CLA and TRAF-1 are Useful in Differentiating Lymphomatoid Papulosis, Cutaneous ALCL and Systemic ALCL.

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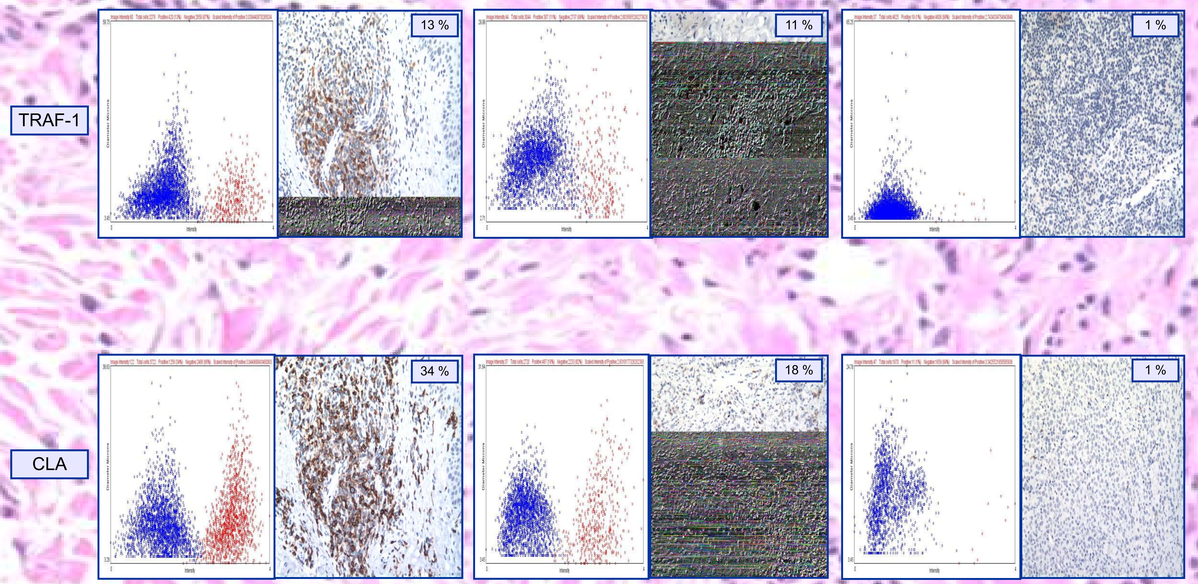
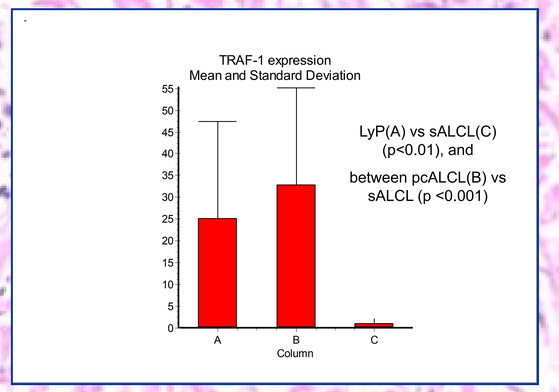
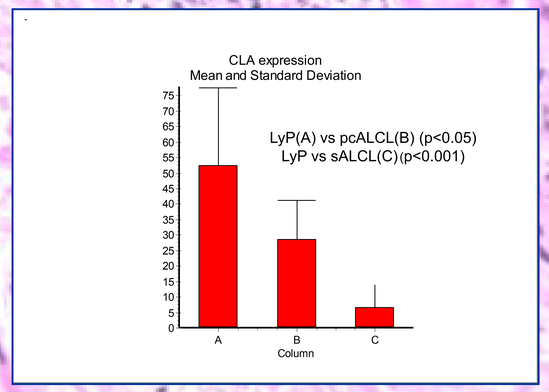
ABSTRACT:
Cutaneous T cell lymphomas and the CD30+ group of T-cell lymphoproliferative disorders are relatively rare and present diagnostic challenges. Lymphomatoid papulosis (LyP), primary cutaneous anaplastic large T-cell lymphoma (pcALCL), and cutaneous infiltrates of systemic anaplastic large T-cell lymphoma (sALCL) are CD30-positive lymphoproliferative disorders of the skin that overlap clinically, histopathologically, and immunophenotypically, but differ in their prognosis. In particular, lesions of LyP regress spontaneously, whereas those of pcALCL persist and may spread to extracutaneous sites; secondary skin lesions confer a poor prognosis in sALCL. In this study we show that differential expression of novel biomarkers may reliably distinguish LyP, primary cutaneous ALCL from systemic ALCL. We used antibodies against TRAF-1(Tumor necrosis Receptor Associated Factor- 1) and CLA (Cutaneous Lymphocyte Antigen) to attempt to differentiate LyP and pcALCL from sALCL. The study included 30 lymphoma biopsies (13 LyP, 9 pcALCL, and 8 sALCL) from patients with CD30-positive lymphoproliferative disorders. Results show an intermediate TRAF-1 expression in LyP and pcALCL with absent expression in sALCL. CLA is higly expressed in LyP and intermediately expressed in primary cutaneous ALCL with very low to absent expression in systemic ALCL. Our findings demonstrate that the extent of TRAF-1 and CLA expression distinguishes LyP/pcALCL from sALCL. TRAF1 and CLA staining, thus, provides diagnostic biomarkers for diagnosis of prognostically divergent CD30-positive lymphoproliferative disorders that affect the skin.

