The DA6-147 monoclonal antibody raide against the HLA-DR alpha chain identifies a cryptic epitope on the BoLA-DR alpha chain

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Short note

The DA6-147 monoclonal antibody raised against the HLA-DRα chain identifies a cryptic epitope on the BoLA-DRα chain

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Summary — By combining immunoprecipitation of BoLA molecules with monoclonal antibodies raised against 2 different major histocompatibility class II antigens, TH14B and TH81A, and western blotting using the anti-HLA DRα monoclonal antibody DA6-147, we characterized an epitope conserved on BoLA- and HLA-DRα chains. This epitope, not accessible on intact cells, was revealed after cell lysis. In addition, these results allowed us to define TH14B as an anti-BoLA-DR monoclonal antibody whereas TH81A, raised against the products of a second MHC locus, is probably an anti-BoLA-DQ monoclonal antibody.

BoLA / MHC / class II product / bovine

Résumé — Mise en évidence d'un épitope cryptique commun à la chaîne DRα des produits BoLA et HLA. *Des anticorps monoclonaux, TH14B et TH81A, dirigés contre des épitopes de classe II du complexe majeur d'histocompatibilité conservés à travers les espèces, précipitent dans la lignée lymphoblastoïde bovine BL-3 des protéines de poids moléculaires différents, notamment une molécule à 33 kDa précipitée par TH14B. Cette molécule est reconnue en Western Blot, seulement après lyse cellulaire, par l'anticorps DA6-147 ; cet anticorps est dirigé contre un épitope porté par la chaîne HLA-DRα non accessible sur cellules intactes. Par ailleurs, aucune des chaînes précipitées par TH81A ne présente de déterminant reconnu par DA6-147. Ces résultats permettent d'affirmer l'existence d'un épitope cryptique commun à la chaîne DRα des produits HLA et BoLA. L'anticorps TH14B définit par conséquent les produits de BoLA-DR et TH81A, les produits d'un second locus du BoLA, vraisemblablement BoLA-DQ.*

BoLA / CMH / antigènes de classe II / bovin

INTRODUCTION

The bovine major histocompatibility complex (BoLA) class II antigens, encoded by at least 2 different subregions (Andersson *et al*, 1986a, b), are constituted by the association of an αß heterodimer glycoprotein with an invariant γ chain. The α chain is a 32-kDa acidic polypeptide non covalently linked to a more basic 29-kDa ß chain, as demonstrated by using anti-murine H2-IA or -IE class II antibodies (Hoang-Xuan *et al*, 1982).

Among monoclonal antibodies (mAbs) characterizing the major histocompatibility class II molecules, we have selected 3 mAbs for this study. The first 2, TH14B and TH81A, are directed against interspecies conserved epitopes on class II major histocompatibility complex (MHC) molecules and identify antigenically distinct polypeptides with slightly different molecular weights (MW) (Davis *et al*, 1987). The third antibody DA6-147, previously described by Guy *et al* (1982), reacts with the α chain of the human HLA-DR molecules; it detects a monomorphic epitope on the 32–34-kDa DRα chain only on lymphoblastoid cell lysates (Cohen *et al*, 1987).

In this paper, we analysed the reactivity of these mAbs on the bovine lymphoblastoid BL-3 cell line (Romano *et al*, 1989). This cell line was selected because it expresses a large amount of 2 different BoLA class II antigens (Janzer-Pfeil and Splitter, 1989). By combining western blotting and radioimmunoprecipitation studies, we were able to demonstrate that TH14B precipitated antigens that express an epitope are found on the HLA-DRα chain.

MATERIALS ET METHODS

Cells

The calf lymphosarcoma B cell line BL-3 (Romano *et al*, 1989), gift of HA Lewin, is homozygous for both DQA and DQB genes (Xu *et al*, 1991). It has previously been shown that this cell line expresses high levels of BoLA class II molecules as assessed by flow cytometry (Davis *et al*, 1987) and immunoprecipitation results (Janzer-Pfeil and Splitter, 1989).

Monoclonal antibodies

The mAbs TH14B (IgG2a) and TH81A (IgG2a) were derived from mice immunized with a mixture of bovine, caprine, canine and rat lymphocytes. They have been shown to react with bovine MHC class II antigens as assessed by their cell distribution in peripheral blood lymphocytes (Davis *et al*, 1987). The mAb DA6-147 (IgG2) obtained from BB Cohen detects a non polymorphic epitope expressed only on lysed HLA-DR heterozygous and homozygous lymphoblastoid cell lines (Guy *et al*, 1982; Cohen *et al*, 1987). This mAb reacts with a single molecule at a MW of 32 000–34 000 after western blotting of human cell lysates (Guy *et al*, 1982). The mAb ALF-2 (IgG1) produced in our laboratory, that reacts only with cat lymphocytes (Le Rhun, personal communication), was used as a negative control for immunoprecipitation experiments. TH14B, TH81A, DA6-147 and ALF-2 were used at 1 mg/ml.

