Immunogenetics 25: 116–122, 1987

Immuno-genetics

Genetic Organization of the Ovine MHC Class II Region

Peter C. Scott, Chung-Lit Choi, and Malcolm R. Brandon

Department of Veterinary Preclinical Sciences, University of Melbourne, Parkville, Victoria, Australia

Abstract. To study the class II genes of the major histocompatibility region of the sheep genome, human *HLA* class II genes corresponding to the known subregions in man (*DR, DQ, DP, DO,* and *DZ*) were used for Southern hybridization analysis of sheep DNA and to probe a sheep genomic library. Hybridizing bands were noted for all probes except DP_α. DQ α and β and DR_β appear to be present as multicopy genes, while DR_α-, DZ_α-, and DO_β-like genes appear to be single copy. All bands detected with the DP_β probe were also detectable with other β chain probes. From eight λ-bacteriophage clones of a sheep genomic library nine distinct class II genes were identified. These genes were characterized by differential hybridization analysis and restriction mapping. Two genes were DR_β-like, three DQ_α-like and four DQ_β-like. The extensive cross-hybridization observed with β chain probes was not seen with α chain probes. The results of this study suggest that the major histocompatibility complex class II region of the sheep has a similar genetic organization to that of man, with the provisional exception of the *DP* subregion.

Introduction

The class II antigens of the major histocompatibility complex (MHC) play a role in immunological function, as they are involved in T lymphocyte, macrophage and T-B cell interactions as restricting elements for T helper (CD 4 positive) cells (Korman et al. 1985). They are expressed predominantly on the surface of B lymphocytes, a subpopulation of T cells and macrophages. Their presence on other tissues is generally limited to the regions corresponding to the location of these cell types (Larhammar et al. 1983). Class II antigens are polymorphic glycoproteins, consisting of two noncovalently linked subunits: an α chain of approximately 35 000 daltons and a β chain of 28 000 daltons (Klareskog et al. 1979). Although polymorphism has been demonstrated in both chains, it is more extensive in the β chain (Wiman et al. 1982). The detailed genetic analysis of the *HLA* MHC class II genes has revealed that the major site of polymorphism is encoded by the second exon, designated $\alpha 1$ or $\beta 1$, which is immediately downstream from the leader peptide encoding signal sequence (reviewed by Korman et al. 1985).

Molecular analysis of the class II region of the MHC of man has revealed a much more complex set of gene clusters than first indicated by classical serological methods. Currently the human HLA class II region consists of at least five subregions termed *DR, DQ, DP, DZ,* and *DO* (Long et al. 1982, Lee et al. 1982, Auffray et al. 1982, Larhammar et al. 1983, Trowsdale et al. 1984, Spielman et al. 1984, Tonnelle et al. 1985). Sequence analysis has revealed two β genes in the *DR* subregion and one α and one β gene in the *DP* region to be pseudogenes, contributing further to the complexity of the human class II region (Trowsdale et al. 1985). In contrast, the mouse class II region (*Ia* region) is less complex than that of man and contains two subregions: *A*, homologous to the *DQ* subregion, and *E*, homologous to the *DR* subregion (Trowsdale et al. 1985). One gene in the mouse *E* subregion is possibly a pseudogene.

Studies of the genetic organization of the MHC class II region in species other than man and mouse are limited. Recent work in the rabbit indicates the presence of several class II genes with an arrangement comparable to man (LeGuern et al. 1985, Sittisombut and Knight 1986), and limited Southern hybridization experiments in sheep using HLA gene probes have detected the presence of several class II-like genes, indicating polymorphism in both the α and β chains (Chardon et al. 1985).

By using a comprehensive range of HLA class II region gene probes evidence is presented here showing at least four equivalent subregions of the class II region are present in the sheep genome, suggesting that the gene organization of this region is as complex as that seen in man.

