

# BETA 2-MICROGLOBULIN IS SELECTIVELY ASSOCIATED WITH H-2 AND TL ALLOANTIGENS ON MURINE LYMPHOID CELLS\*

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$\beta 2\mu$ -microglobulin ( $\beta 2\mu$ )<sup>1</sup> is a carbohydrate-free protein which has a mol wt of 11,700 (1) and a considerable structural homology with IgG, particularly the C3 $\gamma$  domain (2-4). In all species thus far studied,  $\beta 2\mu$  or an analogous 12,000 mol wt peptide appears to be noncovalently bound to antigens coded for by the major histocompatibility complex (MHC) which are present on the surface of a wide variety of cells (5-22). In addition, a 12,000 mol wt polypeptide has also been found to be noncovalently associated with the major 44,000 peptide of two other products of chromosome 17 in the mouse: the thymus leukemia (TL) antigen on thymocytes (9, 10, 23) and a T/t antigen (F9) on sperm and a teratoma line (24). These two antigens are coded for by genes to the right and to the left of the MHC, respectively.

The purpose of this study was to investigate further the identity of the 12,000 mol wt protein associated with H-2 and TL antigens by examining their reactivity with rabbit antirat  $\beta 2\mu$  serum. Because of the great evolutionary conservation of the  $\beta 2\mu$  molecule (2-4, 25-28, and footnote 2), we assumed that this antiserum would cross-react with mouse  $\beta 2\mu$ . Results indicate that virtually all the molecules in detergent lysates of murine lymphoid cells which can be precipitated with this antiserum have mol wts greater than 100,000 and consist of subunits of 44,000 and 12,000. Moreover, virtually all the  $\beta 2\mu$ -reactive molecules can be identified as either H-2 or TL.

## Materials and Methods

*Iodination of Cells.* Thymuses or spleens from 6-8-wk-old A/J mice (The Jackson Laboratory, Bar Harbor, Maine) were prepared, iodinated, and lysed as described previously (9, 29). Small

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<sup>1</sup> *Abbreviations used in this paper:*  $\beta 2\mu$ ,  $\beta 2$ -microglobulin; GAMIg, goat antimouse immunoglobulin; GARIG, goat antirabbit immunoglobulin; 2ME, 2-mercaptoethanol; NRS, normal rabbit serum; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; RAMIg, rabbit antimouse immunoglobulin; SDS, sodium dodecyl sulfate; TL, thymus leukemia.

<sup>2</sup> Poulik, M. D., C. J. Shinnick, and O. Smithies. Partial amino acid sequence of rabbit and rat B2-microglobulin. Submitted for publication.

aliquots of the lysates were precipitated in 5% trichloroacetic acid (TCA) to determine protein-associated radioactivity.

### Immunoprecipitations

#### Antisera

**RABBIT ANTIRAT  $\beta 2\mu$ .** Groups of 10-15 normal rats were treated with chromium chloride to induce tubular damage. The urines were collected daily, pooled, and after dialysis and lyophilization were subjected to gel chromatography on Bio-rad P-100 to obtain fractions containing low molecular weight proteins (28). Such fractions were pooled and then subjected to ion-exchange chromatography (DE-52, Whatmann). A purified protein was obtained, was partially sequenced (42 residues were obtained),<sup>2</sup> and identified by its homology with human  $\beta 2$ -microglobulin. This protein was used for production of specific antisera in New Zealand rabbits. The animals were immunized with 250  $\mu\text{g}$  of protein per rabbit in Freund's adjuvant by three intramuscular injections each given 14 days apart. 7 days after the third injection blood was collected, and the antisera obtained were absorbed with 10 mg of normal rat serum per ml of the antiserum. Using the pure rat  $\beta 2$ -microglobulin, only one line of precipitation was observed (at concentrations ranging from 0.1 to 0.5%) on immunoelectrophoresis. The same antisera tested against lyophilized urine pools of the rats used as a source of  $\beta 2\mu$  also showed a single precipitin line with the mobility of the rat  $\beta 2$ -microglobulin, but with certain antisera two precipitin lines were observed by double diffusion technique. However, the precipitin lines could be absorbed with highly purified rat  $\beta 2$ -microglobulin.

**ANTI-H-2K<sup>k</sup>.** This serum, prepared and assayed as described previously (30), contained specificities against H-2.23, H-2.25, and H-2.11, all of which are present on the same H-2K<sup>k</sup> molecule. The antiserum also reacts with antigens coded for by the IA<sup>k</sup> subregion.

