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Human Leucocyte Antigens

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A. Introduction

The analysis of leucocyte differentiation has been facilitated by developments in two areas of methodology: firstly, by the ability to identify and separate by surface markers cells of different lineages and stages of differentiation, and secondly, by the introduction of new functional assays, both for haemopoietic progenitor cells and mature leucocytes. In humans the production of monoclonal antibodies by somatic cell hybridisation has provided many markers for human leucocytes, whereas previously there was a paucity of reliable antisera.

We have used a number of such antisera to characterise human bone marrow progenitor cells and to trace various stages of human T lymphocyte differentiation.

B. Materials and Methods

I. Cell culture and hybridisation

Cell lines were maintained in RPMI 1640 medium supplemented with 2m*M* glutamine and pyruvate and 10% foetal calf serum (FCS). All fusions were carried out using the P3/NSI/1-Ag4-1 myeloma line. Hybridisation was carried out by a method slightly modified from that of Kohler and Milstein (1975) using 50% w/v polyethylene glycol (PEG 1500, BDH) in medium. Cells were cloned by limiting dilution on normal mouse peritoneal cell feeders.

II. Screening

Culture supernatants were screened on panels of target cells using an indirect I^{125} labeled antiglobulin binding assay (Beverley 1980).

III. Antisera

Serum 2A1 is an IgG1 monomorphic anti-HLA-A and anti-HLA-B serum. DA2 is a monomorphic anti-HLA-Dr serum of IgG-1 class (Brodsky et al. 1979). Anti-HLe-1 (2D1) is an IgG1 antibody derived from an immunisation with peripheral blood mononuclear cells and identifying a determinant present on T and B lymphocytes, monocytes and granulocytes (Beverley 1980, Bradstock et al, to be published). UCHT1 (T28) is an IgG1 antibody derived from an immunisation with thymocytes followed by Sezary cells.

The IgM antibody (TG-1) was derived from a mouse immunised with a thymus membrane glycoprotein fraction eluted from a Con A column (Sullivan and Beverrley, unpublished work). The antiserum stains peripheral blood neutrophils and eosinophils, but not T and B lymphocytes or thymocytes. It stains mature cells of the granulocyte lineage in the bone marrow.

Details of other monoclonal and conventional sera used are given elsewhere (Bradstock et al. to be published). Staining for TdT was kindly performed by Mr W. Verbi of the Membrane Immunology Laboratory, I.C.R.F. London.

IV. Indirect Immunofluorescence and Cell Sorting

Cells were incubated for 30 min on ice using saturating amounts (usually undilute culture supernatant) of monoclonal antiserum, washed twice in Hepes buffered RPMI 1640 with 5% FCS and stained with immunoabsorbent purified and human immunoglobulin absorbed, FITC sheep anti-mouse Ig. In some experiments an $F(ab)_2$ fragment of the anti-Ig was used. Controls included cells stained with second layer only or with culture supernatants from hybridomas of irrelevant specificity but of the IgG1 or IgM class. In some experiments normal sheep serum (10%) was included in the second layer to compete for Fc receptors. Cells were analysed and sorted on a Becton Dickinson FACS-I.

V. Functional Assays

Assays for PHA and Con A responsiveness were carried out in 96 well microtitre trays using 2×10^5 cells per well. Cultures were pulsed for 4 h on day 3 with I^{125} iododeoxyuridine and harvested with a MASH.

MLCs were carried out in a similar fashion using 2×10^5 2000R irradiated E rosette negative cells as stimulators. They were pulsed and harvested on day 5.

In vitro antibody responses to influenza virus were carried out as described previously (Callard 1979).

Assays for myeloid clusters and colonies and erythroid colonies and bursts were carried out as described by others (Pike and Robinson 1970; Burgess et al. 1977; Iscove 1978).

