Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 4451–4454, June 1988
Immunology

HLA class I molecules are associated with CD1a heavy chains on normal human thymus cells

(major histocompatibility complex/intermolecular complexes/T-cell differentiation)

MARTINE AMIOT*, HÉLÈNE DASTOT*, LAURENT DEGOS*, JEAN DAUSSET†, ALAIN BERNARD‡, AND LAURENCE BOUMSELL*§

*Institut National de la Santé et de la Recherche Médicale, U93, Institut de Recherche sur les Maladies du Sang, Hôpital Saint-Louis, 75475 Paris Cedex 10, France; †Centre d'Etude du Polymorphisme Humain, Collège de France, 75005 Paris, France; and ‡Laboratoire d'Immunologie des Tumeurs de l'Enfant, Institut Gustave Roussy, 94805 Villejuif, France

Contributed by Jean Dausset, February 25, 1988

ABSTRACT **The molecules encoded by the major histocompatibility complex play a pivotal role in regulatory interactions between cells of the immune system, which can result in the activation and function of T cells. The function of the CD1 molecules, which are homologous to the major histocompatibility complex-encoded molecules but are encoded on human chromosome 1, is not known. HLA class I molecules and CD1a heavy chains share the ability to associate with several different cell-surface molecules. We show here, by several technical approaches, that HLA class I molecules are associated with CD1a heavy chains on the surface of normal thymus cells. The functional significance of this association during T-cell differentiation is discussed.**

In humans β_2-microglobulin is the light chain of several different cell-surface proteins (1), including HLA-A, -B, and -C class I molecules and the CD1a (2), CD1b (3), and CD1c (4) molecules. Several features distinguish CD1 molecules from HLA class I molecules. Whereas HLA class I molecules are detected on most nucleated cells from the body, the CD1 molecules are expressed almost exclusively by cortical thymus cells and are not displayed by mature peripheral T cells. HLA class I molecules are highly polymorphic; in contrast, the CD1 molecules seem to be rather monomorphic. HLA class I heavy chains are less heavily glycosylated than CD1 heavy chains (5). Whereas HLA class I molecules are all encoded on human chromosome 6, the CD1 molecules may all be encoded on human chromosome 1 (6). Major histocompatibility complex class I molecules play a pivotal role in the immune response as structures that present antigen or antigen-derived peptides to T cells and may also function in general as contexts for ligand binding or as sites of cell–cell interaction. In contrast, the functions of the CD1 molecules are unknown.

However, HLA class I molecules and CD1a heavy chains share the ability to associate with several different cell-surface molecules, thus forming intermolecular complexes. HLA class I molecules have been shown to interact with receptors for peptide hormones or growth factors (7–9). Moreover, HLA class I heavy chains have been shown to form an intermolecular complex with a CD3-associated, T-cell receptor-like structure on the surface of some leukemic T cells (10). Similarly, some of the CD1a heavy chains are covalently associated with some of the CD8 molecules (11, 12), and CD1a heavy chains can also form intermolecular complexes by associating noncovalently either with CD1b molecules or with CD1c molecules on the surface of normal thymic cells (13, 14). In the work described here, we investigated whether HLA class I molecules and CD1a heavy chains can form intermolecular complexes on the surface of normal thymic cells.

MATERIALS AND METHODS

Antibodies. Monoclonal antibodies (mAbs) W6/32 (15, 16) and A1.4 (kindly provided by C. Y. Wang; ref. 10) recognize, respectively, a β_2-microglobulin-dependent, a $NaDodSO_4$-sensitive, and a β_2-microglobulin-independent, $NaDodSO_4$-stable framework determinant of the HLA-A, -B, and -C class I molecules. The rabbit polyclonal antiserum anti-HTA1 (6, 17), raised against purified and denatured CD1a heavy chains, was a gift of C. Milstein. Anti-CD1a mAb L404 has been characterized extensively (1).

Cells. Thymus fragments were obtained from children undergoing cardiac surgery. The thymic cells were grown in tissue culture medium supplemented with purified phytohemagglutinin, recombinant interleukin 2 (kindly provided by Biogen, Geneva), and 20% (vol/vol) normal human AB serum in order to avoid exchanges with xenogeneous β_2-microglobulin, as described (18, 19).

