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THE TH1/TH2 BALANCE IN CLINICAL COURSE OF BURKITT'S LYMPHOMA.

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ABSTRACT

The potential role of host immunity to Burkitt's lymphoma (BL) and responses to sero-therapy with sera from BL patients who had undergone complete regression have been documented. However, this study reports the level of serum immunoglobulin (IgG, IgM, IgA) which are functional signatures of Th2 type of immune function and leucocytes migration inhibition factor (MIF) this is a functional signature of Th1. For homeostasis, this two arm of immune functional unit must act in control of each other. Fifty-seven BL patients were randomly selected from thePediatric Clinic of Ahmadu Bello University Teaching Hospital, Zaria and Kaduna, Nigeria The results show that serum immunoglobulin values (IgA for stage1 BL patients (n=22; 667.27±. 509.16; stage 2:n=11;616.82±408.55;stage3:n=20;485.75±289.99;stage4:n=4;330.11±260..65;IgGstage1:n=22;260.59±232.56;stage2:n=11;195. 18±102.86; stage3:n=20;309.65±276.43; stage4:n=4;244.80±401.93;IgM,stage1:n=22;253.55±104.99;stage2:n=11;217.82±77.80; stage3:n=20;419.80±74.20; stage4: 233.35±108.84) were higher in BL patients compared with control values(IgA: n=28; 330.11±260.65; IgG: n=28; 288.29± 346.93; IgM: n=28; 201.82±64.13).These increases were also influenced by the clinical stages of BL. The MIF values in BL patients were equally higher (stage1: n=22;123.73±26.40; stage 2:n=11;121.0±23.98; stage3: n=20; 118.64±32.09; stage4 : n=4; 126.02±56.66) when compared with the values for the control subject(n=28; 68.53±8.80). The values are progressively depended on the clinical stages. Since Ig values were increasing, indicaing Th2 activity, and MIF values were decreasing in function indicating Th1 activity, then it is reasonable to conclude from this study that there is,Th1 and Th2 imbalance; the level of Th2 being higher. This type of imbalance may not be immunologically beneficial to BL patients.

KEYWORD: Immunoglobulin, Burkitt's Lymphoma, Th1/Th2 balance, MIF

INTRODUCTION

The progression of some diseases may depend on the balance between T-helper lymphocyte1 (Th1) subset and T-helper2 (Th2) subset. A well studied example of this phenomenon is leprosy, which is caused by *Mycobacterium lapre*, an intracellular pathogen that is able to survive within the phagosomes of macrophages. Leprosy is not a single clinical entity, rather the disease presents as a spectrum of clinical responses, with two major forms of disease: tuberculoid and lepromatous, at each end of the spectrum (Abbas, *et al.* 1996). In tuberculoid leprosy, a cell-mediated immune response develops with the formation of granulomas resulting in the destruction of most of the organisms found in the tissue. Although skin and peripheral nerves are damaged, tuberculoid leprosy progresses slowly and patients usually survive(Abbas, *et al.* 1996). In lepromatous leprosy, the cell-mediated response is depressed, and instead humoral antibodies are formed, sometimes resulting in hypergammaglobinaemia. The organisms are widely disseminated in macrophages, often reaching numbers as high as 10^3 per gramme of tissue(Kuby,1997). Lepromatous leprosy progresses into disseminated infection of the bone and cartilage with extensive nerve damage.

The development of lepromatous or tuberculoid leprosy depends on the balance between Th1 and Th2 T-cell subsets(Abbas, *et al.* 1996; Kuby,1997; Sieling, and Modli, 1994). In tuberculoid leprosy the immune response is characterized by Th1 type of responses with delayed type hypersensitivity (DTH) activity and cytokine profile consisting of high levels of interleukin-2(Il-2), interferon-gamma(INF-γ) and tumor necrotic factor alpha(TNF-α(Kuby,1997). In the lepromatous leprosy there is a Th2 type of immune responses which is devoid of DTH with high levels of interleukin-4(il-4), interleukin-5(Il-5) and interleukin-10(Il-10). This cytokine profile explains the diminishing cell-mediated immunity level (i.e DTH) and increased serum antibody level in lepromatous leprosy (Abbas, *et al.* 1996; Kuby,1997; Sieling, and Modli, 1994). There is also evidence of changes in Th1subset activity in HIV/AIDS(Kuby,1997).The aim of this research is to estimate the level of serum immunoglobulin as a measure of Th2 activity and to also estimate migration inhibition factor (MIF) as a measure of Th1 activity in patients with BL. The result of this investigation could enable us understand such phenomena like tumor resistant to drug, metastasis and tumor relapse as well as assessing Th1/Th2 balance in Burkitt's Lymphoma patients.

