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Cytomegalovirus persistence and T-cell immunosenescence in people aged fifty and older

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Review

Cytomegalovirus persistence and T-cell immunosenescence in people aged fifty and older: A systematic review

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ABSTRACT

Introduction: Immunosenescence is the age-related deterioration of immunocompetence which is reflected in a poorer response to new antigens. This process is characterized by decreases in naïve T cells and increases in memory T cells. The highly prevalent β -herpesvirus cytomegalovirus (CMV) is thought to enhance T-cell immunosenescence. The aim of this study was to perform a systematic review on the current evidence regarding the relation between CMV-infection and immunosenescence in Western people aged fifty years and older.

Methods: Studies that investigated the relation between CMV infection and immune parameters in Western people aged 50 years and older were eligible for inclusion. No restrictions were placed on study type. This article focuses on the relation between CMV infections as measured by serology and T cell subsets. A narrative approach to data synthesis was applied.

Results: In the majority of included studies higher levels of Effector Memory (EM) and TEMRA (Effector Memory T cells re-expressing CD45RA) cells were found in the CD4+ and the CD8+ T cell pools in CMV-seropositive elderly compared to CMV-seronegative elderly. No clear evidence was found for lower levels of naïve T cells in CMV-seropositive elderly compared to CMV-seronegative elderly. The total CD8+ T cell pool appeared to be larger in CMV-seropositive elderly in three out of four studies, while the total CD4+ T cell pool appeared to be smaller in CMV-seropositive elderly in two out of four studies.

Discussion: CMV seems to enhance immunosenescence based on the high levels of the highly differentiated EM and TEMRA cells in the CD8+ and CD4+ T cell pools. The relation of the shifts within the T cell compartments in CMV-seropositive elderly in relation to susceptibility to infectious diseases remains to be investigated.

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Abbreviations: CM, Central Memory; EM, Effector Memory; IRP, Immune Risk Profile; TEMRA, Effector Memory T cell Re-expressing CD45RA.

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1. Introduction

Over the last decade, interest in the process of immunosenescence has increased. Immunosenescence is the age-related deterioration of immunocompetence, which is reflected in a poorer response to newly encountered antigens and vaccines (Goronzy and Weyand, 2013; Weng, 2006; Miller, 1996; Zheng et al., 1997). This process is characterized by decreases in naïve T cells and increases in memory T cells (Goronzy and Weyand, 2013; Boraschi et al., 2010; Pawelec, 2012). Immunosenescence is a result of human and cellular aging in combination with environmental stressors and antigen exposure history (Goronzy and Weyand, 2013; Weng, 2006; Miller, 1996; Zheng et al., 1997; Goronzy et al., 2001). The T-lymphocyte compartment has been studied extensively with respect to immunosenescence. T cell differentiation from naïve T cells to memory T cells is characterized by the expression of different surface markers and different functional profiles. Based on their functionality and the expression of different surface markers memory T cells can be found in several different stages of differentiation (Mahnke et al., 2013; Sallusto et al., 2004; Sallusto et al., 1999; Nikolich-Zugich, 2008). Based on the specific markers that they express memory T cells are often referred to as Central Memory (CM), Effector Memory (EM) and TEMRA T cells (Effector Memory T cells re-expressing CD45RA). CM T cells are characterized by a higher sensitivity to antigen stimulation in comparison to naïve T cells and an ability to rapidly differentiate into effector cells (Sallusto et al., 2004). EM T cells are suggested to be generated from CM T cells (Sallusto et al., 1999) and are characterized by an even faster effector function (Sallusto et al., 2004). Furthermore, TEMRA T cells are EM cells that re-express CD45RA. (Sallusto et al., 2004) and are considered late stage memory T cells (Pawelec et al., 2009; Karrer et al., 2009).

The β -herpesvirus cytomegalovirus (CMV) is prevalent worldwide and persists in its host for life (Cannon et al., 2010). CMV-seroprevalence increases with age and is generally higher in populations of lower economic status (Cannon et al., 2010; Cannon, 2009; Manicklal et al., 2013; Korndewal et al., 2015). Nonetheless, CMV-seroprevalence can reach 50% in women of childbearing age in developed countries (Cannon, 2009; Manicklal et al., 2013; Stagno et al., 1982). In the Netherlands, CMV-seroprevalence amongst Western citizens is 51.3% in the age category 50–59 and increases up to 65.6% in the age category of 75–79 (Korndewal et al., 2015). Viral transmission generally occurs through direct contact with body fluids (Cytomegalovirus (CMV) and congenital CMV infection [Internet], 2010). Transplacental transmission of CMV can lead to amongst others, retardation, hearing loss and even death (Leung et al., 2003). In the immunocompromised individual CMV infection can cause severe morbidity and mortality (Reeves and Sinclair, 2008). Conversely, in immunocompetent individuals CMV generally

does not cause overt disease. However, the virus does establish life-long persistence in the human host (Pawelec and Derhovanessian, 2011; Litjens et al., 2011).

The way in which CMV persists in the human body is not yet completely understood. After primary infection, CMV appears to establish some form of latency in bone marrow's myeloid progenitor cells (Sindre et al., 1996; Mendelson et al., 1996). CMV-specific immune cells accumulate during viral persistence and this accumulation is especially apparent in the CD8+ T cell compartment (Nikolich-Zugich, 2008; Pawelec et al., 2010; Moss, 2010; Sylwester et al., 2005). The accumulation of these CMV-specific memory T cells over time is often referred to as 'memory inflation' (Nikolich-Zugich, 2008). It has been suggested that the expansion of these memory CD8+ T cells may 'crowd out' other cells (Pawelec et al., 2010; Ouyang et al., 2003). However, little evidence regarding this hypothesis exists and clinical relevance of memory inflation remains unclear.

