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Functional subsets of human helper-inducer cells defined by a new monoclonal antibody, UCHL1

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SUMMARY

The monoclonal antibody UCHL1 identified an antigen present on most thymocytes, a subpopulation of resting T cells within both the CD4 and CD8 subsets, and on mature activated T cells. The UCHL1 determinant is also present on cells of the myeloid lineage, but not normal B cells or NK cells. Functionally, UCHL1 identifies a subpopulation of T cells which proliferates maximally to soluble antigen and provides maximum help for PWM-stimulated immunoglobulin synthesis. In contrast, the UCHL1$^-$ cells do not induce immunoglobulin synthesis and do not proliferate in the presence of soluble antigen, although both the UCHL1$^-$ and the UCHL1$^+$ fractions of T cells proliferate well in the presence of PHA. By standard immunoprecipitation techniques and SDS page, the antigen recognized by UCHL1 was found to have a molecular weight of 180,000–185,000. Preclearing experiments using antibodies identifying the leucocyte common antigen, LCA, and the lymphocyte function-associated antigen, LFA-1, which have similar molecular weights to UCHL1, showed that the UCHL1 determinant is not biochemically related to these antigens.

INTRODUCTION

Recent studies using monoclonal antibodies have shown that there exists both functional and phenotypic heterogeneity within the two major populations of human T cells defined by CD4 and CD8. Within the helper T-cell subset, the antibodies TQ1 (Reinherz *et al.*, 1982), Leu 8 (Kansas *et al.*, 1985) and HB-11 (Tedder, Cooper & Clement, 1985) all identify populations that induce immunoglobulin synthesis by B cells, while HB-11 also identifies a population that proliferates maximally to soluble antigen. Morimoto *et al.* (1985a, b) have recently described two antibodies, anti-4B4 and anti-2H4, that identify reciprocal populations within the T4 subset, those cells expressing the 4B4 antigen being able to proliferate maximally to soluble antigen and provide help for Ig synthesis, while 2H4$^+$ cells function as inducers of suppression. Antibodies defining populations within the CD4$^+$ subset also reveal phenotypic heterogeneity of the CD8 subset. Leu 8 identifies subpopulations of CD8$^+$ cells, but both Leu 8$^+$ and Leu 8$^-$ CD8$^+$ cells are required for optimal suppression in a PWM-induced Ig response. Functional populations of suppressor and cytotoxic T cells within CD8 have been defined by the monoclonal antibodies 2D2 and Leu 15 (Clement, Dagg & Landay, 1984). This detailed dissection of the helper/inducer and cytotoxic/suppressor T-cell subsets will ultimately facilitate our understanding of the precise function of these cells and the interaction between them. We present here a characterization of another monoclonal antibody, UCHL1, which also subdivides the helper/inducer and suppressor/cytotoxic populations. UCHL1 defines a subpopulation of T cells that proliferates maximally in response to soluble antigens and provides help for pokeweed mitogen-induced B-cell differentiation. The cellular distribution and preliminary molecular characterization of the UCHL1 antigen, which is presented here, shows that UCHL1 appears to differ from all other antibodies that subdivide the CD4 and CD8 populations.

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Abbreviations: BCDF, B-cell differentiation factor; BCGF, B-cell growth factor; E, sheep erythrocyte rosettes; LCA, leucocyte common antigen; LFA-1, lymphocyte function-associated antigen; PBMC, peripheral blood mononuclear cells; PHA, phytohaemagglutinin; PWM, pokeweed mitogen; SDS, sodium dodecyl sulphate.

MATERIALS AND METHODS

Preparation of monoclonal antibody, UCHL1

BALB/c mice were inoculated intraperitoneally with 10^7 cultured T cells from an IL-2- dependent T-cell line (CA1) prepared from human peripheral blood activated with influenza virus as described previously (Fischer, Beverley & Feldmann, 1981), on Days 0, 14 and 28. Spleens were harvested 3–4 days later for hybridization to the BALB/c myeloma cell line P3/NSI/1-Ag4-1 (NSI) (Kohler & Milstein, 1976).

One of the resulting hybridomas produced an antibody, UCHL1, which bound to the immunizing cultured T cells. This was cloned twice by plating at limiting dilutions on BALB/c peritoneal feeder layers. Ascitic fluid, containing high titre monoclonal antibody, was obtained from pristane (2,6,10,14-tetra methyl pentadecane) primed mice after inoculation with 5×10^6 cloned hybridoma cells. The monoclonal antibody UCHL1 was found to be an IgG2a immunoglobulin by Ouchterlony analysis using subclass specific antisera (Miles Laboratory, Slough, Berks).