Abbreviation used in this paper: OLA, ovine major histocompatibility complex

Materials and Methods

Hybridization probes and ^{32}P labeling. All MHC class II probes were cDNA clones, with the exception of DP_β. Clone pDCHI recognizes DQ_α (Auffray et al. 1982); $DR_\beta 1$ (now called p $II_\beta 1$) is a clone of DQ_β (Wiman et al. 1982), pDRH2 a DR_α clone (Lee et al. 1982), $DR_\beta 2$ a DR_β clone (Long et al. 1982), $DR_\beta 10$ a DR_β clone (D. Kappes and J. L. Strominger, unpublished data), 8bα1 a DZ_α clone (Trowsdale et al. 1985), pDAα-13b a clone of DP_α (Trowsdale et al. 1985), and β163 a clone representing the locus DO_β (Tonnelle et al. 1985). A subcloned Pst I fragment of the cosmid clone LCII, designated 11-13, was used as the DP_β probe (Trowsdale et al. 1984). The labeled probes varied not only in representative loci but also with the proportion of exons, corresponding to the molecular domains represented by each probe. The interpretative importance of this is covered in the results. The DQ_α probe covered all representative exons (α1 to the 3′ untranslated region) as did DR_α, DO_β, DR_β, and DP_α. The DQ_β probe was an Ava I fragment that covered the entire coding region except for a few base pairs of the 5′ end. The Pst I fragment of the genomic DP_β gene covers primarily the β1 exon. In a number of cases, 5′ and 3′ specific probes were used to help characterize 5′ and 3′ ends of the sheep genomic clones. Restriction fragments were isolated from vectors by using the appropriate enzyme and eluted onto NA 45 paper (Schleicher and Schüll, Dassel, FRG) or recovered from low melting temperature agarose (Seaplaque). Nick translation of the inserts were undertaken using α-^{32}P-dATP (Amersham, Arlington Heights, Illinois) to a specific activity of approximately 5×10^8 cpm/μg DNA according to the method of Rigby and co-workers (1977).

Cloning and screening of a phage genomic sheep library. A genomic library (kindly supplied by Drs. P. Roche and P. Aldred, Howard Florey Institute, Melbourne, Australia) from a partial Sau 3AI digest of liver DNA obtained from a Merino ewe was constructed in the lambda vector Charon 28 yielding approximately 800 000 unique recombinants. Approximately 6×10^5 recombinant clones from the amplified lambda Charon 28 library were screened by in situ hybridization (Maniatis et al. 1982) using mixed class II probes containing nick translated DQ_α, DQ_β, DR_α, DR_β, and DP_β restriction fragments. Final conditions of washings were 0.2 × standard sodium citrate (SSC), 0.1% sodium dodecyl sulfate (SDS), at 42 °C. Positive clones were isolated, plaque purified and characterized according to standard techniques (Maniatis et al. 1982).

Purification of DNA. High molecular mass DNA was purified from 1 g of sheep thymus as described by Maniatis and co-workers (1982), except for the following modifications. Tissue stored at −70 °C was broken into small fragments and added to 20 ml of buffer (250 m*M* ethylene diaminetetraacetate, 20 m*M* Tris, pH 8.0) and then homogenized using a polytron (Kinematica, Switzerland), after which 1% SDS was added immediately, followed by 20 µg/ml Proteinase K (Boehringer, Mannheim, FRG).

Southern blot analysis of sheep DNA and phage clones. After digestion and electrophoresis of sheep genomic DNA, agarose gels were prepared for Southern hybridization by standard techniques (Maniatis et al. 1982). Transfer to GeneScreen Plus (New England Nuclear Research Products, Boston, Massachusetts) was performed according to the manufacturer's recommendations and as described by Southern (1975). For dot blot analysis, 10 ng of phage DNA was directly placed onto dry GeneScreen Plus, denatured, and neutralized in situ. Prehybridization and hybridization using α-^{32}P nick-translated probes were carried out at 42 °C in 50% formamide, 5 × SSPE (0.9 *M* NaCl, 50 m*M* NaH_2PO_4, pH 7.4, 5 m*M* ethylenediaminetetraacetate, pH 7.0), 5 × Denhardt's solution, 0.5% SDS, and 100-200 µg/ml salmon sperm DNA (Sigma, St. Louis, Missouri). Washing conditions were as described in the figure legends. Removal of the probe was achieved by placing the membrane in 0.4 *M* NaOH with gentle agitation at 42 °C for 30 min and then neutralizing it in 0.2 *M* Tris buffer, pH 7.4, 0.1% SDS, and 0.1 × SSC. Filters were in some cases hybridized up to eight times. For restriction mapping, the phage DNA containing the cloned insert were both singly and doubly digested to completion using a number of restriction enzymes.

Results

Southern blot analysis. Southern blots using eight HLA probes are shown in Figure 1a (α chain probes) and Figure 1b (β chain probes). Due to the complexity of the Southern blot analysis results obtained with this multigene family, the results are tabulated to allow easier interpretation of the significance of individual bands, especially for demonstrating cross-hybridization between subregions (Table 1). Multiple bands are noticeable with the β chain, in particular, DQ_β, DR_β, and DP_β. The Southern blot with DQ_β demonstrates as many as ten bands in each tract, but only three or four are prominent. The multiple bands here may be due to both cross-hybridization and pseudogenes. Generally much of the cross-hybridization with the β chain blots can be resolved by noting the variation in prominence of certain bands depending on the HLA probe used. For example, the presence of a strong Hind III 5.0 kb band observed with DQ_β is associated with the identification of a relatively weak 5.0 kb band in the DR_β blot. Generally the complex cross-hybridization picture associated with the β chain probes is absent with the α chain using similar washing conditions.

