

# Antigenic analysis of human herpesvirus 7 (HHV-7) and HHV-6 using immune sera and monoclonal antibodies against HHV-7

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Using polyclonal and monoclonal (MAbs) antibodies to human herpesvirus 7 (HHV-7), we have studied HHV-7-specific polypeptides. Human sera were obtained during the convalescent phase from patients with exanthem subitum due to HHV-7, and at least 16 HHV-7-specific polypeptides with apparent molecular masses of 26–210 kDa were immunoprecipitated. Sera prepared in mice also precipitated at least 17 HHV-7-specific polypeptides with molecular masses of 26–210 kDa. Among them, the most commonly observed antigenic protein had an apparent molecular mass of 52 kDa. Forty-two clones secreting MAbs against HHV-7-specific proteins, as determined by immunofluorescence assays, were established from BALB/c mice immunized

with HHV-7-infected cell extracts. Seven MAbs which immunoprecipitated HHV-7-specific polypeptides were further characterized. Two of these, MAbs 5E12 and 5F12, reacted predominantly with glycoproteins of 78 kDa and 85 kDa, respectively, and possessed neutralizing activity. This suggests that there are at least two neutralization-inducing proteins in HHV-7. MAb 16B4 reacted with the major immunogenic protein of 52 kDa. Five of the 42 MAbs also reacted in immunofluorescence assays with HHV-6 antigens to the same degree as to HHV-7. Two other MAbs, 7C10 and 10F1, recognized an HHV-7 protein of 40 kDa, and only 7C10 cross-reacted with an HHV-6 protein of 45 kDa.

## Introduction

Human herpesvirus 7 (HHV-7) was first isolated by Frenkel *et al.* (1990) from CD4+ T cells of peripheral blood lymphocytes from a healthy individual, and was subsequently isolated from a patient with chronic fatigue syndrome (Berneman *et al.*, 1992*a*) and saliva of healthy individuals (Wyatt & Frenkel, 1992; Black *et al.*, 1993; Hidaka *et al.*, 1993; Yoshikawa *et al.*, 1993). We previously reported that exanthem subitum (ES) is one of the clinical features of primary infection with HHV-7, since HHV-7 was isolated from the peripheral blood of a child with ES and since antibody to HHV-7 was produced during the convalescent phase (Tanaka *et al.*, 1994). It is now apparent that HHV-7, together with human herpesvirus 6 (HHV-6) (Yamanishi *et al.*, 1988), is one of the

causative agents of ES. Recently, it was reported that CD4 is the cellular membrane receptor for HHV-7 (Furukawa *et al.*, 1994; Lusso *et al.*, 1994). DNA analysis of HHV-7 revealed limited homology between HHV-6 and HHV-7 DNA (Frenkel *et al.*, 1990; Secchiro *et al.*, 1994), and immune sera and/or some monoclonal antibodies (MAbs) against HHV-6 recognized HHV-7 weakly (Wyatt *et al.*, 1991; Berneman *et al.*, 1992*a*; Foà-Tomasi *et al.*, 1994; Black *et al.*, 1996). However, HHV-7 is immunologically distinct from HHV-6. Here, we describe the antigenic properties characterized by sera from immunized mice, human immune sera and MAbs. Furthermore, the antigenic cross-reactivity between the two viruses is discussed.

## Methods

■ **Viruses and cells.** An HHV-7 isolate, KHR strain (7-KHR), and an HHV-6 variant B isolate, HST strain, were used in this study. Both were derived from the peripheral blood of children who had ES (Tanaka *et al.*,

