Clin. exp. Immunol. (1989) **77**, 71–76

Polyclonal proliferation of activated suppressor/cytotoxic T cells with transient depression of natural killer cell function in acute infectious mononucleosis

M. L. WILLIAMS, T. P. LOUGHRAN, JR., P. G. KIDD & G. A. STARKEBAUM

The Departments of Laboratory Medicine and Medicine, Divisions of Oncology and Rheumatology, University of Washington School of Medicine, Seattle, WA, and the Fred Hutchinson Cancer Research Center, Seattle, WA and the Veterans Administration Medical Center, Seattle, WA, USA

(Accepted for publication 1 March 1989)

SUMMARY

In acute infectious mononucleosis large numbers of atypical lymphocytes proliferate in response to B cells infected with Epstein–Barr virus, generally resulting in a self-limited illness. Although both T-cells and NK cells are known to be involved, the precise origin of the large granular lymphocytes in this disorder is incompletely understood. Using two-colour immunofluorescent flow cytometry, we sequentially examined the phenotype of selected T cell and NK cell subsets from nine patients with infectious mononucleosis. In parallel, we determined whether these lymphocytes utilized a restricted repertoire of the T cell receptor gene and also measured their NK activity. Our results show that in acute infectious mononucleosis there was a greater than three-fold increase in T lymphocytes with the phenotype $CD2^+$, $CD3^+$, $CD8^+$ and DR^+. A modest increase in $Leu7(HNK1)^+$ and $CD4^+$ T cells was also seen. In addition, there was a three-fold increase in cells coexpressing $CD3^-$ and $CD16^+$, the phenotype reported to represent most NK cells. In spite of this latter finding, however, a marked decrease in NK function was found at the time of diagnosis, gradually returning to normal by day 28. Finally, Southern blot analysis of DNA from patient lymphocytes showed polyclonal rearrangements of the T cell receptor beta chain gene. These studies indicate that the proliferation of activated suppressor/cytotoxic T lymphocytes in acute infectious mononucleosis is polyclonal and is associated with transient depression of NK function.

Keywords infectious mononucleosis large granular lymphocytes

INTRODUCTION

Infectious mononucleosis (IM) is a self-limited syndrome resulting from infection by the Epstein–Barr virus (EBV) (Henle, Henle & Diehl, 1968). Transformation and proliferation of EBV infected B cells are controlled *in vivo* by a prompt, vigorous and complex cellular immune response (Svedmyr & Jondal, 1975; Tosato & Blaese, 1985). This response is reflected in the transient proliferation of atypical lymphocytes seen in the peripheral blood of IM patients. The atypical lymphocytes are predominantly T cells, since they rosette with sheep red blood cells and possess T cell markers such as CD2, CD3 and CD8 using monoclonal antibodies (Pattengale, Smith & Perlin, 1974; Sheldon *et al.*, 1975; Haynes *et al.*, 1979; Reinherz *et al.*, 1980; De Waele, Thielemans & Van Camp, 1981). Morphologically, many atypical lymphocytes are large granular lymphocytes (LGL) which on Wright's stain have abundant cytoplasm and azurophilic granules; parallel tubular arrays are often present by electron microscopy (McKenna, *et al.*, 1977).

Correspondence: Gordon Starkebaum MD, Department of Medicine (111), Veterans Administration Medical Center, 1660 S. Columbian Way, Seattle, WA 98108, USA.

The precise origin of the LGL in IM, is incompletely understood. Recent studies indicate that normal LGL consist of heterogenous cells that can be divided into $CD3^+$ and $CD3^-$ populations (Lanier *et al.*, 1986a, b). NK cells which lyse target cells in a non-MHC restricted manner are LGL-bearing Fc receptors of IgG (Timonen, Ortaldo & Herberman, 1981) but are $CD3^-$, (Lanier, *et al.*, 1986a). In contrast, cytotoxic lymphocytes such as those activated by viral infections are $CD3^+$LGL (Biron, Natuk & Welsh, 1986). Some *in vitro* studies indicate IM lymphocytes are capable of lysing EBV-infected B cells in a non-MHC-restricted manner (Royston *et al.*, 1975; Seeley *et al.*, 1981), suggesting that these cells do not conform to rules governing conventional cytotoxic lymphocytes (Zinkernagel & Doherty, 1979). Based on these and other findings, some investigators suggest that control of EBV-induced B cell proliferation early in IM is mediated principally by NK cells rather than by cytotoxic lymphocytes (Kaplan & Shope, 1985). Thus, it is likely that the atypical lymphocytosis in IM results from expansion of heterogenous subpopulations of normal lymphocytes in response to EBV.

