Chapter 2
Hematolymphoid Proliferations of the Skin

Carlos A. Torres-Cabala, Jonathan L. Curry, Su S. Chen and Roberto N. Miranda

Introduction

Molecular tests used by practicing pathologists are mostly performed on formalin-fixed, paraffin-embedded tissue specimens. Occasionally, when a more comprehensive molecular analysis is required, the use of fresh tissue or cell suspensions can overcome the limitations of testing on fixed tissue. The range of genetic or molecular tests that can be performed on skin specimens with lymphoid or hematopoietic disorders include, but are not limited to polymerase chain reaction (PCR), fluorescence in situ hybridization (FISH), proteomics, comparative genomic hybridization (CGH), gene arrays, and routine cytogenetics. In this review, we discuss the significance of the molecular testing most frequently used in the evaluation of clinical specimens involved by lymphoid or hematopoietic infiltrates.

Most of the molecular testing performed on cases of lymphoid infiltrates in the skin is done to identify clonality since it is generally thought that the presence of clonality supports a diagnosis of malignancy (i.e., lymphoma) and the lack of clonality excludes malignancy (i.e., reactive lymphoid hyperplasia). This approach is followed because it is considered that skin disorders with lymphoid infiltrates follow the paradigm of lymphoid disorders affecting lymph nodes, where evidence of clon-

C. A. Torres-Cabala (✉)
Departments of Pathology and Dermatology, The University of Texas: MD Anderson Cancer Center, 1515 Holcombe Blvd, Unit 85, Houston, TX 77030, USA
e-mail: ctcabala@mdanderson.org

J. L. Curry
Department of Pathology, Section of Dermatopathology, The University of Texas: MD Anderson Cancer Center, Houston, TX 77030, USA

S. S. Chen
Empire Genomics, LLC 700 Michigan Avenue, Suite 200 Buffalo, NY 14203, USA

R. N. Miranda
Department of Hematopathology, The University of Texas: MD Anderson Cancer Center, Houston, TX, USA

V. G. Prieto (ed.), *Precision Molecular Pathology of Dermatologic Diseases,*
Molecular Pathology Library 9, DOI 10.1007/978-1-4939-2861-3_2

ality has usually been equated with malignancy. However, with further clinical specialization, increasing attention has been paid to lesions and tumors arising at extranodal sites, and differences related with specific anatomic sites have been identified, raising the concern that not all criteria applied to nodal lymphomas perfectly fit in extranodal sites. In dermatologic disorders in particular, whereas clinically typical malignant and benign lymphoid lesions exist and can be easily recognized by the clinician, there are many instances in which fully integration of clinical, pathological, and molecular findings is required to reach a definitive diagnosis. Apparent lack of concordance between these findings is a well-known, and probably not uncommon, phenomenon. Clonality may be detected in clinically indolent lesions (a "false positive," if following the paradigm of clonality being equivalent to malignancy). Conversely, clinically malignant lesions may lack evidence of clonality by molecular methods ("false negative"). Moreover, well-established pathological criteria useful for diagnosis of lymph node entities may not be applicable for their skin counterparts, such as the case of follicular lymphoma (FL), associated with t(14;18)(q32;q21) in approximately 85% of the nodal cases (and usually positive for BCL2 by immunohistochemistry) but commonly negative for BCL2 and harboring the translocation in less than 30% of primary cutaneous follicle centre lymphoma. The need for correlation of molecular findings with clinical and pathological characteristics of the lesions in dermatopathology cannot be overemphasized.