C. Results and Discussion

Because many leukaemias probably originate in the pool of bone marrow progenitor cells, even when the predominant cell type is of a more mature phenotype (Greaves and Janossy 1978), the regulation of this cell pool is clearly of interest. We have, therefore, attempted to develop a method for the isolation of these cells from bone marrow. Ficoll-hypaque separated bone marrow cells are first treated with anti-myeloid antibody (TG-1) and complement, which lyses approximately 50% of the cells, mainly the myelocytes and metamyelocytes.

The remaining cells are stained with anti-HLe-1 for cell sorting. A weakly staining fraction of cells containing less than 2% of the bone marrow nucleated cells is collected. The properties of this population are summarised in Table 1. In addition to containing the myeloid and erythroid progenitor activity, this population contains significant numbers of cells with nuclear TdT (Beverley et al., unpublished work). It is likely that these are lymphoid progenitors (Greaves and Janossy 1978). Thus all these cells share a common phenotype, HLe-1±, TG-1−. Other data suggests that erythroid and myeloid progenitors are also HLA-A, -B, -C+ and HLA-DR+, although pluripotential stem cells may lack HLA-Dr (Moore et al. 1980). The ability to isolate the cells will enable us to study their heterogeneity and regulation in isolation from mature progeny.

Table 1. Properties of cell sorted progenitor fraction

Stains weakly for HLE-1
Contains approximately 2% of marrow nucleated cells
40%–50% blast cells
3%–15% TdT+ cells
20–50× enrichment for myeloid clones[a]
20–50× enrichment for CFU-GM
20–100× enrichment for CFU-E
20–100× enrichment for BFU-E

[a] Compared to Ficoll-Hypaque isolated marrow cells

Recent studies by us and others (Reinherz and Schlossman 1980) show that there are several phenotypically distinct populations within the thymus. Table 2 shows a speculative scheme for T cell differentiation based on double label fluorescence experiments, fluorescence activated cell sorting data and studies of tissue sections by indirect fluorescence.

We identify as the earliest step of intrathymic differentiation a small population (5%) of cells which are larger in size and lack the cortical thymocyte marker HTA-1 (McMichael et al. 1979) and the strong staining with anti-HLe-1 which is characteristic of the major cortical population. Provisionally these cells may also be equated with the earliest stage of differentiation identified by Reinherz and Schlossman (1980). In their scheme a sub-population lacking characteristic thymic antigens but carrying the OKT10 marker is detectable, though it is not yet known whether this cell is HLA− and TdT+ as is the early HTA-1−, HLe-1± cell (Bradstock et al., to be published). The exact location of these early cells within the thymus is not established, but it is suggestive that the cells found nearest to the cortical interlobular connective tissue strain most weakly with another antiserum (F10-89-4) which appears to recognise an antigen which is similar or perhaps identical to HLe-1 (Fabre, personal communication). It is provocative that our data shows that the earliest cortical cells have already lost HLA-A, -B, and -C antigens, whereas the presumed bone marrow progenitor carries these antigens. Whether this loss is the signal for emigration from the marrow or occurs after entry to the thymus is not known.

The major cortical population exhibits further heterogeneity, for example in the expression of TdT enzyme and the OKT9 marker which appears on a small sub-population of

Table 2. T cell differentiation

Antigens	Bone marrow progenitors	Early cortical cells	Late cortical cells	Medulla	Peripheral T cells
HLA	———			———	———
Ia	– – ———				— — —
HLe-1	———	———	———	———	———
TdT	———	———	———		
OKT10	———	———	———	———	
OKT9		– – ———	———		
HTA-1			———		
OKT6			———		
OKT4[a]			———	———	———
OKT5,8[a]			———	———	———
OKT1,3				———	———
UCHT1			.....	———	———

[a] In the cortex OKT 4,5 and 8 are present on all cells, while in the medulla and in the periphery T cells have either OKT 4 or OKT 5 and 8

what are probably early cells. A more dramatic difference in phenotype however is that between the cortical and medullary cells. In many respects the medullary cells approximate in phenotype peripheral T cells, having lost HTA-1 and TdT and regained HLA. With additional markers it is however possible both to distinguish medullary cells from peripheral T cells and furthermore to identify two separate lines of medullary cells which correspond to those identified in the peripheral T cell pool (Reinherz & Schlossman 1980). This is reminiscent of murine data suggesting an early commitment to different lines of T cell development. The heterogeneity of thymic phenotypes is reflected in the heterogeneity of T lineage leukaemia phenotypes which has been described (Reinherz et al. 1979). This heterogeneity also suggests that caution should be used in identifying a phenotype as not seen in normal differentiation, since small populations with rare phenotypes may have been overlooked.

