patients with multiple atypical nevi may someday be monitored with greater confidence than is possible with visual examination alone.

High-frequency ultrasound

High-frequency ultrasound has attained popularity in Europe in recent years as a standard diagnostic technique, but has found limited acceptance in the United States. The ultrasound images are created when high-frequency sound impulses are transmitted into the skin and then reflected, refracted or inflected when tissue of a different acoustic property is encountered. The amplitude of the intensity of the reflections at different skin depths are plotted onto an A-mode (amplitude display) graph. B-mode (brightness display) scanning incorporates the brightness intensities of multiple A-scans to build a 2-dimensional image. The experimental 3-dimensional C-mode (computed) scans may offer superior imaging capabilities than B-scans.

The frequency of the ultrasound transducer dictates the resolution and penetration of the imaging system. Dermatologic ultrasonography generally utilizes the 20 mHz ultrasound and higher frequency transducers (50-100 mHz) to evaluate melanocytic lesions,\(^1\, 6\-10\) Lower frequency 3 to 10 MHz scanners are utilized to examine lymph nodes and subcutaneous tissue.\(^1\, 11\, 12\) The higher frequency scanners offer higher resolution but poorer tissue penetration.\(^1\)

The 20 MHz level is regarded to be the optimal compromise between resolution and tissue depth.\(^1\, 13\) Melanomas typically appear as solid hypoechoic lesions on ultrasound, and ultrasound has been utilized to determine the thickness, volume and vascularity of melanomas to aid in preoperative planning. However, ultrasound can erroneously overestimate tumor thickness due to adjacent obstructive structures such as hair follicles, glands, lymphocytic infiltrates or nevus fragments. Conversely, ultrasound can underestimate the melanoma tumor thickness due to tumor ulceration or if clusters of melanoma cells extend deep into the reticular dermis. These limitations restrict the ultrasound’s role as a consistently reliable diagnostic modality for melanoma.

High-resolution ultrasound (20 MHz B-scan) is not capable of distinguishing between melanoma and benign pigmented lesions because both are typically hypoechoic. Furthermore, ultrasound is not reliable for discriminating between the different types of malignant skin neoplasms, as basal cell carcinoma, squamous cell carcinoma and melanoma all share similar internal echo properties.\(^13\, 14\) One promising technique, called laser Doppler perfusion imaging, combines the use of color Doppler sonography with gray-scale imaging to assess intratumoral and peritumoral vasculature, and may improve on the diagnostic and discriminatory properties of ultrasound in the future.

Computed tomography and magnetic resonance imaging

New imaging modalities recently introduced include spiral computed tomography (CT), total body magnetic resonance imaging (MRI) and positron emission tomography combined with CT (PET-CT). These techniques offer high resolution with a high sensitivity for detection of metastatic disease and are all widely used in various oncologic settings for imaging internal structures. Whereas spiral CT and MRI can detect early metastatic lesions with a diameter of 2-3 mm, PET-CT can recognize disseminated lesions of 1 cm or greater.\(^2\) For skin neoplasms arising in areas where the skin and subcutaneous tissue are relatively thin, allowing early invasion into bone (such as the scalp), CT scan-
ning can be of significant value in discerning bony invasion preoperatively. A high rate of false-positive readings is a shared disadvantage with these techniques. To avoid false-positives, careful comparison with previous images, when available, and follow-up imaging are often required. In particular, PET-CT scans have frequent false-positive findings, and are frequently overutilized in very early stage melanoma — leading to significant patient anxiety with no real gain in improved staging accuracy.

Teledermatology

Since dermatology is among the most visual of the medical specialties, it is well-suited for modern teledermatology techniques. Recent studies have demonstrated that teledermatology conferences highly correlate to the diagnosis and treatment plans derived from face-to-face examinations. Teledermatology has multiple uses, such as: 1) triage; 2) diagnostic and treatment services; and 3) dermatologic consultations for primary care physicians. It is commonly set up as a system for primary care practitioners, nurse practitioners or physician extenders to transmit clinical images and the patient history to a consulting dermatologist as a second opinion or for triage referrals. The technical systems available in teledermatology include real-time videoconferences and store-and-forward (SAF) systems. One disadvantage of real-time videoconferencing, although it is a reliable alternative to a face-to-face consultation, is that it is time-consuming and expensive. SAF systems offer a faster and less expensive method of transmitting crucial data (through email or via a web-based system) to a distant consultant. Teledermatology provides obvious benefits including improved access to dermatologic care and decreased costs of providing medical services. It shortens the waiting time for patients who need to see a specialist as well as alleviating the dermatologist shortage in underserved areas. To ensure the success of teledermatology, the systems used must be easily accessible 24 hours a day and must preserve the confidentiality of the patient medical information.

Teledermoscopy was demonstrated to maintain a 91% concordance between the face-to-face physical examination and the diagnosis achieved by remote access. It is most effective as a triage system. Without patient contact, it has been demonstrated that the examination of lesions (including dermoscopy) was associated with inappropriate management of about 30% of equivocal melanomas. Nevertheless, teledermoscopy shows great promise in the evaluation of suspicious pigmented lesions.

Mobile teledermatology is possible with the advent of wireless telecommunication and takes advantage of the imaging capabilities of mobile devices, such as cellular phones and personal digital assistants (PDAs). This method enlists patient participation and is useful as a triage system for acute dermatologic eruptions. Furthermore, this modality also may prove useful in the management of patients with chronic skin disorders who require systemic therapy, such as psoriatic patients or patients with atopic dermatitis on systemic immunomodulator therapy.

Another system that takes advantage of acquiring clinical images with cellular phones and PDAs is teledermatopathology. Ideally, this modality would enlist the patients with multiple moles to maintain an active role in screening for any suspicious pigmented lesions and to aid in the early detection of malignancies. However, there are numerous legal considerations regarding the standardization of clinical criteria and the definition of the doctor-patient relationship which would have to be resolved before this system is universally implemented.

Teledermatopathology has been performed with both real-time image transmission (utilizing a robotic microscope) and with the SAF procedure. Using the SAF procedure, teledermatopathology has an 80% concordance rate, which increased to 84% when the clinical history was supplied and further increased to 99% with the additional information supplied. The recent advent of the virtual slide systems (VSS) has allowed the slide to be digitized at a high resolution, which can then be stored on a server and made available on the Web.

Melanoma-sniffing dogs

An intriguing story in the April 1, 1989 edition of The Lancet reported that a dog accurately detected a malignant melanoma on its owner. Consequently, experiments were conducted in Tallahassee, Florida testing the olfactory capabilities of a gray-haired schnauzer named George to detect melanoma. After the dog was trained to recognize a tube containing a melanoma sample, it subsequently correctly identified the test tube containing melanoma 99% of the time. Subsequently, the dog correctly identified melanomas in four out of seven patients who were suspected of having suspicious pigmented lesions.
This observation has led to the question whether melanoma emits a specific odor, undetectable by humans, but recognized by dogs. Dogs have 220 million olfactory cells, whereas humans possess only 5 million. Thus, it is not unreasonable to surmise that dogs may possess the capability to be trained to hone in on a specific scent associated with cutaneous malignancy which is completely undetectable by man.