Molecular Tests Commonly Used in the Dermatopathology Practice

T-cell Receptor Gene Rearrangement Analysis

The T-cell receptor (TCR) is present within the CD3 complex that locates on the surface of T cells. Clonal rearrangement of the *TCR* gene is used to determine whether a T-cell lymphoid population is monoclonal or polyclonal [1]. The *TCR* is composed of alpha, beta, gamma, and delta chains. More than 90% of the mature T lymphocytes harbor the alpha-beta TCR [2, 3]. It is important to remember that independently from what TCR (alpha-beta or gamma-delta) is expressed, rearrangement of the gamma gene is the most frequently detected in the T cells [4]. The *TCR* gene rearrangements occur in the following chronological order: delta—alpha—gamma—beta. Since the delta receptor locus is located within the alpha gene, the delta locus is deleted most of the times when the alpha gene is rearranged [5]. PCR-based methods—the most used in daily clinical practice—can detect rearrangements of the delta, gamma, and beta chains [6]. The gamma chain gene has less variation of sequences than the delta and beta genes, thus requiring fewer specific primers for the PCR amplification [7]. Moreover, *TCR* gamma gene rearrangements are found in both gamma-delta and alpha-beta T-cell lymphomas (in the latter, a rearranged gamma allele is retained although not expressed [6]), making the *TCR* gamma gene rearrangement detection the preferred test for detection of T-cell

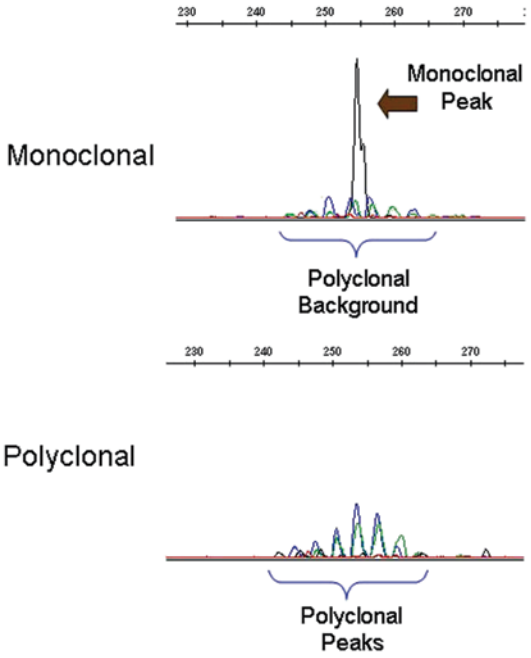

Fig. 2.1 Analysis of rearrangements of the T-cell receptor (*TCR*) beta chain by polymerase chain reaction (*PCR*). The gene scan on the top shows amplification of segments as multiple small peaks and a large predominant peak. This pattern is consistent with a monoclonal population of T-lymphocytes. In the right context, this supports a neoplastic population of T lymphocytes. The *bottom* of the Figure shows a polyclonal pattern characterized by multiple small peaks, consistent with the presence of reactive, nonneoplastic T lymphocytes. *TCR* beta chain analysis is especially useful in the identification of a monoclonal peak in a polyclonal background

clonal populations in dermatopathology. Due to its greater combinational diversity, evaluation of *TCR* beta gene rearrangement seems to be particularly useful in the detection of a monoclonal population in a background of polyclonal reactive cells [8]. Rarely, cases of T-cell lymphomas may present rearrangements of the delta chain as the only evidence of clonality [9].

The PCR products are analyzed using high-resolution capillary electrophoresis. Polyclonal proliferations render multiple peaks (more than five) whereas clonal populations are characterized by one or two peaks whose height exceeds that of the polyclonal background by a ratio of 2:1 to 3:1 [10] (Figs. 2.1 and 2.2).

The sensitivity of PCR-based tests varies depending on the type of sample and technical issues. It is accepted that the minimum percentage of detectable clonal cells by this method is around 1 % [11] and that its overall sensitivity and specificity is around 70 and 97 % for mycosis fungoides (MF), respectively [12]. False negative results may be due to low numbers of malignant T-cells or absence of *TCR* gene rearrangement in the lymphoma cells [13]. False positivity (pseudoclonality) may result from amplification of *TCR* gene rearrangement present in a few T-cells composing a sparse, reactive lymphocytic infiltrate [14]. Duplicate analyses may distinguish reactive from neoplastic proliferations since the dominant peaks detected in reactive conditions vary within the same sample while true clonal peaks are usually reproducible [15].

