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REVIEW

Mast cells: innate attractors recruiting protective CD8 T cells to sites of cytomegalovirus infection

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Abstract Reactivation of latent cytomegalovirus (CMV) in the transient immunocompromised state after hematoablative treatment is a major concern in patients undergoing hematopoietic cell transplantation (HCT) as a therapy of hematopoietic malignancies. Timely reconstitution of antiviral CD8 T cells and their efficient recruitment to the lungs is crucial for preventing interstitial pneumonia, the most severe disease manifestation of CMV in HCT recipients. Here, we review recent work in a murine model, implicating mast cells (MC) in the control of pulmonary infection. Murine CMV (mCMV) productively infects MC in vivo and triggers their degranulation, resulting in the release of the CC chemokine ligand 5 (CCL5) that attracts CD8 T cells to infiltrate infected tissues. Comparing infection of MC-sufficient C57BL/6 mice and congenic MC-deficient *Kit*$^{W\text{-}sh/W\text{-}sh}$ "sash" mutants revealed an inverse relation between the number of lung-infiltrating CD8 T cells and viral burden in the lungs. Specifically, reduced lung infiltration by CD8 T cells in "sash" mutants was associated with an impaired infection control. The causal, though indirect, involvement of MC in antiviral control was confirmed by reversion of the deficiency phenotype in "sash" mutants reconstituted with MC. These recent findings predict that efficient MC reconstitution facilitates the control of CMV infection also in immunocompromised HCT recipients.

Keywords CCL5 · Chemokine · Immune surveillance · Mast cell · Mast cell-specific Cre recombination · Protective immunity

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Introduction

Mast cells (MC) are long-lived, tissue resident cells of the immune system that are derived from committed MC progenitors in the bone marrow and mature in essentially all vascularized tissues. MC are "inbetweeners" in a dual meaning. First, being located beneath endothelial and epithelial surfaces at the boundary between inside and outside the organism, they act as first-line sentinels for environmental antigens, including a wide array of invading pathogens such as viruses, bacteria, protozoa, and even metazoan parasites [1, 2]. Second, MC share properties attributed to cells of the adaptive and the innate arms of the immune system. Their antigen-specific activation and degranulation are mediated through membrane-bound IgE antibody. Aside from the notorious role in allergic inflammatory reactions [3], a

noted protective immune function triggered through MC-IgE is the defense against gastrointestinal helminths [4]. Innate MC effector responses are initiated by ligation of a number of pattern recognition receptors (PRR), including Toll-like receptors (TLR) [5]. Receptor ligation in both the IgE-mediated classical and the alternative innate pathways of MC activation [6] can induce the synthesis of MC cytokines but can also trigger instant effector functions by the release of effector molecules already stored in granules [7].

Besides exerting direct effector functions, MC contribute to immune surveillance by a regulatory cross talk with other cells of the immune system [8]. Of specific interest, sensitized MC release the CC chemokine ligand 5 (CCL5, also known as RANTES), which binds to CC chemokine receptor 5 (CCR5) [9] that is upregulated on sensitized memory CD8 T cells [10], resulting in their recruitment from lymphoid and intravascular compartments to extravascular sites such as peritoneal cavity [11] and lung interstitium [12]. In the primary immune response, naïve CD8 T cells also upregulate CCR5, permitting these cells to be attracted by CCR5 ligands to sites of antigen-specific dendritic cell CD4 T cell interaction, providing CD4 T cell help for promoting CD8 T cell priming [13].

A notable regulatory function of MC in the context of allogeneic HCT is the suppression of graft-versus-host disease (GvHD) by a mechanism independent of $CD4^+CD25^+$ regulatory T cells but involving IL-10 [14]. CMV infection is another most relevant complication in HCT recipients, both clinically (reviewed in [15]) and in the experimental mouse model (reviewed in [16]), and can be controlled by CD8 T cells reconstituted endogenously following HCT or interventionally by adoptive cell transfer immunotherapy [17–24] (reviewed in [16, 25, 26]). It was therefore intriguing to investigate if MC play any role in combating CMV infection.

Here, we review recent work in a murine model [27, 28] implicating infected MC in the recruitment of protective CD8 T cells to the lungs, a predilection site of CMV pathogenesis and disease manifestation in the immunocompromised host [17, 29] as well as of CMV latency and reactivation [30–35] and of CD8 T cell memory inflation [36, 37]. These studies propose a dually beneficial role for MC by moderating two major complications of allogeneic HCT: GvHD and CMV infection.

