T-cell. Subset levels in some Nigerian patients with Burkitts Lymphoma

Umukoro G.E.* and Bonyesom I.***

*Department of Medical Microbiology and Parasitology.
***Department of Medical Biochemistry, Faculty of Basic Medical Sciences. Delta State University, Abraka. Nigeria.

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The levels of CD3, CD4 and CD8 T-cell subsets were assessed in fifty-eight Burkitt’s lymphoma (BL) patients aged 4-14 years at different clinical stages (I-IV) of the Disease progression, and the obtained data were compared with age–matched twenty-eight control subjects in apparently healthy condition. The results show that the level of the anti-leu 3a(CD4+ T-cell) was significantly low (P<0.05) in all the clinical stages of BL when compared with the level obtained for the control subject. The level of anti-leu 2a(CD8+ T-Cell) progressively increases as the tumor condition deteriorates, and the levels for all the four clinical stages of BL were significantly higher (P<0.05) when compared with the level obtained from control subjects. Data shows that BL patients have abnormal immune responses which may likely become more complicated as the disease advances.

Keywords: T-cell, Burkitt’s lymphoma Immune response CD4+ CD8+.
this study attempts to report the levels of T-cell subsets in BL patients at the different stages of the tumour. This is important in order to assess the immunology of BL as the tumour progresses. The role of T-cells in the immunology of BL has not been clearly documented. Therefore, the study attempts to report the levels of T-cell subsets in BL patients at different stages of the tumour. This is important in order to access the immunology of BL as the disease progresses.

MATERIALS AND METHODS

Patients' selection
Fifty-eight Burkitt's lymphoma (BL) patients, aged 4-14 years on their first visit to Almadu Bello Teaching Hospital (ABUTH) between 1993 to 1995 were selected for this study. Diagnosis of BL was made clinically and histologically using the established WHO criteria (Benard, et al 1969). The selected patients were separated into four clinical tumour stages based on Ziegler's method of classification (Ziegler and Kyalmazi 1971) only cases that were yet to be treated were included in the study. Twenty eight apparently healthy children of comparable characteristics age and sex were used as control subjects. Human experimentation conduct protocol was approved by ABUTH Ethical committee (Ref. No.: F-Med/Com-19; project number ESC/95/00075).

Peripheral Blood lymphocyte Separation
Five milliliter of blood was obtained from each patient after an informed consent from the patients' parent by venopuncture technique. The collected blood sample was placed in 20ml culture tubes and diluted with Eagle Minimum Essential Medium (MEM) (1:2) and gently layered on 3ml of ficoll hypaque density gradient in a 20ml centrifuge tube. This was centrifuged at 1800 rpm for 30 minutes at 37°C. The rich lymphocyte layer at the ficoll-MEM interphase was gently removed with a Pasteur pipette and transferred to another 10ml tissue culture tube. The cells were then resuspended in MEM and washed thrice with MEM by centrifuging at 1800 rpm for 30 min. After the final wash, the cells were adjusted to 4 x 10^6 cells per ml of MEM; to obtain the lymphocyte suspension. More than 95% of cells in the suspension were viable as indicated by typhan Blue exclusion test.

T-cell typing and enumeration
Fifty microlitre (µl) of the lymphocyte suspension was placed in a small plastic tubes and 20µl of the monoclonal antibody (antileu, 4a, 3a, and 2a) panel were then used to conjugate the T-cells. The conjugates were incubated at 4°C for 30 minutes, and washed thrice by centrifuging at 1800 rpm for 10 minutes at 4°C. After the final wash the cells were resuspended in two drop of MEM and a drop of the conjugated cells was placed in a glass slide and covered with a cover slip and then sealed with a nail polish hardner. This was further incubated at 4°C for 15 minutes, to allow the cells to settle.

Enumeration of conjugated cells
The conjugated cells were enumerated using fluorescence microscopy (Becton – Dickinson procedures 1985). Percentage count of the conjugated cells from several scanned fields was calculated using the formular.

\[
\frac{a}{100} \times \frac{b}{100} = \frac{X}{Y} \times 10^6 \text{ q/L}
\]

where

- a = % fluorescence positive cells
- b = % peripheral blood lymphocytes
- X = while Blood cell count
- Y = % number of fluorescence positive lymphocyte

Statistical Analysis
The data collected were subjected to statistical analysis using EPI computer package. The various levels of significance differences were computed using analysis of variance (ANOVA), Bartlet test of homogeneity and kruskal – wallis one-way analysis of variance.

RESULTS
The results of the levels of T-cell subsets for the four clinical stages of BL and control and presented in Table 1.

The antileu 3a (CD4^+ T-cells) count for the various clinical stages of BL were significantly lower when compared with the counts for the control subjects (P<0.05). The antileus 2A (CO8^-t-cells) counts in cell the four clinical stages were significantly higher when compared with the count for the control subjects (P<0.05). The CD4^+ and CD8^- cell ratio is significantly reversed when
compared with ratio of the count in the control subjects.