Labeling and Immunoprecipitation of Cell-Surface Proteins. Cells were surface-labeled with ^{125}I [2 mCi (74 MBq) per 2–5 × 10^7 cells] by means of lactoperoxidase. The cells were then lysed in 50 mM Tris·HCl (pH 8.0) containing 1% (wt/vol) Nonidet P-40, 150 mM NaCl, 5 mM $MgCl_2$, 5 mM KCl, and protease inhibitors (1 mM phenylmethylsulfonyl fluoride and 0.2 M iodoacetamide; Sigma). Immunoprecipitations were performed with either W6/32 or L404 mAb crosslinked to protein A-Sepharose CL-4B (Pharmacia, Upsala, Sweden), or with mAb A1.4 and antibody-coated (goat anti-mouse IgG) agarose beads (Sigma), or with the rabbit antiserum and protein A-Sepharose beads. The precipitates were washed extensively before elution in $NaDodSO_4$ sample buffer and electrophoresis. Endoglycosidase F (endo F; mannosylglycoprotein endo-β-*N*-acetylglucosaminidase, EC 3.2.1.96) treatment of immunoprecipitates was as described (5). Endo F-treated (0.5 unit) or sham-treated immunoprecipitates were precipitated with trichloroacetic acid before electrophoresis.

Two-Dimensional Peptide-Map Analysis of Separated CD1 Heavy Chains. After one-dimensional $NaDodSO_4$/polyacrylamide gel electrophoresis, bands corresponding to the heavy chains were digested with pepsin as described (20). Samples (10^4 cpm in 2–4 μl) of the digests were spotted on 20 × 20-cm silica TLC plates. Electrophoresis was in 5% acetic acid/15% formic acid and chromatography was in 40% 1-butanol/8% acetic acid/28% pyridine. ^{125}I-labeled peptides were detected by autoradiography.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: endo F, endoglycosidase F; mAb, monoclonal antibody.
§To whom reprint requests should be addressed.

RESULTS

A Molecule with a 33- to 35-kDa Protein Backbone Is Coprecipitated with HLA Class I Molecules by mAb W6/32. Our starting hypothesis was that HLA class I molecules and CD1a heavy chains are associated on normal human thymus cells. From previous studies (7–10), we knew that HLA class I molecules are recognized by anti-HLA mAbs even when they are associated in a complex with other cell-surface molecules. This is in contrast to our previous findings that CD1a heavy chains in intermolecular complexes with other molecules are not recognized by any of several CD1a mAbs recognizing four epitopes of the CD1a molecules (13, 14). Thus, these associated CD1a heavy chains have previously been identified by immunoprecipitation with mAbs recognizing the cell-surface molecule to which they are associated (e.g., CD1b or CD1c). Therefore, if our hypothesis is correct, anti-HLA class I mAbs should immunoprecipitate HLA class I molecules and CD1a heavy chains from human thymus cells. In an initial experiment, we took advantage of the fact that 45-kDa HLA heavy chains have been shown to have a protein backbone of 41 kDa, and the 45- to 49-kDa CD1a heavy chains, a protein backbone of 33–35 kDa, after endo F treatment (12). We performed endo F treatment of the immunoprecipitates obtained with anti-CD1a mAb L404 and anti-HLA-ABC mAb W6/32 from a lysate of surface-radioiodinated thymus cells. The endo F-treated CD1a immunoprecipitate gave 33-kDa and 35-kDa protein bands. The endo F-treated W6/32 immunoprecipitate was resolved into a major protein band at 41 kDa and two minor protein bands at 33 and 35 kDa (Fig. 1). These results indicate that on thymus cells, HLA class I molecules are associated with molecules with a protein backbone of the same size as CD1a heavy chains.

Similarities in the Peptide Maps of CD1a Heavy Chain and the Molecule Coprecipitated with HLA Class I Molecules. To study the relationship between CD1a and the molecules coprecipitated with HLA class I molecules, we obtained CD1a and W6/32 immunoprecipitates from surface-labeled fresh human thymus cells from the same individual (as shown in Fig. 1) and compared the two-dimensional peptide maps of the isolated 45- to 49-kDa heavy chains. The maps are clearly different; but the peptides observed in the CD1a heavy-chain peptide map (Fig. 2 *Top*) are also observed in the peptide map of the heavy chain isolated from the W6/32 immunoprecipitate (Fig. 2 *Middle*). These experiments were repeated with thymocytes from five unrelated individuals, and the CD1a peptides were always observed in the maps of W6/32 immunoprecipitates. These results strongly suggest that HLA and CD1a heavy chains are associated and therefore coprecipitated by W6/32, since we know from sequential immunoprecipitation experiments (4, 21) and co-capping and cross-blocking experiments (1) that the W6/32 determinant is not detected on CD1a molecules. The CD1a peptides were also observed in the map of the heavy-chain protein band immunoprecipitated by another anti-HLA-ABC mAb, A1.4 (results not shown). mAb A1.4 recognizes a $NaDodSO_4$-stable framework determinant of HLA class I heavy chain (10) that is different from the W6/32 epitope.