Preparation of peripheral blood leucocytes

Human peripheral blood mononuclear cells (PBMC) were prepared from heparinized blood taken from normal donors by Ficoll–Hypaque density (1·077 g/cm^3) centrifugation. E-rosettes were formed using sheep red blood cells (SRBC) treated with S-2-aminoethylisothiouronium bromide hydrobromide (AET) (Aldrich Chemical Co., Gillingham, Dorset) according to the method of Kaplan & Clark (1974). E-rosette forming (E^+) cells were separated from the non-rosetting (E^-) cells by centrifugation over Percoll (Pharmacia, Uppsala, Sweden) at a density of 1·080 g/cm^3. The E^- cells were recovered from the interface, and E^+ cells from the pellet by lysis of the SRBC with Gey's haemolytic solution (Dresser, 1978).

Monocytes were obtained from PBMC by adherence for 30 min at 37° to petri-dishes coated with microexudate from baby hamster kidney cells (fibronectin plates) (Ackerman & Douglas, 1978).

Granulocytes were prepared from the washed Ficoll–Hypaque red cell pellet by diluting with an equal volume of Plasmasteril (Fresenius A.G., Bad-Homborg, FRG) and allowing the red cells to settle for 45 min. Granulocytes in the upper layer were removed and washed twice before staining.

Thymus tissue was obtained from young children undergoing open heart surgery. Tonsils were obtained following tonsilectomy for standard medical indications, and spleen tissue from adults undergoing splenectomy for traumatic rupture. Cell suspensions were prepared from these tissues by teasing with 19 gauge needles. Bone marrow samples were obtained from the iliac crest of normal adult volunteers. Mononuclear cells from tissue preparations were isolated by centrifugation over Ficoll–Hypaque, except in the case of thymocytes which were prepared by washing the teased cell suspension.

Monoclonal antibodies

The following monoclonal antibodies were used to determine cell types present in cell suspensions: UCHT1 (Beverley & Callard, 1981) recognised the CD3 determinant on peripheral T cells and some thymocytes; UCHT4 identified an antigen on suppressor/cytotocic T cells, CD8 (Beverley, 1982); Leu 3a is specific for the CD4 determinant present on helper/inducer T cells; B1 is specific for the CD20 antigen of later B-cell precursors in the bone marrow and all mature B cells (Stashenko *et al.*, 1980); UCHM1 identifies antigens present on the majority of blood monocytes (Hogg *et al.*, 1984); HNK-1 identifies the majority of human NK cells (Abo & Balch, 1981); 2D1 identifies the common leucocyte antigen (Bradstock *et al.*, 1980); MHM 24 and MHM 23 identify the α and βchains, respectively, of the lymphocyte function-associated antigen (LFA-1) (Dongworth *et al.*, 1985).

Cell lines

The human cell lines in Table 1 were used for indirect immunofluorescence studies with UCHL1. CA1 is an IL-2-dependent T-cell line, HA1.4 and 37 are influenza-specific T-helper clones; these were maintained in IL-2-containing medium. All other cell lines were grown in RPMI-1640 with 10% fetal calf serum (FCS). HA1.4 and 37 were kindly provided by Dr J. R. Lamb, HTIG, UCH Medical School, London. Other cells were maintained in our laboratories or obtained from colleagues within ICRF.

Indirect immunofluorescence and cell sorting

Cells were incubated with saturating amounts (determined by titration) of monoclonal antibody (culture supernatant or purified Ig) for 30 min on ice. They were then washed three times in RPMI- 1640 medium containing 2% FCS and stained with fluorescein isothiocyanate (FITC)-conjugated sheep anti-mouse IgG for a further 30 min on ice. After washing three times, samples were analysed and sorted on a fluorescence-activated cell sorter (FACS I or IV) (Becton-Dickinson, Mountain View, CA)

Immunoenzymatic staining of tissue sections

Tissue specimens were snap-frozen in isopentane using liquid nitrogen as coolant and then stored at −70°. Cryostat sections were cut at 6 μm, dried for 1 hr prior to use, and then washed in phosphate-buffered saline. They were then stained by the indirect immunoperoxidase method (Mason & Sammons, 1978).

