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Results of the central data analysis

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Summary This chapter presents the results of blind serological studies carried out by workshop participants on 87 monoclonal antibodies (mAbs) supplied to them as a coded panel. Twenty six mAbs had been studied in the first workshop. Participants were asked to carry out immunohistochemical, immunocytological or flow cytometric analysis on a mandatory panel of target tissues or cells. Central computer analysis and other supporting data allowed the assignment of 33 mAbs to seven clusters. Two of the antigens identified have been cloned while two more have been defined as carbohydrate epitopes. The results allow comparison of new mAbs against lung cancer with existing ones and are beginning to provide a description of the antigenic structure of the SCLC cell surface.

The first and the present workshops on small cell lung cancer antigens (Souhami *et al.*, 1988) have been modelled on the four international workshops on human leucocyte antigens (Knapp *et al.*, 1989). The principal objective of both SCLC and leucocyte workshops has been to provide a means of describing and defining the molecules of a particular cell type which can be identified by monoclonal antibodies. The methodology of both workshop series has been to provide participants with a coded panel of mAbs with which they carry out serological studies on appropriate target cells or tissues. The results of these assays are analysed centrally and the mAbs grouped into 'clusters' for the SCLC workshops (Gilks *et al.*, 1988) or 'clusters of differentiation (CD)' for the leucocyte workshops (Knapp *et al.*, 1989). A variety of other techniques may be used to test and confirm the validity of the serologically defined clusters. Among the more important are immunoprecipitation of the target antigens, cross-blocking of clustered mAbs and recently the use of cloned and expressed gene products. The latter is exemplified by the demonstration that SCLC cluster 1 mAbs identify the human neural cell adhesion molecule (NCAM) transfected into murine fibroblasts (Patel *et al.*, 1989).

While cluster analysis supported by protein chemistry and gene cloning has proved extremely effective in identifying and characterising many leucocyte and some SCLC antigens, there are problems and limitations. Two of these will be mentioned here. The first is that of multi-chain antigens. If for example, several β chains can associate with one α chain of a heterodimer and the β chains are expressed in a tissue specific fashion, confusing results would be obtained by immunoprecipitating with anti-α chain mAb from different cell types. Such complexes also pose problems of terminology (see for example LFA and VLA antigens, Knapp *et al.*, 1989).

A second, and for the present workshop, more pertinent question, is how to deal with carbohydrate antigens. These may be expressed as haptens or epitopes on different protein or lipid backbones. Once again conventional biochemical analysis may be unhelpful as is the transfection approach. However it is possible, using oligosaccharides, to define the specificity of such mAbs in chemical terms; but differences in affinity or fine specificity may still lead to differences in the tissue distribution of ostensibly similar antibodies. Antibodies to carbohydrates have presented a particular difficulty in this workshop. In clustering them we have relied on both biochemical evidence of their oligosaccharide specificity and the tissue distribution of the epitope. These are not always in agreement.

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Date collection and analysis

Eighty-seven mAbs were distributed to participants of which 26 had been studied in the first workshop. In order to make it easier to carry out a complete experiment with adequate controls at one time, the mAbs were divided into two panels of mAbs number 001–050 and 051–098. Each panel contained a known negative control (PBS + 5% FCS) numbers 001 and 051, concealed negatives 032 and 072, as well as concealed duplicates of a cytokeratin mAb (CAM 5.2) numbers 003, 028, 056, 071 and a Cl 1 mAb (NCC-LU-246) numbers 031, 041, 058 and 082. A single sample of MOC-31, 011 and 053, was included in each panel. Prior to distribution mAbs were titrated by indirect immunoperoxidase staining of tissue sections to establish a working dilution. They were then diluted to working dilution in PBS + 5% FCS containing 0.01% sodium azide and shipped at room temperature. Participants were requested to carry out serological analyses on a mandatory list of cells or tissues as well as additional targets of their own choosing (Table I). Methods for data collection and analysis are described in an accompanying paper (Gilks *et al.*, this volume).

As in the first workshop we have retained the 'Cluster w' designation to indicate less firm groupings of antibodies, indicating either that there were few antibodies of a given specificity, or that they were all from one laboratory, or that there was some other reason for uncertainty.

Results and discussion

Figure 1 shows the antibody panel and the results of the cluster analysis. While there are no absolute rules for deciding what is a significant association in clustering, the hidden duplicates and the inclusion of antibodies previously shown to have similar specificity (such as the NCAM mAbs of the first workshop), allow a reasonable cut off to be assigned. The vertical dotted line in Figure 1 indicates such a cut off. It should be noted that using this (admittedly arbitrary) cut off the majority of mAbs do not cluster. Nevertheless several strongly associated clusters of mAbs were observed and will be discussed in more detail.