MATERIALS AND METHODS

PATIENTS: Fifty seven patients aged 4-14 years who presented with BL to Ahmadu Bello University Teaching Hospital, Zaria and Kaduna were studied. Diagnosis was made clinically and histologically using the established criteria (Benard, *et al* 1969). Clinical staging of tumors was based on Ziegler's method of classification Ziegler, and Kyalwaz.1971).Only cases that are yet to start treatment were enlisted in this study. Twenty eight apparently healthy children, matched in age, background and with no BL were used as control subjects. Human experimentations conduct protocol was approved by ABU Hospital Committee, Ref. No. Med-F Med/Comm. 19 of 16/6/95; Project number Esc/95/00075.

IMMUNOGLOBULIN LEVELS: Quantification of serum immunoglobulin levels was carried out using the single radial immunodifussion technique (Mancin, *et al* 1960).

LEUCOCYTES MIGRATION INHIBITION TEST (LMIT) IN BURKITT'S LYMPHOMA PATIENTS:

The method of (Rosemberg and David 1970). was used. Three milliliter of peripheral blood taken by venopuncture was allowed to settle at 37°C for one hour in a plastic tissue culture tube. The supernatant was pipetted and

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washed three times with minimum essential medium (MEM) by centrifugation at 1800rpm for 10minutes at 37°C. The final wash was pipetted and resuspended in 1ml of MEM. This cell suspension was aspirated to approximately 2/3rd of the tube's length leaving about 1/3rd of the tube unfilled. The unfilled portion was then sealed using plasticine. The capillary tubes were then placed in a plastic tube and centrifuged at 1800rpm for 10 minutes at 37°C.The tubes were cut at about one millimeter below the cell/ liquid interface and placed in a migration chamber and anchored firmly with silicone grease. The chambers were filed with MEM containing 15 % of fetal calf serum (FCS) with 100µg/ml purified protein derivative (PPD). The control was as above but without PPD. Cover slips were placed on these chambers and sealed with silicone grease. The tests were performed in duplicate and incubated in perfectly flat position at 37°C for 24 hours. The area of migration was read off using microscopic projection on a piece of graph paper by counting the square covered by the area of migration.
The percentage of migration inhibition (MI) was calculated from the following formula:

$$M.I. = \frac{\text{Area of migration with PPD}}{\text{Area of migration without PPD}} \times 100.$$

STATISTICAL ANALYSIS: This was done by analyzing the various mean differences between immunoglobulin classes, MIF in the various clinical stages of BL using the analysis of variance(ANOVA),Kruskall-Wallis and Bartlet test for homogeneity and the level of significant difference was estimated at 95% confident limit.

RESULT

Table 1 shows these various values of the three major immunoglobulin (IgG, IgA, IgM). This values were compared among the various clinical stages of BL and control subjects, and the level of significant difference was established.

Table 2 contains the various percentages of MIF. These values were compared in the various clinical stages of BL and the significant differences were shown in the table.