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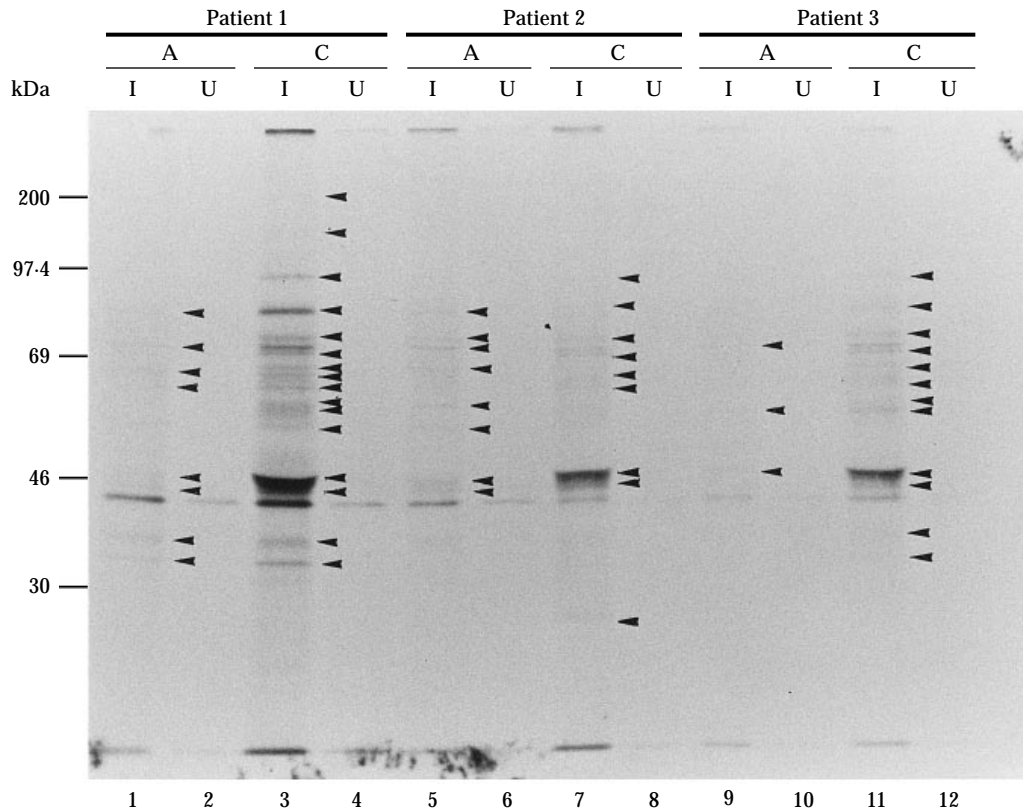


Fig. 1. SDS-PAGE analysis of HHV-7 polypeptides immunoprecipitated by human antibodies against HHV-7. [<sup>35</sup>S]methionine-labelled HHV-7-infected umbilical cord blood mononuclear cell extracts were immunoprecipitated with sera from patient 1 (lanes 1–4), patient 2 (lanes 5–8) and patient 3 (lanes 9–12). A, sera from the acute phase of disease; C, sera from the convalescent phase; I, infected; U, uninfected.

1994; Yamanishi *et al.*, 1988). An HHV-6 variant A, strain U1102, provided by the late R. Honess (National Institute for Medical Research, Mill Hill, London, UK) was also used. Propagation of these viruses in umbilical cord blood lymphocytes has been previously described (Yamanishi *et al.*, 1988). A T lymphocyte cell line, Sup T1, was kindly provided by D. Ablashi (National Institute of Health, Bethesda, Md., USA) and used to test for antibody against HHV-7.

■ **Human sera.** Human sera were obtained from three patients with ES during the acute and convalescent phases of the disease, which was presumed to result from infection with HHV-7 because no antibody (< 1:10) to HHV-6 was detected even in the convalescent phase, and antibody titres to HHV-7 rose from < 1:10 to 1:640 during the convalescent phase, as determined by an immunofluorescent (IF) antibody test.

■ **Preparation of immune sera in mice and MAbs to HHV-7.** BALB/c mice (6-weeks-old) were immunized three times with HHV-7-infected cell lysates as previously described for HHV-6 (Okuno *et al.*, 1990, 1992). The resulting sera were used as murine immune sera against HHV-7. Age-matched mice were immunized with non-infected cell lysates, and the sera were used as a negative control. Hybridomas were established by fusing splenocytes of the hyperimmune mice with a non-producing myeloma cell line, SP-2/0-Ag14, as previously described for those against HHV-6 (Okuno *et al.*, 1990). Cells secreting MAb were screened by an IF assay, and either the culture supernatants of hybridoma

cell lines or the ascitic fluid of mice injected with hybridoma cells were used as sources of MAb.