CMV-persistence is suggested to be a putative enhancer of immunosenescence and CMV-seropositivity is one of the parameters taken into account in many studies on aging of the immune system (Trzonkowski et al., 2003; Derhovanessian et al., 2013a). Furthermore, CMV has been known to impair the immune response and to enhance the age-related changes in the T-cell pool in aging mice (Mekker et al., 2012). CMV has also been related to higher levels of highly differentiated T cells (Pawelec et al., 2009; Derhovanessian et al., 2009) and lower levels of naïve T cells in humans (Karrer et al., 2009; Pawelec and Derhovanessian, 2011; Pawelec et al., 2010; Moss, 2010). These shifts are especially apparent for the CD8+ T compartment. CMV-seropositivity was also described as one of the parameters included in the so called Immune Risk Profile (IRP). The IRP was shown to be related to mortality in elderly in the Swedish OCTA/NONA studies (Olsson et al., 2000; Wikby et al., 2005). However, the IRP is not widely accepted. This profile was established based on a study that included Swedish elderly in a high age category, and therefore its applicability to other populations remains uncertain. Moreover, as IRP is related to increased mortality, the relation between IRP and immunocompetence and response to vaccination remains unclear.

As the world population is aging, more insight into factors that contribute to immunosenescence, including the role of CMV-infection, is needed. Therefore this systematic review was conducted to systematically assess studies that were conducted on CMV's influence on immunosenescence in Western people aged 50 years and older. Based on high levels of highly differentiated EM and TEMRA cells in the CD8+ and CD4+ T-cell pools, we conclude that CMV seems to enhance immunosenescence. Furthermore, this systematic review provides several starting points for further research regarding the role of cytomegalovirus in immunosenescence and to address controversies in the field.

2. Methods

2.1. Search strategy and selection criteria

Databases Medline and Embase were searched by an in-house information specialist for articles published in English or Dutch from 2004 to 31 January 2014 (Medline) and 14 February 2014 (Embase). Supplementary files A and B provide overviews of these search strategies. Only studies that reported on the relation between CMV-persistence and immune parameters in the elderly were considered for inclusion. No restrictions were placed on study design. Articles were eligible if the study included (blood samples of) humans aged 50 years and older and if these humans lived in Western countries. Inclusion of humans aged 50 and older was chosen, as immunosenescence occurs with age, and therefore it was expected the CMV induced immunosenescence would be easier to detect in the elderly. Review papers and papers that included immunocompromised participants or auto-immune diseases patients were not eligible for inclusion.

Two authors were responsible for the selection of articles; MW made a first selection based on title, and subsequently RE checked the excluded articles for agreement. This routine was also performed for subsequent selections based on abstracts and full texts. Discrepancies regarding in- or exclusion of an article could always be solved by discussion. No duplicate studies were found. References of the finally included articles were searched for additional relevant articles.

2.2. Data extraction and risk of bias assessment

Data was extracted via a data-extraction form (Supplementary file C), which was developed by MW. This form also included a pre-specified assessment of quality and risk of bias assessment for the included studies. The form was based on several other data extraction forms and forms assessing articles on quality and risk of bias (Centre for Reviews and Dissemination, 2009; ERBO-Platform, 2014a; ERBO-Platform, 2014b): the Newcastle Ottawa Scale (Wells et al., 2009), the Black and Downs Checklist (Downs and Black, 1998) and the Cochrane Collaboration's Tool for assessing Risk of Bias (Higgins and Green, 2011). Three other epidemiologists were consulted as well. Subsequently, the form was pre-tested by RE and MW by filling out the form for five articles. The form was refined accordingly.

Data collected from studies included study design, exposure, study population characteristics (size, percentages males and females, age ranges) and method of recruitment. The assessment of quality and risk of bias of the included studies regarded the way in which the included studies addressed possible confounders and effect modifiers. In addition, the performed statistical analyses and the representativeness of the participants were assessed. Furthermore, methods for assessment of CMV infection and outcomes measures were appraised. Possible conflicts of interest were checked. Finally, the overall quality of the studies was rated as 'poor', 'acceptable' or 'good'. Data extraction and quality and risk of bias assessment were performed by MW. RE assessed the quality and risk of bias and extracted data of twelve articles independently. Subsequently, the agreement between MW and RE regarding the quality and risk of bias of these twelve articles was assessed. For nine of these twelve assessments there was agreement. Consensus regarding the remaining three articles could be reached after discussion. In addition, based on these discussions the method of quality assessment was refined. Supplementary file D provides an overview of the outcomes from the quality/risk of bias-assessments for each included study.

2.3. Data synthesis: narrative synthesis

As the outcome variables were heterogeneous and no effect measurements were reported, a narrative approach to data synthesis was applied. Findings were reported per outcome variable.

Fig. 1. Flowchart of the selection of articles included in the review. Selection of articles after retrieving a total of 305 articles from databases Medline and Embase. Depicted are the numbers of remaining articles after the selection rounds based on title, abstract and full text. Of the 23 articles that were eligible for the review 14 articles reported on the relation between CMV persistence and naïve and memory CD4+ and CD8+ T cell subsets. The remaining eight articles reported on CMV persistence in relation to other topics. However, for these topics the number of articles per topic was too low to review. Since the vast majority of articles reported on the relation between CMV-persistence and CD4+ and CD8+ T cells this article focuses on this relationship. The results of the articles that reported on other topics than CD4+ and CD8+ T cells can be found in supplementary file F.

