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Brief Communication

Partial Aminoterminal Amino Acid Sequences of Chicken Major Histocompatibility Antigens*

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Partial amino acid sequences of the aminoterminal regions of mouse H-2 and human HLA major histocompatibility antigens (MHAs) have recently been determined (Capra *et al.* 1976, Cunningham *et al.* 1976, Ewenstein *et al.* 1976, Silver *et al.* 1976, Appella *et al.* 1976, Ballou *et al.* 1976, Bridgen *et al.* 1976, Terhorst *et al.* 1976, 1977). The results clearly showed the structural homology of the mouse and human antigens, but presented an unexpected feature: the homology between duplicated genes within a species (*e.g.*, *H-2D* and *H-2K*) was greater than any interspecies homology. This result suggests that the gene duplications giving rise to the *H-2D* and *K* (and *HLA-A*, *B*, and *C*) loci may have taken place late in evolution, after the divergence of mice and humans from a common ancestor (Silver and Hood 1976).

Further information about the evolution of the mouse and human major histocompatibility complexes (MHCs) can be gained from studying their homologs in such nonmammalian species as *Xenopus* (Du Pasquier *et al.* 1975) and the chicken (Hála 1977). The chicken *B* complex and the mammalian MHCs have very similar biological properties, and one class of products of the *B* complex—the B antigens—are clearly homologous to the mammalian MHAs in their apparent molecular weight (about 40,000) and association with a smaller polypeptide (β_2-microglobulin) of molecular weight 11,500 (Ziegler and Pink 1975, 1976). Limited amino acid sequence data on the B antigens of one inbred chicken strain (SC: Vitetta *et al.* 1977) confirm this conclusion.

In this paper, we report partial amino acid sequence data from the aminoterminal regions of the B antigens of the inbred WA and WB chicken strains. The B antigens of these two strains have slightly different apparent molecular weights, suggesting that the two strains may differ at only one locus coding for MHAs [since it is unlikely that the products of duplicated loci would evolve in parallel to give different apparent molecular weights in the two strains (Ziegler and Pink 1975, 1976)]. The

* The following abbreviations are used in this paper: MHA, major histocompatibility antigens; MHC, major histocompatibility complex; PBS, phosphate-buffered saline; PMSF, phenylmethylsulphonyl-fluoride; SDS, sodium dodecyl sulphate.

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results establish the close homology of the avian and mammalian MHAs, and are consistent with the idea that the two strains differ at a single *B* locus only.

Peripheral blood leukocytes from highly inbred WA or WB birds (Hála *et al.* 1966) were isolated by centrifugation over Ficoll-Isopaque (Ziegler and Pink 1976), and 150 × 10^6 viable cells were cultured for 6–8 hours as previously described (Ziegler and Pink 1975) in 30 ml Eagle's Minimal Essential Medium lacking certain amino acids. This was supplemented with 10% dialyzed fetal calf serum, 2 mM glutamine, and the tritiated derivatives of the amino acids missing from the basic medium. Three combinations of tritiated amino acids were used: leucine, alanine, and valine; isoleucine, phenylalanine, alanine, and proline; and tyrosine, histidine, arginine, and lysine. These combinations were chosen because the phenylthiohydantoin derivatives of each group can be separated easily by liquid or thin-layer chromatography (see below). The specific activities of the amino acids were 20–150 Ci/mMol, and 1.5–2.0 mCi of each were added per culture.

Radioactively labeled B antigens were purified from lysates of the cultured cells as described previously (Ziegler and Pink 1975, 1976). The washed cells were lysed in 12–14 ml 0.5% NP-40 in phosphate-buffered saline (PBS) containing 10^{-3} M phenylmethylsulphonylfluoride (PMSF), and labeled immunoglobulins were removed from the dialyzed lysate by addition of 0.65 ml normal chicken serum, plus 10 ml rabbit anti-chicken IgG serum. To the resulting supernatant was added 0.6 ml specific alloantiserum; the preparation and properties of these anti-WA and anti-WB sera have been described previously (Ziegler and Pink 1976). The lysate was incubated at 37°C for 1 hour, and then treated overnight at 4°C with rabbit anti-chicken IgG serum. The specific precipitate was washed in cold PBS containing 10^{-3} M PMSF, and dissolved in 10 M urea (4–5 ml). Aliquots (1–1.5 ml) were made 1% in sodium dodecyl sulphate (SDS) and 5% in β-mercaptoethanol, and applied to 7% polyacrylamide slab gels containing 0.1% SDS, 0.1 M Tris, and 0.1 M Bicine. After electrophoresis, the gels were sliced horizontally into 0.3-cm strips, which were broken into 10–30 pieces and extracted twice (24 hours each extraction) with 2.5 ml water containing bovine cytochrome c (100 μg/ml). The fractions containing radioactive B antigens were dialyzed against 0.005% SDS, lyophilized, and applied to a modified (Wittmann-Liebold 1973) Beckman 890 B sequencer, together with 2–3 mg of an immunoglobulin light chain of known sequence as internal control. The phenylthiohydantoin derivatives were identified by high-pressure liquid chromatography (Frank and Strubert 1973) (for neutral aminoacids) or thin-layer chromatography (Silver and Hood 1975) on Kieselgelplates (Merck, Kieselgel 60 F-254) in chloroform: methanol (85 : 15) (for basic amino acids plus tyrosine).