One viable hypothesis is that the major histocompatibility complex (MHC) genes in tumor cells produce specific organic chemicals that are detectable by dogs. This hypothesis is supported by two bodies of evidence. The first data demonstrate that the human leukocyte antigen (HLA) molecules produce soluble derivatives that are secreted in body fluids including sweat, blood and urine. Secondly, it is known that tumors are associated with changes in HLA expression. The cancer may escape detection by the immune surveillance system through the alteration of HLA molecules. This mechanism is analogous to the immune tolerance phenomenon observed during fetal development. For example, malignant transformation is usually associated with the low expression of classical HLA class I molecules (HLA-A, HLA-B, and HLA-B) but high expression of HLA-G and HLA-E. Studies have recently demonstrated that unique human odors can be detected by an “electronic nose” in serum. Therefore, the fact that there may be odors emitted by melanoma cells and detectable by dogs could someday move out of the realm of tabloid journalism and into a legitimate area of research and development.

**Postbiopsy assessment techniques**

Although the modalities that have been reviewed focus primarily on aiding the clinician in diagnosing melanoma through non-invasive techniques, at times it is near impossible to preemptively make a definitive diagnosis of melanoma prior to the biopsy. Furthermore, it is, at times, challenging for the dermatopathologist to render a diagnosis with complete confidence based on the histologic features alone. Thus, it is helpful to also mention techniques which aid in diagnosing melanoma in biopsy tissue specimens.

**Comparative genomic hybridization**

Over 95% of primary melanomas demonstrate gains or losses of portions of chromosomes, whereas benign melanocytic nevi generally retain their intact genome. Comparative genomic hybridization (CGH) was developed to assess the inherent chromosomal abnormalities present in melanoma and to utilize these differences to distinguish melanomas from benign melanocytic nevi. CGH evaluates the genome from routinely fixed tissue to detect and map the chromosomal aberrancies which result in the change of DNA copy number. The procedure involves obtaining two distinctively fluorescently labeled DNA populations (one from the tumor tissue and one from healthy control donor tissue) and allowing them to hybridize onto a normal metaphase chromosome substrate. A “blocking DNA” population enriched for repetitive genomic regions is also added to prevent unwanted cross-hybridization between repetitive regions in the labeled DNA populations and the substrate.

Amplification of the fluorescence signal occurs if the tumor population demonstrates a copy number gain — the ratio of tumor to reference fluorescence intensity thereby exceeds 1. Amplifications typically correspond to short genomic regions which contain certain oncogenes necessary for tumor growth. Conversely, a ratio of tumor to reference fluorescence intensity at a particular region that is less than 1 indicates a deletion, which may represent lost tumor suppressor genes. The distinct advantage of CGH over conventional cytogenetic analysis is that CGH may be performed on formalin-fixed, paraffin embedded tissue and does not require cell cultures of fresh tumor tissue for chromosomal analysis. One limitation of CGH is that it detects only those genetic mutations which result in DNA copy number changes — for example, point mutations and balanced translocations are not detected.

Although histopathology remains the cornerstone for the diagnosis of melanocytic tumors, CGH has been advocated as a tool in those cases with histologically equivocal diagnoses, such as atypical Spitz nevi, recurrent melanocytic nevi, melanocytic nevi in the genital, acral, or mammary line regions, irritated or sun-exposed nevi and proliferating congenital nevi. Differing CGH analyses patterns have also been associated with various melanoma subtypes, such as mucosal melanomas and acral lentiginous melanomas. These different genetic compositions in the melanoma subtypes are dependent on the anatomical location and sun-exposure pattern and may provide insight into developing future therapeutic approaches.
agents that target certain melanoma subtypes exclusively.24

**Immunohistochemical staining**

Since malignant melanomas do not consistently express melanocytic gene products, the recognition of these potentially deadly carcinomas pose a diagnostic challenge. In general, antigens on melanoma cells include those which are specific for melanoma and those which are nonspecific for melanoma. Examples of highly specific melanoma markers include MAGE-1 and MAGE-3, which belong to the “embryonal cancer testis antigen” family.30, 31 However, the more widely used immunohistochemical markers belong to the latter category of markers which are very sensitive but not specific for melanoma. These particular markers are consistently found on melanoma cells, but are also expressed by other benign and malignant tissues. Historically, the three most popular immunohistochemical markers have been HMB-45, MART-1, and S-100.

Melan-A is a protein product of the MART-1 gene that is expressed on both melanocytes and in melanomas. Consequently, monoclonal antibodies against Melan-A react against both benign and malignant melanocytic lesion.32 Anti-Melan-A immunoreactivity is useful in diagnosing melanomas with spindle-cell differentiation, which is not detected by HMB-45 staining. In addition, some studies have demonstrated that MART-1 expression by melanoma micrometastasis in sentinel lymph nodes correlates with poorer survival.33

Microphthalmia transcription factor (MITF) is a transcription factor of the tyrosinase gene. This nuclear protein plays a vital role in the development, survival and differentiation of melanocytes.34 It is believed that MITF contributes to melanocytic survival by increasing the expression of the BCL-2 gene, which is an antiapoptotic component.35 It also modulates extracellular signals, such as those engendered by alpha-MSH and c-Kit ligand.36 MITF expression is conserved in primary and metastatic malignant melanomas. Studies have utilized a monoclonal antibody generated against human MITF to demonstrate that MITF appears to be a highly sensitive and specific melanocytic marker.37, 38 MITF immunostaining has been demonstrated to be positive in both benign nevi and non-melanocytic tumors in addition to melanomas; thus, MITF does not distinguish between benign and malignant melanocytic lesions.39 Decreased MITF staining intensity was observed in Spitz nevi and neurotized nevi.40 All primary cutaneous melanomas were positive with the exception of desmoplastic melanomas.39 Similar to other melanocyte-associated antigens, the usefulness of MITF in detecting desmoplastic melanomas is limited. However, MITF discriminates between spindle cell nonmelanocytic tumors and melanomas with a spindle cell morphology.34 Hence, the studies demonstrate that MITF is a specific nuclear marker for melanocytes, and the immunostain for MITF has proved to be very useful in diagnosing melanoma when the other traditional melanocytic markers, specifically, HMB-45 and S-100, are negative.41 MITF expression has also been linked to prognosis of intermediate thickness-melanomas.40 The sensitivity and specificity of combined testing for MITF (95%) and Melan-A (100%) was demonstrated to be superior to that of combined S-100 (80%) and HMB-45 (100%).41

**Conclusions**

In essence, it must be stressed that the diagnostic techniques outlined can serve as useful adjunctive (not sole) resources for the practitioner in clinically assessing pigmented tumors. Certainly, they are not mandated in the course of delivering the “standard of care” in the workup of a node or a suspected melanoma. Although there are no randomized trials which objectively assess or compare the efficacies of each of these modalities, it is worthwhile to be aware of them in our arsenal of clinical tests to aid us in our ever-present challenge to diagnose melanoma as accurately and as efficiently as possible.
rativa; l’analisi del gene microphthalmia transcription factor; e cani “che sniffano” melanoma. Sebbene non tutti questi strumenti siano uniformemente accettati né mandatori, una maggiore familiarità con questi sarà utile per ottenere ulteriori dati aggiuntivi circa le loro possibilità di impiego.