INTRODUCTION:
The spectrum of diseases that constitute the CD30 positive lymphomas, with lymphomatoid papulosis (LyP) at one end, and anaplastic large cell lymphoma (ALCL) at the other end, show variable morphology and clinical behavior. The border between various diseases is sometimes difficult to establish. The central problem in the correct diagnosis and classification of this groups of diseases is that there are no reliable histologic criteria to differentiate between the different types of primary and secondary cutaneous CD30+ lymphoproliferations.
This study attempts to use a panel of 6 immunohistochemical stains including TRAF-1 and CLA in differentiating CD30-positive lymphoproliferations involving the skin. The expression profiles of these markers in CD30-positive lymphoproliferations were analyzed and correlated with the pathologic diagnosis for these entities.
In this study we show that tumor necrosis factor receptor (TNFR)-associated factor 1 (TRAF-1) is differentially expressed in LyP and primary cutaneous ALCL compared to systemic ALCL. As a member of the TRAF family, TRAF-1 is involved in the intracellular signal transduction of TNFR family members without death domain. TRAF-1 function is complex and not completely understood. However, it is possible that TRAF1 is involved in the sensitivity of cells to apoptosis. TRAF-1 is expressed mainly in activated lymphocytes and strongly induced by NFk-B2. Therefore, cells with high NFk-B2 activity generally express high levels of TRAF-1. A member of the TNFR family, like CD30, could also mediate intracellular signaling through NFk-B2 activation leading to subsequent TRAF-1 induction.
Cutaneous lymphocyte antigen (CLA) is an E-selectin ligand which facilitates adhesion of T lymphocytes to cutaneous vascular endothelium, initiating egress of T lymphocytes from the skin to systemic circulation. We found that CLA is highly expressed in CD30+ large atypical cells in LyP but expressed at lower level in primary cutaneous ALCL with minimal to absent level in systemic ALCL. The mechanism of decreased CLA expression in extra-cutaneous spread of lymphoma is presently unknown.



Virtual Flow cytometry (Tissue Quantitative Immunohistochemistry of LyP, cALCL, and sALCL.





Manual Morphometry results show a statistically significant difference between LyP (A) , pcALCL (B) and sALCL (C).

