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Hematopoietic stem cell deficiency resulting from cytomegalovirus infection of bone marrow stroma*

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Summary. Cytomegalovirus (CMV) recurrence from latency is a major risk factor in bone marrow transplantation (BMT). Owing to the immunodepletive treatment, ablation of the immune control of latent CMV is responsible for recurrence and cytopathogenic spread of the virus in vital tissues. There is increasing evidence for reconstituting bone marrow being itself a target tissue of CMV. By inhibiting post-transplantation hematopoiesis, CMV is causal for maintenance of the immunocompromised state, which leads to a prolonged phase of persistent virus replication. Based on results in a murine model of BMT and concurrent CMV infection, we discuss possible mechanisms of CMV-mediated bone marrow graft failure. It is concluded that an irremediable damage of bone marrow stroma by CMV is responsible for a reduced rate of regeneration of the marrow-repopulating, pluripotent stem cell.

Key words: Cytomegalovirus – Bone marrow transplantation – Bone marrow stroma – Hematopoietic stem cells – Steel syndrome

Introduction

Primary or recurrent infection with human CMV, the human herpesvirus type 5 (HHV 5), is a frequent and often fatal complication in bone marrow transplant recipients. Interstitial pneumonia is the most prominent manifestation of post-BMT CMV disease [3, 4, 7]. There are clinical observations suggesting that CMV is more than just an opportunistic pathogen profiting from iatrogenic immunodepletion. Rather, CMV appears to contribute actively to the maintenance of the immunocompromised state by inhibiting engraftment [9]. Successful hematopoietic reconstitution requires that long-term repopulating, pluripotent stem cells (S_P) lodge in recipient stroma, self-renew, and differentiate to more committed daughter stem cells from which hematopoietic lineages originate that eventually give rise to all types of mature blood cells [5, 10]. CMV could interfere with reconstitution at any level of the differentiation hierarchy: at the level of the stem cells, the progenitor cells, and the mature cells. There is no cogent reason to assume that CMV targets only one of these levels, but it is clear that disturbance of stem cell self-renewal would have the most profound consequences. Here we present an overview of our recent data showing that CMV infection reduces the rate of stem cell self-renewal, and we discuss possible mechanisms.

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Inhibition of hematopoiesis in long-term bone marrow cultures

With human CMV, experimental approaches are limited to the study of hematopoiesis in long-term bone marrow cultures. The rate of hematopoiesis in culture is reflected by the generation of hematopoietic progenitor cells that can be detected in mature cell colony assays. Human [1, 13] and murine [2] in vitro hematopoiesis is blocked by infection with the respective viruses. Three possibilities were considered to explain the reduced yield of colonies: (a) infection, productive or latent, of progenitor cells; (b) prevention of progenitor cell generation by infection of the preprogenitor or stem cells; and (c) prevention of stem cell renewal by infection of the supporting inductive bone marrow stroma. For human CMV, Apperley et al. [1] documented infection of stromal cells, and Simmons et al. [13] specified this finding by discriminating CMV isolates that differ in their cell tropism: the laboratory strain AD 169 and two thirds of the tested isolates infected stromal cells, whereas one third were able to replicate in hematopoietic cells, thus suggesting different mechanisms of myelosuppression by different isolates of CMV. Murine CMV resembles human CMV AD 169 and the majority of the isolates in its stroma cell tropism [2].

While destruction of the stroma by productive infection is sufficient to explain the block in hematopoiesis, direct infection of stem cells could still be envisaged as a second reason. We were able to refute that possibility by a serial transfer approach [2]: a donor culture was infected with murine CMV, and hematopoietic cells, including preprogenitors, were transferred to a recipient culture consisting of an established inductive stroma. It was found that the rate of generation of progenitor cells in the recipient culture was independent of an infection in the donor cultures. In conclusion, deprivation of stromal support owing to lytic infection of stromal cells is certainly one mechanism by which CMV can inhibit hematopoiesis.