**ANTI-H-2D<sup>d</sup>.** This antiserum was produced by hyperimmunization of (A.BY  $\times$  B10.RIII(71NS)F, hybrids with the spleen, lymph nodes, and thymus of B10.A(5R) mice. The strain combination is such that only antibodies against antigens controlled by the D region of H-2<sup>d</sup> can be produced (the donor and the recipient share the K end). Panel tests done with the antiserum (Table I) indicate that the antiserum contains cytotoxic antibodies against the H-2.4 antigen controlled by the H-2D<sup>d</sup> allele.

**ANTI-TL-1.2.3 (9).** This antiserum was prepared by immunizing A/TL<sup>-</sup> mice with ASL-I leukemia cells and was the generous gift of Dr. E. A. Boyse, Immunology Division, Memorial Sloan-Kettering Cancer Center, New York.

**RABBIT ANTIMOUSE Ig (RAMIg) (31).** RAMIg contained specificities against mouse  $\mu$ -,  $\gamma$ -,  $\alpha$ -,  $\kappa$ -, and  $\lambda$ -chains and was prepared by immunizing rabbits with highly purified myeloma proteins (MOPC-104E, TEPC-15) and serum IgG.

**RABBIT ANTIBACTERIOPHAGE  $\phi\chi$  174 (31).** Its Ig concentration was approximately the same as that found in the RAMIg serum.

**GOAT ANTIMOUSE Ig (GAMIg) (31).** This antiserum contained specificities against mouse  $\gamma$ - and L chains.

**GOAT ANTI-RABBIT Ig (GARIG) (31).** This antiserum contained specificities against rabbit  $\gamma$ - and L chains.

**STAPHYLOCOCCUS AUREUS.** Cowan I strain (ATCC 12598) (*S. aureus*) (32). *S. aureus* was a gift from Dr. Eugene Rosenblum, Department of Microbiology, University of Texas Southwestern Medical School. The washed organisms were heat killed, fixed (33), suspended in phosphate-buffered saline (PBS) at a concentration of 10% (wt/vol), and were frozen in small aliquots. Bacteria were washed once in PBS before use. Several different immunoprecipitation regimens were employed in this study and are described below.

#### Sequential Precipitation of Lysates with Rabbit Antirat $\beta 2\mu$ Followed by Alloantisera

Lysates were treated with saturating amounts of either rabbit antirat  $\beta 2\mu$ , rabbit anti- $\phi\chi$ , or normal rabbit serum (NRS). The latter two served as controls and were used interchangeably. The normal rabbit serum was a prebleeding of the same rabbit that was immunized with rat  $\beta 2\mu$ . In all experiments, twice as much NRS was used as either rabbit anti- $\beta 2\mu$  or rabbit anti- $\phi\chi$ . Complexes were then precipitated with equivalent amounts of GARIG. After centrifugation, the supernate was treated with RAMIg and a slight excess of GARIG. This procedure was routinely performed on both thymic and splenic lysates. After centrifugation, the supernate was treated with saturating

TABLE I  
 Reactivity in the Cytotoxic Test of the Antiserum (A.BY × B10.RIII(71SN)<sub>F<sub>1</sub></sub>-Anti-B10A(5R) with Lymphocytes of H-2-Congenic Lines

Strain	H-2 haplo-type	Reciprocal of titer	Strain	H-2 haplo-type	Reciprocal of titer
B10.A	<i>a</i>	1,064	B10.AKM	<i>m</i>	0
C57BL/10Sn	<i>b</i>	0	C3H.OH	<i>o2</i>	0
B10.D2/n	<i>d</i>	1,064	B10.P	<i>p</i>	0
B10.M	<i>f</i>	0	B10.Q	<i>q</i>	0
HTG	<i>g</i>	0	B10.RIII(71NS)	<i>r</i>	0
B10.A(2R)	<i>h2</i>	0	B10.S	<i>s</i>	0
B10.A(5R)	<i>i5</i>	1,064	A.TL	<i>tl</i>	1,064
B10.WB	<i>j</i>	0	B10.PL	<i>u</i>	1,064
B10.BR	<i>k</i>	0	B10.AQR	<i>yl</i>	1,064

amounts of either anti-TL (thymus), a mixture of anti-H-2D plus anti-H-2K (thymus and spleen), or normal mouse serum (thymus and spleen) and an excess of GAM1g. Precipitates were washed three times with PBS, transferred to new tubes, and the radioactivity determined. In all sequential precipitations, the first wash of the precipitate was pooled with the supernate.

