Biomarkers Provide Clues to Early Events in the Pathogenesis of Breast Implant-Associated Anaplastic Large Cell Lymphoma

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Abstract
Almost 200 women worldwide have been diagnosed with breast implant-associated anaplastic large cell lymphoma (BIA-ALCL). The unique location and specific lymphoma type strongly suggest an etio-pathologic link between breast implants and BIA-ALCL. It is postulated that chronic inflammation via bacterial infection may be an etiological factor. BIA-ALCL resembles primary cutaneous ALCL (pcALCL) in morphology, activated T-cell phenotype, and indolent clinical course. Gene expression array analysis, flow cytometry, and immunohistochemistry were used to study pcALCL and BIA-ALCL cell lines. Clinical samples were also studied to characterize transcription factor and cytokine profiles of tumor cells and surrounding lymphocytes. BIA-ALCL and pcALCL were found to have common expression of transcription factors SOCS3, JunB, SATB1, and a cytokine profile suggestive of a Th1 phenotype. Similar patterns were observed in a CD30+ cutaneous lymphoproliferative disorder (LPD). The patterns of cytokine and transcription factor expression suggest that BIA-ALCL is likely to arise from chronic bacterial antigen stimulation of T-cells. Further analysis of cytokine and transcription factor profiles may allow early detection and treatment of BIA-ALCL leading to better prognosis and survival.

Level of Evidence: 5

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Breast implant-associated anaplastic large cell lymphoma (BIA-ALCL) has been recently described as a CD30+ T-cell derived cancer associated with breast implants used for both aesthetic and reconstructive indications. In keeping with the development of other malignancies, including CD30+ primary cutaneous ALCL (pcALCL), it is likely that there are earlier benign precursors to BIA-ALCL on the pathway towards malignant transformation. Identification of these “premalignant” precursors will provide insight into the pathogenesis of BIA-ALCL leading to earlier diagnosis, treatment, and possibly prevention of this disease.
improved prognosis, and potential to avoid adjuvant therapy. Identifying premalignant steps could potentially allow targeted therapy with monoclonal antibodies, small molecule inhibitors, or monochemotherapy with low dose weekly methotrexate used to treat lymphomatoid papulosis (LyP), a premalignant CD30+ cutaneous lymphoproliferative disorder (LPD).1

BIA-ALCL has many features in common with pcALCL including anaplastic morphology, CD30+, HLA-DR+, TIA-1+, ALK(-) immunophenotype, usual absence of clinical symptoms, localized lesions in most cases, infrequent spread to regional lymph nodes without adverse outcome, and excellent prognosis.2-4 Interestingly, 5 of 19 patients evaluated by Laurent et al presented with erythematous skin eruptions before the diagnosis of BIA-ALCL.5

PcALCL is at the malignant end of a spectrum of CD30+ cutaneous LPD. In some patients pcALCL is preceded by LyP, which is characterized by spontaneously regressing skin lesions.1 In those cases, we have shown that pcALCL and LyP lesions are clonally related.6 Progression of LyP to pcALCL is associated with accumulating genetic abnormalities resulting in activation of STAT3 and SOCS3 among other transcription factors characteristic of ALCL.7 Identification of genetic events during progression of LyP to pcALCL may provide clues to the pathogenesis of BIA-ALCL.

Chronic inflammation is considered to be a precursor of many cancers. There is evidence that cutaneous T-cell lymphomas (CTCL) are preceded by chronic inflammation.8 Lechner et al detected the IL-6 signaling pathway in BIA-ALCL supporting the hypothesis that chronic inflammation initiates BIA-ALCL.9 Examination of excised peri-implant capsules reveals chronic inflammation including fibrosis, plasma cell hyperplasia, and lymphocytic infiltrates. Wolfram et al reported intracapsular T-cells producing IL-17, IFN-γ, IL-6, IL-8, and TGF-β suggesting a Th17/Th1 weighted local immune response in peri-silicone implant capsules with capsular fibrosis.10 They suggested that silicone implants trigger a specific antigen-driven local immune response of activated Th17/Th1 cells with subsequent fibrosis promoted by production of profibrotic cytokines.

