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ARTICLE

Intra-tumoral Cytolytic Cells: Pattern of Distribution in B-cell Non Hodgkin's Lymphoma

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Non-Hodgkin's lymphomas (NHLs) constitute a heterogeneous group of lymphoid neoplasms and a majority of them in India are of B-cell phenotype. Varying numbers of T lymphocytes and natural killer (NK) cells are consistently present within the lymph nodes (LNs). The role of these "reactive" cells is becoming understood. TIA-1 is a cytotoxic granule associated RNA binding protein, the expression of which is restricted to cytotoxic T lymphocytes (CTLs) and NK cells. Snap frozen lymph node biopsies obtained from 41 B-cell NHLs were localized for intra-tumoral TIA-1 + cytolytic cells by immunohistochemistry. Distribution of T cell subsets and NK cells were also quantified. Cells expressing TIA-1 antigen was observed in all the cases, seen as a strong granular cytoplasmic signal. Results indicate significantly higher number of TIA-1 cytolytic cells outside (periphery of the follicle and interfollicular areas) than within the neoplastic follicle in follicular lymphomas ($p<0.001$). In small lymphocytic lymphomas, cytolytic cells were mainly seen as uniformly scattered single cells, distributed throughout the tumor environment. In mantle cell and diffuse large B-cell lymphomas these were most often seen as small clusters and less frequently as singly scattered cells. Higher numbers of $CD4^+$ than the $CD8^+$ T cells were observed in most cases. Contrary to the follicles in follicular hyperplasia, $CD57^+$ NK cells were predominantly observed outside the neoplastic follicle in follicular lymphomas (FLs). These results outline specific interactions between the potential anti-tumoral cytolytic and the malignant cells of B-cell NHLs. (Pathology Oncology Research Vol 6, No 2, 114–117, 2000)

Keywords: lymphoma, T-cells, CD4, CD8, TIA-1, NK cells, tumor infiltrating lymphocytes, cytotoxic cells, B-cell

Introduction

Non-Hodgkin's lymphomas constitute a heterogeneous group of lymphoid neoplasms and a majority of them in India are of B-cell phenotype. Within the LNs, apart from the neoplastic cells, there is a large component of reactive cells, comprising mainly of T lymphocytes. Smaller numbers of macrophages, NK cells and reactive B lymphocytes are also noted. In B-cell NHLs, the number of tumor infiltrating T lymphocytes (TIL "T") vary between the histologic subtypes.[4,11] The TIL "T' are of either helper (CD4) or cytotoxic (CD8) phenotype and may either facilitate the neoplastic process or mount an anti-tumor immune response: some could also be mere bystanders.

TIA-1 is a cytotoxic granule associated RNA binding protein, the expression of which is restricted to cytotoxic T lymphocytes (CTLs) and NK cells. Flow cytometric analysis of purified $CD8^+$ subset has shown TIA-1 antigen in 49–64% of cells.[1] The 15 kd TIA-1 isoform is the major species present in cytotoxic granules and is derived from carboxy terminal) of 40 kd isoform by proteolytic processing.[14] Both species induce DNA fragmentation in digitonin permeabilized thymocytes and are implicated as candidate effectors of cytotoxic lymphocyte mediated apoptosis.

Previous studies in our laboratory indicate the presence of T cells cytotoxic for autologous and allogenic tumor cells in the peripheral blood of B-cell NHL patients and prompted us to undertake this study.[12,13] In the present investigation, we have studied the number and pattern of distribution of intratumoral $TIA\text{-}1^+$ cytolytic cells on snap frozen LN tissues obtained from 41 B-

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Table 1. **T lymphocyte subsets, CD57$^+$ and TIA-1$^+$ cytolytic cells in follicular lymphoma subtype**

Subtype (grade)	*CD45R0$^+$ T cells within/outside follicle*		*CD57$^+$ NK cells within/outside follicle*		*TIA-1$^+$ cells within/outside follicle*		*CD4$^+$:CD8$^+$ T cells outside*
I	1550	4833	101	1616	49	200	10:1
II	2266	5166	108	1853	75	340	4:1
II	2016	4500	345	1783	98	400	8:1
III	2883	4666	106	1641	36	130	1:1
III	2872	4648	200	785	17	194	2:1

The number of CD45R0, CD57 and TIA-1$^+$ cells were scored and represented as number of cells / 10 mm^2 of tissue section as mentioned in materials and methods, immuno-reactive cells were quantified both within and outside (periphery of the follicle and interfollicular areas) the neoplastic follicles of follicular lymphoma.

cell NHLs by immunohistochemistry. These cytolytic cells were also immunophenotyped for CD3, CD4, CD8, CD45R0 and CD57.

