MOLECULAR SIMILARITIES BETWEEN THE Qa-2 ALLOANTIGEN AND OTHER GENE PRODUCTS OF THE 17TH CHROMOSOME OF THE MOUSE*

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Recent immunogenetic analysis of the region between H-2D and Tla on the 17th chromosome of the mouse has revealed the presence of previously unrecognized genes which specify cell surface antigens (1). One part of this area, the Qa-2 region, contains two such genes, one of which codes for an antigen on thymus, spleen, and lymph node, and the other which is expressed on spleen and lymph node only (2). It has been of interest to us to examine biochemically these gene products. We report here that the molecule which carries Qa-2 on lymph node cells (LNC) and spleen is composed of a large subunit of approximately the same molecular weight as H-2 and TL molecules, and a small subunit which can be serologically identified as β2-microglobulin (β2M).

Materials and Methods

Preparation of Radioiodinated Cells. Cells from the thymus, spleen, or lymph nodes were prepared and iodinated as described previously (3). Cells were lysed in phosphate-buffered saline (PBS) containing 0.5% Nonidet P40 (NP40) (Shell Chemical Corp., New York) and the lysates were centrifuged at 1,200 g. Samples were then dialyzed overnight at 4°C against PBS. After dialysis, protein-associated radioactivity was determined (4).

Sera

Rabbit anti-mouse Ig. Rabbit anti-mouse Ig (RAMIg) (5) contained specificities against μ, γ, κ, α, and λ chains and was a pool of several sera prepared against purified myeloma proteins.

Goat anti-mouse Ig. Goat anti-mouse Ig (GAMIg) contained specificities against γ and L chains.

Goat anti-rabbit Ig. Goat anti-rabbit Ig (GARIg) (6) contained antibodies against rabbit γ and L chains.

αK b (7). This serum was a gift from Dr. Jan Klein (University of Texas Southwestern Medical School) and was produced in (D2.GD × B10.D2)F1 mice against C57BL/6 lymphoid cells.

αD b. This serum was produced in (HTI × B6-H-2K)F1 mice against the C57BL/6 leukemia EL4, and detects H-2D b when tested against cells from HTH mice.

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**Results and Discussion**

Using αQa-2 to precipitate antigens from Qa-2+ LNC (C57BL/6, HTH, or B6.K2 mice) two proteins were resolved on 7.5 and 10% acrylamide gels, with apparent mol wt of approximately 43,000 and 12,000 daltons (Fig. 1). The precipitate contained approximately 0.15% of the total labeled protein as compared to 1-4% for H-2. Immunoprecipitates from lysates of spleen cells also show these peaks, but in about 50% of the quantity of Qa-2 found on LNC. No Qa-2 was found on thymocytes by this method. (The reaction of αQa-2 serum with thymocytes in the cytotoxicity assay is probably due to a second antigen specified by the Qa-2 region.) The amounts of Qa-2 recovered from thymus, spleen, and lymph node cells parallel the number of mature T cells found in these lymphoid organs. LNC from Qa-2+ (B6.H-2k or B6.K1) mice did not yield peaks with the αQa-2. Since B6.K1 and B6.K2 are genetically identical except for a small portion of the 17th chromosome adjacent to Qa-2, the peaks detected can...
be ascribed to the Qa-2 antigen which is detected on LNC with the cytotoxicity assay.

Qa-2 was shown to reside on a molecule separate from H-2D by sequential precipitation of HTH LNC lysate with αDβ antiserum followed by αQa-2. The first precipitation removed 85% of the H-2D peak but did not reduce the quantity of the Qa-2 precipitated subsequently (results not shown). By an analogous procedure the Qa-2 molecule was shown to be distinct from H-2K because prior precipitation of Kβ did not reduce the amount of Qa-2 subsequently precipitated (Fig. 2).

Vitetta et al. (5) have recently demonstrated that treatment of splenic or thymic lysates with rabbit anti-rat β2M removes both H-2 and TL molecules. Likewise, prior treatment of lymph node lysates with αβ2M completely eliminated the capacity of αQa-2 to precipitate a Qa-2 peak from the NP40 lysate, while prior treatment with normal rabbit serum had no effect (Fig. 3). In the previous studies performed by Vitetta et al. (5), prior treatment of splenic lysates with anti-H-2 depleted all radioactivity which could be subsequently recognized by αβ2M. However, since the Qa-2 antigen contains less than 5% of the radioactivity found in H-2, these molecules went undetected.

The molecular similarity between H-2D, H-2K, TL, and Qa-2 is remarkable in that they are all molecules of approximately 45,000 daltons which are associated with β2M (5, 8-11). Moreover, to the left of H-2K is the Tl locus in which map the genes specifying the F9 antigen. This antigen also has a mol wt of 44,000 and a 12,000 subunit (12) which does not carry immunologically recognizable β2M determinants (13). This chromosome therefore contains a family of molecules, related by size, subunit structure, genetic linkage, membrane location, and antigenicity, having most likely arisen from a common ancestor gene by tandem duplication.

