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ORIGINAL ARTICLE

Th17 cell inhibition in a costimulation blockade-based regimen for vascularized composite allotransplantation using a nonhuman primate model

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SUMMARY

Vascularized composite allotransplantation (VCA) is challenged by the morbidity of immunosuppression required to prevent rejection. The use of highly specific biologics has not been well explored in VCA. Given that psoriasis is T-cell mediated, as is rejection of skin-containing VCAs, we sought to assess the role of ustekinumab and secukinumab, which are approved to treat psoriasis by inhibiting Th17 cells. We combined these agents with belatacept and steroids in a VCA nonhuman primate model. Group I consisted of belatacept and steroids, group II was belatacept, ustekinumab with steroid taper, and group III was belatacept, secukinumab with steroid taper. Three animals were transplanted in each group. In group I, the mean graft survival time until the first sign of rejection was 10 days whereas in group II and III it was 10.33 and 11 days, respectively. The immunohistochemistry analysis showed that the number of IL-17a$^+$ cells and the intensity of IL-17a expression were significantly reduced in both dermis and hypodermis parts in groups II and III when compared to group I ($P < 0.01$). Ustekinumab and secukinumab led to less T-cell infiltration and IL-17a expression in the allograft but provided no benefit to belatacept and steroids in VCA survival.

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Introduction

An important element for the clinical advancement of vascularized composite allotransplantation (VCA) is the development of immunosuppression regimens that prevent rejection by targeting specific immune mechanisms known to influence the tissues transplanted in a VCA, while avoiding major toxicities of immunosuppressants [1]. Current standard immunotherapies in VCA rely on agents such as calcineurin inhibitors (CNIs) and steroids which are known to cause side effects including nephrotoxicity, hypertension, and diabetes [2].

Recently described and clinically tested methods for immune management have been shown both to prevent the rejection of transplanted organs, while controlling cutaneous autoimmune diseases such as psoriasis [3]. These arguably could directly target the unique components of VCA rejection while tempering the risks

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associated with immune manipulation. Specifically, in recent years, belatacept has become approved by the Food and Drug Administration (FDA) as a replacement for calcineurin inhibitors in kidney transplantation [4,5, though the role of this medication in VCA is under research [6,7].

Belatacept is a high-affinity fusion protein that targets the CD80/CD86 costimulation pathway, which is the best recognized pathway for immune cell activation and proliferation. Prior work has shown that belatacept is able to prolong renal allograft survival in nonhuman primates [8]. Clinically, this agent provides benefits not provided by other agents such as less nephrotoxicity compared to calcineurin inhibitors. However, belatacept is less efficacious than tacrolimus at preventing acute rejection [9]. Due to the drug mechanism of action, belatacept is unable to prevent rejection by memory T cells, which do not require costimulation for activation. Previous work in nonhuman primates (NHP) suggests that drugs designed to treat psoriasis can prolong costimulation blockade-based allograft survival and prevent belatacept-resistant rejection [10,11]. This is important since skin is the most immunogeneic of the tissues transplanted and serves as a harbinger of rejection in a VCA [12].

We sought to determine the role of two recently approved and clinically available immunosuppressive medications in addition to costimulation blockade with belatacept and steroids by utilizing a NHP model of VCA. We interrogated the efficacy of ustekinumab or secukinumab in addition to belatacept as this regimen could allow the assessment of T-cell-mediated rejection with drugs specifically designed to treat cutaneous T-cell-mediated autoimmune diseases.

Materials and methods

Donor-recipient pair selection

The experiments were approved by the Duke Institutional Animal Care and Use Committee (IACUC) and performed in compliance with the principles set forth in The Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, National Research Council, DHHS. Twenty rhesus macaques were obtained from Alpha Genesis Labs (Yemassee, SC, USA). Donor-recipient pairs were chosen to maximize genetic amjor histocompatibility complex (MHC) mismatching at class I and class II alleles. We used our established NHP VCA model utilizing a sensate osteomyocutaneous radial forearm flap that avoids functional impairment even in the case of graft loss, it is well tolerated by NHPs, results in allosensitization, and is responsive to immunosuppression [13]. During the course of the study and to reduce the number of animals, each animal served as both a donor and a recipient of a VCA if the animals could be immunologically paired appropriately. Each VCA recipient was treated with a specific costimulation blockade-based regimen (described below). The NHPs were monitored by daily clinical examination for signs of rejection such as rash or erythema and laboratory tests at the time of the administration of medications, including complete blood count and serum chemistries, to minimize sedation to the animals. Both donor animals and recipient animals maintained functionality of their hands, even in cases of graft rejection or graft loss.