I. Peripheral T Lymphocyte Differentiation

In the peripheral lymphoid system we have studied the properties of cells which carry the antigen defined by the UCHT1 monoclonal antiserum. Table 3 shows the tissue distribution of UCHT1 + cells which suggests that the antigen may be a mature T cell antigen. Additional evidence is provided by experiments in which peripheral blood mononuclear cells (PBM) were separated by sheep red blood cell (E) rosetting into E+ and E-ve fractions before staining with UCHT1 antiserum. Table 4 shows such an experiment. While E+ cells always show high percentages of UCHT1 staining, the E− cells show few UCHT1 positive cells. More intriguingly, when PBM are spearated by cell sorting into UCHT1+ and UCHT1− fractions, the unstained fraction always contains significant numbers (12%–30%) of E rosette forming cells (Table 4).

Table 3. Tissue distribution of UCHT1 positive cells

	UCHT1 (%)	E rosettes (%)
PBM (10)[a]	69	74
Thymus (5)	41	N.D.[b]
Tonsil (4)	31	24
Spleen (2)	36	25

[a] number of samples
[b] conventionally 100%

Table 4. Fractionation of peripheral blood mononuclear

	UCHT1 (%)	E rosettes (%)
Fraction		
E+	91	96
E−	5	7
UCHT1+	98	91
UCHT1−	<0.5	25

Functional studies of UCHT1 separated cells (Beverley & Callard in preparation) show that while the antigen positive cells provide help for an antibody response to influenza virus (Callard 1979) and respond in MLC and to PHA and Con A, the UCHT1 − cells, which include up to a third of E+ cells, fail to respond to mitogens. Thus the E+ UCHT1− cells show as yet little evidence of mature T cell function. They may perhaps correspond to the subset of cells identified as E+, Fcγ+ and monocyte antigen positive (Reinherz et al. 1980), though we have no direct evidence for this.

On the other hand, recent data from studies of neutropenic patients suggests that a true Tγ subset does exist (Bom-van Noorloos et al. 1980). We have studied two similar patients and data from one of these are presented in Table 5. Significant numbers of the patients' PBM have the phenotype E+, UCHT1+, Fcγ+ HLA-Dr+. The presence of HLA-Dr is intriguing, since this has been shown to be expressed on a variety of activated T lymphocytes and in addition, evidence has been presented for the presence of a small subset of Fcγ and Dr+ T cells in normal individuals (Kaszubowski et al. 1980). We would thus suggest in agreement with Cooper (1980) that the E+ Fcγ+ subset includes both T and non-T cells. In the neutropenic patient described there appears to be an expansion of the Fcγ+ T cell subset. Whether the appearance of Fcγ receptors is a consequence of activation, as is that of HLA-Dr, is not yet clear.

At present our data suggest that UCHT1 is a marker for mature T cells, but it should be noted that the functional data is not yet exhaustive. In addition the studies presented here indicate that careful comparisons with existing markers (E and Fcγ receptors) of new monoclonal reagents may not only lead to new definitions for cells but allow clear identification of previously ambiguous cell types.

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Table 5. Phenotype of lymphocytes from a neutropenic patient

	Patient G.G.		Control E.Z. Whole PBM
	Whole PBM	E+ cells	
%E rosettes	80	N.D.	79
%UCHT1	80	91	68
%HLA-Dr	N.D.	62	15
%Fc γ	N.D.	70	N.D.

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