Clin. exp. Immunol. (1991) **83**, 447–451 ADONIS 000991049100082I

Expression of CD45R0 (UCHL1) by $CD4^+$ and $CD8^+$ T cells as a sign of *in vivo* activation in infectious mononucleosis

T. MIYAWAKI, Y. KASAHARA, H. KANEGANE, K. OHTA, T. YOKOI, A. YACHIE & N. TANIGUCHI
Department of Paediatrics, School of Medicine, Kanazawa University, Kanazawa, Ishikawa, Japan

(Accepted for publication 24 September 1990)

SUMMARY

CD45R0 (UCHL1), a member of leucocyte common antigen family, is expressed largely on previously activated or memory T cells. We examined CD45R0 expression of T cell subpopulations in patients with Epstein–Barr virus (EBV) induced infectious mononucleosis (IMN) as a sign of *in vivo* activation. Consistent with the notion that activated $CD8^+$ T cells expand in acute IMN, the majority of $CD8^+$ T cells in patients with acute IMN expressed CD45R0 to the similar extent to HLA-DR expression. Most $CD4^+$ T cells in these patients also demonstrated marked expression of CD45R0 as well as HLA-DR antigens, compared with age-matched controls. Expression of CD45R0 by $CD4^+$ T cells in patients with acute IMN was more notable than their HLA-DR expression. While predominant $CD8^+$ T cells resulted in decreased percentages of $CD4^+$ T cells, $CD4^+$ T cells expressing CD45R0 were shown to be significantly elevated in absolute number. The results suggest that both $CD4^+$ and $CD8^+$ T cells may be activated by stimulation with EBV infection. The appearance of two T cell subpopulations expressing CD45R0 in acute IMN implies their immunoregulatory roles in the control of EBV-infected cells.

Keywords Epstein–Barr virus infectious mononucleosis CD45R0 T cell activation

INTRODUCTION

Infectious mononucleosis (IMN) is an acute and usually self-limiting lymphoproliferative disease caused by a primary infection of Epstein–Barr virus (EBV) (Cheeseman, 1988). The hallmark of acute IMN is the appearance of atypical lymphocytes in the peripheral blood. Although B lymphocytes are primarily the target cells of EBV infection, atypical lymphocytosis results from the expansion of T lymphocytes, especially those of the cytotoxic/suppressor (CD8) phenotype (Reinherz *et al.*, 1980; De Waele, Thielemans & Van Camp, 1981). Blood $CD8^+$ T cells in IMN show the increased expression of HLA-DR antigen, indicative of *in vivo* activation. They exhibit the appreciable cytotoxic activity against a series of EBV-infected cells (Royston *et al.*, 1975; Svedmyr & Jondal, 1975) and the strong suppressor activity on EBV-induced immunoglobulin production by B cells (Tosato *et al.*, 1979). Thus, prominent $CD8^+$ T cells are considered to be the result of the intense *in vivo* stimulation by EBV-infected B cells and to contribute to the control of EBV-infected and transformed B cells during the acute phase of IMN.

Correspondence: T. Miyawaki, Department of Pediatrics, School of Medicine, Kanazawa University, Kanazawa, Ishikawa 920, Japan.

Human T cells express various isoforms of CD45 (leucocyte-common antigen), which arise from alternate mRNA splicing (Thomas & Lefrançois, 1988). The changes in CD45 isoform expression of T cells appear to correspond to their differentiation and activation status. The UCHL1 monoclonal antibody (MoAb) recognizes a 180-kD molecular form of CD45, termed CD45R0 (Terry, Brown & Beverley, 1988). The distribution of CD45R0 is the reciprocal of T cell population to another form (CD45RA) of CD45, identified by the MoAb 2H4 (Morimoto *et al.*, 1985). The current concept is the dissection of human T cells into naive and memory cell populations on the basis of different expression of these CD45 isoforms (Cerottini & MacDonald, 1989). Naive T cells are found in the $CD45R0^-$ $CD45RA^+$ subset, whereas memory T cells are confined to the $CD45R0^+$ $CD45RA^-$ subset. This contention is favoured by the finding that naive T cells lose surface expression of CD45RA and acquire CD45R0 after stimulation *in vitro*, and expression of CD45R0, once acquired, is stable (Akbar *et al.*, 1988).