■ **Immunofluorescence (IF) test.** Sup T1 cells infected with HHV-7, or umbilical cord blood mononuclear cells infected with HHV-6 were fixed with acetone at  $-20^{\circ}\text{C}$  on glass slides. The fixed cells were incubated with supernatants from the hybridomas for 30 min at  $37^{\circ}\text{C}$ , and then for another 30 min with fluorescein isothiocyanate-conjugated  $\text{F}(\text{ab}')_2$  fragments of rabbit anti-mouse immunoglobulin G (Dako). Cells were observed under the fluorescence microscope.

■ **Immunoprecipitation assay.** Umbilical cord blood mononuclear cells were infected with either HHV-7 or HHV-6, and cultured for a few days at  $37^{\circ}\text{C}$ . When 50–60% of cells showed the cytopathic effect, they were radiolabelled with [<sup>35</sup>S]methionine/cysteine (10 mCi/ml, sp. act. 1000 Ci/mmol; NEN) for 16 h in Dulbecco's Modified Eagle Medium (Gibco). Cells were collected by centrifugation and the pellets were solubilized with RIPA buffer (0.01 M Tris-HCl pH 7.4, 0.15 M NaCl, 1% Triton-X 100, 1% SDS, 1 mM phenylmethylsulfonyl fluoride). Radiolabelled antigens were mixed with either immune sera or MAbs, and precipitated by using Protein G/Protein A Sepharose (Oncogene Science). The immunoprecipitated antigens were eluted from the Sepharose using a sample buffer (pH 8.2, 0.125 M Tris, 1% SDS, 15% glycerol) containing 5% 2-mercaptoethanol and analysed by 9% SDS-PAGE, as described previously (Okuno *et al.*, 1983).

■ **Neutralizing assay.** Virus (approximately 100 TCID<sub>50</sub>) was

aliquotted into each well of a 96-well plastic plate either with or without serial-diluted MAb ascitic fluids and incubated at 37 °C for 1 h. Then, Sup T1 cells ( $1 \times 10^4$  cells/well) were added to each well and cultured for 5 days at 37 °C. Cytopathic effects were observed under the microscope, and the reciprocal of the dilution for 50% inhibition of the cytopathic effect was determined to be the antibody titre.

## Results

### HHV-7-specific proteins recognized by human sera and immunized murine sera

Radiolabelled lysates of HHV-7-infected cells were immunoprecipitated with each human serum sample, collected during the acute and convalescent phase of the disease, from three patients with ES. Immunoprecipitates from HHV-7-infected and uninfected cells were analysed by SDS-PAGE. Nine to sixteen virus-specific bands with apparent molecular masses of 26–210 kDa were clearly seen with sera from the convalescent phase of the disease, but only three to eight polypeptides, with apparent molecular masses of 33–78 kDa, reacted faintly with sera from the acute phase (Fig. 1, lanes 1, 5 and 9). Bands which contained immunoprecipitates with the acute phase sera became stronger with sera from the convalescent phase. Among them, the band with molecular mass of 52 kDa was the most prominent.

Next, sera from two immunized mice were used for the immunoprecipitation of HHV-7-infected cell lysates. More than 18 polypeptides, with molecular mass of 26–210 kDa, were recognized (Fig. 2). The most major protein common to the two immune sera had a molecular mass of 52 kDa and was also detected with the human convalescent sera. Other major proteins detected had molecular masses of 40 kDa, 80 kDa, 85 kDa, 140 kDa and 210 kDa. When immunoprecipitates using murine and human sera were run in one gel, proteins with similar molecular masses were detected (Fig. 3).

### HHV-7-specific antigens recognized by MAbs

In order to analyse the virus-specific polypeptides of HHV-7 in more detail, we generated MAbs to HHV-7. Forty-two independent MAbs were isolated which gave positive results in the IF assay, but not all the MAbs immunoprecipitated HHV-7-specific antigens. Seven MAbs showing different immunoprecipitation patterns were selected and analysed in more detail (Table 1). Among them, two MAbs neutralized HHV-7 at 1 : 100 dilutions of ascites in a neutralizing assay.

