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Characterization of Membrane Antigens on Human Cytomegalovirus-Infected Fibroblasts Recognized by Human Antibodies

LUCIA H. M. VAN DER VOORT,†* LOU F. M. H. DE LEIJ, AND T. HAUW THE
Department of Clinical Immunology, University Hospital, Groningen, The Netherlands

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The antigens on the surface of human cytomegalovirus (HCMV)-infected fibroblasts which are recognized by human HCMV antibody-positive sera were characterized. Three HCMV-induced polypeptides, with apparent molecular masses of 53 to 63, 94, and 94 to 120 kilodaltons, were precipitated from ^{125}I-surface-labeled cell extracts with different sera obtained from healthy individuals. Renal transplant recipients who were suffering from active HCMV infections recognized the same set of antigens. By the use of monoclonal antibodies, these antigens were identified as polypeptides belonging to the gcI and gcIII families of HCMV glycoproteins.

In most individuals, infection with human cytomegalovirus (HCMV) proceeds without complications. After infection, the virus remains latently present within its host as a result of an equilibrium established between the virus and the immunological defense of the host (6). In persons with a suppressed immune system, however, HCMV infection may cause severe illness. Acute HCMV infection, either primary or secondary, frequently occurs in renal transplant recipients during the first 6 months after transplantation (3, 15). Different host factors contribute to the recovery from infection (6). One of these factors could be the presence of antibodies against membrane antigens on HCMV-infected cells (10, 12, 17). It has been shown that late HCMV-infected fibroblasts can be killed in a complement-dependent assay by antibodies present in sera obtained from renal transplant recipients during the acute and reconvalescent phase of infection (11). In this study, the molecular nature of the cell surface antigens recognized by human immunoglobulin G (IgG) antibodies was further investigated and described.

Human embryonic lung fibroblasts were infected with HCMV strain AD169 (11). Antigens exposed on the outside of the fibroblast monolayers were labeled by radioiodination in a procedure employing glucose oxidase and lactoperoxidase and were subsequently extracted with lysis buffer (10 mM Tris hydrochloride [pH 8.0], 150 mM NaCl, 1% Nonidet P-40, and 0.5% sodium deoxycholate, supplemented with 0.02 mM phenylmethylsulfonyl fluoride and 200 trypsin-inhibitor units of aprotinine per ml) at 4°C for 20 min (9).

The reliability of the surface iodination technique in the selection of plasma membrane antigens has been demonstrated for a number of cell types (2, 4, 19). To assess this for HCMV-infected fibroblasts, immunoprecipitations were performed using two different HCMV-specific monoclonal antibodies. Monoclonal antibody C10 recognizes an intracellularly located immediate early antigen, which is present in the nucleus and cytoplasm of late-infected fibroblasts as well (18). Monoclonal antibody C40 is reactive with an antigen present on the plasma membrane of late-infected cells.

Monoclonal antibody C40 immunoprecipitated two polypeptides from late-infected fibroblasts which were surface iodinated, one with an apparent molecular mass of 58 kilodaltons (kDa) and one ranging from 94 to 120 kDa (Fig. 1). In contrast, monoclonal antibody C10 did not react with any of the surface-iodinated polypeptides. These results indicate that a specific subset of polypeptides, which are located on the plasma membrane, are labeled by surface iodination. When similar immunoprecipitation experiments were performed using extracts obtained from late-infected cells which were metabolically labeled with [^{35}S]methionine, monoclonal antibody C10 did react with a polypeptide with an apparent molecular mass of 67 kDa whereas monoclonal antibody C40 precipitated three polypeptide bands with apparent molecular masses of 58, 94 to 120, and 150 kDa. Since the latter band was not detected in the experiment in which surface-labeled antigens were used, it can be concluded that the 150-kDa polypeptide was not iodinated. This may indicate that this polypeptide is not present on the surface of infected cells or, at least, that it has no tyrosine residues which are accessible to iodination.