DQ subregion. Ten or more bands were noted for the β probe, while two or three bands were present for the α-probe. Because of cross-hybridization problems, it is difficult to predict the number of β genes; however, the intensity of some of the bands and their multiplicity suggest that there are three or more DQ_β-like genes in the sheep. Similarly it could be predicted that there are at least two DQ_α-like genes. Multiple banding for DQ_α has been observed by Chardon and co-workers (1985) in sheep. In contrast to man and sheep, DQ_α appears to be a single copy gene in the rabbit (Le Guern et al. 1985, Sittisombut and Knight 1986) and mouse (Figueroa and Klein 1986).

DR subregion. Although the HLA-DR_β probe covered the beta-2 transmembrane, 3′ untranslated region, and a small portion of the β1 domain, the complexity of the DR_β-like subregion in the sheep appears to be less than that of DQ_β. This is the reverse of that found in man, although the presence in the sheep of only a single DR_α-like gene is similar to that in man.

DP subregion. The DP_β clone 11-13 only represents the β1 exon (the most variable domain) of the *HLA* gene, however, multiple banding patterns were detected using high stringency washings. Although extensive cross-hybridization with DQ_β and DR_β is present, the comparative intensity of some hybridizing bands on the DP_β Southern blot suggests

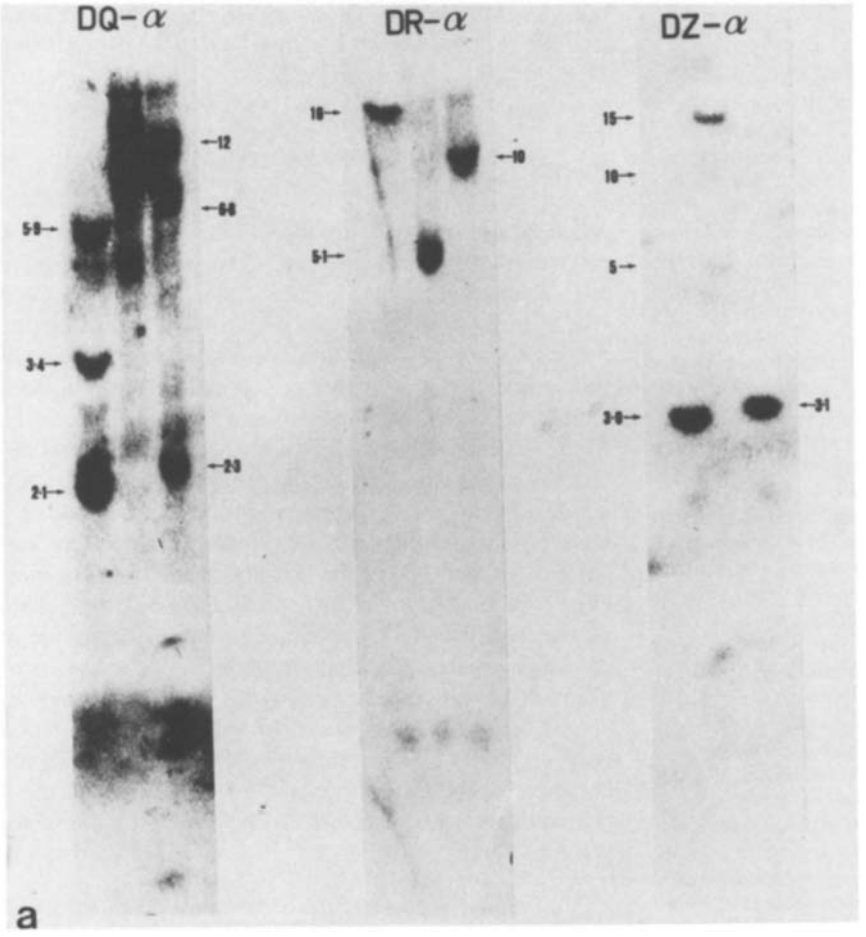

Fig. 1 a and b. Southern hybridization analysis of Eco RI, Bam HI, and Hind III (left to right) restricted sheep DNA, (**a**) probed with *HLA-D* region α chain genes and (**b**) probed with *HLA-D* region β chain genes. Final washing at 55 °C, 0.2 × SSC, 0.1% SDS

