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The age-related increase in low grade systemic inflammation (Inflammaging) is not driven by cytomegalovirus infection

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Abstract

Aging is accompanied by the development of low grade systemic inflammation, termed 'inflammaging', characterised by raised serum C-reactive protein (CRP) and pro-inflammatory cytokines. Importantly, inflammaging is implicated in the pathogenesis of several of the major age-related diseases including cardiovascular disease, type 2 diabetes and dementia and is associated with increased mortality. The incidence of infection with the persistent herpes virus cytomegalovirus (CMV) also increases with age. Cross-sectional studies have proposed CMV infection as a significant driver of inflammaging, but a definitive case for CMV as a causative agent in inflammaging has not yet been made. We studied longitudinally 249 subjects (153 men, 96 women) who participated in the Hertfordshire Ageing Study at baseline (1993/5, mean age 67·5 years) and at 10 year follow up. At both times anthropometric measurements were made and subjects provided blood samples for analysis of inflammatory status and CMV seropositivity. In the cohort as a whole, serum CRP ($p<0·02$) and pro-inflammatory cytokines TNFα ($p<0·001$) and IL-6 ($p<0·001$) were increased between baseline and follow up sampling whereas levels of the anti-inflammatory cytokine IL-10 were decreased ($p<0·001$). These changes to cytokine status over time occurred equally in the 60% of subjects who were seropositive for CMV at baseline and follow up, the 8% who were CMV negative at baseline but who became CMV positive by the 10 year follow up, and also in the 32% who were CMV seronegative throughout. We conclude that CMV infection is not a primary causative factor in the age-related increase in systemic inflammation.

Physiological aging is associated with a chronic sub-clinical systemic inflammatory state, termed inflammaging[1], characterised by elevated levels of serum pro-inflammatory cytokines such as interleukin 6 (IL-6) and TNFα and acute phase proteins such as C-reactive protein (CRP)[1]. Further, the levels of cytokines which counteract the inflammatory state, such as IL-10, are reduced with age[2] compounding the inability to maintain immune homeostasis. Importantly, inflammaging is a predictor of frailty[3] and chronic low grade inflammation is now accepted as a key pathogenic factor in the development of several age-related pathologies including cardiovascular disease[4] and type 2 diabetes[5]. Further, studies in centenarians[6] show that these extremely long lived individuals maintain the cytokine profile of younger adults and do not develop inflammaging. Understanding the causes of inflammaging is therefore important for developing interventions to prevent its occurrence and extend the healthy lifespan of our aging population.

Various factors have been proposed to drive inflammaging including increased adiposity with age[7], and decreased production of sex steroids[8], but one of the dominant theories is that inflammaging is driven in large part by the sustained efforts of the immune system to control infections with persistent herpes viruses, most notably cytomegalovirus[9]. Cytomegalovirus (CMV) is a prevalent β-herpes virus infecting 60-85% of the Western population and approximately 80-90% of those over 65 years[10]. CMV infection has been shown to induce IL-6 and TNFα production by leukocytes[11] and CMV seropositivity is in turn associated with increased all-cause mortality[10,12]. These observations have led to the suggestion that CMV infection is a major driver of inflammaging and that this effect contributes to the increased morbidity and mortality associated with infection. Moreover, if correct, this would suggest that vaccination against CMV in early life would help to prevent inflammaging and therefore

improve health in old age. To date no longitudinal studies have been reported to confirm a link between CMV infection and inflammaging.

The aim of this study was to determine the impact of CMV infection on serum pro- and anti-inflammatory cytokine levels in a 10 year longitudinal study of older adults, comparing individuals who were CMV seronegative throughout, those who acquired CMV during the 10 year period, and those who were CMV seropositive at baseline and follow up. Sera were available from 249 subjects at both baseline and 10 year follow up and the mean age at study entry was 67·5 ± 2·4 years. CMV antibody status was determined on serum at both time points and revealed that 149 (60%) of donors were CMV seropositive at entry and follow up (CMV+/+), 80 (32%) remained CMV seronegative throughout the study (CMV-/-) and 20 subjects (8%) converted from being CMV negative to CMV positive (CMV-/+) during the 10 year study period.

