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Review

αβ and γδ T-cell responses to Epstein-Barr Virus: insights in immunocompetence, immune failure and therapeutic augmentation in transplant patients

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Epstein-Barr Virus (EBV) is a human gamma herpes virus, which causes several diseases in immunocompetent (mononucleosis, chronic fatigue syndrome, gastric cancer, endemic Burkitt's lymphoma, head and neck cancer) and immunosuppressed (post-transplant lymphoproliferative disease, EBV-associated soft tissue tumors) patients. It elicits a complex humoral and cellular immune response with both innate and adaptive immune components. Substantial progress has been made in understanding the interplay of immune cells in EBV-associated diseases in recent years, and several therapeutic approaches have been developed to augment cellular immunity toward EBV for control of EBV-associated malignancy. This review will focus on recent developments in immunosuppressed transplant recipients.

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Introduction

Patients with end-stage organ failure benefit from recent advances in solid organ transplantation (SOT). Immunosuppressive medications are necessary to preserve long-term graft function and avoid rejection. On the downside, this predisposes patients to viral infections, reactivations, and virus-associated malignant complications. This review will focus on Epstein-Barr virus (EBV) as a risk for malignant transformation in immunosuppressed patients, as a target of the cellular immune response and new immunotherapeutic treatment options.

Epstein-Barr virus as risk factor for lymphoproliferation in solid organ transplantation

EBV is a double-stranded virus of the Herpes family with a prevalence of up to 90% worldwide [1]. Its life cycle is defined by primary infection, latency, and a potential lytic reactivation phase. Primary infections appear as asymptomatic or upper respiratory tract infections in children, but may cause infectious mononucleosis in adults. Later on, the virus established latency in memory B cells representing the primary viral reservoir, controlled by the host's immune system. EBV expresses several gene products that act as B cell survival factors (LMP-2), antiapoptotic factors (BHRF-1, LMLP-1) and immunomodulatory factors (BARF-1, BCRF-1; reviewed in [2]), finally enabling uncontrolled lymphoproliferation and malignant transformation in the context of absent or low immunity, which is termed post-transplant lymphoproliferative disease (PTLD).

Pharmacological immunosuppression to preserve organ function is the main pathophysiological reason for PTLD after SOT. However, depending on recipient and donor EBV serostatus the risk is highest in EBV seronegative recipients receiving grafts from seropositive donors [3]. In addition, community-acquired EBV infection after SOT in the status of immune suppression represents a relevant risk factor. Most of the European guidelines suggest to routinely monitor EBV-DNA load to equally guide preemptive therapy and diagnostic work up [4,5]. The serial quantification of EBV-DNA load in plasma, serum or peripheral cells of hematopoetic stem cell transplantation (HSCT) and SOT patients by polymerase chain reaction (PCR)-based assays has therefore become a standard procedure in post-transplant care [6–8]. Some patients experience an (asymptomatic) chronic high viral load

status, which further predisposes to PTLD development, at least in thoracic organ transplantation [9,10]. The concept of EBV dynamics with peak load followed by establishment of equilibrium or setpoint viral load was recently introduced, which may take up to two years until stable equilibrium is reached in immunosuppressed patients [11].

Immune control of Epstein-Barr virus infections

Early viral control is mediated by expansion of various immune cells, such as natural killer (NK) cells, γδ T cell and αβ T cells. Over time and with viral persistence, these responses decrease in magnitude but still remain detectable lifelong [12].

Conventional αβ T cells respond to viral proteins of the lytic (e.g. BZLF-1, BRLF-1, BMLF-1) and latent (e.g. LMP1, LMP2, EBNA 1–4) infection stage [13]. Their T-cell receptors (TCRs), composed of an α- and β-chain, allows the specific recognition of respective viral epitopes, presented by human leucocyte antigen (HLA) molecules. During acute infection, the large majority of EBV-specific T cells are highly activated CD8 T cells (e.g. HLA-DR, CD39, and CD69 positive) that are susceptible for apoptosis [14]. Notably, these T-cell responses are much lower in magnitude in asymptomatic infections [15], but independent of symptom severity, EBV-specific T cells remain detectable lifelong. In healthy EBV carriers, EBV-specific T cells are easily detectable as resting antigen-experience T cells [16–18] that exhibit cytotoxicity and cytokine secretion upon antigen stimulation. Moreover, tissue-resident memory T cells can locally control viral infections (reviewed in [19]). Especially, in long-term EBV carriers virus-specific T cells (VSTs) are enriched in tonsils, characterized by similar TCR repertoires in matched blood samples, which further suggests that no selective recruitment occurred [20,21].