Recently patients have been described who possess a chronically expanded population of LGL with an unusual

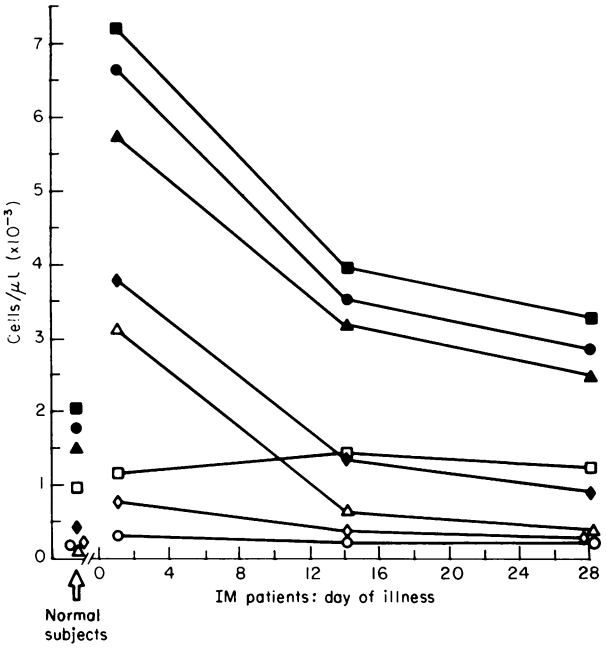

Fig. 1. Lymphocytes subsets during acute IM. Normal values are shown on the left; patients values are shown sequentially over 28 days, on the right. Total lymphocytes (■); $CD2^+$ (●); $CD3^+$ (▲); $CD4^+$ (□); $CD8^+$ (◆); DR^+ (△); $CD16^+$ (◇); $HNK\text{-}1^+$ (○).

Table 1. Lymphocyte subsets in acute infectious mononucleosis

Lymphocyte population	Normal ($n=10$)	Patients ($n=9$) Day 1	Day 14	Day 28
Total lymphocytes	2·05±0·48 (1·22–2·81)	7·19±3·07 (4·18–12·79)	3·96±1·76 (1·93–7·05)	3·28±0·74 (2·03–4·49)
$CD2^+$	1·77±0·44 (1·09–2·47)	6·63±2·91 (3·77–11·64)	3·53±1·68 (1·72–6·63)	2·86±0·73 (1·66–4·22)
$CD3^+$	1·52±0·38 (0·89–2·16)	5·73±2·54 (2·88–9·45)	3·19±1·61 (1·85–6·27)	2·50±0·72 (1·36–4·00)
$CD4^+$	0·97±0·30 (0·57–1·43)	1·17±0·14 (1·02–1·42)	1·43±0·57 (0·83–2·82)	1·24±0·30 (0·73–1·67)
$CD8^+$	0·43±0·11 (0·25–0·54)	3·79±2·40 (1·25–8·06)	1·36±0·96 (0·39–3·48)	0·90±0·36 (0·49–1·71)
DR^+	0·15±0·06 (0·07–0·26)	3·13±1·83 (1·02–6·55)	0·64±0·54 (0·17–1·71)	0·33±0·17 (0·13–0·61)
$HNK1^+$	0·17±0·11 (0·02–0·37)	0·31±0·22 (0·11–0·80)	0·22±0·17 (0·04–0·56)	0·26±0·20 (0·05–0·67)
$CD16^+$	0·21±0·16 (0·06–0·64)	0·77±0·53 (0·35–2·05)	0·37±0·19 (0·10–0·67)	0·36±0·18 (0·17–0·61)
$B4^+$	0·08±0·05 (0·02–0·19)	0·11±0·04 (0·05–0·21)		

Results are expressed as cells/μl $\times 10^{-3}$ (mean ± 1 s.d.) with range in parentheses.

surface phenotype: $CD2^+$, $CD3^+$, $CD8^+$, $HNK\text{-}1^+$ and Fc receptor $(CD16)^+$ (Reynolds & Foon, 1984; Loughran & Starkebaum, 1987). In many patients, substantial evidence indicates that these cells are neoplastic, including the demonstration of clonal cytogenic abnormalities and clonal rearrangements of T cell receptor (TCR) β-chain DNA (Loughran & Starkebaum, 1987). Although the aetiology of this syndrome is presently unknown, in several cases the chronic proliferation of LGL has appeared to follow acute infection with EB virus (Herrod, Semenzato *et al.*, 1984; Wang & Sullivan, 1985; Aronson *et al.*, 1987).

We therefore wondered whether some of the atypical lymphocytes seen in patients with uncomplicated IM might reflect an oligoclonal expansion of a lymphocyte subset similar to that seen in the neoplastic disorder of LGL. In this study, two-colour immunofluorescent flow cytometry was utilized to sequentially define selected subsets of T cells and NK cells during the course of the illness. Clonality was assessed by analysing genomic DNA obtained from these patients' mononuclear cells for evidence of TCRβ gene rearrangement. In parallel, NK activity of patients' lymphocytes was measured serially. Our results show that the early atypical lymphocytosis was primarily due to a polyclonal proliferation of $CD2^+$, $CD3^+$, $CD8^+$, $Leu7^-$, DR^+cells. Although there was an early mild increase in $CD3^-$, $CD16^+$ cells in these patients, NK activity was initially decreased.