Stromal cells permissive for cytomegalovirus replication

Permissive stromal cells are usually referred to as bone marrow fibroblasts [1, 13]. Yet more specific studies on the cell types that constitute bone marrow stroma have revealed that fibroblasts are at best an insignificant component, while two cell types predominate: epithelioid stromal cells (ESC) that are supposed to be related to adventitial reticular cells (ARC) and account for most of the hematopoiesis-supporting activity, and macrophages [11]. We have isolated a stromal cell that shows characteristics of the described epithelioid cell and that is permissive for the productive cycle of murine CMV. This cell expresses the integrin/adhesin cell surface molecules CD4, CD11b, and CD53, which indicates its involvement in cell-cell interactions. While these markers are shared by subpopulations of the myeloid lineage, lack of MHC class-II expression and recipient origin in bone marrow chimeras discriminate this stromal cell from macrophages (manuscript in preparation).

Selective inhibition of pluripotent hematopoietic stem cell regeneration by cytomegalovirus infection in vivo

The in vitro studies have demonstrated that cells of the bone marrow stroma can be permissive for CMV replication, and that an infection of these cells can ablate hematopoiesis. Yet these results cannot be extrapolated to the in vivo reconstitution after BMT without verification, because it is not known whether CMV can enter the hematopoietic compartment by penetrating the marrow sinus wall composed of a luminal layer of endothelial cells and an abluminal coat of adventitial reticular cells. Further, permissivity of a cell for CMV is not only defined by intrinsic, cell-type-specific parameters, but is also dependent upon the stage in the cell cycle and extrinsic cytokine signals that regulate the expression of cell surface receptors and the outfit with cellular transcription factors, all of which may be altered in an unpredictable way when cells are taken into culture.

To study the interference of CMV with hematopoietic reconstitution in vivo we established an animal model, the concurrent infection of the BALB/c mouse with murine CMV after experimental BMT [12]. The course of infection in immunocompetent and immunocompromised BALB/c mice and the role of specific immunity mediated by CD8 T lymphocytes were the theme of a recent review [6]. In essence, immunocompetent mice control the infection and survive, while mice immunocompromised by a sublethal dose of γ-irradiation and not rescued by BMT die of generalized CMV disease characterized by extensive virus replication and tissue lesions in practically all vital organs. We have documented previously that an important, it not the decisive, event responsible for mortality is aplasia of bone marrow resulting from an interruption of endogenous reconstitution that becomes effective as soon as virus replication is detectable in permissive tissues. Notably, without exogenous supply of bone marrow cells, the pool of myeloid intermediate stem cells, the day-14 spleen-colony-forming cells (CFU-S14), was found to be depleted, indicating an early intervention of CMV with reconstitution [8].

Syngeneic BMT modulates the course of concurrent CMV disease by exogenous supply of bone marrow cells in all stages of differentiation that help the animals to withstand an early hematopoietic crisis. Thus, dependent upon the degree of marrow depletion by irradiation, and provided that a sufficient number of stem cells are transplanted, mice survive CMV disease and appear to recover. At this stage, an experiment was performed that shows the value of an experimental model: bone marrow cells retrieved from survivors at 1 month after BMT and concurrent subcritical CMV disease were serially transplanted into irradiated but uninfected *indicator* recipients to assay their capacity to reconstitute, i.e., to measure the content of stem cells in the post-transplantation marrow. For *indicators,* we chose a mutant of BALB/c, strain BALB/c-H-2^{dm2}, in which the gene coding for the MHC class-I cell surface molecule L^d is deleted. Expression of L^d thus served as a *reporter* to track the donor-type hematopoiesis. This approach revealed that the pool of CFU-S is not diminished by CMV, whereas marrow-repopulating ability, reflecting the pool of pluripotent stem cells S_P, is significantly reduced (M. J. Reddehase, manuscript in preparation). The result confirms the distinction between S_P and CFU-S [5, 10] and provides the first example of a viral disease that affects stem cell subpopulations differentially.

