Immunology 1987 **61** 255–260

A new set of monoclonal antibodies to human MHC class II α chains demonstrates that most α epitopes are inaccessible on the living cell surface

B. B. COHEN, D. N. CRICHTON & C. M. STEEL *Medical Research Council, Clinical and Population Cytogenetics Unit, Western General Hospital, Edinburgh*

Accepted for publication 13 February 1987

SUMMARY

When mice were immunized with a mixture of human MHC class II α and β glycoprotein chains, the predominant antibody response was anti-α, and from a subsequent fusion experiment over 60 hybridomas showing anti-α activity were generated, compared with 11 anti-β secretors. These findings contrast with the relative paucity of anti-α monoclonals described previously. Use of a miniaturized Western blot screening protocol was a critical factor in the present study since the anti-α monoclonals do not bind to the surface of living B cells and would therefore be missed in conventional screening assays. After glutaraldehyde fixation of target B lymphocytes or B-cell lines, the majority of anti-α monoclonals do react in a radio-immunobinding assay, although none binds as strongly as pan-reactive anti-β chain antibodies. This suggests that the immunogenic epitopes of α chains are normally concealed by the three-dimensional folding of the αβ dimer. The anti-α monoclonals were all monomorphic but varied in the extent of their reactivity with α chains separated on one-dimensional and two-dimensional IEF gels. The most reactive antibodies identified up to seven distinct components among mature class II antigens from solubilized cell membranes.

INTRODUCTION

Monoclonal antibodies have proved very valuable in the analysis of human MHC class II antigens, which are transmembrane glycoprotein dimers comprising non-covalently linked α and β chains. Almost all antibodies studied in detail, however, have been reactive with the β chain or with intact dimers (Igarashi *et al.*, 1986). The first α-chain specific monoclonal antibody was described from this laboratory (Steel *et al.*, 1982; Guy *et al.*, 1982), and four more have since been reported from other centres (Korman *et al.*, 1982; Adams, Bodmer & Bodmer, 1983; Fermand, Schmitt & Brouet, 1985; Knudsen & Strominger, 1986). This total, however, falls far short of the number required to provide a comprehensive panel of anti-α reagents comparable to that available for MHC class II β chains (Steel, 1984). Such a panel should help to resolve the continuing uncertainty over such questions as the number of α chains expressed in man, the extent of their polymorphism, and the constraints on association between α and β chains encoded at different loci (DP, DQ and DR) within the major histocompatibility complex (Cohen, Deane & Moxley, 1984a; Bach, 1985; Giles & Capra, 1985; Korman *et al.*, 1985). We now report the production of more than 50 monoclonal antibodies specific for MHC class II α chains. All appear to be directed against framework determinants (since they show no allotype restriction), but there is variation in affinity for biochemically distinct subsets of α chains. The results of this study suggest that MHC class II α chains are highly immunogenic, that their antigenic determinants are generally less accessible than those of the β chains on the surface of living human cells, and that the relative paucity of anti-α reagents among monoclonal antibodies described hitherto is a consequence of the screening procedures commonly used.

Correspondence: Dr B. B. Cohen, Medical Research Council, Clinical and Population Cytogenetics Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, U.K.

MATERIALS AND METHODS

Antigen preparation

MHC class II antigens were prepared from the washed, mechanically disaggregated cells of 12 tonsils, yielding 5×10^9 cells, which were lysed in NP40 buffer (Jones, 1980) and the clarified lysate passed twice over an affinity column of CNBr Sepharose 4B to which had been bound monoclonal antibodies CR3.43, DA6.231 (both anti-β chain) and DA6.147 (anti-α chain) (Steel, 1984), each at 14–16 mg Ig/g Sepharose. The column was washed exhaustively with lysis buffer, followed by PBS, centrifuged dry and incubated at 4° with successive 1-ml aliquots of 0·1 M glycine/HCl, pH 2·5, a procedure that not only detaches the antigen, but weakens the bond between α and β chains (Cohen *et al.*, 1984b). Each eluate was tested for content

