JOURNAL OF PURE AND APPLIED MICROBIOLOGY, April 2007. Vol. **1**(1), p. 13-16

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

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(Received: 07 February 2007; accepted: 24 March 2007)

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 $CD4^+$ $CD8^+$.

During the last three decades an increasing number of clinical disorders have been recognized to be T-cell mediated. Uncontrolled T-cell proliferation may result in T-cell malignancies, while disturbed T-cell development may lead to T-cell immunodeficiency. T-cells also play important role in auto immune disease and they have a pivotal function in the control of infection. T-cells are responsible for acute rejection of solid organ transplants. Viral infection can cause dramatic changes in the number and proportion of blood T-cell subsets. Two disease conditions caused by viruses can easily come to focus; infectious mononucleosis due to Epstein – Barr virus (EBV) and Acquired immunodeficiency syndrome caused by Human Immunodeficiency Virus (HIV). These two viral infections are noticed to have two different and opposing effect on Blood T-cell subsets. EBV, a lymphotropic virus, causes infectious mononucleosis. In infectious mononucleosis there is lymphocytosis due to an expansion of $CD8^+$ T-cells leading to disappearance of the infected circulating B-cells and resolution of the clinical signs in four to six weeks, (Groeneveld, *et al.,* 1994; Van Dongen, *et al.,* 1988; Blumberg and Schooley 1985; Epstein and Achomg, 1977) . In AIDS caused by HIV, there is functional abnormalities and quantitative depletion of $CD4^+$ T-Cells which lead to increased disorder of the disease (Groeneveld, *et al* 1994; Centers for Disease control 1992).

There is a report which suggested that there is T-cell colony formation with defective interleukin 2 (il-2) receptor expansion through out all the stages of HIV infection[6]. BL has a multistep etiology. It has a combination of EBV infection and chromosomal translocation involving c-myc.

The role of T-cell in the immunology of BL has not been clearly demonstrated. Therefore,

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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 stags 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 lymph and (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 histological 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 Kyalmazi1971) 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 30minutes at 37^0C. 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 e with MEM by centrifuging at 1800 rpm for 30min. 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 4^0c for 30 minutes, and washed thrice by centrifuging at 1800 pm for 10 minutes at 4^0C. 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 4^0C 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 seanned fields was calculated using the formular.

$$\frac{a}{100} \quad \frac{x\ b}{100} \qquad xX = Yx\ 10\ q/L$$

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 anlaysis 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

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

Age (yrs)	Sex	Control n=28	Stage I n = 20	Stage II n = 14	Stage III n = 21	Stage IV n = 4
(0-5)	Male	4	3	2	1	-
	Female	1	1	12	-	-
(6-10)	Male	13	11	-	11	2
	Female	8	3	-	7	-
(11-15)	Male	2	2	-	3	2
	Female	-	-	-	-	-

Experimental Data obtained

Test		I	II	III	IV	P-value
% Peripheral blood lymph	47.53±13.00 (26-78)	43.05±15.00 (22-77)	38.55±15.18 (14-62)	36.15±15.55 (8-78)	34.75±17.76 (14-54)	AP 0.01* BP-0.95 KP-0.07
% Antileu 4a($CD3^+$)	63.96±2.70 (60-70)	53.82±3.92 (48-65)	51064±4.93 (45-60)	52.55±3.58 (46-60)	50.75±5.12 (45-56)	AP 0.01* BP-0.37 KP-0.001*
% Antileu 3a ($CD4^+$)	44.14±2.99 (25-49)	21.82±3.49 (17-33)	20.00±1.18 (18-22)	20.60±1.93 (18-25)	19.25±2.99 (15-22)	AP 0.01* BP-0.001* KP-0.001*
% Antileu 2a($CD8^+$)	24.61±3.46 (20-34)	34.37±3.90 (23-45)	33.55±4.44 (20-40)	34.95±3.09 (30-44)	35.25±3.18 (30-38)	AP 0.03* BP-0.89 KP-0.0001*
CD 4^+/CD8 ratio	1.8	0.57	0.58	0.59	0.57	AP-0.05*

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 homogensity of variance.
KP – P value using Kruskal – wallis one way analysis of variance.

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 immunogencity, enabling them to escape immune. Surveillance. (Kuby,1997). The central events in the generation of both humoval and cell-mediated immune responses is the activation and expansion of T-helper (T_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_H cell with antigen initiates a cascade of biochemical events that induces the

resting T_H cells to enter the cell cycle (GO to GI transition) and culminated in expression of the high affinity receptor for interleukin 2 (1l – 2) and secretion of 1l – 2. In response to 1l – 2 (and in some cases 1l – 4). the activated T_H – 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 injection 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 mononuclasis. The lymphocytosis was reported to be due to the expansion of $CD8^+$ - cells subset and this high level of $CD8^+$ T-cell subset is said to rapidly reduced as the infected B-cell disappears (Goldstein and Bernstein 1990). The effector function of $CD8^+$ T Cell to some viruses and tissue grafts seem to require the presence of $CD4^+$ T-cells. This complementary effect is required to allow for the generations of 1 l – 2 which is required for the expansion of effector $CD8^+$ T cell clones. $CD8^+$ T-Cell recognize antigen on weakly constmulating cells, may become activated only in the presence of $CD4^+$ T-cells bound to the same APC (Ganeway and Travers2002).

The increase count of $CD8^+$ T cell among BL patients does not seem to be helping the immunology of BL. The observation is that of malfunction of the $CD8^+$ T-cell. Since the increase in the cell count leads to poorer progressis. The level of antileu 3a ($CD4^+$ T-cells) is low when compared among the clinical stages and even when compared with control subjects. In the control subjects the $CD4^+$ T-cell and $CD8^+$ 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.

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