In recent years, great value has been given to the demonstration of the presence of identical T-cell clones at different anatomical sites as a highly specific tool in discriminating between MF and inflammatory conditions [16], the so-called stable clonal pattern [14]. However, this pattern has also been reported in some

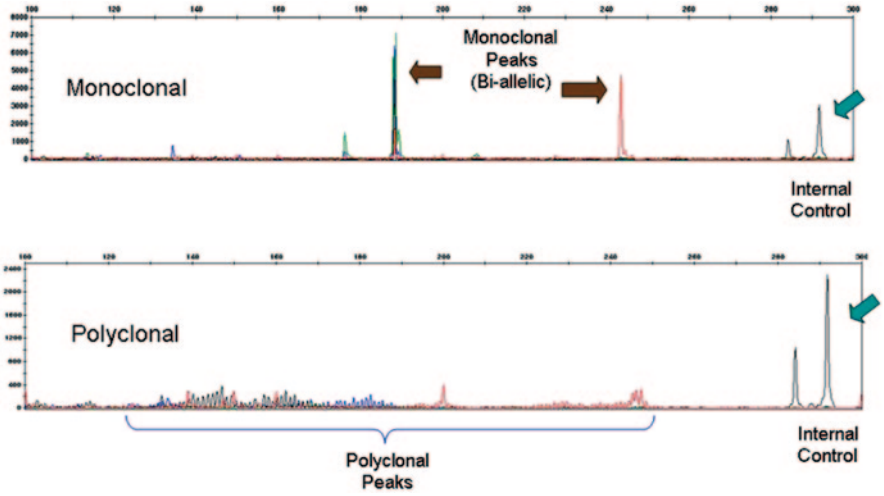

Fig. 2.2 Analysis of rearrangements of the T-cell receptor (*TCR*) gamma chain by polymerase chain reaction (*PCR*). In this case the scan on the top shows that the amplification of segments yielded multiple small peaks and two predominant peaks, consistent with a bi-allelic monoclonal population of T-lymphocytes. The *bottom* shows analysis of *TCR* gamma chain gene rearrangement in a different specimen. It reveals multiple small peaks, consistent with a polyclonal population of likely reactive, nonneoplastic T lymphocytes. As with the *TCR* beta chain gene rearrangement analysis, the correct interpretation of this result should be made in conjunction with clinical and pathological findings. The arrows indicate internal controls

inflammatory and "borderline" processes [17]. Conversely, genetically unstable subclones have been described in T-cell lymphomas [18] and clonal heterogeneity in MF lesions from distinct anatomical sites has been reported [19, 20]. The utility of comparing clones from different anatomical sizes for the diagnosis of T-cell lymphoma needs to be evaluated in the context of the clinical and histopathological findings.

Immunoglobulin Gene Rearrangement Analysis

The genes encoding immunoglobulins (Ig), the antigen receptors in B-cells, include the heavy-chain (*IGH*), kappa light chain, and lambda light chain genes. The *IGH* gene rearrangement starts with the combination of a diversity (D_H) segment with a joining (J_H) segment, which then is joined with a variable (V_H) segment to create a VDJ_H sequence. The kappa gene rearrangement then follows. Depending on the functionality of the rearranged kappa alleles, the lambda gene will be rearranged, usually after deletion of both kappa genes [21].

PCR-based methods for detection of *IGH* gene rearrangement utilize four sets of consensus primers designed to amplify three conserved framework regions within

