Monoclonal Antibodies to Human Cell Surface Antigens

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Köhler and Milstein's method for producing monospecific antibodies by fusing antibody-producing and myeloma cells to derive hybrid cell lines secreting monoclonal antibody considerably facilitates the analysis of the cell surface [1]. In particular, it is possible to produce monoclonal antibodies to a large number of human cell surface antigens. These antibodies will be suitable for immunocytochemical labelling studies, and will also allow the purification of substantial quantities of the major molecules of the cell surface with a view to their characterisation. Furthermore, using interspecific somatic cell hybrids, the genes coding for surface molecules can be assigned to particular chromosomes and conversely a panel of assigned surface antigens would be extremely useful as markers in somatic cell genetics.

We have applied the above approach to an analysis of the cell surface of normal and leukaemic leukocytes using cell membrane for immunization. A

Table 1. Specificity of monoclonal antibodies to human cell surface antigens

Cell fusion experiment reference no.	Immunization		Number of hybrid specificities defined			
	Tissue	Preparation	Species[a] common	Leukocyte associated	Erythrocyte specific	Differentiation antigens
W6[b]	Tonsil lymphocytes	Sucrose gradient purified membrane	4[c]	—	2	—
Gen Ox 4	AML cells	Sucrose gradient purified membrane	8	3	—	—
Gen Ox 5	ALL cells	Tween 40 membrane	2	1	—	—
Gen Ox 7	CLL cells	Tween 40 membrane	5	3	—	B cell associated
Totals:	*Antibody of given specificity*		19	7	2	1
	No of hybrids tested		29	29	29	29

a Some antigens defined were not present on erythrocytes
b Reference [2]
c W6-32 is against an antigenic determinant shared between products of the HLA A, B and C loci