In the immunoprecipitation test, several distinct proteins were precipitated with different MAbs (Fig. 4, Table 1). MAb 16B4 recognized a 52 kDa polypeptide (Fig. 4, lane 1), which was the same size as the most immunogenic polypeptide recognized by the murine and human sera. However, MAb 16B4 did not react with HHV-6-infected cells when examined by the IF test. MAbs 7C10 and 10F1 both mainly reacted with

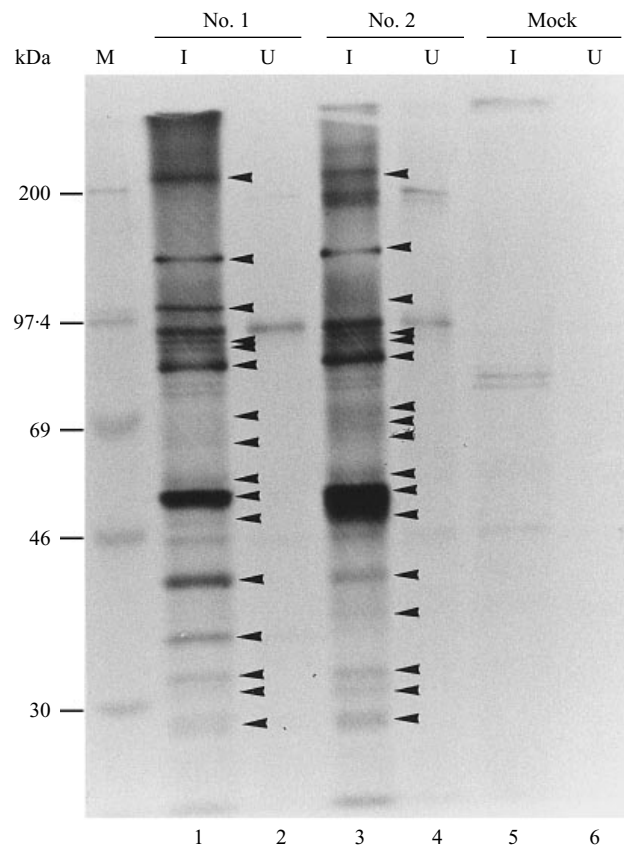
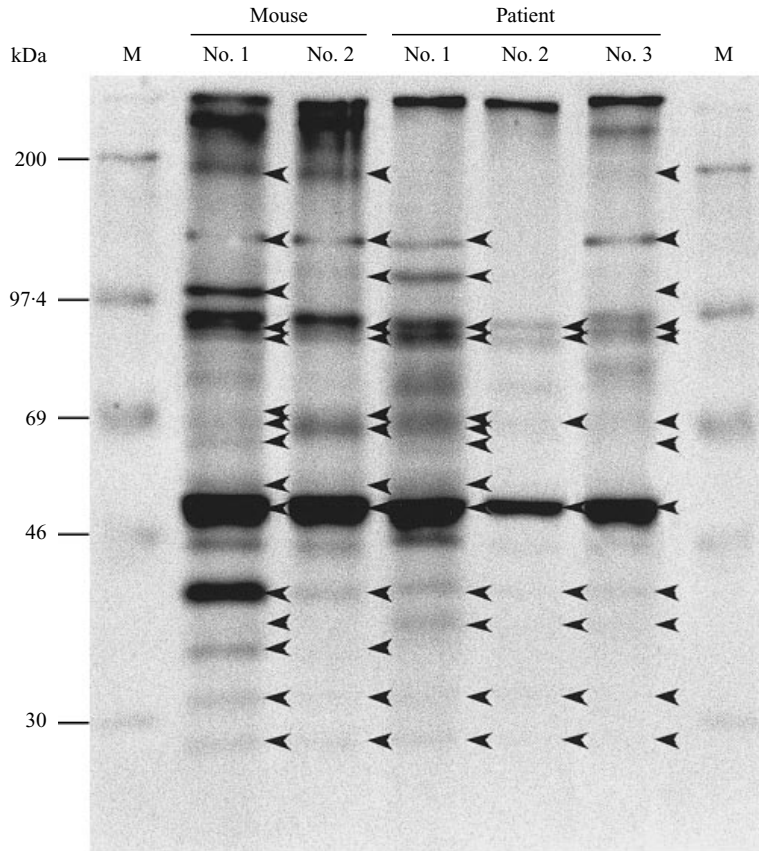