The recovery of nondialyzable radioactivity in the lysate of 150× 10^6 cells cultured in 5–6 mCi of mixed ^{3}H-amino acids was 100–200 × 10^6 cpm (*i.e.*, 2–3% of the isotope input). Addition of specific alloantiserum led to precipitation of about 0.1–0.2% of the nondialyzable radioactivity (*i.e.*, about 1–2 × 10^5 cpm), of which 60–80% could be recovered in the B antigen peak after electrophoresis, extraction from the gel, dialysis, and lyophilization. This amount sufficed for 2–3 runs on the sequencer.

The B antigen specifically precipitated from WA strain lysates migrated on electrophoresis as a single sharp band of molecular weight 40,000 (Fig. 1), which was absent from control precipitates prepared with normal chicken serum instead of alloantiserum. The results of sequencing this material are shown in Figure 2 and Table 1. Amino acids could be placed at 16 of the first 30 positions of the chain, with no evidence of heterogeneity of sequence. However, the assignments of proline and

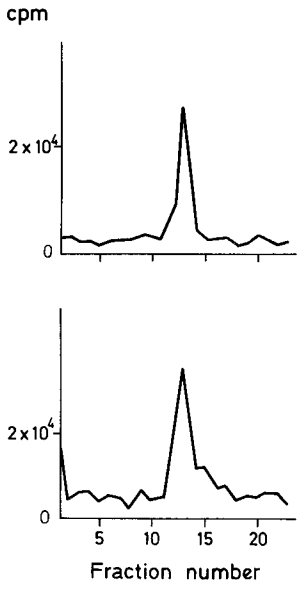

Fig. 1. Purification by preparative SDS-polyacrylamide gel electrophoresis of B antigens precipitated from strain WA (top) and WB (bottom) leukocyte lysates. The leukocytes were cultured in the presence of ^{3}H-leucine, ^{3}H-valine, and ^{3}H-alanine. Immune precipitates containing labeled B antigen were prepared using the appropriate specific alloantiserum, and aliquots (20–25%) of the precipitate were subjected to electrophoresis in SDS-polyacrylamide gels. The gels were sliced and the total radioactivity (cpm) extracted from each slice is given. Material in the peak fractions from 4–5 gels run under identical conditions was pooled and taken for sequencing

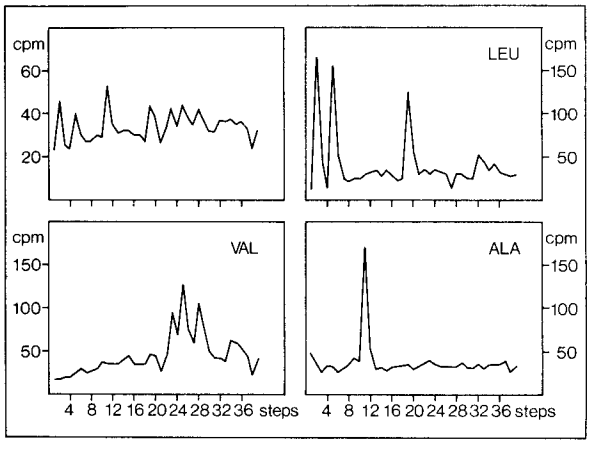

Fig. 2. Sequence analyses of B antigen (52,000 cpm) purified from lysate of WA cells cultured in ^{3}H-leucine, valine, and alanine. An aliquot (10%) of the total material released per step was counted (top left panel), and the remainder was analyzed by high-pressure liquid chromatography. Panels show the total cpm obtained per step in the leucine, valine, and alanine peaks

histidine in the sequence are not certain, since incorporation of these amino acids in culture was relatively poor.