Table1: Serum Immunoglobulin levels for BL patients and control subjects. Changes in serum immunoglobulin

Stages in BL	IgG(IU/ml)	IgA(IU/ml)	IgM (IU/ml)
1(n=22)	260.59 ± 232.56 (42-1134)	667.27± 509.6 (139-1625)	253.55± 104.99 (130-520)
2(n=11)	195.18± 102.86 (63-384)	616.82± 408.55 (95-1625)	217.82± 77.80 (146-412)
3(n=20)	309.65± 276.43 (50-934)	695.65± 699.39 (139-2199)	419.80± 74.20 (112-406)
4(n=4)	244.8± 401.93 (50-934)	485.75± 289.99 (115-789)	233.35± 108.84 (105-348)
Control (n=28)	288.29± 346.93 (75-1260)	330.11± 260.65 (66-1006)	201.82± 64.13 (94-328)
P-value	AP=0.001 *BP=0.001 KP=0.529	AP=0.63 *BP=0.0111 *KP=0.042	AP=0.37 *BP=0.001 KP=0.79

Correlation coefficient (P-value) tested at 95% confidence limit. The values are expressed as mean± standard deviation(SD), n=number of samples, values in parenthesis indicate the ranges and star indicates significant P-values. AP:P- value using ANOVA test for normal distribution; BP:P-value using Bartlett's test for homogeneity of variance and KP: P- value using Kruskal- Walli's one way analysis of variance

Table2: In-vitro Migration inhibition factor percentage values for BL patients and control subjects.

Clinical stage	1 (n=22)	2 (n=11)	3 (n=20)	4 (n=4)	Control (n=28)	P-values
M.I%	123.73±26.98 (88-188)	21.0±23.98 (98-166)	118.64±32.09 (30-166)	126.0±56.66 (122-138)	68.53±8.80 (44-80)	*AP=0.004 BP=0.08 *AP=0.001

Correlation coefficient (P-value) tested at 95% confidence limit. Values are expressed as mean± standard deviation(SD), n=number of samples, values in parenthesis indicate the and star ranges indicates significant P-values. AP:P- value using ANOVA test for normal distribution; BP:P-value using Bartlett's test for homogeneity of variance and KP: P- value using Kruskal- Walli's one way analysis of variance.

DISCUSSION

The immunoglobulin levels in BL patients were significantly higher when compared with the control (Table 1). The results of the *in-vitro* test of cell-mediated immunity(the MIF assays) show that BL patients lack delayed type hypersensitivity (DTH).Migration index far above 88% conforms with T-cell anergy (Mancin, *et al* 1960).
The immune response to a specific antigen must induce an appropriate set of effecter function that can eliminate the particular pathogen involved in the infection. For instance, the neutralization of a soluble bacterial toxin requires antibodies, whereas, the responses to an intercellular virus or bacteria requires cell mediated cytotoxicity or DTH. In the last few years, a large body of evidence has accumulated suggesting that differences in cytokine secretion pattern among T-helper cell subset play a major role in regulating the choice of immune functional modality(Abbas, *et al.* 1996; Kuby,1997). $CD4^{+}$ T-helper cells exert most of its helper function through secreted cytokines which either act on the cells that produce them in an autocrine manner or modulate the responses of other cells through paracrine pathways(Kuby,1997).

The Th1 subset is responsible for classical cell – mediated function (e.g the DTH and cytotoxic T-lymphocytes, CTL).The Th2 subset functions primarily as a helper for B- cell action. The cytokine environment (that is present as antigens) primed T-helper cells differentiate is thought to determine the subset that develops. Two cytokines in particular, interleukin -4 (Il-4) and interleukin -12 (Il-12) play decisive roles in determining whether a Th1 or Th2 response develops. The cytokine produced by Th-1 and Th-2 subset exhibit cross-regulation, that is, the cytokines secreted by one subset can block the production and / or activity of the cytokines secreted by the other subset. For instance, interferon – gamma (IFN-Y) secreted by Th1subset, and interleukin – 10(Il-10) secreted by the TH2 subset down – regulate secretion of IFN-Y and Il-2 by the Th1 subset. (Abbas, *et al.* 1996; (Rosemberg and David 1970).

The phenomenon of cross – regulations provides an explanation for the observation that there is an inverse relationship between antibody production and delayed hypersensitivity, that is, when antibody production is high, delayed type hypersensitivity is low and vice versa.
The immunoglobulin levels are high with a resultant low level of migration inhibition factor (DTH). This is highly indicative of an imbalance in the Th1 and Th2 levels. This may suggest

why BL tends to spread to other organs and subsequent poor prognosis in BL pathogenesis.

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