Subsequently the antigen specificity of human IgG antibodies, which are reactive with the surface of fibroblasts, was analyzed. Antibodies in human sera could, either specifically or unspecifically, react with membrane antigens of the host cell. Therefore, immunoprecipitation experiments were performed with sera from HCMV antibody-positive and HCMV antibody-negative healthy individuals. Furthermore, immunoprecipitations were performed with extracts obtained from uninfected and early-infected as well as late-infected fibroblasts. Both HCMV antibody-positive and HCMV antibody-negative sera precipitated two polypeptide bands with apparent molecular masses of 76 and 46 kDa from all cell extracts (Fig. 2A). In addition to these apparently nonspecifically precipitated bands, HCMV antibody-positive sera precipitated two broad polypeptide bands with apparent molecular masses of 53 to 63 and 94 to 120 kDa from the late-infected cell extract (Fig. 2A, lane 6). The latter band consisted of at least two polypeptides with apparent molecular masses of 94 and 94 to 120 kDa (Fig. 2B). This was shown by immunoprecipitation of a precleared extract followed by analysis of precipitated material on 8% instead of 10% polyacrylamide gels. Both HCMV antibody-positive and HCMV antibody-negative sera precipitated weak 33- and 36- to 39-kDa polypeptides from late-infected cell extracts (Fig. 2A, lanes 5 and 6). After longer exposure (2

* Corresponding author.
† Present address: Gist-brocades, R & D Celbiology, P. O. Box 1, 2600 MA Delft, The Netherlands.

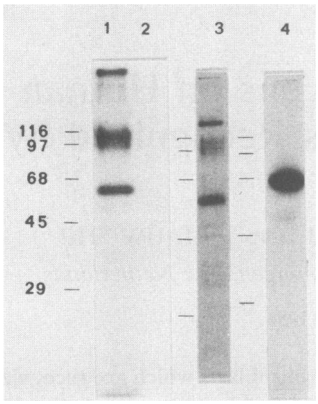

FIG. 1. Polypeptides immunoprecipitated from ^{125}I-surface-labeled and [^{35}S]methionine-labeled late-infected cell extracts with monoclonal antibodies. Lanes: 1 and 2, ^{125}I-labeled polypeptides; 3 and 4, [^{35}S]methionine-labeled polypeptides; Lanes 1 and 3 show immunoprecipitation with monoclonal antibody C40, and lanes 2 and 4 show immunoprecipitation with monoclonal antibody C10. Molecular mass standards (in kilodaltons) are indicated at the left.

months instead of 2 weeks), a weak 67-kDa band became visible with all HCMV antibody-positive serum specimens tested, as well as a weak 150-kDa band with two of the HCMV antibody-positive serum specimens tested (data not shown). Thus at least three, possibly five, HCMV-specific cell-surface polypeptides were precipitated from extracts