Table I Mandatory targets

Immunohistology
One SCLC, one other tumour, one normal tissue
Immunocytochemistry or FACS analysis
One SCLC (cell line or fresh cell suspension, one other tumour (line or fresh cells), one normal cell type (e.g. peripheral blood mononuclear cells)

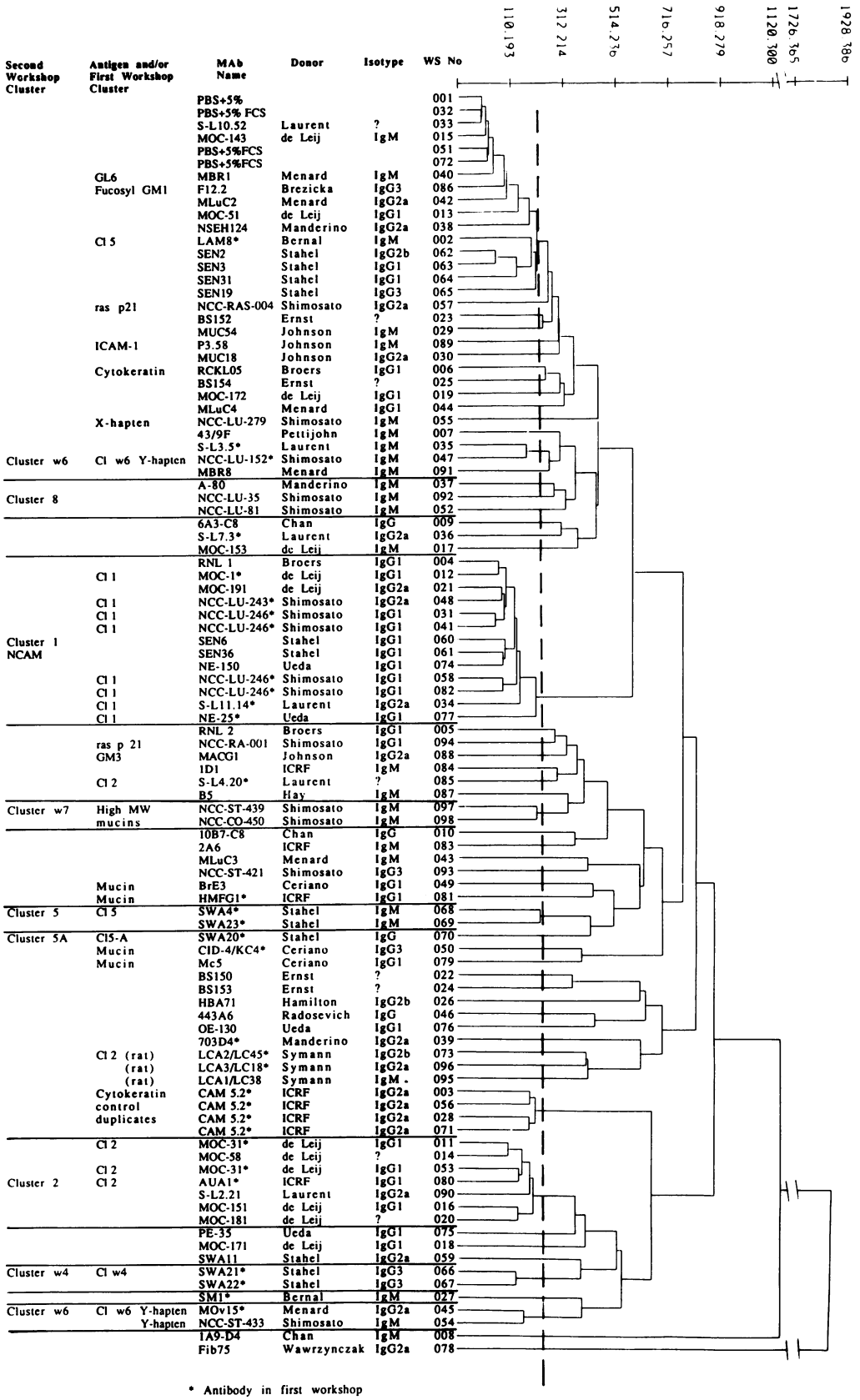

Figure 1 The completed dendrogram (see Chapter 1 for method of construction).

Cluster 1

Thirteen mAbs from the panel grouped together in this cluster, representing ten distinct mAbs, as NCC-LU-246 was included in quadruplicate. Five of these were placed in cluster 1 at the first workshop (Beverley *et al.*, 1988) and were subsequently shown to react with an NCAM transfectant (Patel *et al.*, 1989). All ten were similarly shown to bind to an NCAM transfectant (Table II) using a radiolabel binding assay (Kardamakis *et al.*, 1988). The tissue distribution studies of the present workshop (Figure 2) confirm data of the first workshop in showing that NCAM is principally expressed on neural and muscle cells. The function of human NCAM as an adhesion molecule in muscle development has recently been explored in an *in vitro* model (Dickson *et al.*, 1990). Its role in the biology of SCLC remains to be clarified.

Table II Binding of cluster 1 mAbs to an NCAM transfectant

Antibody	I^{125} *cpm bound* *L cells*	*NCAM transfectant*
Medium	103	148
UCHT1 (CD3 control mAb)	160	248
RNL1	183	971
MOC-1*	72	1,322
MOC-191	115	2,107
NCC-LU-243*	110	1,208
NCC-LU-246*	113	1,207
S-L11.14*	109	709
NE-25*	180	1,132

The table shows mean counts per minute of triplicate samples of 2×10^5 cells exposed to mAbs, followed by I^{125} labelled goat anti-mouse immunoglobulin antiserum. *mAbs included in first workshop.

