

Increased numbers of cells with suppressor T cell phenotype in the peripheral blood of patients with infectious mononucleosis

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SUMMARY

Peripheral blood cells from six patients with acute infectious mononucleosis were studied by two-colour immunofluorescence using antibodies to human T cells, inducer and suppressor–cytotoxic T cell subsets and Ia-like antigens. The absolute number of T cells with the suppressor–cytotoxic phenotype was substantially increased in each case; many of these cells also expressed Ia-like antigens and had the morphology of the large atypical cells characteristic of infectious mononucleosis. These activated suppressor T cells of infectious mononucleosis may therefore represent a control mechanism to prevent viral-induced proliferation of B cells.

INTRODUCTION

Infectious mononucleosis (IM) is a benign, self-limiting lymphoproliferative disease caused by the Epstein–Barr (EB) virus (Henle, Henle & Diehl, 1968), an agent which is aetiologically associated with two geographically restricted human tumours, Burkitt's lymphoma and nasopharyngeal carcinoma (Epstein & Achong, 1979; Epstein, 1978). During IM, EB virus infects B lymphocytes which have specific receptors for the virus (Pattengale, Smith & Gerber, 1973; Jondal & Klein, 1973) and which, when placed in culture, give rise to cell lines with the capacity for unlimited growth (Diehl *et al.*, 1968). By the time of onset of the clinical symptoms of IM, large numbers of atypical lymphocytes are found in the peripheral blood (Downey & McKinlay, 1923; Wood & Frenkel, 1967), and antibodies specific for EB viral antigens are produced (Hewetson *et al.*, 1973). The majority of these atypical cells are T cells (Sheldon *et al.*, 1973); however, a small minority are of B cell origin (Enberg, Eberle & Williams, 1974; Papamichael, Sheldon & Holborow, 1974), and some of these B cells are infected with EB virus (Denman & Pelton, 1974; Klein *et al.*, 1976). These humoral and cellular immune mechanisms are thought to act together to restrict the infection to the region of the oropharynx (Golden *et al.*, 1973; Miller, Niederman & Andrews, 1973) and to a few EB virus genome-carrying B lymphocytes in the circulation (Nilsson *et al.*, 1971). Thus in the immunocompetent patient, the self-limiting nature of the disease is assured, although the virus is not completely eliminated from the body. T cell populations from acute IM patients have been shown to contain cells which are specifically cytotoxic for EB virus genome-containing cell lines (Svedmyr & Jondal, 1975; Royston *et al.*, 1975), and recently increased suppressor activity has been demonstrated in these activated T cell populations (Tosato *et al.*, 1979).

Recently conventional antisera and monoclonal antibodies have become available for the

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detection of T lymphoid cell subsets by immunofluorescence (Reinherz *et al.*, 1979a; Reinherz & Schlossman, 1979; Reinherz *et al.*, 1980a). T lymphocytes reacting with the OKT4 mouse monoclonal antibody include inducer (helper) T cells (Reinherz *et al.*, 1979a). A smaller and discrete subset of circulating T cells reacts in double-staining experiments with both of two other reagents: a horse anti-TH₂ antiserum (Reinherz & Schlossman, 1979), and OKT8, a monoclonal antibody (Reinherz *et al.*, 1980a). This TH₂⁺, OKT8⁺ subset includes T cells with cytotoxic and suppressor functions. Furthermore, it has recently been demonstrated that these markers (mouse OKT4 monoclonal antibody together with horse anti-TH₂ antiserum) can be conveniently used in various combinations using second-layer antibodies labelled with different fluorochromes (Tidman *et al.*, 1980; Janossy *et al.*, 1980). In this paper we have dissected the cellular heterogeneity of the peripheral blood T cell populations during the acute phase of IM with these sensitive techniques.

MATERIALS AND METHODS

Patients. Blood samples were obtained from patients with acute IM of up to 3 weeks' duration. The diagnosis was confirmed by positive serological testing (monospot) and IgM antibodies to EB virus capsid antigen (VCA).

