Primary Brain Lymphoma Cell Turnover Differs in Patients With and Without AIDS: Relationships to bcl-2 Expression and Host Cell Reaction

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Abstract. Primary central nervous system lymphomas (PCNSLs) are more resistant to radiotherapy and chemotherapy in AIDS (A-PCNSLs) than in non-AIDS patients (NA-PCNSLs). We investigated 23 A-PCNSLs and 24 NA-PCNSLs. Lymphoma cell kinetics (i.e. proliferation [mitotic index, MIB-1 and PCNA labeling indices], apoptosis and turnover) were determined and compared with bcl-2 and LMP-1 expression, and to the percentage of tumor-infiltrating T-lymphocytes (T-TILs) and macrophages. A-PCNSLs showed lower proliferation (p < 0.005), less apoptosis (p < 0.0001) and slower cell-turnover (p < 0.0001) than NA-PCNSLs. LMP-1 was detected in 90% of A-PCNSLs and 5% of NA-PCNSLs, a finding correlating positively with bcl-2 expression (p < 0.0007). In contrast, T-TIL counts and CD4/CD8 T-TIL ratios were similar in A-PCNSLs and NA-PCNSLs. T-TIL counts correlated negatively with proliferation indices (from p < 0.05 to p < 0.0005) in NA-PCNSLs, but not in A-PCNSLs. Macrophage counts correlated positively with apoptosis in both groups. We concluded the following: (i) A-PCNSLs are characterized by accumulation of slow-cycling, long-lived cells that might be protected from apoptosis by LMP-1 induced bcl-2 expression, and independently from the host response; (ii) NA-PCNSLs are characterized by a faster cell turnover associated with an insufficient antiproliferative host response; and (iii) A-PCNSLs and NA-PCNSLs constitute 2 entities with distinctive morphology and different kinetic profiles that could account for different responses to therapy.

Key Words: AIDS; Apoptosis; Cell-turnover; Primary brain lymphomas; Proliferation; Tumor-infiltrating T-lymphocytes.

INTRODUCTION

Meta-analysis of the clinical evolution of primary central nervous system lymphomas (PCNSLs) shows that therapy could prolong sevenfold survival of non-AIDS patients (NA-PCNSLs), but does not significantly improve survival time of AIDS patients (A-PCNSLs) (1, 2). A-PCNSLs appear to be resistant to corticosteroids and radiotherapy that usually allow long-term control of NA-PCNSLs (1, 3–7). The long-term benefit of combined radiotherapy/chemotherapy of A-PCNSLs is more difficult to assess (2, 3). Selected A-PCNSL patients have shown response to combined modality treatment but only a few survived up to 13.5 months (4, 8). About 85% of NA-PCNSL patients respond to combined radiotherapy/chemotherapy and up to 45% of them survive for up to 5 years (3, 9, 10). Therapy failure of A-PCNSLs has been attributed to chemotherapy-related complications, concurrent opportunistic infections, poor Karnovsky status, and relapses (1, 3–6). Since lymphoma relative resistance to therapy may critically depend on the rates of tumor cell proliferation and death (11–13), it is plausible that cell turnover, proliferation, and apoptosis could be dissimilar in A-PCNSLs and NA-PCNSLs. Moreover, Epstein-Barr virus (EBV), which is known to influence B-lymphocyte division and apoptosis (14, 15), is consistently detected in A-PCNSLs but rarely in NA-PCNSLs (2).

Ninety-eight per cent of PCNSLs are B-cell non-Hodgkin’s lymphomas (NHLs) (2). B-lymphocytic malignancies are unique in that tumor cells can elicit an immune response and are subject to T-cell regulation (16). The antitumoral host response includes a cytotoxic T-cell reaction (17) and a cytokine-mediated attempt to control the size of the malignant cell population (16). Positive correlations between the numbers of overall T-tumor-infiltrating lymphocytes (T-TILs) (18–20), cytotoxic T-TILs (21, 22), or helper T-TILs (18, 19) with a more favorable outcome has been documented in immunocompetent patients with systemic NHLs. Consistently, immune surveillance failure plays a major role in the pathogenesis of NHLs in immunocompromised hosts (6, 16), and evidence of a deficient TIL-reaction is available in both systemic NHLs (22) and PCNSLs (23).