Cluster 2

Seven workshop samples (6 mAbs) formed a closely grouped cluster. Two of these had clustered in the first workshop. Figure 3 shows the tissue distribution of the antigen. Cluster 2 mAbs react with most epithelia and carcinomas. SCLC is positive as is carcinoid while melanoma is weak and neuroblastoma negative. Recent data indicates that AUA1 (080) reacts with the molecule recently cloned by Strnad (Strnad *et al.*, 1989). This is a 40 kDa transmembrane glycoprotein without obvious sequence homology to molecules of known function.

The members of this cluster exhibited a striking ability to kill target cells in an indirect immunotoxin screening assay (see Derbyshire and Wawrzynczak this volume). Although it did not cluster with the other mAbs in this workshop, perhaps because it is of rat origin, the mAb LCA2/LC45 is a probable member of cluster 2. Other possibly related mAbs are PE-35, which immunoprecipitates an antigen of similar molecular weight and is potent in the immunotoxin screen, and S-L4.20, which clustered in the first workshop but this time appeared to be inactive on many targets perhaps due to excessive dilution or instability in transit. The availability of the cloned gene product should make further functional analysis of this widely distributed gene product possible.

Cluster 3

Two antibodies with largely nuclear reactivity were grouped together in the first workshop. Neither were resubmitted so that this cluster will not be discussed further here.

Cluster w4

As in the first workshop two antibodies from one lab were closely grouped together. These mAbs show reactivity with SCLC, neuroblastoma, carcinoid, adeno and squamous car-

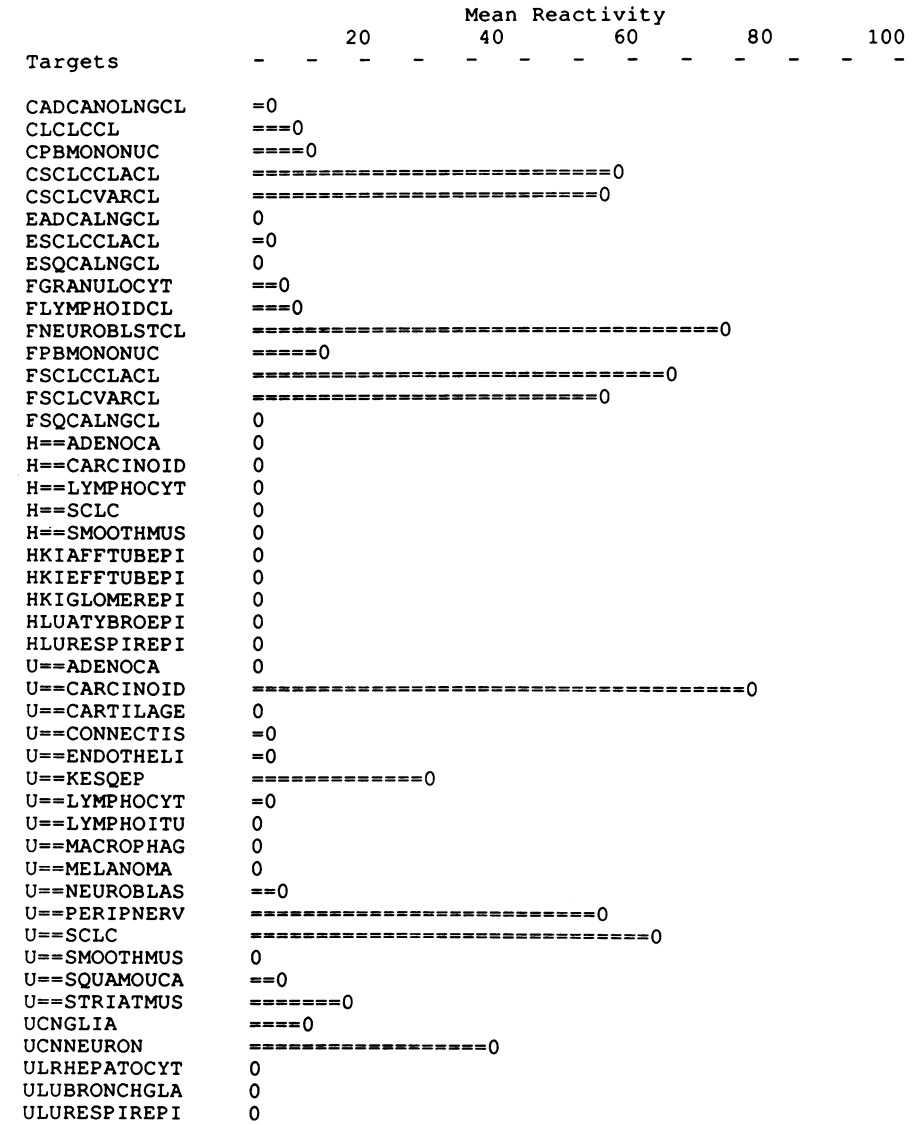

Figure 2 Abbreviated profile plot of reactivity of cluster 1 mAbs. All profile plots in this chapter show data only for targets on which three or more experiments were performed, unless the target was of particular interest (e.g. carcinoid). For full description of targets see Gilks *et al.* Chapter 1.

