Impairment of cytomegalovirus and host balance in elderly subjects

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SUMMARY The titres of IgG antibody against "late antigens", "immediate early antigens", and "early antigens" induced by cytomegalovirus (CMV) and IgM antibody against "late antigens" induced by CMV were analysed in 67 geriatric subjects by immunocytochemical techniques. The titres obtained were compared with those of an adult control population. Significantly increased titres of IgG antibody against induced antigens and a significant increase in CMV reactivated infections occurred in the elderly compared with control subjects.

These findings indicate that the CMV and host balance in the elderly is disturbed, leading to activation of the CMV latent carrier state that follows primary CMV infection.

Cytomegalovirus (CMV) is a member of the herpesviridae family. Although it can cause illnesses ranging from congenital malformations to serious complications in severely immunocompromised hosts, most infections occur asymptotically, and by adulthood most people have serum antibodies to CMV, which are markers of prior infection. Like other herpes viruses CMV is able to remain latent in those infected with it until an appropriate stimulus or a lowering of immune barriers, or both, reactivates the virus.¹

Many studies on man and animals have shown that the efficiency of the immune system declines with age, and it has been suggested that in the elderly the responsiveness of the T cell mediated immune system, both in the effector and in the regulatory compartments, is impaired.²⁻⁴

Cellular immunity has a major role in the control of viral infections, especially herpes virus infections, and the aim of our study was to see if the impaired immune response in the elderly can activate the latent persistent carrier state that regularly follows primary CMV infection.

Material and methods

Serum samples were collected from 67 subjects (41 men, 26 women) aged from 70 to 92 (mean 79) years who lacked antinuclear antibody and rheumatoid factor in their sera. They were inpatients admitted mainly for cerebrovascular problems related to age,

and without evidence of malignant, haematological, endocrine, infectious or immunological disorders. As a control population, 67 sex-matched blood donors aged from 25 to 38 (mean age 33) were tested.

The following determinations were made in each serum sample: (i) IgG against "immediate early antigen" (IEA) induced by CMV; IgG against "early antigen" (EA) induced by CMV; (iii) IgG against "late antigen" (LA) induced by CMV; and (iv) IgM against LA induced by CMV. A serological sign of active or recent infection was the concomitant presence of a titre of IgG anti-CMV induced LA of $\geq 1/320$, anti-EA of $\geq 1/20$, and anti-IEA of $\geq 1/20$; or the presence of IgM against CMV-induced LA of $\geq 1/20$.⁵⁻⁶

To prepare IEA induced by CMV, human embryo fibroblasts infected with the Towne strain of CMV and grown on coverslips were fixed in acetone one hour after infection. EA induced by CMV were prepared by fixing cells infected in the presence of 75 $\mu\text{g/ml}$ of cytarabine, 72 hours after infection, to accumulate all EA synthesised before replication of viral DNA. LA induced by CMV were obtained by fixing infected cells 72 hours after infection.⁷ Two reference sera were used to test antigen preparations: (BE 184, anti-LA titre 1/640, anti-EA titre 1/320, anti-IEA titre 1/160; CF 387, anti-LA titre 1/320, anti-EA and anti-IEA negative titres).

An immunoalkaline phosphatase assay for the detection of IgG to LA induced by CMV, EA, and IEA was performed as previously described.⁸ Briefly, acetone fixed cells were treated with serial twofold dilutions of sera at 37°C for 45 minutes. After three

washes in phosphate buffered saline (PBS) alkaline phosphatase labelled goat immunoglobulins to human immunoglobulin G were added. Cells were then incubated at 37°C for 45 minutes; after a further three washes in PBS the alkaline phosphatase substrate was added. The alkaline phosphatase label was developed with a naphthol salt as a coupling agent and a diazonium salt (fast blue) as a capture agent, forming an insoluble dark blue precipitate at the site of enzyme localisation.

The presence of CMV-induced Fc receptors in infected cells did not interfere with our IgG assays, because Fc receptors are concentrated in the perinuclear region of the cytoplasm while IEA and EA have a nuclear localisation and LA have a nuclear:cytoplasmic distribution.

IgM anti-LA induced by CMV were also titrated by immunoalkaline phosphatase assay. Undiluted serum samples were absorbed with *Staphylococcus* protein A to remove IgG. This pre-treatment avoided the competition of anti-CMV LA IgG molecules with specific IgM in the binding to the antigenic sites. Moreover, this pre-treatment avoided the interference of rheumatoid factor: the removal of IgG from test samples avoids IgM false positive reactions or falsely raised titres of IgM antibody due to the presence of rheumatoid factor.

Serially diluted, absorbed sera were incubated with antigen preparations for three hours at 37°C. Cell smears were carefully washed, monoclonal antibody to human IgM was added, and a second incubation was performed at 37°C for 45 minutes.⁶ After three washes in PBS a further incubation with alkaline phosphatase conjugated immunoglobulins to mouse IgG was carried out, and the reaction was developed as described above.