Parole chiave: Melanoma, diagnosi - Dermoscopia - Microscopio confocale - Tomografia computerizzata - Risonanza magnetica - Irridazione genomica comparativa.

References
Administration of heparanase-III improves the survival and angiogenesis of rat skin autografts

Aim. Heparanase, a glycohydrolase enzyme, cleaves heparan sulfate in the tissue matrix. Heparan sulfate degradation causes the release of angiogenic and growth factors, leading to angiogenesis. The aim of this paper was to evaluate the angiogenic effect of heparanase-III administration on skin autograft healing in rat.

Methods. Four groups of 14 adult male Charles River rats were enrolled. Full thickness skin autografts (15 mm in diameter) were made on the interscapular region of each rat. After 24 hours, 0.1 cc of heparanase, at the three concentrations of 0.1, 0.2, and 0.4 units, were injected intradermally into the grafts of each of the three case groups. The control group received an equal volume of the vehicle (buffered phosphate solution). After 5 days, biopsy specimens from skin grafts of 5 randomly selected rats of each group were submitted for histological studies.

Results. The concentration of vessels (with 5-50 mm in diameter) in the grafts of the groups receiving 0.2 and 0.4 units of heparanase-III was significantly more than that of the control group (100.43±11.24 and 95.85±12.44 vs 71.42±5.22 vessels/mm²; P<0.05). The graft survival time of the group that received 0.2 U heparanase-III was significantly longer than that of the control group (15.43±0.72 vs 13.23±0.69 days; P<0.05).

Conclusion. Heparanase-III administration improves the healing of rat skin autografts through induction of angiogenesis.

Key words: Angiogenesis inhibitors - Heparanase - Survival.

Protoglycan molecules are important components of the connective tissue. They exist in different parts of the extracellular matrix, especially in the dermis and the basement membrane of blood vessels. Protoglycans include chondroitin sulfate, heparan sulfate, dermatan sulfate and keratan sulfate.1

Protoglycans act as mechanical barriers towards the invasion of cells and microorganisms. Different kinds of growth factors such as endothelial growth factor and fibroblast growth factor attach to protoglycan molecules.2 As protoglycans are degraded and broken, these molecules are released and stimulate migration and proliferation of cells, especially endothelial cells. Naturally, in the process of wound healing, endothelial cells produce enzymes that break and proteolyze protoglycans, especially heparan sulfate, and consequently induce angiogenesis and new vessel formation. Heparanase is one of these enzymes, which is also produced and released in tumoral tissues and contributes to the growth of tumors by facilitating angiogenesis.3 Heparanase is an endo-ß-D-glucuronidase that is capable of cleaving heparan sulfate side chains at a limited number of sites, yielding heparin sulfate fragments of appreciable size (5-7 kDa). Heparanase-mediated extracellular matrix degrada-
tion and remodeling traditionally have been correlated with the metastatic potential of tumor-derived cell types. Similarly, heparanase has been shown to facilitate cell invasion associated with autoimmunity, inflammation and angiogenesis. In addition to being directly involved in the enzymatic machinery that orchestrates in cellular invasion, heparanase activity releases extracellular matrix resident, HS-bound polypeptides, converting them into bioactive molecules. The multitude of potential mediators capable of being released by heparanase is of particular importance in the complex setting of wound healing.

The relationship between heparanase and angiogenesis is firmly documented. Most of the research that has been performed about heparanase have focused on the inhibition of it and consequently stopping the process of angiogenesis and limiting the tumor growth.

In this research we used three different concentrations of heparanase-III (heparan sulfate proteolysing enzyme) to evaluate its effect on skin autograft survival and angiogenesis in rat.

Materials and methods

Fifty-six male adult Charls River rats with average weights of 180-220 grams, obtained from the Animal House of the Shiraz Medical School, were enrolled in the study. The animals were kept in standard conditions of temperature (22 °C), humidity (60%), nutrition (ad libidum), ventilation (12 circles/hr), and light (12 hours of light, 12 hours of darkness) for 10 days prior to the study in order to accommodate to this situation. The 56 rats were then divided into four equal groups of 14; three groups served as case groups and one as the control group.

The animals were anesthesized for skin autograft operation using intramuscular ketamine, 90 g/Kg, and xylazine, 5 g/Kg. Then in sterile situations full thickness skin was harvested from the backs of the animals, defatted in sterile petry dish and sutured at its original site with 180 degrees rotation. Dressing with topical tetracycline was applied at the end of the procedure. Heparanase-III was obtained from Sigma Company and diluted in three concentrations, namely 0.1, 0.2 and 0.4 units. Buffered phosphate solution was used as the vehicle. 0.1 cc of each solution was injected subcutaneous into the grafted sites of each of the three case groups one day after the operation. The control group received the same amount of the vehicle.

Five days after the operation, 5 randomly selected rats in each group were sacrificed and biopsy specimens were taken from the grafted sites and prepared for histological study. The rest of the rats were followed and the skin grafts were observed daily for the detection of possible failure.

The histopathlogic slides were reviewed for the concentration of blood vessels with the diameters of 5-50 micrometer and finally the results were analyzed using the SPSS program.

Results

The average skin graft survival time in each group is shown in Figure 1. As shown, using the Tukey HSD test, the survival times of the heparanase receiving groups were more than the control group, being statistically significant in the 0.2 U concentration of heparanase (P<0.05).

Using the oneway ANOVA and Posthoc tests, the concentration of blood vessels measuring 5-50 micrometer in diameter was also increased in the heparanase groups in comparison to the control group (Figure 2), being statistically significant in the 0.2 and 0.4 units of heparanase (P<0.05). All the grafts, however, failed to survive.

Discussion

Our preliminary study shows that heparanase improves rat skin autograft survival time and neovascularization.
Heparan sulfate is the most abundant glycosaminoglycan present in the epidermis. The exact function of heparan sulfate proteoglycans in the epidermis is largely unknown; nevertheless, they are thought to be involved in a variety of processes such as cell-cell interaction, cell adhesion, proliferation, differentiation, morphogenesis, and extracellular matrix structural integrity. Modulation of the physiological functions of heparan sulfate proteoglycans is attributed in part to the activity of the endo-β-D-glucuronidase enzyme, heparanase. Heparanase expression is noted to be restricted to the plantar stratum corneum of human and rat epidermis. In resting conditions, expression of heparan sulfate is essentially confined to the basement membrane between the dermis and epidermis. Heparanase localization is significantly altered during wound healing and appears in most keratinocytes adjacent to the wound margins and in the migrating tip of the wound.4

Heparanase is an endo-β-D-glucuronidase involved in extracellular matrix remodeling and degradation. It is implicated in tumor metastasis, angiogenesis, inflammation, and autoimmunity. The enzyme is synthesized as a latent 65-kDa protein and is processed in the lysosomal compartment to an active 58-kDa heterodimer, where it is stored in a stable form. In contrast, its heparan sulfate substrate is localized extracellularly, suggesting the existence of mechanisms that trigger heparanase secretion.5

Other than the critical role of heparanase in tumor invasion and metastasis, it is believed that heparanase is involved in angiogenesis, another feature of tumor progression which is complicatedly mediated by many molecules, including cyclooxygenase-2 (Cox-2).6 Inhibition of heparanase stopped the growth and progression of tumors by inhibiting angiogenesis.7 Heparanase degrades heparin sulfate, thereby releasing the growth factors attached to it with the resultant induction of angiogenesis and improvement of skin graft survival.1-3 These results show increased blood vessel concentration and graft survival time in the heparanase groups, especially at the 0.2 U concentration of heparanase. Figures 3, 4 are the histologic views of the biopsied specimens of the control and
0.2 U heparanase groups, respectively. Obviously, the concentration of blood vessels in the latter group is significantly more than the former.