Functional assays

Antigen-specific proliferation of T lymphocytes. For the proliferation assays, E^+ cells or the E^+ UCHL1$^+$/E^+ UCHL1$^-$ fractions obtained by sorting were cultured with irradiated E^- cells in the presence of specific antigen or PHA. Antigens used were influenza virus (strain A X31 H3N2, kindly provided by Dr J. Skehel) at an optimum concentration of 1 μg/ml and tetanus toxoid (a gift from Dr M. Hughes, Wellcome Research Laboratories, Beckenham, Kent) at an optimum concentration of 0·3 μg/ml. Cultures were carried out in round-bottomed microtitre trays. Each well contained 10^5 E^+ cells plus 5×10^4 E^- cells in 200 μl of RPMI-1640 containing 2 M L-glutamine, 25 mM HEPES buffer and 10% pooled human A+ serum. The cultures were incubated in a humidified atmosphere of 5% CO_2 in air for 5 days and then pulsed with 1·0 μCi of [^{3}H]TdR (tritiated thymidine, TR120, Amersham International, Amersham, Bucks) for 16 hr. The cells were harvested onto glass fibre discs and [^{3}H]TdR incorporation determined by liquid scintillation counting. All cultures were set up in triplicate.

PWM-induced antibody production. In order to determine the effect of fractionated E^+ cells on PWM-induced antibody production by E^- cells, 2×10^5 unfractionated E^+ cells or the E^+ UCHL1$^+$/UCHL1$^-$ subsets were added to 1×10^5 E^- cells in round-bottomed microtitre trays. Cultures were carried out in 200 μl of RPMI-1640 supplemented with 25 mM HEPES, 10% FCS, 10^{-5} M hydrocortisone plus PWM (Gibco, Paisley, Refrewshire) at a 1/50 dilution. The cultures were incubated at 37° for 7 days in an atmosphere of 5% CO_2 in air. Supernatants were then harvested and assayed for the presence of IgG, IgA and IgM by solid-phase enzyme immunoassay as described by Sigfusson, Babbage & Souhami (1985).

The Ig concentration in the supernatants was calculated from the log/linear regression obtained from serial dilutions of standards of known IgG, IgM and IgA concentrations.

Iodination, immunoprecipitation and electrophoresis

Cells were labelled at the surface by lactoperoxidase catalysed iodination (Walsh & Crumpton, 1977), and lysed with Nonidet P40. Immunoprecipitates were prepared from the lysates as described previously (Owen, Kissonerghis & Lodish, 1980) by using 10^7 cell equivalents with 40 μl of five-fold concentrated hybridoma culture supernatant or 10 μl of ascitic fluid. Immunoprecipitates were analysed in the presence of sodium dodecyl sulphate (SDS) on a 5% polyacrylamide slab gel under reducing conditions (Laemmli, 1970).

RESULTS

Cell and tissue distribution of the UCHL1 antigen

After preliminary screening, supernatants from five hybrids that stained the CA1 T-cell line by indirect immunofluorescence, but not the Burkitt B-cell line RAJI, were further characterized on PBMC. Of these, one (UCHL1) showed preferential staining of T cells and monocytes and was selected for further study.

A number of cell lines were examined for expression of UCHL1 (Table 1). The antigen was present on 8/8 IL-2-dependent T-cell lines and clones (e.g. CA1. HA1.4, 37), and 2/4 (CEM and MOLT4, which have less mature markers) T acute lymphoblastic leukaemia (T-ALL) cell lines. This, together with the large number of UCHL1$^+$ thymocytes, indicates that the antigen appears early in the differentiation of T cells. UCHL1 was present on a small percentage (7%) of the B-lymphoblastoid cell line, GK, but was not present on the other three B-lymphoblastoid lines examined, nor on the Burkitt line RAJ1 or the pre-B cell line NALM-6. It was, however, strongly expressed by the two more differentiated B-myeloma cell lines studied, L6 and HFB-1. It was also present on the promyelocytic (HL60) and monocytic (U937) lines.

UCHL1 binds to all (>95%) monocytes and granulocytes and to a majority (87%) of thymocytes (Table 2). However, only 40% of E-rosette positive T cells from PBM were stained, suggesting that UCHL1 binds to only a proportion of mature T cells. This was confirmed by additive labelling experiments using Leu 3a (CD4) and UCHT4 (CD8) (Table 3). It can be calculated from the results in Table 3 that UCHL1 stained approximately 70% of CD4$^+$ cells and 35% of CD8$^+$ cells.