MATERIALS AND METHODS

Study population

These studies were approved by the Human Subjects Review Committee of the University of Washington. Young adults with acute infectious mononucleosis were recruited through Hall Student Health Center at the University of Washington. The patient group consisted of four females and five males. Criteria for inclusion in the study were positive Monospot test, greater than 10% atypical lymphocytes, fever, pharyngitis, lymphadenopathy, and age between 16 and 30. Blood was drawn from each patient on the day of diagnosis and 14 and 28 days after diagnosis. The interval between onset of clinical symptoms and diagnosis ranged from 4 to 14 days, with a median of 8 days.

Five patients were treated with prednisone, 60–80 mg/day for 5 days at the beginning of their illness, for symptoms of

Table 2. Lymphocyte subsets in two patients: effect of prednisone

Lymphocyte population	Patient B Day 1	Day 14	Day 28	Patient D Day 1	Day 2	Day 28
Total lymphocytes	4·28	3·44	3·79	4·18	4·48	3·55
$CD2^+$	3·77	2·89	3·15	3·89	4·17	3·23
$CD3^+$	3·21	2·37	2·58	2·88	3·45	2·63
$CD4^+$	1·07	1·55	1·67	1·05	1·34	1·28
$CD8^+$	1·80	0·72	0·83	1·25	1·52	0·89
DR^+	2·10	0·45	0·61	1·21	0·94	0·32
$HNK1^+$	0·21	0·28	0·34	0·13	0·13	0·11
$CD16^+$	0·47	0·52	0·61	0·75	0·67	0·53

Results are expressed as cells/μl $\times 10^{-3}$.

Patient B received prednisone early in his illness (see Table 4), patient D received no prednisone.

Table 3. Two-colour analyses of lymphocytes from IM patients

	IM patients ($n=9$)	Normal subjects ($n=10$)
$CD2^+DR^+$	1510 ± 810 (58–3330)	20 ± 8 (4–30)
$CD3^+HNK\text{-}1^+$	140 ± 106 (30–350)	50 ± 38 (10–120)
$CD8^+HNK\text{-}1^+$	110 ± 102 (30–350)	30 ± 28 (6–90)
$CD16^+HNK\text{-}1^+$	60 ± 26 (20–90)	40 ± 40 (5–130)
$HNK\text{-}1^+DR^+$	30 ± 25 (3–80)	2 ± 3 (0–10)
$CD3^+CD16^+$	20 ± 22 (9–70)	4 ± 3 (1–10)
$CD3^-CD16^+$	760 ± 530 (340–1982)	215 ± 160 (59–630)

Results are expressed as cells/μl (mean $\pm$ 1 s.d.) with range in parentheses.

Patients analysed at Day 1 of illness.

respiratory obstruction (Cheesman, 1988). Initial blood samples were obtained in three of these patients prior to beginning prednisone; in the remaining two subjects, initial blood samples were drawn within 24 h of beginning prednisone (patients B and E, Table 4). The normal subject group consisted of 10 volunteers (one male and nine females), aged 16 to 30. One specimen was drawn from each normal subject.

Surface markers

Peripheral blood mononuclear cells (PBMC) were isolated by standard Ficoll-Paque procedures and suspended in RPMI 1640 medium (Patrick *et al.*, 1984; Jackson & Warner, 1986). Cell viability was assessed by exclusion of 0·4% trypan blue. PBMC were analysed for surface markers by direct immunofluorescence and flow cytometry with the following panel of monoclonal antibodies: Leu 3a (anti-CD4), T helper/inducer cells; Leu 4 (anti-CD3), mature T cells; Leu 2a (anti-CD8), T suppressor/cytotoxic cells; Leu 5b (anti-CD2), pan T cells or E-rosetted cells; Leu 7 (anti-HNK-1), LGL; Leu 11c (anti-CD16), IgG FcR^+ cells; Leu 15 (anti-CD-11), monocytes and NK cells; and anti-HLA DR, Class II MHC antigen (all from Becton Dickinson, Mountain View, CA). B cells were identified with monoclonal antibody B4 (anti CD19). LGL subpopulations were analysed by using Leu 7 monoclonal antibody in combination with anti-CD3, anti-CD8, and anti-CD16 monoclonal antibodies. Isotype-specific negative controls were used to test for non-specific binding.

Flow cytometry

Surface membrane immunofluorescence was analysed on a Coulter EPICS-V flow cytometer (Coulter, Hialeah, FL) with a 256-channel analyser interfaced with a multiparameter data acquisition and display computer system. The EPICS-V employs an Innova 90-5 argon ion laser emitting at 488 nm. The two fluorochromes used for two-colour analyses were FITC, producing maximum emission at 520 nm, and phycoerythrin (PE), producing maximum emission at 575 nm. The gating cursors were set to exclude monocytes and other cells, allowing isolation and analysis of the lymphocyte population.