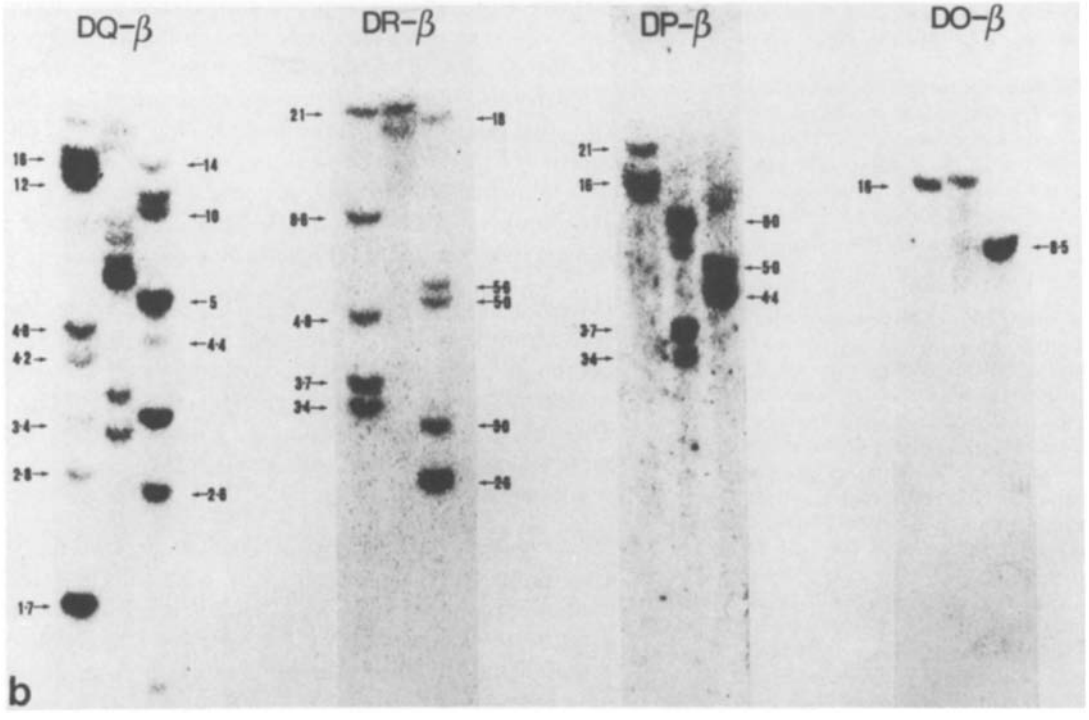

Table 1. Tabulation of all hybridizing bands from a series of Southern blots, probed with a gene representing each of the known *HLA-D* subregions

Eco RI								Bam HI								Hind III							
β				α				β				α				β				α			
Q	R	P	O	Q	R	P	Z	Q	R	P	O	Q	R	P	Z	Q	R	P	O	Q	R	P	Z
								22.0	22.0														
21.0	21.0	21.0																					
								18.0									18.0						
								17.0	17.0		17.0												
16.0		16.0	16.0		16.0																		
															15.0								
14.0		14.0														14.0							
12.0												12.0								12.0			
																11.0		11.0					
															10.0	10.0					10.0		
9.0								9.0															
8.6	8.6							8.0		8.0									8.5				
												7.0											
								6.8)		6.8										6.8			
								6.0)	6.0														
				5.9									5.1		5.0	5.6)	5,6)	5.6)					
																5.0)	5.0)	5.0)					
4.8)	4.8)															4.4	4.4	4.4					
4.2)		4.2																					
	3.7)							3.7)	3.7)	3.7)													
3.4	3.4)			3.4			3.0	3.0)	3.0)	3.0)						3.4	3.4)						3.1
																	3.0)						
2.8				2.1												2.6	2.6			2.3			
1.7		1.7														1.3							
		1.0)																					

All bands are recorded for washing conditions of 50 °C, 0.2 × SSC, and 0.1 % SDS. With the β chain gene probes, little alteration in hybridization patterns occurred until washing conditions were very stringent, 68 °C, 0.1 SSC, and 0.1 % SDS

there are probably two equivalent genes in the sheep. The 1.0 kb band for the Eco RI digest was noted on only one blot with a low stringency wash, the significance of this being uncertain. Although repeated several times and using conditions of low stringency washing (42 °C, 0.2 × SSC), no hybridizing bands were noted for DP_α, although bands were clearly seen on control human DNA Southern blots (data not shown).

The DO and DZ subregions. The presence of a unique 8.5 kb Hind III when hybridizing with the *HLA-DO*$_\beta$ gene supports the existence of a single copy DO_β-like gene in the sheep. For each of the three enzymes, unique bands were noted for DZ_α. The presence of a single band with Eco RI and Hind III supports the existence of a single copy DZ_α-like gene in the sheep.