No evidence was obtained for B-cell staining in additive labelling experiments on tonsil mononuclear cells with anti-CD20 (B1) and UCHL1. This was also true for peripheral blood E$^-$ cells. All of the staining of the PBM E$^-$ cells with UCHL1 was accounted for by the presence of monocytes, i.e. UCHL1 and UCHM1 completely overlapped in additive experiments (results not shown). HNK-1$^+$cells in PBM were not stained with UCHL1.

Specificity of UCHL1 for T cells and cells of the myelomonocytic series was confirmed by histochemical staining of frozen sections from tonsil and spleen (Fig. 1). UCHL1 localized in T-cell areas of tonsil sections, staining both T-cell areas (periarteriolar lymphatic sheath) in the white pulp, and granulocytes and monocytes in the red pulp areas of spleen sections. Scattered labelled cells only were seen in the B-cell areas, i.e. germinal centres and lymphoid follicles of tonsils and spleen. The control pan T antibody, UCHT1, labelled T-cell areas in both tissues.

Table 1. Reactivity of UCHL1 with cultured cell lines

Cell line	Type	% fluorescent positive cells
CA1	T-cell line	>95%
HA1.4	T-cell clone	>95%
37	T-cell clone	>95%
CEM	T-ALL	52
Jurkat	T-ALL	0
MOLT-4	T-ALL	80 (63–95)
HUT 78	T-ALL	0
GK	B-lymphoblastoid	7
WMPT	B-lymphoblastoid	0
C10	B-lymphoblastoid	0
LNAT	B-lymphoblastoid	0
L6	B-myeloma	>95%
HFB-1	B-myeloma	>95%
Raji	B-Burkitt's	0
Nalm-6	Pre-B	0
HL60	Promyelocytic	54
U937	Monocytic	48
K562	Erythroleukaemic	1
KG1	Myeloid	0

Reactivity of UCHL1 was determined by indirect immunofluorescence using a FACS IV. All percentages given are corrected for background staining.

Function of T-cell subsets identified by UCHL1

E-rosette positive (T) cells were separated on the FACS into UCHL1$^+$ and UCHL1$^-$ subpopulations, and tested for proliferation in response to soluble tetanus toxoid and influenza virus antigens and also to PHA. Equivalent responses to PHA were obtained from unfractionated E$^+$ cells and both the UCHL1$^+$ and UCHL1$^-$ subpopulations (Table 4). However, in antigen-stimulated cultures, proliferative responses were only obtained from E$^+$ and E$^+$ UCHL1$^+$ cells. No response was

Table 2. Reactivity of UCHT1 with normal peripheral blood leucocyte cell fractions and cells obtained from various tissues

Cell type	No. tested	% fluorescence positive cells
PBM	10	47 (29–71)
E-rosette +ve	6	40 (27–49)
E-rosette −ve	6	72 (35–85)
Monocytes (fibronectin adherent)	2	>95
Granulocytes	4	>95
Thymocytes	3	87 (79–89)
Spleen mononuclear cells	2	11 (8, 15)
tonsil mononuclear cells	6	23 (18–27)
Bone marrow (F.H. isolated)	2	66 (66, 67)

Table 3. Additive experiments using UCHL1 plus well-defined antibodies on PBM, tonsil cells and the E+ fraction of PBM

Cell type	Antibodies used	% positive cells: Donor 1	2	3	Mean
PBM:					
	HNK1	9	7	16	11
	UCHL1	41	39	29	36
	UCHL1+HNK1	56	46	44	48
Tonsil cells:					
	B1	54	56	86	65
	UCHL1	21	27	14	21
	UCHL1+B1	85	80	94	86
PBM E+ cells:					
	UCHT1 (CD3)	91	92	78	87
	Leu 3a (CD3)	55	56	46	52
	UCHT4 (CD8)	32	39	25	32
	UCHL1	49	49	48	49
	UCHL1+Leu 3a	69	67	55	64
	UCHL1+UCHT4	76	69	63	69
	Calculated % of CD4 and CD8 cells expressing UCHL1				
	% CD4+ UCHL1+	80	53	83	72
	% CD8+ UCHL1+	43	28	36	36

The proportion of cells in the CD4 and CD8 subpopulations bearing UCHL1 was calculated by subtracting the number of cells stained by the two antibodies mixed together from the sum when used separately and expressing this as a percentage of the CD4 or CD8 populations.

obtained with E+ UCHL1− cells. The fractionated T cells were also tested for T-helper activity in pokeweed mitogen (PWM)-induced polyclonal Ig production. UCHL1+ T cells provided equivalent or better T-cell help than unfractionated E+ cells for PWM-induced IgG, IgM and IgA production (Table 5), whereas UCHL1− T cells were unable to provide significant T-cell help.