To determine whether the different clinical behavior of PCNSLs in AIDS and non-AIDS patients could reflect intrinsic or host determined differences in cell kinetics, lymphoma cell proliferation, apoptosis, and cell turnover were determined in A-PCNSLs and NA-PCNSLs and compared with the immunocytochemical expression of bcl-2 and EBV latent membrane protein 1 (LMP-1), on one hand, and to the immune response assessed by the number of T-TILs and macrophages, on the other. Our results indicate that, similar to high grade peripheral
B-cell NHLs, NA-PCNSLs are rapidly proliferating lymphomas susceptible to antitumoral host response, whereas A-PCNSLs represent a distinct entity characterized by an accumulation of longer-lived EBV-infected cells that are poorly affected by the antitumoral host response and that appear intrinsically resistant to chemotherapy and radiotherapy.

MATERIALS AND METHODS

Lymphomas

We studied 47 biopsy (42 needle and 5 open surgery) specimens from consecutive patients with B-cell PCNSLs (A-PCNSLs, n = 23, NA-PCNSLs, n = 24) who had not received antineoplastic therapy prior to surgery. Lymphomas were graded according to the R.E.A.L. classification (24) and according to a modified Working Formulation for clinical usage (WFCU) (25). In this modification the diagnosis of diffuse large noncleaved cell lymphoma (LNCLL) is based on the predominance of large noncleaved cells (>80%) and the diagnosis of immunoblastic plasmacytoid lymphoma (IBPL) on the predominance of large, often polymorphous immunoblasts with plasmacytoid differentiation (>80%). Tumors representing a mixture of these 2 cell types were classified as "immunoblast-rich/large cell lymphoma (26). In our experience this modification is particularly relevant to classifying PCNSLs in immunocompromised patients where a high incidence of IBPLs and immunoblast-rich/large cell lymphomas is observed (27).

Immunohistochemistry

Depending on the availability of paraffin-embedded tissue, primary antibodies were applied to different numbers of specimens; anti-CD20 (1:50) (n = 47), anti-CD 45RO (1:50) (n = 45), anti-CD68 (1:20) (n = 38), anti-CD3 (1:50) (n = 42), anti-bcl-2 (1:20) (n = 36), anti-LMP-1 (1:100) (n = 36), anti-PCNA (1:50) (n = 42) (all from Dako, Glostrup, Danemark), anti-MIB-1 (1:50) (n = 43) (Coulter/Immunotech, Marseille, France), anti-CD8 (1:20) (n = 37) (Mason, Oxford, UK), anti-CD4 (1:30) (n = 23) (Santa Cruz Biotechnology, Santa Cruz, CA). The lower number of cases evaluated for CD4 is due to the fact that reliable immunostaining was obtained in 23/40 samples only. All primary antibodies except anti-CD4 were monoclonal. Paraffin sections (5 μm) of neutral formalin-fixed material on Superfrost slides (Kindler, Freiburg, Germany) were subjected to heat (2–4 5-minute pulses in a microwave oven at 750W in the respective buffer) or proteinase XXIV P(8038) (Sigma, Diesemboren, Germany) digestion-based antigen retrieval as follows: MIB-1 and CD3 (1 mM EDTA-NaOH pH8, 3 × 5 min), PCNA, CD45RO and CD20 (10 mM citrate pH6, 3 × 5 min), CD8, CD68 and bcl-2 (100 mM Tris-HCl pH8, 3–4 × 5 min), LMP-1 (proteinase XXIV, 15 min) and CD4 (proteinase XXIV, 10 min, followed by 1 mM EDTA-NaOH pH8, 4 × 5 min). Non-specific sites were blocked by BSA 3%, applied for 1 hour (monoclonal antibodies) and 4 hours (polyclonal anti-CD3 antibody). Primary antibodies were applied in a humid chamber, at 4°C, overnight (14–16 hours). A ready-to-use streptavidin peroxidase-labeled biotin kit (Dako, Carpinteria, CA) for detection of primary rabbit/mouse antibodies was used according to the manufacturer’s instructions. The immunoreaction was visualized with diaminobenzidine (DAB) (Amresco, Solon, OH), slides were lightly counterstained with Harris’ hematoxyline and mounted with aqueous medium (Shandon, Pittsburgh, Pennsylvania). A nickel/cobalt DAB staining enhancement step (1% NiCl3 and 0.5% CoCl2 in TBS pH 7.6 for 5 min) was carried out in CD4 immunodetection (28). Double labelling for MIB-1, on one hand, and CD20 (n = 40) CD45RO (n = 31), or CD68 (n = 15), on the other, were carried out by a slight modification of the method proposed by Lan et al (29). Briefly, after antigen retrieval in 1 mM EDTA-NaOH pH8, incubation with the anti-MIB-1 antibody, application of the streptavidin peroxidase-labeled biotin second and third layers, and development with DAB to produce a brown color, slides were heated for a second time in the respective buffer to block antibody cross-reactivity and to unmask the second antigen, blocked with BSA 3%, incubated with the second primary antibody, then with a rabbit anti-mouse immunoglobulin antibody (1:25), an APAAP complex (1:50), (Dako, Glostrup, Danemark), (30 min each), developed with Fast Red (Sigma, Diesemboren, Germany) to produce a red color, counterstained, and mounted.