*Sequential Precipitation of the Lysates with Alloantisera Followed by Rabbit Antirat  $\beta 2\mu$*

Preliminary experiments had indicated that there were numerous problems associated with performing sequential precipitations in which alloantisera and GAM1g were used before xenoantisera and GAR1g. For this reason, we chose to substitute *S. aureus* for the GAM1g. This permitted us to avoid the problems of immune complexes and large amounts of goat antibodies in the supernates. It was determined that 0.5 ml of a 10% (wt/vol) *S. aureus* suspension was capable of binding all the immune complexes from the lysate after treatment with 0.05 ml of either allo- or xenoantisera. Hence, in these experiments, lysates were treated with saturating amounts of either anti-TL (thymus), anti-H-2 (thymus and spleen), or normal mouse serum (thymus and spleen) and *S. aureus*. The bacteria were pelleted by centrifugation at 3,000 rpm at 4°C, and the same procedure was repeated a second time. The supernates were then centrifuged at 10,000 *g* for 30 min and treated with rabbit anti- $\beta 2\mu$  (or control rabbit sera) and either *S. aureus* or an excess of GAR1g. Bacterial pellets or immunoprecipitates were washed as described below.

*Analysis of Immunoprecipitates and S. aureus Pellets*

Immunoprecipitates were dissolved in 0.01 M Tris, pH 8.4 containing 8 M urea and 1% sodium dodecyl sulfate (SDS) by incubating samples for 30 min at 56°C or 1-2 min in a boiling water bath. 2-mercaptoethanol (2ME) was added to a final concentration of 0.1 M when reduction was desired. *S. aureus* pellets were boiled for 1-2 min in SDS-urea or SDS-urea-2ME. The mixture was then centrifuged to remove the bacterial pellet. This procedure resulted in >80% solubilization of the bound material. Aliquots of the SDS extracts were mixed with <sup>3</sup>H- $\mu$ - and L chains and were electrophoresed on 7.5% SDS-polyacrylamide gels (PAGE) for 6 h at 4 mA/gel. Gels were fractionated and the radioactivity determined (31).

*G-100 Sephadex Chromatography*

Lysates prepared from radioiodinated splenocytes were mixed with small amounts of dextran blue and cytochrome *c* and applied to a 90 × 2-cm column equilibrated with PBS containing 0.5% Nonidet P40 (NP40) (Shell Chemical Corp., New York). Each fraction was assayed for immun- and acid precipitable radioactivity. The position of the markers was determined visually.

## Results

*Structure of Splenic Cell Surface Molecules that React with Anti- $\beta 2\mu$*

Three approaches were used. First, the detergent lysate was chromatographed and fractions precipitated with anti- $\beta 2\mu$  to determine the molecular

weight of the radioactive material in the lysate. Second, immunoprecipitates were electrophoresed under nonreducing conditions to determine the covalent structure of the molecule. Third, radioactive peaks were eluted from the gel, reduced, and re-electrophoresed. Alternatively, the whole precipitate was dissolved, reduced, and electrophoresed to determine the nature of the subunits.

**MOLECULAR NATURE OF THE ANTIRAT  $\beta 2\mu$ -REACTIVE MATERIAL IN THE CELL LYSATE.** When lysates of the radioiodinated cells were mixed with dextran blue and cytochrome *c* and chromatographed on a G-100 column, all detectable protein-associated radioactivity and the immunoprecipitable material emerged with the dextran blue, indicating the labeled proteins have mol wts greater than 100,000 (Fig. 1). These experiments indicate that either all surface molecules, including those associated with  $\beta 2\mu$ , are of high molecular weight or that molecules form aggregates in the lysates. Alternatively, molecules are incorporated into the detergent micelles and emerge from the column as high molecular weight detergent protein complexes (34). Regardless of the explanation of the large molecular weight of  $\beta 2\mu$ -associated molecules, virtually no free  $\beta 2\mu$  can be found in the detergent lysate.

**MOLECULAR WEIGHT OF COVALENTLY AND NONCOVALENTLY BOUND SUBUNITS.** When immunoprecipitates were dissolved and electrophoresed in 7.5% SDS-PAGE, peaks of 90,000–100,000, 44,000, and 12,000 were observed (Fig. 2). The ratio of the 90,000/44,000 peaks ranged from 1.5–2.5, although in several experiments no 44,000 peak was observed. Pretreatment of cells with iodoacetamide before or during the lysis shifted the ratio of the 90,000/44,000 molecules to 0.7–1.2, suggesting that some dimerization was occurring during lysis. In the majority of experiments, the control serum brought down a small peak of 44,000 which was approximately 5% the size of the 44,000 peak seen in the specific precipitate. This peak is brought down by a large number of normal and hyperimmune rabbit sera and probably represents a natural antibody to a cell surface component. These results indicate that the  $\beta 2\mu$ -reactive material consists of disulfide-bonded dimers of 90,000 and/or monomers of 44,000, both of which are noncovalently bound to subunits of 12,000. The number and arrangements of subunits in the native molecule is unknown.