3. Results

3.1. Selection and quality assessment of articles

The searches in Medline and Embase retrieved 305 articles (Fig. 1), of which 83 articles were duplicates. A total of 222 articles remained eligible for further selection. A first selection based on title and abstract yielded 34 articles. These articles were read in full text, and 23 articles were included in this systematic review. Rationale for exclusion of full text articles are provided in supplementary file E. References of the 23 included articles were checked for additional relevant articles, but none were found. Overall the quality of the studies was acceptable to good, based on the filled in Quality and Risk of Bias Assessment forms (Supplementary file C and D). One article was assessed as 'poor', ten articles were assessed as 'acceptable' and six articles were assessed as 'good'. In addition four articles were assessed as 'acceptable to good' and one article was assessed as 'acceptable to poor'.

The retrieved articles reported predominantly on differences in T lymphocyte subsets between CMV-seropositive and CMV-seronegative elderly. A small number of studies reported on other topics, such as changes in cytokine levels and two studies reported on the control of latent co-infections with Herpes Simplex Virus type-1 (Stowe et al., 2012) and Epstein Barr Virus (Khan et al., 2004). Often only one article per such a topic was found. Since the vast majority of articles reported on CMV-persistence in relation to naïve and memory T cell subsets, this review focuses on the relation between CMV-persistence and these T cell subsets, based on 14 articles. The results of the articles that reported about other topics than naïve and memory T cell subsets can be found in supplementary file F.

3.2. CMV-persistence and shifts in CD8+ T cell subsets

3.2.1. Total CD8+ T cells

Three out of four articles that reported on CD8+ T cell levels reported significantly higher levels of CD8+ T cells in CMV-seropositive elderly compared to CMV-seronegative elderly (Litjens et al., 2011; Strindhall et al., 2013; Kuparinen et al., 2013) (Table 1, Supplementary file G). The fourth study found higher absolute numbers of total T cells in CMV-seropositive elderly, however, this finding was not significant. Kuparinen et al. found significantly lower percentages of CD8+ T cells in CMV-seronegative elderly than in seronegative young controls.

Table 1

Quantitative overview of articles that reported on CMV-seropositivity in relation to T cell subsets. Number of articles that reported significantly lower or higher levels of CD8+ and CD4+ T cell subsets in CMV-seropositive elderly, depicted against the total number of articles that reported about this subset.

T cell subset		Subset was significantly lower in CMV-seropositive elderly (number of studies that found a significant relation / total number of studies reporting on that relation)	Subset was significantly higher in CMV-seropositive elderly (number of studies that found a significant relation / total number of studies reporting on that relation)
CD8+ T cells	Total T cells		3/4
	Naïve T cells	6/8	
	CM T cells	2/4	
	EM T cells		5/7
	TEMRA T cells		5/6
	EM and TEMRA T cells combined		2/2
CD4+ T cells	Total T cells	2/4	
	Naïve T cells	3/6	
	CM T cells	1/5	
	EM T cells		4/4
	TEMRA T cells		2/3
	EM and TEMRA T cells combined		2/2
CD4/CD8 ratio		For CMV-serostatus: 1/2 For CMV-Ab titers: 2/2	

No articles reported on lower (in the left column) or higher (in the right column) on CMV-persistence and T cell levels.

Conversely, they did not find lower percentages of CD8+ T cells in CMV-seropositive elderly compared to seronegative young controls (Kuparinen et al., 2013). This suggests that CD8+ T cells normally decrease with age, but this decrease was not shown in the CMV-seropositive individuals. Northfield et al. analyzed CMV-specific CD8+ T cells as part of the total CD8+ T cell pool. They found the mean percentage of these CMV-specific T cells was 5.2% (sd = 6.2) (Northfield et al., 2005).

3.2.2. Naïve CD8+ T-cells

Eight studies reported on the influence of CMV-persistence on naïve CD8+ T cell levels (Litjens et al., 2011; Strindhall et al., 2013; Almanzar et al., 2005; Alonso Arias et al., 2013; Chidrawar et al., 2009; Derhovanessian et al., 2013b; Lee et al., 2012; Weinberger et al., 2007). These studies reported either on absolute naïve CD8+ T cell numbers or on percentages of naïve CD8+ T cells as part of the CD8+ T cell pool. The majority of these studies reported lower naïve CD8+ T cell levels; two studies reported that CMV-seropositive elderly had lower absolute numbers of naïve T cells (Almanzar et al., 2005; Chidrawar et al., 2009) and four studies reported lower percentages of naïve CD8+ T cells (Strindhall et al., 2013; Derhovanessian et al., 2013b; Lee et al., 2012; Weinberger et al., 2007). Conversely, two other studies found no relation between CMV-seropositivity and naïve CD8+ T cell percentages (Alonso Arias et al., 2013) and absolute numbers (Litjens et al., 2011) (Table 1, Supplementary file G). CMV-antibody titers may reflect the efficacy of the host immune response to control CMV, where higher CMV-antibody titers represent more frequent reactivation and a lesser ability of the immune system to control

such reactivations (Stowe et al., 2007; Vescovini et al., 2010). Alonso Arias et al. also investigated the relation between CMV-antibody titers and naïve CD8+ T cells but found no relation for CMV-antibody titers and lower levels of naïve T cells (Alonso Arias et al., 2013).