Mast cells inversely impact on CD8 T cell infiltration and viral burden in the lungs

First evidence for a contribution of MC to the control of a CMV infection [27] was provided in a murine model by comparing murine CMV (mCMV) infection of immunocompetent and MC-sufficient "wild type" C57BL/6 mice (B6-WT) with the infection of B6 congenic and MC deficient but otherwise immunocompetent $Kit^{W\text{-}sh/W\text{-}sh}$ "sash" mutants (B6-$Kit^{W\text{-}sh}$) [38, 39]. As illustrated in Fig. 1 (groups A vs. B; based on data from Ref. [27]), CD8 T cell infiltration into lung tissue was reduced in the MC-deficient mutants (Fig. 1a) on day 6 after intravenous infection with mCMV, and this corresponded to a subsequently increased virus burden (Fig. 1b). As infection of the lungs is controlled by CD8 T cells localizing to infected lung tissue cells, such as alveolar epithelial cells and interstitial fibrocytes, in nodular inflammatory foci (NIF) [27], it was concluded that MC do not directly control the infection by own effector functions but rather recruit antiviral CD8 T cells to the tissue site of infection. Specifically, depletion of CD8 T cells in B6-WT mice abrogated the control of infection despite the continued presence of MC. In a formal sense, however, this experimental setting did not exclude a "sash" mutation phenotype unrelated to MC. Any doubts about a causal role of MC were solved by restoring lung infiltration of CD8 T cells as well as control of pulmonary infection by reconstituting B6-$Kit^{W\text{-}sh}$ mice with bone marrow-derived, cell culture-selected and thereby purified MC (Fig. 1, group C; based on data from Ref. [27]). The contribution by MC was most prominent in the lungs and not significant in spleen and liver, sites at which residual CD8 T cell recruitment in the absence of MC was already sufficient for controlling infection (authors' unpublished data). It is thus proposed that MC-mediated enhancement of recruitment is required in particular at organ sites where CD8 T cell control is less efficient, such as in the lungs. One may speculate that this relates to the fact that lungs are a known main site of CMV disease manifestation, in particular after HCT.

Infected mast cells degranulate and release the chemokine CCL5

The search for the mechanism by which host infection activates MC for degranulation led to the identification of two kinetically and mechanistically distinct waves [28].

The first wave of degranulation of $CD117^+FcεRI^+$ MC among intraperitoneal exudate cells was observed as soon as 4 h after intraperitoneal mCMV infection and was found to require TLR3–TRIF signaling, as it was absent in both $TLR^{-/-}$ mice and adapter protein $TRIF^{-/-}$ mice [28]. Surprisingly, however, infection of MC-deficient B6-$Kit^{W\text{-}sh}$ mice reconstituted with MC derived from $TLR3^{-/-}$ mice still triggered degranulation of MC [28], indicating that TLR3–TRIF signaling is not required within the MC. Rather, it must be proposed that TLR3–TRIF signaling in another first-line cell type of infection leads to the release of a signal involved in triggering the degranulation of MC.