Radiolabelling of cells

Metabolic labelling of cells with both [^{35}S]-methionine and [^{35}S]-cysteine (Amersham International, UK) was performed by a modification of the method of Jones (1980). Briefly, cell cultures were depleted by incubation in methionine,

cysteine-free medium (3.10^7 cells/15 ml) for 3 h and sedimented by centrifugation (800 *g* for 10 min). After resuspending cells at the same concentration in same medium, 1 mCi [^{35}S]-methionine and 1 mCi [^{35}S]-cysteine were added. The cells were incubated overnight at 37°C and extensively washed with cold phosphate buffered saline (PBS) before use.

Immunoprecipitation of class II molecules

We used a modification of the technique previously described by Kessler (1975). Briefly, 5 µl mAbs (1 mg per ml) were incubated with BL-3 cells (10^6 cells/ml) before or after solubilization in lysis buffer (Tris–HCl, 10 mM, pH 7.2 ; NaCl 150 mM; Nonidet P40, 0.5%). After 1 h incubation at + 4°C, immune complexes were absorbed with protein A Sepharose (Pharmacia, Uppsala, Sweden). After extensive washing, specifically bound materials were eluted by resuspending the protein A Sepharose cell pellet in SDS buffer (Tris, 125 mM; glycerol, 20%; SDS, 4.6%).

SDS–PAGE and western blot

Immunoprecipitated antigens eluted in SDS buffer were run in a 12% acrylamide gel as described by Laemmli (1970) and transferred to nitrocellulose membrane (reinforced cellulose nitrate membrane; Schleicher and Schuell, Dassel, Germany) according to the technique of Towbin *et al* (1979).

Immunodetection

The detection of the immunoprecipitated antigens was performed by a modification of the technique previously described by Liebert *et al* (1985) for ELISA and RIA tests. Transferred antigens were probed for mAb DA6-147 reactivity as follows. After blocking nitrocellulose filters with 3% fish gelatin, 0.1% Tween 20 in PBS, mAb DA6-147 ascites were incubated at a 1/1 000 dilution for 1 h at room temperature. Following 5 washes with blocking buffer, goat anti-mouse IgG peroxidase labelled antibody (Cappel, Organon Teknika Corp, Treyburn, Durham, NC) used at a 1/500 dilution was incubated for 1 h at room temperature. After washing, binding of the secondary antibody was revealed with diaminobenzidine (Sigma) and H_2O_2 as described by Swack *et al* (1987). When antigens were radiolabelled, filters were exposed to X-ray films (Kodak) with intensifying screens (Lithing plus: Dupont de Nemours, Boston, MA).

RESULTS

Confirmation of the reactivity of the mAbs on BL-3 by cytofluorography

TH14B and TH81A gave a strong membrane fluorescence on the vast majority (>95%) of intact BL-3 cells, whereas the DA6-147 mAb had no reactivity under the same conditions. Similarity of fluorescence intensity indicated that TH14B and TH81A have an equal affinity for the BoLA-class II molecules (data not shown).

Analysis of cell surface expressed BoLA-D products

In order to determine which of the TH14B and TH81A mAbs might recognize a BoLA-DR equivalent, whole BL-3 cells were incubated with either TH14B, TH81A or DA6-147. After lysis, electrophoresis (SDS–PAGE) and transfer onto nitrocellulose filters (western blot), the specific bound antigens were probed for DA6-147 reactivity. This mAb recognized a 33-kDa entity only from the TH14B precipitated molecules (fig 1).

Analysis of internal and cell surface expressed BoLA-D products

In another set of experiments, TH14B, DA6-147 and TH81A were incubated with the detergent lysate from [^{35}S]-methionine/

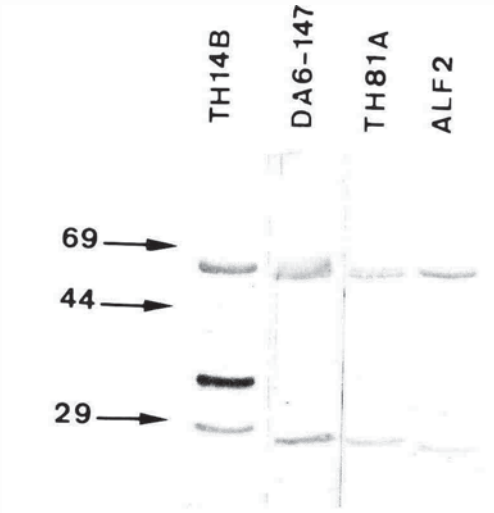

Fig 1. DA6-147 reactivity on western blotting of surface molecules previously recognized by mAbs TH14B, DA6-147, TH81A and ALF-2 (anti-feline lymphocytes, used as negative control). Marker protein relative mass (kDa) is indicated at the side. Fainter entities at 25 and 56 kDa represent heavy and light immunoglobulin chains.