Fig. 2. SDS-PAGE analysis of HHV-7 polypeptides immunoprecipitated by murine antibodies against HHV-7. [ $^{35}$ S]methionine-labelled HHV-7-infected umbilical cord blood mononuclear cell extracts were immunoprecipitated with murine sera No. 1 (lanes 1–2), No. 2 (lanes 3–4) and mock-immunized (lanes 5–6). I, infected; U, uninfected; M, markers.

a protein with molecular mass of 40 kDa (Fig. 4, lane 2), which was the same size as a major protein recognized by the murine sera as shown in Fig. 2. Although the immunoprecipitation patterns of 7C10 and 10F1 were similar, the IF test showed that the localization of antigens was different. MAb 7C10 reacted with an antigen in the nucleus, whereas MAb 10F1 reacted with an antigen in the cell membrane (data not shown). Furthermore, MAb 7C10 reacted with HHV-6 antigen. MAbs 5E12 and 5F12 were observed to have neutralizing activities (Table 1), and very similar IF cytoplasmic staining patterns were observed in both cases. However, they showed different patterns of immunoprecipitation on a gel. MAb 5F12 showed a major band at 85 kDa and several weaker bands, ranging in molecular mass from 42–80 kDa (Fig. 4, lane 3), whereas MAb 5E12 showed a sharp band at 78 kDa and a broad band at 110 kDa (Fig. 4, lane 4). MAb 5A3 reacted with polypeptides with apparent molecular masses of 120 kDa (major), 180 kDa and 210 kDa in HHV-7-infected cells (Fig. 4, lane 5), and also recognized HHV-6 antigen by the IF test. However, the MAb did not precipitate any specific protein in HHV-6-infected cells.



**Fig. 3.** SDS-PAGE analysis of HHV-7 polypeptides immunoprecipitated by human and mouse antibodies against HHV-7. [<sup>35</sup>S]methionine-labelled HHV-7-infected umbilical cord blood mononuclear cell extracts were immunoprecipitated with murine sera No. 1 and No. 2 and sera from patients 1–3. M, markers.

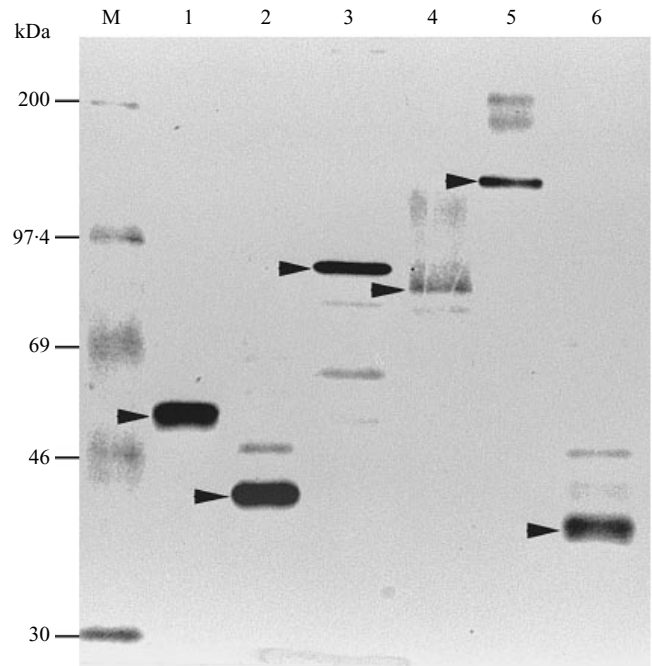
**Table 1.** Characteristics of MAbs against HHV-7

MAb	Isotype	Reacting proteins of HHV-7* (main band, kDa)	Neutralizing activity†	Reactivity with HHV-6 variants‡	
				A	B
16B4	G1	52	–	–	–
7C10	G1	40	–	+	+
10F1	G1	40	–	–	–
5E12	G1	78	+	–	–
5F12	G1	85	+	–	–
5A3	G1	120	–	+	+
2B8	G2a	34	–	–	–

\* Reacting proteins were detected by immunoprecipitation as shown in Figs 4, 5 and 6.

