LYMPHOCYTE CHANGES IN SECONDARY DENGUE FEVER: USE OF THE TECHNICON H*1 TO MONITOR PROGRESS OF INFECTION

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Abstract. Secondary dengue fever as dengue hemorrhagic fever or dengue shock syndrome is a potentially fatal complication of an infection that presents with few clinical signs that help in the diagnosis. Previous workers have reported the value of buffy coat determinations of atypical lymphocytes as an aid to the diagnosis. We report here the use of an automated white blood cell differential counter, the Technicon H*1 in the monitoring of the atypical lymphocyte count as a measure of the progress of the infection in a retrospective study of serial full blood counts in 45 serologically confirmed patients. Technicon H*1 'basophil' and large unstained cell counts and manual atypical lymphocyte counts rose in tandem with the drop in platelets and decreased when the platelets recovered. In a sub-study, the atypical lymphocytes were immunophenotyped and found to be predominantly derived from T lymphocytes. We conclude that the Technicon H*1 is a useful monitor of the activity of the infection in dengue hemorrhagic fever.

INTRODUCTION

Primary dengue fever is an uncomplicated viral fever. A second episode of dengue fever however causes a hyper-sensitization reaction (Halstead *et al,* 1970) and may potentially be fatal particularly when it appears as dengue hemorrhagic fever or as dengue shock syndrome. This usually occurs around the time of defervesence. Clinical diagnosis is often difficult and can usually be confirmed only retrospectively on the basis of serology. The value of the presence of atypical lymphocytes in buffy coat preparations as a diagnostic aid was first raised by Suvatte and Longasamon in 1979. It was felt that the changes seen in these lymphocytes might result in different cytological properties that would enable them to be readily identified by automated white cell differential counters. The Technicon H*1 (Tarrytown, New York) performs a white cell differential count by a simulated morphologic assessment. Changes in the atypical lymphocytes/ H*1 counts as the infection progressed could be useful markers of the activity of the disease. A descriptive study of the changes in the H*1 platelet, basophil, 'Large Unstained Cell' (LUC) and manual atypical lymphocyte counts was thus retrospectively carried out on 45 patients confirmed to have secondary dengue fever by serology.

The origin of the atypical lymphocyte is still unclear. Earlier workers (Boonpucknavig *et al,* 1979) had suggested a B cell ontogeny, contrary to general expectations of a T cell activation and proliferation seen in most viral fevers. The previous immunophenotyping methods used however may not have allowed the restriction of phenotype identification to the atypical lymphocytes. Immunophenotyping was therefore carried out by the alkaline phosphatase anti-alkaline phosphatase method (APAAP) on the atypical lymphocytes of 12 patients.

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MATERIALS AND METHODS

The Technicon H*1 gives a white cell differential count based on 2 separate optical assessments.

The peroxidase channel measures the intensity of the peroxidase staining reaction. White cells are also subjected to a surfactant cell lysate solution to strip off the cell membrane and cytoplasm. The resultant semi-lysed cells are then passed through a laser in the basophil channel and the degree of forward high and low angle light scatter is measured. Basophils are resistant to cell lysis by the lysate solution used for some reason that is as yet unclear and this gives a characteristic pattern on the basophil channel of the H*1. The Technicon H*1 computes a white cell differential by comparing the data obtained from the 2 channels against a set algorithm (Technicon H*1 System, 1986). Big cells that cannot be classified into the usual leucocyte subclasses on the pattern of peroxidase staining are labeled by the H*1 as large unstained cells (LUC).

Over a 6 month period, 45 hospital in-patients were confirmed to have secondary dengue fever by serological criteria as proposed by the World Health Organization (WHO, 1975). Serial changes in the peripheral blood counts performed during the period of hospital stay were retrospectively analysed in relation to the day of defervesence. The H*1 basophil, LUC and platelet counts were noted against a manual atypical lymphocyte count on the peripheral blood smear.

