Brief Definitive Report

HUMAN Ia BETA CHAINS AND THE INVARIANT CHAIN SHARE A COMMON ANTIGENIC DETERMINANT*

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Human Ia antigens, structural and serologic analogues of mouse Ia antigens, are composed of two noncovalently associated polypeptide chains, α (34 kdalton mol wt) and β (28 kdalton mol wt), inserted into the plasma membrane as an oligomeric complex (1–4). A third polypeptide, termed the invariant chain (I), is found noncovalently associated with the intracellular complexes of both human (5, 6) and mouse Ia antigens (7, 8). This polypeptide is basic in character, having a molecular weight of 31 kdaltons in its unprocessed form (I) and 35 kdaltons in its processed form (Ip) (9). The significance of its presence in the α-β chain complexes is still unknown. It has been suggested that the invariant chain may play a role in the association, intracellular processing and insertion of the α and β chains into the plasma membrane (10). The nature and location of the determinants that may regulate these events is so far unknown. We present here our findings that a mouse monoclonal antibody, L227, which was previously shown to recognize a determinant on the light chains of two distinct human Ia molecules (11–13), also recognizes a determinant on the invariant chain found associated with these molecules.

Materials and Methods

Antibodies. L203 and L227 are mouse monoclonal antibodies whose initial isolation and characterization have been previously described (14). Goat α mouse Ig serum #89 has been raised in our laboratory. Affinity-purified rabbit α mouse Ig serum #26 was generously provided by Dr. Luis Cantarero.

Cell Cultures and Radiolabeling of Cells. Human B lymphoblastoid cell lines Raji (DR:3,6) and XNEW (DR:3,10) were cultured in RPMI 1640 medium supplemented with 15% fetal calf serum (FCS). Before radiolabeling the cells were incubated in methionine and cysteine free-medium for 1 h. For continuous labeling 1 mCi of [^{35}S]methionine and 1 mCi of [^{35}S]cysteine (Amersham Corp., Arlington Heights, IL) were added and incubation continued for up to 4 h. For pulse-chase studies cells were labeled for 5 min, diluted, and washed in cold RPMI 1640–20% FCS. Equal aliquots were cultured and harvested at desired chase points and cell extracts made by solubilization in 50 mM Tris-HCl buffer containing 0.5% NP-40.

Immunoprecipitation and Electrophoresis. To generate specific precipitates 100 μl of cell extract was reacted with 200 μl of either L203 or L227 antibody-containing hybridoma supernatants for 30 min at room temperature, followed by an addition of 80 μl of goat α mouse Ig serum #89. The precipitates were allowed to form overnight. Discontinuous

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one-dimensional SDS polyacrylamide gel electrophoresis (1D-PAGE) was performed according to Laemmli (15). For two dimensional gels (2D-PAGE), nonequilibrium pH gradient electrophoresis (NEPHGE) was used to separate the proteins in the first dimension (16).

Electrophoretic Transfer of Proteins and Western Blotting. Unlabeled proteins were transferred from polyacrylamide SDS gels to nitrocellulose membrane (Bio-Rad Laboratories, Richmond, CA) in the Trans-Blot Cell (Bio-Rad Laboratories) using Tris-glycine-methanol running buffer as previously described (references 17, 18, and personal communication with Dr. Neil Burnett, Washington University). Transfer was performed for 4 h. at 25V in the cold. Incubation with specific antibody-containing hybridoma supernatants was performed for 1 h at room temperature with continuous rocking. This was followed by a 1-h incubation with affinity-purified rabbit α mouse Ig antibody #26 and a 1-h incubation with ^{125}I-labeled Staph Protein A at 2×10^6 cpm/ml. The filters were blotted dry and exposed to Kodak XAR-5 x-ray film with an intensifying screen at −70° for 30 min. or 1 h.

Results

2D-PAGE Profile of L227- and L203-Reactive Ia Antigens. We and others have previously reported that antibodies L203 and L227 recognize three distinct populations of Ia molecules on human B cells and cell lines (11–13). These molecules are generated by specific associations of two different heavy chains (α1 and α2) with three different light chains (β1, β2, β3) (19). The α1 chain associated with either β1 or β3 to form two different HLA-DR molecules, both recognized by L203. In contrast, α2 heavy chain associated exclusively with the β2 light chain to form a third molecule seen only by antibody L227. Antibody L227 also reacts with the α1β1 HLA-DR molecule via the shared antigenic determinant between β1 and β2 (11).

Fig. 1 shows a 2D-PAGE profile of these three Ia antigens in a human B cell line XNEW. The cells were pulse-labeled for 5 min with [^{35}S]methionine and [^{35}S]cysteine and at various chase times the individual rates of synthesis and processing of Ia components found in L203 and L227 immunoprecipitates were analyzed.

At the beginning of synthesis (To), one heavy chain (α1), one light chain (β1), and the invariant chain (I) are seen in L203 precipitates. Two heavy chains (α1 and α2), two light chains (β1 and β2) and the invariant chain (I) are present in L227 precipitates. 30 min after the pulse, another light chain (β3) appears, seen only by L203. After 2 h, and more so after 4 h, α1β1 and α1β3 antigens in L203 precipitates appear as mature molecules lacking the invariant chain. L227 continues to recognize the less mature molecules that quantitatively diminish over the 4 h of chase. The invariant chain is still represented by a very strong signal.