Treatment groups

There were three new groups included in this study. Under sedation, experimental infusions were given through temporary 24-gauge intravenous catheters placed in the saphenous vein. All animals received the following medications by injection or infusion:

1. Belatacept (Nulojix; Bristol–Myers Squibb, New York, NY, USA) 20 mg/kg intravenously on days 0 and 4; at 10 mg/kg on postoperative days 7, 14, and every 2 weeks thereafter.
2. Methylprednisolone 15 mg/kg intramuscularly on postoperative days 0, 1, and 2. This dose will then be decreased by half every 2 days until the dose is 1 mg/kg. Methylprednisolone is stopped on postoperative days 30.
3. Heparin 100 μ/kg twice daily subcutaneously on postoperative days 0 through 7, then once daily on postoperative days 8–15.
4. Valganciclovir 60 mg PO twice daily for cytomegalovirus (CMV) prophylaxis starting the day of transplant and continuing for the duration of the study. If CMV viremia was confirmed (greater than 10 000 copies/ml determined by PCR), Ganciclovir 6 mg/kg was administered subcutaneously twice daily for treatment until resolution of infection was determined by repeat PCR assay.

Group I: belatacept and steroids ($n = 3$).

Ustekinumab and secukinumab doses were determined following the clinical standard of care use in humans to treat T-cell-mediated autoimmunity.

Group II: belatacept and ustekinumab with steroid taper ($n = 3$).

Belatacept and steroids as above. Ustekinumab (Stelara, Janssen Biotech, Horsham, PA, USA) given

subcutaneously at 5 mg/kg on POD 0, 4, 7, 14, 21, 28, 35, and 42.

Group III: belatacept and secukinumab with steroid taper ($n = 3$).

Belatacept and steroids as above. Secukinumab (Cosentyx, Novartis, East Hanover, NJ, USA) given subcutaneously at 5 mg/kg on POD 0, 7, 14, 21, 28, and monthly thereafter.

We compared the results of these groups with a historic cohort of standard immunosuppression including a calcineurin inhibitor (tacrolimus 1 mg/kg every 12 h orally with a targeted level from 15 to 20 ng/ml), an antiproliferative agent (mycophenolate mofetil 20 mg/kg every 12 h orally), and methylprednisolone at 15 mg/kg intramuscular for 3 days followed by 7.5 mg/kg for 2 days and a 50% reduction every 2 days until the dose was 1 mg/kg [13].

Histology

All histology specimens were prepared by a histopathologist. Excisional skin biopsy of the transplant was performed at signs of graft rejection (erythema, swelling, rash, desquamation and the appearance of vesicles on gross inspection of the allograft skin). Paraffin-embedded skin was sectioned and stained with hematoxylin and eosin (H&E) for morphology observation. The skin was scored using the VCA Banff grading system [14]. Anti-IL-17a (Novus Biological, Centennial, CO, USA) and CD3 (Agilent, Carpinteria, CA, USA) antibodies were used for the immunohistochemistry (IHC) and dual immunofluorescence staining to evaluate the infiltrating Th17 cells and T lymphocytes. Whole slide digital images were captured by using the Aperio AT Turbo digital slide scanner system (Leica Biosystems, Vista, CA, USA).

Quantitative immunohistochemical analysis was performed using Aperio Imagescope (Leica Biosystems) digital pathology software, and IL-17a expression level was calculated by pixel positivity index of IL-17a staining in the measured areas. Positivity index parameters were computed by the software (Aperio Imagescope) via combining the stained area and intensity values of the staining. Each section was measured 7–12 (depend on the tissue size) areas in dermis and 5–6 areas with infiltrates surrounding the blood vessel in hypodermis. Statistical analysis was performed using GRAPHPAD PRISM (GraphPad Software Inc, San Diego, CA, USA) version eight statistical software. One-way ANOVA test was performed, followed by unpaired *t*-test on significant results. A $P \leq 0.05$ was determined to be statistically significant.