Here we examined the expression of CD45R0 by T cell subpopulations in acute IMN as a sign of activation. We show that the majority of $CD8^+$ T cells in patients with IMN expressed CD45R0 as well as HLA-DR antigen. Marked expression of CD45R0 was also found in most $CD4^+$ T cells in these patients. The results suggest that both $CD4^+$ and $CD8^+$

Table 1. Lymphocyte marker analysis of patients with acute infectious mononucleosis

Patient	Age (year)	Total lymphocyte no. ($\times 10^3/\mu l$)	Total lymphocytes (%)		CD45R0 expression (%) by		HLA-DR expression (%) by	
			$CD4^+$ cells	$CD8^+$ cells	$CD4^+$ cells	$CD8^+$ cells	$CD4^+$ cells	$CD8^+$ cells
A	1	15·41	16·8	55·7	67·4	92·7	27·4	95·0
B	2	7·57	13·4	58·9	40·0	73·4	8·7	68·0
C	4	10·58	7·4	68·0	56·8	84·4	35·2	79·4
D	4	15·40	12·7	63·8	36·8	65·0	11·7	70·1
E	4	8·71	14·0	68·3	55·0	73·0	19·2	89·0
F	6	13·43	7·1	80·1	59·1	71·3	16·8	61·4
G	7	19·18	13·5	63·1	64·1	86·8	25·2	75·2
H	7	9·55	27·3	54·7	51·6	70·4	20·1	67·3
Mean ± s.d.		12·47 ± 4·02*	14·0 ± 6·3*	64·0 ± 8·2*	53·8 ± 0·8*	77·1 ± 9·6*	20·5 ± 8·6*	75·6 ± 11·5*
Age-matched controls ($n = 25$)	Mean ± s.d.	4·73 ± 1·97	36·7 ± 7·1	19·9 ± 5·7	24·5 ± 7·9	17·2 ± 6·9	1·7 ± 0·5	2·5 ± 1·3

*$P < 0·001$ compared with normal controls.

T cell populations may play the immunoregulatory roles in acute EBV infection.

MATERIALS AND METHODS

Patients

Eight patients with IMN aged 1–7 years, were seen at the Paediatric Clinic of the University Hospital. Clinical diagnosis was determined by fever, exudative pharyngitis, lymphadenopathy, splenomegaly and lymphocytosis with atypical lymphocytes in the peripheral blood. Blood samples were obtained after informed parental consent. Some patients were examined until 2 months of the illness. We defined the febrile period (within 10 days after the onset of fever) as the acute phase of the disease. EBV infection was serologically confirmed for all patients as described (Henle, Henle & Horwitz, 1974). In addition, active replication of the EBV genome in the blood was confirmed by polymerase chain reaction amplification (Yokoi *et al.*, submitted for publication). Twenty-five healthy children of the same ages without obvious haematological abnormalities served as the age-matched controls.

Antibodies

The MoAb UCHL1 (IgG2a) against CD45R0 (Terry *et al.*, 1988) was purchased from Dakopatts (Copenhagen, Denmark). PE-conjugated anti-HLA-DR (IgG1) MoAb was obtained from Becton Dickinson (Mountain View, CA). Anti-Leu-3a (IgG1) and anti-Leu-2a (IgG1) MoAbs (Becton Dickinson) against CD4 and CD8, respectively, were used in FITC-conjugated forms.