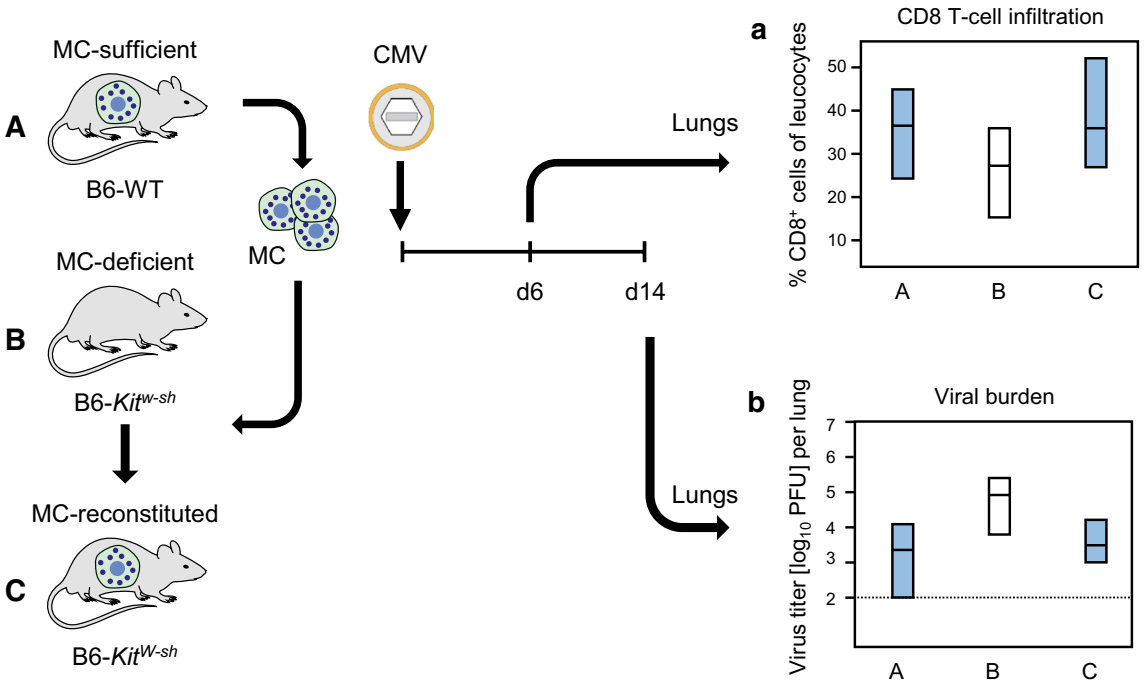

Fig. 1 MC facilitate the recruitment of CD8 T cells to the control of pulmonary CMV infection. Group A, MC-sufficient B6-WT mice. Group B, MC-deficient B6-*Kit*$^{W\text{-}sh}$ mice. Group C, MC-reconstituted B6-*Kit*$^{W\text{-}sh}$ mice. **a** Proportion of CD8 T cells among all leukocytes in lung infiltrates on day 6 after intravenous mCMV infection. **b** Virus titers in the lungs on day 14 after intravenous mCMV infection. *Bar* diagrams represent median values and ranges based on data published in Ref. [27] with permission by *PloS Pathogens*

While suspected candidates, namely macrophages and natural killer (NK) cells, were excluded by their depletion failing to abrogate MC degranulation [28], a positive identification of the cell type involved as well as of the molecular nature of the TLR3–TRIF signaling-dependent paracrine trigger of MC degranulation, awaits further studies.

A second wave of MC degranulation was observed at 24 h [27] and proved to be independent of TLR3–TRIF signaling, as it occurred also upon infection of TLR3$^{-/-}$ or TRIF$^{-/-}$ mice [28]. To test if MC become infected by mCMV in vivo, a conditional reporter virus system was used (Fig. 2), based on Cre recombinase-mediated excision of a stop cassette in the genome of virus mCMV-flox-*egfp* [40, 41] selectively in MC of B6 congenic, *Mcpt5-cre*-transgenic mice that express Cre recombinase under control of the MC-specific MC protease 5 promoter [42]. Thus, upon intraperitoneal infection of *Mcpt5-cre* mice with mCMV-flox-*egfp*, recombination resulting in the expression of enhanced green fluorescent protein (eGFP) labels infected MC (for the principle, see Fig. 2a reproduced from Ref. [28]). A significant proportion of CD117^{+}FcεRI^{+} MC was indeed found to express eGFP, and, most tellingly, degranulation indicated by cell surface exposure of CD107a (also known as lysosomal-associated membrane protein-1, LAMP-1) was restricted to infected, eGFP^{+} MC (Fig. 2b, reproduced from Ref. [28]). These findings not only identified MC as a target cell of mCMV infection, but also indicated that the infection triggers MC degranulation.

Of functional relevance, MC infection was followed by release of the chemokine CCL5 into the serum of infected mice, which strongly peaked around day 2. Notably, besides the high MC-dependent CCL5 levels in infected MC-sufficient B6-WT mice, infection led also to somewhat elevated MC-independent CCL5 levels in the serum of MC-deficient B6-*Kit*$^{W\text{-}sh}$ mice (Fig. 2c, based on data in Ref. [27]). As CCL5 levels in MC-sufficient mice do not significantly increase until day 1, it is proposed that the second wave of MC degranulation, that is the 24-h wave, is functionally more important. The MC-independent fraction of the CCL5 serum level can explain the basal lung infiltration by protective CD8 T cells observed also in infected B6-*Kit*$^{W\text{-}sh}$ mice (Fig. 1a; Ref. [27]).

