Proc. Natl. Acad. Sci. USA
Vol. 74, No. 11, pp. 5135–5139, November 1977
Immunology

Structure of murine Ia antigens: Partial NH_2-terminal amino acid sequences of products of the *I-E* or *I-C* subregion

(H-2 complex gene products/immunoprecipitation/microsequence analysis/sequence homology)

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ABSTRACT **Partial amino acid sequences of the Ia molecule encoded by the *I-E* or *I-C (I-EC)* subregion of the major histocompatibility complex of the mouse are presented. The Ia molecule appears to be comprised of two noncovalently associated polypeptides. The larger subunit, α, has an approximate molecular weight of 35,000 and the smaller subunit, β, an approximate molecular weight of 28,000. Several interesting homology relationships (or the lack thereof) are apparent when the Ia polypeptides from the *I-EC* subregion are compared both with their counterparts from man and guinea pig and with the molecules encoded in the *I-A* subregion. Clearly the most impressive homology relationship is that seen between the α polypeptide from the *I-EC* subregion of mouse and its human counterpart. This is in striking contrast to the β polypeptide, which bears no apparent homology to its human counterpart.**

The major histocompatibility complex (H-2 complex) of the mouse is a genetic region that encodes a variety of cell-surface antigens, many of which seem interrelated with the immune response. Loci that map in the *I* region of the H-2 complex control a series of phenotypic traits that are intimately involved in the vertebrate immune response (1): immune responsiveness to particular antigens (2); cellular interactions among T cells, B cells, and macrophages (3–5); B-cell differentiation (6); and helper or suppressor factors for the immune response (7, 8).

The only gene products from *I* region loci that have been directly identified with alloantisera are the Ia (*I* region *a*ssociated) antigens. Ia molecules are highly polymorphic (9, 10) and are expressed on immunocompetent cells (B lymphocytes, T lymphocytes, and macrophages) and on epidermal cells (11). The relationship between Ia molecules and the phenotypic traits controlled by the *I* region is not clear. Indirect evidence suggests that the Ia gene products mediate at least some of the immunological phenomena controlled by the genes of the *I* region in that antisera against Ia inhibit antibody responses *in vitro* (12), inhibit antigen-induced T-cell proliferation (13), remove antigen-specific T-cell factors (7, 8, 14), and selectively kill (in the presence of complement) lymphocytes that perform different immunological functions (6, 15).

The *I* region of the H-2 complex has been subdivided by recombinational analysis into five subregions, designated *I-A*, *I-B*, *I-J*, *I-E* and *I-C* (16, 17). Three Ia loci (*Ia-1*, *Ia-5*, and *Ia-3*) mark the subregions *I-A*, *I-E*, and *I-C*, respectively. The products of these loci, the Ia antigens, are integral cell-surface glycoproteins (18, 19). In the mouse, Ia antigens generally have two subunits, one approximately 35,000 in molecular weight (α) and one approximately 28,000 (β), that are noncovalently associated (20–22). It is not known whether antisera against Ia recognize one or both subunits. Human Ia molecules isolated from lymphoblastoid cell lines exhibit a similar structure (23). Ia molecules isolated from the spleen cells of guinea pigs have three distinct molecular structures: α and β components noncovalently associated; α and β components disulfide linked, and one or two β components (24).

Because of the genetic and phenotypic complexity of the H-2 complex, detailed analyses of its gene products have been difficult to carry out. One fruitful approach has been the use of the recently developed microsequence techniques for the analysis of picomole quantities of polypeptides. Partial amino acid sequence analyses of the transplantation antigens from mice (25–28), humans (29, 30), and guinea pigs (M. McMillan, B. D. Schwartz, M. J. Waxdal, E. M. Shevach, W. E. Paul, and L. Hood, unpublished data) have demonstrated striking sequence homologies and have raised provocative and intriguing questions about the genetic organization and evolutionary origins of the genes that encode these antigens (31).

Partial amino acid sequence comparisons have also been made among certain Ia polypeptides from humans (32), guinea pigs (33), and *I-A* subregion of the mouse H-2 complex (21, 22). Tentative amino acid sequence homologies were noted between the human and guinea pig β polypeptides. No homologies were demonstrable when α and β polypeptides from humans and a β polypeptide from guinea pigs were compared to their mouse *I-A* region counterparts.