Importantly, the concentration of blood vessels and graft survival time were less in the 0.4 U than the 0.2 U, which may possibly be due to the excessive degradation of proteoglycans by the 0.4 U concentration of the enzyme.

Conclusions

A drawback of this study was that all the grafts were failed to survive. Improper fixation of the grafts and not making incisions on them to prevent the accumulation of wound discharge between the grafts and their beds could be responsible. These points should be considered in future similar animal studies in order to prevent this drawback.

This preliminary study shows that heparanase improves wound healing and paves the way for using this enzyme for the treatment of chronic non-healing human wounds, such as bed sores and diabetic ulcers.

Riassunto

La somministrazione di eparinasi-III migliora la sopravvivenza e l’angiogenesi degli autotrapianti di cute nel ratto

Obiettivo. L’eparinasi, una glicoidrolasi, eliva l’eparan solfato nella matrice tessutale. La degradazione dell’eparan solfato provoca il rilascio di fattori di crescita e angiogenesi, che favoriscono l’angiogenesi. Obiettivo di questo studio era valutare l’effetto angiogenico della somministrazione di eparinasi-III sulla guarigione di un autotrapianto di cute nel ratto.

Metodi. Sono stati arruolati quattro gruppi di 14 ratti adulti di sesso maschile Charles River. Nella regione interscapolare di ogni ratto è stato eseguito un autotrapianto di cute (diametro di 15 mm). Dopo 24 ore è stato iniettato intradermicamente nel trapianto 0,1 cc di eparinasi a tre concentrazioni di 0,1, 0,2 e 0,4, rispettivamente, nei ratti dei gruppi trattati. Al gruppo di controllo è stato somministrato un volume identico di veicolo (fampruno fosfato). Dopo cinque giorni è stata eseguita una biopsia sul trapianto cutaneo su cinque ratti scelti in modo casuale da ognuno dei gruppi. I campioni biotipici sono quindi stati esaminati istologicamente.

Risultati. La concentrazione dei vasi (con diametro do 5-50 µm) nei trapianti dei gruppi ai quali erano state somministrate 0,2 e 0,4 unità di eparinasi-III era significativamente maggiore rispetto a quella del gruppo di controllo (100,43±11,24 e 95,85±12,44 versus 71,42±5,22 vasi/mm², P<0,05). Il tempo di sopravvivenza del trapianto del gruppo al quale erano state somministrate 0,2 U di eparinasi-III è stato significativamente maggiore rispetto a quello del gruppo di controllo (15,43±0,72 versus 13,23±0,69 giorni; P<0,05).

Conclusioni. La somministrazione di eparinasi-III migliora la guarigione degli autotrapianti di cute nel ratto attraverso l’induzione dell’angiogenesi.

PAROLE CHIAVE: Inibitori dell’angiogenesi - Eparanase - Sopravvivenza.

References

Dermatofibrosarcoma protuberans (DFSP) is a relatively unusual, locally aggressive cutaneous tumor of intermediate malignancy. It most frequently occurs on the trunk and proximal extremities of young adults. A small number of cases have been reported in newborns and elderly individuals. Some familial cases have been reported. The tumor arises from the dermis and invades deeper subcutaneous tissues (fat, fascia, muscle, bone). A 54-year-old woman presented with a peculiar clinical presentation of DFSP. Two biopsy specimens, one from the plaque and the other from the left raised vegetation, were obtained. Histological examination of both samples showed in the dermis and subcutis densely packed, plump spindle cells with large and oval nuclei, moderate amounts of eosinophilic cytoplasm, and indistinct cell borders. Immunohistochemically, the tumor cells were strongly positive for CD34 and focally positive for desmin and smooth muscle actin. Despite its local invasiveness, it rarely metastasizes (5% of cases). The case of a patient with a peculiar clinical presentation of DFSP is described.

Case report

A 54-year-old woman presented with a 3-year history of an asymptomatic, red-violaceous lesion on the left submammary area. This lesion, initially small and flat, had slowly extended peripherically, eventually developing a few raised outgrowths. The patient was otherwise healthy. Regional lymph nodes were not detectable and laboratory and instrumental investigations, including computed tomography (CT) scan total body, did not show any abnormalities. Two biopsy specimens, one from the plaque and the other from the left raised vegetation, were obtained. Histological examination of both samples showed in the dermis and subcutis densely packed, plump spindle cells with large and oval nuclei, moderate amounts of eosinophilic cytoplasm, and indistinct cell borders. Immunohistochemically, the tumor cells were strongly positive for CD34 and focally positive for desmin and smooth muscle actin. Despite its local invasiveness, it rarely metastasizes (5% of cases). The case of a patient with a peculiar clinical presentation of DFSP is described.

Key words: Histological techniques - Dermatofibrosarcoma - Subcutaneous tissue.
DE PASQUALE
AN UNUSUAL CASE OF DERMATOFIBROSARCOMA PROTUBERANS

mildly pleomorphic nuclei. These cells were arranged in a storiform pattern and uniformly embedded in the collagen stroma. Mitotic figures were sparse. The degree of cellular atypia was moderate. Moreover there was a diffuse inflammatory infiltration extending into the subcutis (Figures 3-5). Immunohistochemical studies revealed that spindle-shaped dermal dendritic cells were CD34 positive (Figure 6).

On the basis of clinical history, clinical presentation and histological results, a diagnosis of DFSP was made and the patient was referred for surgical excision. At one year follow-up, no local relapses or distant metastases have been detected.

Discussion

Dermatofibrosarcoma protuberans (DFSP) was first described by Darier and Ferrand in 1924 as “Progressive and recurring dermatofibroma”. One year later, Hoffman officially coined the term “Dermatofibroma protuberans”.

DFSP accounts for less than 0.1% of all malignant neoplasms. Incidence has been estimated between 0.8-5 cases per 1 million population per year. It usually occurs between the second and fifth decades of life, rarely it has been reported in newborns and elderly individuals. Some familial cases have been reported. Several studies reveal an almost equal sex distri-
bution, with a slight predominance in men; in a large study of 902 cases of DFSP, 514 patients (57%) were male and 388 (43%) were female. It is a non-inherit-
ed condition that has been reported in all races, with-
out racial predilection, although, an uncommon pig-
mented variant of DFSP, called Bednar tumor, is pre-
dominantly found in black patients.

Currently, the cause of DFSP is unknown. In 10-
20% of patients diagnosed with this tumor, a patho-
genetic role of local trauma has been claimed. Surgi-
cal and old burn scars, as well as sites of vaccinations, have all been reported as sites of DFSP.