Monitoring post-transplant donor-specific alloantibody

Donor-specific alloantibody (DSA) was assessed by flow cross-match. Donor cells ($3–5 \times 10^5$) were blocked with goat IgG (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) and cultured with serially collected recipient serum as previously described [15,16]. Briefly, cells were subsequently stained with FITC-labeled anti-rhesus IgG (KPL, catalog 072-11-021), PerCp-Cy5.5-labeled anti-CD3 mAb (BD, clone: SP34-2), and PE-labeled anti-CD20 mAb (BD, Clone: 2H7) with live/dead cell staining. The DSA level was normalized and expressed as fold increase against the pretransplant MFI value. Samples were collected with BD LSR FORTESSA X-20 (BD Bioscience, San Jose, CA, USA) and analyzed using FlowJo software 9.9. (Tree Star, Ashland, OR, USA).

RhCMV viral monitoring using quantitative real-time PCR analysis

RhCMV viral monitoring was periodically performed by PCR using a previously described and clinically validated technique [6]. In brief, DNA was extracted from whole blood samples and a qPCR reaction was carried out. Transcript copy numbers were determined and compared to a standard curve. Any animal with titers above 10 000 was treated with ganciclovir (6 mg/kg IM twice daily) until the viral load was undetectable.

Results

Graft survival rates per treatment group

Three animals were transplanted in each group. In group I, the mean graft survival time until the first sign of rejection was 10 days (Fig. 1a). The animals showed the first signs of rejection on postoperative days 6, 10, and 14 (Fig. S1). The grafts were excised on postoperative days 6, 13, and 14, respectively. The animal whose graft was ultimately excised on postoperative day 13 received an incisional biopsy on postoperative day 11. No animals were found to have donor-specific antibodies (DSA), or antibodies targeting donor major histocompatibility complex (MHC) in the control or experimental groups. Also, no animals were found to have CMV viremia while undergoing immunosuppression.

In group II, the mean graft survival time was 10.33 days (Fig. 1a). Rejection was diagnosed on

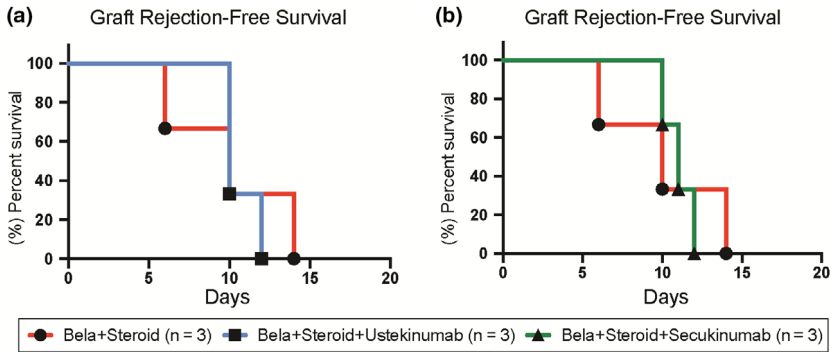

Figure 1 Rejection-free graft survival curves per treatment group. Belatacept/steroids/ustekinumab (a) and belatacept/steroids/secukinumab (b) compared to the belatacept and steroids group.

postoperative days 10, 10, and 12. One animal underwent incisional biopsy on postoperative day 10. Graft excision was performed on postoperative days 10, 11, and 14, respectively (Fig. S2). No animals had DSA at the time of rejection. One animal was found to have CMV viremia with ~14 000 viral copies/ml during treatment with immunosuppression. The animal was treated with ganciclovir until resolution of CMV viremia was confirmed by viral PCR.

In group III, the mean graft survival time until was 11 days (Fig. 1b). Rejection was diagnosed on postoperative days 10, 11, and 12. One animal underwent incisional biopsy on postoperative day 12. All transplanted allografts were excised by postoperative days 10, 11, and 13, respectively (Fig. S3). No animals had DSA at the time of rejection (Fig. 2), and no animals experienced CMV viremia.

The grafts in the historic cohort had an average survival (time from transplant to early signs of rejection) of 31.1 days (seven animals total, range 5–76 days) [13].