Proliferation, bcl-2 Labelling (Lis), Mitotic (Mli), Apoptotic (Ail) and Turnover (Ti) Indices

MIB-1 LI and PCNA LI were defined as percentages of labeled cells of the total cell population on counting of a minimum of 1,000 (1,000–3,000) cells per case (<400). A minimum of 500 (500–1,000) cells were counted to assess the percentage of bcl-2 + cells. The MIB-1 LI of CD20+, CD45RO+ and CD68+ cells were defined on double-labeled sections as the percentages of MIB-1 labeled CD20, CD45RO or CD68 cells. At least 1,000 (1,000–2,500) CD20+, at least 30 (30–400) CD45RO+ and at least 50 (50–400) CD68+ cells were counted (<400). The numbers of MIB-1 positive or negative cells in contact with CD45RO+ (30–100) were recorded on sections double-labeled with CD45RO and MIB-1.

Mitotic and apoptotic indices were assessed on 5 μm-thick hematoxylin and eosin (H&E) sections as described by Spina et al (11, 30, 31) and expressed as percentages of the total cell population on counting of a minimum of 1,000 (1,000–4,000) cells per case with an oil-immersion objective (<1,000x). Both apoptotic and mitotic cells were identified by standardized criteria (32, 33). Apoptosis was assessed by morphology instead of in situ end labeling (ISEL) because a previously described method (34) provided unreliable results in 12 cases, as previously reported in tumors (35). Indeed, the ISEL LI was higher than the apoptotic index assessed in H&E sections by 125% to 600% (p < 0.0001) (Wilcoxon test) and estimates were poorly reproducible. The cell turnover index (TI) as defined by the sum of MI and AI (11, 30, 31) was calculated.

Detection of EBV

LMP-1 was semiquantitatively evaluated (26, 36) (0, no positive cells; 1, scattered positive cells in a middle power field [×<250] [=5% of cells]; 2, few positive cells in some high-power fields [×400] [=10%]; 3, positive cells easily seen in each high-power field [=20%]; 4, many positive cells in each
high-power field (>20%-30%). In situ hybridization (ISH) for EBER-1 was performed as previously described (37, 38).

**T-cell and Macrophage Counts**

T-ILS (CD3+, CD45RO+, CD4+, CD8+) and macrophages (CD68+) populations were defined as percentages of the total cell population on counting of a minimum of 500 (500–3,000) cells per case at high power (X400) using an ocular grid.

Interface between lymphoma and brain parenchyma, and perivascular spaces containing cuffs of lymphoid cells where TILs and macrophages could predominate were not considered because vessels were variably represented in the specimens and border zones were present in only few biopsies. Counts were carried out in well-preserved neoplastic tissue and necrotic foci where avoided. Reliable estimates of the extent of necrosis were not deemed feasible in our small biopsy samples. Attempts were made to count corresponding areas of consecutive sections (23). Each count was performed twice, repeated a third time if the 2 estimates showed a difference greater than 10%, and then the mean was taken.

**Statistics**

Mann-Whitney, Wilcoxon, and Spearman tests (StatView, Abacus Concept) were used. Data are presented as mean ± SD. A p < 0.05 was considered significant.

**RESULTS**

**Histology**

According to the R.E.A.L. classification (24), all tumors were classified as diffuse large B-cell (CD20+; CD3-) lymphomas. According to a modified WFCU (25), A-PCNSLs included 21 lymphomas with immunoblastic features (14 immunoblastic plasmacytoid lymphomas, 7 lymphomas containing a mixture of plasmacytoid immunoblasts and large noncleaved cells: immunoblast-rich/large cell lymphomas), and 2 large noncleaved cell lymphomas. The 24 NA-PCNSLs were large noncleaved cell lymphomas.

