Cytomegalovirus (CMV) research in immune senescence comes of age: overview of the 6th International Workshop on CMV and Immunosenescence

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Abstract Cytomegalovirus (CMV) is one of the most complex and most ubiquitous latent persistent viruses, with a considerable ability to evade and manipulate the immune system. Following an early-life infection, most immunocompetent humans spend several decades living with CMV, and, because the virus in these hosts does not cause manifest disease, CMV can be considered part of normal aging for more than half of humanity. However, there is accumulating evidence that CMV carriage is not a null event and that both potentially harmful and potentially beneficial outcomes emanate from the interaction of CMV with its mammalian hosts. This article provides an overview of the 6th International Workshop on CMV and Immunosenescence, highlighting the advances in the field made in the past two years, as related to CMV epidemiology/geroscience, CMV virology with an accent on latency, and CMV immune evasion and immune recognition of the virus and its antigens.

Keywords Cytomegalovirus · Immunosenescence · T cells · Inflammation

Introduction

Nearly two decades ago, two key findings connected CMV with immune senescence. In 1999, Looney et al. (Looney et al., 1999) showed that cytomegalovirus (CMV)-positive and -negative humans exhibit dramatically different T cell subset ratios and that the effect seems to be increasing with aging. Around the same time, Reddehase and colleagues described “memory inflation” driven by CMV in mice (Holtappels et al., 2000, Holtappels et al., 1998). Since then, numerous studies have been published investigating the associations between human, non-human primate or murine CMV with their respective hosts in the course of aging. The interest in the topic has been so sustained that it led to the establishment of CMV and Immunosenescence Workshops. The five prior workshops were all held in Europe, each of them summarized in corresponding review articles (Solana et al., 2012, Wills et al., 2011, Pawelec et al., 2010, Sansoni et al., 2014, Arens et al., 2015). This overview summarizes the state of the field before and the discussions at the 6th International CMV and Immunosenescence Workshop, held from Nov. 11–14, in Tucson, (AZ), USA. Other articles in this issue deal with presentations and discussions within the thematic sessions of the workshop and are introduced below.

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CMV, a member of beta-herpesvirus family, is the largest human virus, with a genome of nearly 240 kb (Murphy et al., 2003a, Murphy et al., 2003b), and with a potential to encode between 150 and >750 proteins (Stern-Ginossar et al., 2012), many of which are devoted to avoiding elimination by the immune system. As is the case for other herpesviruses, following a brief acute infection period that elicits a typical CD8 T cell response, as well as CD4 and B cell responses, CMV establishes persistence that includes latency. Persistence/latency is established in reservoir cells that are distributed broadly across the organism, and that include hematopoietic stem cells (HSC), cells of the monocyte/macrophage lineage including dendritic cells and endothelial cells lining the surface of blood, and, perhaps, lymphoid vessels (rev. in (Reeves and Sinclair, 2008, Goodrum, 2016) Thereby, CMV has the potential to impact the function of numerous organs and systems, most notably cardiovascular (see the accompanying article by Aiello et al. in this issue). Molecular determinants of latency and reactivation, while still insufficiently characterized, are under intense scrutiny (see the accompanying article by Leng et al. in this issue). However, it is clear that the primary CMV infection is followed by a period of viral shedding and it remains unclear whether the virus, that is a master in immune evasion (as elaborated in the accompanying article by Jackson et al. in this issue), ever really is truly latent in all its reservoirs or whether some cells produce it as a smoldering infection at low levels at most, if not all times. That persistent/intermittent production of viral antigens, if not of fully formed viral particles, is sufficient to prevent contraction of virus-specific T cells that continue to be stimulated periodically by a plethora of viral antigens (as further discussed by the accompanying article by Souquette et al.). As a consequence, a large population of CMV-specific CD8, and to a lesser extent, CD4 T cells, is generated in response to cycles of viral reactivation (aptly called memory inflation, (Karrer et al., 2003)). How exactly this memory inflation impacts the ability of an older immune system to function and provide defense against other infections is one of the key topics of interest. We will briefly review the state of knowledge in these areas as of the beginning the workshop and will then summarize the advances made by the workshop itself. The most exciting and productive part of the workshop was a lively half-day long general discussion that allowed participants to crystallize key recommendations. We therefore finish the overview with a list of these key

recommendations and future directions necessary to fill gaps in our current knowledge.

Current state of knowledge—recap of the 5th CMV and Immunosenescence Workshop

The fifth workshop, held in November of 2014 in Amsterdam, focused on four concrete issues: human cytomegalovirus (HCMV) infection and the aging adaptive immune system, effects of aging and HCMV infection on innate immunity, lessons from experimental models of cytomegalovirus, and clinical aspects of immunosenescence and HCMV infection (Arens et al., 2015). At the workshop, there was a high degree of consensus on the changes that cytomegaloviruses, in different species, induce in the T cell compartment. For CD8+ T cells, the phenomenon of memory cell inflation leading to the emergence of vast populations of resting effector T cells has been extensively documented. At the workshop, the group of Paul Moss showed for the first time the development of HCMV-reactive CD4+ T cells that share phenotype, function, and transcriptomes with inflationary CD8+ T cells (Pachnio et al., 2016). Notwithstanding the common appreciation of the impact of latent HCMV infection on the size and composition of the (circulating) immune system, it is yet unsettled if and how HCMV impacts on responses to other unrelated pathogens. Important in this respect is that HCMV infection leaves the naïve T cell compartment intact and that vaccination studies in CMV+ individuals have yielded mixed results on whether CMV adversely impacts the ability to respond to the vaccine (as further discussed in the accompanying articles by Aiello et al., Jackson et al., and Souquette et al. in this issue).