3.2.3. Central memory CD8+ T cells

Four studies reported on CM CD8+ T cells (Litjens et al., 2011; Almanzar et al., 2005; Lee et al., 2012; Weinberger et al., 2007). In CMV-seronegative individuals absolute numbers of CM CD8+ T cells increased with age, while this increase was absent in CMV-seropositive individuals (Almanzar et al., 2005). Another study reported significantly lower percentages of this subset in CMV-seropositive elderly (Weinberger et al., 2007). In contrast, two studies found no significant difference in CM CD8+ T cell percentages (Lee et al., 2012) and absolute numbers (Litjens et al., 2011) between CMV-seropositive and -seronegative elderly (Table 1, Supplementary file G).

3.2.4. Effector memory and TEMRA CD8+ T cells

Nine studies reported findings regarding EM CD8+ T cells and/or TEMRA CD8+ T cells in relation to CMV-persistence (Litjens et al., 2011; Strindhall et al., 2013; Kuparinen et al., 2013; Almanzar et al., 2005; Alonso Arias et al., 2013; Chidrawar et al., 2009; Derhovanessian et al., 2013b; Lee et al., 2012; Weinberger et al., 2007). Five (Litjens et al., 2011; Strindhall et al., 2013; Kuparinen et al., 2013; Almanzar et al., 2005; Derhovanessian et al., 2013b) out of the seven (Alonso Arias et al., 2013; Lee et al., 2012) studies that reported on EM CD8+ T cells, reported higher levels of this subset in CMV-seropositive elderly (Table 1, Supplementary file G). Kuparinen et al. compared CMV-seropositive nonagenarians and CMV-seronegative nonagenarians to CMV-seronegative young controls. The EM CD8+ T cell percentages were much higher in the CMV-seropositive nonagenarians than in the seronegative nonagenarians (Kuparinen et al., 2013). In contrast to above stated findings, Alonso Arias and colleagues found no significant relation between EM CD8+ T cells and CMV-seropositivity or CMV-antibody titers. However, they did find that most CD8+ T cells in CMV-seropositive elderly were highly differentiated (Alonso Arias et al., 2013). Five (Litjens et al., 2011; Strindhall et al., 2013; Almanzar et al., 2005; Chidrawar et al., 2009; Derhovanessian et al., 2013b) out of six (Lee et al., 2012) studies found significantly higher TEMRA CD8+ T cells in CMV-seropositive elderly. Two studies investigated the relation between CMV-serostatus and EM and TEMRA CD8+ T cell subsets combined. Both studies found significantly higher levels for these subsets combined in CMV-seropositive individuals (Lee et al., 2012; Weinberger et al., 2007) (Table 1, Supplementary file G). Of note, when one of these studies did discriminate between EM and TEMRA cells in an additional analysis, their finding lost statistical significance (Lee et al., 2012). Finally, Litjens and colleagues compared the whole CD8+ T cell memory pool (CM, EM, including TEMRA T cells) of CMV-seropositive elderly to the pool of CMV-seronegative elderly. They reported a significantly higher numbers of memory CD8+ T cells in CMV-seropositive elderly compared to CMV-seronegative elderly (Litjens et al., 2011).

3.2.5. Phenotype of CMV-specific and non-CMV-specific CD8+ T cells in CMV-seropositive elderly

CD8+ T cells are of primary importance in controlling viral infections (Strelkauskas et al., 2010). Several studies investigated the phenotype of CMV-specific CD8+ T cells. Northfield et al. investigated the phenotype of CMV-specific CD8+ T cells. They found lower expression of CCR7 (NS) and a higher expression of CD57 (NS) compared to cells that were not specific for CMV, indicating further differentiated T cells. In addition, Northfield found significantly higher levels of CD85j expression, a protein which Northfield suggests can be regarded as an effector memory marker. In an additional analysis Northfield found that the expression of CD85j on CD8+ T cells that were not specific for CMV was higher compared to similar cells in CMV-seronegative individuals.

Moreover, they found that this marker was expressed at significantly higher levels (18.7%) in the total CD8+ T cell pool in CMV-seropositive individuals, compared to CMV-seronegative individuals (Northfield et al., 2005). These findings suggest that not only CMV-specific CD8+ T cells have a more mature phenotype, but CD8+ T cells that are not specific for CMV are affected as well (Northfield et al., 2005). Hadrup et al. found that 28% of CMV-specific T cells had an EM phenotype and 62% of the CMV-specific T cells had a TEMRA phenotype in nonagenarians (Hadrup et al., 2006). Finally, a study conducted by Litjens et al. investigated expression of the markers CD57 and CD28. Increased expression of CD57 and absence of expression of CD28 indicate later stages of differentiation. They found significantly higher expression of CD57, and lower expression of CD28 on memory CD8+ T cells in CMV-seropositive elderly, compared to CMV-seronegative elderly. Indicating memory T cells of CMV-seropositive elderly are further differentiated.

3.3. CMV-persistence and shifts within CD4+ T cell subsets

3.3.1. Total CD4+ T cells

Two (Litjens et al., 2011; Chidrawar et al., 2009) out of three (Strindhall et al., 2013) studies found no significant differences in absolute numbers of total CD4+ T cells between CMV-seropositive and -seronegative persons. Two studies, that reported in percentages, showed lower percentages of total CD4+ T cells in CMV-seropositive compared to CMV-seronegative individuals (Strindhall et al., 2013; Kuparinen et al., 2013) (Table 1, Supplementary file G).