Immunofluorescence analysis

Peripheral blood mononuclear cells (PBMC) were isolated from heparinized venous blood by Ficoll–Hypaque density gradient centrifugation. Two-colour immunofluorescence was used to examine CD45R0 expression by $CD4^+$ and $CD8^+$ T cells, as described (Miyawaki *et al.*, 1990). PBMC were first treated with anti-CD45R0 MoAb (UCHL1) or isotype-matched mouse IgG, and counter-stained with biotinylated rabbit anti-mouse IgG2a antibody (Zymed Laboratories, San Francisco, CA) and further with PE-conjugated streptavidin (Becton Dickinson). Next, the cells were stained for determination of T cell subpopulations with FITC-conjugated anti-CD4 or anti-CD8 MoAbs. For HLA-DR expression, the cells were incubated simultaneously with PE-conjugated anti-HLA-DR and FITC-conjugated anti-CD4 or anti-CD8 MoAbs. Two-colour staining patterns of lymphocytes gated by forward and 90° light scatters were analysed by an EASY88 data analysing program (Coulter Electronics, Hialeah, FL).

Statistic analysis

The unpaired Student's *t*-test was used to analyse data.

RESULTS

The absolute number of blood lymphocytes in all patients during the acute phase of IMN was significantly elevated ($P < 0·001$) when compared with age-matched normal controls (Table 1). Consistent with previous reports (Reinherz *et al.*, 1980; De Waele *et al.*, 1981), all acute IMN patients had increased percentages of $CD8^+$ T cells in the peripheral blood, concomitant with a decrease in percentages of $CD4^+$ T cells. Increased percentages $HLA\text{-}DR^+$ lymphocytes, exceeding the B cell values, were also observed in these patients (data not shown); this suggested the presence of activated ($HLA\text{-}DR^+$) T cells.

We used two-colour immunofluorescence to examine expression of CD45R0 by two ($CD4^+$ and $CD8^+$) T cell subpopulations from acute IMN patients in comparison with their expression of HLA-DR antigen. Illustrated in Fig. 1 are representatives of immunofluorescence patterns for a patient with acute IMN and a normal control, analysed using corresponding MoAbs. As expected, the percentages of $HLA\text{-}DR^+$ cells within $CD8^+$ T cells were markedly increased in acute IMN patients (Fig. 1d), although expression of HLA-DR antigens by two major T cell subpopulations was negligible in normal controls (Fig. 1 f, h). In addition, it was found that some of $CD4^+$ T cells in these patients co-expressed HLA-DR antigen as well (Fig. 1b). As for CD45R0 expression, some of $CD4^+$ and $CD8^+$ T cells in age-matched controls usually possess CD45R0, corresponding to the age-dependent memory cell pool of the blood (Hayward, Lee & Beverley, 1989). While only a propor-