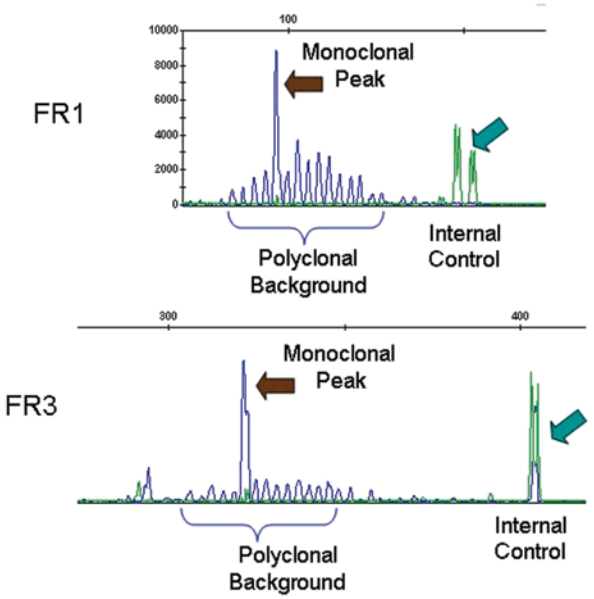

Fig. 2.3 Analysis of rearrangements of the immunoglobulin heavy chain (*IGH*) genes by polymerase chain reaction (*PCR*). Evidence of rearrangements of variable, diversity, and joining (*VDJ*) regions of IGH can be visualized by using pairs of primers at the V and J regions. Each lymphocyte in the analyzed specimen has a rearrangement that renders a segment that is amplified. In B-cell neoplastic processes, the presence of a prominent or distinct peak reflects that a significant number of cells in the specimen have the same size of amplified product. This finding constitutes a monoclonal peak and it usually correlates with a neoplastic expansion of B-cells. The use of three primers for the framework region (*FR*) of the V regions yields a higher chance of identifying a monoclonal population. The top of the figure shows the results of PCR amplification using FR1 and J region primers, while the bottom shows the amplification using FR3 and J region primers. Both analyses yielded the identification of a monoclonal peak amidst several smaller peaks, indicating that the neoplastic clone is admixed with reactive B lymphocytes. The size of the amplified segment is 98 base pairs on the top and is 320 base pairs on the bottom. Internal controls are used to confirm the size of segments and that amplification is taking place. The arrows indicate internal controls

V_H and one within J_H. From these, PCR amplification of framework region III in the V_H and the framework region in J_H segments is the most widely used test in clinical practice, due to the low molecular weight of the resulting amplicon and therefore the possibility of using formalin-fixed paraffin-embedded tissue as DNA source [22].

Same as for *TCR* gene rearrangement analysis, the peaks obtained by capillary electrophoresis are evaluated for the presence of a dominant population or populations (Figs. 2.3 and 2.4). The sensitivity of the method, although usually high, varies according to a number of factors such as the number of reactive B-cells present in the background and tissue fixation, and it can be as low as 47% in formalin-fixed paraffin-embedded tissue [23]. The rate of false negative results seems to be espe-

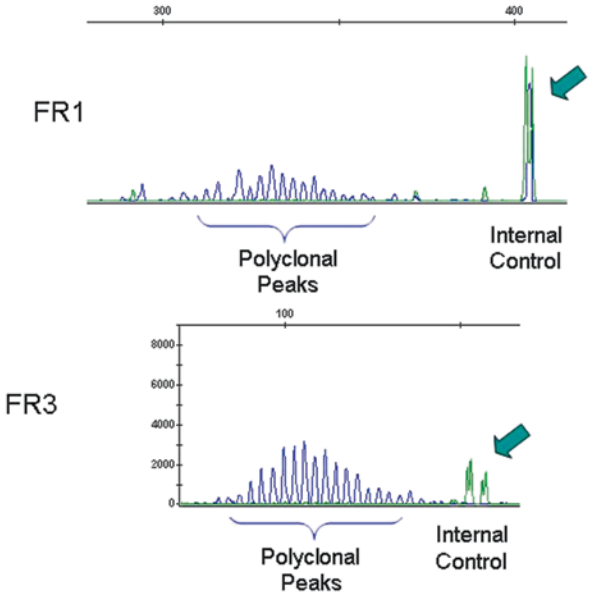

Fig. 2.4 Analysis of rearrangements of the immunoglobulin heavy chain (*IGH*) genes by polymerase chain reaction (*PCR*). In a reactive process, most lymphocytes render different size of amplified product and appear as multiple peaks, interpreted as polyclonal peaks/polyclonal background. In this case, the amplification of segments using FR1 and FR3 primers revealed a polyclonal pattern, consistent with a reactive, not neoplastic infiltrate. Similar results were obtained using FR2 primers. Correlation with clinical and pathological findings is still needed for an adequate interpretation of results of *IGH* gene rearrangement analysis. The arrows indicate internal controls.

cially high in cases of diffuse large B-cell lymphoma (DLBL) and follicular lymphoma, which have a high frequency of somatic mutations [24]. These mutations lead to sequences that are noncomplementary to the primer sequences. Such false-negative result is avoided by using multiple PCR target regions such as FR1, FR2, and FR3 segments of *IGH* gene. Another way to increase the sensitivity to detect clonal B-cell populations is by adding a test for immunoglobulin light chain (IGL) kappa or lambda gene rearrangement [25].