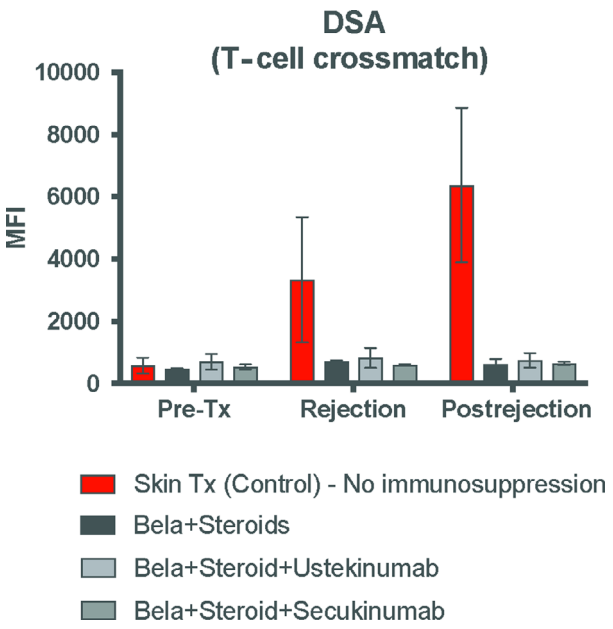

Figure 2 Donor-specific alloantibody (DSA) measurement in all groups. No DSA presents pretransplant, at time of rejection, or postrejection. Serum samples from skin transplantation without immunosuppression (red bar, historic control) showed significantly increased DSA at rejection (D14) and postrejection at one month (D35) while animals treated with belatacept-based regimen showed no sign of post-transplant DSA production.

Histology findings at the time of rejection per treatment group

At the time of rejection in group I, H&E staining of the skin at time of incisional biopsy on postoperative day 11 was classified as Banff II (Fig. 3a) and excisional biopsy on postoperative day 13 was classified as VCA Banff IV. In group II, at diagnosis of acute rejection on postoperative day 14, H&E staining of skin was classified as VCA Banff IV (Fig. 3b). Finally, in group III at the time of rejection on postoperative day 12, H&E staining also revealed a VCA Banff IV rejection (Fig. 3c).

The immunohistochemistry for IL-17a and dual immunofluorescence labeling of IL-17a$^+$ (red)/CD3$^+$ (green)/DAPI (blue) in the skin allografts were performed in all three groups (Fig. 3d–i). IHC analysis result showed there was an increased number of infiltrating cells with strong IL-17a expression in the tissues in group I. The number of IL-17a$^+$ cells and the intensity of IL-17a expression were significantly reduced in both dermis and hypodermis parts in groups II and III when compared to group I, respectively ($P < 0.01$). No significant difference of IL-17a expression between the group II and group III in both dermis and hypodermis areas was detected ($P > 0.05$; Fig. 4a). Dual immunofluorescence staining showed there were more T cells infiltrating and most T cells with stronger co-expression of IL-