Natural killer cell functional assay

PMBC which had been cryopreserved in liquid nitrogen were thawed and incubated overnight in RPMI complete medium plus 20% fetal calf serum at 37°C in an atmosphere of 5% CO_2 to allow recovery of cytotoxic activity. The cells were then used as effectors in a standard 4 h ^{51}Cr-release assay using NK-sensitive K 562 cells as targets (Brunner, Engers & Cerottini, 1974). Effector cells were placed in U-bottomed wells in microtitre plates with 1×10^4 ^{51}Cr-labelled target cells at effector-to-target ratios of 6·25:1, 12·5:1, 25:1, and 50:1. The plates were centrifuged at 200 *g* for 2 min, incubated for 4 h at 37°C in an atmosphere containing 5% CO_2, and centrifuged again at 100 *g* for 2 min. An aliquot (0·1 ml) of supernatant was removed from each well, placed in a plastic vial, and counted in a gamma counter. The percentage specific lysis was calculated by the formula: experimental ct/min − spontaneous ct/min divided by maximum release ct/min − spontaneous ct/min × 100 (Pross *et al.*, 1981).

Blot hybridization analysis

Genomic DNA was extracted as previously described (Brunner *et al.*, 1974) from mononuclear cells of patients at the time of diagnosis of IM and from neutrophils from normal individuals that served as the germ-line control. The DNA samples were then digested with restriction enzymes *Bam*HI, *Eco*RI, or *Hin*d III. Digested DNA was separated on 1·1% agarose gels and transferred onto nitrocellulose filters by the method of Southern (Southern, 1975). Filters were then hybridized to DNA probes ^{32}P-labelled by nick-translation and visualized by autoradiography as previously described (Maniatis, Frisch & Sambrook, 1982). The cDNA clone Jurkat B2 containing the C and J regions of the TCRβ gene (Yoshikai, *et al.*, 1984) was kindly provided by Dr Tak Mak (Ontario Cancer Institute, Toronto, Canada). The insert representing nucleotides 100–870 was isolated on agarose gels and used as the hybridization probe.

RESULTS

At diagnosis, patients with IM had lymphocyte counts over three-fold higher than controls (Fig. 1, Table 1). In the patients, lymphocytes staining for CD2, CD3, CD8 and DR were particularly increased compared to controls. There was also a modest increase in $HNK1^+$ and $CD4^+$ cells early in the illness; these populations of cells remained relatively stable over 28 days (Fig. 1). On the other hand, there was a three-fold increase in $CD16^+$ cells early in illness. The expanded populations of $CD2^+$, $CD3^+$, $CD8^+$, $CD16^+$ and DR^+ cells had fallen to nearly normal levels by day 28 (Fig. 1, Table 1).

Since one half of the patients had received a short course of high-dose prednisone early in their illness the phenotype data were examined in relation to whether or not each patient had received steroids. No significant differences between the two groups were found for any lymphocyte subpopulation by one- or two-colour immunofluorescence at any of the three study times. Individual data from two patients treated with (B) and without (D) prednisone are shown in Table 2 to illustrate this point.

Two-colour analysis clarified the phenotypes of the expanded lymphocyte subsets. Approximately one fourth of

Table 4. Mean natural killer cell activity

Normal subjects		Patients Day 1	Day 14	Day 28
60	A*	5	0	76
57	B*	16	18	30
60	C*	6	33	12
54	D*	17	7	46
57	E*	4	3	13
78	F*	47	5	52
29	G	9	4	9
44	H	3	3	45
80	I	14	27	82
40		(Mean =	(Mean =	(Mean =
(Mean† =		13 ± 13·6)	11 ± 11·9)	41 ± 27·0)
56 ± 15·7)		$P < 0.05$	$P < 0.05$	$P > 0.05$

Results are expressed as percentage lysis at the 50:1 effector to target ratio.

* Received a 5-day course of prednisone.

† ±1 s.d.

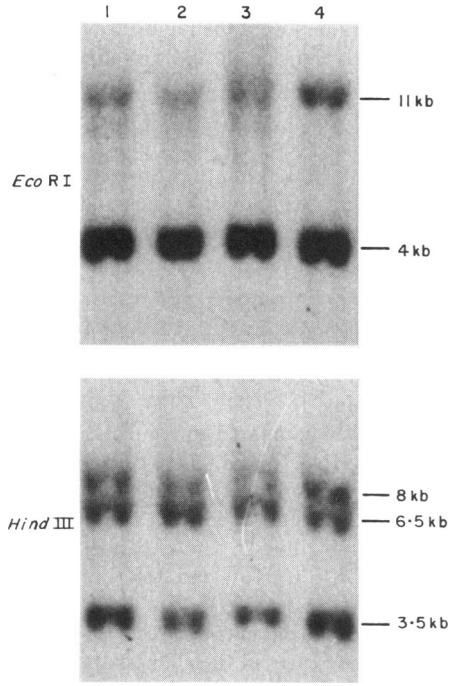

Fig. 2. Southern blot studies. DNA in lanes 1–3 was from patient lymphocytes; lane 4 was from normal human neutrophils (germ-line control). After digestion of lymphocyte DNA from three IM patients with *Eco*RI, marked dimunition of the 11 kb germ-line fragment was found (top panel). After digestion of DNA with *Hind*IIIa, germ-line pattern of 8 kb, 6·5 kb and 3·5 kb was found (lower panel). Similar results were seen with five additional IM patients.