There is accumulating evidence that a second subset of T cells, expressing a TCR formed from a heterodimeric γ-and δ-chain, controls EBV infections. Based on the expressed γδ TCR, these T cells are divided into two main groups: the Vγ9Vδ2 T cells and Vδ1 T cells in humans. The majority of Vγ9Vδ2 T cells develop as preactivated effector cells in the prenatal period to persist into adulthood [22–24]. Their TCR senses small molecular compounds, called phosphoantigens, allowing this γδ T-cell subset to respond to microbial changes, infections and tumor cells in an innate-like fashion (reviewed in [25]). Along that line, aminobisphosphonate expanded Vγ9Vδ2 T cells have the capability to recognize and kill EBV-transformed B cells through TCR and natural killer group 2 member D (NKG2D) receptor triggering [26]. However, this response is donor dependent, presumably due to genetic variability, and might further explain individual γδ T cell- or NK cell-based immune responses to control EBV [27,28].

The Vδ1+ T cells develop as naïve cells in the postnatal thymus [29,30]. Due to their capability to undergo clonal expansion upon reactivation of another virus of the herpes virus family, the cytomegalovirus (CMV), they have been assigned as adaptive-like γδ T cells [31,32]. A handful of studies describe their response capabilities toward EBV. Activated Vδ1 T cells, being HLA-DR and CD38 positive, were increased in the acute and convalescent phase of infectious mononucleosis [33]. Similarly, activated and functional Vδ1 T cells controlled EBV infection after cord blood transplantation, when αβ T cell were not fully active [34]. Thereby, Vδ1 T cells seem to sense ligands of cellular origin on EBV-infected Burkitt lymphoma cells or EBV-transformed lymphoblastoid cell lines, following expansion, activation and cytotoxicity [34,35]. These observations suggest that, similar to anti-CMV or antitumor Vδ1 T cell responses [36–38], inflammation-induced and/or stress-induced ligands are recognized by the $V\delta1^+$ TCR upon EBV reactivation. While the modes of antigen recognition of CMV-induced Vδ1 T cells are discussed in detail by *Prinz and Koenecke* within this issue, it further implies a similar biology of Vδ1 T cells in CMV and EBV immunity. Moreover, and albeit the magnitude of EBV-inducedVδ1 T-cell responses might be individual as not always a repertoire skewing is observed [39], studies from *P. falciparum*-infected [40,41] and HIV infected-individuals [42] give evidence for similarities in the adaptive-like biology (e.g. clonal expansion) and phenotypic responses (e.g. cytotoxicity, CD16 expression and expression of natural killer cell receptors) of Vδ1 T cells in various infectious diseases.

Currently, the majority of cellular anti-EBV therapies in immunosuppressed patients are based on αβ T cells (as discussed below). The fact that γδ T cells can infiltrate/are enriched at tissue sites and function through TCR signaling/costimulation and signaling via NKG2D and natural cytotoxicity receptors broadens and enhances their antiviral and anti-tumor response capabilities, and finally their therapeutic potential. For instance, aminobisphosphonate pamidronate induced expansion of Vγ9Vδ2 T cells with increased expression of NKG2D, FAS ligand (FASL) and perforin/granzyme that associated with increased cytolytic activity against EBV-transformed lymphoblastoid cell lines (EBV-LCLs), migration to tumor sites and prevention of EBV-associated lymphoproliferative diseases [26]. Therefore, there is a high chance that recent advances in γδ T cell-based approaches (Vδ1 and Vγ9Vδ2 based) for cancer therapy can be translated into prevention and treatment options of EBV-associated complications after SOT (reviewed in [43–45]). These

include the adoptive transfer of expanded Vδ1 [46,47] or Vγ9Vδ2 T cells [48], engineered T cells expressing an EBV-responsive γδ TCR [49,50] or the employment of antibody-based constructs that selectively activate and expand γδ T cells [51].

αβ T cell-based antiviral therapies to treat Epstein-Barr virus-associated complications

Reconstitution of the αβ T cell repertoire against EBV under immunosuppression is a lengthy and unfortunately often incomplete process predisposing patients to development of EBV-associated PTLD, representing both clinically and histopathologically heterogeneous lymphoproliferations that may resemble lymphoma histologies in immunocompetent individuals [52–55]. Antiviral therapy alone is insufficient to treat EBV-related lymphoproliferation; strategies focus on restoration of immunocompetence by reduction of immunosuppression, cytoreduction by anti-CD20 antibodies or chemotherapy and, more recently, adoptive immunotherapy using EBV-specific αβ T cells from EBV-seropositive HLA-(partially) matched donors.