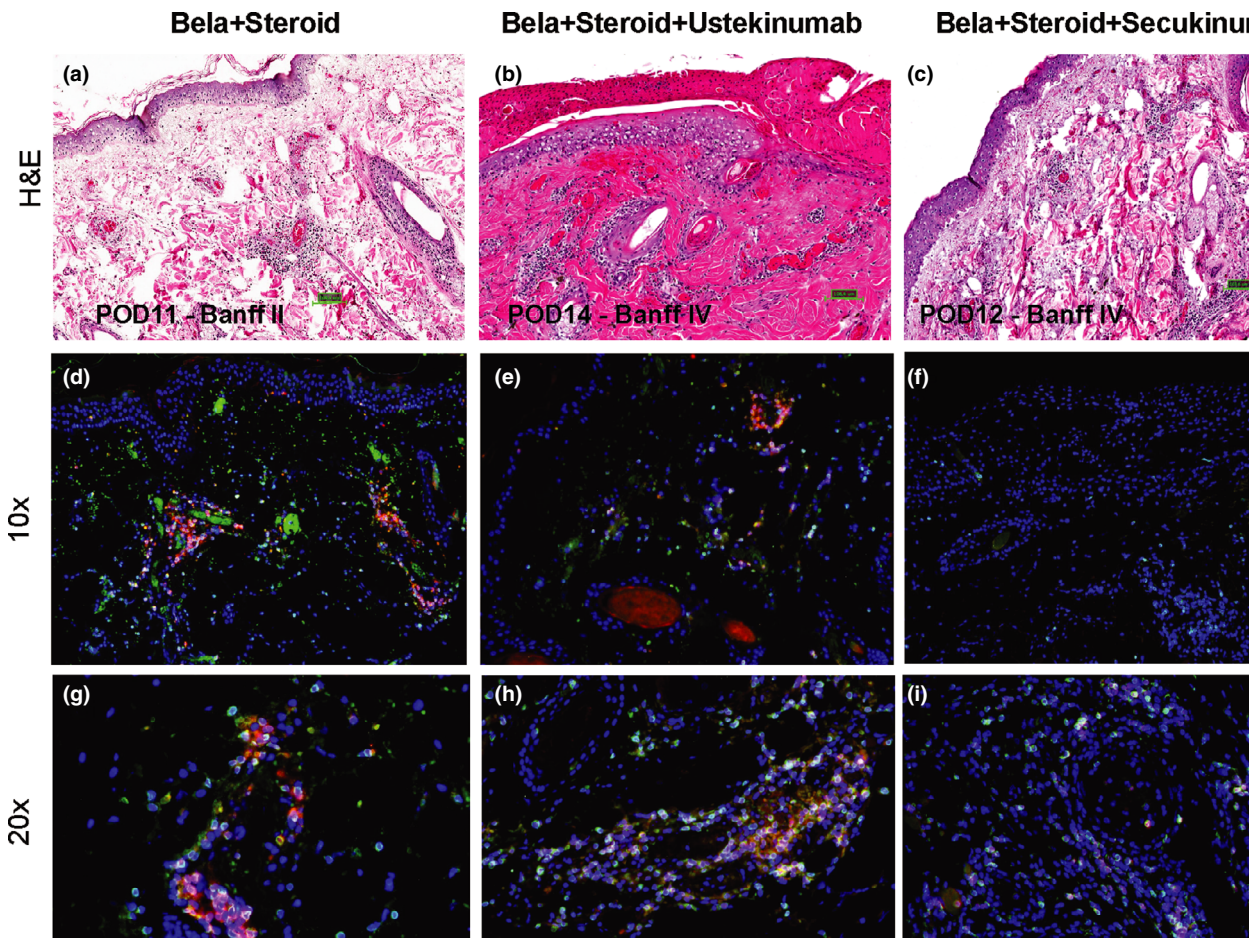

Figure 3 (a) Hematoxylin and eosin of the skin at time of incisional biopsy on postoperative day 11 (VCA Banff II). (b) hematoxylin and eosin of skin at time of excisional biopsy on postoperative day 14 (VCA Banff IV). (c) hematoxylin and eosin skin at time of excisional biopsy on postoperative day 12 (VCA Banff IV). IL17a and CD3 expressions in the skin allograft among all groups. Dual immunofluorescence labeling of IL-17a$^+$ (red)/CD3$^+$ (green) in the skin allografts of belatacept + steroids (d,g), belatacept and ustekinumab (e,h), and belatacept and secukinumab (f,i). Yellow to orange color represents of double stained of IL17a$^+$ and CD3$^+$ cells. Blue color in represents 4′,6-diamidino-2-phenylindole (DAPI). Perivascular infiltrating T cells showed weak or no expression of IL-17a in the belatacept/steroids/ustekinumab and belatacept/steroids/secukinumab groups compared to belatacept/steroids group.

17a in group I. The number of IL-17a$^+$ cells, stain intensity, and the ratios of IL-17a$^+$ cells in infiltrating T cells in both dermis and hypodermis were greatly decreased in group II and III compared to group I (Fig. 4b).

In general, the hypodermis areas had more IL-17a$^+$ infiltrating cells than dermis areas. The addition treatments of secukinumab and ustekinumab significantly reduced the expression of IL-17a in the infiltrating cells in both dermis and hypodermis areas of the skin allografts with some IL-17a$^+$ cells still presented in the perivascular and vascular areas in hypodermis tissues.

In the historic group, the histology in all these animals revealed parakeratotic hyperkeratosis and a moderate dermal, perifollicular, and perivascular mononuclear cell infiltrate. Dermal mononuclear cell infiltrates were predominantly lymphocytes. Moderate multifocal perivascular lymphocytic dermal infiltrates, with mild lymphocytic infiltrates at the dermal-epidermal junction, were also observed. These lymphoid infiltrates were strongly CD3 positive on immunostaining [13].

Discussion

Our focus in this study was to determine the role of ustekinumab and secukinumab in addition to belatacept in a well-established NHP model of VCA. We showed that by adding ustekinumab and secukinumab in a protocol of immunosuppression with belatacept and steroids did not lead to significant increase of mean graft survival time. Similar to prior studies by our team and others, we observed absence of donor-specific antibodies at the time of rejection.