In addition to the possible trigger of BIA-ALCL by silicone particles or a complex of degraded silicone particles and autologous proteins suggested by Wolfram et al, other potential causes of inflammation should be considered. For example, antigens resulting from insect bites have been implicated in the development of ALK+ cutaneous ALCL. The first author (MEK) originally reported the clinical association of insect bite with onset of childhood CD30+ anaplastic lymphoma in skin lesions and regional lymphadenopathy.11 Lamant et al recently postulated that insect bite-associated antigens could result in an influx of T lymphocytes, some bearing the t(2;5) which activates ALK. The subsequent release of cytokines at the site of the bite could act as a “second hit,” eliciting activation of the latter cells.12

Another potential source of antigen stimulation is from bacteria detected on breast implants. Studies of BIA-ALCL clinical samples by Hu et al13 revealed high numbers bacteria analogous to the number of bacteria found in specimens obtained from patients with capsular contracture. Additionally, they showed that the bacterial community profile (microbiome) in these tumor samples was significantly different from the microbiome around non-tumor capsular contracture specimens, suggesting that different bacterial species may preferentially trigger lymphocyte activation. Previously, the authors had shown a linear correlation between the number of activated lymphocytes with increasing numbers of bacteria from breast implant capsules.14 Interestingly, the highest correlation was for CD4 + T lymphocytes, the same phenotype of tumor cells in BIA-ALCL.14 These findings led us to hypothesize that BIA-ALCL tumor cells might be derived from a sustained T-cell immune response to bacterial antigens. CD4 cells are divided into functional subsets according to their transcription factors and the cytokines they produce. As previously reported by Lechner et al15 we found in the current study that BIA-ALCL cells have a phenotype most closely resembling Th1 cells whose main transcription factor is Tbet and signature cytokine is IFN-γ. Non-malignant Th1 cells have a relatively low capacity for self-renewal and undergo senescence, which could explain the typical slow development of BIA-ALCL.3,4,16

METHODS

A panel of potential biomarkers for BIA-ALCL was selected from gene expression arrays of neoplastic T-cell lines, the
literature and known properties of activated and neoplastic T-cells. Three pcALCL from the Kadin lab and three BIA-ALCL lines from the Epstein lab were then studied by flow cytometry and highly expressed biomarkers were selected as targets for immunohistochemical studies. CD30 served as a positive control. Negative controls were non-immune serum for polyclonal antibodies and isotype-matched Isgs for monoclonal antibodies. Clinical specimens of LyP (N = 10), pcALCL (N = 6), BIA-ALCL (N = 4), and non-tumor breast implant capsules taken from grade IV capsular contracture (N = 4) were studied by immunohistochemistry with approval of the Institutional Review Boards of University of Southern California and Roger Williams Medical Center. All patients with BIA-ALCL were women (range, 52-56 years) with clinically indolent disease.

RESULTS

Gene Array Analysis

A gene expression array of T lymphoma lines showed that BIA-ALCL line TLBR3 has similar high gene expression of Th1 cytokine IFN-γ as cutaneous ALCL line Mac-1; TLBR1 and TLBR2 have moderate expression of IFN-γ similar to

Figure 2. Flow cytometry showing intracellular IFN-γ (A) IL-17F (B) and JunB (C). CD30 is a positive control; intracellular staining (D) and extracellular staining (E). Red shaded areas are positive staining, blue indicates isotype control staining.

Figure 3. BIA-ALCL line TLBR3 stained with antibodies against (A) IFN-γ and (B) IL-17F (arrows).

