Angioimmunoblastic lymphadenopathy type of T-cell lymphoma and angioimmunoblastic lymphadenopathy: a clinicopathological and molecular biological study of 13 Chinese patients using polymerase chain reaction and paraffin-embedded tissues

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Abstract The morphological classification of angioimmunoblastic lymphadenopathy (AILD) or T-cell lymphoma of AILD-type (AILD-TCL) is still a subject of considerable difficulty and controversy. The aim of the current study was to examine the value of clinical, morphological, immunohistochemical variables in paraffin-embedded tissues in predicting the clonality of the respective lesion. Fifteen lymph node biopsies derived from 13 patients from Chengdu, China, were diagnosed as AILD or AILD-TCL and included in this study. The specimens were examined using a panel of monoclonal antibodies and a scoring system of morphological features. Clonality of the paraffin-embedded material was investigated using a novel polymerase chain reaction-technique to amplify rearranged T-cell receptor (TCR)-γ sequences. Additional experiments were carried out to investigate the presence of clonal rearrangements of the immunoglobulin heavy chain (IgH) locus. We found clonal rearrangements of the TCR-γ locus in 9 out of 15 lymph node biopsies. In 3 patients, the predominant cell clones carried clonal IgH and TCR-γ rearrangements whereas 1 patient with polyclonal TCR-γ pattern displayed IgH-monoclonality. The statistical evaluation of morphological and immunohistochemical data indicated that no single variable was able significantly to predict the clonality of the lesion. Furthermore, demonstrable clonality for the TCR-γ or the IgH loci of a lesion did not correlate with a bad clinical course. Our data correlate with findings of other studies investigating AILD-TCL in Caucasian populations.

Key words Autoimmunoblastic lymphadenopathy · T-cell receptor γ · Immunoglobulin heavy chain · Clonality · Polymerase chain reaction

Introduction

Since its primary description by Frizzera et al. [10], the histological diagnosis and classification of “angioimmunoblastic lymphadenopathy with dysproteinaemia” (AILD) has been an area of considerable difficulty and controversy. Different designations have been employed to accommodate a spectrum of apparently related lymph node lesions. Lukes and Tindle [22] used the term “immunoblastic lymphadenopathy” (IBL) and Radaszkiewicz and Lennert [29a] advocated the use of “lymphogranulomatosis X” because of similarities to Hodgkin’s disease. Whereas it was first regarded as a reactive or prelymphomatous B-cell disorder, Shimoyama et al. [29b] described cases of similar morphology with clinical courses of peripheral T-cell lymphomas (TCL) which they called “IBL-like TCL”. Morphologically, there exists a continuous spectrum of lymph node lesions. Several groups have devised schemes to subtype the varying appearance of AILD-like lesions. These subdivisions pay attention to the variable content of clear cells, germinal centres and immunoblasts. Again, different nomenclatures exist for the subdivision of AILD: AILD type A, B or C [3, 26] or IBL, IBL-like TCL and AILD [34].

Clinical and molecular studies have revealed that the majority of cases indistinguishable from AILD are in fact clonal and, therefore, can be regarded as neoplastic T-cell proliferations [12, 14, 32]. Interestingly, molecular studies have also discovered clonal rearrangements of the immunoglobulin genes in relatively high frequencies [8]. However, in approximately 30% of the cases no predominant T-cell clone can be detected by molecular studies [23, 35]. These cases might in fact constitute true hyperimmune reactions, the context in which Frizzera originally regarded AILD.
Since the discovery of Epstein-Barr virus (EBV) genomic material in AILD [4], numerous studies have investigated the incidence and possible implication of EBV infection in the pathogenesis of the disease [2, 18, 28, 36]. Using in-situ hybridisation and polymerase chain reaction (PCR), these studies have discovered a significant association of EBV infection with AILD or AILD-TCL – a situation reminiscent of Hodgkin’s disease. Even if nasopharyngeal lymphomas are not taken into account, EBV DNA can be detected in Chinese cases of peripheral TCL more frequently than in cases occurring in the Western populations [37]. Because both monoclonal and polyclonal EBV genomes are known to occur in AILD and AILD-TCL, it is still a matter of controversy whether the high prevalence of EBV reflects the immune deficiency of patients or whether EBV acts as one necessary initiator of the disease.