Analysis of serum CRP and pro- and anti-inflammatory cytokines revealed a profile of increased systemic inflammation during aging in line with previous observations[1] (Figure 1). IL-6 (Figure 1A) concentrations increased 2.3 fold over time ($F(1,154) = 117{\cdot}3$, $p < 0{\cdot}001$; $\eta^2 = 0{\cdot}432$) whilst TNFα (Figure 1B) showed a dramatic 4.3 fold increase over the 10 year period ($F(1,104) = 126{\cdot}5$, $p < 0{\cdot}001$; $\eta^2 = 0{\cdot}549$). CRP (Figure 1C) levels were 1.2 fold higher between follow up and baseline ($F(1,240) = 5{\cdot}0$, $p = 0{\cdot}026$; $\eta^2 = 0{\cdot}021$). In addition, levels of the anti-inflammatory cytokine IL-10 (Figure 1E) decreased by 65 % between the baseline and follow up samplings ($F(1,53) = 42{\cdot}8$, $p < 0{\cdot}001$; $\eta^2 = 0{\cdot}447$). However we saw no change in IFNγ ($F(1,199) = 1{\cdot}7$, $p = 0{\cdot}188$; $\eta^2 = 0{\cdot}009$) (Figure 1D).

When the analysis was repeated for the three different CMV groupings we found that similar increases in both IL-6 and TNFα occurred between baseline and follow up in all three groups (Figure 2A and 2B). IL-6 showed a 2.4, 2.2 and 2.4 fold increase in the CMV-/+ ($p = 0{\cdot}03$), CMV-/- ($p < 0{\cdot}001$) and CMV+/+ ($p < 0{\cdot}001$) groups respectively. A corresponding 6.0, 4.2

and 4.1 fold increase in TNFα was seen in each of these subsets over time (all $p < 0{\cdot}001$). Interestingly, the CRP level did not change over time in the CMV-/- group ($p = 0{\cdot}736$) or the CMV -/+ group ($p = 0.541$) but did show a significant increase in the CMV+/+ ($p = 0{\cdot}004$) group (Figure 2C). As CRP production has a well-defined relationship with IL-6; we assessed the association of IL-6 and CRP to investigate further CRP increases observed in the CMV+/+ group. To determine the relationship of IL-6 with CRP in each group we ran a linear regression analysis with baseline and follow-up IL-6 entered as predictors of follow-up CRP. Follow-up CRP was positively associated with IL-6 in the cohort as a whole ($\beta = .194$, $p = .023$, $\Delta R^2 = .032$) highlighting the physiological relationship between IL-6 and CRP production. Following this we analysed the contribution from each CMV serostatus group. There was no association with IL-6 and CRP in either the CMV-/- ($\beta = .240$, $p = .125$, $\Delta R^2 = .046$) or the CMV-/+ ($\beta = .266$, $p = .443$, $\Delta R^2 = .050$) groups whilst in the CMV+/+ group CRP was positively associated with IL-6 ($\beta = .212$, $p = .049$, $\Delta R^2 = .041$). These data suggest that elevated CRP levels are in part driven by increased IL-6 concentrations if exposed to CMV but this appears to be related to length of CMV exposure. Also as there was no change in the CRP levels for the donors that seroconverted for CMV infection during the study period, this suggests that factors other than CMV infection may also be influencing increases in CRP in the CMV+/+ group.

In our cohort IL-10 showed a 0.8 fold and 0.5 fold reduction in the CMV-/- ($p = 0{\cdot}001$) and CMV+/+ ($p = 0{\cdot}01$) groups respectively and a trend towards a reduction in the CMV-/+ ($p = 0{\cdot}07$) group (Figure 2E). Interestingly, these changes were seen only in male subjects (data not shown). Gender differences for IL-10 production have been suggested previously and may reflect an increased incidence of autoimmunity in females[2]. The serum level of IFNγ did not increase over the 10 year period in the group as a whole ($p = 0.188$) and no change was

seen in any of the three CMV groupings (Figure 2D), CMV-/+ (p = 0·928), CMV-/- (p = 0·401) and CMV+/+ (p = 0·474).

