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HUMAN CYTOMEGALOVIRUS-ASSOCIATED DNASE AND THE SPECIFIC IMMUNE RESPONSE IN DIFFERENT CLINICAL CONDITIONS

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Human cytomegalovirus (HCMV) belongs to the Herpesviridae family and is a human pathogen (1) which has been associated with significant morbidity and mortality in immunocompromised hosts such as organ transplant recipients and patients with acquired immunodeficiency syndrome. Further it can be transmitted *in utero* leading to congenital malformation, mental retardation etc. This virus can give rise to both primary infections and reactivations, being the former generally associated with more severe symptoms; the differential diagnosis between primary and reactivated infections is still uncertain.

We have recently shown (2) that a DNase activity is associated with extensively purified Human Cytomegalovirus (HCMV) virions. This activity is associated with 4 polypeptides: two major species with a MW of 46 and 49 Kd of cellular origin and two minor species of 52 and 54 Kd whose origin has not been determined. We also found that purified IgG from a pool of highly positive human sera to HCMV could neutralize the viral-associated DNase activity while IgG from a pool of negative sera to HCMV had no effect.This evidence led us to investigate in more detail the specific immune response to the HCMV associated DNase.

In the present study we have analyzed by DNase activity neutralization a total of 72 sera, 58 of which were divided in three groups on the basis of their antibody titer to HCMV, while the remainder were 8 pairs of seroconverted sera: 4 from patients with primary HCMV infection and 4 from patients with reactivated infections. All of them came to our diagnostic laboratory for serological monitoring of HCMV infection in renal transplant recipients. The assay procedure for HCMV-associated DNase activity was based on the release of acid soluble nucleotides from double stranded ^{14}C labeled E.coli DNA (Amersham, England) with some minor modifications with respect to the procedure described by Hoffmann and Cheng (3). Briefly purified virus was dissociated in 6M guanidine-HCl for 1 hour on ice, then centrifuged at 10^4 rpm for one hour and the supernatants dialyzed against Tris 10mM pH 7.0, 10% glycerol. The standard reaction mixture contained 0.05M Tris HCl pH 7.5, 10mM $MgCl_2$, 2 mM $CaCl_2$, 3µgs of ^{14}C-E.coli DNA and viral extract in a total volume of 100 µl. The reaction was carried out for 30 min at 37°C and was stopped by chilling and by addition of 10 µg of calf thymus DNA. One unit of DNase activity was arbitrarily defined as the amount of enzyme that converts 1 µg of double-stranded DNA to acid-soluble material in 30 min at 37°C. A 0.1 unit sample of DNase from an extract of purified HCMV in a total volume of 10 µl was incubated with 10 ul of serum at room temperature for 30 min. The activity of the DNase was then determined as described above. The difference of activity in the presence and absence of serum was calculated and expressed as the units of DNase activity neutralized by 1 ml of serum. The presence of endogenous nuclease activity in sera was checked by incubating each serum in the standard reaction mixture: sera with a high nuclease activity were discarded. Serum samples were tested by Enzyme immunoassay (EIA) for the presence of IgG antibodies reacting with HCMV. This was performed using the Cytomegalisa Test kit (M.A.Bioproducts, Walkersville,MD). Absorbance at 405 nm was measured 45 min after the addition of the substrate using a Titertek-multiscan photometer (Flow Laboratories). The sera were divided into 3 groups (ie, strong positive, medium-low positive and negative) by comparison with the three calibration sera which are included in the test kit. Figure 1 shows the correlation of anti HCMV titer with the anti DNase activity in a total of 58 human sera distributed into three groups as described above. Among the 17 HCMV-negative sera we detected a broad range of DNase neutralization activity, namely from zero to 4.9 units per ml of serum. With this in mind only neutralization capacities $\geq$ 5.7 (mean + 2 standard deviation) were considered as significant with respect to HCMV serum positivity. Only 3 out of 19 sera with a low-medium anti-HCMV titer neutralized more than 5.7 units of DNase per ml of sera while among the 21 sera with a high anti HCMV titer 9 could neutralize more than 5.7