Preparation of mononuclear leucocytes. Diluted blood was centrifuged on a Ficoll-Triosil gradient (Böyum, 1968) and the band of mononuclear cells was harvested. The cells were washed twice and resuspended in PBS containing 0.2% albumin (PBSA) at a concentration of 2×10^7 /ml.

Unconjugated antisera

Horse anti-human thymocyte/T cell (HuTLA) serum. This reagent was made against human thymocytes by Upjohn Co., Kalamazoo, USA (Lot 17,923). After extensive absorption with human red cells, liver powder, B chronic lymphatic leukaemia cells and cells from acute myeloid leukaemia the antiserum specifically reacted with human thymocytes and peripheral T cells (Reinherz *et al.*, 1979c).

Horse anti-human suppressor T cells (TH₂) serum. This reagent was made from anti-HuTLA by further absorptions with malignant T cells obtained from a patient with Sézary's syndrome as described by Reinherz and co-workers (Reinherz & Schlossman, 1979). The antiserum identifies a T cell population which also reacts with the OKT8 (Reinherz *et al.*, 1980a) and OKT5 antibodies (Reinherz *et al.*, 1980b) and shows cytotoxic and suppressor activity in culture systems (Reinherz & Schlossman, 1979; Reinherz *et al.*, 1980a).

Rat monoclonal antibody (YE2/36) to human Ia-like molecules was made by Dr I. McConnell and co-workers (Brickell *et al.*, 1980). This reagent reacts with B lymphocytes, as well as some monocytes in blood, and with 4–10% of bone marrow cells which include B lymphocytes, putative precursor cells and most identifiable myeloblasts. Previous studies have also shown that very few T lymphocytes (Johnson *et al.*, 1978; Yu *et al.*, 1980) and a much larger proportion of activated T lymphoblasts developing in cultures stimulated by mixed lymphocyte reaction or T cell mitogens (Reinherz *et al.*, 1979b) also express Ia-like antigens.

Mouse monoclonal antibody (OKT4) (Reinherz *et al.*, 1979a) to inducer (helper) T cells was made by Ortho Pharmaceutical Corp., Raritan, New Jersey, USA.

Conjugated antisera

Swine anti-horse rhodamine conjugate (Sw anti-H TRITC) was obtained from Nordic Immunological Laboratories, Maidenhead, Berkshire, UK.

Goat anti-rat fluorescein conjugate (G anti-Ra FITC) was obtained from Nordic Immunological Laboratories.

Goat anti-mouse fluorescein conjugate (G anti-M FITC). This reagent was prepared by immunizing with purified mouse immunoglobulin. The immune serum was absorbed and eluted from a mouse gammaglobulin column and absorbed once with human liver.

Labelling cells with antisera

Two million cells in 50 μ l of PBSA were incubated for 15 min at 20°C with antisera or culture supernatants containing monoclonal antibodies. After two washes in PBS, cells were incubated with second-layer antibodies conjugated to FITC or TRITC for a further 15 min at 20°C and washed twice again. The cells were examined immediately in suspension sealed under a coverslip using phase-contrast and u.v. light with a standard 14 Zeiss microscope equipped with IV/F epifluorescence condenser, selective filters for FITC and TRITC and a 63-phase oil objective.

Reagent combination used

Total mononuclear cell populations from six IM patients and five control normal subjects were stained with the following antisera:

(i) HuTLA with SW anti-H TRITC second layer in order to determine the proportion of T lymphoid cells in the Ficoll–Triosil-separated mononuclear cell population.

(ii) TH₂ with SW anti-H TRITC and OKT4 with G anti-M FITC in order to determine the ratio of T cells expressing the inducer and suppressor/cytotoxic phenotypes.

(iii) HuTLA with SW anti-H TRITC and YE2/36 with G anti-Ra FITC in order to determine the proportion of cells expressing Ia-like antigens within the T cell population.