**DISCUSSION**

The results of this study indicate reduced number of antileu 3a (CD4⁺) T-cells but increased number of antileu 2a (CD8⁺) T-cell subset in BL patients compared with control values (Table 1)

The basic concept of immune surveillance theory states “that malignant tumour arises only if the immune system is somehow impaired or if the tumour cells, loose their immunogenicity, enabling them to escape immune surveillance. (Kuby,1997). The central events in the generation of both humoral and cell-mediated immune responses is the activation and expansion of T-helper (T\textsubscript{H}) cells. T-cell activations is initiated by interaction of the TCR-CD3 complex with a processed antigenic peptide bound to a class II molecule of major Histo compatibility complex (MHC) or the surface of an antigen presenting cell (APC). This interaction and the resulting activating signals also involve a variety of accessory membrane molecules on the T-cell and APC(Kuby,1997; (Kuby,1997; Smith et al., 1980) interaction of a T\textsubscript{H} cell with antigen initiates a cascade of biochemical events that induces the

**Table 1:** Sex and age distribution of control subjects and BL patients.

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>Sex</th>
<th>Control n=28</th>
<th>Stage I n=20</th>
<th>Stage II n=14</th>
<th>Stage III n=21</th>
<th>Stage IV n=4</th>
</tr>
</thead>
<tbody>
<tr>
<td>(0-5)</td>
<td>Male</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>1</td>
<td>1</td>
<td>12</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(6-10)</td>
<td>Male</td>
<td>13</td>
<td>11</td>
<td>-</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>8</td>
<td>3</td>
<td>-</td>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td>(11-15)</td>
<td>Male</td>
<td>2</td>
<td>2</td>
<td>-</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Experimental Data obtained**

<table>
<thead>
<tr>
<th>Test</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Peripheral blood lymph</td>
<td>47.53±13.00</td>
<td>43.05±15.00</td>
<td>38.55±15.18</td>
<td>36.15±15.55</td>
<td>34.75±17.76</td>
</tr>
<tr>
<td></td>
<td>(26-78)</td>
<td>(22-77)</td>
<td>(14-62)</td>
<td>(8-78)</td>
<td>(14-54)</td>
</tr>
<tr>
<td>% Antileu 4a(CD3⁺)</td>
<td>63.96±2.70</td>
<td>53.82±3.92</td>
<td>51.06±4.93</td>
<td>52.55±3.58</td>
<td>50.75±5.12</td>
</tr>
<tr>
<td></td>
<td>(60-70)</td>
<td>(48-65)</td>
<td>(45-60)</td>
<td>(46-60)</td>
<td>(45-56)</td>
</tr>
<tr>
<td>% Antileu 3a (CD4⁺)</td>
<td>44.14±2.99</td>
<td>21.82±3.49</td>
<td>20.00±1.18</td>
<td>20.60±1.93</td>
<td>19.25±2.99</td>
</tr>
<tr>
<td>% Antileu 2a (CD8⁺)</td>
<td>24.61±3.46</td>
<td>34.37±3.90</td>
<td>33.55±4.44</td>
<td>34.95±3.09</td>
<td>35.25±3.18</td>
</tr>
<tr>
<td></td>
<td>(20-34)</td>
<td>(23-45)</td>
<td>(20-40)</td>
<td>(30-44)</td>
<td>(30-38)</td>
</tr>
<tr>
<td>CD 4⁺/CD8 ratio</td>
<td>1.8</td>
<td>0.57</td>
<td>0.58</td>
<td>0.59</td>
<td>0.57</td>
</tr>
</tbody>
</table>

**Note:** Correlation coefficient (p values) was tested at 95% confident limit. 
This values are given as mean plus or minus standard deviation (.M +SD).
n is number of samples, values in parenthesis indicates the range;
values preceding the asterics indicates statistical significant (P<0.05);
AP – P-value using analysis of variance (ANOVA);
BP – P value using Bartlets list of homogenuity of variance.
KP – P value using Kruskal – wallis one way analysis of variance.

Umukoro & Bonyesom: Levels of T-cell subset in BL.
resting T<sub>h</sub> cells to enter the cell cycle (GO to GI transition) and culminated in expression of the high affinity receptor for interleukin 2 (11 – 2) and secretion of 11 – 2. In response to 11 – 2 (and in some cases 11 – 4), the activated T<sub>h</sub> cells progresses through the cell proliferation and differentiation into memory and/or effector cells. (Kuby, 1997; Royer and Reinberz1987).

BL has a multistep etiology; a combination of viral (Epstein – barr virus) EBV infection and chromosomal translocation involving e-myc an ancogen in chromosome 8 to chromosome 14 at a locus of heavy chain with high immunoglobulin activity (Groeneveld, et al. 1994; Goldstein and Bernstein1990). there is a report that suggested lymphocytosis in EBV induced mononucleosis. The lymphocytosis was reported to be due to the expansion of CD8<sup>+</sup> - cells subset and this high level of CD8<sup>+</sup> T-cell subset is said to rapidly reduced as the infected B-cell disappears (Goldstein and Bernstein 1990). The effector function of CD8<sup>+</sup> T Cell to some viruses and tissue grafts seem to require the presence of CD4<sup>+</sup> T-cells. This complementary effect is required to allow for the generations of 1 1 – 2 which is required for the expansion of effector CD8<sup>+</sup> T cell clones. CD8<sup>+</sup> T-Cell recognize antigen on weakly consmtulating cells, may become activated only in the presence of CD4<sup>+</sup> T-cells bound to the same APC (Ganeway and Travers2002).

The increase count of CD8<sup>+</sup> T cell among BL patients does not seem to be helping the immunology of BL. The observation is that of malfunction of the CD8<sup>+</sup> T-cell. Since the increase in the cell count leads to poorer progress. The level of antileu 3a (CD4<sup>+</sup> T-cells) is low when compared among the clinical stages and even when compared with control subjects. In the control subjects the CD4 <sup>+</sup> T-cell and CD8<sup>+</sup> T-cell ratio was observed to be (1 :1.8). This value was also observed by other researchers in the same laboratory on other disease (tuberculosis); where the CD4+/CD8+ ratio was reported to be (1.8:1). The ratio observed in BL is rather reversed. When compared with control ratio. This reversal is noticed to run along the clinical stages and was found to be statistically significant (P<0.05). Evidence from this study, show that BL patients have abnormal immune response, which becomes more complicated as the tumor or disease condition progresses.

REFERENCES