Figure 4. Immunoperoxidase stain of IFN-γ in cytoplasm of small capsular lymphocytes (left) and large tumor cells (right) in BIA-ALCL tumor.
pcALCL line Mac2A (Figure 1). TLBR1 and Mac-2A have similar elevated expression of Th17 cytokine IL-17F, and TLBR1 and Mac-1 share moderate expression of IL-17A. Gene expression arrays also revealed that SOCS3 and to a lesser degree SOCS2 were highly expressed by cell lines of pcALCL and BIA-ALCL (Figure 1). SOCS3 is highly expressed in systemic ALCL including ALK + ALCL cell lines and most (72%) ALK negative systemic ALCL tumors.17

Flow Cytometry
Flow cytometry confirmed that CD30+ BIA-ALCL cells produce both IFN-γ and IL-17F. Nuclear JunB activity was also demonstrated (Figure 2).

Immunohistochemistry
Immunohistochemistry confirmed expression of IFN-γ and IL-17F proteins in TLBR1 cell line (Figure 3). We next stained clinical samples of BIA-ALCL and benign capsular infiltrates for IFN-γ and IL-17F. Figure 4 shows staining of BIA-ALCL tumor cells and surrounding capsular lymphocytes for cytoplasmic IFN-γ indicating tumor cell production of IFN-γ. Generally weaker expression of IL-17F was detected in tumor cells of BIA-ALCL (Figure 5). T lymphocytes in benign capsular infiltrates usually showed stronger staining for IL-17F (Figure 6). Immunohistochemical studies confirmed tumor cell activity of SOCS3 in both pcALCL and BIA-ALCL (Figure 7). Additionally, preliminary studies reveal comparable high expression of transcription factor JunB in pcALCL (Figure 8) and BIA-ALCL (Figure 9). Similar nuclear activity of Special AT-rich binding protein 1 (SATB1) was found in pcALCL (Figure 10) and in BIA-ALCL (Figure 11).

DISCUSSION
These results are consistent with a Th17/Th1 phenotype of BIA-ALCL tumor cells and benign appearing small intra-capsular T’ lymphocytes.16 Muranski and Restifo have shown a close relationship between Th1 and Th17 polarization and maturational stage of Th memory cells.18 Naïve T-cells which polarize towards Th17 have a high capacity for self-renewal and persistence. Th1 cells have limited capacity for self-renewal and typically undergo senescence. Th17 cells can polarize towards Th1 cells with intermediate Th17/Th1-like cells observed. If antigen activated T-cell precursors of BIA-ALCL cells mimic polarization and maturation of non-malignant Th memory T-cells, their phenotype may determine the time required to initiate BIA-ALCL, ie, a longer time for Th1 polarized cells than for Th17 cells. The observation that BIA-ALCL onset appears around 9 to 10 years after initial implantation19 is consistent with these findings.

Helicobacter pylori (H. pylori) infection has been shown to initiate and contribute to the progression of a B-cell lymphoma from gastric mucosa-associated lymphoid tissue (MALT).20 Increasing evidence shows that eradication of H. pylori with antibiotic therapy can lead to regression of gastric MALT lymphoma and can result in a 10-year sustained remission. Recent findings of Deva et al suggest that bacterial antigens may be involved in the pathogenesis of BIA-ALCL.13 Similarities between BIA-ALCL and pcALCL...
shown here appear to support the hypothesis that bacterial antigens are linked to the pathogenesis of BIA-ALCL. Staphylococcal superantigen endotoxins (SE) colonizing the skin and nares have been incriminated in the pathogenesis of cutaneous T-cell lymphomas. A direct effect on malignant T-cells and indirect mechanisms involving cytokine-driven cross-talk between malignant- and non-malignant T-cells have been demonstrated. Eradication of staphylococci from the skin with antibiotic treatment was associated with clinical improvement. Superantigens of S. aureus are particularly efficient in stimulating IL-17 production and the cytokines produced are from memory T-cells. Similar to BIA-ALCL cells studied here, a set of malignant T-cell lines established from patients with cutaneous T-cell lymphoma (CTCL) spontaneously secrete IL-17F. Inhibitors of Janus kinases and Signal transducer and activator of transcription 3 (STAT3) were able to block that secretion suggesting that IL-17 cytokines and their receptors may serve as therapeutic targets. Moreover, TLBR cell lines expressing phosphorylated STAT3 and are killed by STAT3 inhibition in a dose-dependent manner.