Infection of mast cells is productive, and viral progeny disseminates to other cell types

Expression of eGFP in peritoneal exudate MC of *Mcpt5-cre* mice infected intraperitoneally with mCMV-flox-*egfp* has verified viral entry into MC as well as subsequent Cre-mediated recombination of the viral genomes. Completion of the viral productive cycle with release of recombined

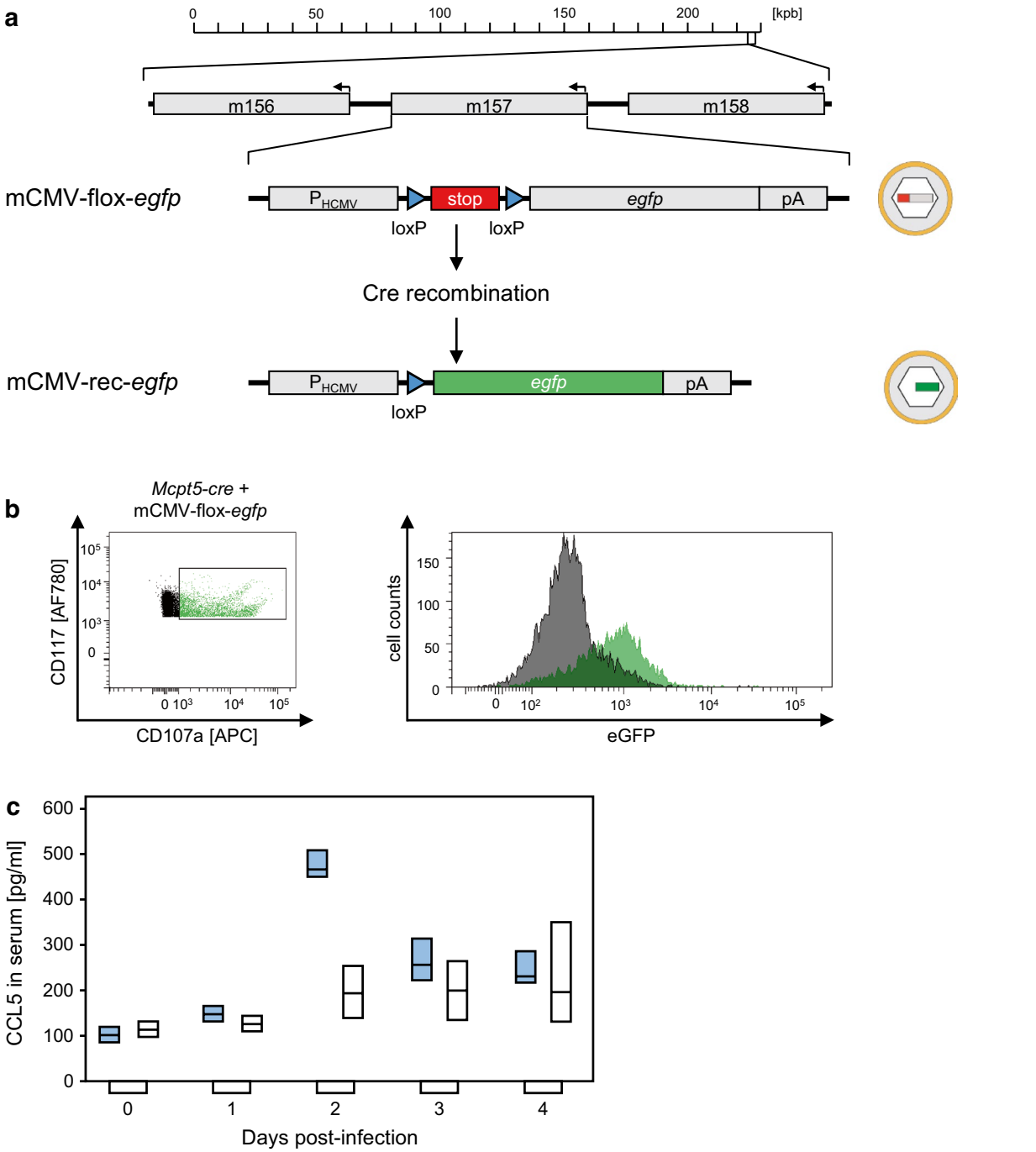

Fig. 2 Infection of MC leads to MC degranulation and release of the chemokine CCL5. **a** Gene map and genetic principle of Cre recombination-based conditional expression of the reporter protein eGFP in *cre*-transgenic *Mcpt5-cre* mice expressing Cre recombinase selectively in MC. Virion pictograms represent stop (*red*) and go (*green*) for eGFP reporter protein expression upon infection. **b** Selective degranulation of infected (eGFP$^+$) CD117$^+$FcεRI$^+$ MC as indicated by expression of CD107a (**a** and **b** reproduced from Ref. [28] with permission by *Molecular and Cellular Immunology*). **c** MC-dependent (*blue bars*) and MC-independent (*open bars*) serum levels of chemokine CCL5 in intravenously infected MC-sufficient B6-WT and MC-deficient B6-*Kit*$^{W\text{-}sh}$ mice, respectively. Bar diagrams represent median values and ranges based on data published in Ref. [27] with permission by *PloS Pathogens*