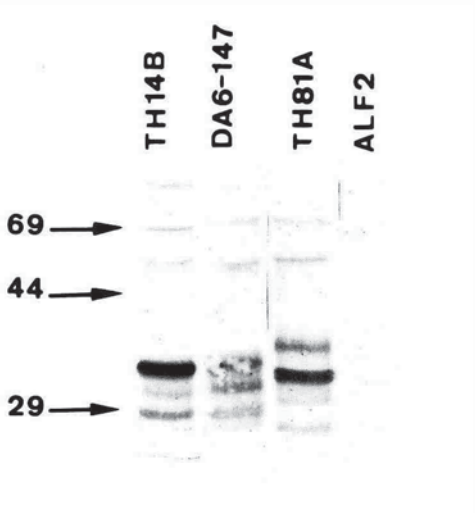

Fig 2. Autoradiography of labelled molecules precipitated after cell lysis by TH14B, DA6-147, TH81A, ALF-2 mAbs. Marker protein relative mass (kDa) is indicated at the side.

cysteine biosynthetically–labelled BL-3 cells. Specific radioactive bound antigens were electrophoresed, transferred onto nitrocellulose filters, probed for DA6-147 reactivity, and finally, exposed to X-ray films with intensifying screens. DA6-147 as well as TH14B precipitated 3 radioactive bands at 33, 31 and 29 kDa (fig 2). Among them, only the 33-kDa molecule was also detected by western blotting with the DA6-147 mAb (fig 3).

TH81A precipitated radioactive bands at 35, 32, 30 and 27 kDa (fig 2). None of them was recognized by DA6-147 (fig 3). These data confirmed that TH81A precipitated molecules different from those precipitated either by TH14B or DA6-147.

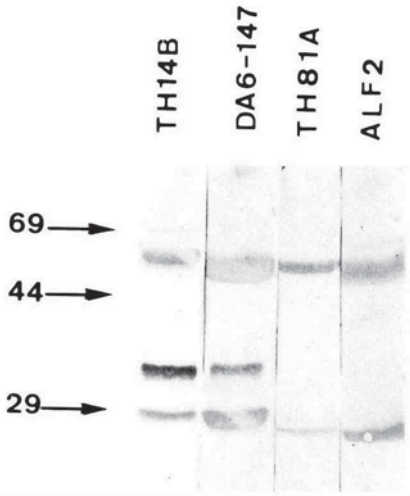

Fig 3. Immunodetection with mAb DA6-147 of nitrocellulose filters run in figure 2. Marker protein relative mass (kDa) is indicated at the side (Fainter entities at 25 and 56 kDa represent heavy and light immunoglobulin chains).

DISCUSSION

By combining immunoprecipitation and western blotting techniques, we were able to define TH14B as an anti BoLA-DR mAb. HLA and BoLA-DRα gene and gene products were found to possess a strong homology (Van der Poel *et al*, 1990). In ad-

dition, the BoLA-DRα chain was found to be monomorphic both biochemically (Joosten *et al*, 1989) and genomically (Van der Poel *et al*, 1990) as described for HLA-DRα (Charron and McDevitt, 1980). Consequently, the TH14B-precipitated 33-kDa polypeptide, specifically labelled by DA6-147, was considered to represent the BoLA-DRα chain. Immunoprecipitation experiments performed before and after cell lysis demonstrated that the determinant detected with TH14B is present on the peptide (BoLA-DRα) that expresses the cryptic epitope recognized by DA6-147 by western blotting on cell lysates. The 31 and 29-kDa radioactive entities, also precipitated by TH14B and DA6-147 but not probed with DA6-147 mAb after western blotting, respectively represent the invariant and the β DR chains (Hoang-Xuan *et al*, 1982). On the other hand, no DA6-147 reactivity was observed for the TH81A precipitated molecules that also had different relative masses compared with the DA6-147 and TH14B precipitated molecules. TH81A thus recognizes the products of another class II locus on the following criteria. First, apparent molecular weights were obviously different. Second, none of the chains recognized by TH81A present in the cytoplasm or at the cell surface possessed the epitope revealed by DA6-147. As no DP-like region has been evidenced to date in the bovine MHC locus and no messenger RNA found for the BoLA-DY (Andersson *et al*, 1988), it is likely that TH81A recognizes BoLA-DQ products. Such studies are important as they provide tools allowing the preparation of purified molecules for microsequencing studies.

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