**MOLECULAR WEIGHT OF THE DISULFIDE-BONDED SUBUNITS.** When the 44,000 or 90,000 peak was eluted from the gel, reduced and re-electrophoresed, a single peak of 44,000 was obtained. When the immunoprecipitate was reduced, peaks of 44,000 and occasionally 22,000 were observed in addition to the 12,000 subunit. The 12,000 peak is noncovalently bound to the larger subunits, since it can be observed after SDS-PAGE of the precipitate without reduction (Fig. 3). The 22,000 peak was usually, but not exclusively, observed in the specific precipitate. Taken together, these experiments indicate that the 90,000 subunit consists of two disulfide-bonded subunits of 44,000, and that both monomers (44,000) and dimers (90,000) of the 44,000 subunit are attached to an unknown number of 12,000 subunits. The nature of the 22,000 peak is not known. It could be a proteolytic product of the 44,000 protein.

#### *Reaction of Antirat $\beta 2\mu$ with Lysates of Thymocytes*

In four separate experiments, 1.3–2.4% of the protein-associated radioactivity could be specifically immunoprecipitated with antirat  $\beta 2\mu$  serum. The ratio of

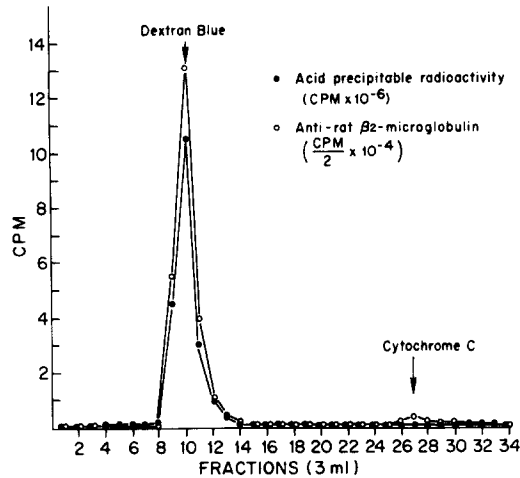


FIG. 1. Chromatography of NP40 lysates on G-100 Sephadex. Lysates from radioiodinated splenocytes were mixed with dextran blue and cytochrome *c* and sieved on a G-100 column equilibrated with 0.5% NP40 in PBS. The acid and immunoprecipitable radioactivity from each fraction was determined. The position of the markers was assessed visually.

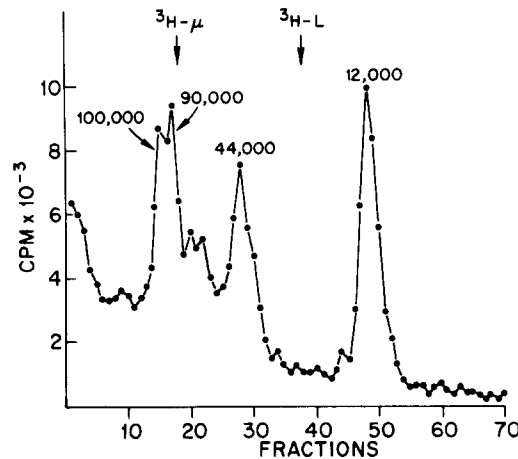


FIG. 2. SDS-PAGE of the unreduced anti- $\beta_2\mu$  precipitate prepared from radioiodinated splenocytes. The dissolved precipitate was mixed with  $^3\text{H}$ - $\mu$ - and L chain and electrophoresed for 16 h at 4 mA/gel.

radioactivity in the precipitate compared to the control was 1.8–2.3. The results of SDS-PAGE of reduced and unreduced anti- $\beta_2\mu$  immunoprecipitates were similar to those described for splenocytes with two exceptions: First, the ratio of 90,000/44,000 mol wt material was 0.3–0.6 on the thymocytes in contrast to 1.5–2.5 on splenocytes (not shown), suggesting that covalent dimerization of the 44,000 subunits was less frequent. The ratios obtained with spleen and thymus were not altered when unlabeled lysates of one were mixed with labeled lysates of the other before precipitation, suggesting that molecules in the lysate were not promoting disulfide bond formation or reduction. In addition, when iodoacet-