The histogenesis of this tumor is still controversial. Although commonly regarded as belonging to the fibrohistiocytic group of tumors, histologic typing techniques have provided conflicting results and there is evidence that this tumor may arise from histiocytes, fibroblasts, and perineural or endoneural cells. Therefore, several investigators have postulated that DFSP may derive from an undifferentiated mesenchymal cell. Ultrastructurally this theory is supported by the presence of cells sharing some features of both fibroblasts and histiocytes. Moreover, it has been theorized that the direction of cell differentiation may reflect on the biologic behaviour of DFSP. Thus, tumors with a predominant fibroblastic component would be more likely to remain locally aggressive, whereas tumors with histiocytic foci or histiocytic predominance would have a greater propensity to metastasize.

Cytogenetic studies have shown that chromosomal

Figure 4.—Characteristic spindle cells in a storiform pattern at higher magnification (HE 25×).

Figure 5.—Histological examination (HE 10×) of vegetant and hanging proliferative lesions showing the same aspect of the plaque.

Figure 6.—Immunohistochemical studies revealing CD34 positivity of the spindle-shaped dermal dendritic cells.
aberrations may contribute to the pathogenesis of DFSP. These studies have revealed specific alterations in DFSP tumor cells, such as reciprocal translocations of chromosomes 17 and 22, t(17;22), and supernumerary ring chromosomes composed of interspersed sequences from chromosomes 17 and 22. These rearrangements fuse the platelet-derived growth factor B-chain (PDGFB, c-sis proto-oncogene) and the collagen type 1 alpha 1 (COL1A1) genes. PDGFB and COL1A1 fusion noted in DFSP may contribute to tumor development through the ectopic production of PDGF-BB and the formation of an autocrine loop, as increased cell proliferation has been demonstrated in cultured DFSP cells exposed to PDGF-BB.7,12,13

DFSP is a very slowly growing tumor. Location is predominantly on the trunk and proximal extremities, but occurrences on most body sites have been reported (face, neck, scalp and sole).14,15 It clinically appears as an asymptomatic, indurated plaque that may be violaceous, red-brown, or flesh-colored. Although DFSP typically ranges in size from 1 to 5 cm, neglected lesions may grow as large as 20 cm in diameter and have multiple satellite nodules. Characteristically, the tumor is fixed to overlying skin and not to deeper structures. Recurrent or long-standing tumors, however, may invade fascia, striated muscle, and bone.7 During pregnancy it has an accelerated growth.4

DFSP typically arises as a solitary lesion but multiple primary lesions have also been reported. Over a period, which varies from a few months to decades, DFSP slowly and relentlessly enlarges and develops protuberant nodules within the plaque. Once nodules appear, growth is often accelerated and the tumor may ulcerate, bleed, or become painful in 10% to 25% of cases.1,5

Uncommon clinical variants of DFSP are atrophic or morphea-like DFSP and pigmented DFSP or Bednar tumor. The atrophic variant of DFSP is characterized by dermal atrophy of more than 50% of the locoregional dermis, and may clinically appear as an atrophic or depressed plaque.16,17 The Bednar tumor is a rare neoplasm (1-5% of all cases of DFSP) characterized by spindle cells arranged in a storiform pattern admixed with a variable population of melanin-containing dendritic cells that may clinically appear as a pigmented lesion.18,19 The former is more often observed in females without age predilection, whereas the latter is found predominantly in infants.

The histological diagnosis of DFSP is often easy. The more characteristic histologic findings include the high cellularity and irregular short intersecting bands of tumor cells forming a storiform pattern. Cells radiating from a central hub of fibrous tissue forming a cartwheel pattern are also typical. The degree of cellular atypia is moderate. Some cases may be difficult to distinguish from other fibrohistiocytic neoplasms and, in such instances, the unique immunohistochemical staining patterns of DFSP may help to clarify the diagnosis. Immunohistochemistry studies have shown that DFSP is CD34 positive and factor XIIIa and S-100 negative. These findings differentiate this neoplasm from factor XIIIa positive dermatofibroma and S-100 positive neurofibroma.20

Conclusions

In the literature we were unable to find any case of DFSP showing raised hanging outgrowths within the plaque as we observed in our patient. Interestingly, this peculiar clinical aspect was not found on histopathology to be related to fibrosarcomatous changes neither to an aggressive behaviour or metastatic potential. Therefore, it should be regarded only as an atypical clinical manifestation of DFSP without any negative prognostic implications.

Riassunto

Dermatofibrosarcoma protuberans

Il dermatofibrosarcoma protuberans (DFSP) è un raro tumore cutaneo localmente invasivo di malignità intermedia che si localizza principalmente al tronco e alle estremità soprattutto in giovani adulti di sesso maschile. Il DFSP origina dal derma e invade i tessuti sottocutanei profondi (tessuto adiposo, facce, muscoli, ossa), ma, a discrezione della notevole invasività locale, raramente metastatizza (5% dei casi). Attualmente, l’eziologia non è nota. Una donna di 54 anni giungeva alla nostra osservazione per la presenza nella regione sottomammaria sinistra di una placca ovale di colore ros-
so-violaceo, asintomatica. La lesione presentava bordi net-
ti e due proliferazioni pendule sovrastanti, di cui una color
carne e a superficie liscia e l’altra di colore rosso e a super-
ficie mammellonata. L’esame istologico e immunistoichi-
metrico confermavano la diagnosi di DFSP: questo caso atipi-
co di DFSP è presentato e discusso.

PAROLE CHIAVE: Tecniche istologiche - Dermatofibrosarcoma - Tessuto sottocutaneo.

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Disseminated herpes simplex infection in a HIV+ patient

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Genital herpes, a viral infection caused by Herpes simplex virus (HSV), is the most common cause of genital ulceration. Patients with a severe decrease in cellular immunity, such as patients positive for Human immunodeficiency virus (HIV) infection, are more likely to develop atypical, severe, disseminated and/or chronic HSV infections. On the other hand, there is an increase incidence of HIV detection among patients positive for HSV infection, as genital ulcers represent a potential portal of entry of HIV into the host. A case of a 52-year-old homosexual man with a two-month history of multiple erythematous ulcerative lesions on the perianal area, the buttocks, and the third left finger is presented. According to the clinical history, the clinical findings and the laboratory results, a diagnosis of HSV infection was made and treatment with valaciclovir was started, which led to complete regression of lesions 30 days later. The atypical features of the herpetic lesions, along with a past history of atypical pneumonitis one year prior to our observation, prompted to a diagnosis of concurrent HIV infection, later confirmed by laboratory results. Atypical and disseminated HSV infections occur relatively often in HIV+ patients. This article discusses clinical presentation, diagnosis and management of HSV infection in such cases.

Key words: Herpes genitalis - HIV infections - Immunity, cellular.

Case report

A 52-year-old homosexual man presented with multiple erythematous ulcerative lesions that had appeared two months earlier on the perianal area, the buttocks and on the third left finger.

Family history was unremarkable. Past medical history revealed an atypical pneumonitis one year before, totally regressed six months later following adequate treatment.

At physical examination multiple coalescing ovalar, oozing and partly crusted painful ulcerations were present on the perianal area and the buttocks (Figure 1). Similar lesions were also present on the ventral and the dorsal aspects of the medial phalanx of the third left finger, that appeared swollen (Figures 2, 3).

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Inguinal and axillary lymph nodes were palpable and painful.