SOCS proteins inhibit cytokine signaling. SOCS3 is expressed at relatively lower levels in Hodgkin lymphoma characterized by numerous tumor derived cytokines and abundant inflammatory cells. Hodgkin lymphoma patients often have clinical symptoms of fever, night sweats, and weight loss attributable to cytokine effects that are commonly lacking in patients with pcALCL and BIA-ALCL. Therefore the high expression of SOCS3 by tumor cells in BIA-ALCL could explain the fewer inflammatory cells and usual absence of clinical symptoms of BIA-ALCL patients.

JunB is a transcription factor regulating gene activity after primary growth factor responses. Overexpression of
JunB is associated with neoplastic transformation. High expression of JunB and CD30 is a hallmark of malignant cells in Hodgkin lymphoma and ALK + ALCL. JunB regulates the CD30 promoter. Silencing JunB leads to decreased CD30 activity and suppresses ALCL growth. We found JunB nuclear activity is increased in cell lines and clinical samples of cutaneous and BIA-ALCL. Figure 8 shows JunB expression also is expressed in LyP but by fewer cells and at lower intensity than in pcALCL. This is consistent with a role of JunB in the progression of LyP to ALCL. The finding of high levels of JunB in BIA-ALCL is therefore consistent with malignant transformation. Future studies are planned to determine if JunB is active at lower levels in a precursor of BIA-ALCL.

SATB1 is a thymocyte specific chromatin organizer, which is over-expressed in lymphoma cells in CD30+ cutaneous LPD; its expression is up-regulated during disease progression. SATB1 promotes proliferation of CD30+ lymphoma cells by direct transcriptional repression of cell cycle inhibitor p21. We found that SATB1 is over-expressed in LyP but to a lesser degree than in pcALCL (Figure 10). SATB1 also is expressed by tumor cells in BIA-ALCL (Figure 11). Its possible role in progression from activated T-cells to ALCL is under investigation.

Lechner et al described a regulatory T-cell (Treg)-like suppressive function for TLBR2 and TLBR3 cell lines manifested by transcription factor FoxP3 and secretion of suppressive cytokines IL-10 and TGF-β. We also reported TGF-β secretion by pcALCL lines Mac-1, Mac-2A, and Mac-2B. Figure 12 shows similar FoxP3 expression in Mac-2A and TLBR-2. These findings suggest that BIA-ALCL is capable of suppressing the local immune response. Our conclusions and hypothesis are limited by the small numbers of cell lines and samples of clinically indolent disease analyzed to date due to the rarity of the disease. Further studies should account for the more aggressive clinical presentations, different pathologic stages, variable histopathologies, and immunophenotypes of BIA-ALCL.

**CONCLUSIONS**

These preliminary studies suggest that BIA-ALCL cells may be derived from Th1/Th17 cells in capsular tissues.
Figure 11. SATB1 antibody stains BIA-tumor cells (A, C) and negative controls (B, D).

Figure 12. Cytospins showing similar FoxP3 activity in multinucleated giant cells of BIA-ALCL line TLBR2 (A) and pcALCL line Mac-2A (B).

Figure 13. Working hypothesis for progression of immune responding T lymphocytes to BIA-ALCL.
and surrounding seromas. Th1/Th17 cells are antigen driven memory T-cells and our findings are consistent with the hypothesis that BIA-ALCL results from chronic bacterial antigen stimulation, sustained T-cell proliferation, and subsequent genetic events (Figure 13). Further investigations to determine whether select biomarkers (eg, JunB, SATB1) will identify non-malignant precursors to BIA-ALCL are planned. This will potentially allow earlier detection of patients at risk of BIA-ALCL and also provide the opportunity to initiate earlier treatment with the hope of avoiding the need for adjuvant therapy and improving both prognosis and survival.

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