Twelve other subjects also with serologically confirmed secondary dengue fever had buffy coat preparations made from the peripheral blood when the atypical lymphocyte count was greater than 10% of the total white cell count. Immunophenotyping of the atypical lymphocytes for pan T-cell CD2, CD7, pan B-cell CD19, T-helper CD4, and T-suppressor CD8 surface markers was done using monoclonal antibodies obtained from Dakopatts of Denmark by the APAAP method (Cordell *et al,* 1984) with hematoxylin as a nuclear counter-stain. The atypical lymphocytes were identified on the basis of morphological features visible under light microscopy and only cells which had features resembling those of atypical lymphocytes as seen with the classical Romanowsky stains were counted.

RESULTS AND DISCUSSION

Atypical lymphocytes in dengue fever appear as cells with abundant and intensely basophilic cytoplasm resembling plasma cells.

Patients with raised atypical lymphocyte counts had raised H*1 LUC and 'basophil' counts as well. The Technicon H*1 apparently identifies the atypical lymphocyte as LUCs in the peroxidase channel and in addition, classifies some of the atypical lymphocytes as 'basophils' in the basophil channel. This combination of a raised LUC and 'basophil' count on the peroxidase and basophil cytograms formed a picture which was rather characteristic of the atypical lymphocyte (Fig 1) The elevation in the 'basophil' count was a 'false' elevation as the manual basophil count was consistently less than 1%. Why a proportion of the atypical lymphocytes should be represented as 'basophils' in the basophil channel is uncertain. The problem is not one of coincidence as patients with chronic lymphocytic leukemia do not have a falsely elevated basophil count (Drewinko, 1985) and dilution studies, in addition, do not eliminate the reading. The finding suggests that despite similar morphological appearances, some of the atypical lymphocytes have acquired different physical properties which conferred resistance to cell lysis.

Manual atypical lymphocyte counts were already raised 2 days before defervesence and peaked sharply on day 1 (day of defervesence being represented as day 0). They remained high for about a week before settling. The H*1 LUC and

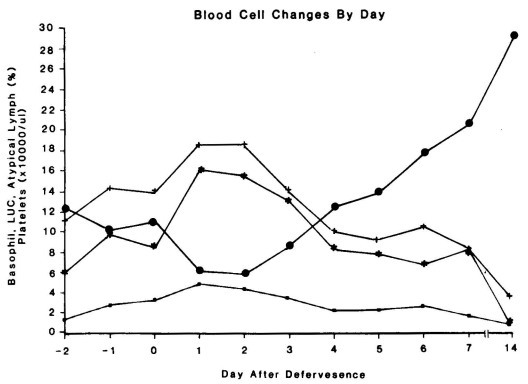

Fig 1—Technicon H*1 cytograms in A) normal subject and B) patient with dengue fever. (LUC - large unstained cells, P - polymorph, Mono - monocytes, Lymph - lymphocytes, Eos - eosinophils, MN - mononuclear cells, PMN - polymorphonuclear cells)

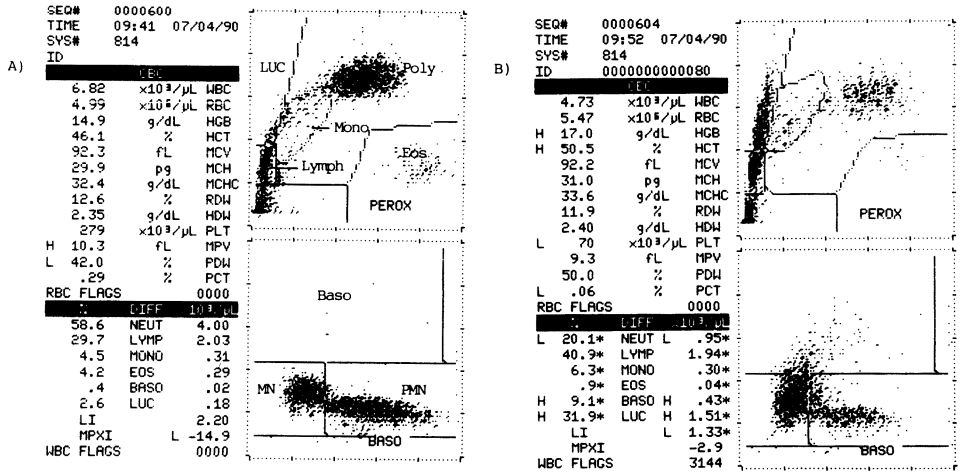

Fig 2—Changes in basophil, LUC, atypical lymphocyte and platelet counts against day after defervesence. (●-● platelet, + – + LUC, *– * atypical lymph, ■-■ basophil).