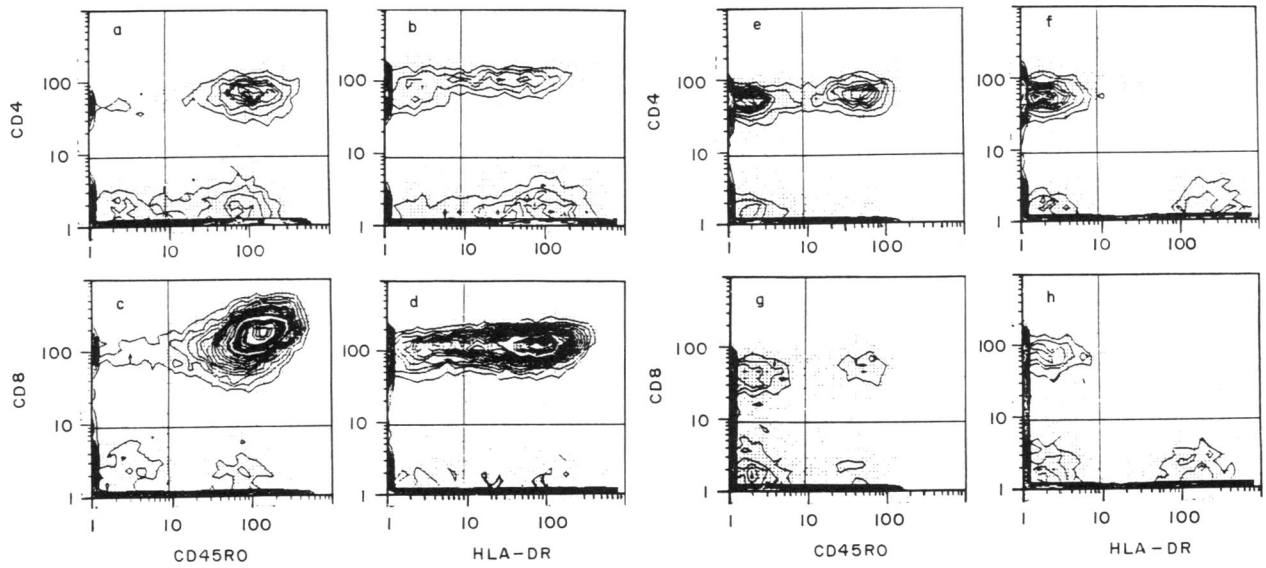

Fig. 1. Two-colour immunofluorescence analysis of CD45R0 and HLA-DR expression by $CD4^+$ and $CD8^+$ T cells in a patient with acute infectious mononucleosis (a–d) and an age-matched control (e–h). Contour maps show two-colour staining patterns of lymphocytes gated by forward and 90° light scatters. Immunostaining was performed as described in Materials and Methods.

Table 2. Increased numbers of $CD4^+$ and $CD8^+$ cells expressing CD45R0 in patients with acute infectious mononucleosis

	No. of lymphocytes ($\times 10^3/\mu l$)	
Cell population	Patients ($n=8$)	Age-matched controls ($n=25$)
CD45R0+$CD4^+$	0·93±0·19*†	0·43±0·13
CD45R0$^+$ $CD8^+$	6·15±0·77*	0·16±0·06
HLA-DR$^+$ $CD4^+$	0·35±0·15*	0·03±0·01
HLA-DR$^+$ $CD8^+$	6·03±0·92*	0·02±0·01

Results are expressed as mean ± s.d.
*$P<0·001$ compared with normal controls.
†$P<0·001$ compared with HLA-DR$^+$ $CD4^+$ cells of patients.

tion of $CD8^+$ T cells in normal controls were CD45R0$^+$ (Fig. 1g), the majority of $CD8^+$ T cells were found to express CD45R0 on their surfaces (Fig. 1c). Interestingly, there was a distinction between acute IMN patients and normal controls for CD45R0 expression by $CD4^+$ T cells (Fig. 1 *a versus* e). In a similar manner to $CD8^+$ T cells, most $CD4^+$ T cells in acute IMN patients were demonstrated to express CD45R0 strongly. Table 1 summarized the results of CD45R0 and HLA-DR expression by each T cell subset in acute IMN patients. It should be noted that the expression of CD45R0 by two T cell subpopulations in acute IMN patients more marked than that of HLA-DR antigen.

The absolute numbers of $CD4^+$ and $CD8^+$ T cells expressing CD45R0 or HLA-DR antigen were calculated as shown in Table 2. The numbers of $CD8^+$ and $CD4^+$ T cells expressing HLA-DR antigens in acute IMN patients were significantly increased ($P<0·001$) when compared with normal controls. $CD8^+$ T cells expressing CD45R0 in these patients demonstrated the dramatic increase in absolute number. The absolute values of $CD4^+$ T cells coexpressing CD45R0 in acute IMN patients were significantly greater ($P<0·001$) than those in age-matched controls. These observations indicated the actual circulation of activated ones of $CD4^+$ as well as $CD8^+$ T cells during the acute phase of IMN.

The evolution of CD45R0$^+$ $CD4^+$ and $CD8^+$ T cells as a function of disease time is shown in Fig. 2. The percentages of CD45R0$^+$ $CD8^+$ T cells remained markedly elevated around 5 weeks after the onset of symptoms and subsequently declined. Although the frequency of CD45R0$^+$ $CD4^+$ T cells became normal beyond 6 weeks after the onset of the disease, $CD4^+$ T cells from some IMN cases still showed higher percentages of CD45R0$^+$ cells between the week 3 and 5 of the illness, as compared with age-matched control.