Taken together our data suggest that mechanisms other than the immune response to CMV infection are driving the increase in systemic inflammation that is observed with normal physiological aging. The longitudinal OCTO-Immune and NONA-Immune studies followed small cohorts of the very elderly (>85 years) for up to 6 years and correlated various immune markers with mortality. These studies were the first to define a set of parameters termed the "immune risk profile" which was associated with increased mortality during follow up. Interestingly, these included CMV seropositivity and a profile of raised pro-inflammatory markers[12,13]. However, these studies did not compare the inflammatory status of individuals who remained CMV-seronegative or who seroconverted in the 6 year study period. Similarly, Roberts *et al*[14] conducted a prospective analysis of CMV titres and found that increased titre was associated with increased TNFα, IL-6 and raised mortality but did not consider longitudinal differences between CMV seronegative and seropositive individuals. Our study is thus the first to address the impact of CMV serostatus on the increase in inflammatory markers during aging using longitudinal data and reveals that chronic systemic inflammation occurs independently of CMV infection.

Several genetic and environmental factors may contribute to inflammaging. IL-6 polymorphisms (174G>C) are associated with elevated IL-6 levels and increase in prevalence in the over 65 year olds[15], possibly reflecting an evolutionary benefit of a robust pro-inflammatory response in early life which is not conducive to a healthy old age. Indeed raised inflammatory cytokines such as IL-6 are not seen in centenarians[6] or members of families with extended longevity[16], supporting the notion that an anti-inflammatory genotype is beneficial in reaching extreme old age. Amongst lifestyle factors that could influence

inflammageing are the age-related increase in adiposity[7], reduced physical activity[17], the loss of sex hormones during menopause[18] and andropause[8] which are all known to increase systemic inflammation.

Our study has some limitations. Firstly, we did not consider the impact of other latent viral infections such as Epstein Barr virus and Varicella Zoster Virus, which might possibly contribute to inflammation in the CMV negative grouping. Secondly, due to the relatively small size of the cohort as a whole and the low seroconversion rate for CMV during the study period, the size of the CMV-/+ and CMV -/- groups are quite small and the impact of serostatus on serum markers should be addressed in a larger study. Finally, study participants were lost to follow-up between the 1993/5 baseline and 2003/5 follow-up clinics due to a variety of reasons (including 121 to mortality, loss to follow-up, refusal to participate) and we have previously shown that a healthy participant effect is, unsurprisingly, evident in HAS[19]. In the current study, the 153 men who went on to have CMV status classified at the 2003/5 HAS follow-up were significantly ($p<0{\cdot}05$) younger, less likely to be current smokers, were of higher social class, and had lower IL-6 and CRP levels at the baseline clinic than the 258 men who only participated in the baseline study. Selection effects were less evident for women; the 101 women who were sampled at both time points were significantly ($p<0{\cdot}05$) less likely to be current smokers than the 205 women who only participated in the baseline study, but were otherwise similar. These selection effects have the potential to bias our results. However, our analyses were internal to the HAS sample; bias would only be introduced if the associations between CMV status and inflammaging were systematically different among those who participated in our study, and those who did not; this seems unlikely.

In conclusion, CMV infection does not appear to determine the age-related increase in serum markers of inflammation. This suggests that interventions to control CMV infection incidence

in the population would have little or no impact on the inflammatory profile of aging individuals. However, it is well established that the cellular immune response against CMV is extremely immunodominant and increases further in older adults[20], occupying valuable "immune space" and potentially impairing the response to other pathogens and to vaccination in old age. Further epidemiological analyses of the impact of CMV infection on health status in the elderly thus remain warranted.