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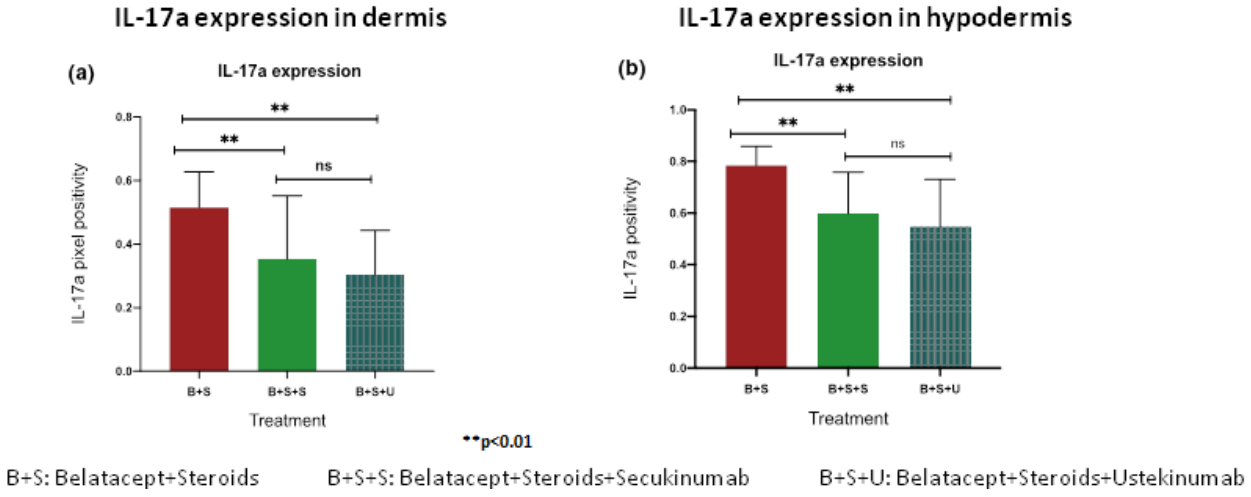

Figure 4 (a) IL17a expressions in the skin allograft among all groups: There was a significantly reduced IL-17a expression in groups II and III in both dermis and hypodermis areas compared to group I, respectively ($P < 0.01$). (b) There was no significantly difference of IL-17a expression between the group II and group III in dermis and hypodermis ($P > 0.05$). **statistical significance.

Vascularized composite allografts offer the opportunity for life-improving function to patients with tissue loss unable to be reconstructed through traditional techniques. Currently, most immunosuppressive regimens reported to date in clinical VCA use CNIs, with most of the VCA recipients experiencing the corresponding side effects [17]. We have previously shown that costimulation blockade regimens can improve rejection-free survival of VCAs when compared to a standard CNI-based therapy [6]. Although rejection may have been reversed, treatment of rejection was outside the scope of this study. In the same frame, de novo sirolimus started on the day of transplant has been correlated with increased wound healing complications ultimately leading to VCA engraftment failure [13].

Belatacept has been approved for kidney transplantation. It inhibits graft-specific immune responses by blocking CD28/CTLA-4 signals on T cells and offers significantly improved long-term graft function and fewer toxicities compared to calcineurin inhibitors. However, belatacept is associated with a high incidence of pathologically severe acute rejection episodes [18]. This finding generated the concept of costimulation blockade resistance. The mechanism of this phenomenon is not fully elucidated and mandates higher resolution understanding of the functional characteristics of pathologic T-cell subsets in order to more effectively modulate pathological T-cell responses [19,20].

Belatacept has been extensively studied by our group in vitro, in NHPs, and in clinical transplantation, showing a delayed onset of rejection in transplant recipients [21,22]. In clinical hand transplantation, we have previously reported that conversion from a standard maintenance regimen (tacrolimus, mycophenolate mofetil, and steroids) to belatacept and sirolimus led to improving renal function in the absence of calcineurin inhibitors long term [23]. In a stepwise progression, we have also shown that hand transplantation can be performed using a de novo belatacept-based treatment without CNIs long-term, resulting in sufficient prophylaxis from rejection, reversible rejection when occurred, and reduced side effects [24].

