

THE SMALL SUBUNIT OF HL-A ANTIGENS IS
 β 2-MICROGLOBULIN*

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HL-A antigens prepared from human lymphoid cells by treatment of either intact cells or membranes with papain or nonionic detergents have been shown to contain two polypeptide chains. The antigen prepared by treatment of cells or cell membranes with papain contains polypeptide chains with a mol wt of about 34,000 and 11,000 (1), whereas the detergent-solubilized antigen has polypeptides of mol wt about 43,000 and 11,000 (2). Another membrane-bound protein found on human lymphocytes is β 2-microglobulin, an 11,700 mol wt polypeptide which was first isolated from the urine of patients with renal tubular disease (3) and which has subsequently been sequenced and shown to possess a moderate degree of sequence homology to constant region domains of immunoglobulin polypeptide chains (4, 5).

The purpose of the present work was to determine if any relationship existed between the 11,000 dalton peptide chain found in HL-A antigens and β 2-microglobulin. The data obtained indicate that these two peptides are in fact identical as assessed by immunologic techniques.

Materials and Methods

Source of Cells.—Thoracic duct lymphocytes were obtained from chronic drainage, and lymphocytes from patients with this disease who had high lymphocyte counts. Lymphoblastoid cell line RPMI 4265 and BA were also studied.

Antisera.—Antisera specific for β 2-microglobulin were prepared in rabbits by immunization with 0.5–1 mg of purified β 2-microglobulin in complete Freund's adjuvant over a period of 3 mo. Antisera were absorbed with lyophilized normal human urine, serum, and red blood cell antigen. Turkey anti- β 2-microglobulin serum was a gift from Mr. Harvey Faber, University of Wisconsin. HL-A antisera of the specificities and source previously described (1) were used.

Radioiodination of Lymphocyte Surfaces.—Lymphocytes were labeled with ^{125}I by the lactoperoxidase method as previously described (6). Lysates were prepared by treating la-

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beled lymphocytes with 0.5% Nonidet P-40. Immunoprecipitation of the lysates was accomplished by the indirect technique using goat antirabbit γ -globulin as coprecipitating agent.

Radiolabeling of Lymphoblastoid Cell Lines and Papain Digestion.—RPMI 4265 cells were labeled with [^3H]amino acids in amino acid-free medium and digested with papain as previously described (1).

RESULTS

Immunoprecipitation of Surface-Radioiodinated Lymphocyte Proteins with Anti- β 2-Microglobulin Serum.—Lymphocytes were radiolabeled by the lactoperoxidase catalyzed reaction and lysed with Nonidet P-40. Indirect immunoprecipitation was performed on the cell lysates using 10 μl of rabbit anti- β 2-microglobulin or normal rabbit serum as the primary reagent and 250 μl of goat antirabbit γ -globulin as the coprecipitating agent. Of the total acid precipitable counts in the lysate, 1.1–2.4% of counts were precipitated with the anti- β 2-microglobulin serum compared with 0.4–0.6% with normal rabbit serum control (1.6–3.4% was precipitated with anti-Ig sera). The immune precipitates obtained with thoracic duct lymphocytes were completely reduced and alkylated and electrophoresed on 5% polyacrylamide gels containing 0.1% sodium dodecyl sulfate (SDS). The precipitate obtained with anti- β 2-microglobulin serum contained two peaks of radioactivity, one of which electrophoresed slightly ahead of a cytochrome *c* marker, in an identical position to that of β 2-microglobulin purified from urine, and another peak which migrated slightly ahead of a γ -heavy chain marker (Fig. 1). This larger peptide had a calculated mol wt of about 45,000. The normal rabbit serum control showed only a small peak in the general area of 70,000 daltons. The two peaks observed with the anti- β 2-microglobulin precipitates were uniformly seen in all lymphocyte populations studied, i.e., normal peripheral blood, tonsils, thoracic duct, lymphoblastoid cell lines, and chronic lymphatic leukemia cells. The ratios of label in the two peaks varied somewhat from one cell source to the next, but most often approximated 1:1. Partial absorption of the anti- β 2-microglobulin serum with small amounts of purified antigen diminished the capacity of the antiserum to precipitate the

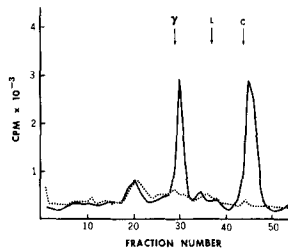


FIG. 1. SDS-polyacrylamide gel electrophoresis of reduced and alkylated anti- β 2-microglobulin immune precipitate of a cell lysate of ^{125}I -labeled thoracic duct lymphocytes (solid line); normal rabbit serum control (dotted line). Arrows indicate position of marker proteins: γ , heavy chain of IgG; L, light chains; C, cytochrome *c*.

two peaks to an equal extent (e.g., treatment of antiserum with 0.1 mg/ml of purified antigen resulted in a 50% decrease in each peak). Absorption with larger amounts (0.5–1 mg/ml of antiserum) completely removed the capacity of the antiserum to precipitate either peak, indicating that the presence of the first peak was not merely due to contaminating antibodies to cell protein unreactive with β 2-microglobulin.