Biochemical analysis of UCHL1 antigen

Five-fold concentrated UCHL1 hybridoma culture supernatant precipitated a single somewhat diffuse band of about 185,000 molecular weight under reducing conditions from the cell lines MOLT 4 and HFB-1 (Fig. 2a and b, Lanes 1 and 3). In MOLT 4, a similar result was obtained under non-reducing conditions except that the band had a molecular weight of about 180,000. This molecular weight is similar to that of the leucocyte common antigen (LCA), suggesting that the UCHL1 monoclonal antibody may be directed against this antigen. Figure 2 (a and b, Lanes 2 and 4) shows the compositions of immunoprecipitates of LCA prepared from MOLT 4 and HFB-1 using the monoclonal antibody 2D1. The LCA from MOLT 4 co-migrated with the UCHL1 band, whereas LCA from HFB-1 comprised two bands, one of 185,000 and a second band of about 190,000 molecular weight, a difference that could be explained by variation in glycosylation, a characteristic of LCA (Thomas *et al.*, 1985). As shown in Fig. 2b, lysates of MOLT 4 and HFB-1 cells were apparently completely depleted of LCA by five successive precipitations with 2D1. A similar amount of radioactivity was, however, precipitated by the UCHL1 monoclonal antibody from these depleted lysates (Lanes 6 and 8) as from the original lysates (Lanes 1 and 3). It was concluded that UCHL1 and 2D1 recognize different molecules.

The possibility that the UCHL1 antigen was related to another high molecular weight surface antigen LFA-1 (Sanchez-Madrid *et al.*, 1983) was explored by depletion experiments using a combination of antibodies MHM24 and MHM23 specific for the α and β chains of LFA-1, respectively. As can be seen in Fig. 3, Lane 1, the mobility of the α chain of LFA-1 (177,000 MW) precipitated from HFB-1 was distinct from that of UCHL1 (Lane 4). Furthermore, the UCHL1 antigen was still precipitated (Lane 3) from lysates depleted of LFA-1 (Lane 2), and LFA-1 (Lane 6) was still present in lysates depleted with UCHL1 (Lane 5). The faint high molecular weight band in Lane 6 may be residual UCHL1 antigen carried over with UCHL1 antibody from the depletion step. Taken in concert with the difference in mobility, these results indicate that the UCHL1 antigen is not LFA-1.

DISCUSSION

UCHL1 indentifies an antigen that appears early in the differentiation of T cells. It occurs on most thymocytes and some T-ALL cell lines (in our sample of four lines, those expressing less mature T-cell antigens). Only a proportion of mature T cells (40%) express UCHL1, but it is present on most activated T cells, including IL-2-dependent T-cell lines and PHA-activated T-cell blasts. The antigen is, therefore, re-expressed upon activation of the T cells. The UCHL1 determinant is also present on cells of the myeloid lineage, but not NK cells or a readily detectable fraction of normal B cells. Interestingly, the antigen is present on the more differentiated B-myeloma cell lines, indicating that the antigen may be expressed when B cells are in a late stage of maturation, although stimulation of UCHL1− B-lymphoblastoid cell lines with TPA does not induce the expression of UCHL1 (results not shown). The lack of staining of most B cells was confirmed by the examination of frozen sections of tonsil and spleen which showed staining only of T-cell zones and of monocytes and granulocytes in the red pulp of the spleen. UCHL1 stains T cells in formalin-fixed and paraffin-embedded tissue, which makes this antibody useful in pathology. It has already been shown to stain several T-cell lymphomas (P. G. Isaacson, University College, London).

Experiments on the function of T subsets separated with UCHL1 indicate that this antibody identifies a functionally distinct population of T cells. The E+ UCHL1+ cells were found to proliferate maximally to soluble antigens, while the E+ UCHL1− fraction gave no response. Similarly, in a PWM system, help for Ig synthesis was provided by the E+ UCHL1+ cells only. Subpopulations of T cells that preferentially induce B-cell differentiation have been previously described, including 9·3+ (Lum *et al.*, 1982), CD4+ TQ1−, CD4+ Leu 8−, CD4+ HB-11− and CD4+ 4B4+. The CD4+ TQ1+ and CD4+ Leu 8+ populations do induce some B-cell differentiation, whereas the