It is of interest that one of the two antigens determined by the Qa-2 region appears to be expressed on peripheral T cells and is absent from thymocytes (2), thus being the first known alloantigen with this pattern of expression. The transition from thymocyte to peripheral T cell in a TL+, Qa-2+ mouse therefore involves the loss of TL and the acquisition of Qa-2. This is reminiscent of the loss
Fig. 3. The effect of preclearing radioiodinated lysate of C57BL/6 LNC with αβ2M on Qa-2 antigen precipitation with αQa-2. (A) Precleared with normal rabbit sera (control). (B) Precleared with αβ2M.

of F9 and gain of H-2 (14) which occurs during embryogenesis, and of the reduction of H-2D which accompanies the expression of TL during thymocyte development (15). Presumably, all of these molecules are not only biochemically but functionally analogous. In light of evidence suggesting that F9 functions in cellular recognition in the early embryo (16) and that the H-2 region is important in cellular interaction in the immune response (17, 18) it is tempting to speculate that a variety of cell to cell interactions may be mediated by the family of molecules to which Qa-2 belongs.

Summary

The alloantigen Qa-2, whose gene is located on the 17th chromosome between H-2D and Tla, is identified as a molecule of 43,000 daltons which is associated with β2-microglobulin. Qa-2 comprises approximately 0.15% of the iodinateable cell surface protein of lymph node cells. Sequential precipitations demonstrated that Qa-2 is distinct from H-2D and H-2K molecules.

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References

1. Stanton, T. H., and E. A. Boyse. 1976. A new serologically defined locus, Qa-1, in the Tla-region of the mouse. Immunogenetics. 3:525.
2. Flaherty, L. 1976. The Tla region of the mouse: identification of a new serologically defined locus, Qa-2. Immunogenetics. 3:533.
3. Baur, S., E. S. Vitetta, C. J. Sherr, I. Schenkein, and J. W. Uhr. 1971. Isolation of heavy and light chains of immunoglobulin from the surfaces of lymphoid cells. J. Immunol. 106:1133.

4. Vitetta, E. S., J. Forman, and J. R. Kettman. 1976. Cell surface immunoglobulin. XVIII. Functional differences of B lymphocytes bearing different surface immunoglobulin isotypes. J. Exp. Med. 143:1055.

5. Vitetta, E. S., M. D. Poulak, J. Klein, and J. W. Uhr. 1976. Beta 2-microglobulin is selectively associated with H-2 and TL alloantigens on murine lymphoid cells. J. Exp. Med. 144:179.

6. Vitetta, E. S., J. W. Uhr, and E. A. Boyse. 1975. Association of a $\beta_2$-microglobulin-like subunit with H-2 and TL alloantigens on murine thymocytes. J. Immunol. 114:252.

7. Capra, J. D., E. S. Vitetta, D. G. Klapper, J. W. Uhr, and J. Klein. 1976. Structural studies on protein products of murine chromosome 17: partial amino acid sequence of an H-2K$^b$ molecule. Proc. Natl. Acad. Sci. U.S.A. 73:3661.

8. Silver, J., and L. Hood. 1974. Detergent-solubilised H-2 alloantigen is associated with a small molecular weight polypeptide. Nature (Lond.). 249:764.

9. Natori, T., M. Katagiri, N. Tanigaki, and D. Pressman. 1974. The 11,000-dalton component of mouse H-2: isolation and identification. Transplantation (Baltimore). 18:550.

10. Rask, L., J. B. Lindblom, and P. A. Peterson. 1974. Subunit structure of H-2 alloantigens. Nature (Lond.). 249:833.

11. Ostberg, L., L. Rask, H. Wigsell, and P. A. Peterson. 1975. Thymus leukemia antigen contains $\beta_2$-microglobulin. Nature (Lond.). 253:375.

12. Vitetta, E. S., K. Artzt, D. Bennett, E. A. Boyse, and F. Jacob. 1975. Structural similarities between a product of the T/t-locus isolated from sperm and teratoma cells, and H-2 antigens isolated from splenocytes. Proc. Natl. Acad. Sci. U.S.A. 73:3215.

13. Dubois, P., M. Fellous, G. Gachelin, F. Jacob, R. Kemler, D. Pressman, and N. Tanigaki. 1976. Absence of a serologically detectable association of murine $\beta_2$-microglobulin with the embryonic F9 antigen. Transplantation (Baltimore). 22:467.

14. Artzt, K., and D. Bennett. 1975. Analogies between embryonic (T/t) antigens and adult major histocompatibility (H-2) antigens. Nature (Lond.). 256:545.

15. Boyse, E. A., E. Stockert, and L. J. Old. 1968. Isoantigens of the H-2 and Tla loci of the mouse: interactions affecting their representation on thymocytes. J. Exp. Med. 128:85.

16. Artzt, K., D. Bennett, and F. Jacob. 1974. Primitive teratocarcinoma cells express a differentiation antigen specified by a gene at the T-locus in the mouse. Proc. Natl. Acad. Sci. U.S.A. 71:811.

17. Shearer, G. M. 1974. Cell-mediated cytotoxicity to trinitrophenyl-modified syngeneic lymphocytes. Europ. J. Immunol. 4:527.

18. Zinkernagel, R. M., and P. C. Doherty. Restriction of in vitro T cell-mediated cytotoxicity in lymphocytic choriomeningitis within a syngeneic or semiallogeneic system. Nature (Lond.). 248:701.