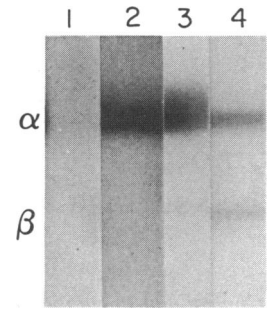

Figure 1. Assay of mouse sera, by Western blotting, for antibodies against MHC class II antigens. MHC class II glycoproteins from a lysate of a human B-lymphoblastoid cell line (10^7 cells/ml in NP40 buffer) were separated in 12·5% SDS–PAGE gels (Laemmli, 1970) and transferred to cellulose nitrate (Towbin *et al.*, 1979; Cohen *et al.*, 1984a). Strips were cut and probed with serum or with control antibodies. Lane 1: preimmune serum; Lanes 2 and 3: sera from the same animal taken on Days 56 and 92, respectively, of the immunization protocol described. Lane 4: mixture of monoclonal antibodies DA6.147 and CR3.43 (anti-MHC class II α and β chains, respectively). Mouse sera were diluted 1:40 in PBS; monoclonal antibodies were used as undiluted hybridoma supernatant. Bound antibody was detected with ^{125}I-rabbit anti-mouse Ig (Cohen *et al.*, 1984a) followed by autoradiography for 16 hr. Note that immunized mouse showed anti-α chain response alone on Day 56 with additional (weak) anti-β chain activity by Day 92. An identical pattern was observed in sera from the second animal.

of MHC class II antigens by Western blotting with DA6.147 and CR3.43 (Towbin, Staehelin & Gordon, 1979; Cohen *et al.*, 1983a). The first five eluates were strongly positive for both chains, yielding a total of 1·87 mg protein, 60% of this being in the second fraction, which was then used to immunize two adult BALB/c mice.

Immunization protocol

Protein eluate (10 μg in complete Freund's adjuvant) was injected subcutaneously on Day 1, and the same amount, in incomplete adjuvant, subcutaneously on Day 27. By Day 45 there had been no detectable antibody response, so a further subcutaneous injection of 250 μg protein, in incomplete Freund's adjuvant, was given. Thereafter an antibody response developed (Fig. 1) and on Day 93 one animal was given an intravenous boost of 100 μg protein without adjuvant. It was killed on Day 96 and the spleen used for hybridoma production.

Hybridization and screening

Mouse spleen cells ($4·6 \times 10^7$) were fused with 10^7 cells from the non-secreting mouse plasmacytoma line × 63.NS1 using polyethylene glycol in a standard protocol (Kennett *et al.*, 1978). The fusion products were distributed in 100-μl aliquots in six flat-bottomed 96-well microtitre plates, with mouse peritoneal macrophages as feeders. Culture medium was RPMI-1640 with 10% fetal calf serum plus HAT. Between 7 and 20 days after fusion, growth was observed in the majority of the 576 wells. Supernatant fluid from each well was screened by indirect radio-immunobinding to glutaraldehyde-fixed cells from human B- and T-lymphoid lines using, as the second layer, ^{125}I-labelled $F(ab')_2$ fragment of rabbit anti-mouse Ig (Williams, Galfre & Milstein, 1977; Guy *et al.*, 1982) and by a miniaturized Western blotting procedure using a series of 2 mm wide nitrocellulose strips bearing one of three different preparations of human MHC class II antigens; intact αβ dimers, α and β chains separated by boiling in SDS with mercaptoethanol, or α and β chains split by acid treatment (Cohen *et al.*, 1983a; Cohen *et al.*, 1984b). This procedure required only 100 μl of hybridoma supernatant per blot (Fig. 2).

Further characterization of antibodies

Hybridomas of interest were cloned by limiting dilution and grown up to yield larger volumes that were tested for Ig class and subclass by Ouchterlony double diffusion. Specificity was investigated further by repeat Western blotting and by indirect radio-immunobinding to a panel of glutaraldehyde-fixed MHC homozygous typing B-lymphoblastoid cell lines representing the DR allotypes 1 to 8 inclusive. Each assay was carried out in duplicate; human T-cell lines and the class II-negative leukaemia cell line K562 served as negative control targets. Positive control monoclonals included well-characterized monomorphic antibodies directed against MHC class II α and β chains, leucocyte common antigen and β_2 microglobulin. Negative control antibodies were mouse monoclonals directed against cortisol and mouse sperm (Crichton & Cohen, 1983; Crichton *et al.*, 1985). In order to standardize binding levels between experiments, the counts obtained with hybridoma supernatants under test were always compared with those given by saturating concentrations of two pan-reactive anti-β chain monoclonals, DA6.231 and CR3.43. These two reagents always gave very similar results and their mean value was taken as the 100% binding level.