Materials and Methods

Patients and Classification

41 patients diagnosed as NHL at the Tata Memorial Hospital, Mumbai (Bombay), India were evaluated in this study. To delineate the T cell responsive component unambiguously, we selected lymphomas of B-cell lineage for the study. Patients were classified according to the Revised European American Lymphoma (REAL) classification[8] by routine histopathological examination and immunophenotyping using a panel of monoclonal antibodies (*Table 1*) and polyclonal CD3 (DAKO, Denmark). The study included 10 small lymphocytic lymphomas (SLL), 21 follicular center lymphomas, follicular grade I (n=7), grade II (n=10) and grade III (n=4), 4 mantle cell and 6 diffuse large B-cell lymphomas. The age of the patients ranged from 22 to 77 years (median age 53) and the male to female ratio was 8:1.

Monoclonal antibodies

CD3, CD4 and CD8 antibodies were from NFATCC, Pune, India. The CD5, CD10, CD20, CD23, CD30, CD45R0, CD57, PC10 and 120 antibodies were from DAKO, Denmark. Antibody to TIA1 cytotoxic granule (2G9) was a kind gift from Dr. Paul Anderson, Boston, USA.

Immunohistochemical Study

4 μm thick formalin fixed paraffin embedded LN tissue sections were immunostained for memory/activated T lymphocytes (CD45R0) and NK cells (CD57). T cell subsets (CD4 and CD8) and intratumoral TIA-1 cytolytic cells were localized on 5 μm, thick snap frozen LN tissue sections. Briefly, sections were incubated with relevant monoclonal antibodies followed by biotinylated anti-goat immunoglobulin (DAKO, Denmark).

Sections were finally incubated with avidin-biotin complex conjugated to horseradish peroxidase (DAKO, Denmark) and a color reaction was developed using diaminobenzidine as chromogen. Tissue sections were counterstained with haematoxylin.

Enumeration of lymphocyte subsets and TIA-1$^+$ cytolytic cells

In each case, ten microscopic fields (400x) were evaluated for cell counting. The number of CD45R0, CD57 and TIA-1 was scored and represented as number of cells/10 mm^2 of tissue section using microscopic grid. Immunoreactive cells were quantified both within and outside (periphery of the follicle and interfollicular areas) the neoplastic follicles of follicular lymphoma and in random areas of other NHL subtypes. Quantitation was performed independently by two pathologists to exclude any bias.

Statistical analysis

Analysis was performed by Statsview software (MS windows 6.0). Statistical significance between the number of TIA-1$^+$ cytolytic within and outside the neoplastic follicles in follicular lymphomas was analyzed by 2 tailed "t" test for paired samples.

Results

Expression in small lymphocytic lymphomas

Specific patterns of distribution of cytolytic cells were observed in different histologic subtypes. Positivity for TIA-1 antigen was seen as strong granular cytoplasmic signal. In SLL, cytolytic cells were singly scattered throughout the tumor and were rarely seen in clusters. In only 2/10 cases were they seen in small clusters (2–3 cells/cluster). TIA-1$^+$ cytolytic cells infiltrating the tumor environment were fewer (range 27–266, median 65) as compared to CD 57$^+$ NK cells (range 86-1932, median 391) and CD45R0$^+$ T lymphocytes (range 847–4017) per 10 mm^2 of tissue section. The ratio of CD4: CD8$^+$ T cells was ≥1 in 9/10 cases. In patient 3, despite higher numbers of CD8$^+$ T cells, the TIA-1$^+$ cytolytic cells were few (*Figures 1a,b*).