In this paper we present partial amino acid sequence data on α and β polypeptides coded by the *I-E* or *I-C* (*I-EC*) subregion of the mouse H-2 complex. The new data demonstrate that striking homology exists between the α polypeptide of mice and the larger Ia polypeptide (p34) of humans. This striking homology is absent when the β polypeptide of mice is compared to the smaller Ia polypeptide (p29) of humans. We have published a preliminary report of the α polypeptide sequence elsewhere (22).

MATERIALS AND METHODS

Mice and Antisera. The genetic constitutions of the mice used in the preparation of the alloantiserum and in the isolation of the Ia antigens are given in Table 1.

Isolation of Ia Antigens. Splenic lymphocytes (2×10^8) were incubated for 5 hr in 4 ml of Hanks' balanced salt solution with 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes), 5% dialyzed fetal calf serum, and 1 mCi each of tritiated alanine, tyrosine, valine, isoleucine, phenylalanine, and leucine. The cells were then lysed with 4 ml of 10 mM Tris/0.15 M NaCl/0.5% Triton X-100 at pH 7.4. The debris was removed by centrifugation. The supernatant containing the radiolabeled Ia antigens was incubated with the specific alloantiserum and the resulting immune complexes were precipitated by either the gamma globulin fraction of rabbit anti-mouse IgG or by

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Abbreviations: $NaDodSO_4$, sodium dodecyl sulfate; PTH-amino acid, phenylthiohydantoin-amino acid; *I-EC*, *I-E* or *I-C*.

Table 1. Genetic constitution of recipient-donor-target strain combinations of mice used to prepare alloantiserum and Ia polypeptides

Strain	Haplotype	Regions of H-2* K	I A	I B	I J	I E	I C	I S	D
A.TH (recipient)	t/2	s	s	s	s	s	s	s	d
A.TL (donor)	t/1	s	k	k	k	k	k	k	d
BIO.HTT (target)	t/3	s	s	s	s	k	k	k	d

* Boxed regions indicate specificity of antiserum against target.

Staphylococcus aureus (34, 35). The immunoprecipitates were run on 10% sodium dodecyl sulfate ($NaDodSO_4$)/polyacrylamide gels. The Ia polypeptides were eluted from the gels, mixed with 2 mg of carrier ovalbumin, lyophilized, and dialyzed extensively against 0.001% $NaDodSO_4$.

Microsequence Analysis. The α and β polypeptides were each submitted to automatic sequence analysis twice with 2 mg of sperm whale apomyoglobin each time as a carrier and to monitor the performance of the sequenator. In one run, a Beckman model 890A sequenator was used and the individual labeled phenylthiohydantoin (PTH)-amino acid derivatives were separated on a Waters Associates high-pressure liquid chromatograph. In the second run, the Beckman model 890A sequenator had been modified as described by Wittman-Liebold (36). The labeled PTH derivatives were separated on a Du Pont 830 high-pressure liquid chromatograph with a Zorbax O.D.S. column. The radioactivity of the individual labeled PTH-amino acids fractions was determined on a Beckman model LS 230 scintillation counter.

RESULTS AND DISCUSSION

Ia Molecules of Different Subregions of Mice Exhibit Structural Similarities. The A.TH anti-A.TL antiserum, when tested against B10.HTT lysates, has potential reactivity with gene products encoded by the *S* and *G* (not shown) regions of the H-2 complex as well as the *I-E* and *I-C* subregions. Since Ia-like membrane antigens have not been demonstrated for the *S* or *G* regions in lymphocytes, this antiserum is probably reacting principally with *I* region molecules. Our antiserum cannot distinguish between molecules coded by the $I\text{-}E^k$ or $I\text{-}C^k$ subregions, and we will denote this ambiguity as the $I\text{-}EC^k$ subregion.‡ The two Ia polypeptides isolated by using this antiserum appear to be noncovalently associated because they migrate as two components on $NaDodSO_4$/polyacrylamide gel electrophoresis under reducing and nonreducing conditions. A typical gel profile is shown in Fig. 1. The larger component, designated α, migrates with an apparent molecular weight of 35,000, whereas the smaller component, denoted β, migrates with an apparent molecular weight of 28,000. These components migrate as relatively broad peaks, often with shoulders on them. This molecular weight heterogeneity may be due to the presence of additional polypeptides that have blocked NH_2 termini, or alternatively it may have its origins in carbohydrate heterogeneity or in complexities, not as yet understood, in the serology of the *I* region. However, since our antiserum putatively detects gene products encoded in the *I-EC* subregion, we tentatively conclude that the products we have isolated are Ia antigens. The Ia molecules encoded in the *I-EC* subregion, as well as those coded by the *I-A* subregion of mice appear to have α and β polypeptides of similar molecular weights. Thus, the Ia molecules from mice, humans, and guinea pigs demonstrate homology in their gross polypeptide structures.