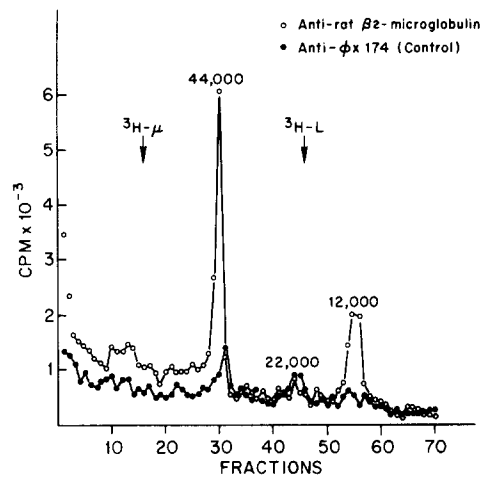


FIG. 3. SDS-PAGE of the reduced anti- $\beta 2\mu$  precipitate prepared from radioiodinated splenocytes. See Fig. 2 for details.

amide was added to the thymocytes before lysis, no change in the ratio of 90,000/44,000 molecules was observed for either H-2 or TL. Second, the 22,000 peak was consistently absent from the gels of the thymic anti- $\beta 2\mu$  precipitate even though it was frequently found in lysates of splenocytes. To test for the possibility that the 22,000 peak in the splenic lysate was generated by proteolysis, labeled thymic lysates were mixed with unlabeled splenic lysates to determine if a 22,000 peak would be found. The 22,000 peak could not be found in these experiments.

#### *Does Antirat $\beta 2\mu$ React with H-2 and TL?*

In these experiments, lysates of spleen or thymus cells were depleted of  $\beta 2\mu$ -reactive molecules and of Ig as described in Materials and Methods. The supernates of these precipitates were treated with saturating amounts of anti-H-2 or anti-TL. As can be seen in Figs. 4 and 5, the anti- $\beta 2\mu$  removed more than 80% of the radioactivity that could be immunoprecipitated with either anti-H-2 or anti-TL, respectively. In four such experiments, the depletion of TL and H-2 was 68–83%, indicating that the majority of these molecules are associated with  $\beta 2\mu$ . The failure to deplete completely suggests that either a portion of the H-2 or TL molecules lose their  $\beta 2\mu$  subunit during these procedures, that some molecules lack  $\beta 2\mu$ , or that the conformation of  $\beta 2\mu$  is different in some molecules. It should be noted that the 30,000 peak, which other studies (30) have suggested is Ia, is not lost after depletion with anti- $\beta 2\mu$ . It is, therefore, unlikely that Ia contains  $\beta 2\mu$ . In addition, the 22,000 peak is not depleted, suggesting that it also is not associated with  $\beta 2\mu$ .

#### *Are There Molecules Other Than H-2 or TL Which Contain $\beta 2\mu$ ?*

In these experiments, splenic or thymic lysates were depleted of TL and H-2 as described in Materials and Methods. The supernates were then examined for  $\beta 2\mu$ -reactive molecules. As shown in Fig. 6, panel C, when splenic lysates were treated with anti-H-2 before anti- $\beta 2\mu$ , little or no radioactivity could be subsequently precipitated with anti- $\beta 2\mu$ . In contrast, removal of H-2 had no effect on

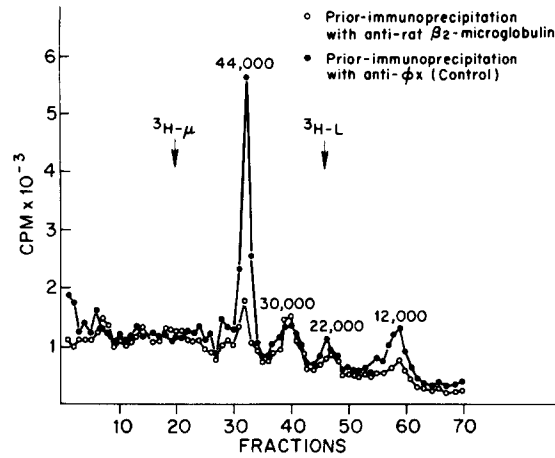


FIG. 4. Depletion of H-2 antigen from lysates of radioiodinated splenocytes by prior treatment with anti- $\beta 2\mu$ . Lysates were treated with saturating amounts of either anti- $\beta 2\mu$  or anti- $\phi\chi$  (control) and GAR Ig. The supernates were precipitated with anti-H-2 and GAM Ig (shown here). Anti-H-2 precipitates were reduced and electrophoresed as described in Fig. 2.

