

Abstract

Interleukin-21 (IL-21) has been described as a potent stimulator of antitumor T cell immunity, but also of autoimmune reactions and oncogenesis. Antigen presenting cells genetically modified to release IL-21 allow for the expansion of tumor- specific T cells exhibiting favorable effector and growth characteristics and a minimal risk of detrimental side effects.

Keywords: DC vaccination, adoptive cell transfer, IL-21, common gamma-chain cytokine receptor family, antigen presenting cell

Cytokines belonging to the common γ chain (γ_c) family are central regulators of the development, proliferation, survival and differentiation of multiple cell lineages of the innate and adaptive immune system, and as such are of high interest for anticancer therapeutic applications. Clinically employed γ_c cytokine family members encompass interleukin (IL)-2, IL-7, IL-15 and IL-21, with IL-21 being the most recently introduced into the clinic. IL-21 is produced by a variety of activated CD4+ T cells including T_H1 and T_H17 cells, natural killer T (NKT) cells and follicular helper T cells. IL-21 plays a role in the differentiation and proliferation of B cells as well as of CD4+ and CD8+ T lymphocytes. Moreover, IL-21 exerts potent antitumor effects due to its ability to induce and expand cytotoxic CD8+ T cells, NK cells and NKT cells, as well as to its capacity to suppress FOXP3 expression and the expansion of regulatory T cells (Tregs). In line with this notion, IL-21 has been associated with clinical antineoplastic activity.[1] However, at high concentrations, IL-21 can also lead to dose-limiting side effects including grade 3/4 granulocytopenia and liver toxicities.[1] Moreover, by driving an inflammatory response sustained by IL-6 and IL-17, as it occurs during chronic colitis, IL-21 can also contribute to oncogenesis.[2] In this commentary, we discuss our recent work on the use of IL-21-transduced or -transfected antigen-presenting cells (APCs) for anticancer immunotherapy, demonstrating that the local or *ex vivo* application of these cells promotes antitumor immunity but neither unwarranted systemic side effects nor IL-6/IL-17-driven inflammation (Fig. 1).

Effective cancer immunotherapy relies on high numbers of tumor-specific T lymphocytes with appropriate phenotypic characteristics, homing capacity, self-renewal potential and effector functions. One strategy in this sense involves adoptive cell transfer (ACT). Until now, IL-2 has been the cytokine of choice for the expansion of tumor-infiltrating lymphocytes (TILs) for ACT. Combined with lymphodepleting therapies, this approach has shown remarkable efficacy in patients affected by metastatic melanoma. Given that IL-2 can also have a negative impact on anticancer responses (as it can promote activation-induced cell death, the progressive differentiation of T cells and/or the accumulation/activation of Tregs), alternative γ_c cytokines have been investigated as potential alternatives for the expansion of TILs *ex vivo*. In a direct comparison with IL-2 and IL-15, we have recently demonstrated that IL-21 secreted by artificial K562 APCs (aAPCs) that were genetically modified to express 4-1BB ligand (4-1BBL) can promote the expansion of TIL-derived CD8+ T cells exhibiting a less differentiated, “young” (i.e., CD27+CD28+) phenotype and superior cytotoxic effector characteristics (i.e., high granzyme B and perforin expression levels), while avoiding collateral expansion of Tregs.[3] From previous studies we know that a poorly differentiated CD8+ T cell phenotype (CD27hiCD28hiCD45RA+CD62L+) associated with stem cell-like self-renewal capabilities guarantees the persistence of adoptively transferred T cells *in vivo*, and hence the elicitation of long-term immunological memory.[4] The fact that IL-21 appears to marry this phenotype with superior cytolytic effector functions[3,5] makes it highly suited for ACT strategies. Indeed, the clinical efficacy of IL-21-stimulated WT1-specific CD8+ T cell clones has recently been demonstrated in high-risk leukemia patients following allogeneic hematopoietic cell transplantation.[6] Importantly, these clones persisted over prolonged periods of time *in vivo* and adopted traits of long-lived memory cells.[6]