Clone isolation and characterization. From the phage Charon 28 library, 33 hybridizing clones were identified. By restriction analysis with Eco RI, eight of the clones were unique. Two of these clones, C8-1 and C17-2, hybridized with an α chain probe, five clones, C5-2, 4/1, A15-2, B16-2, and B10-1, with a β chain probe, and C13-4 with both α and β chain probes.

Dot blot analysis of the eight unique clones (Fig. 2) using a range of washing conditions indicates that there are

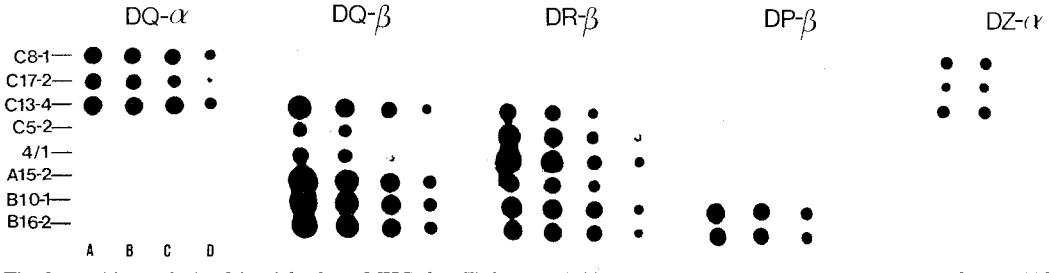

Fig. 2. Dot blot analysis of the eight sheep MHC class II clones. Washings were carried out in 0.2 × SSC at A, 42 °C; B, 55 °C; C, 63 °C; and D, 68 °C for DQ_α, DQ_β, DR_β, DP_β, and DZ_α. No hybridization was noted when probed with DR_α, DP_α, and DO_β

two DQ_α-like clones, one clone containing both DQ_α- and DQ_β-like genes. In addition, there are three DQ_β- and two DR_β-like gene containing clones. The use of heterologous human probes and the extensive homology between the β chain make it difficult to allocate these clones precisely to comparable human loci. On the basis of hybridization studies, the DR_β-like clone, C5-2, demonstrates more divergence from the HLA-DR than does the DR_β clone 4/1. The two DQ_β-like clones B10-1 and B16-2 differ from the other DQ_β representative clones C13-4 and A15-2 in that the former strongly hybridize with the HLA-DP_β probe while the latter show no homology at all. This divergence within the DQ_β subregion is similar to that found in the rabbit for DR_β-like clones (Sittisombut and Knight 1986). Study of the blot analysis demonstrates that there is little difference between the hybridization stringencies of the clones B10-1 and B16-2 with the *HLA-DQ*$_\beta$ and *-DR*$_\beta$ genes. Probing with 5′ and 3′ fragments (data not shown) indicates that stronger hybridization was observed with the 3′ fragment of the *HLA-DQ*$_\beta$ gene.

Restriction fragment analysis of these clones is shown in Figure 3 a and b. Three clones, C13-4, B16-2, and A15-2, appear to contain truncated genes. The β gene in the phage clones C13-4 and A15-2 and the α gene in the clones C8-1