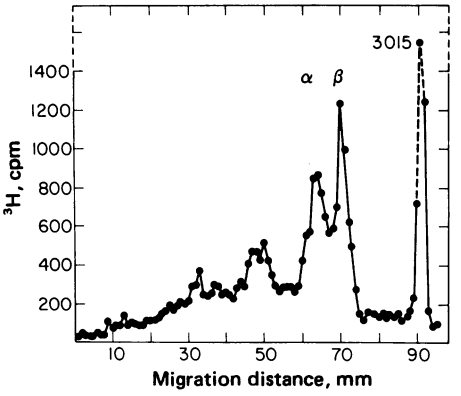

FIG. 1. $NaDodSO_4$/polyacrylamide electrophoresis pattern of $I\text{-}EC^k$ Ia molecules under reducing conditions. Migration distance in the gel is plotted on the abscissa and radioactivity of tritiated amino acids incorporated into protein is plotted on the ordinate. *Staphylococcus aureus* was used in this precipitation. Peak at 90 mm = 3015 cpm.

Microsequence Analysis of Ia Molecules. The repetitive yields of the carrier myoglobin throughout these runs ranged between 90 and 95%. The repetitive yields of the Ia polypeptides were comparable to those of their myoglobin carrier.

Typical amino acid sequence data obtained by the microsequencing technique are illustrated in Fig. 2. The sequence data from the Ia molecules exhibit a peculiarity not present in the application of this methodology to the presumably homogeneous H-2K and H-2D transplantation antigens in that minor peaks of radioactivity are sometimes observed, occasionally distributed over several residues. The same peculiarity was observed with the *I-A* subregion polypeptides (22). It is quite possible that these minor peaks of radioactivity result from unresolved technical problems. Alternatively, they may reflect heterogeneity in the Ia polypeptides. In this regard, it is interesting to note that with the admittedly limited number of residues incorporated into these polypeptides, we have never identified two major residues at a single position. Thus, a single major sequence is clearly discernible from these data (Table 2). However, because of the minor peaks and the limited sequence data available, no firm conclusions can be reached about the heterogeneity (or homogeneity) of Ia molecules.

One technical point should be stressed. During the production of the PTH-isoleucine residues, some isomerization to PTH-allo-isoleucine occurs (37). PTH-allo-isoleucine cochromatographs with PTH-phenylalanine under the conditions of high-pressure liquid chromatography. Thus, PTH-isoleucine also appears in the PTH-phenylalanine portion of the high-pressure liquid chromatogram (see Fig. 2, compare the isoleucine at positions 1, 7, and 8 with the phenylalanine at position 12).

The partial NH_2-terminal amino acid sequences of several Ia polypeptides from mice, humans, and guinea pigs are shown in Table 2. Three types of amino acid data are presented. (*i*) At certain positions major residues are unambiguously identified (e.g., tyrosine at position 13 in EC^k_α). (*ii*) At other positions positive identification has not been made although we are certain that the residue(s) identified in other Ia polypeptides

‡ An informal nomenclature committee at the Ir Gene Workshop at Asilomar in December, 1976, suggested that the Ia polypeptides of the mouse should be designated by a capital letter denoting the appropriate subregion with the haplotype designated by a superscript and the subunit component by a subscript. Thus the α polypeptide isolated from the *I-A* subregion of mice carrying the $H\text{-}2^k$ haplotype would be denoted A^k_α. The larger component of the human Ia molecule is designated p34 (polypeptide of 34,000 molecular weight), whereas the smaller component is designated p29.