DISCUSSION

Activation of T cells induces increased expression of a variety of cell surface molecules. Some molecules such as HLA-DR antigens and interleukin-2 (IL-2) receptors (IL-2R) are transiently induced *in vivo* as well as *in vitro* (Yachie *et al.*, 1983). Marked HLA-DR expression of T cells is often found during the acute phase of IMN, but IL-2R (Tac antigen or p55) bearing T cells are hardly identified in the blood of IMN patients, although a recent development of MoAbs against another non-Tac (p70) peptide of IL-2R has demonstrated a definite but weak expression of this molecule by acute IMN T cells (Kamio *et al.*, 1990). In contrast to these transient activation antigens, there are other surface molecules whose expression is stably increased after activation and serves as markers of previously activated or memory T cells (Sanders *et al.*, 1988). Among them, CD45R0 (UCHL1) appears to be particularly a useful marker for discrimination between antigen-primed and virgin T cells, as its expression by adult T cells exhibits a clear bi-modal distribution (Cerottini & MacDonald, 1989). It has been demonstrated that most T cells in inflammatory compartments

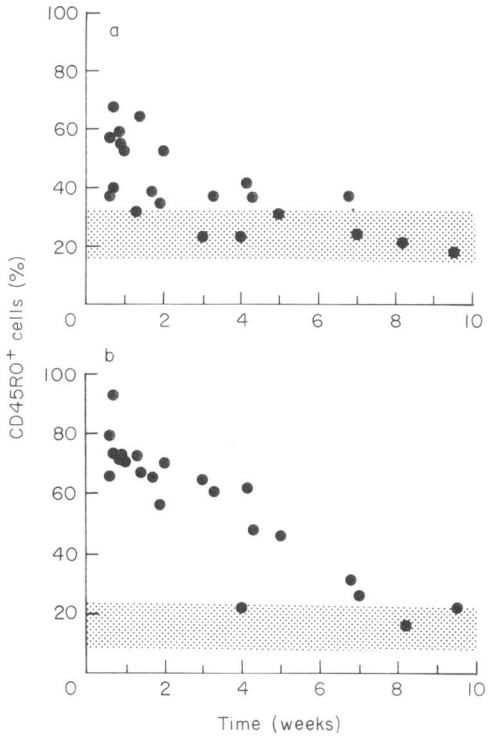

Fig. 2. Sequential changes in CD4+ (a) and CD8+ (b) T cells with CD45R0 expression in patients with infectious mononucleosis. The percentages of CD45R0+ cells in each T cell subset were plotted as a function of time after the onset of symptoms, on the basis of obtainable data of time-points. The shaded area shows the mean value ± s.d. of normal age-matched controls.

such as cerebrospinal fluids, synovium, or intestinal epithelium in various disease conditions are enriched for CD45R0+ cells, compared with the peripheral blood, indicating the local accumulation of activated populations of T cells (Pitzalis *et al.*, 1988; Chofflon *et al.*, 1989). However, increased expression of CD45R0 by circulating T cells has not been documented.

We investigated whether T cells from patients with acute IMN could express CD45R0 (UCHL1), reflecting an *in vivo*-activated situation by EBV-infected B cells. It was found that the majority of CD8+ T cells expressed CD45R0, similar in extent to HLA-DR expression. The CD45R0 expression of most IMN CD8+ T cells was in marked contrast to the finding that only a small proportion of CD8+ T cells in age-matched controls expressed CD45R0. To our surprise, most CD4+ T cells in acute IMN patients also indicated the remarkable expression of CD45R0, compared with normal controls. While IMN CD4+ T cells expressed HLA-DR antigens to some extent, their expression of CD45R0 was more prominent. Although predominance of CD8+ T cells in IMN patients result in decreased percentages of CD4+ T cells, the absolute number of CD45R0+ CD4+ T cells was increased in these patients. These observations suggested that both CD4+ and CD8+ T cells may be activated by stimulation with EBV infection and mobilized into the circulating pool during the acute phase of IMN. In this regard, Tomkinson *et al.* (1987) have demonstrated by HLA-DR expression that activated lymphocytes expanded in acute EBV-induced IMN consisted of a heterogeneous population, including CD4+ as well as CD8+ T cells. Although functional studies (Royston *et al.*, 1975; Svedmyr & Jondal, 1975; Tosato *et al.*, 1979) suggest a role of activated CD8+ T cells for the control of EBV-infected B cells, the immunoregulatory function of activated helper/inducer (CD4) T cells in IMN has not yet been defined. Further functional studies should focus on the roles of these activated CD4+ as well as CD8+ T cells for clinical manifestations of EBV-induced IMN.