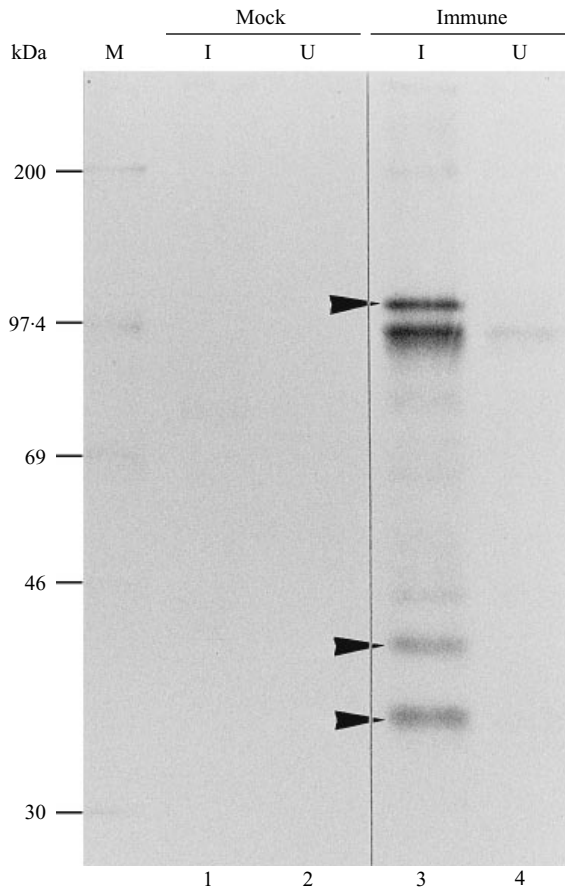
† Neutralizing activity was detected as described in Methods.

‡ Reactivity with HHV-6 variants A and B was detected by immunoprecipitation and immunofluorescence assays.



**Fig. 4.** SDS-PAGE analysis of HHV-7 polypeptides immunoprecipitated by MAbs against HHV-7. [<sup>35</sup>S]methionine-labelled HHV-7-infected umbilical cord blood mononuclear cell extracts were immunoprecipitated with MAbs 16B4, 7C10, 5F12, 5E12, 5A3 and 2H3 (lanes 1–6, respectively). M, markers.

Finally, MAb 2B8 precipitated a polypeptide with molecular mass of 34 kDa, together with several minor polypeptides of greater molecular mass (Fig. 4, lane 6).