The significance of results was determined with the χ^2 test and with Student's two-tailed *t* test.

Results

The distribution of IgG antibody titres against LA, EA, and IEA induced by CMV in 67 elderly people and in the control population is shown in fig 1. All 67 were seropositive for IgG anti-CMV-induced LA; 55 of the control population (82.0%) had IgG anti-LA. Thirty nine of the 67 (58.0%) had anti-LA IgG with titres of $\geq 1/320$; only 10 (18.0%) of the 55 positive control subjects had values of $\geq 1/320$.

The geometric mean titre of IgG anti-LA in the elderly was 1/245 with a 95% confidence interval between 1/188 and 1/309. The mean titre of IgG anti-LA was 1/79 in CMV positive control subjects with a 95% confidence interval between 1/60 and 1/105. Statistical analysis with Student's *t* test gave a *t* value of 5.645 ($p < 0.001$) for both groups.

Fig 1 Distribution of IgG against LA, EA, and IEA induced by CMV in 67 elderly subjects (●) and 67 adult control subjects (○).

Antibodies against EA induced by CMV were present with titres of $\geq 1/20$ in 30 old subjects (45.0%), compared with 11 of the 55 (20%) control CMV positive subjects ($p < 0.01$). Twenty six elderly subjects (39%) had titres against IEA of $\geq 1/20$, only eight of 55 CMV positive control subjects (14.5%) had antibody against IEA $\geq 1/20$ ($p < 0.01$).

The distribution of IgM antibody titres against CMV-induced LA is shown in fig 2. In the elderly population 10 of 67 subjects (15.0%) had IgM titres of $\geq 1/20$ and in the CMV positive control subjects six of 55 (11.0%) had IgM titres of $\geq 1/20$. The statistical analysis of IgM values did not show significant differences between the elderly and the control populations.

Serological signs of CMV active or recent infection (IgG anti-LA of $\geq 1/320$, IgG anti-EA of $\geq 1/20$, IgG anti-IEA of $\geq 1/20$; or the presence of IgM anti-LA of $\geq 1/20$) were present in 26 elderly subjects (39%) and in seven (13%) control subjects ($p < 0.005$). No subjects with serological signs of active or recent infection showed raised IgM without concomitant presence of high IgG titres.

Fig 2 Distribution of IgM against LA induced by CMV in 67 elderly subjects (●) and 67 adult control subjects (○).

Discussion

In the sera of elderly subjects we have shown that there is a significant increase of IgG antibody titres to CMV-induced LA, EA, and IEA and an increased prevalence of CMV recent or active infections compared with adult controls. On the other hand, IgM anti-LA values did not show a significant increase in the elderly. The low positivity and the low titres of IgM in the elderly can be explained by the presence of reactivated and not primary CMV infections. In fact, IgM anti-CMV-LA, which are normally present in primary infections, may or may not be present in CMV reactivations, and then only at very low titres.⁹ In our study all serum samples from the elderly were positive for IgG to LA and presumably the subjects who were CMV-LA positive had been previously infected with CMV.

The high prevalence of CMV reactivated infections in the elderly was similar to that seen in a variety of malignant and benign diseases, all of which have immunosuppressive effects or require immunosuppressive treatment.¹⁰⁻¹³ Our CMV-immune response results in the geriatric population were also consistent with the results obtained by Glaser *et al*, who studied the immune response against Epstein-Barr virus

Musiani, Zerbini, Zauli, Cometti, La Placa

(EBV), another member of herpesviridae, which can be reactivated following immunoregulatory defects.¹⁴ In fact, these authors showed a significant increase of IgG against different antigens induced by the virus in an elderly population compared with a young population. Moreover, given that IgG-1 and IgG-3 are the principal anti-CMV and anti-EBV subclasses in CMV and EBV reactivations,¹⁵⁻¹⁶ the increased titres of IgG, especially of the IgG-1 and IgG-3 subclasses, shown in elderly people's sera,¹⁷ could be partly attributed to CMV and EBV reactivations.

In conclusion, our findings suggest that there is a disturbance of the CMV and host balance in the elderly, which could be a consequence of the age related impairment of the cell-mediated immune response. Moreover, the high titres of CMV reactivated infections in the elderly may reflect the development and perpetuation of the atherosclerotic process. Indeed, a recent report confirmed high titres of CMV antibody in clinically manifest atherosclerosis and suggested that periodically reactivated virus may have a role in the pathogenesis of atherosclerosis.¹⁸

A practical problem, which may also arise from reactivated CMV infection in the elderly, is the prevention of CMV infection acquired through blood transfusions in high risk patients. In view of a re-evaluation of the role of the elderly in routine blood donations¹⁹ we emphasise the need to screen serologically for antibodies against CMV-induced antigens to exclude potential donors with high IgG titres.

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