As the preparation of cells for ACT is laborious and time-consuming and since TILs

often are not available due to tumor accessibility issues, the development of clinically efficient active immunization approaches is warranted. Dendritic cells (DCs) play a crucial role in the induction of adaptive immune responses and are therefore the most frequently used cells for anticancer immunotherapy. To study whether IL-21 may further increase the T cell stimulatory capacity of DCs, we co-transfected mature monocyte-derived DC (MoDCs) with codon-optimized IL-21- and MART1-encoding mRNAs through electroporation, and then assessed the induction of functional MART1-specific CD8⁺ T cells *in vitro*. [7] IL-21 significantly enhanced the priming efficiency of DCs, enhancing the generation of tumor-specific CD8⁺ T cells *in vitro*. Similarly to IL-21-expanded TILs, tumor-antigen-specific CD8⁺ T cells primed by IL-21-expressing DCs exhibited very high expression levels of granzyme B and improved cytolytic functions. [7] IL-21 thus makes a valuable addition to active as well as passive immunotherapy strategies (Fig. 1).

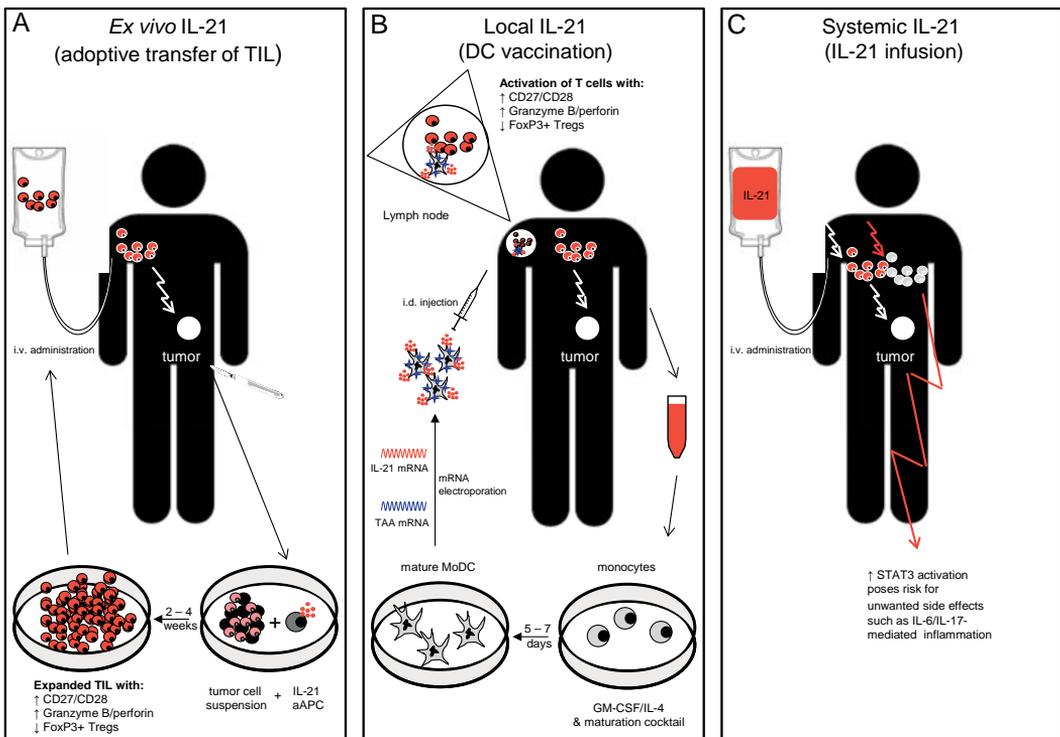


Figure 1. Possible applications of interleukin-21-releasing antigen presenting cells in cancer immunotherapy. Interleukin (IL)-21-releasing antigen presenting cells (IL-21-APCs) can be used *ex vivo* to expand tumor-infiltrating lymphocytes (TILs) for adoptive cell transfer (ACT) (A), or locally, as an mRNA-transfected dendritic-cell vaccine (B). (C) the systemic administration of IL-21 *i.v.* may cause unwanted inflammatory side effects, including a potentially tumor-promoting inflammatory response mediated by IL-6 and IL-17.

