Brief Definitive Report

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)F_1 mice against C57BL/6 lymphoid cells.

αD^b. This serum was produced in (HTI × B6-*H-2*^{K})F_1 mice against the C57BL/6 leukemia EL4, and detects H-2D^b when tested against cells from HTH mice.

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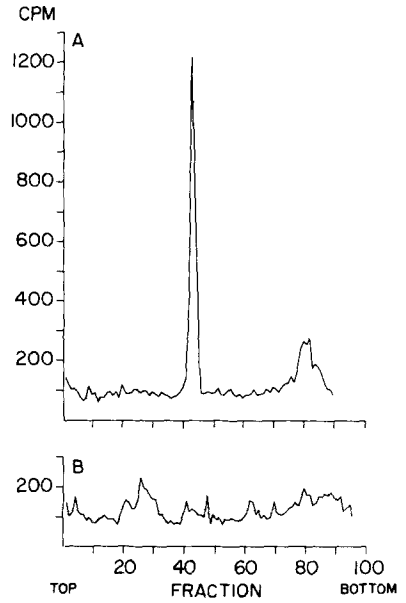

FIG. 1. SDS-polyacrylamide-gel electrophoresis of radioiodinated Qa-2 antigen. Immunoprecipitates were reduced and electrophoresed on a 7.5% gel. (A) Precipitate of αQa-2 and C57BL/6 LNC. (B). Precipitate of αQa-2 and B^6-*H-2*^{k} LNC (control).

αQa-2. This serum was produced by immunizing B6.K1 mice with C57BL/6 lymphocytes and thymocytes (2).

αβ2M. Rabbit anti-rat β2M (5) was produced as described previously and was cross-reactive with mouse β2M. Previous studies (5) have shown that this serum can precipitate the β2M-containing molecules H-2 and TL.

Immunoprecipitation. Dialyzed lysates were depleted of B-cell Ig by treatment with RAMIg and GARIg as described previously (5, 6). The alloantigens were then precipitated with alloantisera and GAMIg. Precipitates were washed and solubilized at 56°C in 1% sodium dodecyl sulfate (SDS) containing 2% 2-mercaptoethanol (2ME) and 6 M urea at pH 6.8. Samples were electrophoresed along with markers of ^{3}H-μ and L chains on 10 cm SDS polyacrylamide gels at 25 V for 18 h. Gels were sliced into 1-mm fragments with a Mickle gel slicer (Brinkmann Instruments, Inc., Westbury, N. Y.). Samples were counted as described previously (3).

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-2*^{K} 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

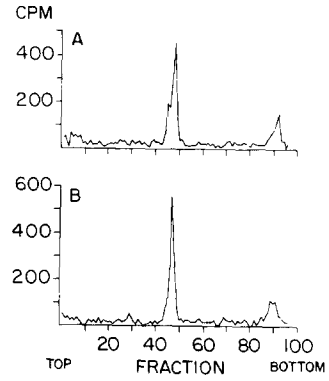

FIG. 2. The effect of preclearing radioiodinated lysate of C57BL/6 LNC with αK^b on Qa-2 antigen precipitated with αQa-2. (A) Precleared with αK^b. (B) Precleared with normal mouse serum (control).

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 αD^b 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 K^b 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 $\alpha\beta$2M 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 $\alpha\beta$2M. 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 *T/t* 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

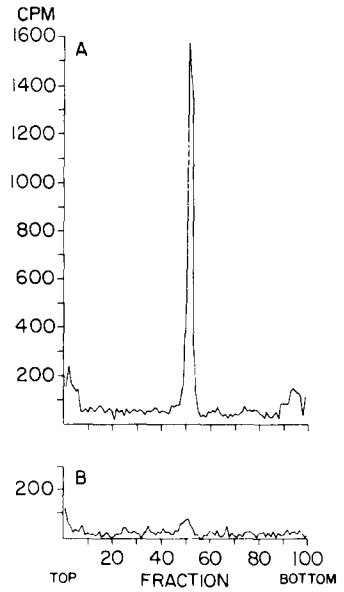

FIG. 3. The effect of preclearing radioiodinated lysate of C57BL/6 LNC with $\alpha\beta$2M on Qa-2 antigen precipitation with αQa-2. (A) Precleared with normal rabbit sera (control). (B) Precleared with $\alpha\beta$2M.

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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