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Legends to Figures

Figure 1. Cytokine and CRP concentrations at baseline and 10 year follow up in subjects in the Hertfordshire Ageing Study. Significant increases over time were observed for serum concentrations of IL-6 (**A**), TNFα (**B**) and CRP (**C**) whilst a decrease was observed for IL-10 (**E**). No differences over time were observed for IFNγ (**D**). Results are mean ± SEM analysed by mixed model repeated measures ANOVA. *p<0·03, **p<0·001 for baseline compared with 10 year follow up.

Figure 2. Impact of CMV serostatus and aging on serum cytokine and CRP concentrations. Subjects were grouped by CMV serostatus as either negative at baseline and positive at 10-year follow-up (CMV-/+), negative at both time points (CMV-/-), or positive at both time points (CMV+/+). Serum IL-6 (**A**) and TNFα (**B**) concentrations increased in all 3 CMV groups. CRP (**C**) was only increased in the CMV+/+ group, while the anti-inflammatory cytokine IL-10 (**E**) was decreased in the CMV-/- and CMV+/+ groups and a trend (p=.07) towards a reduction was observed in the CMV-/+. IFNγ (**D**) was unchanged for all groups. Results are mean ± SEM analysed by mixed model repeated measures ANOVA with Bonferroni corrected pair wise comparisons. * p<0·05, **p<0·01, ***p<0·001 for baseline compared with 10 year follow up.

Figure 1.

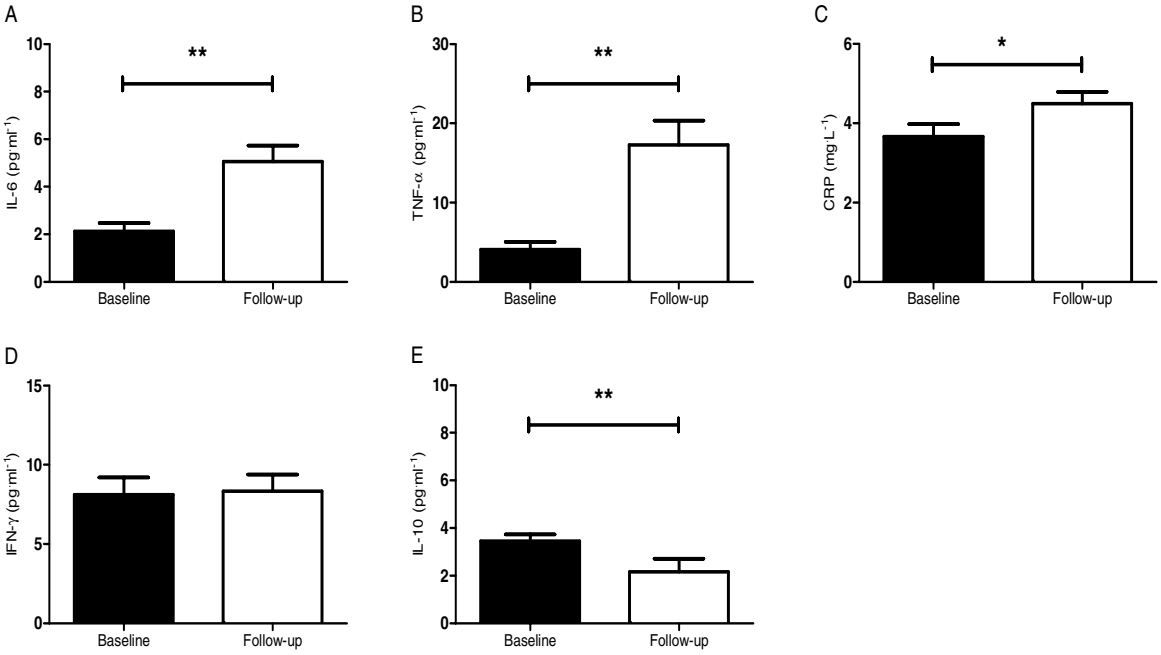

Figure 2.