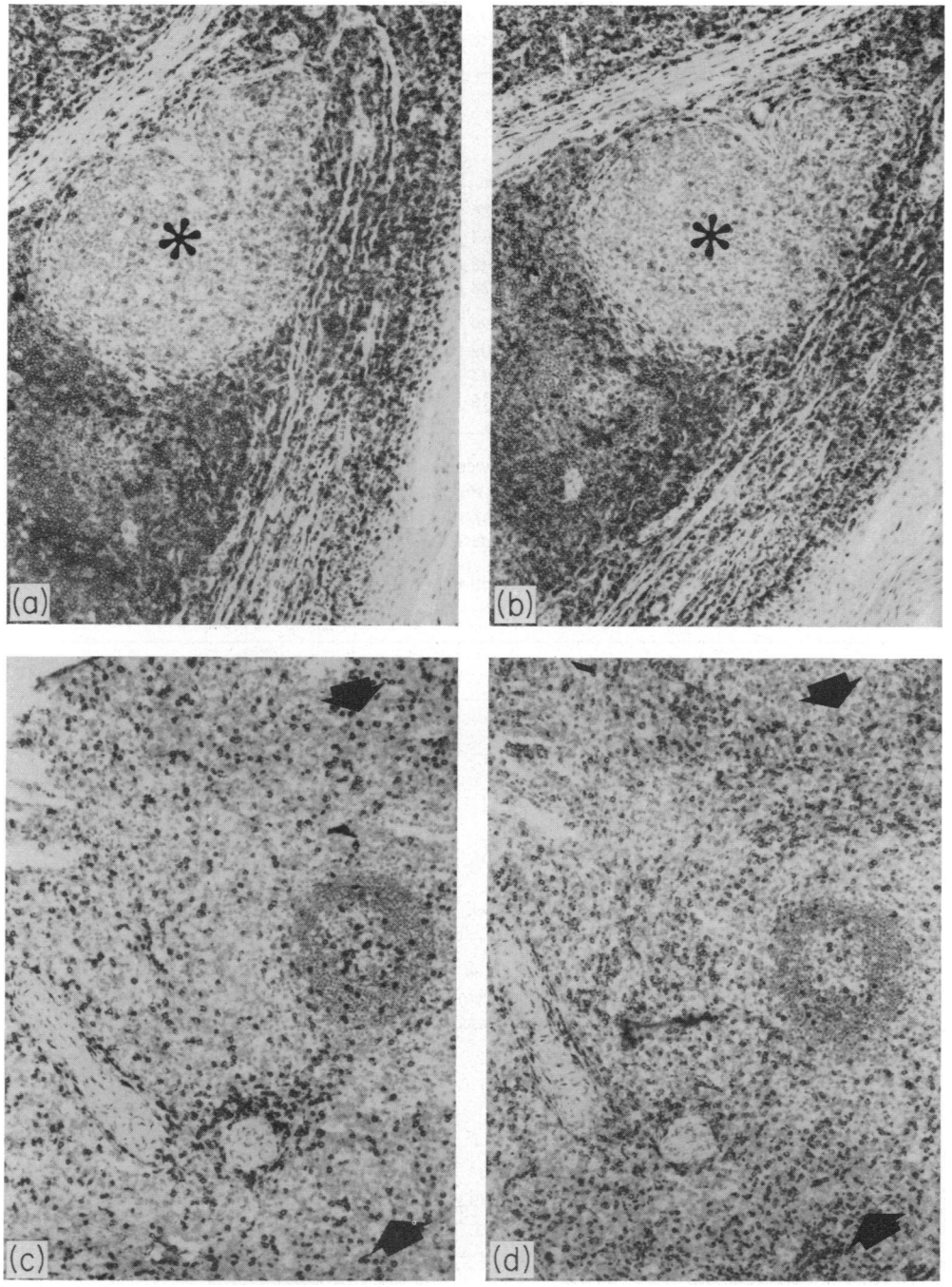

Figure 1. (a and b) Serial sections of a tonsil stained with (a) UCHT1, and (b) UCHL1. The germinal centre is marked with an asterisk. Similar staining patterns are seen with both antibodies. (c and d) Serial sections of a spleen stained with (c) UCHT1, and (d) UCHL1. Both antibodies stain cells in the peri-arteriolar lymphatic sheath, but UCHL1 stains more cells in the red pulp (arrowed). All sections were stained by indirect immunoperoxidase and counterstained with haematoxylin, magnification × 100.

