Published in final edited form as:

Immunotherapy. 2013 April ; 5(4): 353–355. doi:10.2217/imt.13.22.

Targeting EBV's Achilles' heel with antigen-specific T cells

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Abstract

Adoptive transfer of donor-derived EBV-specific T cells is an effective strategy for the prevention and treatment of EBV-associated post-transplant lymphoproliferative disease (PTLD). However, the time-consuming process of EBV-specific T-cell generation using standard protocols has limited their broader use. *Ex vivo* IFN- γ capture assay is an attractive alternative for the rapid isolation of EBV-specific T cells. In the present study, Icheva *et al.* employ this method to rapidly isolate clinical-grade T cells that are specific for EBNA1. Adoptive transfer of EBNA1-specific T cells was safe and resulted in clinical benefit in 7 out of 10 patients with EBV-viremia and/or PTLD. Thus, T-cell therapy targeting a single EBV antigen, EBNA1, which is critical for EBV episome maintenance, may be sufficient for EBV-PTLD therapy in hematopoietic stem cells transplantation recipients.

Keywords

EBNA1; EBV; Epstein–Barr virus; post-transplant lymphoproliferative disease; stem cell transplantation; T-cell immunotherapy

EBV belongs to the family of human γ -herpes viruses with the unique ability to latently persist in B cells and reactivate in immunocompromised patients, resulting in post-transplant lympho-proliferative disease (PTLD). EBV-PTLD remains a life-threatening complication after hematopoietic stem cell transplantation (HSCT) and solid organ transplantation with an incidence of 1–25% depending on the type of graft and degree of immunosuppression. EBV-PTLD represents type 3 EBV latency in which infected B cells express all nine latency-associated proteins, including EBNA1, 3A, 3B and 3C, which are very immunogenic [1,2]. However, in the absence of adequate EBV-specific T-cell immunity, EBV-infected B cells undergo uncontrolled proliferation, which can develop into lethal EBV-PTLD.

Standard systemic therapy for EBV-PTLD includes reduction of immunosuppression, CD20 monoclonal antibody (Rituxan[®], Genentech, CA, USA) and chemotherapy $\pm$ Rituxan [3,4]. Adoptive transfer of EBV specific T cells is an attractive alternative strategy, especially for

competing interests disclosure

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

EBV-PTLD post-HSCT. In HSCT recipients, infused EBV-specific T cells reconstitute EBV-specific T-cell immunity and are effective in the prevention as well as treatment of EBV-PTLD [5]. However, the current standard method to generate EBV-specific T cells takes 12–16 weeks and relies on live virus. Several strategies have been pursued to overcome this problem. Using banked, closely HLA matched EBV specific cells overcomes the T 'production time hurdle', but does not eliminate the use of live virus [6]. Several groups have developed peptide- or plasmid-based methods to rapidly isolate/generate virus specific T cells [7,8], and Icheva *et al.* demonstrate in their study that the infusion of EBNA1 specific T cells, rapidly isolated by IFN- γ capture, is feasible, safe and results in clinical benefit[9].

Summary of methods & results

In the present study, Icheva *et al.* describe a fast and simple procedure to generate EBNA1 specific T cells for patients with EBV-viremia and/or EBV-PTLD from their healthy EBV-seropositive stem cell donors [9]. EBNA1 is a latency-associated EBV antigen universally expressed in EBV-PTLD. It plays a crucial role in viral replication and persistence [10]. Both CD4- and CD8-restricted epitopes have been described for EBNA1 in healthy donors [11]. In this study, the authors used their previously published protocol for producing adenovirus and CMV specific T cells to generate EBNA1 specific T cells [12,13]. To determine which EBV antigen to target, the authors determined the precursor frequency of T cells specific for one early EBV antigen (p54) and two latent EBV antigens (EBNA1 and LMP2) in the peripheral blood of healthy donors. Peripheral blood mononuclear cells were stimulated with overlapping peptide libraries containing 15 amino acid peptides covering the entire length of the corresponding protein with an 11 amino acid overlap. Based on intracellular IFN- γ staining, LMP2 specific T cells showed the highest precursor frequency followed by EBNA1 and then p54. Since LMP2 pepmixes contain many hydrophobic peptides, which are difficult to purify by high performance liquid chromatography, the authors focused on generating EBNA1 specific T cells, since a good manufacturing practice grade pepmix is readily available.