$CD2^+$ cells coexpressed DR, representing a marked increase over the normal levels (Table 3, $P < 0.0005$). Two-colour analysis utilizing Leu 7 in combination with anti-CD3, anti-CD8 or anti-DR showed modest increases compared to normal subjects. Two-colour studies indicated that almost all of the expanded population of $CD16^+$ cells were $CD3^-$ (Table 3), a phenotype reported to correspond to activated NK cells (Lanier, *et al.*, 1986b).

Despite increased numbers of cells with an NK cell phenotype, however, NK activity of patient cells was low at day one. At the 50:1 effector to target ratio, mean NK activity in the acute phase was 13% compared to 56% in the normal subjects. NK activity remained low 2 weeks after diagnosis, but was nearly normal 1 month after diagnosis (Table 4). Similar results were obtained at the other effector: target ratios examined (not shown). In those patients who took prednisone, no effect on NK activity was found (Table 4).

Results of Southern blot hybridization analyses using the TCRβ gene probe are shown in Fig. 2. Analysis after digestion of patient cell DNA with *Hind* III showed the germ-line pattern as did analysis after *Bam*HI digestion (results not shown). In contrast, analysis of patient cell DNA after *Eco*RI digestion showed marked diminution in intensity of the 11·0 Kb germ fragment, indicating polyclonal rearrangement of the C_B1 gene (Fig. 2). These results are consistent with polyclonal proliferation of T-cells in patients with IM with multiple individual rearrangements (Flug *et al.*, 1985).

DISCUSSION

These results demonstrate that the transient proliferation of atypical lymphocytes in acute IM was due mainly to expansion of $CD2^+$, $CD3^+$, $CD8^+$, DR^+ T cells, as previously reported (Reinherz *et al.*, 1980; De Waele, Thielemans & Van Camp, 1981). Our two-colour analysis demonstrated a marked increase in cells coexpressing CD2 and DR antigens, indicating that these cells were activated. Many of the atypical lymphocytes in acute IM morphologically are LGL (McKenna *et al.*, 1977), a population which normally contains most NK cells (Timonen, Ortaldo & Herberman, 1981). Therefore we examined patient cells for reactivity with monoclonal antibodies Leu 7 and Leu 11 (CD 16), which stain the majority of LGL (Abo, Cooper & Balch, 1982; Phillips & Lanier, 1985). Both one- and two-colour analyses, however, indicated that Leu 7+ cells were only minimally increased during the course of IM. On the other hand, the two-colour analyses revealed a three-fold increase in $CD3^-$, $CD16^+$ cells early in IM. This population of cells, which may be Leu 7^-, has been found to possess potent NK activity (Phillips & Lanier, 1985). Nevertheless, this population of cells represented only a minority (approximately 10%) of the total lymphocytes early in IM. These findings are consistent with the atypical lymphocytes in IM, reflecting for the most part expansion of presumably antigen-specific, activated cytotoxic/suppressor T-lymphocytes. Biron *et al.*, (1986) found similar results in the acute phase of viral infections in mice and emphasized that LGL morphology could correlate more closely with a cell's lytic ability than with its lineage or surface markers.

The proliferation and activation of suppressor T cells during acute IM appear to be responsible for controlling outgrowth of EBV-infected B cells (Svedmyr & Jondal, 1975; Tosato & Blaese, 1985). However, these T cells have been shown to also

induce a profound and diverse state of transient immunologic deficiency. For example, during acute IM, B cell responses to non-specific mitogens are markedly impaired (Reinherz *et al.*, 1980; Tosato, *et al.*, 1979). The T-cell-dependent B cell response to the neoantigen bacteriophage ΦX174 is markedly reduced during acute IM but returns to normal after 4–6 weeks (Junker *et al.*, 1986). T cell responses to mitogens (Mangi *et al.*, 1974), soluble, or allo-antigens, are also depressed *in vitro* (Reinherz., *et al.*, 1980). Consistent with these findings, skin tests to antigens become negative during acute IM (Mangi *et al.*, 1974).

It is possible that suppressor T cells activated in IM could also inhibit NK cells. Tarkkanen *et al.* (1983) recently described a subset of T cells which inhibit normal NK cells. Injection of mice with *C. parvum* results in inhibition of NK function, partly due to activated T cells (Lotzova & Savary, 1986). Consistent with these findings, we found a dramatic decrease in NK activity during acute IM and at day 14, which had returned to normal by day 28. It is unlikely that this finding reflected dilution of NK cells due to the three-fold increase in total lymphocytes, since patient lymphocytes also demonstrated a three-fold increase of $CD3^-$, $CD16^+$ cells noted above. These results of decreased NK activity are similar to those reported by Svedmyr *et al.*, (1984) in a longitudinal study of one patient with IM. Also, in an earlier study by Svedmyr and Jondal (1975), three of 12 patients with IM had markedly reduced NK function. In contrast, however, Sullivan *et al.*, (1980) and Brewster, Byron & Sullivan (1985) found normal NK activity in two groups of patients with acute IM.