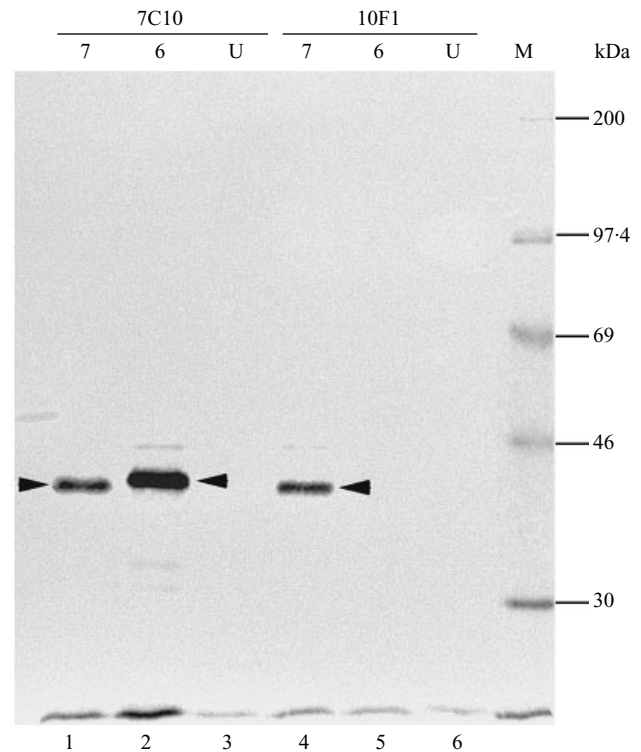


**Fig. 5.** SDS-PAGE analysis of HHV-6 polypeptides immunoprecipitated by murine antibodies against HHV-7. [<sup>35</sup>S]methionine-labelled HHV-6-infected umbilical cord blood mononuclear cell extracts were immunoprecipitated with murine non-immune (lanes 1–2) and immune sera (lanes 3–4). I, infected; U, uninfected; M, markers.

### Cross-reactive antigens between HHV-7 and HHV-6

With the human immune sera, positive staining was observed by IF in HHV-7-infected cells, but not in HHV-6-infected cells. On the other hand, the murine immune sera reacted with antigens in HHV-6-infected cells when examined by IF (data not shown). Furthermore, with HHV-6-infected cells, the murine sera immunoprecipitated radiolabelled antigens with molecular masses of 34 kDa, 45 kDa, 88 kDa and 100 kDa (Fig. 5). However, the 88 kDa polypeptide was also observed very faintly with non-immune sera (Fig. 5, lanes 3 and 4). Therefore, at present, it is not clear whether this polypeptide is virus-specific or not. These results suggest that at least three proteins share antigenic domains common to both HHV-6 and HHV-7.

The IF test showed that five of the 42 MAbs reacted with HHV-6-specific antigens in infected cells (data not shown). Although MAb 7C10 and 10F1 reacted with a 40 kDa polypeptide of HHV-7, only MAb 7C10 recognized HHV-6A- and HHV-6B-specific antigens by IF (Table 1). An immunoprecipitation experiment revealed that MAb 7C10 precipitated a



**Fig. 6.** SDS-PAGE analysis of HHV-6 and -7 polypeptides immunoprecipitated by MAbs against HHV-7. [<sup>35</sup>S]methionine-labelled HHV-6- or HHV-7-infected umbilical cord blood mononuclear cell extracts were immunoprecipitated with MAbs 7C10 and 10F1. 7, HHV-7-infected cell extract; 6, HHV-6-infected cell extract; U, uninfected cell extract; M, markers.

45 kDa protein from HHV-6-infected cells (Fig. 6, lane 2), whereas it reacted with an HHV-7-specific polypeptide of 40 kDa (Fig. 6, lane 1). MAb 5A3, which reacted with polypeptides with molecular masses of 120 kDa, 180 kDa and 210 kDa in HHV-7-infected cells, recognized some HHV-6 antigens by the IF test, but did not precipitate any specific protein in HHV-6-infected cells. Three MAbs, which recognized HHV-6 antigens by the IF test, did not precipitate any specific polypeptides from either HHV-7- or HHV-6-infected cells.

### Discussion

In this report, we have analysed HHV-7-specific polypeptides using human immune sera, sera from immunized mice and MAbs. In relation to this, we previously reported that HHV-7 causes exanthem subitum, as does HHV-6 (Tanaka *et al.*, 1994). Since it was reported that there is antigenic cross-reaction between HHV-7 and HHV-6 (Wyatt *et al.*, 1991; Berneman *et al.*, 1992*b*; Foà-Tomasi *et al.*, 1994, 1996; Black *et al.*, 1996), for the present study, we have chosen human sera having antibodies specific to HHV-7. The sera were taken from patients with ES whose antibody titre to HHV-7 increased