**LMP-1, EBER, and bcl-2 Expression**

EBV was detected in 22/22 A-PCNSLs (18 were positive for LMP-1 and 4 were equivocal for LMP-1 but positive for EBER-1 ISH). Positivity for LMP-1, confirmed by EBER-1 ISH, was found in 1/18 NA-PCNSLs. This single EBV-positive specimen was from an immunosuppressed patient with a renal graft.

Various degrees of bcl-2 immunopositivity were found in all A-PCNSLs (18/18) and all NA-PCNSLs (18/18). The percentage of neoplastic bcl-2 + cells was much higher in A-PCNSLs than in NA-PCNSLs (47.5 ± 18.0 vs 25.0 ± 18.3, p < 0.0007) (Mann-Whitney test). More than 30% of bcl-2 + cells were observed in 15/18 A-PCNSLs and in only 4/18 NA-PCNSLs. A significant positive correlation (r = 0.76, p < 0.0003) (Spearman test) was found between LMP-1 and bcl-2 in A-PCNSLs (n = 18) (Table 1; Fig. 1a, b).

**TABLE 1**

<table>
<thead>
<tr>
<th>Case LMP-1 bcl-2%</th>
<th>Case LMP-1 bcl-2%</th>
<th>Case LMP-1 bcl-2%</th>
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<tbody>
<tr>
<td>1 1 22 7 2 19 13 4 53</td>
<td></td>
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</tr>
<tr>
<td>2 1 25 8 2 30 14 4 55</td>
<td></td>
<td></td>
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<tr>
<td>3 1 36 9 2 39 15 4 55</td>
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<td></td>
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<tr>
<td>4 1 40 10 2 51 16 4 58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 1 41 11 3 55 17 4 71</td>
<td></td>
<td></td>
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<tr>
<td>6 1 42 12 3 72 18 4 80</td>
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</tr>
</tbody>
</table>

*1, less than 5%; 2, less than 10%; 3, less than 20%; 4, more than 20–30% of cells.

**Proliferation, Apoptosis, and Turnover Indices**

Positive correlation among proliferation indices was moderate to strong (0.54 ≤ r ≤ 0.87, p < 0.0005). Apoptotic index was only weakly correlated with MIB-1 and PCNA LI (r ≤ 0.4, p ≤ 0.01), but not with MI (r = 0.26, not significant [NS]).

Compared with NA-PCNSLs, A-PCNSLs had lower proliferation, apoptotic and turnover indices (Table 2) (Mann-Whitney test). A scattergram of turnover and MIB-1 LI values established as previously described (30) discriminated A-PCNSLs and NA-PCNSLs as 2 clusters with minimal overlap (Fig. 2). Interestingly, the A-PCNSLs with the highest turnover indices (cases a and b) were those 2 classified as LNCLs, and the EBV-positive NA-PCNSL from the immunosuppressed patient was also plotted into the overlap zone (case c). No significant difference was detected between the frequencies of MIB-1 positive or negative cells in direct contact with CD45RO+ cells (Wilcoxon test).

**Correlation between Apoptosis and bcl-2 Expression**

Expression of bcl-2 was strongly and inversely correlated with the apoptotic index in the whole series (r ≤ -0.68, p < 0.0001). The correlation coefficients were very similar in A-PCNSLs and NA-PCNSLs (Fig. 3a, b). No association could be demonstrated between bcl-2 and any of the proliferation indices.

**Tumor Infiltrating Lymphocytes and Macrophages**

A-PCNSLs and NA-PCNSLs had similar numbers of CD3+, CD45RO+, CD4+, CD8+ TILs, similar numbers of CD CD4+/CD8+ ratios, but NA-PCNSLs had significantly more macrophages (Table 3). In our series of A-PCNSLs, CD45RO labeling was seen exclusively in cells with the morphology and distribution of CD3+ cells. The MIB-1 LI of CD45RO+ cells (6.1 ± 6.0%) was invariably and several-fold lower than the MIB-1 LI of CD20+ cells (p < 0.0001) (Wilcoxon test) and a close positive correlation between the CD3+ and the CD45RO+ counts were present in both NA-PCNSLs and
A-PCNSLs ($r_c \geq 0.92, p \leq 0.0001$). Some CD45RO+ cells with anaplastic cytologic features, as reported in peripheral AIDS-related NHLs, were seen in 1 case only (39). The MIB-1 LI of CD68+ cells was less than 2%.