Th17 cells, and their signature cytokine IL-17, are evident that in many models of autoimmunity and cancer acting as the principal driver of inflammation [3]. Ustekinumab is a monoclonal antibody that neutralizes IL-12/IL-23p40 and has been shown to inhibit cutaneous Th17 cell proliferation and maturation in the skin [25]. Similarly, secukinumab, a fully human monoclonal anti-IL-17A antibody, inhibits activation of neutrophils and synergy with TNF and IL-1 which are known to promote inflammation. This drug has been used to treat plaque psoriasis, psoriatic arthritis, and ankylosing spondylitis [26].

It is now well established that alloreactive memory T cells accelerate allograft rejection and prevent transplant tolerance [27]. The observation that Th17 cells from renal transplant recipients are resistant to belatacept combined with the association of elevated Th17 memory cell frequencies with acute cellular rejection strongly suggests that Th17 memory cells play a role in clinical belatacept-resistant graft rejection [28]. IL-12 also plays a key role in Th1 differentiation, and IL-23 stabilizes the Th17 phenotype. These cytokines share a common

p40 subunit. Neutralization of IL-12/IL-23p40 is shown to reduce Th1 and Th17 differentiation [29].

In our study, however, there was no reduction in the onset and severity of clinical rejection, in spite of both broad and targeted immunosuppressive therapy, as well as histologic demonstration of a reduction of Th17 cells. One possible arm not affected by the immunosuppressive approach is the regulatory T-cell compartment [30]. The promotion of immunologic ignorance, even in the absence of immunosuppression, can prolong survival in some allograft models [31]. It also appears to be independent of the IL17 [31].

Our findings did not support the concept of Th17 cell inhibition in addition to costimulation molecule-focused therapies to directly targeting the unique components of T-cell-mediated rejection of vascularized composite allografts in nonhuman primates.

Authorship

AA, DM, MM and LC: cendales designed and performed experiments, analyzed data and co-wrote the paper. FL and KW: performed experiments. MS, LS and JK: performed bioinformatic analyses. WP and ARC: critically revised the paper. ADK and LCC: critically revised the paper and supervised the research.

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Conflicts of interest

The authors of this manuscript have no conflicts of interest to disclose.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Belatacept/steroids group.
Figure S2. Belatacept/steroids/ustekinumab group.
Figure S3. Belatacept/steroids/secukinumab group.

REFERENCES

1. Kollar B, Pomahac B, Riella LV. Novel immunological and clinical insights in vascularized composite allotransplantation. *Curr Opin Organ Transplant* 2019; **24**: 42.
2. Ojo AO, Held PJ, Port FK, *et al.* Chronic renal failure after transplantation of a nonrenal organ. *N Engl J Med* 2003; **349**: 931.
3. Maddur MS, Miossec P, Kaveri SV, Bayry J. Th17 cells: biology, pathogenesis of autoimmune and inflammatory diseases, and therapeutic strategies. *Am J Pathol* 2012; **181**: 8.
4. Adams AB, Goldstein J, Garrett C, *et al.* Belatacept combined with transient calcineurin inhibitor therapy prevents rejection and promotes improved long-term renal allograft function. *Am J Transplant* 2017; **17**: 2922.
5. Kirk AD, Guasch A, Xu H, *et al.* Renal transplantation using belatacept without maintenance steroids or calcineurin inhibitors. *Am J Transplant* 2014; **14**: 1142.
6. Freitas AM, Samy KP, Farris AB, *et al.* Studies introducing costimulation blockade for vascularized composite allografts in nonhuman primates. *Am J Transplant* 2015; **15**: 2240.
7. Grahammer J, Weissenbacher A, Zelger BG, *et al.* Benefits and limitations of belatacept in 4 hand-transplanted patients. *Am J Transplant* 2017; **17**: 3228.
8. Lo DJ, Anderson DJ, Weaver TA, *et al.* Belatacept and sirolimus prolong nonhuman primate renal allograft survival without a requirement for memory T cell depletion. *Am J Transplant* 2013; **13**: 320.
9. Muduma G, Hart WM, Patel S, Odeyemi AO. Indirect treatment comparison of belatacept versus tacrolimus from a systematic review of immunosuppressive therapies for kidney transplant patients. *Curr Med Res Opin* 2016; **32**: 1065.
10. Weaver TA, Charafeddine AH, Agarwal A, *et al.* Alefacept promotes co-stimulation blockade based allograft survival in nonhuman primates. *Nat Med* 2009; **15**: 746.
11. Hadaya K, Ferrari-Lacraz S, Fumeaux D, *et al.* Eculizumab in acute recurrence of thrombotic microangiopathy after renal transplantation. *Am J Transplant* 2011; **11**: 2523.
12. Cendales LLM, Bartlett S, Cheeseman J, *et al.* Skin as a harbinger of rejection of underlying structures in vascularized composite allografts: concordance or discordance? *Am J Transplant* 2016; **16** (suppl 3): 433.
13. Cendales LC, Xu H, Bacher J, Eckhaus MA, Kleiner DE, Kirk AD. Composite tissue allotransplantation: development of a preclinical model in nonhuman primates. *Transplantation* 2005; **80**: 1447.
14. Cendales LC, Kanitakis J, Schneeberger S, *et al.* The Banff 2007 working classification of skin-containing composite tissue allograft pathology. *Am J Transplant* 2008; **8**: 1396.
15. Page EK, Page AJ, Kwun J, *et al.* Enhanced de novo alloantibody and