$CD4^+$ $HB\text{-}11^+$ and $9{\cdot}3^-$ populations are similar to the $UCHL1^-$ population of T cells in that they do not induce any significant production of antibody by B cells. The possibility that the $UCHL1^-$ fraction contains a subset of suppressor cells has not been eliminated. The $CD4^+$ $HB\text{-}11^-$ population of T cells is also similar to the $UCHL1^+$ population of T cells, in that they respond by proliferation to soluble antigen while the $CD4^+$ $HB\text{-}11^+$ subset does not. Both the TQ1 and Leu 8 positive and negative fractions of the $CD4^+$ subset proliferate to soluble antigen. Therefore, the functional subsets identified by TQ1 and Leu 8 appear to be distinct from those identified by UCHL1. It would seem that HB-11 identifies a reciprocal population of lymphocytes to UCHL1 both functionally and from cell surface distribution. HB-11 only reacts with a small percentage (10%) of relatively mature $T3^+$ thymocytes and 60% of peripheral blood T cells. It is present on normal B cells but not mature plasma cells, and is absent from cells of the myeloid lineage. T-cell maturation or activation results in the loss of the

Table 4. Proliferative responses of unfractionated E+ cells and E+ UCHL1+ or E+ UCHL1- subpopulations to PHA or antigenic stimulation

	Proliferative stimulus	[³H]TdR uptake (c.p.m.)		
		E+ cells	E+ UCHL1+	E+ UCHL1-
Exp. 1	None	1086±316	821±395	282±164
	PHA	33,137±2010	31,147±2298	27,316±2530
	Tetanus toxoid	10,644±327	14,140±903	389±65
	X 31 (influenza virus)	4628±1781	7668±1983	574±420
Exp. 2	None	829±322	1003±1040	477±109
	PHA	32,402±1444	40,371±1477	20,568±2429
	Tetanus toxoid	13,597±927	18,013±1243	819±167
	X 31 (influenza virus)	7615±490	13,089±406	1220±225

Populations of E+ cells (1×10^5) were cultured with 5×10^4 irradiated E- cells plus antigen or PHA for 6 days. Proliferation was measured by [³H]TdR incorporation during the final 16 hr of culture. Values are expressed as mean ± standard deviation of triplicate samples.

Table 5. Helper function provided by E+ cells and fractionated E+ cells in PWM-induced antibody production by B cells

Responding cell population	IgG (ng/ml)		IgM (ng/ml)		IgA (ng/ml)	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp.2
E-+E+	1564±484	301±18	265±229	399±476	12±0	91±13
E-+E++PWM	5047±3227	1349±403	11,642±6748	27,826±20,622	47±14	2521±358
E-+E+ UCHL1+	1626±712	300±39	238±43	6585±10,240	13±01	90±358
E-+E+ UCHL1++PWM	4043±2418	1089±386	15,902±5696	>40,000	24±0	3401±176
E-+E+ UCHL1-	1782±884	330±73	142±52	638±478	12±0	90±8
E-+E+ UCHL1-+PWM	1357±384	467±36	116±41	5645±1589	12±0	17±39

E- cells (1×10^5) were cultured with unfractionated E+ cells or the E+ UCHL1+/E+ UCHL1- fractions (2×10^5) for 7 days with or without PWM. The amount of IgG, IgM and IgA in the supernatants was determined by solid-phase ELISA. Results are expressed as mean ± standard deviation of triplicate samples.