It should be noted that all our IMN patients were children. It is accepted that EBV-induced IMN generally occurs in adolescence and adults, but is not uncommon in young infants and childhood (Cheeseman, 1988). Although young children with IMN have been described (Horwitz *et al.*, 1981; Weigle, Sumaya & Montiel, 1983), we often encounter children with typical manifestations of EBV-induced IMN in paediatric clinics, possibly from epidemiological backgrounds in Japan or other Asian areas where primary EBV infection occurs mainly in early childhood (Hinuma *et al.*, 1969). We observed that remarkable expression of CD45R0 by two T cell subsets was not demonstrable in other common viral diseases such as measles, rubella, varicella or mumps (unpublished observations). Usually, the percentages of CD45R0+ cells in each T cell subset in young children are lower as a function of age than those seen in adults (Hayward *et al.*, 1989). Thus, evaluation of CD45R0 expression by T cell subsets seems to be valuable for clinical diagnosis of EBV-induced IMN, especially in young children.

ACKNOWLEDGMENTS

This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture in Japan and grants from the Ministry of Welfare in Japan. We thank Miss Noriko Yoshida and Miss Sayuri Tai for the excellent technical assistance.

REFERENCES

Akbar, A.N., Terry, L., Timms, A., Beverley, P.C.L. & Janossy, G. (1988) Loss of CD45R and gain of UCHL1 reactivity is a feature of primed T cells. *J. Immunol.* **140,** 2171.

Cerottini, J.-C. & MacDonald, H.R. (1989) The cellular basis of T-cell memory. *Annu. Rev. Immunol.* **7,** 77.

Cheeseman, S.H. (1988) Infectious mononucleosis. *Semin. Hematol.* **25,** 261.

Chofflon, M., Gonzalez, V., Weiner, H.L. & Hafler, D.A. (1989) Inflammatory cerebrospinal fluid T cells have activation requirements characteristic of CD4+ CD45RA− T cells. *Eur. J. Immunol.* **19,** 1791.

De Waele, M., Thielemans C. & Van Camp, B.K.G. (1981) Characterization of immunoregulatory T cells in EBV-induced infectious mononucleosis by monoclonal antibodies. *N. Engl. J. Med.* **304,** 460.

Hayward, A.R., Lee, J. & Beverley, P.C.L. (1989) Ontogeny of expression of UCHL1 antigen on TcR-1+ (CD4/8) and TcRδ+ T cells. *Eur. J. Immunol.* **19,** 771.

Henle, W., Henle, G. & Horwitz, C.A. (1974) Epstein-Barr virus specific diagnostic tests in infectious mononucleosis. *Hum. Pathol.* **5,** 551.

Hinuma, Y., Ohta-Hatano, R., Suto, T. & Numazaki, Y. (1969) High incidence of Japanese infants with antibody to a herpes-type virus associated with cultured Burkitt lymphoma cells. *Jpn. J. Microbiol.* **13,** 309.

Horwitz, C.A., Henle, W., Henle, G., Goldfarb, M., Kubic, P., Gehrz, R.C., Balfour, H.H., Fleisher, G.R. & Krivit, W. (1981) Clinical and laboratory evaluation of infants and children with Epstein-Barr virus-induced infectious mononucleosis: report of 32 patients (aged 10–48 months). *Blood,* **57,** 933.