Reactivity of L227 Antibody with a Determinant on β1, β2, and I

We demonstrated previously the ability of antibody L227 to specifically precipitate both β1 and β2 light chains labeled by lactoperoxidase-catalyzed surface iodination and eluted separately from 1D-PAGE gels (11). The antibody's reactivity with the invariant chain was not seen at this time, consistent with previously reported observation that this polypeptide either does not exist on the cell surface or does not contain tyrosines accessible to iodination (21). Electrophoretic transfer of unlabeled proteins onto nitrocellulose and Western blotting was then used as a method of choice for determining the location of L227-specific antigenic

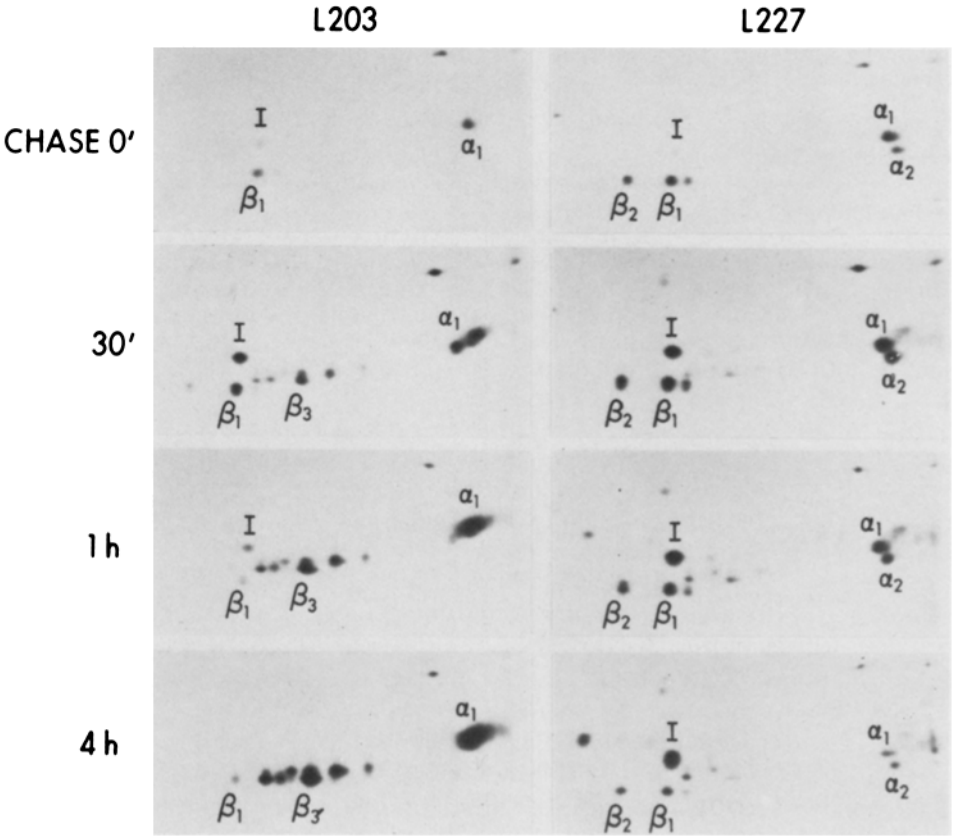

FIGURE 1. 2D gel profile of Ia antigens immunoprecipitated from XNEW cells with antibodies L203 and L227 after a 5-min [^{35}S]methionine and [^{35}S]cysteine pulse and different time intervals of chase labeling.

determinants. The proteins were first run on two-dimensional gels to achieve greater separation of the light chains and the invariant chain. We routinely used immunoprecipitates in place of whole cell lysates in order to concentrate the antigens of interest.

Fig. 2 illustrates results obtained when we immunoprecipitated L203- and L227-reactive Ia antigens from unlabeled Raji cells, resolved them by 2D-PAGE, and electrophoretically transferred the proteins to nitrocellulose. Filters containing either L227- or L203-precipitated proteins were incubated with L227 antibody, followed by rabbit α mouse Ig. The specific spots were developed using ^{125}I-labeled Protein A. Three major spots were detected on the filters that originally contained L227-reactive molecules. They correspond to the invariant (I) chain, β1 light chain, and β2 light chain (Fig. 2*B*). Slightly different pattern is seen on the L203 filter, which should contain only the β1 light chain. Accordingly, when this filter is reacted with L227, the β2-specific signal is absent from the Western blot, and the β1 and the I chain spots are present (Fig. 2*D*). The rabbit serum alone does not react with any of these three spots. It does react with the heavy and light chains of the precipitating antibodies, but the

signals are found outside the area of interest. We have included the L203 (Fig. 2*C*) and L227 (Fig. 2*A*) precipitates from Raji cells labeled for 4 h with [^{35}S] methionine and [^{35}S]cysteine, and it is evident that the I, β1, and β2 spots on the Western blots can be superimposed on the corresponding spots from labeled precipitates.