(iv) TH₂ with SW anti-H TRITC and YE2/36 with G anti-Ra FITC for analysing the proportion of cells with Ia-like antigens within the population of TH₂⁺ cells.

(v) Normal donkey serum with SW anti-H TRITC and normal mouse serum with G anti-M FITC as controls for non-specificity of the second layer.

Finally, these staining procedures were carried out on cells from one IM patient (No. 1) taken during the acute disease and convalescent period in order to determine the time course of the abnormalities observed.

RESULTS

The total white blood cell (WBC) counts from the six IM patients ranged from 8.6–22.5 $\times 10^9$ /litre (Table 1). All but one patient (No. 5) had raised total WBC counts (over 10.0 $\times 10^9$ /litre), and all patients had raised total lymphocyte counts (over 4.0 $\times 10^9$ /litre). The total number of atypical cells varied from 4.9–22.2 $\times 10^9$ /litre (Table 1). These haematological findings taken together with the positive monospot tests and the presence of IgM to virus capsid antigen (VCA) in the sera establishes the diagnosis of IM in all six patients.

In Ficoll–Triosil-separated preparations of mononuclear cells, over 60% of cells were found to be HuTLA⁺ in all IM patients and control subjects. Of these HuTLA⁺ cells, 36–94% were TH₂⁺ in the IM patients, whereas the range for normal subjects was 16–25% (Table 2). In the same cell

Table 1. Haematological data from six IM patients and control subjects

Patient No.	Total WBC ($\times 10^9$ /l)	Total lymphs ($\times 10^9$ /l)	Atypical lymphs ($\times 10^9$ /l)
1	10.1	5.5	5.5
2	12.6	8.5	4.9
3	16.5	13.9	13.4
4	15.0	11.7	6.7
5	8.6	6.4	6.3
6	22.5	22.0	22.0
Controls*	4.4–7.5	0.9–1.9	<0.1

* Range from five normal subjects.

populations from the IM patients, 2–16% were found to be OKT4⁺, whereas 36–58% of HuTLA⁺ cells were OKT4⁺ in normal subjects. In these double-stained preparations most cells were labelled singly; however, a small number (up to 5%) of double-labelled cells were found in both the IM and the control subjects (Table 2). From these findings the total numbers of HuTLA⁺, TH₂⁺ and OKT4⁺ cells can be calculated (Fig. 1). All six IM patients showed an absolute increase in the number of cells expressing TH₂ antigen (cells with suppressor–cytotoxic phenotype). These cell numbers ranged from 3.1–14.9 × 10⁹/litre as compared to the normal range of 0.2–0.4 × 10⁹/litre. In contrast, the number of OKT4⁺ cells in the IM peripheral blood remained within the normal range (0.2–1.1 × 10⁹/litre).

A high percentage of Ia⁺ cells was observed in the mononuclear cell populations from all six IM patients, and most of these cells were T cells (HuTLA⁺). The percentage of HuTLA⁺ cells which also expressed Ia ranged from 23–83% in the IM patients, whereas control values were less than 7% (Table 2). In three IM patients it was shown that the TH₂⁺ subset of T cells included 38–60% of Ia⁺

Table 2. Percentages of total mononuclear cells from peripheral blood of IM patients and control subjects expressing immunological markers

Patient No.	HuTLA ⁺	HuTLA ⁺ Ia ^{**}	TH ₂ ⁺ OKT4 ⁻	TH ₂ ⁻ OKT4 ⁺	TH ₂ ⁺ OKT4 ⁺
1	74	40	55	15	2
2	93	23	36	12	1
3	80	36	70	10	1
4	74	68	70	2	1
5	70	40	50	16	1
6	93	83	68	3	3
Controls†	62–85	4–7	16–25	36–58	0–5

* In three cases TH₂, Ia double-staining showed that the vast majority of the Ia⁺ cells were TH₂⁺.

