Anaplastic Large Cell Lymphomas lack the expression of T-cell receptor molecules or molecules of proximal T-cell receptor signaling

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Abstract

Anaplastic large cell lymphoma (ALCL) designates a heterogeneous group of CD30+ (systemic or primary cutaneous) peripheral T-cell lymphomas (PTCL). A subgroup of systemic ALCL is transformed by anaplastic lymphoma kinase (ALK).

We compared 24 ALK+, 15 ALK− systemic and 7 cutaneous ALCL with 29 non-anaplastic PTCL in terms of T-cell receptor (TCR) rearrangements, expression of TCRs and TCR-associated molecules (CD3, ZAP-70).

Despite their frequent clonal rearrangement for TCRβ, only 2/47 ALCL (4%) expressed TCRβ protein, while TCRs were detected on 27/29 non-anaplastic PTCL. Moreover, both TCRβ+ ALCL lacked CD3 and Zap-70, i.e. molecules indispensable for the transduction of cognate TCR signals.

Defective expression of TCRs is a common characteristic of all types of ALCL which may contribute to the dysregulation of intracellular signalling pathways controlling T-cell activation and survival. This molecular hallmark of ALCL is analogous to defective immunoglobulin expression distinguishing Hodgkin lymphoma from other B-cell lymphomas.
Introduction

Anaplastic large cell lymphoma (ALCL) of T-cell type comprises distinct entities of lymphomas expressing CD30. The WHO-classification of malignant lymphomas distinguishes systemic and primary cutaneous forms with systemic ALCL comprising about 26% of all peripheral T-cell lymphomas (PTCL). While CD30 expression is a consistent but not defining feature of ALCL (since other PTCL can express CD30 as well, the diagnosis of ALCL either rests on morphology or the detection of over-expressed anaplastic lymphoma kinase (ALK). Morphologically, the large and polymorphic tumor cells sometimes resemble Reed Sternberg cells and their sheet-like growth pattern is distinct from most other PTCL that often exhibit a minor tumor cell population in an inflammatory background. ALK is overexpressed in about 60% to 85% of systemic ALCL and defines a clinically homogeneous group with good prognosis.

Recent research has focused on the molecular mechanisms of ALK-overexpression and its biological consequences. In ALK cases the exact mechanism of transformation is still unknown. Therefore, a unifying concept for both ALK+ and ALK- as well as systemic and cutaneous ALCL is still missing. Our finding that both ALK+ and ALK- ALCL lack T-cell receptor (TCR) expression may provide this unifying feature.

Material and methods

We have studied the expression of T-cell receptors (TCR) αβ (βF1; Serotec, NC, USA) in 46 ALCL (24 ALK+, 15 systemic ALK-, 7 cutaneous) by immunohistochemistry. We also analyzed CD30, ALK1, CD8 (all DAKO Cytomation, Hamburg, Germany), CD3ε, CD4, Perforin (Novocastra, Newcastle, UK), ZAP-70 (Upstate, NY, USA), TIA1 (Coulter Immunotech, Marsaille, France), and GranzymB (Monosan, Am Uden, The Netherlands).

Frozen material of 12 ALK+, 5 ALK-systemic and 1 primary cutaneous ALCL was studied in more detail including the expression of TCRγδ (δF1; T-cell diagnostics, MA, USA) and the NK-cell receptor CD94 (DAKO Cytomation, Hamburg, Germany). To detect weak expression levels at higher sensitivity, coexpressions of CD30 and βF1 or CD30, CD3 and ZAP-70, respectively, were investigated by double and triple...
immunofluorescent stains and confocal laser scanning microscopy. Rearrangements of the TCRβ and TCRγ gene families were analyzed according to the Biomed2 protocol.

Twenty-two not otherwise specified PTCL (PTCL-NOS) and seven angioimmunoblastic T-cell lymphomas (AILT) were studied in comparison. Immunophenotypes were compared by χ² tests or Spearman’s rank correlation (ZAP-70) using Statistica for Windows® (Statsoft GmbH, Hamburg, Germany) to adopt the comparison to the scales used.

Results and Discussion

Only two of 46 ALCL (4%) expressed the TCRβ chain on tumor cells (Figure 1, Table 1) and all were negative for TCRδ and the NK-cell receptor CD94. In both βF1⁺ cases, TCRβ expression was restricted to a subpopulation of the tumor cells. By contrast, TCR molecules were detected in 20/22 PTCL-NOS and in 7/7 AILT.

CD3 molecules are associated with the TCR and transduce the signal of TCR engagement to ZAP-70, a TCR-associated tyrosine kinase that integrates cognate and costimulatory signals to guide downstream signaling. CD3 was lacking in all but one systemic ALK1⁺ ALCL and in 40% of ALK1⁻ systemic ALCL in our study. In most other cases, its expression was restricted to a subset of the tumor cells and much weaker than in accompanying small T-cells. The frequent lack of TCRs and of CD3 and/or βF1 has been demonstrated previously in ALCL. CD3 was not detected in 15/17, 5/6 and 47/70 cases of ALCL.