**Correlation between Proliferation, Apoptosis, T-TILs or Macrophage Counts**

CD3+ and CD45RO+ cell-counts were inversely correlated with all proliferation in NA-PCNSLs but not in A-PCNSLs (Table 4; Figs. 1c, d, 3c, d). Notably, since this inverse correlation was equally strong with the MIB-1 of CD20+ cells, it was not attributable to the presence of abundant CD45RO+ and CD68+ cells with low proliferation indices in the overall analyzed population. CD68+ counts were positively correlated with apoptosis in both NA-PCNSLs and A-PCNSLs (Table 4; Fig. 3c, f).

**DISCUSSION**

In the present study, A-PCNSLs had slower cell-turnover than NA-PCNSLs. Although an increase in apoptosis in various tissues is known to occur in the course of AIDS (40), A-PCNSLs showed less apoptosis than NA-PCNSLs and a positive correlation between LMP-1 and the apoptosis-inhibiting bcl-2. Moreover, contrary to what might be expected from the clinically rapid growth of opportunistic tumors in AIDS (41), proliferation in A-PCNSLs was also lower. Despite the progressive T-cell depletion in the course of AIDS, A-PCNSLs and NA-PCNSLs showed similar T-TIL counts and similar CD4+ /CD8+ T-TIL ratios, another somewhat unexpected observation. In addition, unlike NA-PCNSLs, A-PCNSLs proliferation could not be correlated to the host T-cell response.

The finding of lower proliferation, apoptosis and turnover in A-PCNSLs suggests that they represent a separate biologic category of PCNSLs. In 1 large series, A-PCNSL specimens obtained by autopsy were found to have MIB-1 LI 2-fold than NA-PCNSLs specimens obtained by biopsy, but the finding was attributed to the different fixation of the samples (42).

It has also been stated that lymphomas with immunoblastic features are characterized by strong, presumably LMP-1 induced, expression of bcl-2 (25). Indeed, bcl-2 expression in A-PCNSLs, most of which contained plasmacytoid immunoblasts was high, in keeping with previous reports (25, 36, 43). However, we were unable to confirm the claim that bcl-2 expression is exclusively limited to A-PCNSLs with immunoblastic features (25). A variable degree of bcl-2 immunopositivity was present...
in all NA-PCNSLs, and in 80% of the cells in 1 of them. Such a pattern of bcl-2 immunostaining was recently reported in NA-PCNSLs (44, 45).

bcl-2 expression was inversely correlated with apoptosis and this negative correlation was strikingly similar in both NA-PCNSLs and A-PCNSLs, similar to what has been observed in some high-grade systemic NHLs (46, 47). A recent study failed to demonstrate an association between bcl-2 and DNA nick-end labeling (TUNEL) in PCNSLs (48). This lack of association might have possibly been due to the specificity limitations of TUNEL apoptosis detection (49). We and others (35) share the feeling that ISEL likely overestimates the true apoptotic rate of tumor cells.

The EBV-encoded LMP-1, detected in 90% of A-PCNSLs was positively correlated with bcl-2 expression. Our study supports previous evidence of concomitant high expression of LMP-1 and bcl-2 in A-PCNSLs (36, 43). Since in vitro studies have shown the selective capability of LMP-1 to upregulate bcl-2 (14), it is conceivable that LMP-1-induced high levels of bcl-2 are responsible for the lower apoptotic rate of A-PCNSLs.

Expression of bcl-2 was not correlated with cell proliferation. Although a negative correlation between bcl-2 upregulation and proliferation indices has been reported in some studies on systemic NHLs (12, 13), other reports have failed to establish such a relationship (46). Notably, no study on PCNSLs has so far documented an inverse relationship between bcl-2 and proliferation (42, 45, 48).

The resistance to treatment of some systemic NHLs may reflect slow cell turnover (11, 12). In the same way, the fact that A-PCNSLs grow by accumulation of longer-lived, slower-cycling cells may contribute to their resistance to therapy modalities that are known to control NA-PCNSLs, but not A-PCNSLs.