Case #	TRAF-1 (%)	CLA (%)
LyP-1	6	42
LyP-2	16	13
LyP-3	14	15
LyP-4	6	63
LyP-5	8	27
LyP-6	70	76
LyP-7	60	80
LyP-8	46	82
LyP-9	32	43
LyP-10	24	42
LyP-11	37	81
LyP-12	1	74
LyP-13	6	43
pcALCL-1	7	21
pcALCL-2	30	20
pcALCL-3	40	30
pcALCL-4	50	40
pcALCL-5	37	49
pcALCL-6	4	10
pcALCL-7	77	42
pcALCL-8	20	24
pcALCL-9	30	21
sALCL-1	0	1
sALCL-2	<1	3
sALCL-3	1	22
sALCL-4	<1	2
sALCL-5	4	6
sALCL-6	0	14
sALCL-7	0	1
sALCL-8	0	3

DESIGN:
After obtaining SRC and IRB approval, formalin-fixed and paraffin-embedded biopsy specimens from well-characterized diagnostically confirmed CD30 positive lymphomas, both systemic and involving the skin, examined at the Moffitt Cancer center during years 2000 to 2007 were selected from the pathology department of Moffitt Cancer and Research Institute database. All cases were stained with NFk-B2, Fascin, Bcl-2, EMA, ALK-1 and CD56 as well as TRAF-1 (Imgenex IMG 5757) and CLA (HECA 452). Manual morphometry of 500 lymphocytes counted under high power view (40x HPF) and virtual flow cytometry[3] were performed to quantitate the degree of antibody expression. The antibody expression was scored as negative (less than 5%), weak positive (5 to 50%), intermediately positive (50-80%) and strongly positive (80 –100%). Immunohistochemistry was performed on paraffin fixed samples at the University of South Florida Core Laboratory Department of Pathology & Cell Biology according to the manufacturer's protocols.
The study initially included 40 sections from cases with CD30 positive lymphoproloferative disorders. All skin and lymph node samples were examined using hematoxylin–eosin staining and immunohistochemical staining. After pathology review, 10 case were excluded from the study due to scant tissue for adequate diagnosis. The reminder included 13 LyP, 9 cALCL, and 8 sALCL. All diagnosis were confirmed by expert hematopathologists.

RESULTS:
The result shows unclear differential utility for many of the members of the panel (data not shown) except for TRAF-1 and CLA expression. TRAF-1 is expressed in more than 5% in 12 out of 13 LyP cases and in all nine pcALCL. TRAF-1 expression is virtually absent in sALCL with or without skin manifestations (Kruskall Wallis statistic). There was no significant difference of TRAF-1 staining between LyP and pcALCL analyzed for TRAF1 expression (p>0.05), but significant betweeen LyP vs sALCL (p<0.01), and between pcALCL vs sALCL (p <0.001). The tumor cells of all cases of sALCL case were negative for TRAF-1 (p= 0.0003). CLA expression shows extremely significant differences between LyP vs pcALCL (p<0.05) as well as LyP vs sALCL (p<0.001) and insignificant differences between pcALCL vs sALCL(p>0.05). Mean expression of TRAF-1 is 25% in LyP vs 32% in pcALCL and 0.87% in sALCL. CLA had a mean of 52% for LyP, 28.5% for pcALCL and 6.5% for sALCL (see graphs). These results indicate statisticaly significant differences in TRAF-1 expression in cases with LyP / pcALCL vs sALCL, but no significant difference between LyP and pcALCL. CLA on the other hand shows significant differences between LyP vs pcALCL and sALCL. Cutaneous Lymphocyte Antigen (CLA), showed strong expression in LyP with variable expression in primary cutaneous ALCL and low expression in systemic ALCL (see table).

CONCLUSION
There is significant differential expression of TRAF-1 and CLA in lymphomatoid papulosis, primary cutaneous anaplastic large T cell lymphomas and systemic anaplastic large T cell lymphoma. CLA can differentiate LyP from pcALCL. Strong TRAF-1 expression is almost restricted to CD30-positive cells in LyP and intermediate to high expression in pcALCL. sALCL is uniformly negative for TRAF-1.
This study, though of limited sample size, indicates potential utility of TRAF-1 and CLA expression data in separating systemic from primary cutaneous CD30-positive lymphoproliferative disorders.

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