Kamio, M., Uchiyama, T., Hori, T., Kodaka, T., Ishikawa, T., Onishi, R., Uchino, H., Yoneda, N., Tatsumi, E. & Yamaguchi, N.

(1990) Selective expression of the p70 subunit of the interleukin-2 receptor on lymphocytes from patients with infectious mononucleosis. *Blood*, **75,** 415.

MIYAWAKI, T., KASAHARA, Y., TAGA, K., YACHIE, A. & TANIGUCHI, N. (1990) Differential expression of CD45R0 (UCHL1) and its functional relevance in two subpopulations of circulating TCR-γ/δ^+ lymphocytes. *J. exp. Med.* **171,** 1833.

MORIMOTO, C., LETVIN, N.L., DISTASO, J.A., ALDRICH, W.R. & SCHLOSSMAN, S.F. (1985) The isolation and characterization of the human suppressor inducer T cell subset. *J. Immunol.* **134,** 1508.

PITZALIS, C., KINGSLEY, G., HASKARD, D. & PANAYI, G. (1988) The preferential accumulation of helper-inducer T lymphocytes in inflammatory lesions: evidence for regulation by selective endothelial and homotypic adhesion. *Eur. J. Immunol.* **18,** 1397.

REINHERZ, E.L., O'BRIEN, C., ROSENTHAL, P. & SCHLOSSMAN, S.F. (1980) The cellular basis for viral-induced immunodeficiency: analysis by monoclonal antibodies. *J. Immunol.* **125,** 1269.

ROYSTON, I., SULLIVAN, J.L., PERIMAN, P.O. & PERLIN, E. (1975) Cell-mediated immunity to Epstein–Barr-virus-transformed lymphoblastoid cells in acute infectious mononucleosis. *N. Engl. J. Med.* **293,** 1159.

SANDERS, M.E., MAKGOBA, M.W., SHARROW, S.O., STEPHANY, D., SPRINGER, T.A., YOUNG, H.A. & SHAW, S. (1988) Human memory T lymphocytes express increased levels of three cell adhesion molecules (LFA-3, CD2, and LFA-1) and three other molecules (UCHL1, CDw29, and Pgp-1) and have enhanced IFN-γ production. *J. Immunol.* **140,** 1401.

SVEDMYR, E. & JONDAL, M. (1975) Cytotoxic effector cells specific for B cell lines transformed by Epstein-Barr virus are present in patients with infectious mononucleosis. *Proc. natl Acad. Sci. USA*, **72,** 1622.

TERRY, L.A., BROWN, M.H. & BEVERLEY, P.C.L. (1988) The monoclonal antibody, UCHL1, recognizes a 180,000 MW component of the human leukocyte-common antigen, CD45. *Immunology*, **64,** 341.

THOMAS, M.L. & LEFRANÇOIS, L. (1988) Differential expression of the leucocyte-common antigen family. *Immunol. Today*, **9,** 320.

TOMKINSON, B.E., WAGNER, D.K., NELSON, D.L. & SULLIVAN, J.L. (1987) Activated lymphocytes during acute Epstein–Barr virus infection. *J. Immunol.* **139,** 3802.

TOSATO, G., MAGRATH, I., KOSKI, I., DOOLEY, N. & BLAESE, M. (1979) Activation of suppressor T cells during Epstein-Barr-virus-induced infectious mononucleosis. *N. Engl. J. Med.* **301,** 113.

WEIGLE, K.A., SUMAYA, C.V. & MONTIEL, M.M. (1983) Changes in T-lymphocyte subsets during childhood Epstein–Barr virus infectious mononucleosis. *J. clin. Immunol.* **3,** 151.

YACHIE, A., MIYAWAKI, T., UWADANA, N., OHZEKI, S. & TANIGUCHI, N. (1983) Sequential expression of T cell activation (Tac) antigen and Ia determinants on circulating human T cells after immunization with tetanus toxoid. *J. Immunol.* **131,** 731.