3.3.2. Naïve CD4+ T cells

Six studies reported on naïve CD4+ T cells in relation to CMV-persistence (Table 1, Supplementary file G). Three of these studies found significantly lower percentages of naïve CD4+ T cells in CMV-seropositive elderly (Alonso Arias et al., 2013; Weinberger et al., 2007; Libri et al., 2011). In addition, one of these studies found that not only CMV-seropositivity, but also CMV-antibody levels were related to lower naïve CD4+ T cell percentages (Alonso Arias et al., 2013). Three studies found no significant association between CMV-serostatus and naïve CD4+ T cells (Litjens et al., 2011; Chidrawar et al., 2009; Derhovanessian et al., 2013b). Another study found that expression of CD27+, CD28+ and CCR7+ T on CD4+ T cells was significantly lower in CMV-seropositive individuals, which could mean lower levels of naïve CD4+ T cells and/or lower levels of CM CD4+ T cells (Pourghesari et al., 2007).

3.3.3. Central memory CD4+ T cells

Of the five studies that reported on CM CD4+ T cells, one study found that CMV-seropositivity and anti-CMV-antibody titers were significantly correlated to a decrease in CM CD4+ T percentages (Alonso Arias et al., 2013) (Table 1, Supplementary file G). Conversely, Weinberger et al. (2007) and Chidrawar et al. (2009) found lower levels of CM CD4+ T cells in CMV-seropositive elderly, however, their findings were not statistically significant. Two studies conducted by Libri et al. and Litjens et al. found no association between CMV-persistence and CM CD4+ T cell numbers (Litjens et al., 2011; Libri et al., 2011).

3.3.4. Effector memory and TEMRA CD4+ T cells

All four studies that reported on EM CD4+ T cells in CMV-persistence reported a positive association between CMV-seropositivity or CMV-antibody titers and EM CD4+ T cells (Litjens et al., 2011; Kuparinen et al., 2013; Derhovanessian et al., 2013b; Libri et al., 2011) (Table 1, Supplementary file G). In addition, two of these studies showed a significant relation between CMV-seropositivity and increased TEMRA CD4+ cells in CMV-seropositive individuals (Derhovanessian et al., 2013b; Libri et al., 2011). In contrast, Alonso Arias et al. found no significant relation between CMV-antibody levels and TEMRA CD4+ T cells (Alonso Arias et al., 2013). Two studies did

not discriminate between EM and TEMRA CD4+ T cells and reported significant increases in these two subsets combined in CMV-seropositive individuals (Weinberger et al., 2007; Pourghesari et al., 2007). Litjens and colleagues compared absolute numbers of all memory T cells (EM and CM combined) for CMV-seropositive and -seronegative elderly, but found no difference. In addition, they investigated the expression of the markers CD57 and CD28 on memory CD4+ T cells in CMV-seropositive and -negative elderly. They found significantly higher percentages of CD4+ T cells that had lost expression of CD28, indicating a later stage of cell differentiation. For the expression of CD57, however, no significant difference between the CMV-seropositive and -seronegative group was found (Litjens et al., 2011). Of note, Weinberger et al. showed a significant negative correlation between EM CD4+ T cells and CM CD4+ T cells in CMV-seropositive elderly only.

3.3.5. CD4+ T cells specific for other antigens than CMV

Two studies investigated whether CMV-seropositivity affected differentiation of CD4+ T cells specific for antigens other than CMV. Fletcher et al. found that CMV-seropositivity had a profound impact on the differentiation status of non-CMV specific CD4+ T cells based on the loss of CD27 and CD28 expression. CD4+ T cells specific for tuberculin purified protein derivative (PPD), Varicella Zoster Virus (VZV), Epstein-Barr Virus (EBV) and Herpes Simplex Virus (HSV) from CMV-seropositive individuals appeared to have a higher differentiated pattern, compared to these cells retrieved from CMV-seronegative individuals (Fletcher et al., 2005). Another study found that *in vitro* expansions of EM CD4+ T cells specific for EBV, HSV and VZV were significantly larger in CMV-seropositive elderly than in CMV-seronegative elderly (Libri et al., 2011). In addition, the proportion of EBV-specific TEMRA CD4+ T cells was significantly higher in CMV-seropositive elderly than in CMV-seronegative elderly. Proportions of TEMRA CD4+ T cells specific for HSV and VZV did not differ significantly. These findings indicate a possible influence of CMV-seropositivity on the cellular defense to pathogens other than CMV.

3.4. CMV-persistence and the CD4/CD8 ratio

An inverted CD4/CD8 ratio is the best predictor of the so called immune risk profile (IRP) and CMV-persistence has been related to inversion of this ratio (Karrer et al., 2009; Olsson et al., 2000; Wikby et al., 2005). Three out of four studies found a lower CD4/CD8 ratio in CMV-seropositive individuals, however only the finding by Litjens's study was statistically significant (Litjens et al., 2011; Alonso Arias et al., 2013; Chidrawar et al., 2009) (Table 1, Supplementary file G). Conversely, one of these studies and a study by Strindhall et al. did find a significant negative relation between CMV-antibody titers and the CD4/CD8 ratio (Strindhall et al., 2013; Alonso Arias et al., 2013). In addition, Strindhall found CMV-seropositivity was significantly more common in elderly with a CD4/CD8 ratio less than one (Strindhall et al., 2013).