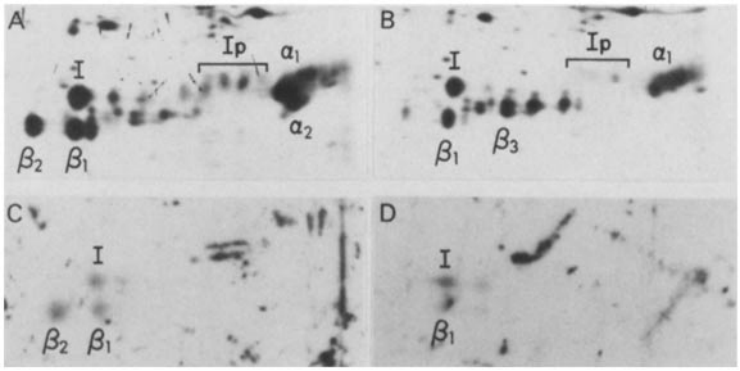

FIGURE 2. The invariant chain (I) and the β1 and β2 light chains share an antigenic determinant recognized by antibody L227. (*A*) L227 precipitate from [^{35}S]methionine labeled Raji cells. (*B*) L203 precipitate from [^{35}S]methionine labeled Raji cells. (*C*) Western blot of unlabeled L227 precipitate probed with L227 and ^{125}I-protein A. (*D*) Western blot of unlabeled L203 precipitate probed with L227 and ^{125}I-protein A.

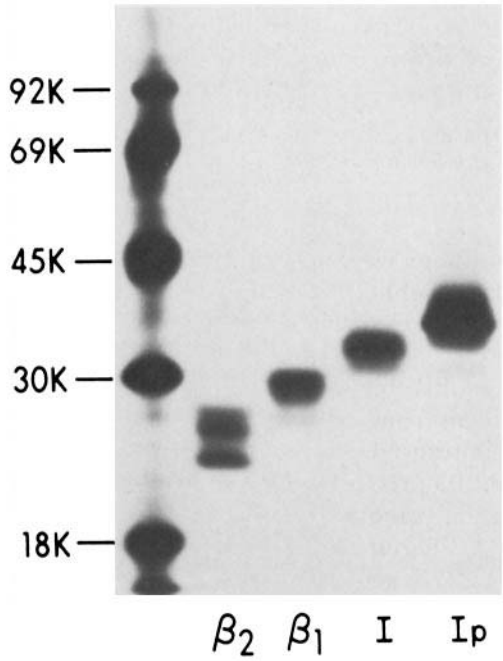

FIGURE 3. Recognition by L227 of isolated light chains and invariant chains. [^{35}S]Methionine- and [^{35}S]cysteine-labeled Raji cell extract was electrophoresed on NaDodSO$_4$/10% polyacrylamide gel under reducing conditions. The invariant chain and heavy and light chains were eluted separately and reprecipitated with L227. The resulting precipitates were analyzed on NaDodSO$_4$/10% polyacrylamide gel. *K*, kdaltons.

To further test the specificity of L227 we attempted to precipitate each of these molecules individually following their separate elution from an SDS gel. [^{35}S]Methionine-labeled Raji whole cell lysate was electrophoresed on a 10% polyacrylamide tube gel. 1-mm slices were collected, the proteins eluted, and amounts of radioactivity determined. The desired peaks corresponding to the light chains, the invariant chain and the heavy chain were located using ^{14}C-labeled molecular weight markers electrophoresed in parallel. In Fig. 3 we show that the antibody L227 precipitates in addition to the two light chains, the unprocessed I (31 kdaltons) and the processed Ip (35 kdaltons) form of the invariant chain (9). All of these polypeptides were subsequently identified on 2D gels (data not shown).

Discussion

This paper provides the first evidence that the invariant chain and the polymorphic chains of human Ia antigens share common antigenic determinants. These determinants are present on both unprocessed (I) as well as the processed (Ip) forms of the invariant chain as evidenced by the capacity of L227 antibody to precipitate both forms independently. On the Western blots, however, we repeatedly obtain positive signal for the unprocessed form (I) only, which may indicate a difference in the relative amounts of those two forms present in the Ia precipitates.

We do not know yet whether the determinants detected by L227 have any relationship to the determinants involved in the formation of α-β complexes and their intracellular processing. We are now trying to isolate and compare the peptides bearing these determinants from the invariant chain and the light chains. We are also using antibody L227 to look for the presence of the invariant chain in cells that do not normally express Ia molecules, as well as on the plasma membrane.

Summary

We have presented evidence that a mouse monoclonal antibody L227, which was previously shown to recognize a determinant on the light chains β1 and β2 of human Ia antigens, also recognizes a determinant on the invariant chain (I) associated with these molecules. Ia-specific proteins were resolved by two-dimensional (2D) PAGE and electrophoretically transferred onto nitrocellulose filters. The presence of a determinant shared between β1, β2 and I was established using "Western blotting" technique. In addition, we demonstrated that L227 can immunoprecipitate isolated β1, β2, and the invariant chain proteins following their separate elution from SDS-PAGE.

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