The Heavy Chains Associated with HLA Class I Molecules Are CD1a Heavy Chains. If the heavy chain associated with HLA class I molecules on fresh human thymus cells are CD1a heavy chains, they should not be coprecipitated from CD1a-negative cells from the same individual. To test this, we cultured thymic cells in medium containing phytohemagglu-

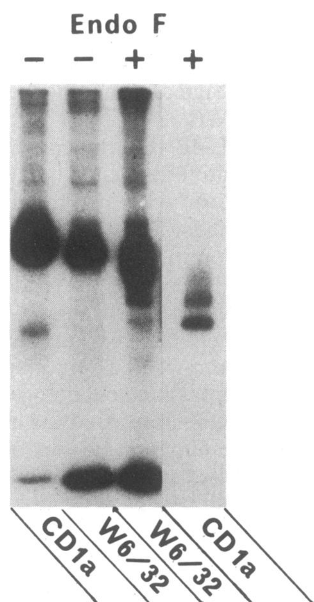

FIG. 1. $NadDodSO_4$/polyacrylamide gel electrophoresis of the molecules precipitated from radioiodinated thymus-cell lysate by mAb W6/32 or CD1a mAb L404. Immunoprecipitates were either sham-treated (−) or treated with endo F (+). Samples were electrophoresed under reducing conditions.

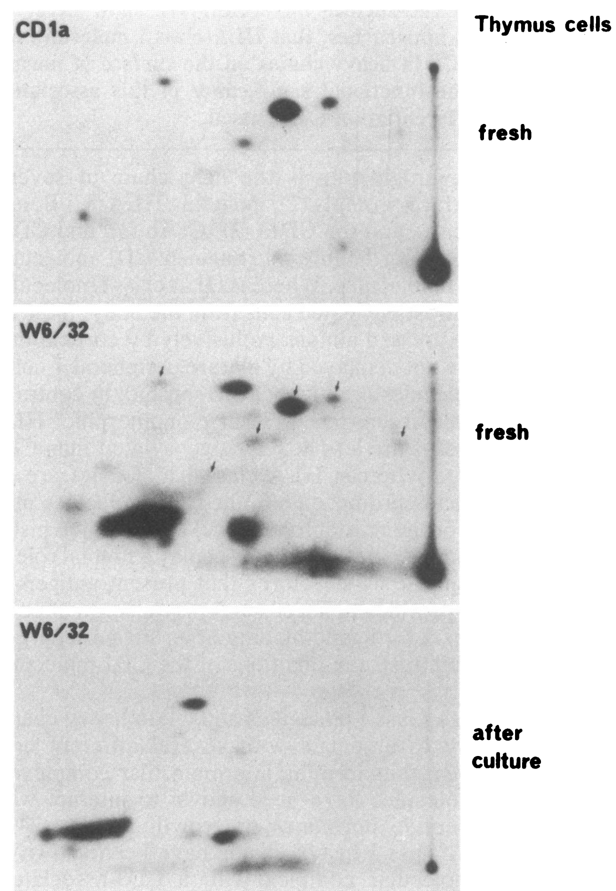

FIG. 2. Comparison of pepsin digests of the 45-kDa protein bands precipitated by W6/32 or CD1a mAb from lysates of surface-radioiodinated thymus cells. The protein bands were isolated from slab gels (see Fig. 1), and two-dimensional peptide maps were prepared. Shown are peptide maps of the heavy chains precipitated by CD1a mAb L404 (*Top*) or anti-HLA mAb W6/32 (*Middle*) from fresh thymus cells or by W6/32 from the thymus cells after culture (*Bottom*). The fresh and cultured thymus cells came from the same individual. Electrophoresis was from right to left and chromatography was from bottom to top. Arrows indicate the CD1a peptides that are observed in fresh thymus cells (*Middle*) but not after culture (*Bottom*).