Formation of Immune Complexes with Radioactive Papain Digests of Human Lymphocytes.—In a second type of experiment, cultured human lymphoid cells (cell line RPMI 4265, HL-A 2, 7, 12) were labeled with tritiated amino acids. The cells were treated with papain to release surface proteins including HL-A which were isolated by gel filtration on Sephadex G-150 (1). Treatment of this material with turkey anti- β 2-microglobulin serum resulted in the formation of an immune complex in a much larger amount than that which had previously been obtained with any individual anti-HL-A serum (Fig. 2, compare to Fig. 2

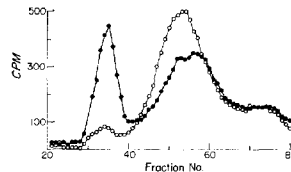


FIG. 2. Immune complex formed with anti- β 2-microglobulin serum. Elution profiles on Sephadex G-150 of [3 H]amino acid-labeled material (prepared by papain digestion of RPMI 4265-labeled cells and previously purified by Sephadex G-150 chromatography [1]) are shown: ●—● = the supernatant from 10^6 cells after incubation with turkey anti- β 2-microglobulin serum (10 μ l); ○—○ = after incubation with turkey anti- β 2-microglobulin serum (10 μ l) previously absorbed with pure unlabeled β 2-microglobulin (11 μ g).

in reference 1). The turkey anti- β 2-microglobulin serum was treated with β 2-microglobulin to remove specific antibodies. When this absorbed serum was employed with the radioactive papain product the amount of immune complex formed was reduced by 80% (Fig. 2). When the immune complex formed with the anti- β 2-microglobulin serum was subjected to SDS gel electrophoresis it was found to contain two subunits of mol wt 34,000 and 11,000 identical in size and relative amounts to the materials which had been obtained using anti-HL-A sera (Fig. 3 A).

The material which remained uncomplexed after treatment with turkey anti- β 2-microglobulin serum was treated with a mixture of anti-HL-A 2, 7, and 12 sera. The small amount of complex formed yielded no discrete polypeptide bands on SDS gel electrophoresis. The material which remained uncomplexed after treatment with the absorbed anti- β 2-microglobulin serum was also treated with the mixture of anti-HL-A sera. By contrast it yielded an immune complex containing polypeptides of mol wt 34,000 and 11,000 (Fig. 3 B).

Quantitatively, the inhibition by HL-A antigen preparations of binding of

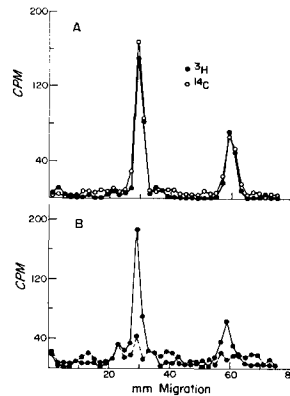


FIG. 3. (A), SDS gel (10% acrylamide) electrophoresis of a mixture of the [^3H]amino acid-labeled anti- β_2 -microglobulin complex formed in Fig. 2, and a [^{14}C]amino acid-labeled HL-A7 complex prepared using Jackson (HL-A7) serum as described previously (1). (B), SDS gels of complexes formed with a mixture of HL-A 2, 7, and 12 sera of [^3H]amino acid-labeled material which failed to complex with turkey anti- β_2 -microglobulin (●---●), and turkey anti- β_2 -microglobulin absorbed with purified β_2 -microglobulin (●---●). The gels were cut into 2-mm slices and the slices shaken overnight at 30°C in 8 ml of 5% Protosol in toluene scintillation fluid before counting.

[^{125}I] β_2 -microglobulin by rabbit anti- β_2 -microglobulin serum indicated that at least 20–25% of the purified HL-A antigen was β_2 -microglobulin (in accord with the percent of the total HL-A molecule represented by the small subunit) and moreover the shape and extent of the inhibition curves indicated that the inhibiting material in HL-A was antigenically identical with purified β_2 -microglobulin. Similarly, anti- β_2 -microglobulin sera were found to lyse human lymphocytes; the inhibition of lysis by β_2 -microglobulin and either papain or detergent HL-A antigen preparations was qualitatively and quantitatively identical. Both β_2 -microglobulin and HL-A antigen preparations gave lines of complete identity with either rabbit or turkey anti- β_2 -microglobulin serum and the small subunit of HL-A was identical to β_2 -microglobulin when they were electrophoresed together in SDS gels. Details of these additional experiments will be presented elsewhere.

DISCUSSION

The experiments reported above indicate that cell-associated β_2 -microglobulin is bound to another polypeptide chain with a mol wt of about 45,000. That this macromolecular complex is related to the HL-A antigen system was demonstrated by the finding that anti- β_2 -microglobulin added to a papain digest containing HL-A antigens removed all detectable HL-A antigen activity. Furthermore, the labeled material complexed to the anti- β_2 -microglobulin serum had an identical profile on SDS gel electrophoresis to that of the HL-A antigen isolated by complex formation with anti-HL-A sera. It has also been

shown that HL-A antigen purified by physicochemical techniques contained 20–25% β 2-microglobulin, as assayed by immunologic procedures.

These findings lead to the conclusion that the small subunits of HL-A antigens and β 2-microglobulin are identical, or so closely related that they cannot be distinguished by immunological means. β 2-microglobulin is a protein of mol wt 11,700 which is found in human urine and has already been sequenced (3–5). It is homologous in structure (both with regard to amino acid sequence and the position of an intrachain disulfide bridge) to the constant region domains of immunoglobulin polypeptide chains. It will be of great interest to learn whether or not the large subunits of HL-A antigens may also be structurally related to immunoglobulin heavy chains. Both are glycoproteins, HL-A having a mol wt of 43,000 and the heavy chains of IgG 50,000. In view of the fact that immune response (*Ir*) genes in man and in the mouse have been shown to be genetically linked to HL-A and *H-2* respectively (7, 8), it seems possible that the HL-A antigen is somehow functionally involved in the immune response and inevitably leads to the speculation that they could be part of an antigen receptor complex, as has also been suggested for the *Ir* gene product.

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