In addition to the evaluation of the EBV-DNA load, the accurate monitoring of the EBV-specific T-cell immunity (i.e. absolute lymphocyte count, frequency and functionality of EBV-specific T cells) has revealed beneficial for evaluating the patient's capacity to endogenously clear EBV infections and reactivations. This monitoring enables the identification of patients with a deficient cell-mediated immunity and immune surveillance, thus being at high risk of developing EBV-associated PTLD (reviewed in [56]). It moreover allows an efficacious treatment of overt or emerging EBV infections and -reactivations in immunocompromised transplant patients as well as the assessment of the individual patient's benefit of treatment [57–65]. Several monitoring techniques have been applied to evaluate T-cell immunity, such as interferon-gamma (IFN-γ) EliSpot assay, intracellular staining and peptide-major histocompatibility complex (MHC) multimers. They have been reviewed with regard to their potential to manage the immunosuppressive regimen and to direct the administration of adoptive immunotherapy, with special regard to its time point. Recently, reference values for EBV-specific T cells in a large cohort of healthy donors have been established providing guidance for functional EBV-specific T-cell monitoring [66]. Correspondingly, immunomonitoring of T cells specific to targets of interests, such as EBV, is aimed to be translated into routine clinical screening practice [62,63,65]. Using the methods mentioned above and considering newer technologies for determining cell type-specific markers to determine immunodominant TCRs (by single-cell sequencing) metabolic fitness and epigenetic profiles, patients can be early identified who are in need for adjustment of T-cell functionality.

Restoring the EBV-specific cellular immunity of patients represents an effective approach to both prevent and successfully treat these EBV-associated manifestations. In patients without sufficient EBV-specific immunity adoptive transfer of EBV-specific T cells has evolved over the last decades. The aim is to reconstitute and reinforce the impaired EBV-specific cellular immunity by the transfer of *ex vivo* manufactured functional ab EBV-specific T cells. In general, clinical-grade and functional EBV-specific T cells are meant to recognize viral antigens, to elicit or reconstitute a functional EBV-specific immunity as well as to persist via long-lasting T-cell engraftment [67–70]. However, the high antigenic diversity, which is characteristic of EBV, turned out to be a limiting factor of this cell-based immunotherapy. The latter is due to the different gene and protein expression profiles of the respective latency stages, different HLA restrictions as well as a pronounced variability within patient and donor cohorts. Clinical studies have unveiled that these T cells expand subsequent to their adoptive transfer and effectively lyse and eliminate EBV-infected cells. Clinical studies and case series did not reveal relevant side effects related to the adoptively transferred EBV-specific T cells, either at the systemic level or by affecting the organ graft, which is especially important to patients with relevant comorbidity [67–69,71,72].

The investigational focus has shifted towards the transfer of cytotoxic T lymphocytes (CTLs) directed against specific virus-associated antigens to further minimize the risk of graft versus host disease (GvHD) development. Among others, this shift is based on the above-stated findings by Haque et al., Doubrovina et al. and Icheva et al. [67,69,73]. These antigen-specific CTLs rapidly reconstituted the antiviral immunity with only limited increase in GvHD risk and no significant systemic or organ toxicity in immunosuppressed patients [67–69,71,72]. Furthermore, generated CTLs were found to persist long-term, as revealed by the detection of gene-marked cells several years after adoptive transfer [74]. Patients' need for EBV-specific T cells is usually urgent, thus, attempts have been made to rapidly generate CTL from partially HLA-matched, EBV-seropositive third-party donors [75,76]. In order to enable the generation of easily accessible donor-derived and highly frequent VSTs) allogeneic T-cell banks [52,77,78] and donor registries have been established, such as the allogeneic T-cell donor (*alloCELL)* registry at Hannover Medical School [79]. They are meant to facilitate the adoptive immunotherapy with T cells from third-party donors by offering a means for a more rapid identification of available best-suited T-cell donors. As for the *alloCELL* registry, this identification is done in

accordance with virus serology, virus-specific cell-mediated immune response and HLA type [59,63,75,76].

With respect to the efficacy of preemptive therapeutic approaches, a case-control study by Rooney et al. demonstrated that none of the patients, who had received preemptively transferred EBV-specific αβ CTLs, developed PTLD as compared to 11.5% within the control group. In addition, a significant reduction of EBV-DNA load was observed in the preemptively treated patients [80]. They have been successfully employed in both prophylaxis and treatment of EBV infections, -reactivations and EBV-associated PTLD, notably in patients at high risk or after failed first-line treatment [67–70,72,74,80].