4. Discussion

To the best of our knowledge this is the first systematic review examining the relation between CMV-seropositivity and T-cell immunosenescence in Western people aged fifty years and older. The studies included in this review reported primarily on shifts within the CD8+ and CD4+ T cell compartments. Overall higher levels of highly differentiated (EM/TEMRA) cells were seen in both the CD4+ (Litjens et al., 2011; Kuparinen et al., 2013; Derhovanessian et al., 2013b; Weinberger et al., 2007; Libri et al., 2011; Pourghesari et al., 2007) and the CD8+ (Litjens et al., 2011; Strindhall et al., 2013; Kuparinen et al., 2013; Almanzar et al., 2005; Chidrawar et al., 2009; Derhovanessian et al., 2013b; Lee et al., 2012) T cell compartments in CMV-seropositive elderly compared to CMV-seronegative elderly (Fig. 2). For CD8+ T cells we know that a large part of these cells are CMV-specific (Khan et al., 2004; Northfield et al., 2005). Moreover,

CD4+ and CD8+ T cells with a specificity for antigens other than CMV-antigens appeared to be further differentiated in CMV-seropositive individuals (Northfield et al., 2005; Libri et al., 2011; Fletcher et al., 2005). It has been hypothesized that CMV-persistence triggers a pro-inflammatory environment (Pawelec, 2012; Pawelec et al., 2009; Moss, 2010; Trzonkowski et al., 2003) which increases differentiation of T cells (Fletcher et al., 2005). CMV-induced IFN- α has indeed been found to induce loss of CD27 and CD28 receptors of non-CMV specific CD4+ T cells (Fletcher et al., 2005) and on CD8+ T cells *in vitro* (Borthwick et al., 2000). Therefore, higher levels of differentiated CD28-, CD27- T cells may be a result of higher levels of pro-inflammatory cytokines in CMV-seropositive elderly.

The reviewed studies indicated a relation between CMV-persistence and levels of total CD8+ T and CD4+ T cells (Fig. 2). Levels of total CD8+ T cells appear to be higher in CMV-seropositive elderly, which can be explained by the high numbers of memory cells in this subset. Uncertainty remains regarding the level of total CD4+ T cells. For CD4+ T cell levels, two out of three studies that reported in absolute numbers showed no difference in total CD4+ T cell numbers between CMV-seropositive and -seronegative elderly. Conversely, both studies that reported percentages of total CD4+ T cells, found lower levels of total CD4+ T cells in CMV-seropositive elderly. Therefore absolute numbers of total CD4+ T cells may be similar in CMV-seropositive and CMV-seronegative elderly, but percentages of total CD4+ T cells may be lower in CMV-seropositive elderly. This relative decrease in total CD4+ T cells can be explained by the increase of total CD8+ T cells. A recent study by Benedetto and colleagues confirmed a lower percentage of total CD4+ T cells in CMV-seropositive individuals, regardless of gender and age (Benedetto et al., 2015). Strindhall et al. showed that an inverted CD4/CD8 ratio was associated with the IRP (Strindhall et al., 2013). However, whether CMV-persistence is directly related to a lower CD4/CD8 ratio is unclear. The two studies that investigated CMV-antibody levels in relation to the CD4/CD8 ratio did find significantly lower CD4/CD8 ratio's in CMV-seropositive elderly (Strindhall et al., 2013; Alonso Arias et al., 2013). However, CMV-persistence per se may not be related to a lower ratio, but a poorer control over CMV, as indicated by higher levels of CMV-antibodies may be related to a lower CD4/CD8 ratio. Moreover, the use of IRP and the CD4/CD8 ratio as indicators of immunocompetence is controversial. The IRP is based on the mortality of a Swedish population of very old individuals. Obviously, mortality cannot be seen as a direct indicator of immunosenescence, and the representability of this population in questionable. For both the CM CD8+ and CD4+ T cells no conclusion can be drawn. The inconsistent findings regarding CM T cells can be attributed to the intermediate status of this subset, harboring cells in transition

from the naïve to the EM/TEMRA pool (Sallusto et al., 2004; Sallusto et al., 1999).

Most studies included in the review reported lower levels of naïve CD8+ T cells subsets in CMV-seropositive elderly compared to CMV-seronegative elderly. However, two studies of good quality did not report significantly lower levels of this subset in CMV-seropositive elderly. Again, the difference in reporting in percentages and absolute numbers between studies may be an explanation why some studies found lower levels of naïve CD8+ T cells while others did not. The decrease of naïve CD8+ T cell levels might be merely relative, and not absolute, since CD8+ memory T cell levels are higher in CMV-seropositive elderly. In agreement with this are the findings of a recent study by Wertheimer et al. Wertheimer reported no influence of CMV-seropositivity on absolute naïve CD8+ T cells levels (Wertheimer et al., 2014). Moreover, Wertheimer's study population was considerably larger than the study populations of the reviewed studies. In addition, Wertheimer performed a simulation study that showed the influence of cohort size on study outcome. They reported that undersampling easily leads to erroneous conclusions for the relation between CMV-serostatus and naïve CD8+ T cells (Wertheimer et al., 2014). Nevertheless, if CMV-persistence actually reduces populations of naïve T cells, the mechanism behind this is unknown. However, none of the studies discussed this, and this topic remains to be investigated. Lastly, gender may be an important factor in the influence of CMV on immunomodulation; a recent study by Benedetto and colleagues found CMV only to be related to lower naïve CD8+ T cells in elderly women, and not in men (Benedetto et al., 2015). However, none of the included studies in this review took gender into account.

