PARTIAL AMINO ACID SEQUENCES OF THE HEAVY CHAINS OF HUMAN HLA HISTOCOMPATIBILITY ANTIGENS

Appella, E., Tanigaki, N., Fairwell, T. and Pressman, D.

Laboratory of Cell Biology, National Cancer Institute and Molecular Diseases Branch, National Heart and Lung Institute, Bethesda, Maryland 20014, Department of Immunology Research, Roswell Park Memorial Institute, Buffalo, New York 14263

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SUMMARY: HLA antigens are composed of two polypeptide chains, the heavy chain carrying the alloantigenic determinants and the light chain identified as β_2-microglobulin. By the use of microsequencing techniques, the amino terminal sequences of two HLA heavy chain preparations carrying different HLA allospecificities (A2 and B7, 14) have been determined through position 22. With the exception of one position, the two are identical through 22 residues. There is also a considerable degree of homology with mouse H-2 antigens. These data support the hypothesis that the HLA-A and B gene products have evolved by gene duplication from a common ancestral gene and that these gene products have great homology in the primary structure.

Human HLA histocompatibility antigens are composed of two polypeptide chains, one heavy chain of molecular weight about 45,000 carrying the alloantigenic specificity (1, 2) and one light chain of molecular weight 11,500 that has been identified as β_2-microglobulin (3, 4, 5). It has been shown that β_2-microglobulin has marked homology in the amino acid sequence with C_H3 domain of immunoglobulin IgG (6). Recent preliminary studies have also suggested the presence of immunoglobulin-like domains in the HLA and H-2 heavy chains (7). On this structural homology and on several other similarities, for example, their extreme polymorphism, their cell surface location and their role in regulating the immune response, it has been proposed that the immunoglobulin genes descended from the histocompatibility genes (8). A more detailed chemical analysis, however, is necessary to support such a proposal and to provide information on the structure of their alloantigenic sites.

Although HLA antigens are highly polymorphic, accumulating evidence from several sources has suggested only a limited degree of heterogeneity (9, 10).

We have isolated chemically and immunologically pure preparations of papain-solubilized HLA antigens from cultured human lymphoid cells (10, 11). The HLA heavy chain preparations of a molecular weight 33,000 obtained from such two HLA preparations differing in the evident HLA allospecificity (i.e. A2 and B7, 14) contained the same amino-terminal (glycine) and the same carboxyl-terminal (serine). The carbohydrate content of the heavy chain was 12.9% making the polypeptide molecular weight of approximately 30,000. The two heavy chain preparations have been found to have small but significant differences in the amino acid compositions and tryptic peptide maps (10). We now report the partial amino-terminal sequence of the two HLA heavy chain preparations. These data will allow some conclusions about the origin and the diversity of the major histocompatibility antigens.

MATERIALS AND METHODS: HLA antigens were prepared as described (11). The preparations used in this study were from a human lymphoid cell line, RPMI 1788. HLA specificities of this cell line are HLA-A2, B7 and B14. The HLA-C specificities have not been identified. Two different preparations, HLA-A2 and HLA-B7, were isolated. The indicated HLA specificity of the preparations is the evident specificity followed during isolation. The HLA-B7 preparation contains also B14 specificity since the preparation has been found to bind to anti-B14 alloantiserum (Fe 122/10). The HLA-C specificities may be present in these preparations but were not followed. Separation of the heavy and light polypeptides by acid dissociation of papain-solubilized HLA antigens have been described in detail (12). NH_2-terminal sequences on 10-30 nmoles quantities of the heavy chain preparations were determined in an up-to-date Beckman 890B sequencer using a modified program for 0.1 M Quadrol and Me_2-allylamine (13). Phenylthiohydantoins were identified both by high pressure liquid chromatography (14) and by hydrolysis with HI (15). The mass spectral analysis (chemical ionization) of the phenylthiohydantoins was performed on a Finnigan 1015 mass spectrometer equipped with a direct inlet solid-probe and PDP-8e digital computer. Isobutane was used as the reagent gas and the source

was maintained at 225°C and the probe was heated up to 250°C over a 90-120 sec. period (16).