Laboratory investigations showed the following abnormalities: hemoglobin 8.5 g/dL (n.v.: 14-18 g/dL), erythrocyte sedimentation rate (SER) 82 mm/h (n.v.: <15 mm/h), total proteins 8.7 g/dL (n.v.: 6.4-8.3 g/dL); (↓ Alb, ↑ β2 and γ).

Swab from the lesions on the perianal area and the third left finger was positive for Staphylococcus aureus.

A cytologic examination from two lesions located respectively on the buttocks and on the finger revealed in both samples several multinucleate, polymorphic and giant cells, histiocytes and neutrophils, and a few lymphocytes and plasmocytes.

Search for anti-HSV immunoglobulins (Ig) performed by ELISA and Western blot, was positive for anti-HSV2 Ig (IgM and IgG). Search for anti-HIV immunoglobulins (Ig) performed by ELISA and Western blot was also positive.

X-ray examination of the left hand showed marked bone resorption in all the phalanges of the third finger.

Histological examination of two biopsy specimens taken from the buttocks and the left hand, showed in both samples a massive granulocytic infiltrate with prevalence of neutrophils (Figure 4), multinucleate, polymorphic giant cells with a typical “frosted glass” aspect; some of these cells contained basophilic or eosinophilic nuclear inclusion bodies (Figure 5). Acantholysis and ballooning degeneration were present.
A diagnosis of HSV infection in a HIV+ patient was done and treatment with valaciclovir (1 g t.i.d. for 30 days) and topical antibiotic therapy was started, with complete clinical resolution at two months (Figures 6-8). The patient was referred to the Department of Infectious Diseases for follow-up.

Discussion and conclusions

The occurrence of genital herpes simplex lesions in HIV+ patients, as observed in our case, has frequently been reported in the literature, and this phenomenon is regarded as frequent in the immunocompromised patient. The severity of a concomitant HSV infection in HIV+ patients relies on its association with increased morbidity and mortality, as HSV may, although not frequently, disseminate hematogenously to visceral organs, causing pneumonitis, hepatitis, pancreatitis, adrenal necrosis or life-threatening disseminated infections. Genital HSV infection in HIV+ patients may present with atypical clinical features, namely genital severe superinfected ulcers, often
extremely painful, hemorrhagic and necrotic, or exophytic verrucous lesions resembling anogenital warts.\textsuperscript{5} Genital herpes has also been found to develop more often in the perianal and anal area in HIV\textsuperscript{+} patients with a history of anal intercourse. Patients may show perianal or anal pain, tenesmus, altered bowel habit, and mucoid or bloody anal discharge. Altered sensation, urinary retention and impotence may also occur. Inguinal lymphadenopathy is often present.\textsuperscript{6} Symptoms are those of a viral infection with proctitis.

Herpetic whitlow represent an HSV infection of the fingers may be observed in HIV\textsuperscript{+} patients as a result of direct inoculation or of direct spread from mucosal sites. It is usually caused by HSV1, but HSV2 whitlow may occur as consequence of manual-genital contact with an infected partner. The infected area becomes erythematous and edematous. Lesions are usually present at the fingertip and can be pustular, ulcerative and very painful. Fever and local lymphadenopathy are common.\textsuperscript{4}

Several studies have been performed in order to elucidate the pathogenesis of HSV infection in HIV\textsuperscript{+} patients. Studies on humans and mice have implicated a role for both CD8\textsuperscript{+} and CD4\textsuperscript{+} T-lymphocytes, for natural killer (NK) cells, and for inflammatory cytokines, like IFN-\(\gamma\), in mediating protection against HSV.\textsuperscript{7} Therefore, subjects with severe decrease in cellular immunity, such as HIV\textsuperscript{+} patients, are more likely to develop disseminated and chronic HSV infections. On the other hand, several epidemiological and laboratory studies have indicated that sexually transmitted diseases, especially those characterized by development of genital ulcers, such as HSV infection, enhance the transmission of HIV infection by increasing the amount of HIV shedding through genital lesions and by providing an easier portal of entry for the virus into the host.\textsuperscript{8} As a result, there is an increase in the incidence of HIV infection among patients with herpetic disease.\textsuperscript{7}

These findings highlight the need to consider a concurrent HIV infection in case of patients presenting with severe, hemorrhagic, necrotic and/or superinfected herpetic ulcerations that spread locally involving adjacent areas and that do not show any tendency to spontaneous regression. The occurrence of herpetic whitlow in HIV\textsuperscript{+} patients, as painful non-healing pustular or ulcerative lesions located on the fingertips, should also be considered. These patients need specific antiviral treatment schedules designed to prevent the recurrence of HSV infection.\textsuperscript{4, 9} HSV2 suppression may also represent a strategy to reduce the incidence and the transmission of HIV infection.\textsuperscript{10} Antiviral drugs currently available for the treatment of genital herpes include acyclovir, valacyclovir and famciclovir.

The case herein described is remarkable and deserves consideration due to the atypical features of the herpetic lesions, which were persistent and severe, resembling those of a neoplastic ulcerative skin disorder. Such clinical data, along with those of past medical history, revealing an atypical pneumonitis one year prior to our observation, suggested to rule out a concurrent immune depression in this patient, allowing to disclose a previously undetected HIV infection.

**Riassunto**

**Infezione disseminata da Herpes simplex in un paziente affetto da HIV\textsuperscript{+}**

L’herpes genitale, un’infezione virale indotta dall’\textit{Herpes simplex} virus (HSV), è la causa più frequente di ulcerazione genitale. Pazienti con grave deficit dell’immunità cellulare, come i pazienti HIV\textsuperscript{+}, sviluppano più facilmente infezioni da HSV atipiche, severe, disseminate e/o croniche. Inoltre, vi è un incremento nell’incidenza di infezioni da HIV nei pazienti con infezioni da HSV, poiché le ulcerazioni genitali rappresentano una potenziale porta d’ingresso dell’HIV. Un paziente omosessuale di 52 anni presentava da circa due mesi lesioni eritemato-ulcerative multiple localizzate alla regione perianale, ai glutì e al terzo dito della mano sinistra. In accordo con l’anamnesi, le manifestazioni cliniche e i risultati di laboratorio, veniva posta diagnosi di infezione da HSV e iniziata terapia sistemica con valaciclovir che conduceva alla completa regressione della sintomatologia dopo 30 giorni. Gli aspetti atipici delle lesioni erpetiche, insieme ad un pregresso episodio di polmonite atipica verificatosi circa 1 anno prima, facevano soprattutto per la diagnosi di una concomitante infezione da HIV, successivamente confermata dai risultati di laboratorio. Infezioni atipiche e disseminate da HSV si verificano relativamente spesso in pazienti HIV\textsuperscript{+}. Il presente lavoro tratta l’aspetto clinico, la diagnosi e il trattamento dell’infezione da HSV in tali casi.

**Parole chiave:** Herpes genitale - HIV, infezione - Cellule, immunità.

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Papillomatosis cutis lymphostatica

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Dear Sir,

We describe here two different cases of papillomatosis cutis lymphostatica.

The first case is reported in a 65-year-old man who presented with a two year history of chronic lymphedema of lower legs due to venous insufficiency and altered lymphatic system. The medical history revealed hypertension, with left-side heart failure.