The basis for this discrepancy is unclear, but in part, experimental conditions could be responsible for the reported differences. Similar to the study by Svedmyr *et al.*, (1984), we cryopreserved mononuclear leucocytes from IM patients. These cryopreserved cells were then incubated overnight at 37°C before assay of NK activity. Brewster *et al.*, (1985), on the other hand, used freshly obtained cells that were incubated overnight at 37°C before NK assays. Further studies using cryopreservation *versus* short term incubation of cells, as well as studies mixing patient and normal T cells are necessary to resolve this question. Of interest, Moss *et al.* (1985) recently noted that T cells from patients with acute IM rapidly died when cultured *in vitro*. This finding could affect analysis of T cell function.

In other studies we used Southern blot techniques to analyse the TCRβ gene in proliferating T cells from IM patients. T lymphocytes respond to antigens through a cell-surface heterodimer consisting of alpha and beta chain proteins (Kronenberg *et al.*, 1986). The genes encoding these proteins undergo rearrangement during T cell differentiation to yield the functional TCR gene (Kronenberg *et al.*, 1986). In case of the β chain, separate variable (V), diversity, and joining region segments rearrange into a continuous V-β gene, which in turn joins with a constant region segment (Toyonaga, 1985). By analysing the pattern of rearrangement of TCR-β genes, the clonality of proliferating T cells can be inferred (Flug *et al.*, 1985; Waldmann *et al.*, 1985). Of interest, T cell responses to certain well-defined antigens utilize relatively few TCR-β genes (Hochgeschwender *et al.*, 1986; Beall, 1987). Since some EBV-specific cytotoxic T cells have been found to respond to a 10 amino acid peptide comprising an EBV-encoded membrane protein on infected B cells (Thorley-Lawson & Israelsohn, 1987), we wondered whether an oligoclonal TCRβ repertoire could be detected in IM patients. The Southern blots indicate that in each patient the TCRβ DNA was rearranged but in no case was a monoclonal pattern seen. These findings clearly demonstrate that reactive T cells in IM use polyclonal TCR genes. As suggested by several recent reports (Herrod, Wang & Sullivan, 1985; Aronson *et al.*, 1987; Semenzato *et al.*, 1984), however, it appears possible for an occasional patient to develop a chronic monoclonal proliferation of LGL T cells in response to EBV infection. Whether such clonally expanded T cells are reactive with EBV antigens, however, has not been determined. Further studies are needed to analyse the potential role of EBV infection in such lymphoproliferative syndromes.

ACKNOWLEDGMENTS

We thank Dr Gordon G. Bergy, Director, Hall Health Center, for his co-operation in these studies, Joe Aprile for excellent technical assistance and Roberta Ruimy for typing the manuscript. This investigation was supported by Grant HL 36444 awarded by the National Heart, Lung, and Blood Institute, by the Leukemia Research Foundation, by PHS grant CA 18821, and by the Veterans Administration. Dr Loughran is a Special Fellow of the Leukemia Society of America.

REFERENCES

ABO, T., COOPER, M.D. & BALCH, C.M. (1982) Characterization of HNK-1$^+$ (Leu-7) human lymphocytes. *J. Immunol.* **129,** 1752.

ARONSON, F.R., DEMPSEY, R.A., ALLEGRETTA, M., ANDRE-SCHWARTZ, J., POLDRE, P.A., HILLYER, C.D., PARKINSON, D.R., RUDDERS, R.A., SCHWARTZ, R.S. & MIER, J.W. (1987) Malignant granular lymphoproliferation after Epstein-Barr virus infection: partial immunologic reconstitution with interleukin-2. *Am. J. Hematol.* **25,** 427.

BEALL, S.S., LAWRENCE, J.V., BRADLEY, D.A., MATTSON, D.H., SINGER, D.S. & BIDDISON, W.E. (1987) β-chain gene rearrangement and Vβ gene usage in DPw2-specific T cells. *J. Immunol.* **139,** 1320.

BIRON, C.A., NATUK, R.J. & WELSH R.M. (1986) Generation of large granular T lymphocytes *in vivo* during viral infection. *J. Immunol.* **136,** 2280.

BREWSTER, F.E., BYRON, K.S. & SULLIVAN, J.L. (1985) Immunoregulation during acute infection with EBV: dynamics of interferon and 2′, 5′-oligoadenylate synthetase activity. *J. infect. Dis.* **151,** 1109.

BRUNNER, K.T., ENGERS, H.D. & CEROTTINI, J.C. (1974) The ^{51}Cr-release assay as used for the quantitive measurement of cell mediated cytolysis *in vitro*. *Adv. Immunol.* **18,** 67.