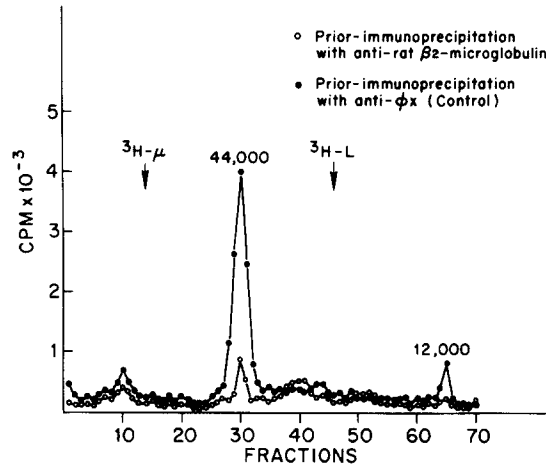


FIG. 5. Depletion of TL antigen from lysates of radioiodinated thymocytes by prior treatment with anti- $\beta 2\mu$ . Lysates were treated as described in Fig. 4 and the supernates precipitated with anti-TL and GAM Ig (shown here). Anti-TL precipitates were reduced and electrophoresed as described in Fig. 2.

subsequent recovery of cell surface IgM and IgD (panel B). Moreover, the control serum (anti- $\phi\chi$ ) did not bring down radioactivity from the samples that had been depleted by either anti-H-2 or NMS. These results indicate that all the  $\beta 2\mu$  is associated with H-2 in lysates of radioiodinated splenocytes.

As shown in Fig. 7, panel A, depletion of thymic lysates with a combination of anti-H-2 and anti-TL also removed all the  $\beta 2\mu$ -reactive molecules. Moreover, as shown in panels B and C, the majority of  $\beta 2\mu$  was removed with anti-H-2 treatment alone. Only 10-20% was removed with anti-TL. Since experiments described in the previous section indicate that TL can be removed by precipitation with anti- $\beta 2\mu$ , we are left with three possible interpretations of these

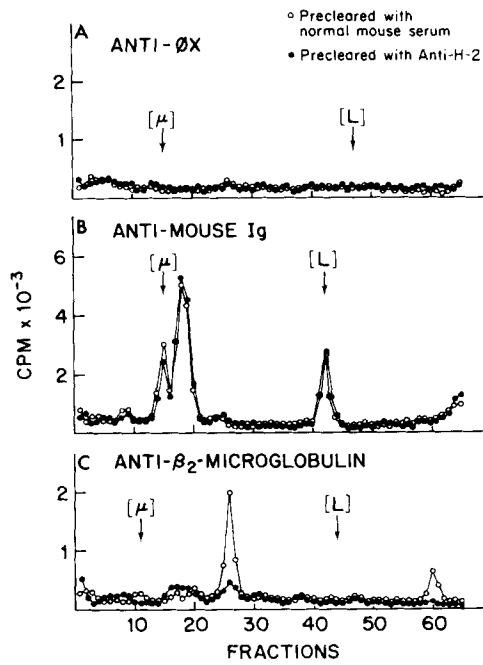


FIG. 6. Depletion of anti- $\beta_2\mu$ -reactive molecules from lysates of radioiodinated splenocytes by prior treatment with anti-H-2. Lysates were treated with saturating amounts of anti-H-2 or NMS (control) and *S. aureus*. The supernates were precipitated with anti- $\beta_2\mu$  (C), RAMIg (B), or anti- $\phi\chi$  (A) and *S. aureus*. Bacteria were treated with SDS-urea-ME, and the extracts were electrophoresed as described in Fig. 2.

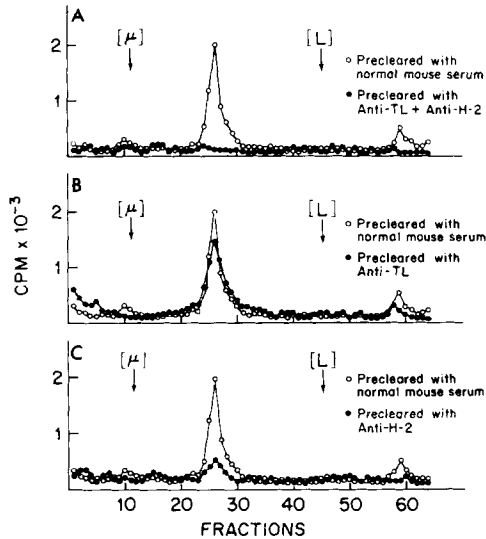


FIG. 7. Depletion of anti- $\beta_2\mu$ -reactive molecules from lysates of radioiodinated thymocytes by prior treatment with anti-H-2 (C), anti-TL (B), or both (A). See Figs. 2 and 6 for details.



results. First, the thymus contains much less TL- $\beta 2\mu$  than H-2- $\beta 2\mu$ . Second, there are similar amounts of H-2 and TL "heavy chains" but fewer  $\beta 2\mu$  molecules per TL H chain than per H-2 H chain. Third, the TL- $\beta 2\mu$  is less heavily labeled than the H-2- $\beta 2\mu$ . None of these possibilities can be excluded.