On examination, brownish grey discolouration plaques, 7 cm in diameter, with indurated, verrucous hyperkeratoses and desquamation were observed on both lower legs. Both lower leg showed a non-pitting edema. Peripheral arterial pulses were palpable (Figure 1).

A 60-year-old woman presented with a history of synovial sarcoma of right heel treated 10 years ago. The right kidney was resected for a chronic interstitial glomerulonephritis 6 years ago. Clinical examination revealed edematous right foot with large hyperkeratotic plaques, made up of multiple small coalescing pink papules, on the dorsum of second and third foot-fingers (Figure 2).

Laboratory examination-clinical chemistry, hematology and urinanalysis showed no abnormality.

A biopsy from a plaque was taken for both histologic examination and human papilloma virus (HPV)-DNA research with polymerase chain reaction PCR.

Histologic examination showed thickening of the epidermis, hyperkeratosis with orthokeratosis and hyperacanthosis, and localized epidermal spongiosis. The papillary dermis showed lymphangiectasia; homogeneous connective tissue fibers and a lymphocytic infiltrate in the dermis.

HPV research with PCR was negative.

The papillomatosis cutis lymphostatica is a chronic hyperproliferative verrucous skin disorder, localized on lower leg or feet. It is caused by chronic lymphatic congestion or relapsing infection.1

The pathogenesis of papillomatosis cutis involves cutaneous inflammation, epidermal proliferation and abnormal keratinisation. The lymphatic disorder which underlines the

Figure 1.—Non-pitting edema with peripheral arterial pulses.

Figure 2.—Edematous right foot with large hyperkeratotic plaques, made up of multiple small coalescing pink papules, on the dorsum of second and third foot-fingers.
cutaneous response can be visualized and diagnosed with a non-directive lymphogram. Such an examination reveals pathologically widened lymphatics with valvular insufficiency.²

The clinical differential diagnosis of papillomatosis cutis is wide. In fact, on a vulnerable site as a lymphoedematous leg, also called "locus minoris resistentiae", other verrucous processes can appear.³ Several clinical and experimental observations demonstrate that the regional immune defect, which occur on the limb subsequently to the hampered circulation of the lymphocytes, facilitates the onset and development of tumors and infections.⁴

Butcher’s warts are benign epithelial proliferations of the skin and mucosa caused by infection with papillomaviruses. They are verrucous papules, usually multiple, on the dorsal palmar, or finger of hands and feet. The diagnosis of viral warts is made by PCR that confirm the presence of HPV-DNA in the lesion.

The cutaneous verrucous carcinoma is a subtype of low-grade squamous cell carcinoma (SCC) that is a distinct clinicopathologic entity and not simply SCC with verrucous clinical features. It may initially resemble a common wart but it is locally aggressive and only rarely metastasizes.

The distinction between verrucous carcinoma, SCC with verrucous clinical morphology, and a large persistent verruca vulgaris may be very difficult, both clinically and histologically. In fact, the presence of markedly displastic cells makes for the diagnosis of a cutaneous SCC and not for the subtype called verrucous carcinoma. A remarkable clinical improvement was observed within 2 months of treatment with a natural diuretic drugs like orthosiphon stamineus, ananas sativus, betula alba, tilia platyphyllos.

The classical therapeutic approach consists of decongestion therapy, using compression-bandages and/or a decompression pump. Superinfection is treated with antibiotics. ⁵ Topical treatment with urea-containing keratolytics improves the hyperkeratotic aspect of the papillomatosis.⁶

The cases here reported demonstrate a the remarkable therapeutic potential of natural diuretic drugs for this condition. Within 2 months of treatment, the severe expression of papillomatosis cutis lymphostatica remitted to a normal skin appearance. So far, i.e. 10 months after discontinuation of papillomatosis cutis lymphostatica remitted to a normal skin appearance. So far, i.e. 10 months after discontinuation of natural treatment, no relapse of the papillomatosis has been observed.

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Human papillomavirus type 16 detected in four periungual squamous cell carcinomas from the same patient

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Dear Editor,

Although the correlation between human papillomavirus (HPV) and cancer of skin and mucosae has been studied extensively, the etiological role of the virus has been demonstrated only in cervical carcinoma and other anogenital carcinomas and a subgroup of head-and-neck cancers.¹ On the other hand, high-risk HPV genotypes, in particular HPV-16, have been detected in different cases of squamous cell carcinoma (SCC) of the nail bed and periungual region,² ³ suggesting a possible autoinoculation from the genital tract.² ³

We describe the case of an Italian woman who developed 4 periungual SCCs in 8 years. In all 4 specimens, HPV-16 has been detected using two different polymerase chain reaction (PCR)-enzyme-linked immunosorbent assay (ELISA) methods.

A 52-year-old Italian woman was referred to our clinical service because the growth of a hyperkeratotic verrucous lesion on the right thumb. The mass extended from the proximal nail fold to the distal portion of the nail bed. The overlying nail plate appeared partially destroyed (Figure 1). There was no history of trauma, nor the patient was in discomfort. Her recent medical history revealed that she had been previously operated for three periungual SCCs. The first tumour had developed on the left thumb in 1999 and the surgery
performed had led to the amputation of the distal phalanx and disarticulation of the digit. The second and the third SCCs, respectively, had developed at the 5th left finger in 2003 and at the 2nd right finger in 2004, and both had been excised and repaired with a split-skin graft. Radiologic studies had been negative for bone involvement and no relapses or complications had been reported.

At the time of her clinical attendance, the examination of hands, foots and anogenital area were negative for warts or condylomas, nor the patient revealed any history of previous HPV infections. A gynecologic visit was negative for cervical dysplasia or other related problems. Hematologic studies, including routine blood tests and CD4/CD8 ratio were within normal value. The roentgenography of the finger was normal. A biopsy from the area affected revealed a poorly differentiated SCC with bowenoid features. Histologic examination showed a squamous keratinocyte proliferation, characterized by irregular acanthosis and papillomatosis with marked keratinocytes atypia and pleomorphism. High-power view of the tumour revealed dyskeratotic and vacuolated cells, numerous mitoses and consistent lymphocytic infiltrates.

The surgical procedure consisted in the complete excision of the nail bed and the nail matrix up to the level of the underlying distal phalanx. The area healed completely and there are no evidence of recurrence at this time.

HPV DNA detection was performed using two different PCR-ELISA methods, namely MY-PCR and GP-PCR, that we have been using routinely in our department, for identification of low risk HPV genotypes (HPV-6, 11, 34, 40, 42, 54, 55, 57, 61, 83 and 84), high-risk HPV genotypes (HPV-16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73 and 82), and undefined risk HPV genotypes (HPV-13, 30 and 32), as previously described. In all specimens from four periungual SCCs analyzed, HPV-16 DNA sequences were detected, both with MY and GP primers.

Although HPV has been rarely detected in extragenital SCC, demonstration of the virus in SCC of the nail bed and periungual region is a frequent outcome. In particular, reviewing the English-language literature, the occurrence of HPV-16 is restricted to SCC of the genital tract and finger, suggesting a venereal transmission to the periungual region of the virus.2-4

Our patient developed a sequence of four SCCs HPV-16 positive at the periungual region on four different fingers in the space of eight years but, unexpectedly, without any pre-existing history of warts or trauma at the site of the tumours. On the other hand, the relatively long space of time in which the different tumours developed, demonstrate that a latent HPV infection puts patients at risk for longterm periods.