Alloantiserum directed against the products of the WB strain *B* complex precipitated, in addition to the B antigen of apparent molecular weight 42,000, varying amounts of material of slightly lower molecular weight (Fig. 1). The nature of this material is unknown, but it may be a protein(s) homologous to the mouse Ia

Table 1. Partial Amino Acid Sequences of Major Histocompatibility Antigens[a]

Antigen	Allele	1				5					10					15					20					25			
Mouse[b] H-2	K[d]	M		H			R	Y			(T)				R	(P)					(P)	R	F					Y	
	K[k]	M	P	H		L	R	Y	F	H		A	V			P		L		K	P							Y	
	D[d]	M		H		L	R	Y	F	V		A	V		R	P		F			P	R	Y					Y	
Human[c] HLA	A2	G	S	S	S	M	R	Y	F	F	T	S	V	S	R	P	G		G	E	(S)	B	F	I	A	V		Y	
	B7	G	S	H	S	M	R	Y	F	Y	T	S	V	S	R	P	G	R	G	E	P	P	F	I	A	V	G	Y	V
	A1	G	S	H	S	M	R	Y	F	F	T	A	M		R	P	G	R	G	E	P	P	F	I		V		Y	
	+B8									D			V									R							
Guinea pig[d] GPLA	B1			H		L	R	Y	F	Y		A	V			P							F	(V)				Y	
	+S					I			L														L						
Chicken B	WA		L	(H)		L	R	Y	I	F		A				(P)		(P)		L			F	V		V		Y	V
	WB[e]		L	H		L	R	Y	I			A				(P)													
	SC[f]			H			R	Y	(F)	R			M										(Y)					Y	

[a] Sequences are given in the one-letter amino acid code of Dayhoff (1976). Residues in brackets are doubtful. Gaps indicate that no residue has been assigned to that position. Residues definitely present in all four species are boxed

[b] Data from Capra *et al.* 1976, Cunningham *et al.* 1976, and Silver *et al.* 1976

[c] Data from Appella *et al.* 1976, Ballou *et al.* 1976, Bridgen *et al.* 1976, and Terhorst *et al.* 1976, 1977

[d] Data from Schwartz *et al.* 1976

[e] Only the first 20 positions of the WB antigen were examined

[f] Data from Vitetta *et al.* 1977

antigens, like the B-L antigen previously isolated from cultures of CC-strain chicken leukocytes (Ziegler and Pink 1976). Contamination of WB B antigen with this material was evident in some preparations; for this reason, we could not obtain sequence data beyond residue 20 for the WB protein.

Table 1 presents the sequence data accumulated on the B antigens of the WA and WB strains, and compares them with published data on mouse, human, and guinea pig major histocompatibility antigens. The sequences of the two B antigens are very similar to each other: the only well-established differences between them are the phenylalanine and leucine at positions 9 and 19 in the WA antigen, which are absent from the WB antigen. It was previously found that the two antigens differ by about 3000 daltons in apparent molecular weight, as calculated from their mobilities in SDS-polyacrylamide gel electrophoresis (Ziegler and Pink 1976). The present results make it very unlikely that this difference is due to an aminoterminal deletion or addition to one of the chains. The WA and WB antigens show less similarity in sequence to the B antigen of the SC chicken strain studied by Vitetta and coworkers (1977), since these authors did not identify leucine or alanine in any position of the SC chain, but found an arginine at position 9 and a methionine (for which we did not test) at position 12. Vitetta and coworkers (1977) also tentatively identified phenylalanine and tyrosine residues at positions 8 and 22, respectively, whereas the WA and WB antigens have an isoleucine at position 8, and the WA antigen has a phenylalanine at position 22.

The sequences obtained for the chicken B antigens are clearly homologous to the known sequences of mammalian MHAs (Table 1). The only positions in which the chicken antigens differ from all the mammalian sequences are positions 2, 8, 9, and 19, where residues found in the B antigens have not been observed in the mammalian MHAs. Also, in position 12, the B antigens lack a valine found in most of the mammalian proteins.

The finding of an apparently homogeneous sequence at the aminoterminus of the WA antigen and the lack of strong evidence for heterogeneity of the WB B protein suggest that the antisera used in the precipitation procedure react with a single B antigen from each strain, rather than reacting with the products of duplicated loci (such as the *H-2D* and *K* loci). It may be that the chicken *B* complex does include duplicated loci coding for MHAs, but that the antisera used detect only one of the products—perhaps because the WA and WB strains [which are congenic lines derived from a single, partially inbred W strain (Hála *et al.* 1966)] differ at only one of the loci. On the other hand, a more interesting possibility, which we favor, is that the chicken MHC actually contains only one locus coding for MHAs. This suggestion is consistent with the fact that, of the various examples of possible recombination thought to have taken place within the *B* complex (Schierman and MacBride 1969, Hála *et al.* 1976, Pink *et al.* 1977, Briles and Briles 1977), none has been demonstrated to separate two loci coding for B antigens; and with the observation of Kubo and coworkers (1977) that inbred strain-2 chicken B antigens were less heterogeneous than human HLA antigens in a two-dimensional gel electrophoresis system. The idea that the duplications in the *H-2* and *HLA* complexes arose by independent events from a single precursor locus (Ziegler and Pink 1976), as suggested by sequence studies on mammalian MHAs (Silver and Hood 1976), would be entirely in accord with our results.

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