Another issue that remained to be settled is the functional state of inflationary (CD8+) T cells and their possible contribution to the maintenance to CMV latency. Although these cells have been termed “terminally differentiated,” using proper activation stimuli, these cells can divide, secrete cytokines, and execute superb cytotoxicity. In other words, these cells do not appear to be functionally exhausted which is supported by the notion that they are generally low in PD1 expression.

Finally, the concept of HCMV-induced immunosenescence was a major subject of debate. Perhaps the major reason for the continuation of this discussion is the fact that large epidemiological studies (perhaps except in the very elderly) do not give

unequivocal evidence for dominant detrimental effects of HCMV infection on, e.g., responses to vaccines, cardiovascular diseases, and inflammatory disorders.

The absence of evident clinical signs in immunocompetent people in relation to the vast change of the immune system remains somewhat a paradox. The fact that the HCMV responses appear to be compartmentalized (changes are mainly seen in the circulating pool and in the spleen) may form part of the explanation. Yet it is not unlikely that in the co-evolution with cytomegaloviruses specific adaptations have occurred that allow accommodation of these large pools of CMV-directed effector T cells.

Goals and structure of the 6th CMV and Immunosenescence Workshop

The 6th International Workshop on CMV and Immunosenescence was organized with a primary goal to fill a gap identified at several recent meetings. Specifically, in addition to the topics reviewed above from the fifth workshop, it was felt that stronger attention must be focused on the biology of the virus itself and on its interactions with the host in the course of latency and reactivation. In fact, one of the greatest weaknesses of research into HCMV and immunosenescence comes from our inability to control and measure the state of viral activity. Clearly, in cross-sectional, and even in longitudinal, human studies, it is difficult to determine the time, dose, and frequency of exposure. Even if one were to undertake a heroic effort to follow a CMV-naïve population in a longitudinal design of lifelong duration, only the timing of infection could be approximated. Reason for optimism, however, comes from the recent techniques to enrich for viral transcripts and bioinformatically deduce the level of activation of CMV in naturally infected, healthy hosts (Cheng et al., submitted). The first session, on CMV virology, latency, and reactivation, addressed challenges and advances in this area, and these are described in more details in the article by Leng et al.

Another area of major interest is the impact of CMV on diseases on aging—whether and how the virus may be involved in modulating frequent age-related morbidities, in particular cardiovascular diseases (CVD), where there are strong epidemiological associations between CMV infection and morbidity and mortality from CVD. Associations with diabetes, inflammation, and other outcomes are also both interesting and clinically

important. To that effect, the session on CMV, human health and epidemiology (CMV geroscience) was convened and its results summarized in the article by Aiello, Chen, and Frasca (this issue).

The last two articles tackle the interplay of the virus and the immune system. The first one, by Jackson et al., takes a more virus-centric view, discussing how the virus manipulates and evades the immune system, including the discussion of the possible use of CMV as a vaccine vector against HIV and other diseases. In the second one, Souquette and colleagues discuss the impact of anti-CMV immunity on immune senescence during aging, including the impact on the immune repertoire and function.

Dos and don'ts for the future: what issues we need to tackle and how

While presentations themselves brought a lot of unpublished, original, and stimulating science, the most valuable part of the workshop was the general discussion that allowed vigorous exchange of ideas and opinions. Along the customary highlights of key questions and gaps in knowledge, participants were asked to specifically answer two questions regarding current practices in the field: (1) What terms we should not be using and what studies we definitely should not be doing (any more)?; and (2) What conceptually and technically new studies/approaches we really need if we want to address grand challenges in CMV and immunosenescence? Parts of these questions from general discussion, as they pertain to the session themes described above, will be covered in individual review/opinion articles. At the risk of being potentially redundant, we summarize here the key ones.

What we definitely should not be doing

- Using terms like exhaustion related to CMV-specific cells—without functional validation of the capacity of involved cells, particularly in light of evidence that there is little, if any, exhaustion of T cell responses against CMV even in the oldest old (Cicin-Sain et al., 2011)
- Performing immune (particularly immune phenotyping) studies without functional and/or viral measurements

- Reporting percentages of T cell and other cell subsets without counts and claiming a change in numbers (rather than ratios)
- Just measuring IE1 and pp65 responses vs using a broader array (12–19) pools (see discussion in the accompanying article by Souquette et al.)

What we should be doing more

- Persist in standardizing methods, cohorts, and reagents (Ab, peptides, ELISA)—(use guidelines from the Human Immune Profiling Consortium, Human Immunology Centers, etc.)
- Apply geriatric and epi confounder analysis to correct for biases
- Perform parallel analysis to connect in vitro to in vivo cellular infection models and mouse to human models (non-human primates and humanized mice as intermediate models)
- Develop whole-body imaging/in vivo reporter mice to assess rare/discrete events in viral reactivation and immune activation
- Use high-resolution, high-throughput analysis of viral and cellular transcriptome and analysis of global as well as CMV-specific TCR repertoire
- Sample human tissues and not only blood
- Elucidate metabolic activity of the virus and the interplay between CMV and host metabolism
- Define a few (2–3) key questions that can be simultaneously and in a standardized manner addressed in several independent laboratories

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