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Article (Accepted Version)

Pera, Alejandra, Broadley, Iain, Davies, Kevin A and Kern, Florian (2017) Cytomegalovirus as a driver of excess cardiovascular mortality in rheumatoid arthritis: a red herring or a smoking gun? *Circulation Research*, 120 (2). pp. 274-277. ISSN 0009-7330

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Cytomegalovirus as a driver of excess cardiovascular mortality in rheumatoid arthritis: a red herring or a smoking gun?

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Key words: Cardiovascular Disease, Rheumatoid Arthritis, Cytomegalovirus, CD4 T-cells

Disclosures: The authors report no conflict of interest.

Sources of funding: KAD and FK hold substantive appointments at Brighton and Sussex Medical School, a joint venture of the Universities of Sussex and Brighton. AP is a BSMS-funded post-doctoral research fellow; she is also an Honorary Lecturer in the Cell Biology, Physiology and Immunology Department, Faculty of Medicine, University of Córdoba-IMIBIC). IB was a University of Sussex (School of Life Sciences) final year student at the time of writing.

Introduction

Rheumatoid arthritis (RA) is the most common inflammatory arthritis worldwide. Apart from its well known manifestations involving the joints (e.g. deformity, swelling, synovial inflammation), there are widespread, extra-articular manifestations that also involve the cardiovascular (CV) system.¹ The consequences of CV involvement are often serious; cerebrovascular disease, ischemic heart disease and CV disease (CVD)-associated death are up to 50% higher in the context of RA leading to a reduction of life-expectancy in these patients of 3-10 years compared with the general population.¹ *To date, no satisfactory explanation of this phenomenon has been found, or maybe, it has been overlooked?*

This Viewpoint argues that Cytomegalovirus (CMV) is a major contributor to the excess of CVD observed in RA. For over two decades CMV has been implicated in both RA and CVD; however, no direct, mechanistic explanations for its role in either condition have been found. At the same time, in both conditions investigators have observed the expansion of CD4⁺ T-cells that lack a surface marker called CD28 ('CD4⁺CD28⁻ T-cells', sometimes referred to as 'CD28^{null}' cells). Numerous reports have shown not only a direct contribution of this subset to vascular pathology but also a positive correlation between the numbers of these cells and the severity of disease/complications, both in regards to RA and CVD.^{2,3} *Is this simply a coincidence?*

It is clearly not, since research has also revealed that expansions of this 'pro-atherogenic' CD4⁺CD28⁻ subset only occur in CMV-seropositive (CMV⁺) individuals; they do not occur as a result of ageing in CMV-seronegative (CMV⁻)

people, suggesting that ageing contributes to them only inasmuch as it is associated with a higher prevalence of CMV infection.⁴⁻⁷ Expansions of CD4⁺CD28⁻ T-cells reported in RA and other autoimmune diseases frequently exceed 5-10% (when *not* stratified by CMV infection status) and so vastly exceed the percentages observed in CMV⁻ individuals where reported (about 1-2% in the literature). A small number of studies in RA and granulomatosis with polyangiitis (GPA) were actually stratified by CMV serology and found that the CD4⁺CD28⁻ T-cell subset was increased by 10 to 20-fold in the CMV⁺ population.^{4, 6, 7} It is becoming increasingly obvious that the expansions of CD4⁺CD28⁻ T-cells observed in RA are explained by the CMV⁺ population of RA patients; they may also explain the increased incidence of CV morbidity and mortality. This is, because, to make things worse, CMV infection in RA produces larger expansions of these cells than in otherwise healthy people, suggesting RA itself might accelerate these expansions.⁸ *But, have studies investigating CV complications in RA ever considered the CMV infection status of participants?*

The answer is no. This Viewpoint combines evidence from seemingly independent lines of investigation to demonstrate that CMV is likely to be a major driver of cardiovascular mortality in RA.

The beginning: Cytomegalovirus (CMV) infection in brief

CMV is best known for causing congenital abnormalities but has been implicated in (accelerated) immune senescence, diabetes mellitus (DM), and CVD among other conditions. Infection usually occurs in early childhood but may occur at any age. Latent infection is present in 40% - 90% of the world's population. Older

people are more likely to carry CMV, however, prevalence depends on a number of other factors including race and education.⁹ Lower socioeconomic status in particular is associated with a higher prevalence of CMV infection but also a higher prevalence of dyslipidaemia, higher cholesterol, and smoking, which are all risk factors for CVD. Despite this complex interrelatedness of risk factors, epidemiological evidence supports that CMV infection independently contributes to CVD and more recent work identifying CMV as the major driver of CVD in HIV-infected people supports this view.⁹ *But how does CMV actually cause vascular pathology?*