Similar to the relation of CMV-serostatus and naïve CD8+ T cells levels, the relation between CMV-seropositivity and naïve CD4+ T cell levels remains unclear. Three out of six studies reported significantly lower numbers or percentages of this subset in CMV-seropositive elderly (Alonso Arias et al., 2013; Weinberger et al., 2007; Libri et al., 2011). In addition, two studies that did not find a significant relation between CMV-seropositivity and lower levels of naïve CD4+ T cells did find trends towards lower levels of this T cell subset in CMV-seropositive elderly (Chidrawar et al., 2009; Derhovanessian et al., 2013b). Wertheimer's recent study reported a significant relation between CMV-seropositivity and lower absolute numbers of naïve CD4+ T cells (Wertheimer et al., 2014). Moreover, Alonso Arias found a significant inverse relation between CMV-antibody levels and naïve CD4+ T cell levels (Alonso Arias et al., 2013; Derhovanessian et al., 2013b). This may be indicative of a more detrimental effect on naïve CD4+ T cells in individuals with a poorer control of CMV. While TEMRA and EM CD4+ T cells were higher in CMV-seropositive elderly, the levels of total CD4+ T cells were not simultaneously increased in these individuals. On the contrary, levels of total CD4+ T cell levels might even be lower in CMV-seropositive elderly. In short, this may indicate an increase of EM and TEMRA CD4+ cells at the expense of other CD4+ T cell subsets, such as naïve and/or CM CD4+ T cells. Furthermore, Weinberger et al. found a significant correlation between an increase in EM CD4+ T cells and a decrease in CM CD4+ T cells, which indeed may indicate an increase of EM CD4+ T cells at the expense of CM CD4+ T cells (Weinberger et al., 2007). Whether the increases in EM and TEMRA T cells and the possible decreases of naïve T cells are inter-related, remains to be investigated.

While lower levels of naïve CD8+ T cells are a characteristic of immunosenescence (Nikolich-Zugich, 2008; Wertheimer et al., 2014; Fulop et al., 2013; McElhaney and Effros, 2009), lower levels of naïve CD4+ T cells do not appear to be age-related (Wertheimer et al., 2014). Wertheimer's study, as one of the first, nicely discriminated the difference between changes in T cell subsets due to aging and due to CMV-seropositivity (Wertheimer et al., 2014). They found no relation between lower numbers of naïve CD4+ T cells and age. Conversely, they did find a significant relation between CMV-seropositivity and lower numbers of naïve CD4+ T cells. Therefore, lower levels of naïve

Fig. 2. T-cell differentiation in CMV-seropositive elderly. Higher numbers of total CD8+ cells are seen in CMV-seropositive elderly. The relations of CMV persistence to total CD4+, naïve CD4+ and naïve CD8+ T cells are unclear, numbers of these cells may be equal or lower in CMV-seropositive elderly.

CD4+ T cells appear not to be a feature of aging, but may be a unique feature of CMV-persistence. In the case that CMV-seropositivity is indeed related to lower numbers of naïve CD8+ T cells, then CMV-persistence can be regarded as an enhancer of immunosenescence. However, much uncertainty remains regarding naïve CD8+ and CD4+ T cell levels in relation to CMV-seropositivity. Hence, further research upon this relation is needed. In contrast, the relation between CMV-persistence and higher levels of differentiated CD4+ and CD8+ T cells seems evident. Higher levels of differentiated T cells, has generally been accepted as a parameter of the aging immune system. However, based on our findings, CMV-persistence appears to be related to increases of differentiated memory T cell subsets. Findings by Wertheimer gave initial insight to this whilst investigating the separate relations of aging and CMV with T cell subsets (Wertheimer et al., 2014). Our research highlights the need of taking CMV-status into account in research on the aging immune system. The clinical relevance of the shifts within the T cell compartments is unclear. Lower levels of naïve T cells may be detrimental to immunity as less naïve cells are available to fight off new infections. In accordance, lower levels of naïve T cells have been related to poorer responses to vaccination in elderly primates (Cicin-Sain et al., 2010). Conversely, a study by Derhovanessian et al. found, unexpectedly, that lower levels of naïve CD8+ T cells corresponded with longer survival in a Dutch cohort aged 85 years and older (Derhovanessian et al., 2013b). The lower levels of naïve CD8+ T cells appeared to be significantly related to higher levels of EM and TEMRA T cells. The authors suggested a high level of immune surveillance by EM and TEMRA T cells to previously encountered pathogens might be more beneficial in the current society, than having high levels of naïve T cells available to fight off novel pathogens (Derhovanessian et al., 2013b). Of note, these participants were very old and therefore less representative of the whole elderly population. More research is needed to confirm lower levels of naïve CD4+ and CD8+ T cells in CMV-seropositive elderly and to investigate the clinical impact of these lower levels on susceptibility to infections and vaccine responsiveness. In contrast to the above stated possible beneficial effect of EM and TEMRA T cells, it has often been hypothesized that increases in EM and TEMRA T cells may “crowd” other T cell subsets (Almanzar et al., 2005; Alonso Arias et al., 2013; Chidrawar et al., 2009; Weinberger et al., 2007; Koch et al., 2007; Saurwein-Teissl et al., 2002). It has also been suggested that their generation and maintenance is energy consuming which may be at the expense of other parts of the immune system (Fulop et al., 2013). We are not aware of studies that have investigated the influence of these increased subsets on susceptibility to infectious diseases. However, studies have been conducted that investigated the relation between these subsets and responsiveness to influenza vaccination. Two of these studies found more highly differentiated EM and TEMRA CD8+ T cells present in elderly who had a poor humoral response to influenza vaccination (Trzonkowski et al., 2003; Saurwein-Teissl et al., 2002). Likewise, Goronzy et al. found EM/TEMRA CD8+ T cells to be a predictive marker of poorer response to influenza vaccination (Goronzy et al., 2001). Conversely, they did not find such a relation for CD4+ differentiated subsets. A study by Xie, found highly differentiated CD8+ T cells were related to a poorer cellular immune response to influenza vaccination (Xie and McElhaney, 2007). In contrast, another study did find that highly differentiated CD4+ T cells were negatively associated with responsiveness to influenza vaccination, but this study did not find that EM/TEMRA CD8+ T cells were related to poorer immune responsiveness (Derhovanessian et al., 2013a). Clearly, many questions remain and studies are needed to confirm the shifts within the T cell compartments as stated in this review and the relation of these shifts to susceptibility to infectious diseases. These kinds of studies are difficult to perform due to ethical and practical constraints, therefore vaccine-responsiveness studies could be a first step.