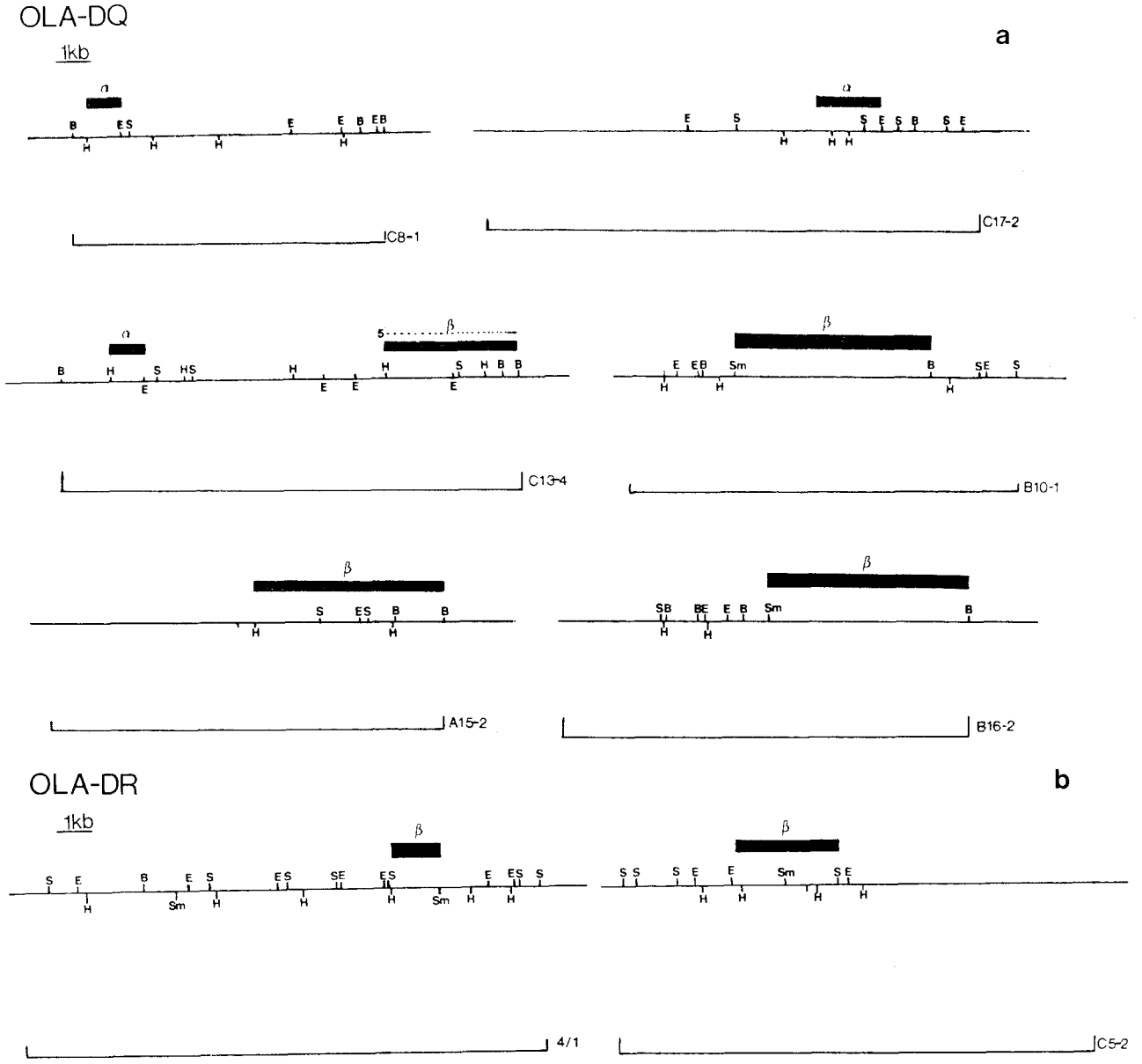

Fig. 3 a and b. Restriction enzyme maps of (**a**) *OLA-DQ* and (**b**) *OLA-DR*-like class II genes: Solid boxes represent the smallest unique hybridizing fragment. B, Bam HI; H, Hind III; E, Eco RI; S, Sac I; Sm, Sma I

and C13-4 probably represent the allelic forms of a single locus, as there is minimal variation in the restriction sites. By probing with fragments of the HLA cDNA clones it was possible to determine a probable 5′ orientation. Many restriction fragment sizes for both the α and β chain clones correlate with restriction fragment sizes in the sheep genomic DNA Southern blots, for example, the 2.3 kb Hind III fragment of the DQ_α-like clone C8-1 and the 3.4 kb Eco RI fragment of the DR_β-like clone C5-2. A number of clones have internal restriction fragments that do not correlate (see *Discussion*).

No clones for DR_α were found (DZ_α, DP_α, and DO_β were not used in the original screening), although Southern analysis from Figure 1a and b suggests the existence of these loci in the sheep. For an understanding of the *DP* subregion, further investigation is required (see *Discussion*).

Examination of the shortest unique hybridizing fragments in the restriction map suggests that the α chain genes appear to be smaller than those of the β chain. These findings are similar to those for the rabbit (Sittisombut and Knight 1986).

Discussion

The existence of unique strongly hybridizing band(s) for six of the *HLA-D* subregions (*DQ* α and β, *DR* α and β, *DO* β and DZ α) suggests sheep have MHC class II genes homologous to those found in man. The presence of only one unique hybridizing band with the *HLA-D* region probes DZ_α and DR_α suggests that only one gene is present in sheep for each locus, which is similar to the results found for DR_α in man and rabbit (Le Guern et al. 1985, Sittisombut and Knight 1986).

Although DO_β shares similar size bands with DQ_β, 16 kb for Eco RI and 17 kb for Bam HI, the existence of a unique 8.5 kb Bam HI band and the lack of any significant cross-hybridization in the dot blot analysis with the DQ_β- and DR_β-like genes in the sheep support the notion that a single copy DO_β-like gene does exist in the sheep.