'basophil' counts rose in tandem with and paralleled the changes in the atypical lymphocyte count (Fig 2). Both the manual atypical lymphocyte and H*1 'basophil' counts had dropped to normal levels by day 14 and the LUC was less than 4%.

Immuno-phenotyping using the APAAP technique showed the composition of the atypical lymphocytes to be of T-lymphocyte origin predominantly, with most cells carrying markers for both CD2 and CD7 (Table 1). It is interesting to note that despite a plasmacytoid appearance, the atypical lymphocytes immunophenotype as T-cells. B-cell markers were relatively infrequent. The T-cell response was however rather heterogeneous and was not restricted to either the CD4+ or CD8+ subsets. This is reflected in the variable CD4/CD8 ratios. These findings are at odds with those obtained by earlier workers. Boonpucknavig *et al* (1979) and Sarasombath *et al* (1988) suggested the identity of these atypical lymphocytes to be B cells as they found an increased number of B lymphocytes in the peripheral blood around the time of appearance of these cells using mouse rosetting and immunofluorescence techniques. Sarasombath *et al* (1988) in addition, showed a reversal of the normal CD4/CD8 ratio in their immunophenotyping of peripheral blood lymphocytes.

We feel that immuno-phenotyping by the APAAP technique has some advantage over the methods used in the 2 earlier studies as it allows the simultaneous visualization of cellular morphology and permits a positive identification of the atypical lymphocytes.

The role of the atypical lymphocyte in the pathogenesis of the disease is rather uncertain. Dengue viruses have been shown to infect and replicate in peripheral blood monocytes enhanced by the production of alpha-interferon (Kurane *et al,* 1984). Transformed T-lymphocytes appearing as atypical lymphocytes could be responsible for the production of interferon and the atypical lymphocytes could well be a direct reflection of the activity of the underlying immune process. Our data also suggest that the immune response varies in different individuals as reflected by the differing CD4/CD8 ratios with some showing predominantly CD4 responses and others, a CD8 response. The numbers are however too small for any firm conclusions to be drawn.

A sudden drop in platelets preceding the onset of shock had been previously reported (Nimmanitya *et al,* 1969). In our series, patients with dengue fever sustained a sharp drop in the platelet counts on day 1 after defervesence, coinciding with the

Table 1

Immunophenotyping of atypical lymphocytes in dengue fever.

Patient	Day after defervesence	CD2	CD4	CD8	CD7	CD19	CD4/CD8 ratio
d17	0	40	23	10	20	3	2.30
d16	1	77	47	36	63	4	1.31
d11	1	21	22	10	27	0	2.20
d7	0	50	35	19	51	24	1.84
d8	1	60	8	25	62	2	0.32
d10	0	51	16	28	68	12	0.57
d6	1	60	38	10	50	8	3.80
d4	1	40	30	12	0	3	2.50
d1	1	nd	7	27	35	0	2.59
d3	2	nd	16	35	52	20	0.46
d14	2	32	20	16	33	1	1.25
d2	3	nd	8	17	26	1	0.47
d12	3	67	36	14	41	6	2.57
d13	3	60	30	20	43	6	1.50
	mean	52.5	22.5	22.9	39.9	5.9	1.34
	1 sd+	16.5	11.4	13	17.5	6.7	1.0

* nd = not done
\+ 1 sd = 1 standard deviation

sharp rise in the manual atypical lymphocyte and LUC counts (Fig 2).

The manual atypical lymphocyte, H*1 LUC and 'basophil' counts significantly, were raised even on day 2 when the platelets were > 100,000/μl.

There was a general inverse relationship between the atypical lymphocyte/LUC/'basophil' counts and that of the platelet count. No correlation between the mean platelet volume and the platelet count was found. The raised atypical lymphocyte-/LUC/'basophil' counts were useful in alerting the clinician to the need for closer monitoring over the next few days.

We conclude that the Technicon H*1 is a useful and convenient tool for the monitoring of the progress of the infection in dengue fever. Its applicability in the monitoring of other viral infections with similar changes is a possibility which requires further studies.

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