First line of investigation: CMV, T-cells, and vascular injury

The connection between CMV and vascular damage was first made when CMV was isolated from atherosclerotic lesions (vascular endothelium is in fact a primary target tissue of CMV). We now understand that following CMV infection of endothelial cells (EC), their class-II MHC expression is reduced, hampering direct CMV-antigen presentation to CD4⁺ T-cells. However, CMV-infected EC can release non-infectious exosomes that are replete with CMV proteins and uptake of these by antigen-presenting cells allows effective presentation to and activation of CMV-specific CD4⁺ T-cells.¹⁰ CD4⁺ T-cells are directly involved in coronary artery injury, which may be driven by the presence of CMV antigens in the vasculature.²

Work in mouse models has confirmed additional roles for T-cells in causing vascular injury, for example by driving hypertension. Mice lacking both T-cells and B-cells (Rag -/-) show blunted hypertension in response to angiotensin-II infusion or (DOCA)-salt (which are popular mouse models of hypertension). Rag -/- mice

also exhibit decreased vascular reactive oxygen species (ROS) production with reduced nitric oxide (NO, 'relaxing factor') consumption. Adoptive transfer of T-cells (but not B-cells) restores these responses to normal.¹¹ Additional work in mice has revealed that persistent murine CMV (MCMV) infection of EC induces renin expression, which subsequently increases local angiotensin-II (ATII). This in turn leads to an increase of ROS production by ATII receptor-positive infiltrating T-cells. This mechanism causes hypertension within weeks, independently of atherosclerotic plaque formation but at the same time contributes to (aortic) atherosclerosis.¹²

Second line of investigation: CMV and autoimmune disease

A connection between CMV and autoimmune/chronic inflammatory disease has been discussed since the early 1990s. CMV infection not only exacerbates inflammation in RA, higher anti-CMV antibody levels also associate with more frequent surgical procedures and more severe joint damage.⁶ Moreover, CMV-specific T-cells are present in RA affected joints. Apart from RA, CMV has been directly or indirectly implicated in several other autoimmune and chronic inflammatory conditions, including for example Granulomatosis with Polyangiitis, Systemic Lupus Erythematosus, or systemic sclerosis.

Conventional CV risk factors including smoking, physical inactivity, hypertension and DM contribute to CV mortality in RA but have reduced predictive value compared to patients without RA. RA might itself accelerate atherogenesis, e.g. as a consequence of chronic inflammation, related to therapy, or, due to other, as yet poorly identified factors.

Third line of investigation: CD4⁺CD28⁻ T-cells in CVD and autoimmune disease

As mentioned above, expansions of CD4⁺CD28⁻ T-cells have been implicated in both CVD and RA. Judging by their combination of adhesion molecules, chemokine receptors, and cytolytic molecules, they have a highly cytotoxic, 'effector' phenotype; they are also resistant to the 'dampening' effects of regulatory T-cells. This dangerous combination translates into an increased potential for causing tissue damage including in the vasculature.^{2, 3} In acute coronary syndrome (ACS) and myocardial infarction, increased numbers of these cells are associated with increased immediate mortality and recurrence. Some authors found nearly 10-fold higher levels of CD4⁺CD28⁻ T-cells in patients with unstable angina (UA) compared to those with stable angina. They so identified the percentage of CD4⁺CD8⁻ T-cells as an independent predictor of future acute coronary events, implying that these cells are key-players in mediating atherosclerotic plaque injury/instability.¹³ The number of studies investigating CD4⁺CD28⁻ T-cells in autoimmune and chronic inflammatory disease is impressive. They were most extensively characterized in RA patients, where their frequencies correlate positively with disease severity and the extent of extra-articular involvement. The properties of CD4⁺CD28⁻ cells and their damaging effects on CV and other tissues were the subject of extensive recent reviews.^{2, 3}

CD4⁺CD28⁻ T-cells are the 'missing link' between CMV infection and increased CVD-associated mortality in RA

The observation that expansions of CD4⁺CD28⁻ T-cells exclusively occur in CMV infected people inevitably joins up the three lines of investigation we have explored and provides a straightforward and convincing explanation for how CMV infection drives excess cardiovascular damage in RA.

Several review articles have distracted from CMV infection as the exclusive trigger of CD4⁺CD28⁻ T-cell expansions by stating that such expansions may also be induced in the presence of other infections or be related to cellular senescence.^{2,3} It is surprising though, that none of the clinical studies allegedly supporting a role of other pathogens in driving CD4⁺CD28⁻ T expansions investigated the CMV infection status of the participants. While some authors have described the induction of CD4⁺CD28⁻ T-cell during in vitro proliferation experiments, to the best of our knowledge, no study has ever reported the presence of CD4⁺CD28⁻ T-cell expansions in CMV⁻ individuals.^{2,3}