CMV-mediated steel syndrome: a provocative working hypothesis

A failure in stem cell renewal can have basically two alternative reasons: (a) the S_P itself can be a target for CMV gene expression and can be destroyed or made anergic, or (b) it could be deprived of essential hemopoietins owing to infection of stromal cells in the microenvironment. In molecular terms, either S_P fail to respond to hemopoietins or stromal cells fail to express hemopoietins. A series of recent reports (reviewed in [14]) has disclosed the ligand-receptor interaction between a stem cell factor (SCF) encoded in the Sl (steel) locus and expressed by stromal cells, and the c-kit transmembranal tyrosine kinase encoded in the W locus and expressed by stem cells. Phenotypically, the defect observed in post-BMT CMV

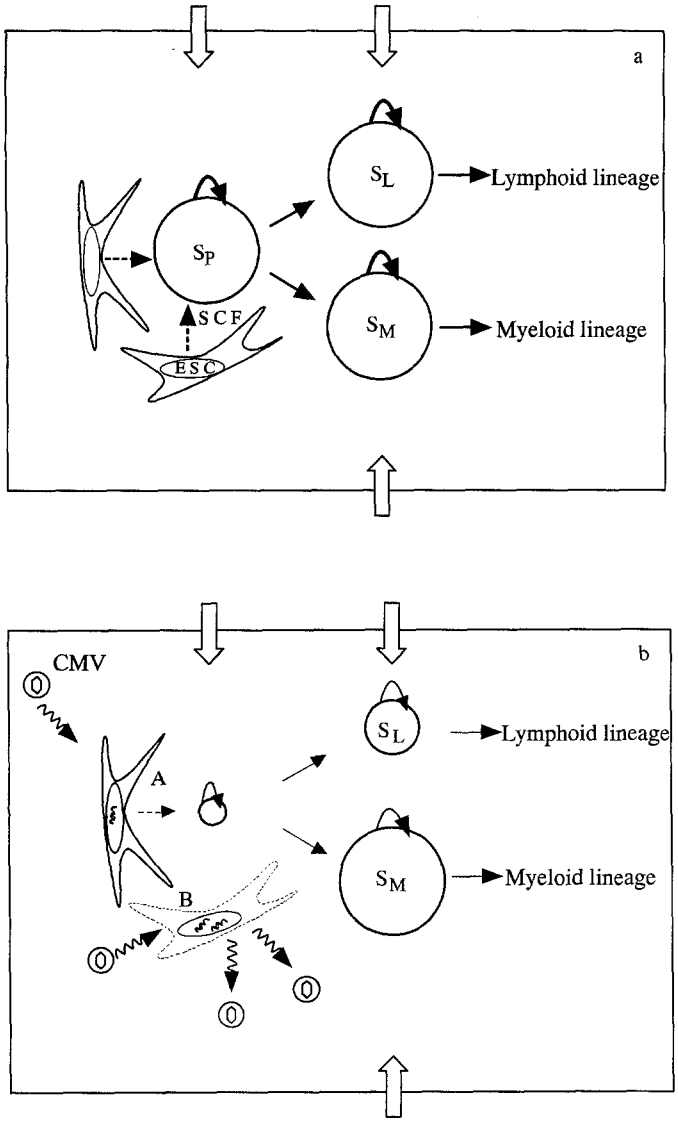

Fig. 1. a BMT in absence of CMV. The bone marrow compartment (*box*) is repopulated by stem cells (S_P, pluripotent stem cell; S_L, lymphoid stem cell; S_M, myeloid stem cell) supplied by BMT (*open arrows*). Intact epithelioidal stromal cells (*ESC*) provide hemopoietins, such as stem cell factor (*SCF*). In response, S_P renew and differentiate to replenish the pools of the daughter stem cells, S_L and S_M, over the long term. **b** BMT and concurrent CMV disease. The renewal rate of S_P, and thus its pool size, is reduced by a relative deprivation of stromal support, which is apparently only partial in survivors, but can explain mortality when it is absolute. A partial functional failure of stroma may be caused (*A*) by nonreplicative infection of ESC with limited viral gene expression reducing the expression of hemopoietins or (*B*) by lytic, productive infection of some, but not all ESC. The pools of S_L and S_M are replenished, at least for some time, by intrinsic renewal, which then must be proposed to be less stroma dependent than S_P renewal. There ist preliminary evidence that the S_M pool is more independent of the S_P than is the S_L

disease resembles the defect in the mouse mutant Sl/Sl^d, in which CFU-S are about normal, and is distinct from the defect in the mouse mutant W/W^v, in which CFU-S are deficient.

Very recent results from our lab (M. J. Reddehase, manuscript in preparation) show that the defect cannot be cured by a second *in-row* BMT with healthy stem cells. This finding points to an irremediable damage of stroma.

Our current knowledge and view are summarized in the Fig. 1.

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