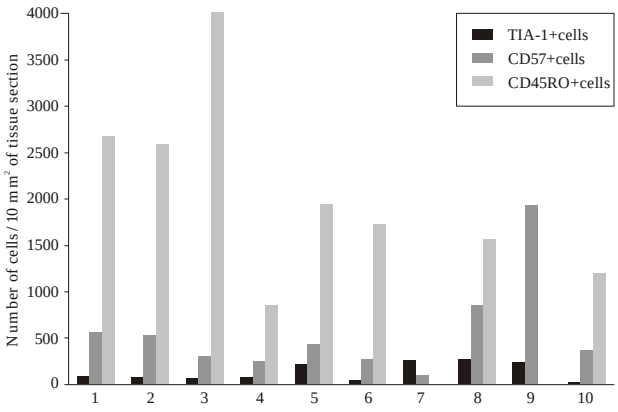

Figure 1a. Expression of TIA-1, CD57 and CD45R0 cytolytic cells in small lymphocytic lymphomas.

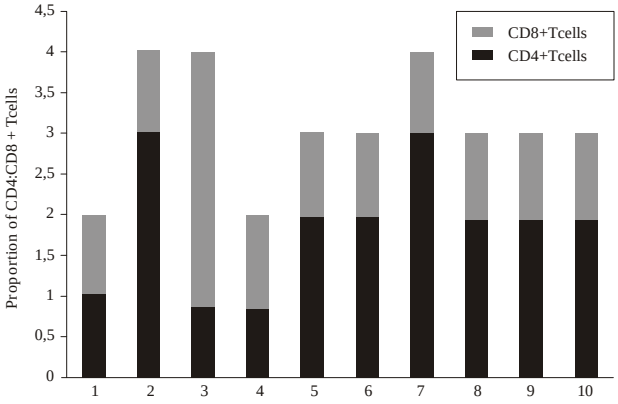

Figure 1b. Proportion of CD4⁺ and CD8⁺ T lymphocytes in small lymphocytic lymphomas.

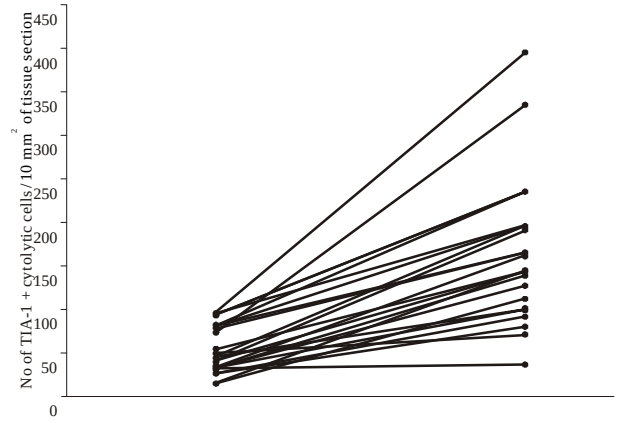

Figure 2. Expression of Intra-tumorai TIA-1⁺ cytolytic cells in follicular lymphomas. Statistical significance ($p < 0.001$) as analyzed by 2 tailed 't' test for paired samples.

Expression in follicular lymphomas

TIA-1⁺ cytolytic cells were predominantly localized outside (at the periphery of the follicle and interfollicular areas) than within the neoplastic follicle in FLs (*Figure 2*, $p < 0.001$). TIA-1 staining highlighted the follicular pattern in majority of these cases. Addtionally, in 5 cases the number of CD45 RO, CD4, CD8 and CD57 positive cells were also quantified (*Table 1*). The CD4/CD8 ratio was consistently higher within the neoplastic follicles. Similarly, CD57⁺ NK cells were also predominantly observed outside the neoplastic follicle in FLs.

Expression in mantle cell and diffuse large B-cell lymphomas

TIA-1⁺ cytolytic cells were mainly seen as uniformly distributed clusters in mantle cell and diffuse large B-cell lymphomas (*Figure 3*). Unlike SLLs they were rarely observed as singly scattered cells. The number of TIA-1⁺ cytolytic cells infiltrating the tumor environmemt were fewer (range 27–618, median 245) as compared to CD57⁺ NK (range 121–1069, median 560) and CD45R0⁺ lymphocytes (range 1942–5312) per 10 mm² of tissue section. The ratio of CD4:CD8⁺ T cells was = 1 in 9/10 cases.

