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The Role of Cytotoxic and Regulatory T-Cells in Relapsed/ Refractory Hodgkin Lymphoma

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Abstract

Recent data suggests the presence of cytotoxic (TIA-1 and granzyme B+) and regulatory T-cells (FOXP3+) in classical Hodgkin lymphoma (cHL) tissues has been shown to correlate with poor overall survival in mainly diagnostic biopsies. By tissue microarray analyses we extend this observation to a cohort of relapsed/refractory cHL tissue biopsies and analyze immunohistochemical expression of FOXP3, TIA-1 and granzyme B in the inflammatory background and the tumor microenvironment. High expression of TIA1 (>50%) correlated with poor overall survival (p<0.0001), low expression of FOXP3 (<25%) correlated with poor overall survival (p<0.01) and combined high TIA1 (>50%) and low FOXP3 (<25%) correlated with poor overall survival (p<0.0001). Expression of cytotoxic and regulatory T-cells shows prognostic significance in the relapsed/refractory clinical setting of cHL.

INTRODUCTION

In histologic sections of Hodgkin lymphoma, neoplastic cells constitute <1% of the total cell population with a pronounced reactive inflammatory background. Regulatory T-cells within the inflammatory background may play a role in the antitumor host immune response and the role of tumor microenvironment is of interest.(1–4) The prognostic role of cytotoxic T-cell markers in cHL has been shown as well as the role of chemokines in attracting cytotoxic and regulatory T-cells.(5) Although earlier data suggested no prognostic significance to TIA1 expression alone in cHL,(6) recent data show correlation between the presence of cytotoxic (TIA-1+ and granzyme B+) and regulatory T-cells (FOXP3+) and poor overall survival(7–9), however, one report studied only four cases of relapsed biopsies(7). The use of tissue microarrays has been used in the study of cHL(10–12) and we were therefore interested in analyzing expression of these markers for specific cell types in the inflammatory background in a larger series of relapsed/refractory cHL biopsies to determine if there was any correlation with clinical factors and survival outcome.

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MATERIALS AND METHODS

Cases of relapsed/refractory cHL were consecutively ascertained from the files of Memorial Sloan-Kettering Cancer Center (MSKCC) and use of tissues was approved by the Institution's Human Biospecimen Utilization Committee (HBUC) and an Institutional Research Board (IRB) waiver. Patients were treated on one of 3 sequential IRB-approved clinical trials of high dose therapy/autologous stem cell transplant (HDT/ASCT) at MSKCC between September 1994 and December 2003 that required biopsy confirmation of relapsed/refractory HL. Clinical characteristics of patients with relapsed Hodgkin lymphoma who underwent autologous transplantation on two sequential prospective studies as well as data on histologic subtypes are summarized in Table 1. The cohort was comprised of a total of 63 patients, 22 females and 41 males. The median age was 29 yrs (range from 17–62 yrs). Clinical outcomes have already been reported as noted above.(13, 14) A tissue microarray (TMA) of the reactive inflammatory background of relapsed/refractory cHL cases was constructed using a fully automated arrayer Beecher instrument, ATA-27. TMAs were stained in the Pathology Core Immunohistochemistry Laboratory at MSKCC using the following conditions: FOXP3, a monoclonal antibody from ABCAM Inc (Cambridge, MA) used pretreatment steaming with EDTA pH 8.0 for 30 minutes with a 1:1000 dilution of the primary antibody overnight incubation at 4°C, a secondary anti-mouse from Vector Labs (Burlingame, CA) for 1 hour, Streptavidin from DAKO (Carpenteria, CA) at 1:500 dilution for 1 hour and DAB for 5 minutes. TIA-1, MAB, at a 1:400 dilution from Immunotech Inc. (Beckman Coulter, Miami, FL) performed on the Ventana Discovery XT(Tuscon, AZ). Granzyme B, a monoclonal antibody, from ABCAM Inc (Cambridge, MA) was used at a 1:10 dilution and performed on Ventana's Discovery XT(Tuscon, AZ). Stained TMA slides were evaluated by 2 pathologists (AFK and JTF) using the Olympus BX41 double headed microscope using 20X/0.50 and or 40X/0.75 objectives, and scored using the following criteria: 0=negative; 1+=<25%; 2+=25–50%; 3+=50–75%; 4+>75%. A total of 63 evaluable cases of relapsed/refractory cHL with tissue core availability and stain results for TIA-1, granzyme B and FOXP3 were obtained. Results were linked with clinical factors and statistically analyzed using SPSS v15.0 and SAS software. Clinical characteristics, including age at ASCT, presence of bulky disease > 10cm, presence of B symptoms, high risk factors, number of involved sites, disease progression, and survival. Histologic features were reviewed; all cases were diagnosed as classical Hodgkin lymphoma according to the World Health Organization classification scheme. Histologic subtype (nodular sclerosis, mixed cellularity, lymphocyte-rich, lymphocyte-depleted) was noted as well as presence of CD20 positivity of RS cells. Radiologic status including PET scan after ICE chemotherapy was also noted. Histologic and immunohistochemical images for figures 1–4 were obtained with the Olympus AH2 microscope Camera from Center Valley, Pennsylvania. Image acquisition and processing software was performed using Adobe Photoshop 7.0 and Olympus DP12 software. Survival analysis was performed using failure-free survival (FFS) and overall survival (OS) as clinical end points and plotted as Kaplan-Meier survival curves. Statistical analysis was performed using SPSS version 12.0 (SPSS Inc, Chicago IL).