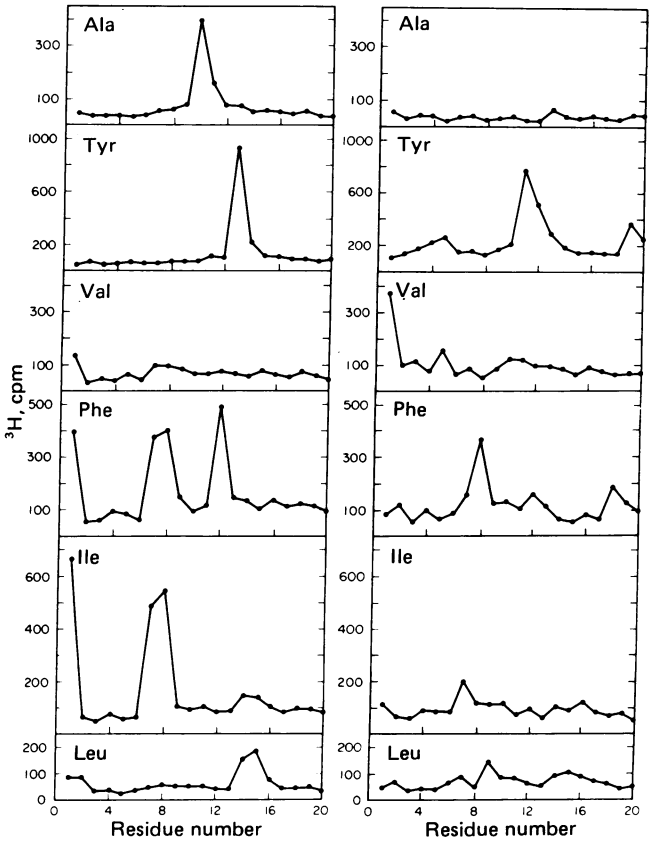

FIG. 2. (*Left*) Amino acid sequence data from the EC_α^k polypeptide. (*Right*) Amino acid sequence data from the EC_β^k polypeptide. Residue number is plotted on the abscissa and the radioactivity of individual tritiated PTH-amino acids is plotted on the ordinate. We have assigned residues using the following criteria: (*i*) A sharp rise in radioactivity at one position is followed by a more gradual decline at subsequent positions, e.g., Ala at position 10 in the α polypeptide. (*ii*) The amount of radioactivity in a particular PTH-residue must be comparable in the α and β polypeptides, i.e., Tyr at position 13 in the α polypeptide and Tyr at position 11 in the β polypeptide. We do not, therefore, assign Ile at position 7 in the β molecule because it is less than 25% that of the Ile residues in the α polypeptide. We believe there is a Leu at position 9 in the β molecule (the counts between α and β are comparable in two sequence runs) but have *not* assigned it due to technical difficulties in one run. (*iii*) All assigned peaks of radioactivity must conform to the 90–95% repetitive yield observed for myoglobin, e.g., Phe at positions 8 and 18 in the β molecule. We do not, therefore, assign Phe at position 12 or Val or Tyr at position 5 in the β molecule. We believe these small peaks of radioactivity are due to minor components or to unidentified technical difficulties.

is not present at this same position in a particular component. This is indicated by a dash (e.g., there is no tyrosine at position 16 in EC_α^k). (*iii*) Finally, there are positions in which no residue has been identified (e.g., position 2 in EC_β^k). These data are very limited in nature, but they do allow us to draw certain preliminary conclusions about homology relationships among the various Ia genes.

Partial Amino Acid Sequence Analyses Can Reveal Three General Types of Homology Relationships. To illustrate the types of conclusions we will draw from our data, the NH_2-terminal sequences of three known pairs of proteins of varying relatedness are given in Table 3. Below the complete sequences are partial sequences which would be obtained using the methodology employed in this paper. Three points can be made about these partial sequence comparisons.