Accordingly, Icheva et al. successfully developed a protocol applying an EBV nuclear antigen 1 (EBNA1)-specific peptide pool for the rapid isolation of polyclonal EBNA1-specific T cells by the use of an IFN-γ-based capture system [73]. This approach revealed to be feasible and well tolerated by the patients, resulting in a fast reconstitution of their impaired EBV-specific immunity with clinical and virological responses in 70% of them. In contrast to Icheva et al., Moosmann et al. isolated donor-derived EBV-specific T cells stimulated overnight with a peptide pool of 23 peptides derived from eleven different derivational EBV proteins. Following transfusion, three of their patients, who were of early stage, achieved complete and stable remission [72].

Recently, Gottlieb et al reported all 3 patients treated for EBV-associated PTLD by third-party EBV-specific T cells achieved sustained complete remission. Infusion of αβ EBV-CTLs generated after short peptide stimulation and based on expression of CD137 was associated with rapid recovery of CD8 +CD45RA-CD62L- and a slower recovery [81]. Expansion of VSTs in the blood is frequently seen in patients after adoptive T-cell transfer [71,73,82]. It has been questioned whether partially HLA-matched EBV-specific T cells will persist in the recipient's circulation or be rejected by the immune system. Using TCR αβ sequencing, we approached this topic in a pediatric liver transplant patients receiving partially matched EBV-specific CTLs [71]. Comparison of clones retrieved from the T cell product and from the patient's blood after transfer indicated that there were donor-derived clones, which expanded and were detectable at 180 days post transfer, as well as newly arising clones from endogenous patient production. Other groups similarly found evidence that partially matched VSTs persist and are detectable for up to 120 days after transfer [81,83]. Certainly, these initial results are intriguing to prompt studies on the fate of transferred cells, the endogenous EBV-specific T cell priming and expansion and the potential help by other (innate) immune components in patients after adoptive T-cell transfer.

Conclusion

Adoptive immunotherapy with EBV-specific αβ T cells has been investigated in several studies and among others, these studies have focused on the obviation of alloreactive T lymphocytes inherent in the adoptively transferred cell product and on strategies to rapidly generate VSTs. By providing the required T-cell subsets and immunophenotypes in sufficient cell numbers as functional substitutes, the adoptive transfer of donor-derived EBV-specific T cells has proven to offer a personalized, targeted and nontoxic immunotherapeutic strategy. In this way, both the immediate and long-term EBV-specific immunity can effectively be restored in immunocompromised patients [72,79,84]. Despite success in clinical studies, critical variables still have to be clarified. One of these unknown variables is the optimal dose of adoptively transferred T cells, which (1) results in a successful in vivo expansion as well as in a clinically and virologically effective immune control in patients and (2) obviates the risk of inducing GvHD. With respect to the targeted virus, the mode of manufacturing and the resulting different antigens being used, the number of adoptively transferred T cells considerably varied in recent studies, for example, due to the diverse individual therapeutic requirements and the difference between haploidentical and HLA-matched T-cell administration. In studies using *ex vivo* expanded CTL numbers usually range from 1 to 2×10^6 CD3+ cells/kg [67,69,85]. Cell numbers are usually much lower if directly isolated EBV VSTs are used [71,73]. Icheva et al. demonstrated that already 150 EBNA1-specific $CD3^+$ T cells per kg manufactured by direct isolation proved sufficient to induce T-cell expansion in vivo [73]. This kind of expansion was discerned in 80% of the patients with EBV viremia and/or PTLD. Concerning the number of cells meant for infusion, a starting population of on average 5.79 $CD3^+$ T cells per kg revealed effective despite the known low in vivo immunogenicity of EBNA1 [73]. The aforementioned study disclosed that the therapeutic success of the adoptively transferred T cells and their effectiveness in vivo are not exclusively linked to a high T-cell dose. This highlights that adoptive immunotherapy with VSTs does not follow a linear dose–effect relation [86]. In consequence, the potential of the transferred T cells to expand in vivo might be more essential to an effective immune response than the applied T-cell dose and might therefore lead to a better protective reconstitution of immunity. Furthermore, a low dose administration of EBV-specific T cells was said to be beneficial to the obviation of GvHD because it reduces the number of contaminating unrestricted,

potentially alloreactive $CD3^+$ T cells remaining in the adoptively transferred cell product despite strict purification [86]. Furthermore, modifications in the cell product composition, such as addition of innate immune cells like γδ T cells, or genetic modifications by TCR transfer or chimeric antigen receptor (CAR)-transduction, may help even more to advance therapeutic efficacy of EBV-specific therapeutic cell products.

Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

No data was used for the research described in the article.

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