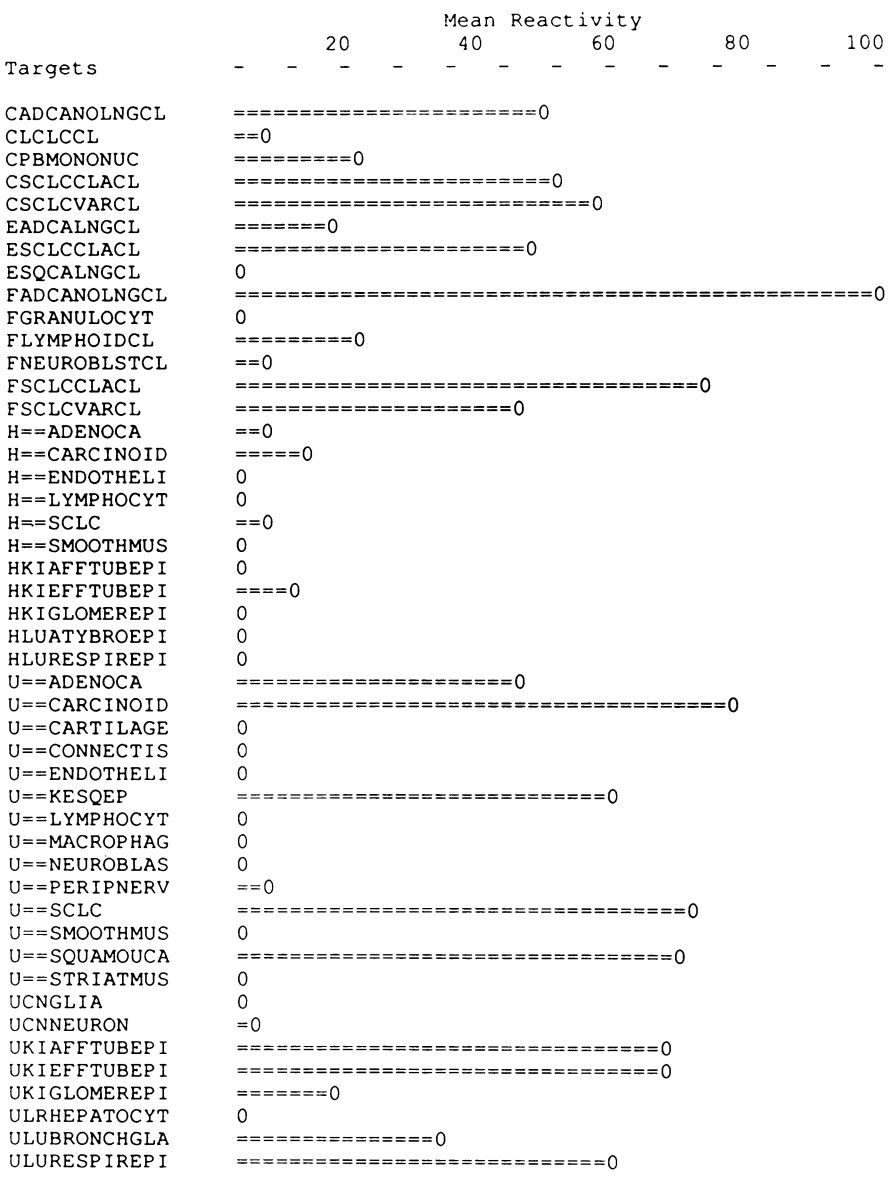

Figure 3 Profile analysis for cluster 2.

cinoma, renal tubules, granulocytes and peripheral blood mononuclear cells. They do not stain nerve, endothelium and connective tissue (Figure 4). The antigen reacts unpredictably after fixation. It is heavily glycosylated protein of molecular weight 45 kDa, which is reduced to 17 kDa by deglycosylation (Waibel *et al.*, this volume). The mAb SWA 11 clustered close to Cl w4 in the workshop analysis (Figure 1) and has been shown to cross compete with Cl w4 mAbs (Smith *et al.*, 1989). It is therefore a possible member of the cluster.

Cluster 5

Two mAbs from the first workshop clustered again. These mAbs react with SLCL, carcinoid, nerve, renal tubules and adenocarcinomas but more weakly with squamous carcinomas (Figure 5). The mAbs detect antigens of 90–135 and 200 kDa which are neuraminidase and periodate sensitive. It is likely that these are sialogycoproteins (Maier *et al.*, 1989). In this workshop LAM 8 failed to cluster in Cl 5, but had done so in the first workshop. It is a probable member of Cl 5, but had done so in the first workshop. It is a probable member of Cl 5 and may have lost activity in this workshop.

Cluster 5A

MAb SWA20 clustered adjacent to Cl 5 in this workshop as it had in the first workshop. It has very similar tissue distribution although identifying antigens of 40, 100 and 180 kDa which are also sialogylcoproteins. The mAbs SEN3 and SEN31 (063, 064) share the idiotype of SWA20 (Barth *et al.*, 1989) but failed to cluster with Cl 5 or 5A. They may have lost activity as they clustered close to the negative controls.