False positive results may be seen in cases in which the skin biopsy shows only sparse infiltrate and the PCR products amplified from the few B-cells appear as a distinct peak. Again, as for T-cell pseudoclonal cases, repeated testing may help in discriminating false clonality from true clonal B-cell expansion. Cases of cutaneous lymphoid hyperplasia have been demonstrated to be clonal for *IGH* gene rearrangement [26], and clonal *IGH* gene rearrangement has been reported in T-cell proliferations [27]. Other causes of false-positive results include immune disorders and infections showing a predominant B-cell population.

It is important to emphasize that the demonstration of monoclonal *IGH* or *TCR* gene rearrangement in a cutaneous lymphoid proliferation by itself does not make

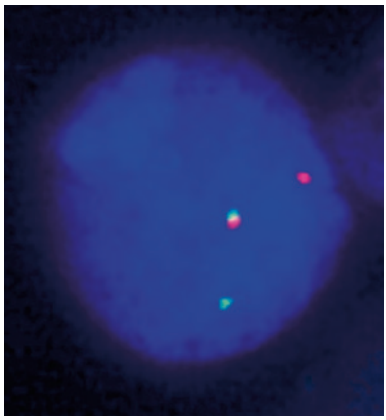

Fig. 2.5 Break-apart fluorescence in situ hybridization (*FISH*) analysis for identification of translocations involving *DUSP22-IRF4* gene locus. The picture shows a nucleus of a cutaneous anaplastic large cell lymphoma (*C-ALCL*) cell in which there is separation of *red* and *green* signals flanking the locus containing the *DUSP22* and *IRF4* genes, indicating a translocation involving this locus. This finding is most often seen in primary cutaneous ALCL, though it may be seen occasionally in systemic ALK-negative ALCL. (Courtesy of Dr. Andrew L. Feldman, Mayo Clinic, Rochester, MN, USA)

the diagnosis of lymphoma, and that both positive and negative results need to be interpreted in the appropriate clinical and histopathological context.

Detection of Chromosomal Translocations

From the several chromosomal translocations identified in hematolymphoid proliferations only a few are routinely investigated in the dermatopathology practice.

The t(14:18)(q32;q21) *IGH*/*BCL2* translocation can be analyzed by FISH and PCR. Fluorescence in situ hybridization (FISH), using appropriate probes, can detect almost all *BCL2* translocations [28]. This translocation occurs in more than 85% of cases of follicular lymphoma [29] but in only a small number of primary follicular lymphomas of the skin [30], and therefore its presence should suggest secondary involvement of the skin by systemic follicular lymphoma.

Translocations associated with extranodal marginal zone lymphoma rarely occur in the primary cutaneous tumors. The t(14:18)(q32;q21) *MALT1/IGH* has been identified in a subset of extranodal marginal zone lymphomas of the skin [31].

The t(2;5)(p23;q35)*ALK/NPM,* found in most of the cases of systemic anaplastic large cell lymphoma (ALCL) [32], has been rarely reported in primary cutaneous ALCL [33]. The demonstration of *ALK* gene rearrangements by FISH is thus very suggestive of secondary cutaneous involvement by systemic ALK+ ALCL [34]. A subset of primary cutaneous ALCL harbors (interferon regulatory factory-4) translocations; [35] FISH testing in this setting may be of potential diagnostic utility (Fig. 2.5) .

In situ Hybridization Studies

Demonstration of cytoplasmic immunoglobulin (Ig) light chain mRNA in plasma cells can be achieved by in situ hybridization (ISH) studies performed on formalin-fixed paraffin-embedded tissue sections [36]. Restricted Ig lambda or kappa light chains support a diagnosis of extranodal marginal zone lymphoma.