RESULTS

The TMA scores for TIA1, FOXP3 and granzyme are summarized in Table 2. Representative H&E sections of cHL cases that showed an increase in TIA-1+ cells and decrease of FOXP3+ cells are shown in Figure 1. Representative images and examples of 1+, 2+, 3+, and 4+ staining for TIA-1 from the immunohistochemical stained TMA slide are shown in Figure 2. Representative images of high and low staining for granzyme B from the immunohistochemical stained TMA slide are shown in Figure 3. The inflammatory cells showed characteristic cytoplasmic granular staining and localization for TIA1 and granzyme B. TIA-1 was noted to show localization to myeloid and neutrophil cells as well as lymphoid

cells, where as granzyme B was noted to localized to lymphoid cells only, presumably cytotoxic T cells. The extent of reactivity for granzyme B (89% with score of 1) was far less than that observed for TIA1 (82% with score of 2 or greater). Cases with predominant myeloid and neutrophil cytoplasmic localization of TIA-1 could account for differences in reactivity between granzyme B seen localized in general to lymphoid cells, presumably cytotoxic T cells. The remote possibility of technical artifact in staining technique cannot also be ruled out. Representative images and examples of FOXP3 scored as 1+, 2+, and 3+ from the immunohistochemical stained TMA slide are shown in Figure 4 with the inflammatory cells showing nuclear localization. TIA-1 staining showed significant inverse correlation with FOXP3 in relapsed/refractory biopsies ($p < 0.0002$). No significant correlation was seen with granzyme B expression, high LDH, and relapse in prior radiation site. High expression of TIA1 (>50%) correlated with poor overall survival ($p < 0.0001$) as shown in Figure 5A, low expression of FOXP3 (<25%) correlated with poor overall survival ($p < 0.01$) as seen Figure 5B and combined high TIA1 (>50%) and low FOXP3 (<25%) correlated with poor overall survival ($p < 0.0001$) as shown in Figure 5C.