Cluster w6

Two mAbs clustered together as Cl w6. One of these (MOV15) was designated as Cl w6 in the first workshop. Analysis of the oligosaccharide specificity of these mAbs showed them to be directed to the Le^y blood group antigen (Andrews *et al.*, this volume). An additional mAb (NCC-LU-152, 047) which had clustered with MOv15 in the first workshop but not in this one, also showed Le^y binding and is therefore a probable member of the cluster. Figure 6 shows the predominantly epithelial reactivity of cluster w6 mAbs.

The differences in fine specificity detected in immunohistology of mAbs showing similar oligosaccharide binding emphasise the difficulties of clustering mAbs to carbohydrates. Similar variation in tissue distribution has been noted with mAbs to the x-hapten in leucocyte antigen workshops (see Knapp *et al.*, 1989).

Cluster w7

Two mAbs from the same source clustered closely together and showed a predominantly epithelial distribution (Figure

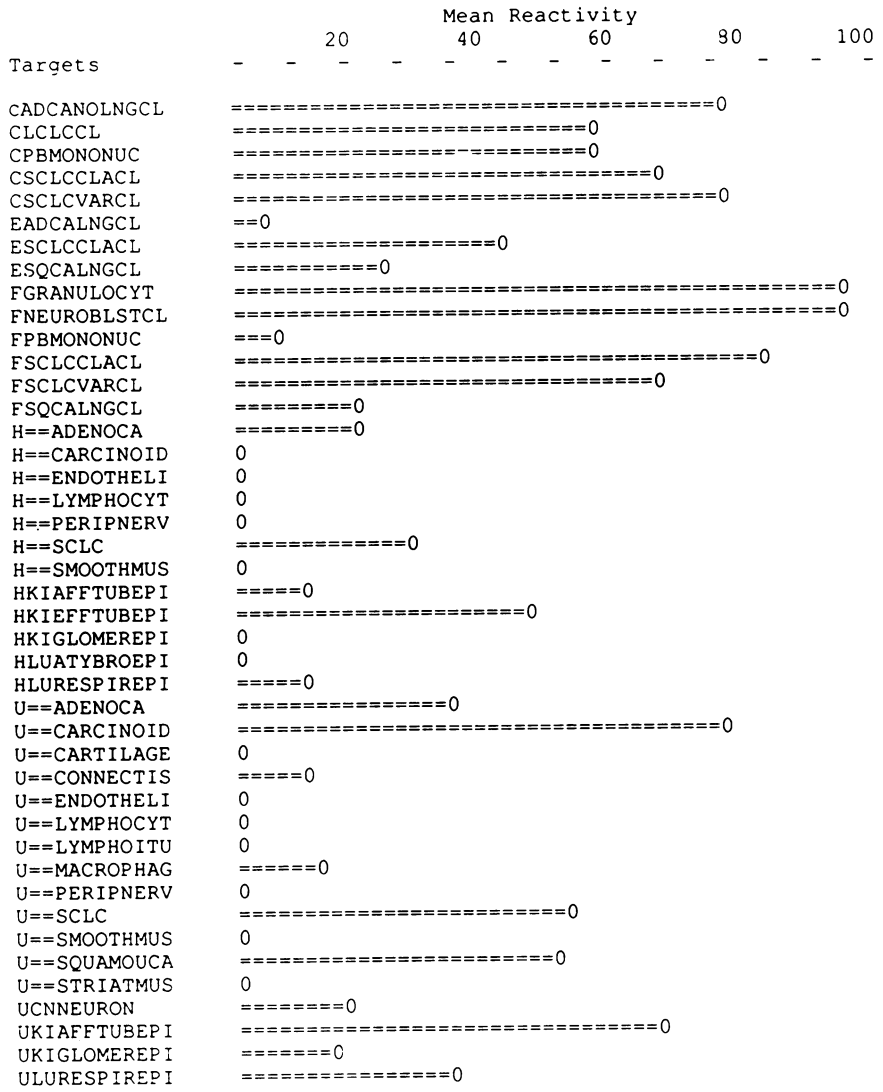

Figure 4 Profile analysis for cluster w4.

7). These two mAbs react with high mw mucins but it should be noted that they do not cluster with several other mAbs against mucins (Figure 1). This is in line with evidence that there is a family of mucin genes with tissue specific expression of different members and that different mAbs against the same molecule may show distinct binding patterns because the glycosylation of mucin molecules is cell type specific (Burchell *et al.*, 1989).

Cluster w8

Three mAbs were grouped, although somewhat weakly, into this cluster (Figure 1). Their tissue distribution is shown in Figure 8 and again shows a predominantly epithelial pattern. Figure 9 compares a w8 mAb, NCC-LU-35 and w6 and shows the differing reactivity of the two clusters. Cluster w8 mAbs all showed binding to the blood group A trisaccharide in oligosaccharide binding assays (Andrews *et al.*, this volume), and NCC-LU-35 was also shown to bind to blood group A_1 erythrocytes (Ernst & Sonneborn, this volume). The relatively poor clustering of the three mAbs may be because of differences in fine specificity or affinity but the identical oligosaccharide binding supports the cluster analysis.

Conclusions

Of the 87 mAbs, 28 were placed firmly in clusters and another six were probably or possibly part of the clusters. The workshop analysis revealed the predominance of two specificities Cl 1 and Cl 2.