tinin and recombinant interleukin 2. After a week, the cells were large blast cells that gave a homogeneous peak of brightly stained cells upon flow cytofluorimetric analysis with several anti-HLA mAbs and CD3 or CD5 mAb. In contrast, none of the anti-CD1 mAbs stained these cells (results not shown). The peptide map of the heavy chain isolated from the W6/32 immunoprecipitate of the iodinated CD1-negative cultured thymus cells (Fig. 2 *Bottom*) lacks the CD1a peptides (arrows in Fig. 2 *Middle*). Thus, after the loss of expression of the CD1 molecules from the thymic cell surface, the CD1a peptides are not observed in the W6/32 immunoprecipitate. This result confirms that the molecules associated with HLA class I molecules on normal human thymus cells are CD1a heavy chains.

To demonstrate this further, we tested whether the W6/32 immunoprecipitate from fresh thymus cells contains heavy chains that are recognized by the rabbit polyclonal anti-HTA1 antiserum, raised against purified and denatured CD1a heavy chains (6). Indeed, the rabbit antiserum precipitated a portion of the material from the denatured W6/32 immunoprecipitate. Moreover, the HLA heavy chains recognized by mAb A1.4 gave 45-kDa protein band (Fig. 3, lane C), whereas the CD1a heavy chains immunoprecipitated by the rabbit antiserum to CD1a heavy chains gave a fainter band, of slightly higher molecular mass (Fig. 3, lane B).

DISCUSSION

We have used several technical approaches to show that HLA class I molecules and CD1a heavy chains are associated on normal human thymus cells. The association is noncovalent, as one-dimensional $NaDodSO_4$/polyacrylamide gel electrophoresis of W6/32 immunoprecipitate gave identical results under reducing and nonreducing conditions (data not shown).

The reactivity of W6/32 with the HLA molecules associated with CD1a heavy chain shows that the β_2-microglobulin from the HLA molecule is present in this intermolecular complex (16). We do not know whether CD1a heavy chains are associated with β_2-microglobulin molecules in these complexes. This could explain why none of the CD1a mAbs recognizing four different epitopes of CD1a molecules reacted with CD1a heavy chain noncovalently associated with HLA class I, CD1b, or CD1c molecules (13), whereas these mAbs react only poorly with the CD1a heavy chain covalently associated with CD8 (11, 12). In contrast, the rabbit antiserum can also recognize purified denatured or *in vitro* translated (unglycosylated) CD1a heavy chains. Thus, CD1a heavy chains would be conformationally modified when present in these intermolecular complexes, as two-dimensional peptide maps of associated and unassociated CD1a heavy chains are very similar. Alternatively, free and associated CD1a heavy chains may represent different posttranslational products of a single gene or of highly homologous genes (6, 17). The glycosylation of one of the CD3 polypeptides differs when they are associated with the α/β or with the γ/δ T-cell receptors (22). Also of note is our finding that although CD1a molecules are present on the surface of malignant T cells, we did not find CD1a heavy chains associated with CD1b or CD1c molecules (14). Alterations of posttranslational modifications of these molecules on human tumor cells, such as anomalous glycosylations, which are well-documented for tumor cells (23), may be responsible for the lack of association of the CD1 molecules on malignant cells.

It is quite puzzling that CD1a heavy chains can associate with two different molecules, CD8 and HLA class I, which are thought to interact as associative recognition structures for the T-cell receptor–nominal antigen–HLA complex. The interaction between CD8 and HLA class I molecules during antigen presentation would occur between CD8 molecules from the mature, antigen-receptor-positive T cell and the HLA class I molecules from the antigen-presenting cell. We do not know whether the CD1a intermolecular complexes occur between molecules from the same thymic cell or between molecules from different thymic cells. The function of CD1a intermolecular complexes could be to modulate positively or negatively the function of the molecules while they are in the complex. It is of interest that the cells bearing CD1 molecules in the thymic cortex do not show any of the typical functions of T cells while they are acquiring their major histocompatibility complex-restricted antigen-recognition structures.