DISCUSSION

Cytotoxic and regulatory T-cells play an important role in the antitumor host immune response in relapsed/refractory cHL and shows clinical prognostic significance. This study confirms the observation of increased TIA-1, a marker of cytotoxic T cells (abbreviated as TIA-1 throughout the remainder of the discussion) and decreased FOXP3, a marker of regulatory T cells (abbreviated as FOXP3 throughout the remainder of the discussion) correlates with a poor outcome in a larger series of relapsed/refractory cHL biopsies. This observation was previously reported mainly in diagnostic specimens of cHL(7–9), with one report analyzing only four cases of relapsed biopsies(7). Our data also confirms that high FOXP3, a marker of regulatory T-cells suggests better overall survival as indicated in a series of diagnostic specimens of cHL. (9) Recent gene expression profiling and correlative immunohistochemistry expression on tissue also show that high TIA-1 expression was associated with poor or adverse survival and a high FOXP3 correlated with a favorable influence.(15) Schreck et al. showed that a high frequency of tumour-infiltrating c-Maf (Th2) cells was associated with an improved disease-free survival and event-free survival. They also showed that a high ratio of FOXP3 (Treg) over c-Maf (Th2) cells resulted in a shortened disease-free survival. Although they do not report on the prognosis of FOXP3 alone, their data indirectly implies a high FOXP3 may correlate with a favorable response. (16) The recruitment of Tregs to tumors is poorly understood but might involve chemokines. (17) Some data suggest an increase of Tregs is associated with loss of EBV immunity and that EBNA1 up-regulates the expression CCL20 in HL cells and that this leads to increased chemotaxis of Tregs.(18) Prior studies have not found any association with EBV infection and the proportion of FOXP3 and TIA1 positivity(7) or a survival difference between EBV positive and negative patients although data is controversial in the literature.(8) Recent data suggest Hodgkin and Reed-Sternberg (HRS) cells express IL-21 which upregulates MIP-3alpha which in turn attracts CCR6+CD4+CD25+FoxP3+CD127lo regulatory T cells toward HRS cells, which might favor their immune escape.(19) Gal1 treatment of activated T cells favored the secretion of Th2 cytokines and the expansion of CD4+CD25highFOXP3+ Treg cells, therefore, Gal1 may represent a potential therapeutic target for restoring immune surveillance in cHL.(20) The increase of Treg cells seems to be a characteristic feature in most tumors, with much recent interest in the molecular and cellular mechanisms responsible for the increase and maintenance of elevated levels of Treg cells in cancer and as a potential therapeutic agent, so much so, with an entire May issue of *Immunity* devoted to the subject.(21–28) Further insights into the molecular mechanisms and association with other signaling pathways and relation with EBV will be of interest in

the clinicopathologic significance in HL and provide additional insight into novel therapeutic strategies. (4, 29)

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Figure 1.
Representative H&E sections of cHL cases that showed an increase in TIA-1+ cells and decrease of FOXP3+ cells (400×) showing nuclear localization to mainly lymphoid cells, presumably T cells.

Figure 2. Representative images and examples of 1+, 2+, 3+, and 4+ staining for TIA-1 from the immunohistochemical stained TMA slide (400×) showing cytoplasmic localization to myeloid, neutrophils, and lymphoid cells.

Figure 3.
Representative images of high and low staining for granzyme B from the immunohistochemical stained TMA slide (400×) showing cytoplasmic localization to lymphoid cells.

Figure 4.
Representative images and examples of FOXP3 scored as 1+, 2+, and 3+ from the immunohistochemical stained TMA slide (400×) showing nuclear localization.

Figure 5.

Kaplan-Meier survival plots showing high expression of TIA-1 (>50%) correlated with poor overall survival ($p < 0.0001$) as shown in Figure 5A, low expression of FOXP3 (<25%) correlated with poor overall survival ($p < 0.01$) as seen Figure 5B and combined high TIA-1 (>50%) low FOXP3 (<25%) correlated with poor overall survival ($p < 0.0001$) shown in Figure 5C.

Table 1

Characteristics of 63 patients with relapsed Hodgkin's lymphoma who underwent autologous transplantation on two sequential prospective studies.

Feature	Number	Percent (%)
Patients (10/94-9/03)	63	
Age at ASCT, median (range)	29 (17–62)	
Bulky disease >10 cm	11/56	20
CD20+	6/53	11
Refractory	31/62	50
High risk (2 or 3 risk factors)	38/64	59
B symptoms at relapse	21/59	36
Extranodal disease	33/61	54
PET negative CR after ICE	49/63	78
Type of cHL		
NSHL	41	65
MCHL	3	5
LRCHL	0	0
LDHL	1	2
CHL unspecified	18	29

Table 2

TIA1, FOXP3, and Granzyme B Stain Results in patients with relapsed Hodgkin's lymphoma who underwent autologous transplantation.

Staining	Score	No. of positive cases	Percent %
TIA1	1	16	25
	2	24	38
	3	14	22
	4	9	14
FOXP3	0	11	17
	1	38	60
	2	12	19
	3	2	3
Granzyme	0	3	5
	1	56	89
	2	4	6