It is not clear whether these are immunodominant entities or whether their strong reactivity with many cells or tissues leads antibody producers to pick out hybrids producing them. In any case several new mAbs were added to each cluster. A considerable amount of information on Cl 1 (Patel *et al.*, 1989) and Cl 2 (Strnad *et al.*, 1989) has accumulated since the first workshop though their functions in lung cancer remain unclear.

There has been less advance in understanding of Cl w4, 5 and 5A though the biochemical nature of the 5 and 5A antigens is more clear-cut (Waibel *et al.*, 1988). All three antigens appear in the workshop analysis to be widely distributed although all may be more strongly expressed on tumour than normal tissues.

In the second workshop two groups analysed the blood group or oligosaccharide binding specificity of the mAbs and the results of these studies were taken into account in defining clusters w6 and w8. It must be recognised however that apparently identical sugar specificity does not necessarily lead to identical results in immunohistology. In a future workshop it would be useful to pre-screen mAbs for carbo-

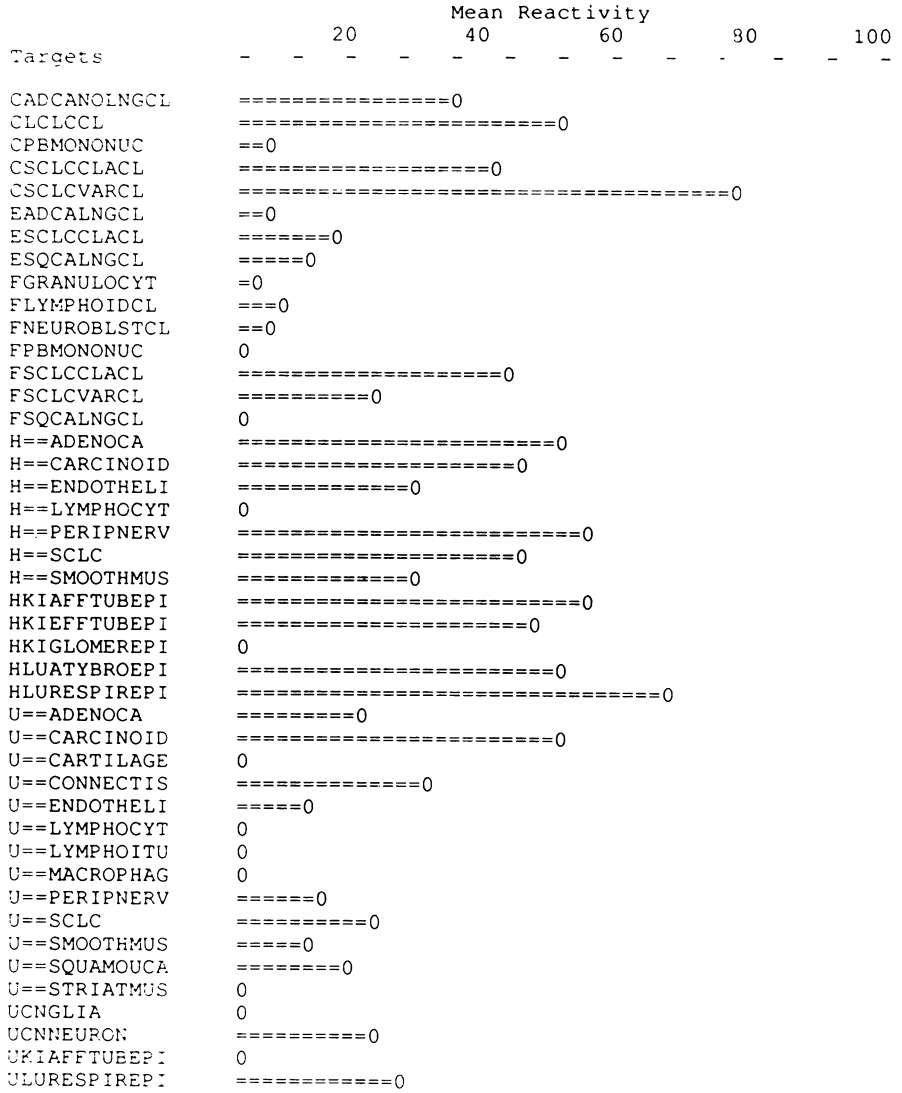

Figure 5 Profile analysis for cluster 5.