In these experiments hybridoma supernatants were used undiluted but each was also tested in a series of doubling dilutions for quantitative binding to glutaraldehyde-fixed cells from a pool of human B-lymphoblastoid lines.

Peripheral blood lymphocytes depleted of T cells by 'E' rosetting (Hutchins & Steel, 1983) were also used, both fresh and after glutaraldehyde fixation, as targets for antibody binding. In addition to the radio-immunobinding assay described above, indirect immunofluorescence was used in some experiments, the second layer being a FITC-conjugated $F(ab')_2$ fraction of rabbit anti-mouse Ig (Sigma, Poole, Dorset). For fresh cells, the incubations, each of 30 min, were carried out on crushed ice and

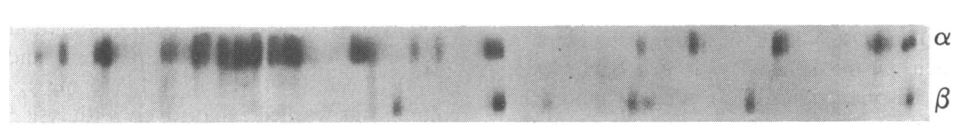

Figure 2. Western blot screening of hybridoma supernatants. Culture supernatants (100-μl aliquots) were incubated with 'mini-strips' (20 × 2 mm) of cellulose nitrate to which had been transferred the MHC class II antigen region from SDS–PAGE gels as described in the text. This representative autoradiograph shows the activity of 84 hybridoma supernatants against acid-split antigen preparations. Note that the great majority of positive supernatants contain anti-α chain activity.

the cells were fixed in 1% paraformaldehyde before examination by flow cytopherometry (FACS 4; Bector-Dickinson, Mountain View, CA) or by eye, using a Leitz Ortholux fluorescent microscope with Ploem's incident illumination.

A subgroup of the monoclonal antibodies, selected to represent a wide range of cell-binding characteristics, was examined further by Western blotting onto cellulose nitrate transfers of human MHC class II antigens separated by isoelectric focusing in a 7% polyacryamide gel, using a 4–6·5 pH gradient (LKB 'Ampholine') in 1% NP40 and 8 M urea. [Subsequent studies have shown that more consistent separation of human MHC class II α chains is achieved by substituting a pH gradient of 2·5–5 ('Pharmalyte', Pharmacia, Uppsala, Sweden).] Three antibodies that gave different patterns of reactivity in this analysis were then examined by blotting onto solubilized class II antigens separated in two dimensions: IEF in pH 4–6·5 tube gels with NP40 and urea as above, followed by electrophoresis in 12·5% polyacrylamide with SDS (Laemmli, 1970). The previously described anti-α monoclonal DA6·147 (Cohen *et al.*, 1983a) was also included in the analysis.

RESULTS

From the initial Western blot screen, 62 supernatants appeared to contain antibody reactive only with α chains; 11 others had anti-β chain activity alone; seven bound to both chains, and four recognized α chain plus unidentified lower molecular weight components. Of the 62 that appeared α-chain specific, six were positive in the indirect radio-binding assay with both T- and B-lymphoid cells. Multiple reactivity in the screening tests could have been due to the presence of more than one clone in some wells or to the production of an antibody recognizing epitopes shared by more than one polypeptide. As yet, the question has not been investigated since priority has been given to characterizing the 56 antibodies reactive only with α chain on Western blotting and which did not bind to T cells. The majority were Ig-γ1, three were γ2a, one γ2b and two μ. The antibody activity of each clone was confirmed by repeating the Western blot screening procedure. Five of the hybridomas either failed to grow or stopped secreting specific antibody at this stage. The remaining 51 monoclonals were tested further by indirect radio-immunobinding to the homozygous typing cell panel.