Bashir et al (23) reported lower CD3+ and CD4+ cell counts in 6 A-PCNSLs than in 7 NA-PCNSLs, and absence of CD8+ cells in NA-PCNSLs. Our results obtained in a larger series and with similar methodology do not confirm these 2 findings. Published studies of T-TILs in systemic NHLs are similarly discrepant (22, 39). Our results are in keeping with previous evidence that circulating T-cell counts poorly correlate with tissue T-cell

<table>
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<th>TABLE 2</th>
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<tr>
<td>Apoptosis and Proliferation Indices in PCNSLs</td>
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<tr>
<td><strong>AIDS</strong></td>
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<tr>
<td>---</td>
</tr>
<tr>
<td>Apoptotic index%</td>
</tr>
<tr>
<td>n = 23</td>
</tr>
<tr>
<td>Mitotic index %</td>
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<tr>
<td>n = 23</td>
</tr>
<tr>
<td>Turnover index %</td>
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<tr>
<td>n = 23</td>
</tr>
<tr>
<td>MIB-1 labeling index %</td>
</tr>
<tr>
<td>n = 20</td>
</tr>
<tr>
<td>MIB-1 LI of CD20+ cells %</td>
</tr>
<tr>
<td>n = 19</td>
</tr>
<tr>
<td>PCNA labeling index %</td>
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<tr>
<td>n = 21</td>
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Mean ± SD; range
content in AIDS patients (50). Furthermore, it is possible that similar to what has been documented in Hodgkin's disease, EBV-related cytokine production (IL-10) by the tumor cells may be responsible for the abundant, inactivated TILs in A-PCNSLs (51, 52).

The relationship between host cell reaction and tumor cell proliferation in PCNSLs has not so far been investigated, possibly because proliferation indices have been considered of no prognostic value in NA-PCNSLs (42, 53). We observed a negative correlation between TILs and lymphoma cell proliferation in NA-PCNSLs but not in A-PCNSLs. The results in NA-PCNSLs are similar to those reported in high-grade systemic NHLs, where the number of TILs was inversely correlated with the percentage of cells in S-phase (18). Accordingly, small cell NHLs could be subdivided into lymphomas with abundant T-TILs and low Ki-67 LI and those with the reverse phenotype (19).

In the present study, CD8+ T-cell counts did not correlate with apoptosis and TILs were not selectively observed in contact with MIB-1 negative or apoptotic cells. It is likely, therefore, that the antineoplastic effects of T-cells in NA-PCNSLs are mediated by secreted products rather than by cell-cell contact induced cytotoxicity or proliferation inhibition. A number of cytokines belonging to the repertoire of CD45RO+ cells, either CD4+ or CD8+ (54), including IFNγ, TNFα, and IL-4, can impair lymphoma cell proliferation in vivo and in vitro (55–57). Lymphoma cells can respond to cytokines by an increase or inhibition of proliferation in an unpredictable fashion (55, 58). And therefore growth inhibitors may differ from case to case (55). Secretion of proliferation inhibiting cytokines by T-cells depends on specific engagement of the CD3 and TCR receptor (56, 59). Tumor-induced TNFα secretion by B-clone-specific T-TILs has been demonstrated in some NHLs (60).

Similar to other estimates, proliferation of CD45RO+ T-TILs assessed by MIB-1 LI was low, (61). This finding is consistent with previous evidence that autologous B-cell lymphoma cells do not evoke strong T-cell proliferation (62). Nevertheless, TILs proliferate in specific response to autologous lymphoma B-cells in a number of NHLs, and these NHLs have a better prognosis (63). Tumor proliferation in NA-PCNSLs remained high, suggesting that the TILs response was insufficient to strongly inhibit NA-PCNSL growth. Indeed, lymphomas may use a variety of mechanisms to ultimately subvert the immune response (17) and may even employ TILs to sustain their growth in certain lymphoma types (64–66).

Apoptosis positively correlated with macrophage counts both in A-PCNSLs and NA-PCNSLs. That macrophage-induced apoptosis could be part of the host reaction against PCNSLs has been suggested in systemic NHLs (67, 68). However, the correlation between macrophages and apoptosis was equally strong in NA-PCNSLs and A-PCNSLs; c) and d) negative correlation between CD3 counts in NA-PCNSLs but not in A-PCNSLs; e) and f) positive correlation between macrophage counts in NA-PCNSLs and in A-PCNSLs.