antibody-mediated injury in rhesus macaques. *Am J Transplant* 2012; **12**: 2395.
16. Kwun J, Page E, Hong JJ, *et al*. Neutralizing BAFF/APRIL with atacicept prevents early DSA formation and AMR development in T cell depletion induced nonhuman primate AMR model. *Am J Transplant* 2015; **15**: 815.
17. Cherikh WS, Cendales LC, Wholley CL, *et al*. Vascularized composite allotransplantation in the United States: a descriptive analysis of the Organ Procurement and Transplantation Network Data. *Am J Transplant* 2019; **19**: 865.
18. Vincenti F, Blancho G, Durrbach A, *et al*. Five-year safety and efficacy of belatacept in renal transplantation. *J Am Soc Nephrol* 2010; **21**: 1587.
19. Espinosa J, Herr F, Tharp G, *et al*. CD57(+) CD4 T cells underlie belatacept-resistant allograft rejection. *Am J Transplant* 2016; **16**: 1102.
20. Cortes-Cerisuelo M, Laurie SJ, Mathews DV, *et al*. Increased pretransplant frequency of CD28(+) CD4(+) TEM predicts belatacept-resistant rejection in human renal transplant recipients. *Am J Transplant* 2017; **17**: 2350.
21. Larsen CP, Pearson TC, Adams AB, *et al*. Rational development of LEA29Y (belatacept), a high-affinity variant of CTLA4-Ig with potent immunosuppressive properties. *Am J Transplant* 2005; **5**: 443.
22. Larsen CP, Knechtle SJ, Adams A, Pearson T, Kirk AD. A new look at blockade of T-cell costimulation: a therapeutic strategy for long-term maintenance immunosuppression. *Am J Transplant* 2006; **6**(5 Pt 1): 876.
23. Cendales L, Bray R, Gebel H, *et al*. Tacrolimus to belatacept conversion following hand transplantation: a case report. *Am J Transplant* 2015; **15**: 2250.
24. Cendales LC, Ruch DS, Cardones AR, *et al*. De novo belatacept in clinical vascularized composite allotransplantation. *Am J Transplant* 2018; **18**: 1804.
25. Liu XK, Clements JL, Gaffen SL. Signaling through the murine T cell receptor induces IL-17 production in the absence of costimulation, IL-23 or dendritic cells. *Mol Cells* 2005; **20**: 339.
26. Baeten D, Sieper J, Braun J, *et al*. Secukinumab, an interleukin-17A inhibitor, in ankylosing spondylitis. *N Engl J Med* 2015; **373**: 2534.
27. Benichou G, Gonzalez B, Marino J, Ayasoufi K, Valujskikh A. Role of memory T cells in allograft rejection and tolerance. *Front Immunol* 2017; **8**: 170.
28. Krummey SM, Cheeseman JA, Conger JA, *et al*. High CTLA-4 expression on Th17 cells results in increased sensitivity to CTLA-4 coinhibition and resistance to belatacept. *Am J Transplant* 2014; **14**: 607.
29. Muranski P, Restifo NP. Essentials of Th17 cell commitment and plasticity. *Blood* 2013; **121**: 2402.
30. Issa F, Wood KJ. The potential role for regulatory T-cell therapy in vascularized composite allograft transplantation. *Curr Opin Organ Transplant* 2014; **19**: 558.
31. Pilat N, Wiletel M, Weijler AM, *et al*. Treg-mediated prolonged survival of skin allografts without immunosuppression. *Proc Natl Acad Sci USA* 2019; **116**: 13508.