RESULTS AND DISCUSSION: The yields of PTH derivatives from three separate sequenator runs are shown in Fig. 1. The repetitive yields were calculated

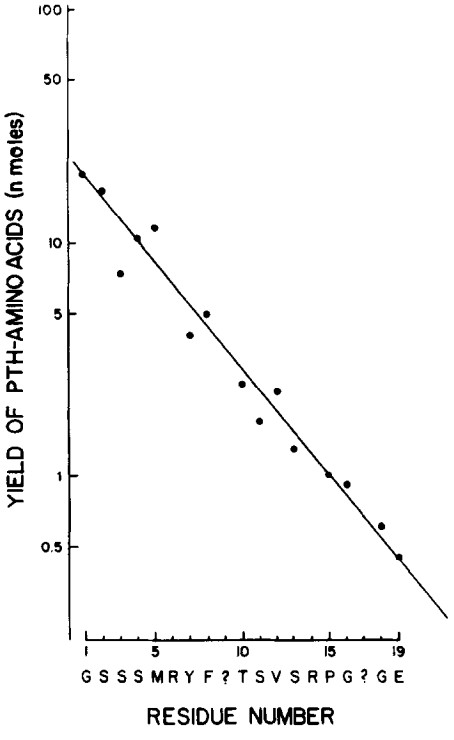

Fig. 1. Yields of PTH amino acids obtained by degrading HLA-A2 preparation (30 nmoles) in the presence of PTH norleucine.

to be 84-86%. MTH-Ala of PTH-nLeu were added to the heptafluorobutyric acid to prevent losses during extraction and conversion of PTHs (17). In the experiments in which 10 nmoles were used, succinylated polyornithine was added to the cup as a carrier (18). Fig. 2 shows some representative mass spectra of PTHs obtained during automated Edman degradation. At each step a clear pattern was obtained. At position 9 traces of Tyr and other small peaks were found indicating some degree of microheterogeneity. Position 17