Each anti-α monoclonal showed a consistent level of binding to the entire panel, i.e. none showed any DR allotype preference. Fifteen failed to bind at all (counts not significantly different from negative controls). Almost all of the remainder could be classified as 'weak' binders (mean counts bound 6–15% of those obtained with the pan-reactive anti-β chain monoclonals) or 'strong' binders (mean counts 30–50% of DA6.231 and CR3.43 values). Nine of the 15 'weak' and ten of the 18 'strong' cell binders contained at least four times the minimum saturating concentration of antibody. A definite plateau binding level could not be demonstrated in the dilution curves for the remaining supernatants, which included the three whose cell binding levels placed them between the 'weak' and 'strong' categories defined above. The Ig γ1 monoclonals were distributed almost equally between negative, 'weak' and 'strong' cell binders, but all six antibodies of other Ig classes were in the 'strong' category. The pattern of reactivity for the different monoclonal antibodies was the same when glutaraldehyde-fixed T-depleted peripheral blood lymphocytes were substituted for lymphoblastoid cell lines in the radio-immunobinding assay, although the counts bound per cell were five- to ten-fold lower.

None of the anti-α chain antibodies bound to living (unfixed) peripheral blood lymphocytes as judged by radio-immunobinding or by indirect immunofluorescence. Unfixed cells from B-lymphoblastoid lines did give positive results in radio-immunobinding assays with the antibodies classified as 'strong' binders to fixed cells, but the counts were highly variable from one experiment to another, and on indirect immunofluorescence it was apparent that only a minority of cells was reacting, 1–15%

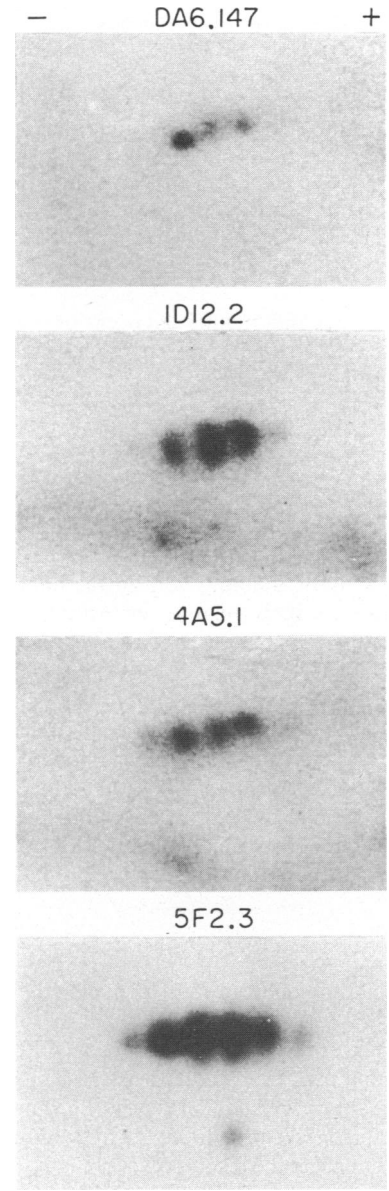

Figure 3. Binding of representative monoclonals to MHC class II antigens separated by two-dimensional gel electrophoresis. Acid-treated lysate from a human B-lymphoblastoid cell line was concentrated 30-fold in the Centricon Miniconcentrator (Amicon, MA). Twenty-microlitre aliquots of concentrate were focused in pH 4–6·5 Ampholine polyacrylamide tube gels, followed by SDS–PAGE in the second dimension (Deane *et al.*, 1983) and Western blotting with the monoclonal antibodies indicated. The figures show the α chain regions of four typical autoradiographs.