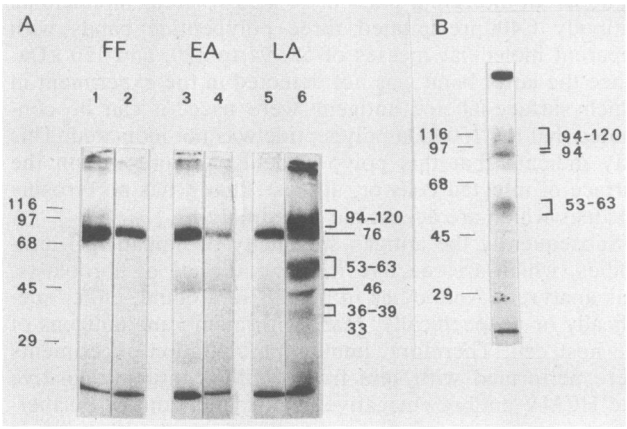

FIG. 2. Immunoprecipitation of ^{125}I-surface-labeled cell extracts with human antibodies. (A) Immunoprecipitations were performed without prior treatment of the extract with HCMV antibody-negative serum. The extracts were obtained from uninfected (FF, lanes 1 and 2), early-infected (EA, lanes 3 and 4), and late-infected (LA, lanes 5 and 6) fibroblasts. Serum specimens were obtained from healthy laboratory personnel. To reduce the volume of serum in the immunoprecipitation mixture, the IgG antibodies in the serum were immobilized by binding to protein A-Sepharose beads (Pharmacia, Uppsala, Sweden) prior to the immunoprecipitation experiment. Lanes: 1, 3, and 5, polypeptides immunoprecipitated with an HCMV antibody-negative serum; 2, 4, and 6, polypeptides immunoprecipitated with an HCMV antibody-positive serum. SDS-PAGE was on a 10% gel. Molecular mass standards (in kilodaltons) are indicated at the left. (B) The cell extract was precleared twice with HCMV antibody-negative sera prior to immunoprecipitation with an HCMV antibody-positive serum. The cell extract was obtained from late-infected fibroblasts. SDS-PAGE was on an 8% gel. Molecular mass standards are indicated at the left.

obtained from late-infected cells by IgG antibodies obtained from HCMV antibody-positive individuals. HCMV-specific polypeptides were not detected in extracts obtained from early-infected cells. The presence of HCMV-induced early membrane antigens has been indicated by membrane fluorescence with sera (10), and by electrophoretic analysis of plasma membranes obtained from early-infected cells (16). A possible explanation for these apparently contradictory results is that these early membrane antigens are not accessible to iodination or are not present in sufficient amounts to be detected in the immunoprecipitation experiments described here. In all subsequent experiments, extracts were precleared twice with HMCV antibody-negative serum. This was done to reduce the presence of the apparently nonspecifically precipitated 76- and 46-kDa polypeptides.

In previous studies using both antibody-dependent complement-mediated cytotoxicity and membrane fluorescence techniques, antibodies to surface antigens on late-infected cells were usually detected in sera from renal transplant recipients which are obtained during or shortly after active HCMV infection and not in sera from healthy HCMV antibody-positive individuals (10–12, 17). This might indicate that a specific set of membrane-directed antibodies is induced during active infection. Therefore, the surface polypeptides which are precipitated by sera from renal transplant recipients during a period of active HCMV infection were studied. Initial immunoprecipitation experiments indicated that the apparent molecular masses of the HCMV-specific polypeptides which were detected by these sera were generally similar to those seen after immunoprecipitation with sera from healthy HCMV antibody-positive individuals (Fig. 3A, lane 3). To further investigate whether antibodies are induced during active HCMV infection, sequential immunoprecipitations with paired serum specimens from renal transplant recipients were performed. One serum specimen was obtained at least 2 weeks before and the other was obtained at least 3 weeks after onset of secondary HCMV infection. These experiments also failed to reveal a specific set of antibodies which are reactive with formerly unrecognized antigens (data not shown). The possibility remains, however, that antibodies are induced during active HCMV infection which are reactive with new epitopes on the same membrane antigens, the new epitopes being located extracellularly.

The cell-surface polypeptides, which are recognized by human HCMV antibody-positive sera, were identified by the use of monoclonal antibodies. Recently, three families of glycoprotein complexes, designated gcI, gcII, and gcIII, have been identified in the envelopes of HCMV virions (5). Monoclonal antibodies reactive with antigens belonging to two of these families were used. Monoclonal antibody C40 is reactive with the HCMV homolog of herpes simplex virus glycoprotein B, as was determined by immunoprecipitation experiments with the cloned gene product (by courtesy of M. P. Cranage, Cambridge, United Kingdom). This antigen belongs to the gcI family of glycoproteins (1, 5). Monoclonal antibody 1G6 has been used for the identification of the gcIII family of glycoproteins (a kind gift of L. E. Rasmussen, Stanford University, Stanford, Calif.) (5, 13, 14).

Whereas monoclonal antibody 1G6 precipitated two surface-labeled polypeptides with apparent molecular masses of 94 and 116 to 130 kDa, monoclonal antibody C40 precipitated two polypeptide bands with apparent molecular masses of 58 and 94 to 120 kDa (Fig. 3A, lanes 1 and 2). The latter band is clearly distinguishable from the 116- to 130-kDa band precipitated by monoclonal antibody 1G6.