This systematic review has some limitations. When conducting a systematic review, randomized controlled trials (RCT's) are the gold standard (Higgins and Green, 2011). However, due to ethical and

practical constraints, no RCT's have been conducted on the relation between CMV-seropositivity and immunosenescence in humans. Consequently, only observational studies could be included in this systematic review, which are prone to bias. In addition, most studies were cross-sectional studies, due to which no conclusions regarding causality could be drawn. Moreover, the included studies used different methodologies, which makes comparisons between studies more difficult. Furthermore, no consensus regarding the use of markers to identify the different stages of T cell exists (Mahnke et al., 2013). As a result, different studies used different combinations of markers to identify T cell subset. The use of different markers in different studies makes comparison of studies less appropriate. Consequently, this review highlights the need for standardization of markers to be used for identification of different T cell subsets. An overview of the markers used in the different studies is provided in Supplementary file H. In addition, during the period in which articles eligible for this review were published (January 2004 to January/February 2014), laboratory techniques have developed. Therefore, results obtained using techniques applied at the beginning of this 10-year period are not fully comparable with results obtained at the end of this time period.

The included studies suffered a few shortcomings. One of these limitations was that many studies excluded elderly who suffered from a chronic or acute illness. Elderly who did not live independently were also often excluded. Since many elderly do suffer from illnesses and do not live independently, the exclusion of these individuals negatively affects the representability of the study population. In addition, the method of recruitment of participants was described briefly in many studies, or was not stated at all. Consequently, the assessment of risk of bias of these studies was hindered. Many studies included volunteers, which have some disadvantages, for example, participants with a particular interest in health may be overrepresented. In addition, many articles did not provide information regarding socioeconomic status and other demographic indicators of the participants. Due to these shortcomings, the risk of selection bias could not always be assessed. More detailed features of the studies and their limitations are stated in Supplementary file D.

Duration of CMV-persistence may be of influence in the CMV-related modulation of the T cell pool. CMV is known to induce a prompt expansion of EM and TEMRA cells in both CD8+ (Chidrawar et al., 2009; Waller et al., 2008; Wills et al., 2002; Lilleri et al., 2008) and CD4+ T cells (Chidrawar et al., 2009) after initial infection. CMV-specific T cells are known to often have a highly differentiated phenotype (Moss, 2010; Koch et al., 2007; Lilleri et al., 2008; Pawelec et al., 2006) and cumulative expansion of CMV-specific T cells is thought to occur with the repeated reactivation and desilencing of viral genes (Moss, 2010; Seckert et al., 2012). This suggests that an early in life acquired infection may result in more pronounced CMV-induced immunomodulation later in life, in comparison to an infection acquired later in life. However, since initial CMV-infection generally occurs unnoticed, information regarding time of infection is difficult to obtain. Consequently, the studies included in this review did not take duration of CMV-persistence into account. Despite the difficulties regarding obtaining this information, duration of CMV-persistence might be an important factor to consider.

A strength of our review was the systematic approach. Our searches in Medline and Embase were developed and conducted by an information specialist. We believe our search was thorough, as we did not find any new articles when screening the references of included articles. The agreement between the two researchers during assessment of study quality and data-extraction was high. Furthermore, as the strengths and limitations of the included studies are stated in Supplementary file D, the quality of the included studies has been made transparent.

5. Conclusion

The current review found that CMV-seropositivity is related to shifts within the T cell compartment. We found strong evidence of increased

levels of EM and TEMRA cells in both the CD4+ and CD8+ T cell pools. Nonetheless, the clinical relevance with respect to susceptibility to infectious diseases of these increased EM and TEMRA levels remain unclear. Uncertainty remains regarding the relation between CMV-seropositivity and lower levels of naïve T cells in both the CD4+ and CD8+ T cell compartments. Future research should investigate whether the increases in EM and TEMRA T cells and the possible decreases of naïve T cells are interrelated. Moreover, research is needed to investigate whether these shifts increase susceptibility to infectious diseases. This review also highlights the need for standardization of markers for the identification of T cell subsets, the reporting of absolute T cell numbers instead of percentages, and taking CMV-status into account when researching on the aging immune system. To conclude, CMV seems to enhance immunosenescence at least based on the high levels of the highly differentiated EM and TEMRA cells in the CD8+ and CD4+ T cell pools. Many questions remain, however, this review provides several starting points for further research regarding cytomegalovirus.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.exger.2016.02.005>.

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