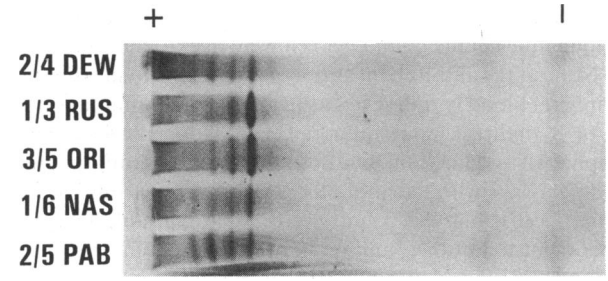

Figure 4. Western blot analysis following IEF of cell lines of different DR allotypes. Gels were 7% acrylamide 8 M urea, 1% octylglycoside and 1·9% 2·5–5 Pharmalyte (Pharmacia). Samples were applied in slots in the same mixture except that 2% NP40 was substituted for the octyglycoside. Samples contained lysate prepared from 10^6 cells (prepared by lysing 3×10^7 cells in 1 ml of 1% NP40). Samples were applied at the anode. Following electrophoresis overnight at 1500 V, the gels were transferred directly and blotted using 5F2.3 followed by peroxidase-conjugated sheep anti-mouse Ig (Miles, Slough, Berks). Note that 5F2.3 appears to react with α chains of the same isoelectric points in the five cell lines, but there is clear variation in the relative intensities of the individual bands in different lines.

(from different lines and on different occasions) giving a strong signal on FACS analysis while the remainder were negative. On direct microscopic examination, the positive cells were seen to be damaged and the fluorescence appeared to be on the inner aspect of the cell membrane, spreading into the cytoplasm.

A representative sample of anti-α chain antibodies has been characterized further by 'Western blotting' onto cellulose nitrate transfers of human MHC class II antigens separated by isoelectric focusing in one-dimensional slab gels. At least three patterns of reactivity, differing both in the numbers of bands recognized and in their relative intensities, could be distinguished and these differences were confirmed on two-dimensional blots (Fig. 3). There was no evident correlation between performance in cell binding assays and the pattern of bands produced by different antibodies in Western blots. The monoclonal 5F2.3 consistently gave the largest number of bands or spots in these assays, and it has proved useful in demonstrating the limited but real polymorphisms of human MHC class II α chains in Western blots following IEF separation of solubilized membrane glycoproteins in one dimension (Fig. 4).

DISCUSSION

The most broadly reactive anti-α monoclonals in this series, exemplified by 5F2.3, recognize at least five major and two minor α-chain components, which agrees with the extent of variation among expressed MHC class II α chains predicted from analysis of the corresponding genes (Spielman *et al.*, 1984; Bach, 1985; Giles & Capra, 1985; Korman *et al.*, 1985) and considerably improves the resolution of α-chain spots identified in two-dimensional gels of immunoprecipitates from biosynthetically labelled antigen preparations (Markert & Cresswell, 1982; Adams *et al.*, 1983; Crumpton *et al.*, 1984). Furthermore, biosynthetic labelling generates metabolic intermediates which increase the complexity of gel patterns, whereas Western blotting detects, almost exclusively, components derived from more mature αβ dimers (Cohen *et al.*, 1983b; Crumpton *et al.*, 1984). Hence, the monoclonal antibodies described in this report, in combination with anti-β chain reagents previously shown to be broadly reactive in Western blotting (Cohen *et al.*, 1984a), promise to be extremely useful in dissecting the repertoire of MHC class II gene products expressed at the cell surface.

The failure of anti-α monoclonals to recognize determinants on the surface of living MHC class II-positive cells has been noted previously (Steel *et al.*, 1982; Guy *et al.*, 1982; Adams *et al.*, 1983; Guy, Ritchie & van Heyningen, 1984; Liebold & Gatti, 1984; Kaufman *et al.*, 1984; Knudsen & Strominger, 1986), but comment on the difference between anti-α and anti-β chain monoclonals in this regard has been inhibited by the very small numbers of the former available for study. We have now shown that inability to bind to unfixed B lymphocytes is a consistent feature of more than 50 anti-α monoclonal antibodies of different Ig classes and with a range of target specificities (deduced from their varying reactivity with glutaraldehyde-fixed B cells and from the Western blotting results). In some previous studies, the α chain used as immunogen was denatured by vigorous chemical and/or enzyme treatment (Adams *et al.*, 1983; Knudsen & Strominger, 1986): hence, it could be argued that the epitopes characteristic of the intact molecules had been destroyed, although it was conceded that in the case of the β chain some antigenic determinants are present on both intact and denatured forms (Kaufman *et al.*, 1984). In the present study, care was taken to prepare the immunogen by a very gentle procedure in order to preserve as many epitopes as possible. Although acid-split α and β chains run separately in SDS gels, immunoprecipitation of acid-treated solubilized class II antigens with either an anti-α or an anti-β monoclonal antibody brings down both chains, suggesting that at this stage the bond between them is only weakened, not broken (B. B. Cohen, unpublished results). It therefore seems likely that a substantial portion of the immunogen in the present case was in the form of αβ dimers, and it is of interest that the dominant antibody response appeared to be anti-α.