We conclude that (i) A-PCNSLs are characterized by accumulation of slow-cycling, long-lived cells likely from protected from apoptosis by LMP-1-induced expression of bcl-2 expression and are independent from the proliferation-restrictive host response; (ii) NA-PCNSLs are characterized by a faster cell turnover associated with an insufficient proliferation-restrictive host response; and (iii) A-PCNSLs and NA-PCNSLs constitute 2 entities with distinctive morphology and markedly different kinetic profiles that could account for some differences in their response to therapy.
### TABLE 3
T-cell Populations and Macrophages in PCNSLs

<table>
<thead>
<tr>
<th></th>
<th>AIDS</th>
<th>Non-AIDS</th>
<th>p values</th>
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</thead>
<tbody>
<tr>
<td>CD3+ lymphocytes %</td>
<td>31.1 ± 12.7</td>
<td>27.8 ± 16.8</td>
<td>Not significant</td>
</tr>
<tr>
<td>(range)</td>
<td>(9.2–50.2)</td>
<td>(2.6–60.0)</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>19</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>CD45RO+ lymphocytes %</td>
<td>23.3 ± 12.0</td>
<td>20.4 ± 16.1</td>
<td>Not significant</td>
</tr>
<tr>
<td>(range)</td>
<td>(3.8–43)</td>
<td>(2.0–56.3)</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>22</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>CD4+ lymphocytes %</td>
<td>15.4 ± 7.9</td>
<td>16.6 ± 7.5</td>
<td>Not significant</td>
</tr>
<tr>
<td>(range)</td>
<td>(7.0–33.2)</td>
<td>(8.0–27.8)</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>10</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>CD8+ lymphocytes %</td>
<td>13.1 ± 6.6</td>
<td>14.5 ± 11.3</td>
<td>Not significant</td>
</tr>
<tr>
<td>(range)</td>
<td>(4.8–25.8)</td>
<td>(0.5–39.2)</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>16</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>CD4/CD8 ratio</td>
<td>1.1 ± 0.5</td>
<td>1.7 ± 1.6</td>
<td>Not significant</td>
</tr>
<tr>
<td>(range)</td>
<td>(0.5–2.0)</td>
<td>(0.2–5.7)</td>
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</tr>
<tr>
<td>n</td>
<td>10</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>CD68+ cells %</td>
<td>17.6 ± 11.3</td>
<td>28.2 ± 8.3</td>
<td>p = 0.003</td>
</tr>
<tr>
<td>(range)</td>
<td>(2.8–40.3)</td>
<td>(11.7–39.0)</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>18</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

### TABLE 4
Correlations Between Density of T-cells (CD3+ and CD45RO+) and Macrophages with Proliferation and Apoptosis Indices in A-PCNSL and NA-PCNSL

<table>
<thead>
<tr>
<th></th>
<th>CD3 %</th>
<th>CD45RO %</th>
<th>CD68 %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-AIDS</td>
<td>AIDS</td>
<td>Non-AIDS</td>
</tr>
<tr>
<td>Mitotic Index %</td>
<td>r = −0.65</td>
<td>NC</td>
<td>r = −0.51</td>
</tr>
<tr>
<td>p</td>
<td>&lt; 0.003</td>
<td>p &lt; 0.017</td>
<td>p &lt; 0.003</td>
</tr>
<tr>
<td>MIB-1 LI %</td>
<td>r = −0.66</td>
<td>NC</td>
<td>r = −0.74</td>
</tr>
<tr>
<td>p</td>
<td>&lt; 0.003</td>
<td>p &lt; 0.007</td>
<td>p &lt; 0.007</td>
</tr>
<tr>
<td>CD20 MIB-1 LI %</td>
<td>r = −0.68</td>
<td>NC</td>
<td>r = −0.70</td>
</tr>
<tr>
<td>p</td>
<td>&lt; 0.003</td>
<td>p &lt; 0.007</td>
<td>p &lt; 0.007</td>
</tr>
<tr>
<td>PCNA LI %</td>
<td>r = −0.45</td>
<td>NC</td>
<td>r = −0.57</td>
</tr>
<tr>
<td>p</td>
<td>&lt; 0.005</td>
<td>p &lt; 0.011</td>
<td>p &lt; 0.005</td>
</tr>
<tr>
<td>Apoptotic Index %</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>p</td>
<td>NC</td>
<td>+0.65</td>
<td>p &lt; 0.003</td>
</tr>
</tbody>
</table>

Spearman’s rank correlation test, NC = no correlation.

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