Only a small number of studies on CD4⁺CD28⁻ T-cells in autoimmune diseases divided participants by CMV infection status. For example, Pierer et al. showed 22-fold higher and Hooper et al. 10-fold higher numbers of these cells in CMV⁺ compared to CMV⁻ RA patients.^{6,7} Morgan et al. reported a 24-fold increase of CD4⁺CD28⁻ T cells in CMV⁺ compared CMV⁻ GPA patients. In the same study, CD4⁺CD28⁻ T-cell numbers were 'only' 14-fold higher in CMV⁺ compared to CMV⁻ healthy controls, suggesting that, as with RA, the underlying condition accelerated these expansions.^{4,8} In all studies, CMV⁻ patients had low numbers of CD4⁺CD28⁻ T-cells (in the order of 1%) and only CMV⁺ patients showed expansions of these

cells. A compelling observation in renal allograft recipients further confirms the role of CMV in driving such expansions; increasing numbers of CD4⁺CD28⁻ T-cells were observed in CMV⁻ recipients of renal allografts only when they received a CMV⁺ graft but not if the graft was CMV⁻.⁵ *If only CMV-infection drives the expansion of CD4⁺CD28⁻ T-cells, then what is the antigen-specificity of these cells?*

It remains unclear to what extent CD4⁺CD28⁻ T-cells are CMV specific

Since expansions of CD4⁺CD28⁻ T cells are induced by CMV infection, they are likely to be responsive to CMV antigens. Alternatively, CMV infection might trigger cross-reactivity to constitutively expressed antigens, or facilitate reactivity to antigens whose expression is induced after CMV infection or as a result of tissue damage. In agreement with our own (unpublished) data, it was shown that CD4⁺CD28⁻ T-cells proliferate in response to CMV antigens.⁵ Others have reported, however, that in patients with ACS a proportion of these cells recognize human heat shock protein 60 (HSP60) but not a CMV lysate.² Of note, non-responsiveness to a CMV lysate does not rule out CMV reactivity (CMV-lysates do not contain all relevant T-cell antigens in sufficient amounts to stimulate effective T-cell responses). Interestingly, antibody cross-reactivity between HSP60 and the CMV UL122 and US28 proteins has been reported as an indirect mechanism by which CMV infection might facilitate EC injury.¹⁴ Cross-reactivity of antibodies suggests cross-reactivity at the level of T-cells, too. It is important to note, however, that the question if CMV-infection drives CD4⁺CD28⁻ T-cell expansions is not predicated on whether these cells are CMV-specific.

Should we target CMV and/or CD4+CD28⁻ T-cells in CMV⁺ RA patients?

Experimental evidence in man suggests that anti-CMV treatment may be able to reduce both reactivity and numbers of CMV-specific T-cells. For example, low dose acyclovir (ACV) therapy, used to treat herpes simplex virus, decreases the response of CD4⁺ T-cells to pp65 CMV protein, probably by diminishing the CMV-antigen load, turnover, and uptake by APCs.¹⁵ If CMV-specific T-cells were indeed instrumental in mediating CMV-driven vascular damage, anti-viral drugs might be a way of slowing down this process. Therapies targeting CD4⁺CD28⁻ T-cells directly have not been investigated to date. Since biologic agents for cell-specific treatment are designed to target specific molecules expressed on relevant tissues, a molecule specifically expressed on CD4⁺CD28⁻ T-cells would have to be identified. Interestingly, however, anti-TNF therapy appears to reduce the risk of myocardial infarction and, along with abatacept has been shown to reduce the number of CD4⁺CD28⁻ T-cells.³ Our knowledge of how other drugs used in RA affect CV complications or CD4⁺CD28⁻ T-cells is, however, limited.

What next?

Studies systematically evaluating these factors are clearly indicated. We believe that CMV infection status has not been given sufficient attention in studies investigating CVD, in particular in the context of RA. This may be due to a lack of awareness of the damaging effects of CMV beyond congenital disease and specific end-organ disease in the immunocompromised or immunosuppressed. The widespread clinical consequences of CMV infection and the burden on the immune system that it represents are still being underestimated. One important goal of this

Viewpoint is to encourage researchers to stratify future CV studies by CMV infection status. This would be one important step forward. Future research may identify promising new targets for drugs, which could be used adjunctively in selected RA patients to reduce CMV-associated CV mortality.

Single display item: Facts Box**CMV as a driver of excess CVD in RA: The facts in short**

- CMV infection has been implicated in CVD and RA for >20 years and is associated with increased disease severity and higher numbers of complications.
- CMV infection drives the expansion of CD4⁺CD28⁻ T-cells, which are highly potent, cytotoxic CD4⁺ T-cells with an increased propensity to infiltrate and destroy vascular tissue. Only very small numbers of CD4⁺CD28⁻ T-cells occur in CMV-uninfected people.
- CVD and RA are associated with often major expansions of these cells. Their numbers correlate with both disease severity and the incidence of complications, however, most clinical studies investigating the role of CD4⁺CD28⁻ T-cells were not stratified by CMV infection status.
- Published research suggests that RA and other chronic inflammatory diseases accelerate CD4⁺CD28⁻ T-cell expansions within the CMV-infected patient population.
- Routinely stratifying studies in CVD by CMV infection status would be a major step forward in raising awareness and addressing the indirect, detrimental effects of CMV infection on the CV system.

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