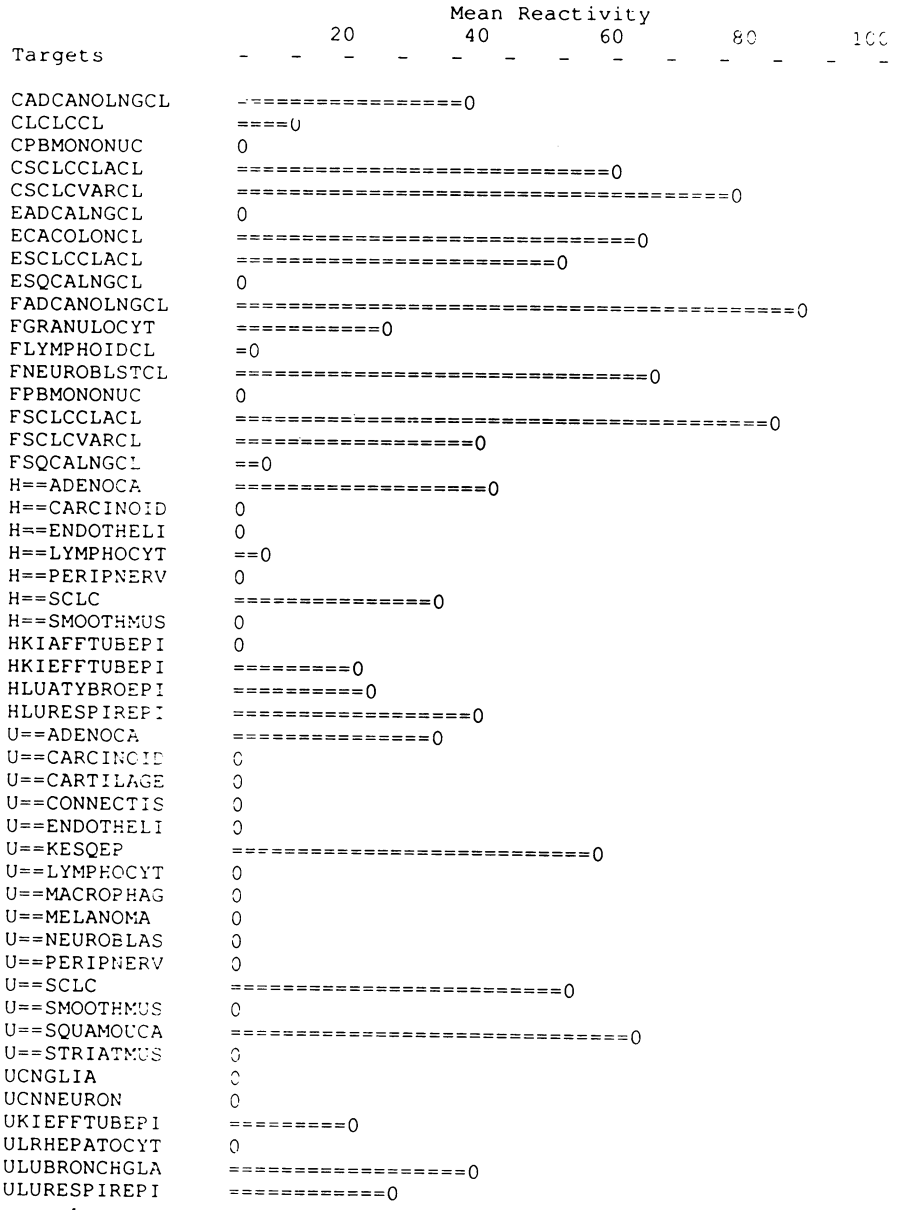

Figure 6 Profile analysis for cluster w6.