mCMV-rec-*egfp* progeny virions (sketched in Fig. 3a) was previously demonstrated by detection of green fluorescent plaques in mouse embryo fibroblast cell cultures inoculated with tissue homogenates from liver and lungs of *Mcpt5-cre* mice, which were immunocompromised 2 days after infection with mCMV-flox-*egfp* to facilitate dissemination of

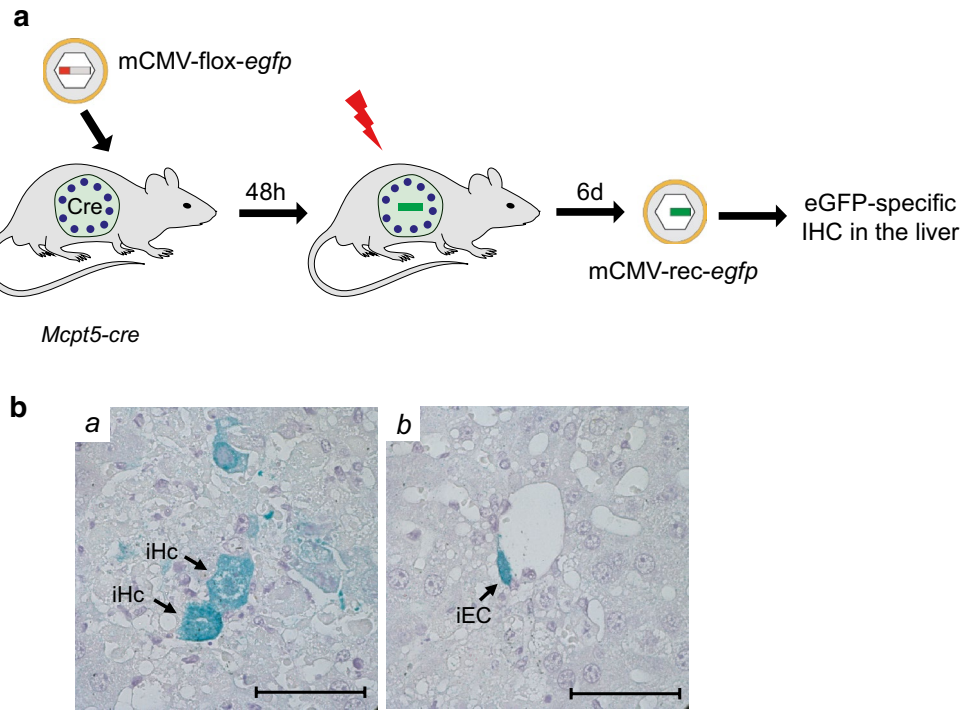

Fig. 3 Infection of MC is productive, and recombined reporter virus disseminates to other cell types. **a** Principle of the generation of MC-derived recombined reporter virus in *Mcpt5-cre* mice expressing Cre selectively in MC. After allowing 48 h for initiation of host infection and genetic recombination in MC, mice were immunocompromised by 7 Gy of γ-irradiation to facilitate virus dissemination for another 6 days. **b** Immunohistological (IHC) images of liver tissue sections taken on day 8 after the initial infection. To identify cells infected by originally MC-derived recombined virus mCMV-rec-*egfp*, the reporter protein eGFP was stained in turquoise color (method essentially as described in Ref. [27], except that eGFP was labeled with polyclonal rabbit antibodies directed against all variants of GFP, and with peroxidase-conjugated anti-rabbit Ig as the second antibody). iHc, infected hepatocyte; iEC, infected vascular endothelial cell. *Bar markers* represent 50 µm

mCMV-rec-*egfp* for a period of 6 further days [28]. Dissemination from the peritoneal cavity to other cell types in distant organs, however, was not formally proven, because mCMV-flox-*egfp* might have infected MC resident in liver and lungs [43, 44]. Here, we provide new data as firm evidence for dissemination of MC-derived mCMV-rec-*egfp* to cell types other than MC. Immunohistochemical (IHC) images (Fig. 3b) demonstrate infected $eGFP^+$ hepatocytes (Fig. 3b, image *a*) and an infected $eGFP^+$ vascular endothelial cell (Fig. 3b, image *b*) in liver tissue sections taken on day 8 after intraperitoneal infection of *Mcpt5-cre* mice with mCMV-flox-*egfp*.