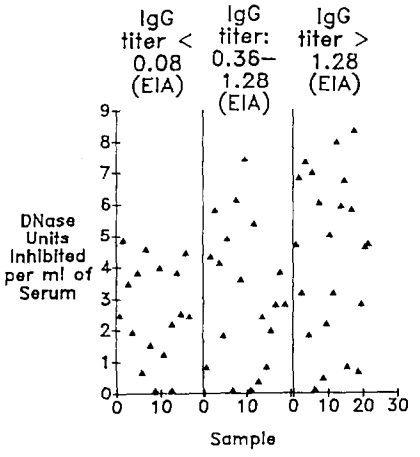
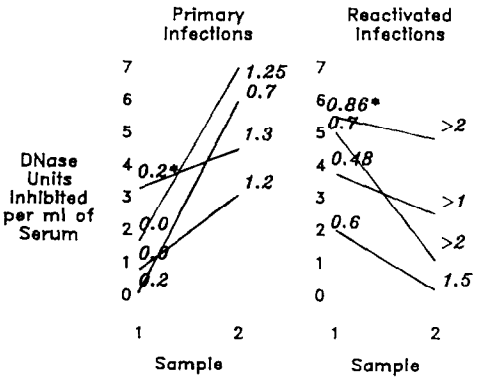


Fig 1.: Anti DNase titers in 58 sera divided in 3 groups on the basis of their antibody titer (EIA) to HCMV.



** EIA Antibody Titer to HCMV*

Fig 2.: Anti DNase titers in serum pairs from patients with primary or reactivated infections.

units of DNase activity. The difference between the results obtained with the two groups of HCMV-positive sera is statistically significant (χi^2=4.89). Nevertheless, only 42% of the HCMV highly positive sera had anti DNase activity. This led us to hypothesize the existence of a subpopulation of sera with a common feature at the basis of their high anti DNase titer. Therefore we tested 4 serum pairs from patients with primary HCMV infection and four pairs from patients undergoing a viral reactivation. The first sample of each serum pair was taken before seroconvertion and was HCMV-negative by EIA in the case of primary infections, medium positive in the case of reactivations. The second sample was taken at the time of seroconvertion and was strongly positive both in primary and reactivated infections. In the latter case we considered as indicative of a current infection a rise of 1.5 times in the value of EIA titer. As shown in Fig. 2, high anti DNase titers were observed only in parallel with the appearance of a primary response to HCMV infection, strongly suggesting that the observed subpopulation of sera with high anti DNase titer among highly positive sera to HCMV was constituted by samples from patients responding a primary infection.
The presence of a normal cell constituent has already been demonstrated within HCMV particles (4,5) and may explain immunological abnormalities of autoimmune nature that often develop and become resolved in concomitance to HCMV disease during acute primary infection. The induction of rheumatoid factor, anti nuclear antibodies (6) anti smooth muscle antibodies as well as hemolytic anemia and thrombocytopenia are among these immunological aberrations. In this respect Revello and coworkers (7) recently found that primary HCMV infection can be identified by the detection of IgM antibodies against the membrane of uninfected fibroblasts. In the present report we demonstrate that antibodies to a DNase activity which is of cellular origin and which is associated with purified HCMV are preferentially present during acute primary HCMV infection. Diagnosis of primary HCMV infection is still essentially based on the demonstration of seroconversion or the presence of HCMV-specific IgM at a high titer. In fact it is difficult to obtain serum samples in the acute phase of the disease because most of the post-natal HCMV infections in the immunocompetent host are clinically asymptomatic; therefore it is often impossible to detect the time of appearance of HCMV-specific IgG. Other diagnostic procedures such as virus isolation or detection of specific IgM give positive results both with primary and recurrent infections. Our data suggest that the presence of antibodies to a normal cellular component such as a cellular DNase might be a marker of primary HCMV infection.

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