As a T_H17-derived pro-inflammatory mediator, IL-21 can exacerbate tumor progression by activating signal transducer and activator of transcription 3 (STAT3).[2] Nevertheless, IL-21 also exerts clear anti-tumor functions through the induction of cytolytic tumor-reactive CD8⁺ T cells, a process that is mediated by the T_H1-associated transcription factor Tbet. These diverging effects call for a spatial control of therapeutic IL-21 applications. To avoid unwanted side effects, IL-21 should be administered locally, rather than systemically, or employed *ex vivo* (Fig. 1). IL-21 has been shown to affect immune effectors through short-range cognate interactions[8] or even as a membrane-bound isoform.[9] The use of IL-21-secreting aAPCs or DCs may hence recapitulate the physiological mode of action of IL-21 while avoiding detrimental off-target effects. Timing is also essential when it comes to the transfection of DCs with IL-21-coding sequences, as the activation of immature DCs is hindered by IL-21,[10] whereas mature DCs, such as those used in our study, appear unaffected.[7] In view of these considerations, the targeted use of IL-21 *in vivo* for the reprogramming of tumor-conditioned, terminally differentiated and exhausted effector T cells may be one of the future challenges for the field.

In summary, IL-21-expressing APCs are powerful tools for the generation of tumor-reactive CD8⁺ T cells with powerful cytolytic effector functions and elevated self-renewal capability and should therefore be exploited for anticancer immunotherapy.

References

1. Petrella TM, Tozer R, Belanger K, Savage KJ, Wong R, Smylie M, *et al.* Interleukin-21 has activity in patients with metastatic melanoma: a phase II study. *J Clin Oncol* 2012; 30:3396-401; PMID:22915661; [http:// dx.doi.org/10.1200/JCO.2011.40.0655](http://dx.doi.org/10.1200/JCO.2011.40.0655)
2. Stolfi C, Pallone F, Macdonald TT, Monteleone G. Interleukin-21 in cancer immunotherapy: Friend or foe? *Oncoimmunology* 2012; 1:351-4; PMID:22737612; <http://dx.doi.org/10.4161/onci.19122>
3. Santegoets SJ, Turksma AW, Suhoski MM, Stam AG, Albelda SM, Hooijberg E, *et al.* IL-21 promotes the expansion of CD27+CD28+ tumor infiltrating lymphocytes with high cytotoxic potential and low collateral expansion of regulatory T cells. [Epub ahead of print]. *J Transl Med* 2013; 11:37; PMID:23402380; <http://dx.doi.org/10.1186/1479-5876-11-37>
4. Gattinoni L, Klebanoff CA, Restifo NP. Paths to stem- ness: building the ultimate antitumour T cell. *Nat Rev Cancer* 2012; 12:671-84; PMID:22996603; <http:// dx.doi.org/10.1038/nrc3322>
5. Yang S, Ji Y, Gattinoni L, Zhang L, Yu Z, Restifo NP, *et al.* Modulating the differentiation status of *ex vivo*- cultured anti-tumor T cells using cytokine cocktails. *Cancer Immunol Immunother* 2013; 62: 727-36; PMID:23207483
6. Chapuis AG, Ragnarsson GB, Nguyen HN, Chaney CN, Pufnock JS, Schmitt TM, *et al.* Transferred WT1- reactive CD8+ T cells can mediate antileukemic activ- ity and persist in post-transplant patients. *Sci Transl Med* 2013; 5:74ra27; PMID:23447018; <http://dx.doi.org/10.1126/scitranslmed.3004916>
7. Turksma AW, Bontkes HJ, Ruizendaal JJ, van den Heuvel H, Scholten KB, Santegoets SJ, *et al.* Increased cytotoxic capacity of tumor antigen specific human T cells after *in vitro* stimulation with IL21 producing dendritic cells. *Hum Immunol* 2013; 74:506–13; PMID:23376456; <http://dx.doi.org/10.1016/j.humimm.2013.01.014>
8. Yoshizaki A, Miyagaki T, DiLillo DJ, Matsushita T, Horikawa M, Kountikov EI, *et al.* Regulatory B cells control T-cell autoimmunity through IL-21-dependent cognate interactions. *Nature* 2012; 491:264-8; PMID:23064231; <http://dx.doi.org/10.1038/nature11501>
9. Nara H, Rahman M, Araki A, Jin L, Takeda Y, Asao H. IL-21 isoform is a membrane-bound ligand and activates directly interacted cells. *Cytokine* 2013; 61:656-63; PMID:23312859; <http://dx.doi.org/10.1016/j.cyto.2012.12.010>
10. Ansén S, Butler MO, Berezovskaya A, Murray AP, Stevenson K, Nadler LM, *et al.* Dissociation of its opposing immunologic effects is critical for the optimization of antitumor CD8+ T-cell responses induced by interleukin 21. *Clin Cancer Res* 2008; 14:6125-36; PMID:18829491; <http://dx.doi.org/10.1158/1078-0432.CCR-08-1146>