Our data, support the theory that SCC of the nail unit must be considered a HPV-induced neoplasm, and that a high index of suspicion, whereas occurring multiple periungual warts, should be required in predisposed individuals.2

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Figure 1.—Grey-brownish verrucous growth covering the lateral nail fold and nail bed, with partial onycholysis.
L-carnitin: a potential treatment for hyper-IgE syndrome

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Dear Editor,

Hyper-IgE syndrome (previously Job’s syndrome) is a complex disorder characterized by extreme elevation of serum IgE level, chronic dermatitis, repeated lung and skin infection, atopic dermatitis 1 and Staphylococcal pneumonia.2 These patients are also at increased risk of osteoporosis and spontaneous bone fracture.1 Ulceration is also frequently present and lymphadenopathy may be complicated by lymph node abscess.2

Non-immunological features of the condition include coarse facies with a wide nasal bridge, large head, joint laxity and a high incidence of scoliosis.2 After the newborn period, skin findings include retroauricular fissure, external oitis, infected dermatitis of axilla and groin, and folliculitis of the upper back and shoulders.1

Peripheral blood lymphocyte subsets are generally normal and no consistent abnormality of T-cells has yet been identified.3 In a significant proportion of patients there is a failure of antibody response to polysaccharide antigens, which contributes to the susceptibility to infection.2 Patients are not neutrophenic and their neutrophils ingest and kill bacteria normally. However, neutrophil chemotaxis is reduced and it has been shown that mononuclear cells exposed to head-killed staphylococci inhibit neutrophil chemotaxis.3

The mainstay of treatment is long term anti-Staphylococcal antibiotic prophylaxis.1, 4 Intravenous immuno-globulin, which is an expensive treatment modality, is also employed successfully for the treatment of this illness.5 Severe cases may need bone marrow transplantation.1

Cimetidine is often given to those who respond poorly to antibiotics alone, for its stimulatory effects on neutrophil chemotaxis.1, 6 Recently, Namazi and Handjani speculated the potential efficacy of lithium, which enhances PMN motility, for treatment of this order.1

L-carnitin is a natural biomolecule found in all animal tissues, which acts as an essential cofactor in fatty acid metabolism.7 It has been shown that L-carnitin restored lymphocyte proliferative response 8 and the lytic activity of macrophages in aged rats.9 L-carnitin increases human lymphocyte proliferative response following mitogenic stimulation and increases polymorphonuclear chemotaxis.10 Furthermore, it improved decreased neutrophil chemotactic activity in aging rats.7

In conclusion, therefore, given the safety of L-carnitin, we suggest to our colleagues to consider L-carnitin treatment while facing a patient suffering from this conundrum.

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Carlo Luigi Meneghini

Il 27 dicembre del 2008 è deceduto il Professore Carlo Luigi Meneghini, il Padre della Dermatologia Allergologica e Professionale Italiana. L’attività pionieristica in questo importante settore della Dermatologia risale ai primissimi anni cinquantà dello scorso secolo, quando nella Clinica Dermatologica dell’Università di Milano il Professore Meneghini ha intrapreso gli studi sull’“eczema esogeno da contatto”. Studi che sono di volta in volta culminati in centinaia di lavori sulle più importanti riviste italiane e straniere ed in particolare con la pubblicazione di due monografie: “Morbilità cutanea esogena (non microbica) e fattori di localizzazione” (Minerva Dermatologica 31, supp n. 4, 1956) e “Le Dermatiti eczematose professionali” (edita dal Giornale Italiano di Dermatologia nel 1961). Nella seconda monografia, una delle prime al mondo sul tema, la dermatite da contatto da noxae chimiche per la prima volta viene trattata in maniera organica dal punto di vista eziopatogenetico e clinico-diagnostico.

Il Professore Meneghini aveva all’epoca anche intuito e dimostrato sperimentalmente i meccanismi patogenetici immunitari e non alla base dell’affezione, superando e annullando così la teoria patogenetica nervosa, allora condivisa da molti studiosi italiani e stranieri. Un altro pionieristico aspetto dello studio della dermatite da contatto è stato il particolare approccio al tema: trattandosi infatti di patologia allora diffusa in particolare in ambiente professionale, per meglio studiarne tutte le sfaccettature (comprese quelle sociali e previdenziali), il Professore Meneghini l’ha affrontata direttamente sul “posto di lavoro”. Con i suoi continui “sopraluoghi” in tutte le fabbriche dell’interland Milanese ha potuto così fotografare e studiare tutti gli aspetti più salienti della patologia occupazionale: “gesti” lavorativi, agenti chimici impiegati nelle singole attività lavorative, “cicli lavorativi”, “ambiente di lavoro” (inteso nel significato più ampio del termine, fisico, sociale e igienico-sanitario). Questo studio diretto “dal vivo” dell’ambiente di lavoro, insieme alla proficua collaborazione con medici del lavoro e medici assicurativi, ha permesso al Professore Meneghini di sviluppare l’argomento nel suo più giusto e organico inquadramento.

Questi studi hanno fatto sì che a Milano fosse per la prima volta istituita la Cattedra di Dermatologia Allergologica e Professionale, Cattedra che il Professore Meneghini ha istituito anche a Bari e che con l’avvento della Associatura è sorto poi in tutte le sedi Universitarie italiane.

Grazie a queste pionieristiche ricerche, nel 1966 il Professore Meneghini, insieme ad altri studiosi del settore di 6 Paesi Europei (Inghilterra, Germania Occidentale, Svezia, Danimarca, Olanda, Finlandia) è stato cofondatore dell’International Contact Dermatitis Research Group (ICDRG), gruppo internazionale che ha poi standardizzato organicamente la dermatite da contatto con regole seguite da allora in tutto il mondo.


Durante la sua presidenza della SIDEV il Professore Meneghini ha avuto un’altra brillante idea, quella di istituire i Gruppi di studio, tra cui il Gruppo Italiano di Ricerca Dermatiti da Contatto e Ambientali (GIRDCA), che grazie alla sua guida ha potuto assurgere ad alti livelli scientifici, in Italia e all’estero.


Ho lavorato per oltre 30 anni sotto la sua guida ed ho poi continuato per molti anni ancora un bellissimo rapporto umano con il Professore Meneghini. E a parte il giudizio mio personale (che potrebbe sembrare dettato da retorica emulazione), questa lunga “vicinanza” mi ha permesso di toccare con mano il grande rispetto e la massima stima che circondavano il Professore Meneghini, sentimenti continuamente espressi dai suoi amici e Colleghi, da allievi e studenti, da collaboratori e vari e mondo accademico. La grande umanità, la correttezza, la gentilezza e la signorilità del Professore Meneghini sono state e rimarranno proverbiali. Il suo attaccamento al lavoro e la sua costante onestà di condotta e probità hanno fatto del Professore Meneghini, con unanime consenso, un grande Maestro e un integerrimo Uomo.

I suoi dotti insegnamenti di vita e professionali, ne sono certi, ci accompagneranno sempre e ci accomuneranno nell’indelebile e caro ricordo di un Signore della Dermatologia.

G. Angelini