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

DE WAELE, M., THIELEMANS, C. & VAN CAMP, K.G. (1981) Characterization of immunoregulatory T cells in EBV-induced infectious mononucleosis by monoclonal antibodies. *N. Engl. J. Med.* **304,** 460.

FLUG, F., PELICI, P.G., BONETTI, F., KNOWLES, D.M. & DALLA-FAVERA, R. (1985) T-cell receptor gene rearrangement as markers of lineage and clonality in T-cell neoplasms. *Proc. natl. Acad. Sci. USA.* **82,** 3460.

HAYNES, B.F., SCHOOLEY, R.T., GROUSE, J.E., PAYLING-WRIGHT, C.R., DOLIN, R. & FAUCI, A.S. (1979) Characterization of thymus-derived lymphocyte subsets in acute EBV induced infectious mononucleosis. *J. Immunol.* **122,** 699.

HENLE, G., HENLE, W. & DIEHL, V. (1968) Relation of Burkitts tumor-associated herpes-type virus to infectious mononucleosis. *Proc. natl. Acad. Sci. USA.* **59,** 94.

HERROD, H.G., WANG, W.C. & SULLIVAN, J.L. (1985) Chronic T-cell lymphocytosis with neutropenia. *Am. J. Dis. Child.* **139,** 405.

HOCHGESCHWENDER, U., WELTZIEN, H.U., EICHMANN, K., WALLACE, R.B. & EPPLEN, J.T. (1986) Preferential expression of a defined T-cell receptor beta-chain gene in hapten-specific cytotoxic T-cell clones. *Nature,* **322,** 376.

JACKSON, A.L. & WARNER, N.L. (1986) Preparation, staining and analysis by flow cytometry of peripheral blood leukocytes. In: *Manual of Clnical Laboratory Immunology* 3rd edn (eds. N.R. Rose, H. Friedman & J.L. Fahey) pp. 226-235. Am. Soc. Microbiol. Washington DC.

JUNKER, A.K., OCHS, H.D., CLARK, E.A., PUTERMAN, M.L. & WEDGWOOD, R.J. (1986) Transient immune deficiency in patients with acute Epstein-Barr virus infection. *Clin. Immunol. Immunopathol.* **40,** 436.

KAPLAN, J. & SHOPE, T.C. (1985) Natural killer cells inhibit outgrowth of autologous Epstein-Barr virus-infected B lymphocytes. *Nat. Immun. Cell Growth Regul.* **4,** 40.

KRONENBERG, M., SIU, G., HOOD, L.E. & SHASTRI, N. (1986) The molecular genetics of the T-cell antigen receptor and T-cell antigen recognition. *Ann. Rev. Immunol.* **4,** 529.

LANIER, L.L., LE, A.M., CWIRLA, S., FEDERSPIEL, N. & PHILIPS, J.H. (1986a) Antigenic, functional, and molecular genetic studies of human natural killer cells and cytotoxic T lymphocytes not restricted by the major histocompatibility complex. *Fed. Proc.* **45,** 2823.

LANIER, L.L., PHILLIPS, J.H. HACKETT, J., TUTT, M. & KUMAR, V. (1986b) Natural killer cells: definition of a cell type rather than a function. *J. Immunol.* **136,** 2735

LOTZOVA, E. & SAVARY, C.A. (1986) Regulation of NK cell activity by suppressor cells. In: *Immunobiology of Natural Killer Cells (Vol II),* (eds. E. Lotzova & R.B. Herberman) pp. 163-177. CRC Press, Boca Raton, Florida.

LOUGHRAN, T.P. JR. & STARKEBAUM, G. (1987) Large granular lymphocyte leukemia. *Medicine,* **66,** 397.

MANGI, R.J., NIEDERMAN, J.C., KELLEHER JR., J.E., DWYER, J.M., EVANS, A.S. & KANTOR, F.S. (1974) Depression of cell-mediated immunity during acute infectious mononucleosis. *N. Engl. J. Med.* **291,** 1149.

MANIATIS, T., FRISCH, E.F. & SAMBROOK, J. (1982) *Molecular cloning: A Lab Manual.* Cold Spring Harbour, New York.

MCKENNA, R.W., PARKIN, J., GAJL-PECZALSKA, K.J., HERSEY, J.H. & BRUNNING, R.D. (1977) Ultra-structural, cytochemical, and membrane surface marker characteristics of the atypical lymphocytes in infectious mononucleosis. *Blood,* **50,** 505.

MOSS, D.J., BISHOP, C.J., BURROWS S.R. & RYAN, J.M. (1985) T lymphocytes in infectious mononucleosis. I. T cell death *in vitro. Clin. exp. Immunol.* **60,** 61.

PATRICK, C.W., SWARTZ, S.J., HARRISON, K.A. & KELLER, R.H. (1984) Collection and preparation of hematopoietic cells for cell marker analysis. *Lab Med.* **15,** 659.