First, the human and mouse β_2-microglobulin are highly homologous with ~80% sequence identity over their NH_2-terminal 18 residues. Five out of seven residues (~70%) are identical in the partial sequence comparisons (see boxed residues in Table 3). Second, human β_2-microglobulin and a constant region homology unit from the heavy immunoglobulin chain show ~34% identity after the appropriate placement of sequence insertions or deletions. Since sequence gaps cannot be placed in partial amino acid sequences, only 1/7 (~15%) of the residues that can be compared are identical. Thus low levels of sequence identity may indicate low levels of homology or it may represent a statistical fluctuation in the comparison of two unrelated samples. Finally, two unrelated proteins, human β_2-microglobulin and lysozyme, with less than 10% identity overall, show no identities in nine residues compared. Thus partial sequence data reveal three general types of homology relationships with somewhat arbitrary boundaries—striking sequence homology (50% or greater), questionable homology (10–50%), and no apparent homology (<10%).

Several Homology Relationships Are Evident from Partial Amino Acid Sequence Comparisons of Ia Polypeptides. (*i*) The EC_α^k polypeptide shows striking homology with its human counterpart (p34). Six of the nine positions that can be compared between the mouse EC_α^k polypeptide and the human p34 polypeptide are identical. That six out of nine positions are identical suggests that these two polypeptides are going to be very similar, at least in their NH_2-terminal regions. This observation raises several interesting points. First, homology relationships have now been demonstrated between two categories of genes in the major histocompatibility complex of humans and mice—certain Ia molecules and the transplantation antigens. Moreover, the genes encoding the transplantation antigens and certain Ia polypeptides appear to have maintained a close linkage within the major histocompatibility complex at least over the period of 75 million years during which mice and humans (i.e., mammals) have diverged from one another. Second, the Ia homology relationship raises interesting questions as to the location of the gene coding for the human p34 polypeptide. Is this gene in the *HLA-D* region which lies outside the genes coding for the human transplantation antigens, or is it located between the *HLA-A* and *B* genes? In the mouse, the *I* region is located between the *K* and *D* transplantation genes.

(*ii*) In contrast to the EC_α^k polypeptide, the EC_β^k polypeptide shows no identical residues in the four positions that it can be compared with in its human counterpart, p29. This apparent lack of homology must be treated with caution since the data are limited. Moreover, it is possible that homology exists in other parts of the molecules.

(*iii*) Both the EC_α^k and EC_β^k polypeptides are very different, respectively, from the A_α^k and A_β^k polypeptides. The α polypeptides differ at each of the ten positions that can be compared and the β polypeptides differ at each of the nine positions compared. The fact that the Ia molecules of the *I-A* and *I-EC* subregions are both comprised of two polypeptides of 35,000 and 28,000 molecular weights that are noncovalently associated, and the fact that serological crossreactions have been demonstrated between products of these two subregions (38, 39), suggest they may have diverged from common ancestral genes. However, if the *I-A* and *I-EC* subregion genes did share a common ancestor, then the sites for serological crossreactivity must be located in other parts of the molecule.

(*iv*) The α and β polypeptides of the *I-EC* subregion show no identity over the seven residues that can be compared. A comparison of the α and β molecules from the *I-A* region of two different haplotypes shows 0–29% sequence identity (22). Clearly, much more amino acid sequence data will have to be

Table 2. Amino acid sequences of the α and β polypeptides of Ia molecules*

	α polypeptide					β polypeptide					
			Mouse						Mouse		
	Mouse EC^k_α	Human p34	EC^k_α	A^b_α	A^k_α	Mouse EC^k_β	Human p29	Guinea pig 4,5	EC^k_β	A^b_β	A^k_β
1	Ile	Ile	Ile	—	—	Val	Gly	Ile	Val	—	—
2		Lys					Asp	Tyr			
3		Glu					Thr				
4		Glu	—	Ile	Ile		Pro	Pro			
5		(Arg)					Glu			Arg	
6		Val	—	Ala	Ala		(Arg)				
7	Ile	Ile	Ile	—	—	—	Phe	Phe			
8	Ile	Ile or Leu	Ile	—	(Val)	Phe	Leu	Leu	Phe	Val	Val
9		Gln		Val			Glu	Phe	—	Tyr	—
10	Ala	Ala	Ala	—	—		Gln				
11		Glu				Tyr	Val	Phe	Tyr	—	—
12	Phe	Phe	Phe	Tyr	Tyr						
13	Tyr	Tyr	Tyr	—	—						
14	Leu	Leu	Leu								
15	Leu	Asn	Leu								
16	—	Tyr		Val	Val				—	Tyr	Tyr
17		Asp	—	Tyr	Tyr			Tyr			
18	—	Phe				Phe			Phe		
19		Gln				Tyr			Tyr	—	—
20		Gly									
21											
25											
26			—	Tyr	Tyr				—	Tyr	—
27						Tyr			Tyr	—	—
28											
29											
30									—	Tyr	Tyr