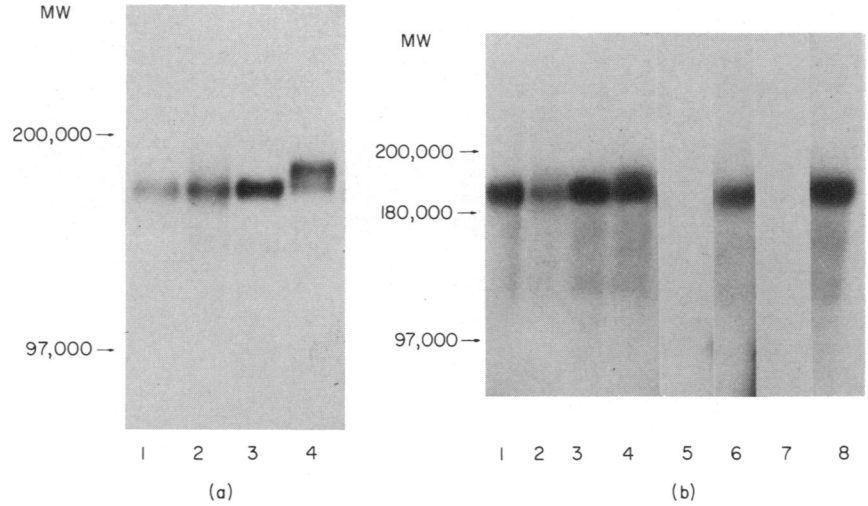

Figure 2. ^{125}I-labelled cells were solubilized using Nonidet P40, and immunoprecipitated antigens analysed on a 5% polyacrylamide SDS gel under reducing conditions. (a) Lysates from MOLT 4 cells precipitated with UCHL1 (Lane 1) and 2D1 (anti-Leucocyte common) (Lane 2) or from HFB-1 cells precipitated with UCHL1 (Lane 3) and 2D1 (Lane 4). (b) Represents a separate experiment. Lanes 1–4 are as for (a). Lysates from MOLT 4 were depleted five times with 2D1 and precipitated with 2D1 (lane 5) or UCHL1 (Lane 6). HFB-1 lysate was also depleted five times with 2D1 and precipitated with 2D1 (Lane 7) or UCHL1 (Lane 8) (Lanes 5 and 7 are from a different gel run under identical conditions with the same labelled lysate as the other lanes).

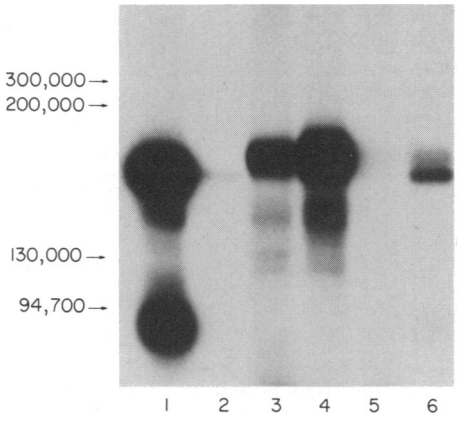

Figure 3. SDS gel of ^{125}I lysates from HFB-1 cells precipitated with anti-LFA-1 (Lane 1) or UCHL1 (Lane 4). Lanes 2 and 3 were depleted four times with anti-LFA-1 and then precipitated with anti-LFA-1 (Lane 2) or UCHL1 (Lane 3). Lanes 5 and 6 were pre-cleared four times with UCHL1 and then precipitated with UCHL1 (Lane 5) or anti-LFA-1 (Lane 6).

HB-11 antigen, whereas UCHL1 is expressed strongly at this stage. Tedder *et al.*.(1985) suggest that the less mature T cells that express HB-11 are able to produce BCGF, while on activation with antigen and loss of HB-11, they acquire the ability to produce BCDF and exhibit memory proliferative responses to soluble antigen. Since UCHL1$^+$ T cells proliferate to antigen and the determinant is present on activated T cells, it is likely that UCHL1 is also present on memory T cells which have the ability to produce BCDF. Both the Leu 8 and TQ1 antigens probably appear at a similar stage of maturation of T cells to HB-11 since they are expressed on about 10% of thymocytes and are lost as the T cells mature.

Biochemical analysis by immunoprecipitation, using either MOLT 4 or HFB-1 cells shows that UCHL1 identifies an antigen of molecular weight 180,000–185,000. Preclearing experiments indicated that UCHL1 antigen is not related to two well-characterized antigens of similar molecular weight, the leucocyte common and function-associated antigens. Among antibodies identifying subsets of CD4 and CD8 cells, only anti-2H4 appears to identify an antigen of similar molecular weight to UCHL1 (Morimoto *et al.*, 1985b), but this antigen is expressed on inducers of suppression, not helper cells. UCHL1 appears, therefore, to identify an antigen not so far detected by other monoclonal antibodies.

Monoclonal antibodies to T cells have been used to determine changes in T-cell subsets in certain disease states, for example, in chronic infections and certain immunodeficiences an increase in the ratio of CD8$^+$ to CD4$^+$ T cells is seen (reviewed by Goldstein, Lifter & Mittler, 1982). Monoclonal antibodies that separate subsets of CD4 and CD8 are beginning to be used to show disturbances in the balance of these further subsets in certain diseases (see, for example, Nichloson *et al.*, 1984). The monoclonal antibody UCHL1, with the use of two-colour fluorescence, may be significant in identifying abnormalities of functional T-cell subsets in immune disorders.

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