Immunology Letters 162 (2014) 141–144

Contents lists available at ScienceDirect

Immunology Letters

journal homepage: www.elsevier.com/locate/immlet

Review

The interaction between cytomegalovirus and the human immune system

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ARTICLE INFO

Article history:
Available online 16 October 2014

Keywords:
cytomegalovirus
CMV
CD8 cells
antiviral immunity

ABSTRACT

Studies on antiviral immunity in man are hampered by the impossibility to standardize the infection as is done in experimental animal studies. An exception is the occurrence of cytomegalovirus infection transmitted by a donor organ into a transplant-recipient, where the time-point of infection is exactly known. Moreover, its strong interaction with the human immune system during evolution and the strong immunogenic properties of this persistent virus, as well as the need for intervention e.g. by vaccine development, all make studies towards the immune response against just this virus very attractive and relevant. In this work, we will present an overview of the studies on this topic that were performed in the departments of Experimental and Clinical Immunology in the AMC and Sanquin in Amsterdam.

Cytomegalovirus (CMV) is a persistent β-herpesvirus affecting approximately 75% of healthy individuals, globally. Its main tropism is in the white blood cells and endothelial cells. Transmission occurs by body fluids. In healthy individuals primary infection is often asymptomatic, but the infection may cause severe morbidity and even mortality in immunocompromised patients.

CMV has been within the human population for thousands of years, enabling a continuous mutual adaptation. Many players are involved in the immune response against CMV, like antibodies, NK cells, γδ T cells as well as CD4 and CD8 positive T cells.

In the later years, we have used the transplantation of a kidney graft from a CMV seropositive donor into a CMV seronegative recipient as a model to study the immune response against CMV. We collected blood samples from these patients longitudinally in time and studied changes in phenotype, function and transcriptomes of the CMV specific T cells as a direct consequence of the infection. Typically, one week after the peak of CMV replication, virus-specific $CD4^+$ T cells emerge in the circulation, which synthesize the T helper 1 type (Th1) cytokines IFNγ and TNFα. After that, IgM and IgG anti-CMV antibodies become detectable and moreover CMV specific $CD8^+$ T cells appear in the peripheral blood [1,2].

After infection, not only effector/memory cell populations are formed, but also a large pool of dormant $CD8^+$ effector T cells which remain circulating during latency. They are $CD45RA^+CD27^-$, thus having the phenotype of far differentiated effector-type cells [3–5]. These cells are apparently resting, but have the potential to produce IFNγ and are armed with cytotoxic granules, ready to become active as soon as viral replication occurs (Fig. 1). We called them vigilant resting effector cells [6]. In contrast to the memory cells found after infection with cleared viruses, these cells do not need IL7 for their persistence, because of the persistence of antigen. Indeed, CMV appeared to leave a strong fingerprint on our immune system. The population of CMV-specific T cells increases with age and in situations of immunosuppression, these may be instrumental to maintain latency to immune-evasive CMV [2,7–9]. In vitro stimulation of CMV specific $CD45RA^+CD27^-CCR7^-CD8^+$ T cells with their cognate peptide together with either $CD4^+$ help or IL-2, IL-15 or IL-21 induces massive clonal expansion. Concurrently, these stimulated effector T cells change cell surface phenotype from CD45RA to CD45R0 and regain CCR7, while effector functions are maintained. These data imply that these CMV specific effector-type T cells contribute to immunity not only by direct execution of effector functions, but also by yielding progeny in situations of viral reinfection or reactivation [8,10].

Next, we were interested in the molecular pathways involved in activation, differentiation and maintenance of this large population of CMV specific $CD8^+$ T cells. For that, we used the tetramer staining technique to select cells at the peak of viral infection and at latency. We studied gene expression at these different time points following primary infection. Naive cells of CMV seronegative patients were used as reference. We found upregulation of activation molecules and downregulation of differentiation markers during the peak of infection, which remained so during latency. However, only

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http://dx.doi.org/10.1016/j.imlet.2014.10.009
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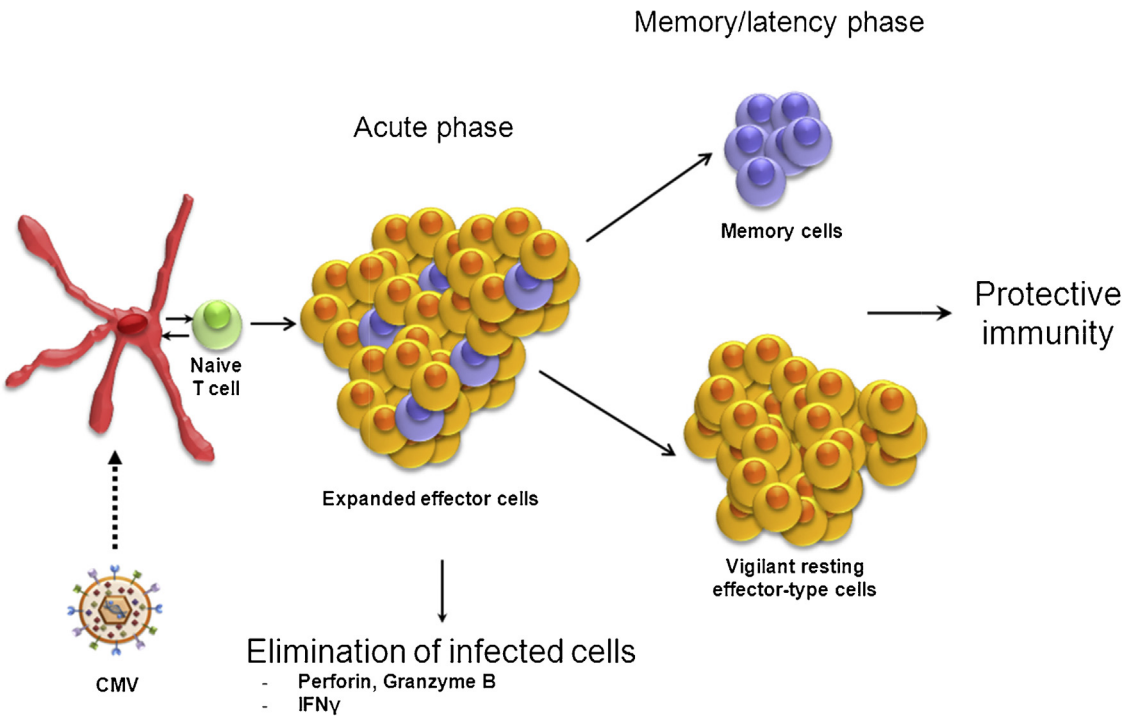