The extreme rarity of anti-α monoclonals before the present study can be explained by the fact that, in most protocols, hybridoma supernatants are screened by indirect binding assays, with intact cells as targets. Only one of the anti-α monoclonals described so far has been reactive with unfixed peripheral blood B cells (Fermand *et al.*, 1985; Fermand, Chevalier & Brouet, 1986), while the levels of binding to fresh or fixed lymphoblastoid lines have been, at the very most, only about half that achieved by pan-reactive anti-β reagents (Guy *et al.*, 1982; Adams *et al.*, 1983; and present data). The Western blot screening procedure adopted in the present study obviously introduces a bias towards antibodies directed against epitopes remaining intact following SDS gel electrophoresis. Whether this favours anti-α monoclonals is uncertain. There is an additional *N*-linked oligosaccharide side-chain on the membrane-proximal domain of the α chain, not found on the β chain (Giles & Capra, 1985; Korman *et al.*, 1985). It could be argued that this is the immunogenic region of the α chain and that its position relative to the rest of the dimer makes it inaccessible to antibody while the cell is alive and the membrane intact. Such an explanation, however, is unsatisfactory for a number of reasons. Firstly, anti-α monoclonals retain the ability to bind to denatured class II antigens from which carbohydrate side-chains have been removed by treatment with neuraminidase and endoglycosidases (B. B. Cohen, unpublished observations) or which have been synthesized in the presence of tunicamycin to

block glycosylation (Adams *et al.*, 1983). Secondly, β_2 microglobulin, which is non-covalently bound to the heavy chain of MHC class I antigens, is conventionally represented as projecting no further from the membrane than the proximal domain of the class II α chain (Kaufman *et al.*, 1984), yet β_2M is highly immunogenic (when intact cells are used as immunogen), and both polyclonal and monoclonal antibodies to β_2M react well with living cells (Sanderson, 1977; Trucco, Stocker & Ceppellini, 1978). Thirdly, from what is known of their amino-acid sequences (Giles & Capra, 1985; Korman *et al.*, 1985), there is no *a priori* reason why the 'framework' epitopes recognized by monoclonal antibodies should be distributed differently on class II α and β peptides.

The findings are more readily explained by postulating that the MHC class II dimer, in its undenatured state, is folded with the β chain on the outside and much of the α chain thereby rendered inaccessible. This explanation is incompatible with the currently accepted representation of the dimer structure, in which the α and β chains are equally exposed (Kaufman *et al.*, 1984; Giles & Capra, 1985; Korman *et al.*, 1985). It is, however, consistent with the observation that most of the MHC class II allotype diversity in man is associated with β-chain polymorphism (Bach, 1985; Giles & Capra, 1985; Korman *et al.*, 1985). It may be significant that the only anti-α monoclonal known to be reactive with living B cells is directed against an epitope on a DQ gene product. Unlike the DR-encoded counterpart, the DQ α chains are polymorphic (Kaufman *et al.*, 1984), which could imply a difference in the three-dimensional configuration of DR and DQ αβ dimers and hence in the 'display' of the respective α chains on the cell surface.

The cross-linking action of glutaraldehyde may distort the cell membrane in such a way as to increase exposure of the α chain. It is also possible that, in the course of cellular interactions involving MHC class II antigen, unfolding of the dimer occurs so that the α chain plays a significant part, hence the inhibitory effect of anti-α reagents in some functional assays (Palacios *et al.*, 1982; Steel *et al.*, 1985). The present findings direct attention to tertiary structure as a determinant of function among cell surface molecules and contribute new reagents for studies on the biochemistry of an important group of membrane antigens.

ACKNOWLEDGMENTS

The authors acknowledge the expert technical assistance of Ms Marion Moxley and of the staff of the Animal Unit, Western General Hospital, Edinburgh. We are grateful to Dr Keith Guy for helpful discussions. Supplies of some control monoclonal antibodies were generously provided by Dr D. Y. Mason and Professor J. Fabre, and homozygous typing cell lines by Professor Hilliard Festenstein and colleagues. The figures were prepared by Sandy Bruce.