ZAP-70 was lacking in more than 70% of all ALCL cases studied. Interestingly, the both TCRβ⁺ cases lacked both CD3 and ZAP-70 indicating that proximal TCR-signaling may be impaired in all ALCL investigated. A low protein expression of ZAP-70 has also been reported in ALCL as compared to other PTCL.

In the present series of 19 cases with frozen material, TCRβ and TCRγ genes were clonally rearranged in 74% each. One case each was rearranged for TCRβ but not TCRγ and vice versa. Only three cases, all ALK⁺, showed a Gaussian distribution of peak signals in gene scans. The tumor cells in these cases expressed cytotoxic granules, indicating their T-cell derivation. As only a minor tumor cell population
was present in an inflammatory background, existing TCR rearrangements may have not been detected.

TCR-expression on the tumor cell surface in ALCL has only rarely been investigated: Early studies found TCRβ expressed in 7/713 and 9/157 ALCL studied. In a more recent series, TCRβ was detected in only 4/19 cases but TCRδ or NK cell receptor expressions were not investigated14. Barry and colleagues reported on five PTCL containing small pleomorphic and large CD30+CD15+ Reed-Sternberg-like tumor cells, in which TCRβ was expressed in the small cell population but missing in the large cells15. On the genomic level, TCRβ rearrangements have been detected previously in 90% of ALCL of both T- and null cell type14 and many ALCL negative for pan-T-cell markers have somatically rearranged TCR genes10.

Until today the distinction between ALK1– ALCL and CD30+ cases of PTCL-NOS has not been conceptionally clarified. Three of our 22 PTCL-NOS (14%) were CD30+. In contrast to the ALCL, all of them expressed the TCRβ chain. Although currently there is no difference in treatment options, the lack of TCR expression may well help to further delineate ALK– ALCL.

Considering the presence of somatic TCRβ rearrangements, the normal counterpart of ALCL appears to be an αβ T-cell in most, if not all cases. Further studies are needed to understand the mechanisms underlying the failure of ALCL to express TCRs on the cell surface. Among cell lines derived from ALK+ ALCL, Karpas299 expresses TCRβ RNA by northern blot analysis16, while both Sup-M2 and Su-DHL-1 lack full-length TCRβ RNA in spite of rearrangements for the TCRβ genes17.

Mutations in coding or regulatory regions of the gene or a lack of TCR-specific transcription factors may thus underlie the defective TCR expression, i.e. mechanisms that have been implied to explain the lacking immunoglobulin expression in Hodgkin lymphoma18,19. Alternatively, post-transcriptional mechanisms affecting RNA processing or protein stability are also conceivable, as described recently for the defective TCRβ protein expression in GATA-3-deficient murine thymocytes20.

While the effects of ALK overexpression are well studied, the exact mechanism of transformation in ALK– cases is still unknown3. ALK mediates mitogenicity via
phospholipase C-γ21 and Cyclin D322 and inhibits apoptosis via the phosphatidylinositol 3-kinase/Akt23 and the JAK/STAT pathways24,25. Physiologically, STAT3 and CyclinD3 activation are partially counterbalanced by the effects of TCR signaling: In activated cytokine dependent T-cells, TCR ligation may downregulate CyclinD3 and block cytokine activity by inhibiting signal transduction via the JAK-STAT pathway26. Thus, the inability of the tumor cells in ALCL to cognate TCR interactions, as suggested by our data, may perhaps contribute to the transformation in ALK− cases because of lacking feedback inhibition via TCR signaling.

In summary, both cutaneous and systemic as well as ALK+ and ALK− ALCL lacked TCRs on the cell surface. Both causes and consequences of this observation require further study. The absence of TCR expression in concert with the positivity for CD30 and cytotoxic granules such as perforin12, may now allow to delineate ALCL more precisely from large cell variants of PTCL-NOS, which can also express CD30.
Acknowledgement
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Table 1: Expression of TCRs and molecules involved in proximal TCR signaling in ALCL and other PTCL.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>ALK1</th>
<th>N</th>
<th>βF1</th>
<th>δF1</th>
<th>CD3</th>
<th>ZAP-70</th>
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<td>ALCL</td>
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<td>80%</td>
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<tr>
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<td>0%</td>
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<td>71%</td>
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</tr>
<tr>
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<td></td>
<td>7</td>
<td>100%</td>
<td>0%</td>
<td>80%</td>
<td>0%</td>
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</table>

Differences between ALCL and both PTCL-NOS and AILT are statistically significant (p<0.001) for the expression of βF1 and CD3 in the χ² test and for ZAP-70 expression in Spearman’s rank correlation test. The expression of these antigens was not significantly different between systemic and cutaneous ALCL (p>0.05).

Among systemic ALCL, CD3 expression was significantly more frequent in the ALK1− than in the ALK1+ group, but the expression of TCRβ and ZAP-70 was not significantly different.
Figure 1: Anaplastic large cell lymphoma stained for H&E (upper left), Immunostains for βF1 (upper right), CD3 (middle left) and ZAP-70 (middle right) show negativity of the tumor cells with strong expression of the respective antigens in reactive small T-cells. Immunofluorescent stains for CD30 (green) and βF1 (red) (lower left) or CD30 (green), ZAP-70 (red) and CD3 (blue) (lower right) confirm the negativity of CD30+ tumor cells for the respective antigens.
References


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