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Humoral immune response to human cytomegalovirus infection: diagnostic potential of immunoglobulin class and IgG subclass antibody response to human cytomegalovirus early and late antigens

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Summary. For the development of effective prophylaxis (hyperimmune globulins) and improvement of serological testing for human cytomegalovirus (HCMV) infection in immunocompromised patients it is essential to characterize the viral encoded proteins and the humoral immune response in terms of neutralizing antibodies and immunglobulin class and IgG subclass reactivity to "early" and "late" HCMV proteins. The major neutralizing epitopes have been identified and screening of donor sera for neutralizing antibody by either conventional neutralization assays or enzyme-linked immunosorbent assay using recombinant antigens may help to improve the efficacy of hyperimmune globulin prophylaxis. The humoral response to individual HCMV proteins has been thoroughly investigated in immunocompromised patients. Antibodies against HCMV induced "early" antigens are not exclusively associated with active infection but may indicate an elevated risk for cytomegalic inclusion disease in immunocompromised patients. With a sensitive western blot technique. IgM and IgA antibodies against HCMV "late" proteins can be detected in sera from healthy seropositive individuals. Serum samples from subjects suffering from cytomegalic inclusion disease show significantly larger broader immune responses compared with healthy HCMV antibody carriers. Promising results using recombinant antigens corresponding to immunodominant epitopes for the detection of HCMV specific antibodies have been published.

Key words: Human cytomegalovirus – Early antigens – Late antigens – Recombinant antigens – Immunglobulins G_1–G_3, A and M – Western blot

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Abbreviations: AIDS = acquired immunodeficiency syndrome; CID = cytomegalic inclusion disease; ELISA = enzyme linked immunosorbent assay; HCMV = human cytomegalovirus; PCR = polymerase chain reaction

Dedicated to Prof. Dr. K. Munk, Heidelberg, on the occasion of his 70th birthday

Human cytomegalovirus (HCMV) is the most frequent known cause of congenital viral infections in humans and an important cause of morbidity and mortality in organ transplant recipients and patients with acquired immunodeficiency syndrome (AIDS). Immunocompromised patients often fail to react serologically in an appropriate way to an acute HCMV recurrence or even primary infection [68, 69, 71]. Cell-mediated immunity to HCMV is probably the most important form of defense in immunocompetent individuals, but this is not exclusively the case in severe immunocompromised organ transplant recipients and AIDS patients. There is increasing evidence that specific antibody (hyperimmune globulin) may protect against cytomegalic inclusion disease (CID) in transplant patients but do not prevent infection [4, 5, 6, 54].

Before an effective prophylaxis (vaccines and passive immunization) and serological diagnosis can be developed, it is imperative that the cellular and humoral immune mechanisms involved in limiting the severity of CID and the antigens inducing these responses should be thoroughly characterized. Especially for passive immunization and serological diagnosis of HCMV infection it is mandatory to determine the role of virus encoded proteins in humoral immunity and the type of humoral immune response. Since studies are now under way for the detection of HCMV-specific antibodies using recombinant antigens, it is of interest to review the response of neutralizing antibody, immunoglobulin classes G, M, and A, and IgG subclasses to both nonstructural ("early") and structural ("late") proteins of HCMV in immunocompetent individuals (primary infection) and in different series of immunocompromised patients (AIDS patients and organ transplant recipients).

Neutralizing antibody response to HCMV

Neutralizing antibody plays an important role in defense against HCMV infections. In murine CMV

infections, neutralizing CMV antibodies reduce the virus dissemination during the acute phase in immunocompromied mice [2, 31, 56] and protect mice from lethal infections with murine CMV [29].

The HCMV envelope glycoproteins gB and gH are important targets for neutralizing antibodies [3, 12, 15, 16, 30, 63]. The majority of neutralizing antibodies detected in human immune sera react with gB [14]. There are conflicting reports concerning the influence of strain variation on the neutralizing antibody activity against individual patient strains. Both murine monoclonal antibody and human sera which have good neutralizing activity against HCMV strain AD169 from cell culture have been shown to have no reactivity against urine virus [44]. In contrast, sera from transplant recipients suffering from primary or secondary infection have nearly identical neutralizing antibody titers against the virus isolate passaged in vitro and strain AD169 [14, 70].