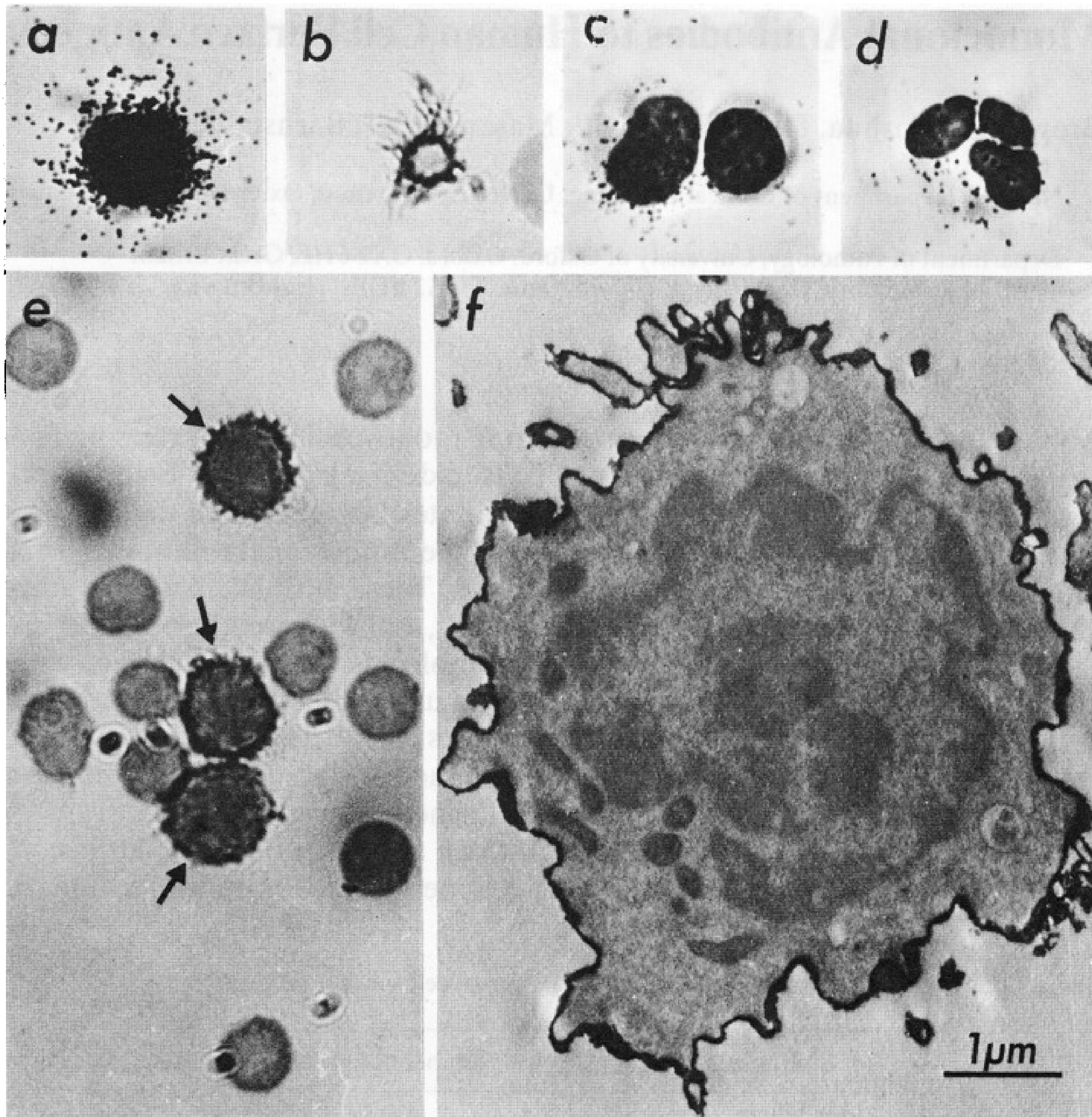

Fig. 1. Reactivity of the monoclonal antibody to HLA with lymphoid and bone marrow cells.
a) Bone marrow lymphocyte
b) Platelet
c) myelocyte and normoblast
d) polymorphonuclear leukocyte
e) thymocytes, positive cells are indicated by the arrows
f) tonsil lymphocytes
a), c) and d) are autoradiographs and the binding is demonstrated in b), e) and f) using an immunoperoxidase procedure. f) shows the surface membrane distribution of HLA, the cells were fixed in paraformaldehyde and glutaraldehyde before incubating with the monoclonal antibody

number of hybrids secreting antibody have been identified and their individual specificities characterized. The results are shown in Table 1. Many of the antibodies recognize antigens of wide tissue distribution, but a number of leukocyte associated, erythrocyte specific and putative lymphocyte specific antigens have been identified. Further clones are at present being investigated.

The monoclonal antibody W6-32 is against an antigenic determinant shared between products of the HLA, A, B and C loci [2]. By immunofluorescent,

immunoenzymatic and autoradiographic techniques, the cellular distribution of HLA A, B and C antigens, both on cell suspension and tissue sections, has been investigated. The differential expression of these antigens on developing haemopoietic and lymphoid cells has been semi-quantitatively analysed by grain counting of autoradiographs [3]. The results are shown in Fig. 1 and Table 2. Lymphocytes and platelets were very densely labelled and myeloid

Table 2. The distribution of HLA on human lymphoid. bone marrow and peripheral blood cells

Tissue	Reactivity
Peripheral blood	
Polymorphs	+ +
Lymphocytes	+ + + +
Erythrocytes	–
Platelets	+ + + +
Bone marrow	
Lymphocytes	+ + + +
Myeloid precursors	+ + +
Erythroid precursors	±
Thymus	
Lymphoid cells	85%–/15%+ + +
Tonsil lymphoid cells	+ + + +

precursors showed more labelling than mature neutrophils. Erythroid precursors, although very weakly labelled, were clearly positive, in comparison with bone marrow erythrocytes which were negative except for a small percentage (11%) which were presumed to be reticulocytes. In the thymus HLA negative, thymocyte-antigen positive cells [4] can be distinguished from HLA positive, thymocyte-antigen negative cells. By using immunofluorescent techniques on tissue sections, Mason, Christonsson and Biberfeld have shown the former to be cortical thymocytes and the latter medullary cells. The immunoperoxidase technique has the advantage that antigens can be demonstrated at the electron microscope level. Fig. 1f shows a continuous surface membrane distribution of HLA on tonsil cells.

These antibodies will also allow the purification of substantial quantities of the major molecules of the cell surface. W6-32 antibody precipitates radioactive molecules of apparent m.wt 43000 daltons and 12000 daltons from lysates of BR18 cells which have been lactoperoxidase labelled with I^{125} [2]. Furthermore, an antibody affinity column of W6-32 bound equally the HLA A, B and C antigens (Parham, Barnstable and Bodmer: In preparation) and can be used to isolate substantial quantities of the antigen.

By using monoclonal antibodies against cell surface antigens, genes or gene clusters coding for surface molecules can be identified. Rodent-human somatic cell hybrids tend to undergo a relatively rapid loss of human chromosomes and then become karyotypically stable. Human genes can, therefore, be assigned to specific chromosomes by correlating the presence or absence

Table 3. Genetic analysis of cell surface markers

Antibody	Chromosome
W6-32	6
W6-34, 45, 46 Gen Ox 4-17, 21	11
NA/1-33	13
Gen Ox 4-1	14
Gen Ox 4-7, 24	22

Monoclonal antibodies have also been produced against β_2-microglobulin, a marker for chromosome 15 (Brodsky, Parham, Barnstable and Bodmer. In preparation)

of a particular gene product with the presence or absence of a particular cytologically identified chromosome. The results presented in Table 3 represent a summary of the characterisation of a number of monoclonal antibodies on a panel of somatic cell hybrids.

Ten antigens have been assigned, to date, to particular chromosomes. Four different specificities are coded for by chromosome 11 and markers are also available for chromosomes 6, 13, 14 and 22. The gene coding for the antigen SA-1, described by Buck and Bodmer, is on chromosome 11 and is probably a glycosyl transferase. Of obvious interest is whether a cluster of genes involved in cell surface carbohydrate structure is located on chromosome 11.

Monoclonal antibodies against assigned surface markers could also be used to select hybrid derivatives with or without particular chromosomes, for example by the use of the fluorescence activated cell sorter. The efficiency of these selections and the reliance that can be placed on their results depend upon the quality of the reagents detecting the cell surface antigens. Monoclonal antibodies are ideal in this respect.

These results clearly demonstrate that it is possible to produce monoclonal antibodies to a large number of human cell surface antigens suitable for immunocytochemical and genetic studies. However, many of the antibodies recognize antigens of wide tissue distribution if cell membrane is used for immunization. Therefore, alternative sources of antigen, for example purified glycoproteins, may provide monoclonal antibodies against differentiation antigens of greater interest.

References

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