† Control range from five normal subjects.

cells, and phase-contrast microscopy showed that many of these cells were large with a blast-like morphology. No OKT4⁺, Ia⁺ doubles were seen. Thus these findings show that the large atypical lymphocytes in IM are HuTLA⁺, TH₂⁺ cells which include many Ia⁺ cells indicating activated T blasts of the suppressor–cytotoxic phenotype.

Mononuclear cells from patient 1 were studied at 2, 3, 4 and 8 weeks after the onset of clinical illness. The total white cell, lymphocyte and T (HuTLA⁺) cell numbers were all raised in the initial sample, with increased numbers of atypical cells; these had all returned to normal by the 3rd week of the illness and remained within the normal range thereafter (Fig. 2). Numbers of OKT4⁺ cells remained within the normal range throughout. In contrast, the numbers of TH₂⁺ cells were greatly increased for 4 weeks after the onset of IM, causing an inversed helper/suppressor–cytotoxic cell ratio. This had returned to within the normal range by 8 weeks (Fig. 2). Similarly, the percentages of HuTLA⁺ cells which expressed Ia antigens were increased during the first 4 weeks of the illness being 54, 83 and 31% at 2, 3 and 4 weeks respectively; no increase in Ia⁺, HuTLA⁺ cells was found by 8 weeks. The patient was clinically well by the 4th week.

DISCUSSION

By the use of two-colour immunofluorescence and double-staining techniques with a pair of antibodies specific for human inducer (OKT4) and suppressor (TH₂) T cell subsets we have been able to measure the proportion of cells expressing the phenotype of cells with inducer or suppressor–cytotoxic function in the peripheral blood of IM patients and control normal subjects (Table 1).

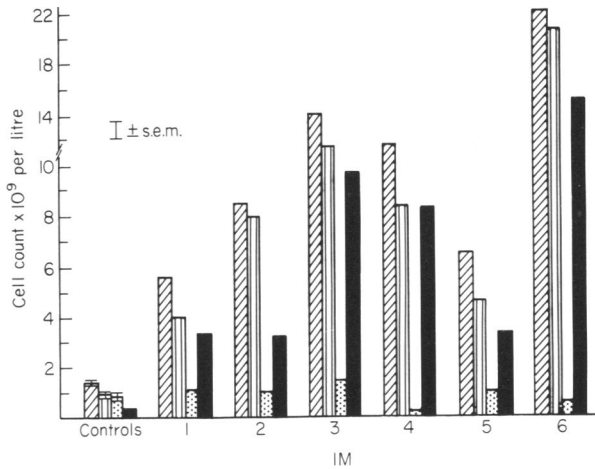


Fig. 1. Total numbers of lymphocytes (▨) and cells expressing the phenotype of T cells (■), T suppressor-cytotoxic cells (TH₂⁺; ■) and T helper cells (OKT4⁺; ▨) in the peripheral blood of six IM patients and control subjects.

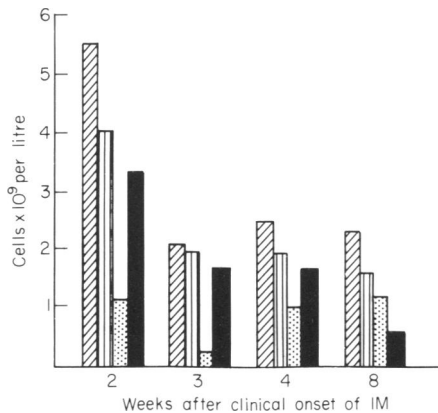


Fig. 2. Total numbers of lymphocytes (▨) and cells expressing the phenotype of T cells (■), T suppressor-cytotoxic cells (TH₂⁺; ■) and T helper cells (OKT4⁺; ▨) in the peripheral blood of IM patient 1 at 2, 3, 4 and 8 weeks after the onset of clinical illness.