It has been shown that neutralizing antibodies and IgG-class antibody detected by enzyme-linked immunosorbent assay (ELISA) are not correlated to each other [9, 57]. AIDS patients and immunocompromised renal transplant recipients show higher ELISA antibody titers but lower neutralizing antibody titers than healthy seropositive controls [8, 18].

To improve the quality of hyperimmune globulins for the prophylactic treatment of CID it would be of interest to use the neutralizing antibody titer as selection criterion of donor sera. Until now donor sera have been selected on the basis of their reactivity to whole HCMV-infected cell lysates using the ELISA assay. Because of the abundance of highly antigenic HCMV-encoded phosphoproteins (pp150, pp65, and pp28) within infected cell lysates used as antigen source for the ELISA, commercial hyperimmune globulin preparations have only slightly higher neutralization titer per milligram IgG than normal globulin preparations [42]. As suggested by Mach and Britt [42], screening of donor sera should be performed by an ELISA specific for antibodies against known neutralizing epitopes using recombinant or synthetic antigens in order to ameliorate the quality of immune globulin preparations.

Neutralizing monoclonal antibody reacting with a 34-kDa polypeptide found in purified virions of all strains of HCMV [11, 58] cross-reacts with a glycoprotein of 94 kDa immunoprecipitable from uninfected fibroblasts [47]. The possible existence of cellular determinants in virus particles could be of significance in the pathogenesis of HCMV disease and could be the cause of autoimmune reactions including the presence of anti-nuclear [66] and anti-smooth-muscle antibodies [1], lymphocytosis, hemolytic anemia [73], and thrombocytopenia [13].

IgG antibody reactivity to HCMV-induced early antigens detected by immunoblotting

Immunogenic HCMV-infected cell proteins made before viral DNA synthesis have been sought mostly by immunoprecipitation with human serum [4]. Analysis by immunoblotting of purified HCMV early polypeptides with human sera having different HCMV antibody titers show a total of 11 electrophoretically distinct early polypeptides with a molecular weight ranging between 23 and 79 kDa [25]. Sera from immunocompetent individuals suffering from CID, AIDS patients, and renal transplant recipients suffering from primary or recurrent HCMV infection present approximately the same percentage of reactive immunoblots. In contrast to healthy HCMV antibody carriers, sera from subjects at elevated risk of CID react with a significantly higher amount of HCMV early polypeptides [25].

Some authors suggested that antibody reaction to HCMV early antigens is of short duration, and the presence of antibodies directed against early antigens is considered as a marker of an active HCMV infection [23, 24, 49, 62]. However, more recent studies demonstrate that early antigen antibodies can be detected in seropositive healthy individuals without evidence of active infection [19–22, 25, 48]. In patients suffering from an active HCMV infection, antibodies directed against early antigens may still be detected up to 7 months after convalescence [25]. The discrepancies among these studies may be attributed to differences in sensitivity of the Western blot techniques.

In most cases, however, active HCMV infections can be differentiated from nonactive infections by the intensity of protein-specific immune reactions. Sera from the majority of individuals with nonactive HCMV infection merely react with the 66-, 59-, 56-, 50-, or 26-kDa proteins, whereas sera from most of the subjects suffering from active HCMV infection recognize, in addition, the 79-, 70-, 43-, 38-, 30-, or 23-kDa proteins [25].

IgG subclass specific antibodies to HCMV induced early antigens

Few reports deal with the reactivity of immunoglobulin G subclasses against HCMV early proteins. Hamann and Doerr [26] investigated the IgG subclass reactivity pattern to early antigens in 217 sera obtained from immunocompetent and immunosuppressed individuals. All IgG subclasses are involved in the IgG immune response to

HCMV early antigens. IgG1 is the major subclass reacting with early antigens, and it is present in sera from HCMV-seropositive immunocompetent persons, healthy persons, renal transplant recipients, and AIDS patients. Antibody responses of IgG isotypes 2, 3, and 4 are observed with lower frequency and lower activity levels [26].

While only minor differences in the subclass reactivity pattern can be detected within the different stages of human immunodeficiency virus infection, the prevalence of IgG1 antibodies to the 70-kDa protein is lower in individuals infected with the human immunodeficiency virus than in renal transplant recipients and immunocompetent healthy persons [26]. No significant differences are observed in the subclass-specific early antigen antibody formation in primary and reactivated or in clinically manifested and asymptomatic HCMV infections. Furthermore, there is no correlation between the duration of early antigen antibody formation and the state of the HCMV infection [26].