In the present study, we have investigated the morphology, immunohistochemistry and rearrangements of all four functional variable (V)-γ-gene families of the T-cell receptor-γ (TCR-γ) as well as the immunoglobulin heavy chain (IgH) genes together with clinical data of 13 families variable (V)yI (Vy-) cell receptor (TCR)-γ locus. In the University of Cologne. The specimens were re-assessed according to the updated Kiel classification [30] and the diagnostic criteria for AILD published by Frizzera et al. [11] were meticulously applied. In 15 lymph node biopsies derived from 13 patients sufficient material was available for molecular biological studies. In these cases the following morphological features were evaluated: obliteration of lymph node architecture, involvement of capsular and pericapsular tissue, patency of sinuses, alteration of germinal follicles, extracellular deposition of amorphous periodic acid-Schiff (PAS)-positive substances, small vessel proliferation, presence and distribution of pale cells as well as basophitic immunoblasts and mitotic figures per ten high power fields (HPF).

Clinical data were available for seven patients. Medical records were reviewed for the clinical history, duration of symptoms at initial presentation, clinical and laboratory investigations and treatment.

Immunohistochemistry was performed with a panel of monoclonal antibodies (Table 1) using the streptavidin-biotin method [15]. Biotinylated rabbit-anti mouse monoclonal antibodies and streptavidin-biotin-complex were obtained from Dako (Hamburg, Germany). New fuchsin served as a chromogen and sections were counterstained with haematoxylin.

To amplify rearranged TCR-γ sequences sections of 10 μm thickness were cut using a new microtome blade for each case. They were dewaxed in xylene and following ethanol precipitation, the material was desiccated in a SpeedVac centrifuge and incubated with PCR-buffer (Gibco, Eggenstein, Germany) and protease K at a concentration of 1 mg/ml for 12–24 h. The protease K was inactivated by heating to 96 °C for 10 min and samples were stored at 4 °C until further investigation. DNA was extracted from these samples by phenol-chloroform extraction and precipitation with absolute ethanol. The quality of the DNA and the level of degradation was tested by agarose gel electrophoresis.

Amplification was performed as previously described [21]. Briefly, primers specific for the four functional Vγ-gene families [16] and a consensus 3′-primer for the junctional (J1)- and J2-genes of the TCR-γ [33] were used. According to their sequence homologies, the Vγ-genes can be grouped into four families. Where-as family I comprises of 8 different Vγ-genes, the Vγ-families II to IV are made up of a single gene each. During rearrangement, a specific Vγ-gene is brought into proximity to a Jγ-gene (Fig. 1). Random nucleotides are inserted into the N-segments. These rearranged sequences can be amplified by PCR using primers for the Vγ- and Jγ-genes. A stretch of high homology between the different genes 120 bp upstream of the Jγ-gene was chosen to place

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**Material and methods**

Formalin-fixed and paraffin-embedded material were taken from the files of the Department of Pathology, West China University of Medical Sciences, Chengdu. From a selection of 34 specimens collected during the period from 1985 to 1992 with features suggestive of AILD-TCL, paraffin blocks were reembedded and sections of 4 μm thickness were cut at the Department of Pathology, University of Cologne. The specimens were re-assessed according to the updated Kiel classification [30] and the diagnostic criteria for AILD published by Frizzera et al. [11] were meticulously applied. In 15 lymph node biopsies derived from 13 patients sufficient material was available for molecular biological studies. In these cases the following morphological features were evaluated: obliteration of lymph node architecture, involvement of capsular and pericapsular tissue, patency of sinuses, alteration of germinal follicles, extracellular deposition of amorphous periodic acid-Schiff (PAS)-positive substances, small vessel proliferation, presence and distribution of pale cells as well as basophitic immunoblasts and mitotic figures per ten high power fields (HPF).

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**Fig. 1** Rearrangement of the T-cell receptor (TCR)-γ locus. In germ line configuration, the TCR-γ locus comprises of the four gene families variable (V)γI (Vγ-gene 1–8), VγII (Vγ9), VγIII (Vγ10) and VγIV (Vγ11) together with the pseudogenes VγSP, A and B. These genes are separated by at least 16 kb from the closest joining (Jγ)-gene, JP1. During T-cell development, these genes are rearranged so that one Vγ-gene is brought into the proximity of one Jγ-gene. Between these gene sequences, random nucleotides are inserted to form N-regions, as indicated in the insert. Using primers for Vγ- and Jγ-genes, polymerase chain reaction (PCR)-amplification can only take place after rearrangement. The length of the amplification products varies between of the variable length of the respective N-regions.