Discussion

Varying numbers of TIL-'T' are consistently present in LNs of B-cell NHL.[6,10] These non-neoplastic T cells could either be residual elements of the LN, or cells attracted from circulating pool as a reaction to the malignant process and may represent an immune response to the tumor. Hence, we have quantified and studied the distribution of T-cell subsets, NK cells and intratumoral cytolytic cells using a specific marker, TIA-1.

Previous studies have reported greater number of activated T-cells in NHLs than in benign lymphoid hyperplasias (BLH,[9]). Three color flow cytometric analysis heve shown significantly higher percentege of activated TILs in B cell lymphomas of intermediate and high grade as compared to low grade lymphomas and BLH.[2] As in the previous studies, we heve observed higher numbers of CD4⁺ TILs than the CD8⁺ TILs in most cases.[7]

Among the reactive components studied, the proportion of CD57⁺ NK cells were significantly lower compared to the T-cell subsets. In FLs, though the CD45R0⁺ T and CD57⁺ NK cells were abundant, TIA-1⁺ cytolytic cells were relatively few. In an earlier study on FL, nearly 50% of CD56⁺ NK cells co-expressed TIA-1.[9] In the current study CD57⁺ NK cells outnumbered the TIA-1⁺ cytolytic cells. This suggests that a higher proportion of CD57⁺ NK cells in FL lack the TIA-1 antigen. It is of interest to note that the pattern of infiltration by CD57⁺ cells of reactive follicles in benign LNs is

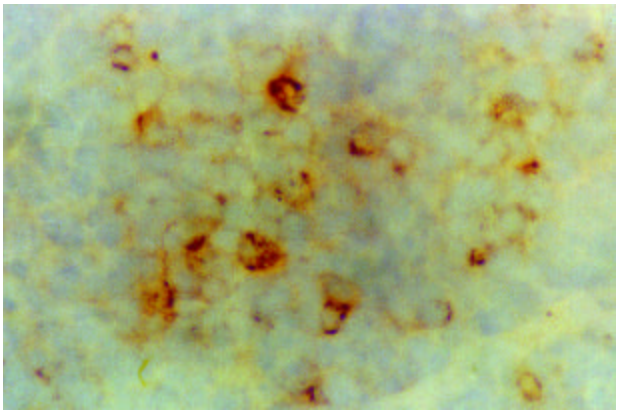

Figure 3. Frozen section immunostaining with monoclonal antibody to TIA-1 antigen in mantle cell lymphoma.

remarkably different. While the $CD8^+$ T cells are infrequent, large numbers of CD57 NK cells are seen within the reactive follicles (unpublished observation). Similar findings have also been reported in two independent studies.[3,9]

Felgar et al have reported the expression of TIA-1 antigen in all cases of T-cell rich large B-cell lymphomas and diffuse large B-cell lymphomas as in our study on forty one B-cell NHLs.[5] We have noted variable patterns in distribution of TIA-1^+ cytolytic cells in different NHL subtypes. In the current study on twenty one FLs, we report significantly higher number of TIA-1^+ cytolytic cells outside (periphery of the follicle and interfollicular areas) than within the neoptastic follicle ($p<0.001$). TIA-1 staining highlighted the fotlicular pattern in majority of these cases. The TIA-1^+ cytolytic cells were seen mainly as small clusters and less frequently as singly scattered cells in mantle cell and diffuse large B-cell lymphomas. In contrast in SLLs, they were seen most often as uniformly scattered single cells. In one case, despite higher proportion of $CD8^+$ T cells, the TIA-1 + cells were few (*Figures 1a,b*). Probably, the majority of $CD8^+$T cells lack the TIA-1 antigen in this case.

In conclusion, our results outline specific interactions between potential anti-tumoral cytolytic and the malignant cells of B-cell NHL. These interactions are heterogeneous among various histopathological subtypes and the basis of this heterogeneity should be further analyzed to determine their impact on pathogenesis, evolution and prognosis.

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