REFERENCES

ADAMS T.E., BODMER J.G. & BODMER W.F. (1983) Production and characterisation of monoclonal antibodies recognising the α chain subunits of human Ia allo-antigens. *Immunology*, **50,** 613.

BACH F.H. (1985) The HLA class II genes and products: the HLA D region. *Immunol. Today*, **6,** 89.

COHEN B.B., DEANE D.L., VAN HEYNINGEN V., GUY K., HUTCHINS D., MOXLEY M. & STEEL C.M. (1983a) Biochemical variation of human Ia-like antigens detected with monoclonal antibodies. *Clin. exp. Immunol.* **53,** 41.

COHEN B.B., DEANE D.L. & MOXLEY M. (1984a) Analysis of antibody specificity by Western blotting. *Dis. Markers*, **2,** 135.

COHEN B.B., DEANE D.L., MOXLEY M. & STEEL C.M. (1983b) Methods of detecting mature human Ia-like antigen and their metabolic precursors. *J. immunol. Meth.* **61,** 91.

COHEN B.B., MOXLEY M., CRICHTON D., DEANE D.L. & STEEL C.M. (1984b) A mild procedure for separating polypeptide chains prior to immunoprecipitation and Western blotting. *J. immunol. Meth.* **75,** 99.

CRICHTON D.N. & COHEN B.B. (1983) Analysis of the mouse sperm surface with monoclonal antibodies. *J. reprod. Fert.* **68,** 497.

CRICHTON D.N., GRATTAGE L., MCDONALD A., CORRIE J.E.T., STEEL C.M., HUBBARD A.L., AL DUJAILI E.A.S. & EDWARDS C.R.W. (1985) The production and assessment of monoclonal antibodies to cortisol. *Steroids*, **45,** 503.

CRUMPTON M.J., BODMER J.G., BODMER W.F., HAYES J.M., LINDSAY J. & RUDD C.E. (1984) Biochemistry of class II antigens: workshop report. In: *Histocompatibility Testing* (eds E.D. Albert, M.P. Baur and W.R. Mayr). Munksgaard, Copenhagen.

DEANE D.L., COHEN B.B., MOXLEY M. & STEEL C.M. (1983) Detection of Ia epitopes by monoclonal antibody blotting. *Immunol. Lett.* **6,** 93.

FERMAND J.-P., CHAVALIER A. & BROUET J.-C. (1986) Characterisation of a monoclonal antibody recognising a monomorphic determinant of the alpha chain of class II DQ antigens. *Scand. J. Immunol.* **24,** 313.

FERMAND J.-P., SCHMITT C. & BROUET J.-C. (1985) Distribution of class II DQ antigens on normal and leukaemic lymphoid cells. *Eur. J. Immunol.* **15,** 1183.

GILES R.C. & CAPRA J.D. (1985) Structure, function and genetics of human class II molecules. *Adv. Immunol.* **37,** 1.

GUY K., VAN HEYNINGEN V., COHEN B.B., DEANE D.L. & STEEL C.M. (1982) Differential expression and serologically distinct subpopulations of human Ia antigens detected with monoclonal antibodies to human Ia α and β chains. *Eur. J. Immunol.* **12,** 942.

GUY K., RITCHIE A.W.S. & VAN HEYNINGEN V. (1984) Anomalous expression of MHC class II antigens on human monocytes. *Dis. Markers*, **2,** 283.

HUTCHINS D. & STEEL C.M. (1983) Phytohaemagglutinin-induced proliferation of human T lymphocytes: differences between neonates and adults in accessory cell requirements. *Clin. exp. Immunol.* **52,** 355.

IGARASHI M., IMBERTI L., MAIO M., TSUJISAKI M., PERORA F., SAKAGUCHI K., NATALI P.G. & FERRONE S. (1986) In: *HLA Class II Antigens* (eds B. G. Solheim, E. Moller and S. Ferrone), p. 224. Springer-Verlag, Berlin.