during the convalescent phase of ES, while that to HHV-6 did not. When human sera were used for immunoprecipitation, sera from the acute phase reacted with two to three polypeptides which varied with each serum. In contrast, the convalescent sera reacted with 7–17 polypeptides of different molecular masses (Fig. 1). To further characterize the immunogenic HHV-7 antigens, we produced antisera in mice. When the murine immune sera were tested using HHV-7-infected cell lysates, more than 20 polypeptides reacted. The most antigenic polypeptide detected with both human and mouse sera had an apparent molecular mass of 52 kDa. Foà-Tomasi *et al.* (1994) also reported that human immune serum and sera prepared in rabbits and mice precipitated 20 proteins specific to HHV-7, and that seven of them were glycoproteins. They also reported that a polypeptide of 85 kDa was the major determinant of the immune response. Wyatt *et al.* (1992) reported that an HHV-7 protein of 91 kDa was mainly detected in Western blotting with human serum. Foà-Tomasi *et al.* (1996) recently reported that several HHV-7 polypeptides with apparent molecular masses of 85 kDa, 70 kDa, 61 kDa and 52 kDa were detectable by immunoprecipitation tests using sera positive for HHV-7 and negative for HHV-6. In our study, the 85 kDa polypeptide was also detected using human serum (Fig. 1, lane 3), although the 52 kDa polypeptide was the major protein detected with every serum. Foà-Tomasi and colleagues used sera from normal adults, while we used the convalescent sera from patients with ES caused by HHV-7. Therefore, the sera from adults may have a higher antibody titre than ours because of boosting after the primary infection.

We established 42 independent hybridoma clones which produced MAbs specific to HHV-7, and analysed further the immunoprecipitation patterns and biological characteristics of seven of these MAbs. These MAbs reacted mainly with HHV-7-specific polypeptides with molecular masses of 34 kDa, 40 kDa, 52 kDa, 78 kDa, 85 kDa and 120 kDa. Foà-Tomasi *et al.* (1994) reported that they obtained four MAbs which reacted specifically with HHV-7 polypeptides with molecular masses of 51 kDa, 85 kDa and 121 kDa. However, their MAbs did not neutralize HHV-7 infectivity. In contrast, two of our MAbs showed neutralizing activities to HHV-7. Both recognized glycoproteins. However, the reactivities to HHV-7 polypeptides were different: one reacted with the 85 kDa protein and the other with the 78 kDa protein. This result indicates the existence of two separated neutralizing epitopes, each present in a different glycoprotein. It was reported that at least seven glycosylated polypeptides with molecular mass of 41–100 kDa were recognized by human immune sera (Foà-Tomasi *et al.*, 1994). However, it is not clear whether the glycoproteins of 78 kDa and 85 kDa, which reacted with MAbs 5E12 and 5F12, respectively, were among those glycosylated polypeptides.

The most antigenic protein of HHV-7 detected by all of our immune sera was the 52 kDa protein, which also reacted with HHV-7-specific MAb 16B4. Another major protein which one

immune murine serum recognized had a molecular mass of 40 kDa and possessed a common epitope with HHV-6, although the molecular mass of the HHV-6-specific polypeptide recognized by MAb 7C10 was 45 kDa.

Using sequence analysis, HHV-7 is more closely related to HHV-6 than to other human herpesviruses (Berneman *et al.*, 1992*b*; Mukai *et al.*, 1995; Nicholas, 1996). It was also reported that HHV-7 shares common epitopes with HHV-6 (Wyatt *et al.*, 1991). Furthermore, HHV-6 antibody was boosted during HHV-7 infection (Ueda *et al.*, 1994; Tanaka-Taya *et al.*, 1996). In our study, mouse sera against HHV-7 also precipitated antigens of HHV-6 with molecular mass of 36 kDa, 41 kDa, 95 kDa and 110 kDa, and the IF test showed that two MAbs were able to react with HHV-6A and HHV-6B antigens. It was also observed, using IF, that two MAbs to HHV-7 capable of reacting with the HHV-7 polypeptide of 85 kDa reacted weakly to HHV-6 antigen (Foà-Tomasi *et al.*, 1994). Similarly, some MAbs to HHV-6 reacted weakly with HHV-7 antigens (Wyatt *et al.*, 1991). Thus, there is cross-reactivity between the two herpesviruses. However, each viral infection seems to occur independently, because a second episode of ES was caused in quite a few cases by HHV-7, as we have previously reported (Tanaka *et al.*, 1994). Therefore, HHV-7 infection is possible in the presence of antibody to HHV-6. It is, however, not clear whether there is some modification of clinical symptoms due to the prior infection, because the cross-reactivity of cellular immunity has also been reported (Yasukawa *et al.*, 1993).

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