Synopsis and outlook

The current view on how MC are involved in the immune control of CMV infection in the mouse model is sketched in Fig. 4. MC are cellular targets of mCMV infection and degranulate in the course of the productive viral replication cycle. The chemokine CCL5 is released upon degranulation of MC and binds to CCR5 upregulated on CD8 T cells. The CCL5 chemokine gradient attracts the CD8 T cells in an initially antigen-independent manner to infected tissues, where viral epitope-specific CD8 T cells form nodular inflammatory foci (NIF) around infected tissue cells [45] of various cell types, depending on the type of tissue. In the NIF, CD8 T cells control the infection by their effector functions exerted upon recognition of cognate peptide–MHC class I complexes (pMHC) presented at the cell surface of infected cells. An immediate question may be why infection of MC in the net effect leads to reduction in viral burden although, as shown by the conditional reporter virus studies, MC are a source of infectious virus. The answer is that many different cell types are permissive for productive mCMV infection, which applies also to clinical isolates of human CMV [46, 47], and account for most of the virus production in infected tissues with little contribution coming from infected MC.

Regarding a mechanistic understanding, future work will focus on the question if MC degranulation relates to cytopathogenic infection of MC associated with cell death

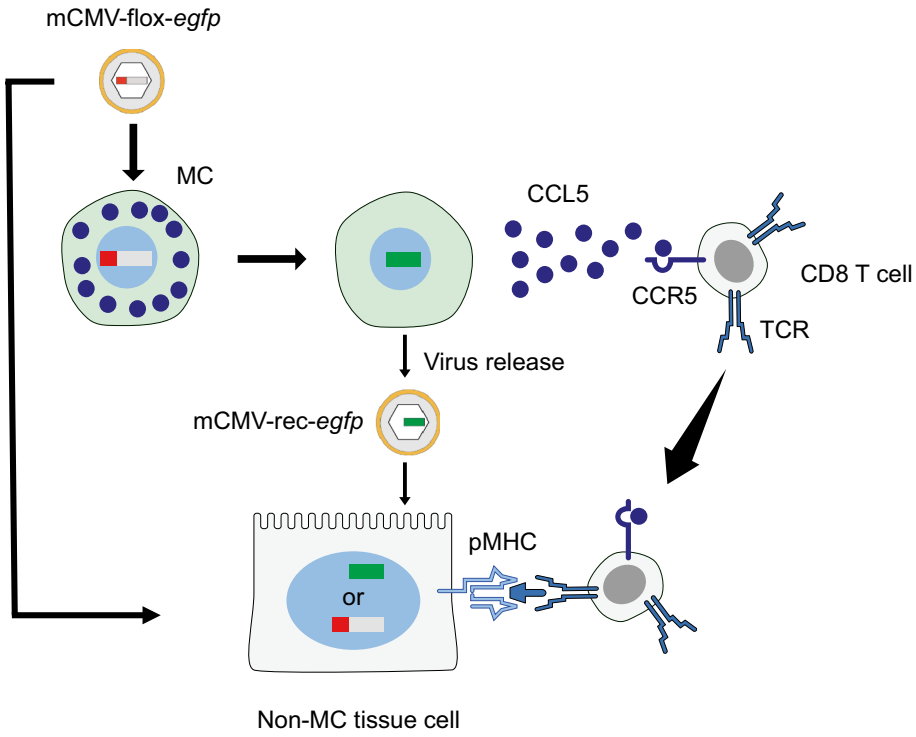

Fig. 4 Synopsis explaining how infection of MC enhances the antiviral CD8 T cell response against CMV

or if degranulation of MC can more specifically be attributed to a particular viral gene product. Regarding clinical relevance to be studied in a preclinical model, we are fascinated by the emerging evidence that MC functions converge in moderating GvHD in an allo-HCT mouse model [14] and improve the control of CMV infection (this report and [27, 28]), the two major complications in clinical allo-HCT. We will therefore extend our previous studies in the immunocompetent host mouse models to investigate the role of MC in the control of CMV infection in experimental HCT [16] using MC-sufficient and MC-deficient mice mutually as hematopoietic cell donors and recipients to define the roles of recipient-resident MC and transplanted donor MC progenitors in the control of CMV infection following HCT.

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