The above results indicate that (a) *S. aureus* alone cannot bind to TL or H-2 since these molecules were present in the control samples (treated with NMS). (b)  $\beta 2\mu$  is associated only with H-2 and TL on both thymus and spleen, and (c) some H-2 and TL H chains may lack  $\beta 2\mu$ .

### Discussion

We have used rabbit antirat  $\beta 2\mu$  to investigate mouse  $\beta 2\mu$  on murine splenocytes, assuming from the close phylogenetic relationship between rat and mouse and the conservation in  $\beta 2\mu$ s of known sequence, that the antisera would cross-react with mouse. The purity of the immunogen used to prepare the rabbit antirat  $\beta 2\mu$  was firmly established by sequence analyses.<sup>2</sup> Thus, Poulik et al<sup>2</sup> have demonstrated that 32 out of 42 of the residues at the N-terminal portion of the rat protein are identical with human  $\beta 2\mu$ . When this purified protein was used as an immunogen, all of the reactivity of the resulting antiserum could be absorbed with the purified antigen. Nevertheless, one reservation concerning the specificity of the antiserum should be mentioned: the antiserum could cross-react with the major subunit of H-2 and TL. Although this latter possibility is unlikely, it cannot be disproved unless the individual 44,000 and 12,000 mol wt subunits are separated and individually tested with the anti- $\beta 2\mu$  serum.

Assuming that the specificity of this antiserum is solely to  $\beta 2\mu$ , a number of conclusions can be drawn from the present studies in the mouse: (a) All the molecules with which this serum reacts have subunits of 44,000 and 12,000 mol wt. (b) The major subunits recovered from the cell lysate appear to be noncovalently bound to  $\beta 2\mu$  and are themselves either disulfide-bonded dimers of 90,000 mol wt or monomers of 44,000. The ratio of radioactivity of the 90,000 to the 44,000 molecule was approximately 2 for spleen and 0.4 for thymus. (c) All of the  $\beta 2\mu$  that could be identified by immunoprecipitation in lysates of thymocytes and splenocytes is bound to H-2 or TL. (d) The Ia molecule in lysates of radioiodinated splenocytes lacks the  $\beta 2\mu$  subunit. (e) Neither TL nor H-2 molecules bind directly to *S. aureus*.

*Association of  $\beta 2\mu$  with H-2 and TL Antigens.* When lysates are pretreated with saturating amounts of either anti- $\beta 2\mu$  or a control serum, and H-2 or TL were immunoprecipitated from the supernate, there was a substantial (68–83%) depletion of both molecules from the supernate of the anti- $\beta 2\mu$  precipitate. The experiments indicate, therefore, that the H-2 and TL molecules contain  $\beta 2\mu$ . These studies, therefore, confirm earlier reports that TL (35) and H-2 (36) contain a 12,000 subunit which is immunologically and structurally analogous to human  $\beta 2\mu$ . The depletion of H-2 and TL was never complete, however, suggesting that either the unreactive H-2 and TL molecules lacked a  $\beta 2\mu$  subunit in vivo or that they lost it during isolation.

A surprising result to emerge from these studies is that all the  $\beta 2\mu$  found in lysates of either radioiodinated splenocytes or thymocytes is associated with

either H-2 or TL. This was established by showing that after removal of H-2 from splenic lysates and H-2 and TL from thymic lysates no  $\beta 2\mu$ -reactive molecules remained. In contrast, two other surface molecules on splenocytes, IgM and IgD, were not removed. This observation suggests that  $\beta 2\mu$  is not present in large amounts as free molecules; moreover, the attachment of  $\beta 2\mu$  to H-2 is not fortuitous. Rather,  $\beta 2\mu$  appears to be a subunit of virtually only H-2 on splenocytes and H-2 and TL on thymocytes. Our results are in contrast to the finding that anti-HLA does not cap all  $\beta 2\mu$  on human lymphocytes (19, 22). There are several possible explanations for the differences between the two studies. (a) HLA on the cell surface may not be completely available to anti-HLA antibody but might be more readily capped with anti- $\beta 2\mu$ . (b) HLA and  $\beta 2\mu$  may dissociate during capping. (c) The cocapping assay may be more sensitive than the immunoprecipitation assay, i.e., we might not detect 10% of the putative non-H-2- $\beta 2\mu$ -containing molecules by precipitation, whereas these might be detected by immunofluorescence.