IgG-, IgM-, and IgA-class specific antibody response to HCMV structural antigens

Since HCMV structural proteins were described by Stinski in 1977 [60] and more recently reviewed by Landini and Michelson [33], more than 120 late proteins have been described in a great number of reports dealing with HCMV, although its genome is known to code only for some 35 late antigens. With the immunoblot technique, antibodies to 25 late antigens have been reproducibly detected [23, 34]. The major antigens recognized by human sera are polypeptides of molecular masses of 150, 115, 100, 66, 58, and 50 kDa [23]. Nevertheless, variable amounts of secondary bands have been described by various authors [16, 21, 27, 51]. These differences may reflect strain variations, the source of viral proteins, differences in technique, or variations in conditions used for the single technique.

The number of polypeptides recognized by human sera and the intensity of reaction correlates closely to serum IgG titers as determined by ELISA [34]. IgG antibodies react with 17–20 polypeptides [33]. IgM and IgA reactivities are less broad; these immunoglobulins preferentially bind a few polypeptides (150, 66, and 38 kDa). One of the most reactive proteins with IgG (28 kDa) fails to elicit IgM response [34, 58]. In primary infection of renal transplant recipients, the first IgG to appear is specific for a polypeptide of 66 kDa and appears either alone or together with other immunoglobulin G antibodies against a polypeptide of 82 kDa (23, 35, 37]. IgG to the major immunogenic structural protein pp150 appears later in the course of natural infection [35]. In primary infection as well as in reinfection, the first IgM to appear reacts preferentially with a 38- and a 66-kDa polypeptide [28, 35].

The major structural matrix phosphoprotein pp150 is recognized by nearly 100% of the sera from HCMV-seropositive subjects. The response to pp150 persists for years after convalescence, even when antibody to other HCMV proteins are no longer detectable [28, 34, 35]. Controversy persists concerning the apparent molecular mass of pp150. It is of note that the protein's calculated molecular weight on the basis of nucleotide sequencing seems to be 123 kDa and not 150 kDa as observed with the PhastSystem sodium dodecyl sulfate polyacrylamide gel electrophoresis [7].

The polypeptide p82 may play a role in inducing a protective immune response, since renal transplant recipients with antibody to this protein at the time of transplantation have a lower incidence of virus reactivation [28]. The matrix phosphoprotein (pp 65/66 kDa) is the most important consistent of the complement binding antigen. This phosphoprotein is highly reactive to both IgG and IgM in the early onset of primary HCMV infection [35, 53] but is also detected by IgA antibody in recurrent HCMV infection and in healthy seropositive individuals [35].

In 100% of subjects over 13 years of age and in 75% of those aged 2–6 years recovering from primary HCMV infection, a membrane protein of 60 kDa which seems to be present on human fibroblasts is recognized by IgM [36]. The mp60-affinity purified IgM were shown to react with a protein of 38 kDa exclusively present in HCMV infected cell extracts. A region of homology between mp60 and HCMV assembly protein p38, which is the major IgM-reactive polypeptide of HCMV, can be localized approximately in a linear determinant between amino acid 226 and the carboxy terminus of the viral protein [36]. The presence of this cross-reactive antigen on the membrane of human red blood cells gives a possible explanation for the pathogenesis of hemolytic anemia that may appear during primary HCMV infection. Furthermore, primary HCMV infection can be diagnosed by the appearence of IgM directed against this epitope [36].

The IgA and IgM antibodies produced during active HCMV infection in renal transplant recipients and AIDS patients exhibit different patterns of reactivity with HCMV-encoded polypeptides compared to HCMV positive healthy individuals

[10]. Immunoreactions of healthy individuals are restricted to viral polypeptides with approximate molecular weights of 68 kDa and 150 kDa. Additional bands are found in the actively infected patients only and are observed more frequently with IgA than IgM [10]. Porath et al. [53] described immunoglobulin A reactivities to HCMV proteins of molecular masses of 66–70, 150, 205 and 235 kDa in 80–100% of sera drawn from patients with HCMV mononucleosis.

The frequent detection of HCMV-specific IgM in sera of healthy HCMV-seropositive individuals by immunoblot supports the evidence of a continuous age- and sex-related endogenous HCMV reactivation [45] and does not seems due to the persistence of low levels of IgM antibody for many years.