Epstein-Barr virus-encoded mRNA (EBER) ISH studies are important in a number of T-cell and B-cell lymphoproliferative disorders and lymphomas. Latent Epstein-Barr virus (EBV) infection can be revealed by ISH studies due to the large load of EBERs present in the nuclei of infected cells [37].

T-cell Lymphoid Proliferations of the Skin

Mycosis Fungoides

Mycosis fungoides (MF) is the most common cutaneous T-cell lymphoma. Clinical features are crucial for diagnosing this entity, especially on its early stages. The initial presentation is that of small scaly patches, usually on sun-protected areas such as the buttocks, lower abdomen, and thighs. In a minority of patients the patches disseminate and indurated raised plaques appear. The third stage is called tumor stage, which results in nodules that frequently ulcerate and may involve sun-exposed skin. Extracutaneous dissemination may be seen as the disease progresses. The histopathological findings on early, patch-stage mycosis fungoides are subtle and can be easily overlooked. The lymphocytic infiltrate is usually composed of small lymphocytes with minimal or no atypia, arranged around vessels within the papillary dermis and superficial plexus. Epidermotropism by lymphocytes, considered a strong sign for suspecting MF (especially in the absence of important spongiosis), may be very focal. As the lesion progresses, the lymphocytes start to display more obvious cytologic atypia. Plaques of MF usually exhibit cells with "cerebriform" nuclei and often show Pautrier's microabscesses. Dense infiltrates occupying the reticular dermis characterize tumor lesions. The lymphocytes in tumor stage MF may display an anaplastic morphology making the distinction between MF and ALK negative anaplastic large cell lymphoma (ALCL) impossible without clinical information. Many clinical and histopathological variants of MF have been described. Although scoring systems using clinical, histopathological, immunophenotypic, and molecular characteristics are in use [38], the diagnosis of early MF can still be cumbersome.

Staging of MF, mainly based on clinical and histopathological findings, correlates with prognosis [39]. Immunophenotyping is used as an adjunct for diagnosis. Most of the cases display a CD3+, CD4+, CD8− phenotype. Demonstration of loss of CD2, CD3, CD5, or CD7 may help in the diagnosis [11]. However, some controversy still exists about the exact role of immunohistochemistry in the diagnosis of

early MF, since overlapping findings may be seen in inflammatory conditions [40] and the prognosis of early MF does not seem to be influenced by phenotype [41].

Molecular studies can be helpful in the diagnosis of MF, if taken in conjunction with clinical and histopathological findings. T-cell receptor (*TCR*) gene rearrangement studies are used to demonstrate T-cell clonality in MF [42]. Although Southern blot analysis is still considered the gold standard for determination of T-cell clonality, [43] PCR-based methods are routinely used for this purpose. Investigation of TCR-γ chain gene rearrangements is the most commonly used test in clinical laboratories, due to its high sensitivity [44, 45]. Evaluation of *TCR*-β gene rearrangements is being increasingly performed in clinical settings. It is expected that the assessment of both *TCR*-γ and *TCR*-β gene rearrangements will increase the sensitivity of T-cell clonality detection in MF [8].

When interpreting *TCR* gene rearrangement studies the dermatopathologist should be aware of a few caveats. Inflammatory conditions in which the lymphocytic infiltrates are scant not uncommonly yield oligoclonal or even monoclonal bands [3]. Duplicate analyses are needed to rule out the possibility of pseudoclonality in these cases [14]. On the other hand, the sensitivity of the PCR-based methods used for *TCR* gene rearrangement determination widely varies: it is not uncommon that patch MF lesions show oligoclonality [14]. Therefore, a negative or oligoclonal *TCR* gene rearrangement pattern does not rule out MF.