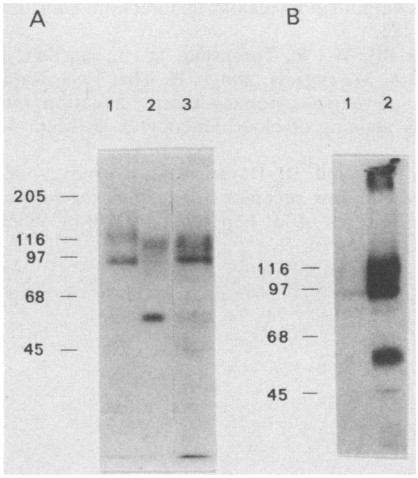

FIG. 3. Immunoprecipitation of extracts obtained from ^{125}I-surface-labeled late-infected fibroblasts with monoclonal antibodies and HCMV antibody-positive human sera. (A) Lanes: 1, immunoprecipitation with monoclonal antibody 1G6; 2, immunoprecipitation with monoclonal antibody C40; 3, immunoprecipitation with an HCMV antibody-positive human serum specimen obtained from a renal transplant recipient during reconvalescence. (B) Sequential immunoprecipitation of an extract obtained from ^{125}I-surface-labeled late-infected fibroblasts. Lanes: 1, polypeptides precipitated by an HCMV antibody-positive human serum specimen after a previous immunoprecipitation of the extract with a mixture of monoclonal antibodies C40 and 1G6; 2, polypeptides precipitated directly from the extract by the human serum. Molecular mass standards are indicated (in kilodaltons) at the left.

Comparison of the polypeptide bands obtained after immunoprecipitation with monoclonal antibodies to those obtained after immunoprecipitation with serum specimens from healthy HCMV antibody-positive individuals and renal transplant recipients showed that the polypeptide bands with apparent molecular masses of 58, 94, and 94 to 120 kDa were recognized both by human sera and monoclonal antibodies (Fig. 3A). The 58-kDa band was however less intense in experiments with human sera. It is not entirely clear whether the 116- to 130- kDa band recognized by monoclonal antibody 1G6 is also recognized by human antibodies.

To further establish the identity of these three polypeptides recognized by human antibodies, a sequential immunoprecipitation experiment was performed. After immunoprecipitation with a mixture of monoclonal antibodies C40 and 1G6, the cell extract was used for immunoprecipitation with a human HCMV antibody-positive serum specimen obtained from a healthy individual. The reactivity of human antibodies with the three polypeptides was considerably reduced, indicating that these antigens had been removed from the immunoprecipitation mixture by the monoclonal antibodies (Fig. 3B). These results indicate that the 58-, 94-, and 94- to 120-kDa bands precipitated by human sera are at least partially identical to the surface antigens precipitated by monoclonal antibodies reactive with the family of gcI and gcIII glycoproteins. Immunoprecipitation of surface-labeled cells with HCMV antibody-positive sera did not reveal antigens with apparent molecular masses comparable to those of gcII polypeptides. Nevertheless, it has been shown that human sera contain antibodies reactive with gcII glycoproteins (8). It is concluded that these antigens may not be present on the surface of infected cells or may not be accessible to iodination.

The minor 150- and 67-kDa surface polypeptides recognized by HCMV antibody-positive sera, which were observed only after longer exposure of the gels, have not been identified yet.

Recently, it was shown in immunoelectron microscopy studies using a polyclonal anti-gcI hyperimmune serum, that the plasma membranes had reacted with this serum only on spots where the virus and dense bodies budded through the plasma membrane (7). Since gcIII glycoproteins have been shown to be present on the envelopes of virions as well, it is possible that at least part of the major surface polypeptides recognized by HCMV antibody-positive sera are present on the surface of late-infected cells as budding virions (5).

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