JONES P.P. (1980) Analysis of radiolabelled lymphocyte proteins by one- and two-dimensional polyacrylamide gel electrophoresis. In: *Selected Methods in Cellular Immunology* (eds B. B. Mishell and S. M. Shiigi), p. 398. Freeman, San Francisco.

KAUFMAN J.F., AUFFRAY C., KORMAN A.J., SHACKELFORD D.A. & STROMINGER J.L. (1984) The class II molecules of the human and murine major histocompatibility complex. *Cell*, **36,** 1.

KENNETT R.H., DENIS K.A., TUNG A.S. & KLINMAN N.R. (1978) Hybrid plasmacytoma production: fusions with adult spleen cells, monoclonal spleen fragments, neonatal spleen cells and human spleen cells. *Curr. Top. Microbiol. Immunol.* **81,** 77.

KNUDSEN P.J. & STROMINGER J.L. (1986) A monoclonal antibody that recognises the α chain of HLA-DR antigens. *Human Immunol.* **15,** 150.

KORMAN A.J., BOSS J.M., SPIES T., SORRENTINO R., OKADA K. & STROMINGER J.L. (1985) Genetic complexity and expression of human class II histocompatibility antigens. *Immunol. Rev.* **85,** 45.

KORMAN A.J., KNUDSEN P.J., KAUFMAN J.F. & STROMINGER J.L. (1982) cDNA clones for the heavy chain of HLA-DR antigens obtained after immunopurification of polysomes by monoclonal antibody. *Proc. natl. Acad. Sci. U.S.A.* **79,** 1844.

LAEMMLI U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. *Nature (Lond.)*, **227,** 680.

LIEBOLD W. & GATTI R.A. (1984) Binding patterns of monoclonal antibodies with specificities against MHC-class II determinants on HLA-DR homozygous lymphoblastoid cell lines. *Dis. Markers*, **2,** 263.

MARKERT M.L. & CRESSWELL P. (1982) Human B cell alloantigens: α subunit variability. *J. Immunol.* **128,** 1999.

NEEFJIES J.J., DOXIADIS I., STANN N.J., BECKERS C.J. & PLOEGH H.L. (1986) An analysis of class I antigens of man and other species by one-dimensional IEF and immunoblotting. *Immunogenetics*, **23,** 164.

PALACIOS R., CLAESSON L., MOLLER G., PETERSON P.A. & MOLLER E. (1982) The al/ha chain, not the β chain of HLA-DR antigens participates in activation of T cells in autologous mixed lymphocyte reaction. *Immunogenetics*, **15,** 341.

SANDERSON A. (1977) HLA 'help' for human β_2 microglobulin across species barriers. *Nature (Lond.)*, **269,** 414.

SPIELMAN R.S., LEE J., BODMER W.F., BODMER J.G. & TROWSDALE J. (1984) Six HLA-D region α chain genes on human chromosome 6: polymorphisms and associations of DC α-related sequences with DR types. *Proc. natl. Acad. Sci. U.S.A.* **81,** 3461.

STEEL C.M. (1984) Table of workshop antibodies and their characteristics. *Dis. Markers*, **2,** 363.

STEEL C.M., VAN HEYNINGEN V., GUY K., COHEN B.B. & DEANE D.L. (1985) Influence of monoclonal anti-Ia-like antibodies on activation of human lymphocytes. *Immunology*, **47,** 597.

STEEL C.M., VAN HEYNINGEN V., GUY K., COHEN B.B., DEANE D.L., CRICHTON D.N. & HUTCHINS D. (1982) Monoclonal anti-B cell antibodies: their effects on human mixed lymphocyte reaction. *Proc. Biol. Fluids*, **29,** 695.

TOWBIN H., STAEHELIN T. & GORDON J. (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. natl. Acad. Sci. U.S.A.* **76,** 4350.

TRUCCO M.M., STOCKER J.W. & CEPPELLINI R. (1978) Monoclonal antibodies against human lymphocyte antigens. *Nature (Lond.)*, **273,** 666.

WILLIAMS A.F., GALFRE G. & MILSTEIN C. (1977) Analysis of cell surfaces by xenogeneic myeloma-hybrid antibodies: differentiation antigens of rat lymphocytes. *Cell*, **12,** 663.