Sézary Syndrome

Sézary syndrome (SS) is characterized by erythroderma, lymphadenopathy, and the presence of the so-called Sézary cells in peripheral blood. Controversy still exists about the distinction between erythrodermic MF (a condition that may evolve through patch/plaque disease and has variable levels of blood involvement) and SS [46]. Some evidence suggests that these are two different processes and that they constitute malignancies of thymic memory T cells (SS) and skin resident effector memory T cells (MF) [47]. The prevailing view of SS as the leukemic counterpart of MF seems therefore not completely accurate. On clinical grounds, however, there is frequent overlapping between these two entities and a diagnosis of SS should be made under strict criteria, such as the demonstration of a T-cell clone in peripheral blood, peripheral blood lymphocytosis with a CD4+/CD8+ ratio greater than 10, and circulating Sézary cells greater than 1×10^9/L.

Histologically, the changes seen in SS may be indistinguishable from those seen in MF. However, lack of epidermotropism and low grade cytologic atypia are common in SS [48]. Most of the cases exhibit a CD3+, CD4+, CD8− phenotype.

Clonal *TCR* gene rearrangements are commonly demonstrated in peripheral blood of patients with SS [1]. These monoclonal *TCR* gene rearrangements can be identical to those detected in skin [49] and this finding has been used as evidence of multiple site involvement by the same malignant process. However, different clonal *TCR* gene rearrangements in peripheral blood and skin have been detected in a number of patients, suggesting clonal heterogeneity in some cases [14, 19].

Moreover, monoclonal T-cell populations in peripheral blood have been associated with autoimmune disorders [17], advanced age [50], and can be identified in association with other non cutaneous lymphomas and inflammatory skin conditions [1]. Patients with SS may be negative for T-cell clones in peripheral blood [51].

Primary Cutaneous CD30-Positive T-cell Lymphoproliferative Disorders

Primary cutaneous CD30-positive T-cell lymphoproliferative disorders (CD30+ TLPD) constitute the second most common cutaneous T-cell lymphoma/lymphoid proliferations. The WHO classification recognizes three types: primary cutaneous anaplastic large cell lymphoma (C-ALCL), lymphomatoid papulosis (LyP), and borderline lesions. It is recognized that these disorders represent a clinical and histological spectrum of entities going from LyP, a recurrent, benign diffuse eruption of papulonodular lesions that regress spontaneously, to C-ALCL, usually presenting as one or multiple localized ulcerated nodules they may in some cases regress [52]. Distinction should be made from systemic ALCL with secondary involvement of the skin and from other lymphomas showing CD30 expression, such as mycosis fungoides with large cell transformation [53].

LyP has been classified classically in three histological types [54]. Type A, that resembles Hodgkin lymphoma, i. e., shows aggregates of large Reed-Sternberg-like CD30-positive cells in a background of inflammatory cells; type B, or papular mycosis fungoides-like; and type C, or ALCL-like in which the large CD30-positive cells are arranged in sheets with minimal inflammatory background. Recently, a "type D" denomination has been proposed for CD8-positive lesions [55]. Cutaneous anaplastic large cell lymphoma (C-ALCL) reveals a dermal infiltrate composed of large anaplastic, pleomorphic cells, most of them expressing CD30.

The demonstration of clonal rearrangements of TCR in CD30+ TLPD has been variably reported [56, 57]. It is important to take in consideration that identical clones can be detected in different CD30+ TLPD lesions [58] as well as in LyP and concomitant lesions of mycosis fungoides [57]. When dealing with ALCL lesions, it is important to distinguish C-ALCL from involvement of skin by systemic ALCL. Cutaneous anaplastic large cell lymphoma (C-ALCL) cases usually lack t(2;5) and ALK expression by immunohistochemistry [59]. Translocations involving *IRF4* detected in C-ALCL but not in systemic ALCL, have been reported as useful in this differential diagnosis [35].

Other Primary Cutaneous T-Cell and NK Lymphomas

This group encompasses rare lymphomas in which a diagnosis of MF has been excluded, clinically and histopathologically.

http://www.springer.com/978-1-4939-2860-6

Precision Molecular Pathology of Dermatologic Diseases

Prieto, V.G. (Ed.)
2015, VIII, 136 p. 40 illus., 39 illus. in color., Hardcover
ISBN: 978-1-4939-2860-6