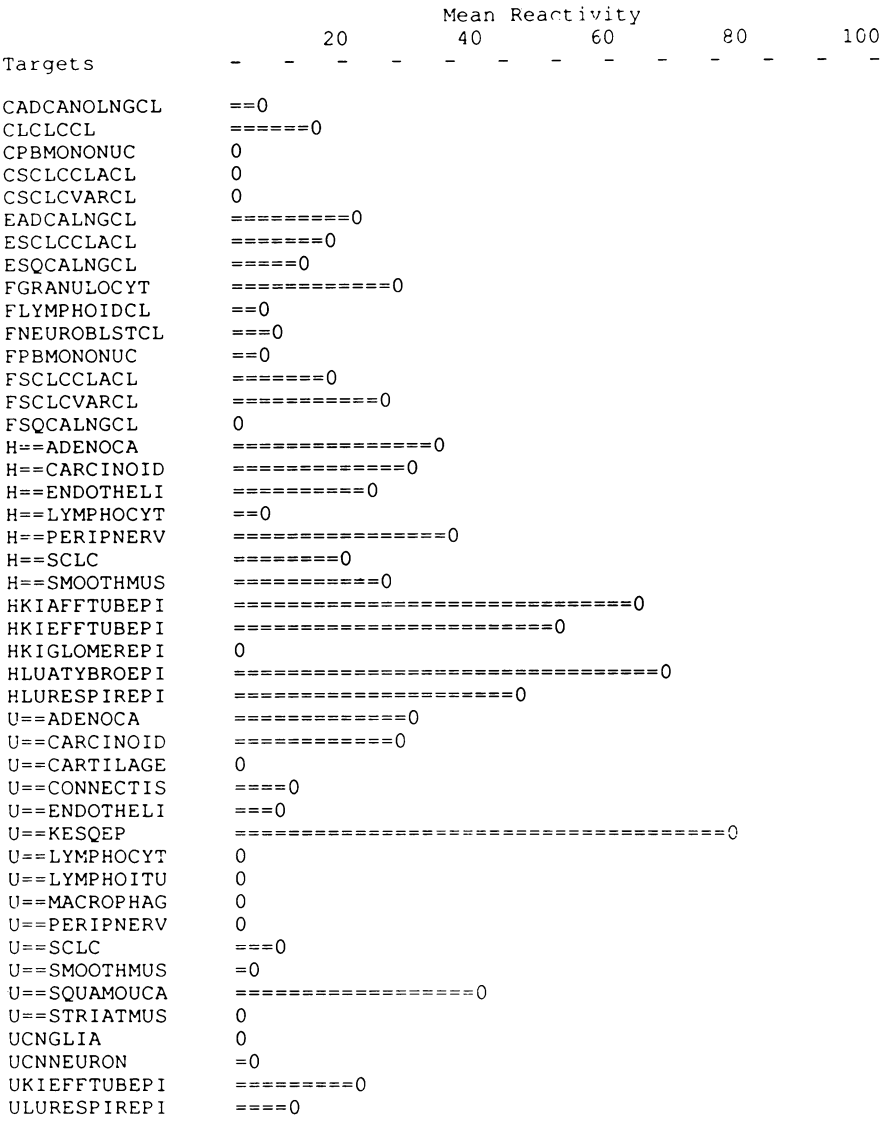

Figure 7 Profile analysis for cluster w7.

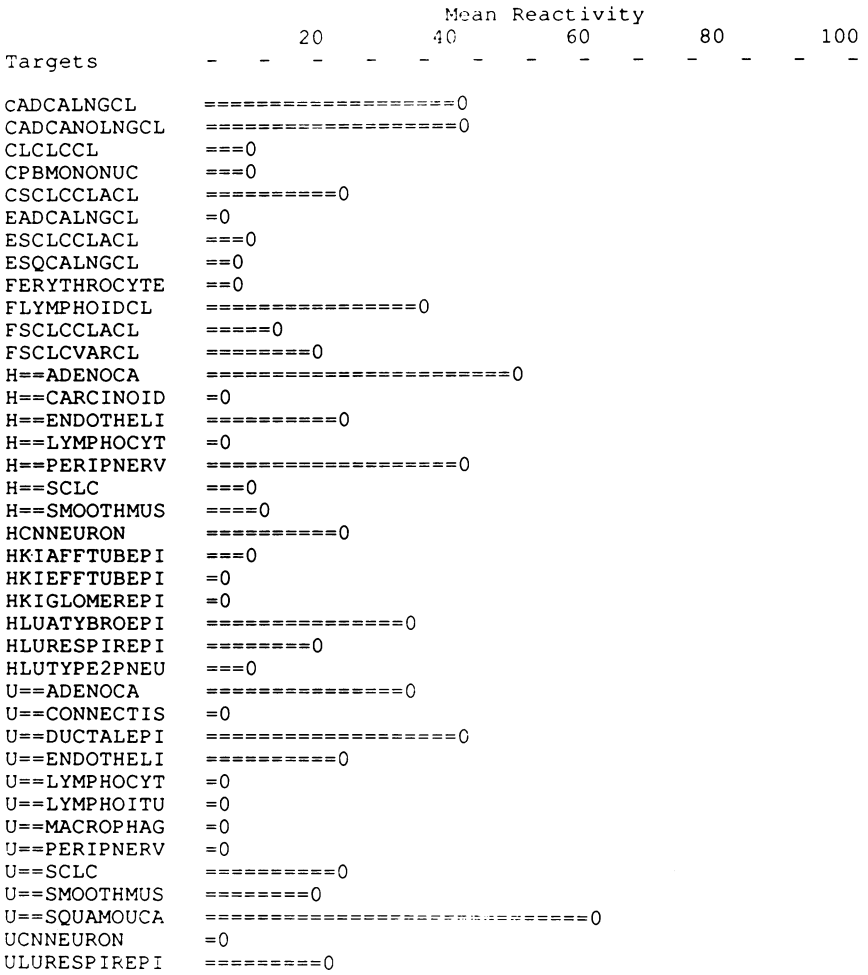

Figure 8 Profile analysis for cluster 8.

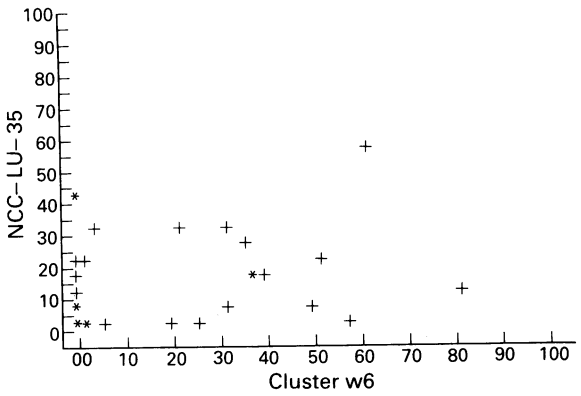

Figure 9 The symbol + represents reactivity of the mAb and cluster on a single tissue,* indicates coincident reactivity on more than one tissue. If the two mAbs had similar reactivity all points should fall on the diagonal. That they do not indicates that Clw6 and Clw8 are distinct.

hydrate binding and compare the tissue specificity of panels of mAbs of defined oligosaccharide specificity.

While the second workshop defined only two new clusters, it focussed attention on the problem of carbohydrate antigens and provided a panel of antibodies which threw up several interesting observations. Among the most intriguing was the finding that Cl 2 antigen appears to be a particularly good target for immunotoxin cytotoxicity (Derbyshire and Wawrzynczak, this volume).

We are grateful to all workshop participants for supplying mAbs and to Professor F. Walsh for supplying NCAM transfected L cells.

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