Fig. 1. Protective immunity against cytomegalovirus.

minimal change in the level of CD27mRNA was observed in the course of CMV infection, suggesting regulation at the (post-) translational level. Next, we found marked expression of effector molecules already from the acute phase on as was in agreement with what we already observed at the protein level. In addition, we found a differential regulation of chemokine receptor expression already at the peak of infection: CCR7 was strongly downregulated; CX3CR1 was upregulated at the peak, and remained so during latency. In summary, we showed that a number of key features of CMV specific $CD8^+$ T cells is already installed during the primary response in this population, in particular: IFNγ production, cytolytic potential, and migration potential [11,12]. This process appeared governed by stable changes in transcription factor expression, in particular genes coding for t-Bet and Eomes. The latter was quite recently confirmed at the protein level (van Aalderen et al., 2014, submitted).

One of the remarkable findings was the strong upregulation of CX3CR1 both at the peak of viral load, and at one year after primary infection, which was again confirmed at the protein level. CX3CR1 is the receptor for fractalkine, which is induced on endothelial cells (EC) by inflammatory cytokines like TNFα and IFNγ. This finding led us to a model for involvement of CMV specific T cells in vascular damage: we showed that CMV infection induces a systemic Th1 response with increased serum levels of a.o. IFNγ and TNFα [13]. These cytokines were shown to induce an upregulation of fractalkine on endothelial cells in vitro, and CX3CR1-positive CMV specific effector T cells were shown to bind to these fractalkine-expressing endothelial cells. This is followed by migration of these cells to activated EC in vitro, followed by damage and apoptosis of these endothelial cells [14,15]. These data confirm a role of these immune cells in CMV induced immunopathology, and are in line with epidemiological data on an association of CMV infection and atherosclerotic disease. Moreover, we showed shortened telomere length of these cells [14], which was by others described to be associated with the occurrence of vascular diseases, and was even proven to be predictive for vascular disease [16]. In conclusion, the immunopathology caused by CMV might very well be an etiological factor in development of atherosclerotic disease (Fig. 2). So, apart from maintaining lifelong protection against immune-evasive CMV, we also showed that this same population of CMV specific $CD8^+$ effector T cells is associated with immunopathology and exert damaging effects on activated endothelial cells, explaining – at least in part – their involvement in atherosclerotic vascular disease and their involvement in immunosenescence.

That CMV infection leaves a strong fingerprint on our immune system, is also illustrated by the fact that CMV seropositive individuals have twice as many circulating total CD8+ T cells than do CMV seronegative individuals [17]. Moreover, a high percentage of these circulating CD8 T cells, up to 40%, can be directed against one CMV epitope. Considering this huge impact of CMV on the circulating CD8 T cell compartment, and the known impaired immune capacity of the older CMV seropositive population, we next questioned whether the circulating pool is related to lymph node populations. We hypothesized that CMV infection might limit immunological space at sites where immune reactions are initiated, such as in lymph nodes (LN), if indeed these CMV specific cells in LN would be as abundant as in PB. We collected LN from the para-iliac area from CMV seropositive individuals during the renal transplant procedure. From those LN and paired PB samples, we isolated and compared the CMV specific T cells. We found that CMV specific cells are much less dominant in LN than in PB. In addition, the CMV specific T cells in LNs contained less $CD45RA^+CD27^-$ effector cells and less granzyme B^+ cells than in the paired PB samples. Actually, we found that LN CMV specific T cells lacked the typical effector cell traits, and resemble more memory-type cells, as was also shown by their increased capacity to produce IL-2 and by their capacity to secrete IFNγ, TNFα, MIP1β and CD107a, so called polyfunctionality, which was clearly higher than in the PB compartment. Moreover, these LN CMV specific T cells appeared to have a unique homing potential, showing increased expression of the LN homing marker CCR7 and decreased expression of CX3CR1, which was in sharp contrast to their PB counterparts. The expression of the chemokine receptor CX3CR1 on PB cells probably directs these cells to (stressed) endothelium rather than to LN, where they may bind