From this data and the haematological observations, the absolute numbers of these cells could be calculated (Fig. 1). We have revealed a massive increase in T cells of the suppressor-cytotoxic phenotype occurring during acute IM. These results corroborate those of Tosato *et al.* (1979) who found that IM T cells co-cultured with normal B cells in the presence of pokeweed mitogen caused severe suppression of T cell-dependent B cell activation as demonstrated by failure of immunoglobulin production.

By double-staining techniques we have also demonstrated that a substantial number (23–83%) of IM mononuclear cells express both T and Ia antigens (Table 2), a finding which has previously been demonstrated serologically (Johnson *et al.*, 1978). We have further shown that these Ia⁺, T⁺ cells are contained within the population of suppressor-type (TH₂⁺) cells (Table 2) and are the large atypical cells characteristic of IM. It has been demonstrated by immunofluorescence that a small percentage (average 2.6%) of peripheral blood T cells from normal individuals express Ia antigens

(Yu *et al.*, 1980), and that this percentage is increased in certain diseases, including rheumatoid arthritis, systemic lupus erythematosus and active bacterial infection (Yu *et al.*, 1980). Recently, analysis of lymphocytes after mitogen or soluble antigen stimulation or mixed lymphocyte reaction has shown increased numbers of T⁺, Ia⁺ cells (Reinherz *et al.*, 1979b). In contrast to our findings in IM, however, after soluble antigen stimulation the T⁺, Ia⁺ cells were exclusively OKT4⁺ (inducer cell phenotype) (Reinherz *et al.*, 1979b). Thus in different situations T cells of either helper and suppressor phenotypes can selectively express Ia antigens.

During acute IM a transient polyclonal hypergammaglobulinaemia may occur (Wollheim & Williams, 1966). This hypergammaglobulinaemia, which occurs early in the disease, is associated with increased numbers of immunoglobulin-secreting cells in the circulation (Tosato *et al.*, 1979). This clinical finding is thought to reflect the ability of EB virus to cause polyclonal stimulation of B cells with the production of IgG, IgM and IgA in short-term cultures. Although this B cell activation is T cell-independent *in vitro* it cannot be excluded that T-inducer cells may facilitate the B cell activation *in vivo* which probably occurs in the lymphoid organs very early in IM before the activation of the immune responses and the onset of clinical symptoms. Our results show that by the 2nd or 3rd week of the disease large numbers of T cells with the suppressor-cytotoxic phenotype are present in the circulation and no increase in the absolute numbers of inducer cells could be detected. This finding could represent a regulatory mechanism with increased numbers of T suppressor cells inhibiting the B cell activation caused by EB virus infection. IM T cells have been shown to be specifically cytotoxic for EB virus genome-carrying target cell lines (Svedmyr & Jondal, 1975; Royston *et al.*, 1975) and to be capable of reducing the efficiency with which EB virus-infected fetal lymphocytes give rise to cell lines (Rickinson, Crawford & Epstein, 1977). Moss, Rickinson & Pope (1978) find evidence for T cell memory in seropositive individuals and convalescent IM patients leading to the generation of cells in culture which are cytotoxic for EB virus-infected cells. Paradoxically, the same group fails to find any memory T cell cytotoxicity in cultures from acute IM patients (Rickinson *et al.*, 1980). This finding suggests that the immunological mechanisms by which primary infection by EB virus is controlled may differ from the long-term immunity by which cells infected with this putative oncogenic virus are controlled.

It is likely that the large increase in peripheral blood suppressor T cells which occurs in IM plays an important role in assuring the benign course of IM. The importance of these T cells in normal individuals is exemplified by the recent reports of fatal cases of IM in individuals who failed to mount this characteristic T cell response (Crawford *et al.*, 1979; Robinson *et al.*, 1980). Hypogammaglobulinaemia is a rare sequel of IM (Provisor, Iacoue & Chilcote, 1975), and in these cases a persistence of the suppressor T cells (TH₂⁺) may play a pathogenic role.

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