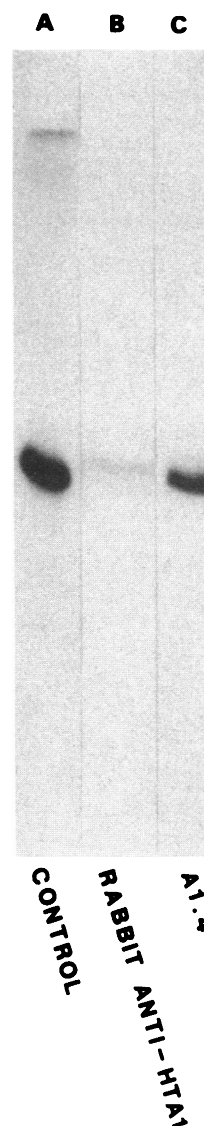

FIG. 3. $NaDodSO_4$/polyacrylamide gel electrophoresis of denatured heavy chains precipitated by W6/32 from fresh human thymus cells. W6/32 immunoprecipitate was denatured and then divided into three aliquots, which were precipitated with trichloroacetic acid (lane A), immunoprecipitated with a rabbit anti-HTA1 (anti-CD1a heavy chain) antiserum (lane B), or immunoprecipitated with A1.4 (anti-HLA heavy chain) mAb (lane C).

This work was supported by Institut National de la Santé et de la Recherche Médicale, by Association pour la Recherche sur le Cancer, and by Fondation pour la Recherche sur la Leucémie.

1. Amiot, M., Bernard, A., Raynal, B., Knapp, W., Deschildre, C. & Boumsell, L. (1986) *J. Immunol.* **136**, 1752–1757.
2. McMichael, A. J., Pilch, J. R., Galfre, G., Mason, D. Y.,

Fabre, J. W. & Milstein, C. (1979) *Eur. J. Immunol.* **9**, 205–210.
3. Olive, D., Dubreuil, P. & Mawas, C. (1984) *Immunogenetics* **20**, 253–264.
4. Knowles, R. W. & Bodmer, W. F. (1982) *Eur. J. Immunol.* **12**, 676–681.
5. Van De Rijn, M., Lerch, P. G., Knowles, R. W. & Terhorst, C. (1983) *J. Immunol.* **131**, 851–855.
6. Calabi, F. & Milstein, C. (1986) *Nature (London)* **323**, 540–543.
7. Due, C., Simonsen, M. & Olsson, L. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 6007–6011.
8. Lafuse, W. & Edidin, M. (1980) *Biochemistry* **19**, 49–54.
9. Schreiber, A. B., Schlessinger, J. & Edidin, M. (1984) *J. Cell Biol.* **98**, 725–731.
10. Bushkin, Y., Posnett, D. N., Pernis, B. & Wang, C. Y. (1986) *J. Exp. Med.* **164**, 458–473.
11. Ledbetter, L. A., Tsu, T. T. & Clark, E. A. (1985) *J. Immunol.* **134**, 4250–4254.
12. Snow, P. M., Van de Rijn, M. & Terhorst, C (1985) *Eur. J. Immunol.* **15**, 529–532.
13. Amiot, M., Dastot, H., Fabbi, M., Degos, L., Bernard, A. & Boumsell, L. (1988) *Immunogenetics* **27**, 187–195.
14. Amiot, M., Dastot, H., Schmid, M., Bernard, A. & Boumsell, L. (1987) *Blood* **70**, 676–685.
15. Parham, P. C., Barnstable, J. & Bodmer, W. F. (1979) *J. Immunol.* **123**, 342–349.
16. Kahn-Perles, B., Boyer, C., Arnold, B., Sanderson, A. R., Ferrier, P. & Lemonnier, F. A. (1987) *J. Immunol.* **138**, 2190–2196.
17. Martin, L. H., Calabi, F. & Milstein, C. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 9154–9158.
18. Bernabeu, C., Van de Rijn, M., Lerch, P. J. & Terhorst, C. (1984) *Nature (London)* **308**, 642–645.
19. Kefford, R. F., Calabi, F., Fearnley, I. M., Burrone, O. R. & Milstein, C. (1984) *Nature (London)* **308**, 641–642.
20. Acuto, O., Meuer, S. C., Hodgdon, J. C., Schlossman, S. F. & Reinherz, E. L. (1983) *J. Exp. Med.* **158**, 1368–1373.
21. Cotner, T., Mashimo, H., Kung, P. C., Goldstein, G. & Strominger, J. L. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 3858–3862.
22. Kranger, M. S., Bierer, B. E., Devlin, P., Clabby, M., Strominger, J. L., McLean, J. & Brenner, M. B. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 3817–3821.
23. Fiezi, T. (1985) *Nature (London)* **314**, 53–57.