*EC^k polypeptides are compared independently to human and guinea pig Ia antigens and to *I-A* subregion products. Dashes indicate the absence of a particular amino acid at a postion in which that amino acid has been assigned in another molecule. For example, the EC^k_β molecule does not have tyrosine at position 16. Accordingly, the dashes, as well as identified residues, are useful in homology comparisons. A box indicates identical residues in two molecules. A residue in parentheses indicates some uncertainty as to its identification. Sequence data were obtained from the following sources: human (32), guinea pig (33), and *I-A* subregion (22).

accumulated before the low levels of homology between α and β polypeptides in the *I-A* subregion and their apparent lack of homology in the *I-EC* subregion can be interpreted.

(*v*) Finally, it should be remembered that the *I* region comprises a multigenic system in which only a few gene products have been examined, and it is possible that, in time, new sets of molecules may be isolated from these same subregions which show quite different homology relationships.

New microsequence techniques are being developed that promise to provide far more detailed sequence analyses on these gene products. In the near future we will begin to unravel some of the intriguing problems posed by these partial amino acid sequence data.

M.M. is a Senior Gosney Fellow (California Institute of Technology). J.M.C. is supported by National Institutes of Health Fellowship AI05276. D.B.M. is a Fellow of the Cancer Research Institute. This work was supported by Grants AI10781, GM06965 and AI07757 from the National Institutes of Health.

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Table 3. Comparison of conventional NH_2-terminal amino-acid sequence data with partial NH_2-terminal sequence data for human β_2-microglobulin and proteins of varying relatedness*

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1. Mouse β_2-microglobulin	Ile	Gln	Lys	Thr	Pro	Gln	Ile	Gln	Val	Tyr	Ser	Arg	His	Pro	Pro	Glu	Asn	Gly
Human β_2-microglobulin	Ile	Gln	Arg	Thr	Pro	Lys	Ile	Gln	Val	Tyr	Ser	Arg	His	Pro	Ala	Glu	Asn	Gly
Mouse	Ile		—				Ile		Val	Tyr		Arg			—			
Human	Ile		Arg				Ile		Val	Tyr		Arg			Ala			
2. β_2-Microglobulin	Ile	Gln	Arg	Thr	Pro	Lys	Ile	Gln	Val	Tyr	Ser	Arg	His	Pro	Ala	Glu	Asn	Gly
Eu C_H3 (residues 342-359)	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr
β_2-Microglobulin	Ile		Arg				Ile	—	Val	Tyr		Arg		—	Ala			
Eu C_H3			Arg				Val	Tyr	—	Leu		—		Arg				
3. β_2-Microglobulin	Ile	Gln	Arg	Thr	Pro	Lys	Ile	Gln	Val	Tyr	Ser	Arg	His	Pro	Ala	Glu	Asn	Gly
Lysozyme	Lys	Val	Phe	Gly	Arg	Cys	Glu	Leu	Ala	Ala	Ala	Met	Lys	Arg	His	Gly	Leu	Asp
β_2-Microglobulin	Ile	—	Arg		—		Ile		Val	Tyr	—	Arg		—	Ala			
Lysozyme		Val	—		Arg			Leu	Ala	Ala	Ala	—		Arg	—		Leu	

* The sequence data were obtained from the sources: murine β_2-microglobulin (40), human β_2-microglobulin and C_H3 region of immunoglobulin Eu (41), and lysozyme (42).

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