At least three genes comparable to those of the *HLA-*DQ_β subregion and at least two DR_β-like genes exist in the sheep. Some of these, like in man, may be pseudogenes. Although only limited data are available, evidence from clone C13-4 suggests that MHC class II genes in the sheep are tandemly arranged. Future use of overlapping cosmid clones will confirm this. Chardon and co-workers (1985) found a constant Eco RI band of 3.2 kb in the sheep genome when using both the DQ_α and DR_β probes. Examination of Table 1 reveals a similar 3.4 kb band for the Eco RI digest. The use of three restriction enzymes, Eco RI, Bam HI, and Hind III, combined with examination of the restriction maps of the available clones, indicates that this results from two restriction fragments that are coincidentally of comparable size. Examination of hybridization patterns in the Southern blots demonstrates that most of cross hybridization of DP_β occurs with DQ_β. Comparisons between the different subregions of the *HLA-D* region have shown that the DP_β chain gene has approximately 75% homology with both DQ_β and DR_β (Gorski et al. 1984). Recent reports have noted that the *HLA-*DP_β gene is most homologous to the mouse gene $\psi A\beta_3$ (equivalent to the *DQ* subregion), thus suggesting some common ancestry with these two subregions (Korman et al. 1985). Our findings in the sheep indicate that two of the DQ_β-like genes from the clones B10-1 and B16-2 are homologous to *HLA-*DP_β, while the DQ_β-like genes of the clones A15-2 and C13-4 are not. Not only does this suggest some sequence divergence of the sheep *DQ*-like subregion, it also contrasts with the rabbit, where a subset of the DR_β-like genes hybridized strongly with the DP_β probe (Sittisombut and Knight 1986). It is also interesting that the DQ_α-like clones in the sheep, although demonstrating some cross-hybridization with HLA-DZ_α, show none with DR_α, indicating greater divergence of the latter. This is different from the situation in man, where the gene structure and sequence of DZ_α conforms equally to all the α chain genes (Trowsdale and Kelly 1985).

The absence of a detectable hybridizing DP_α-like gene in the samples of sheep DNA examined, in spite of the presence of prominent hybridizing bands with the HLA-DP_β probe, has a number of possible explanations. The lesser homology of the α chain genes with the β chain genes may suggest that the probe utilized for the *DP* locus is too divergent to allow detection of a similar locus in sheep. It is also possible that there is no equivalent of the DP product in the sheep MHC class II, because of the absence of any coordinately expressed DP_α-like gene with that of DP_β. This would compare to the recently recognized DO_β subregion in man, where a corresponding α chain gene may not exist (Tonelle et al. 1985)

The extensive cross-hybridization between the *DQ, DR* and *DP* β chain genes (see Table 1) further complicates the interpretation of the *DP*-like subregion in the sheep. Some of this can be resolved by probing the sheep DQ_β and DR_β-like genes back to sheep genomic DNA and washing under stringent conditions. The future use of a HLA-DP_β gene probe representing more than just the β1 exon will also be advantageous.

In *Results* it was noted that for a small number of sheep class II clones the size of internal restriction fragments did not correlate with the respective hybridizing bands seen on the genomic Southern blots (see Table 1, Fig. 3 a and b). This is in part attributable to the use of two sources of sheep DNA, which introduces complications due to restriction fragment length polymorphism. This has been demonstrated for sheep class II genes (P. C. Scott, unpublished data; Chardon et al. 1985). In addition, the extensive cross-

hybridization of the β chain genes limits the ability to predict their exact locus specificity and number, and therefore the number of genes remaining to be cloned. With respect to genes coding for the DQ_α-like chains where the hybridizing patterns are less complicated, it would appear that there is one DQ_α gene which remains to be cloned.

The results presented herein indicate that the genetic organization of the sheep MHC class II region is similar to that of man, with the presence of comparable subregions, tandemly arranged genes, and the existence of homology between subregions (particularly the β chain genes). It will now be possible, by DNA sequencing, to precisely define sheep α and β chain genes and also examine polymorphism for each subregion.

Acknowledgments. The authors are most grateful to Mr. Ken Snibson for his assistance in photography and to Drs. T. Adams and N. Puri for valuable discussions in the preparation of this manuscript.