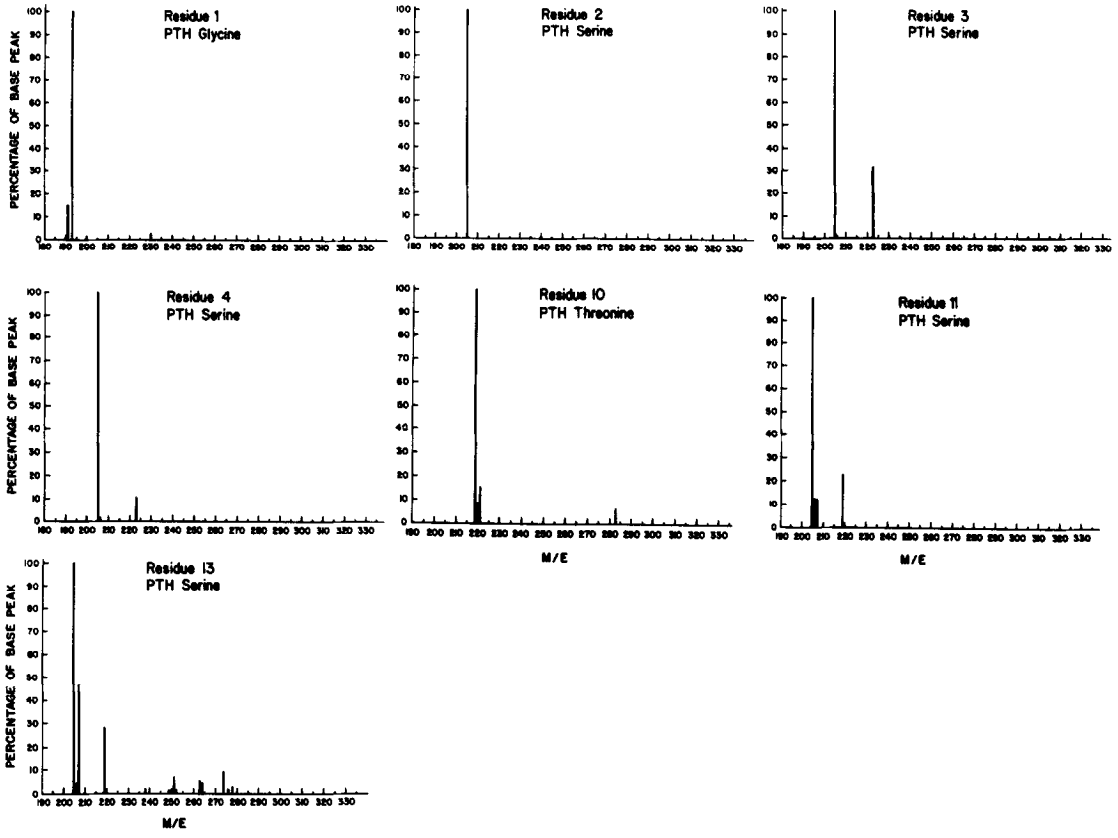

Fig. 2. Mass spectra of PTH amino acids obtained during automatic Edman degradation of HLA-A2 preparation.

could not be identified, and position 20 has been assigned tentatively to be Ser, due to the low yield of the PTH derivative.

In the chemical ionization mass spectral analysis, the serine and threonine phenylthiohydantoins are identified as the dehydro derivatives only because of the relative ease with which these derivatives undergo molecular rearrangement with the elimination of a molecule of water at the temperatures used. However, in many instances (residues 3 and 4) both serine and threonine produce a molecular ion (m/e 223 for serine and 237 for threonine).

Partial NH_2-terminal sequences have been also obtained from a second preparation which contained B7 and B14 allospecificities. A comparison with the

Table 1. Partial N-Terminal Sequences of Human and Mouse Transplantation Antigens

	Residue Position																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
HLA-2	G	S	S	S	M	R	Y	F	?	T	S	V	S	R	P	G	?	G	E	(S)	B	F
HLA-7, 14	—	—	—	—	—	—	—	—	Y	—	—	—	—	—	—	—	?	—	—	—	—	—
[a] HLA-7, 12	—	—	—	—	—	R/V	—	—	—	—	A	—	—	—	—	—	?	—	—	?	?	—
[b] H-2K^k	M	P	H	?	L	R	Y	F	H	?	A	V	?	I	P	?	L	?	K	P	F	A
[b] H-2K^d	—	?	—	?	—	—	—	—	V	?	—	—	?	R	—	?	?	?	?	P	?	Y

[a] Data from reference 19

[b] Data from references 20, 21 and 22

? No information is available on the residue at this position

- Indicates identical residue at this position

preparation containing A2 specificity reveals a close identity (Table 1), with one exception at position 9. At position 9 HLA-A2 sample had only a trace of Tyr and no other identifiable PTH-amino acids, whereas the HLA-B7, 14 sample had a clear Tyr. Position 17 had in both cases a PTH-amino acid which could not be identified. These results confirm the observations by Terhorst et al. of a unique sequence for the heavy chains from three different HLA antigens (19). It is noteworthy to mention that their preparation containing specificity, B7, 12 had more than one residue at position 6 (Arg plus Val) and differed from HLA-B7 of an homozygous individual at position 11 with an interchange of Ala for Ser, whereas our HLA-B7, 14 mixture is indistinguishable from HLA-A2 preparation for the first 22 residues. From these limited sequence data it is clear, as it was from the amino acid compositions and tryptic peptide maps (10) that primary structures of the HLA specificities studied here are very similar. The differences in the first 22 amino acids do not suggest a variable region as is present in the immunoglobulins. A comparison with the available sequences from mouse histocompatibility antigens (Table 1) indicates that there are more differences between H-2 specificities than between HLA specificities (20, 21, 22). There is, at the moment, no explanation for such striking difference but this extent of diversity among the polymorphic forms of the human and mouse transplantation systems may in the future help in understanding the different types of genetic or evolutionary mechanisms which have been suggested for rat and rabbit immunoglobulin allotypes (23).

The question whether HLA antigens have structural homology with immunoglobulin molecules remain unanswered. The very limited regions examined preclude any significant analysis of the type carried out by Fitch and Margoliash (24) and more data have to be collected in order to examine such homology.

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