PATTENGALE, P.K., SMITH, R.W. & PERLIN, E. (1974) Atypical lymphocytes in acute infectious mononucleosis. *N. Engl. J. Med.* **291,** 1145.

PHILLIPS, J.H. & LANIER, L.L. (1985) A model for the differentiation of human natural killer cells. *J. exp. Med.* **161,** 1464.

PROSS, H.F., BAINES, M.G., RUBIN, P., SHRAGGE, P. & PATTERSON, M.S. (1981) Spontaneous human lymphocyte-mediated cytotoxicity against tumor target cells. IX. The quantitation of natural killer cell activity. *J. clin. Immunol.* **1:**51.

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.

REYNOLDS, C.W. & FOON, F.A. (1984) T-lymphoproliferative disease and related disorders in humans and experimental animals: A review of the clinical, cellular and functional characteristics. *Blood,* **64:** 1146.

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.

SEELEY, J., SVEDMYR, E., WEILAND, O., KLEIN, G., MOLLER, E., ERIKSSON, E., ANDERSSON, K. & VAN DER WAAL, L. (1981) Epstein-Barr virus selective T cells in infectious mononucleosis are not restricted to HLA-A and B antigens. *J. Immunol.* **127,** 293.

SEMENZATO, G., PIZZOLO, G., RANUCCI, A., AGOSTINI, C., CHILOSI, M., QUINTI, I., DE SANCTIS, G., VERCELLI, B. & PANDOLFI, F. (1984) Abnormal expansions of polyclonal large to small size granular lymphocytes: Reactive or neoplastic process. *Blood,* **63,** 1271.

SHELDON, P.J., HEMSTEAD, E.H., PAPAMICHAIL, M. & HOLBOROW, E.J. (1975) Thymic origin of atypical lymphoid cells in infectious mononucleosis. *Lancet,* **ii:** 55.

SOUTHERN, E.M. (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* **98,** 503.

SULLIVAN, J.L., BYRON, K.S., BREWSTER, F.E. & PURTILO, D.T. (1980) Deficient natural killer cell activity in X-linked lymphoproliferative syndrome. *Science.* **210,** 543.

SVEDMYR, E., ERNBERG, I., SEELEY, J., WEILAND, O., MASUCCI, G., TSUKUDA, K., SZIGETI, R., MASUCCI, M.G., BLOMGREN, H., BERTHOLD, W., HENLE, W. & KLEIN, G. (1984) Virologic, immunologic, and clinical observations on a patient during the incubation, acute, and convalescent phases of infectious monoucleosis. *Clin. Immunol. Immunopathol.* **30,** 437.

SVEDMYR, E. & JONDAL, M. (1975) Cytotoxic effector cells specific for B-cell lines transformed by EBV are present in patients with infectious mononucleosis. *Proc. natl. Acad. Sci. USA.* **72,** 1626.

TARKKANEN, J., SAKSELA, E., VON WILLEBRAND, E. & LEHTONEN, E. (1983) Suppressor cells of human NK activity: characterization of the cells and mechanism of action. *Cell. Immunol.* **79,** 265.

THORLEY-LAWSON, D.A. & ISRAELSOHN, E.S. (1987) Generation of specific cytotoxic T cells with a fragment of the Epstein–Barr virus-encoded p63/latent membrane protein. *Proc. natl. Acad. Sci. USA.* **84,** 5384.

TIMONEN, R., ORTALDO, J.R. & HERBERMAN, R.B. (1981) Characteristics of human large granular lymphocytes and relationship to natural killer and K cells. *J. exp. Med.* **153,** 569.

TOSATO, G. & BLAESE, R.M. (1985) Epstein-Barr virus infection and immuno-regulation in man. *Adv. Immunol.* **37,** 99.

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,** 1133.

TOYONAGA, B., YOSHIKAI, Y., VADASZ, V., CHIN, B & MAK, T.W. (1985) Organization and sequences of the diversity, joining, and constant region genes of the human T-cell receptor β chain. *Proc. natl. Acad. Sci.* **82,** 8624.

WALDMANN, T.A., DAVIS, M.M., BONGIOVANNI, K.F. & KORSMEYER, S.J. (1985) Rearrangements of genes for the antigen receptor on T cells as markers of lineage and clonality in human lymphoid neoplasms. *N. Engl. J. Med.* **313,** 776.

YOSHIKAI, Y., ANATONIOU, D., CLARK, S.P., YANAGI, Y., SANGSTER, R., VAN DEN ELSEN, P., TERHORST, C. & MAK, T.W. (1984) Sequence and expression of transcripts of the human T-cell receptor β chain genes. *Nature,* **312,** 521.

ZINKERNAGEL, R.M. & DOHERTY, P.C. (1979) MHC-restricted cytotoxic T-cells: Studies on the biological role of polymorphic major transplantation antigens determining T-cell restriction-specificity, function and responsiveness. *Adv. Immunol.* **27,** 51.