References

Auffray, C., Korman, A. J., Roux-Dosseto, M., Bono, R., and Strominger, J. L.: cDNA clone for the heavy chain of the human B cell alloantigen DCI: Strong sequence homology to HLA-DR heavy chain. *Proc. Natl. Acad. Sci. U.S.A. 79:* 6337–6341, 1982

Chardon, P., Kirszenbaum, M., Cullen, P. R., Geffrotin, C., Auffray, C., Strominger, J. L., Cohen, D., and Vaiman, M.: Analysis of the sheep MHC using HLA class I, II, and C4 cDNA probes. *Immunogenetics 22:* 349–358, 1985

Figueroa, F. and Klein, J.: The evolution of MHC class II genes. *Immunol. Today 7:* 78–81, 1986

Gorski, J., Rollini, P., Long, E., and Mach, B.: Molecular organization of the HLA-SB region of the major histocompatibility complex and evidence for two SB β-chain genes. *Proc. Natl. Acad. Sci. U.S.A. 81:* 3934–3938, 1984

Klareskog, L., Tragardh, L., Rask, L., and Peterson, P. A.: Isolation and characterization of detergent-solubilized human HLA-DR transplantation antigens. *Biochemistry 18: 1481–*1489, 1979

Korman, A. J., Boss, J. M., Spies, T., Sorrentino, R., Okada, K., and Strominger, J. L.: Genetic complexity and expression of human class II histocompatibility antigens. *Immunol. Rev. 85:* 45–86, 1985

Larhammar, D., Hyldig-Nielsen, J. J., Servenius, B., Andersson, G., Rask, L., and Peterson, P. A.: Exon-intron organisation and complete nucleotide sequence of a human major histocompatibility antigen DCβ gene. *Proc. Natl. Acad. Sci. U.S.A. 80:* 7313–7317, 1983

Lee, J. S., Trowsdale, J., and Bodmer, W. F.: cDNA clones coding for the heavy chain of human HLA-DR antigen. *Proc. Natl. Acad. Sci. U.S.A. 79:* 545–549, 1982

LeGuern, C., Marche, P. N., and Kindt, T. J.: Molecular evidence for five distinct MHC class II α genes in the rabbit. *Immunogenetics 22:* 141–148, 1985

Long, E. O., Wake, C. T., Strubin, M., Gross, N., Accolla, R. S., Carrel, S., and Mach, B.: Isolation of distinct cDNA clones encoding HLA-DR-β chains by use of an expression assay. *Proc. Natl. Acad. Sci. U.S.A. 79:* 6465–6469, 1982

Maniatis, T., Fritsch, E. F., and Sambrook, J.: *Molecular Cloning,* Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1982

Rigby, P. W. J., Dieckmann, M., Rhodes, C., and Berg, P.: Labelling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. *J. Mol. Biol. 113:* 237–251, 1977

Sittisombut, N. and Knight, K. L.: Rabbit major histocompatibility complex 1. Isolation and characterization of three subregions of class II genes. *J. Immunol. 136:* 1871–1875, 1986

Southern, E. M.: Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol. 98:* 5003–5117, 1975

Spielman, R. S., Lee, J., Bodmer, W. F., Bodmer, J. G., and Trowsdale, J.: Six HLA-D region α-chain genes on human chromosome 6: Polymorphisms and associations of DCα related sequences with DR types. *Proc. Natl. Acad. Sci. U.S.A. 81:* 3461–3465, 1984

Tonnelle, C., DeMars, R., and Long, E. O.: DOβ: A new β chain gene in the HLA-D region with a distinct regulation of expression. *EMBO J. 4:* 2839–2847, 1985

Trowsdale, J. and Kelly, A.: The human HLA/Class II α chain gene DZ-α is distinct from genes in the DP, DQ and DR subregions. *EMBO J. 4:* 2231–2237, 1985

Trowsdale, J., Kelly, A., Lee, J., Carson, S., Austin, P., and Travers, P.: Linkage map of two HLA-SBβ and two HLA SBα related genes: An intron in one of the SBβ genes contains a processed pseudogene. *Cell 38:* 241–249, 1984

Trowsdale, J., Young, J. A. T., Kelly, A. P., Austin, P. J., Carson, S., Meunier, H., So, A., Erlich, H. A., Spielman, R. S., Bodmer, J., and Bodmer, W. F.: Structure, sequence polymorphism in the HLA-D region. *Immunol. Rev. 85:* 5–43, 1985

Wiman, K., Larhammar, D., Claesson, L., Gustafsson, K., Schenning, L., Bill, P., Bohme, J., Denaro, M., Dobberstein, B., Hammerling, U., Kvist, S., Servenius, B., Sundelin, J., Peterson, P. A., and Rask, L.: Isolation and identification of a cDNA clone corresponding to an HLA-DR antigen β-chain. *Proc. Natl. Acad. Sci. U.S.A. 79:* 1703–1707, 1982

Received August 15, 1986; revised version received September 17, 1986