IgG subclass specific antibodies to HCMV structural antigens

There are conflicting observations with regard to the distribution of IgG subclasses in HCMV infections [17, 29, 32, 41]. The differences in the results of these studies are probably related to the differing designs of the studies. IgG1 and IgG3 are the most frequent and active subclasses in HCMV-seropositive healthy persons, renal transplant recipients, and AIDS patients. These antibodies are highly reactive with all the major proteins (150, 68, 67.5, 28 kDa). The general reactivity pattern of IgG1 is similar to that seen with total IgG [34, 65, 72] although the reported apparent molecular weights differ among different authors. Although virus-specific IgG1 is the dominant antibody in several virus diseases [32], HCMV-specific IgG3 appears to possess neutralizing activities [22] and to be restricted in advanced HCMV disease [61]. Reactivities against IgG3 are quantitatively and qualitatively most important in renal transplant recipients.

Detection of antibodies against HCMV using recombinant or synthetic peptides

A number of studies using recombinant proteins from HCMV proteins in immunoblots have shown that it is possible to identify human sera containing antibodies against HCMV by using recombinant antigens from only a few viral proteins [38, 39, 40, 46, 55, 67]. Recently, fusion proteins containing immunodominant epitopes of pp150 have been obtained using recombinant DNA techniques and have been shown to react efficiently with human

Table 1. Possibilities and applications of serologic testing for HCMV antibody detection using different antigens and methodologies

Test system	HCMV antigens	Antibody detected	Clinical relevance
ELISA	Viral lysate	IgG	Assessment of immunity Epidemiological marker
	Recombinant pp150	IgG	Assessment of immunity
	Viral lysate	IgM	Acute primary and recurrent infection
	Viral lysate	IgA	Recurrent infection
		IgG_3	Acute primary infection
	Recombinant glycoprotein B	IgG	Monitoring of HIG[a] prophylaxis
Western blot	Late antigens:		
	p66, (p82)	IgG	Early seroconversion
	p82	IgG	Protective immunity?
	pp150[b]	IgG	Late seroconversion
	p38, p66	IgM	Early seroconversion
	p68, pp150	IgA	Recurrent infection
	p28, p68, pp150	IgG_3	Primary and secondary infection Neutralizing activity?
	Early antigens	IgG	Active infection, CID risk[c]
		IgG_1, IgG_3	CID risk[c]
	Early antigen p70	IgG_1	Reduced prevalence in immunocompromised patients
Neutralization assay	Infectious virions	Neutralizing antibodies	Monitoring of HIG[a] prophylaxis Prognostic marker

[a] Hyperimmune globulin
[b] Antibodies against pp150 appear late in the course of primary infection
[c] Broader immune reactions (> 5 polypeptides) with sera from immunocompromised patients

antibody [46, 55, 59, 67]. Using ELISA with recombinant or synthetic peptides, it appears that peptides from a single protein of HCMV may be sufficient to identify HCMV-seropositive individuals [52]. Nevertheless, at present time, to prevent transfusion associated CID in persons at risk, HCMV antibody screening of blood donations should be performed with serologic tests using more than one recombinant antigen. The overall sensitivity of IgM detection using single peptides is still under 85% [38].

Conclusion

In view of the development of subunit vaccines and ELISA assays using single recombinant or synthetic peptides for the detection of immunoglobulins to HCMV, it is of importance to give a concise review of the most relevant immunogenic proteins encoded by HCMV genome. Especially, to improve the efficacy of hyperimmune globulins it is necessary to test donor sera for the presence of neutralizing antibodies, either by a rapid neutralization assay [8] or by ELISA using recombinant glycoprotein B. Table 1 summarizes the possibilities and applications of serologic testing for HCMV antibody detection using different antigens and methodologies.

Despite the numerous reports dealing with immunoblotting of HCMV proteins in different patient series, controversy persists concerning the temporal and distribution pattern of the immunoglobulins and immunoglobulin subclasses to HCMV proteins; there is no evidence for a reliable serologic marker of acute infection. Using recombinant antigens corresponding to immunodominant epitopes, serologic testing of HCMV infection in immunocompromised patients may be substantially improved.

Today, the diagnosis of active HCMV infection is best accomplished by virological methods. HCMV p65 antigen detection and HCMV DNA detection by polymerase chain reaction permit a very sensitive and rapid laboratory diagnosis of HCMV infection [50, 64, 72]. Recent studies have shown the early detection of HCMV in human fibroblasts with monoclonal antibodies (shell vial culture) to be more reliable for the diagnosis of CID in immunocompromised patients than serologic testing [43, 71].

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