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SESSION 2

Chemistry of I-Region Gene Products

CHAIRMAN: DR. JONATHAN UHR

Presence of a Non-polymorphic Polypeptide Chain in Ia Immunoprecipitates. Patricia P. Jones, Donal B. Murphy, and Hugh O. McDevitt. Department of Medicine/Division of Immunology and Department of Genetics, Stanford University School of Medicine, Stanford, Calif. 94305.

All immunoprecipitates prepared from NP-40 extracts of ^{35}S-methionine-labeled mouse lymphocytes using anti-Ia alloantisera contain a common polypeptide chain, MW 31,000 daltons. The electrophoretic mobility of this molecule in two-dimensional polyacrylamide gel electrophoresis is the same for Ia antigens coded for by loci in the *I-A* and *I-E* subregions and for molecules obtained from mice of different *H-2* haplotypes and genetic backgrounds. Immunoprecipitates obtained with anti-H-2K, anti-H-2D, and anti-immunoglobulin antisera do not contain this polypeptide chain. This molecule therefore appears to be a nonpolymorphic protein unique to Ia immunoprecipitates. Because it is nonpolymorphic, this chain probably is not recognized by alloantisera and hence may be precipitated only because it is complexed to polymorphic Ia proteins. Thus the occurrence of this invariant chain with Ia antigens appears to be analogous to that of β_2 microglobulin, a nonpolymorphic polypeptide chain coprecipitated with H-2K and H-2D antigens.

Chemical Characterization of Murine Ia Antigens. M. McMillan, J. M. Cecka, J. G. Frelinger, D. Murphy, J. A. Frelinger, H. O. McDevitt, and L. Hood. Division of Biology, California Institute of Technology, Pasadena, Calif. 91125; [1] Department of Pathology, Yale School of Medicine, New Haven, Conn. 06510; [2] Department of Microbiology, USC School of Medicine, Los Angeles, Calif. 90033; and [3] Division of Immunology, Department of Medicine, Stanford University School of Medicine, Stanford, Calif. 94305.

Several products of the I region of the mouse H-2 complex have been characterized by micropeptide analysis, microsequence analysis, and two-dimensional gel (IEF/SDS-PAGE) analysis. Ia antigens have been isolated using the biosynthetic radiolabeling technique together with highly specific alloantisera and indirect immunoprecipitation. Chromatography of tryptic peptides prepared from the α and β polypeptides of Ia antigens encoded by the I-A and I-EC subregions will be compared from both spleen and epidermal cell preparations. These data, in conjunction with partial N-terminal amino acid sequence data on Ia antigens from the $I\text{-}A^b$, $I\text{-}A^k$, $I\text{-}A^d$, $I\text{-}A^s$, $I\text{-}EC^k$ and $I\text{-}EC^d$ subregions and haplotypes will be discussed in terms of homology relationships and haplotype-associated differences in the structures of these Ia polypeptides. Two-dimensional gel patterns of isolated α and β polypeptides will also be presented. The implications of these data will be discussed as they relate to the genetic organization of the *I*-region.

Detection of Two Independent Ia-Like Antigens Determined by Genes Linked to Rat MHC. Nobukata Shinohara and David H. Sachs, Immunology Branch, National Cancer Institute, NIH, Bethesda, Md. 20014.

The mouse alloantiserum B10.D2 anti-B10.BR ($H\text{-}2^d$ anti-$H\text{-}2^k$) crossreacted with rat lymphocyte surface glycoproteins with characteristics of Ia antigens. Sequential precipitation analysis on solubilized radiolabeled Lewis rat spleen cell alloantigens pretreated with BN anti-Lewis (Ag-B3 anti-Ag-B1) alloantiserum revealed that the Ia-like antigens detected by the mouse alloantiserum also reacted with the rat anti-Ia antibodies. The rat alloantiserum also detected another set of Ia-like antigens which did not crossreact with the mouse alloantibody. Precipitation analysis using congenic rat strains confirmed that all Ia-like antigens precipitated by the rat alloantibody were encoded by Ag-B-linked genes. Thus the shared Ia-like antigen must also be the product of Ag-B-linked gene(s) or be physically associated with such products. The existence of two separable Ia antigens suggest the existence of more than one sublocus coding for Ia antigens within the rat MHC. The determinant detected on the crossreactive molecule by the mouse alloantibody is probably distinct from that detected by the rat alloantibody since the mouse alloantibody reacted similarly with both Lewis and BN lymphocytes.

Comparison of Primary Structures of Ia Products from the *I-A* and *I-E/C* Subregions. John H. Freed. Johns Hopkins University School of Medicine, Baltimore, Md. 21205.

The primary structures of α and β chains from both *I-A* and *I-E/C* subregions of several haplotypes were examined by comparative ion exchange chromatography of tryptic peptides. The materials examined were obtained from NP-40 extracts of biosynthetically radiolabeled normal spleen cells. The preparations were subjected to LcH-Sepharose affinity chromatography prior to immunoprecipitation with specific alloantisera; α and β chains were separated by polyacrylamide gel electrophoresis and/or hydroxylapatite chromatography in SDS. Comparative chromatographies of tryptic peptides from α and β chains reveal that corresponding chains from the highly cross-reactive $I\text{-}A^k$ and $I\text{-}A^r$ gene products share approximately 65–75% of their peptides. Preliminary results suggest that for the $I\text{-}E/C^k$ and $I\text{-}E/C^r$ products the α chains share all peptides, whereas the β chains share only 65% of their peptides. This latter result, considered in light of the proposed assignment of Ia.7 to the *I-E* subregion (David and Cullen, in press), suggests that the $I\text{-}E^k$ specificity Ia.22 (which $I\text{-}E^r$ lacks) may be carried specifically on the E_β^k chain. In addition, homologous chains (α or β) from *I-A* and *I-E/C* subregions share fewer peptides than do H-2K and H-2D molecules of a given haplotype implying that if *I* subregions arose by gene duplication, they have diverged more than have the *K* and *D* regions. These peptide mapping results are completely compatible with the N-terminal amino acid sequence analyses obtained by other laboratories and represent a valuable extension of sequence observations because the present method allows sampling of the entire molecule and not just the N-terminal 25 amino acid residues.