Another interesting finding to emerge from these studies is that anti-TL is less effective at depleting  $\beta 2\mu$ -reactive molecules than anti-H-2. Thus, there is less TL- $\beta 2\mu$  or less  $\beta 2\mu$  per TL heavy chain than there is H-2- $\beta 2\mu$  or  $\beta 2\mu$  per H-2 heavy chain on thymocytes. This finding is consistent with previous observations that radioiodination of TL always gave lower  $\beta 2\mu$ /TL ratios on SDS-PAGE than those observed for H-2 (9).

*Lack of a  $\beta 2\mu$  Subunit on Cell Surface Ia.* Ia was not depleted by prior treatment with anti- $\beta 2\mu$  suggesting that cell surface Ia lacks a  $\beta 2\mu$  subunit. This result is in agreement with a number of studies which have failed to detect a 12,000 mol wt subunit on Ia molecules isolated from cell lysates (37-39). The results indicating lack of  $\beta 2\mu$  on cell surface Ia, however, are in striking contrast to other reports that Ia isolated from serum (40) or culture supernates (41) contains  $\beta 2\mu$ . It is possible that serum Ia is derived from secreting cells rather than being shed from the cell surface. If so, secreted Ia could be in a different molecular form compared to the cell surface. Secreted and cell-associated immunoglobulins represent such an example in which the secreted form may have additional subunits (J chain or secretory piece). Another possibility is that the association of Ia and  $\beta 2\mu$  may occur extracellularly.

*Structure of Cell Surface H-2 and TL Molecules.* The covalent structure of H-2 and TL is difficult to assess for a number of reasons. Firstly, it is possible that either reduction and/or disulfide exchange may occur after cell lysis, thus promoting either dimerization or breakdown of natural dimers into monomers. In this regard, pretreatment of splenocytes with alkylating reagents before lysis, as described previously by Henning et al. (42) and also in these studies, tends to favor recovery of the monomeric H chain rather than the H-chain dimer of H-2. However, in our studies, both forms of H-2 were recovered in all experiments. Secondly, as described in these studies, H-2 from splenocytes always contained both single and disulfide-bonded H chains, whereas H-2 prepared from thymocytes contained, almost exclusively, single H chains. Mixing experiments did not alter this result, suggesting that different forms may exist in the thymus and spleen or perhaps on T cells vs. B cells. Thirdly, TL prepared from detergent lysates contained single H chains, while TL prepared

by EDTA extraction (35) consisted of H-chain dimers. Thus, the extraction method may favor the recovery of one molecular form over another.

With regard to the noncovalent structure of TL and H-2, i.e. the number of  $\beta 2\mu/H$ , this can only be deduced from the difference between the molecular weight of the native molecule and that of the H chains. Based on a native mol wt of 100-300,000 the majority of studies have suggested an  $H_2L_2$  structure for both H-2 (24, 37, 44) and TL (35). With regard to a native mol wt  $>100,000$ , Hart (34) has shown that when cells are lysed in detergent a number of membrane proteins bind to, or are partially intercalated into the detergent micelles. This may result in an artificially large molecular weight when the molecules are evaluated by chromatography on sieving columns. This could have been the explanation for a mol wt of  $>100,000$  for the H-2 molecules described in these studies. Since both H-2 and TL are insoluble after removal of detergent, it is not possible to investigate the true molecular weights of the native molecules. Moreover, studies using papain cleavage of the hydrophilic portion of H-2 (42, 44, 45) are subject to the criticism that cleavage may occur distal to a disulfide bond, and that the native molecules may really exist as even larger molecules representing multiples of a four-chain structure.

Thus, in conclusion, while it is clear that H-2 and TL contain 44,000 and 12,000 subunits ( $\beta 2\mu$ ) and that the molecules are anchored in the plasma membrane by a hydrophobic tail (42, 44, 45) at the C-terminal end of the H chain (42, 45), it is not at all certain that an  $H_2L_2$  structure is, indeed, the native one or, in fact, the only one.

### Summary

We have used a rabbit antiserum prepared against purified rat  $\beta 2$ -microglobulin to immunoprecipitate molecules from lysates of radioiodinated murine thymocytes and splenocytes. All the molecules that are reactive with this serum have subunits of 44,000 and 12,000 and can be identified as H-2 and TL antigens. Thus, the anti- $\beta 2\mu$  serum can deplete lysates of the majority of the TL and H-2 antigens which can be subsequently recognized by alloantisera. If TL and H-2 are precipitated from the lysates before the addition of anti- $\beta 2\mu$ , no  $\beta 2\mu$ -reactive molecules remain. Our results indicate that Ia antigens cannot be depleted from the lysates with anti- $\beta 2\mu$ . The studies also suggest that TL and H-2 heavy chains can exist as both monomers and dimers. These observations are discussed with regard to previous studies concerning the native structure of H-2 and TL antigens.

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