EBNA1-specific T-cell isolation was carried out from donor peripheral blood mononuclear cells in a good manufacturing practice-compliant manner. Briefly, EBNA1-specific T cells were stimulated with EBNA1 protein (seven donors) or an overlapping peptide library (three donors) overnight and isolated by IFN- γ capture assay using the CliniMACS® (Miltenyi Biotec, Bergisch Gladbach, Germany) device. The isolated EBNA1-specific cells were 57% pure [$\pm 22\%$] and contained more CD4⁺/IFN- γ ⁺ than the CD8⁺/IFN- γ ⁺ T-cell subset. The freshly isolated cells were infused the same day without any further *in vitro* expansion. Since the majority of EBNA1-specific T cells were CD4⁺, three MHC class II-restricted EBNA1-derived peptides were used to determine their specificity. A total of 80% of EBNA1-specific CD4⁺ T cells elicited responses to at least one of the three peptides. A significant proportion of T cells produced more than one cytokine suggestive of polyfunctionality.

EBNA1-specific T cells were infused into ten patients with EBV-viremia and/or EBV-PTLD. Three patients received a second dose since they did not respond to the first. The adoptive transfer was safe as no dose-limiting toxicity was observed. One patient developed grade 1–2 acute skin graft versus host disease that completely resolved with treatment. The authors determined the frequency of EBNA1-specific T cells postinfusion in peripheral blood using intracellular IFN- γ staining. *In vivo* T-cell expansion correlated strongly with clinical outcome. Seven of eight patients with T-cell expansion had a decrease in viral load and/or disease control in contrast to two patients with no T-cell expansion. Of note, even a very low T-cell dose (150 cells/kg) resulted in efficient expansion *in vivo*.

However, the infused cells were predominantly CD4⁺, the expansion in CD8⁺ compartment was observed for all positive responders whereas expansion of CD4⁺ population was seen in six of eight responders. At the end of the follow-up period, three of seven clinical responders were disease free, three succumbed to complications due to other infections and one died because of EBV-PTLD recurrence.

Significance & discussion

The study demonstrates that the infusion of donor derived, rapidly selected EBNA1-specific T cells is safe and results in antiviral and antitumor effects. Thus, it presents an important milestone in the field of T-cell therapy for EBV-PTLD, which started in the 1990s with the infusion of donor derived or EBV-specific T cells [14,15]. While past products contained T cells specific for multiple EBV antigens, this study suggests that targeting one EBNA1 alone might be sufficient. Due to its critical role in EBV's life cycle, EBNA1 is EBV's 'Achilles' heel' or 'Linden leaf spot' and thus presents an ideal target not only for T-cell therapies but also for other targeted therapies [10]. Besides directly targeting EBNA1⁺ cells, EBNA1-specific CD4⁺ T cells most likely also provided cognate help to CD8⁺ EBNA1-specific cells and T cells specific for other EBV antigens as shown in the article for LMP2 [9]. Thus, the study highlights the importance of EBNA1-specific T-cell responses to control EBV, supporting epidemiological evidence that patients with decreased EBNA1-specific T-cell responses are at higher risk for EBV-associated malignancies [16].

The authors confirm previous studies that only a small cell dose needs to be given to achieve *in vivo* T-cell expansion post-HSCT [5,13]. Unfortunately, outside the transplant setting, significant *in vivo* expansion of adoptively transferred T cells has in general only been observed postaggressive lymphodepletion using chemotherapy and/or radiation [17]. The study should be an impetus to explore other strategies including the use of monoclonal antibodies and/or vaccines to boost adoptively transferred T cells.

Future perspective

The study provides the rationale to explore the use of rapidly selected EBNA1-specific T-cells for EBV-associated malignancies outside the transplant setting. T-cell therapies with EBV-, LMP1/LMP2- or EBNA1/LMP1/LMP2-specific T cells are actively being explored for EBV⁺ non Hodgkin's lymphomas and nasopharyngeal carcinoma; however, all these studies rely on protocols that use *ex vivo* culture [18–20]. Extending the developed protocol to include multiple EBV antigens for the T-cell therapy of EBV⁺ malignancies or antigens for other viruses that are associated with peritransplant morbidity/mortality would be an obvious next step.

Acknowledgments

Financial

The authors were supported by grants from the NIH (P01 CA094237) and Cancer Prevention Research Institute of Texas (RP110172).

No writing assistance was utilized in the production of this manuscript.

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- of interest

■ ■ of considerable interest

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Executive summary

- EBV post-transplant lymphoproliferative disease (PTLD) remains a life-threatening complications in transplant recipients.
- T-cell therapy has shown promise in the prophylaxis and therapy of EBV-PTLD posthematopoietic stem cell transplantation.
- IFN- γ capture assay allows for the rapid, good manufacturing practice-compliant isolation of EBNA1-specific T cells.
- Adoptive transfer of donor-derived, EBNA1-specific T cells is feasible and safe, and infused T cells have anti-EBV-PTLD activity.