Inhibitory Effects of Dapirolizumab Pegol, a Monovalent Anti-CD40L PEG-Conjugated Antigen-Binding Fragment Lacking a Functional Fc Domain, on *In Vitro* T Follicular Helper/B Cell Interactions and Cytokine Production in Systemic Lupus Erythematosus

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Objective

To investigate the effect of dapirolizumab pegol (DZP) on *in vitro* T cell and B cell interactions, and cytokine and immunoglobulin production, relevant to systemic lupus erythematosus (SLE) pathogenesis.

Background

- The pivotal role of CD40-CD40L interactions in SLE pathogenesis stems from the orchestration of a range of immune and inflammatory responses involving T cells, B cells, and other antigen-presenting cells (APCs), which result in the production of pro-inflammatory cytokines and autoantibodies (**Figure 1**).[1]
- DZP is a novel, polyethylene glycol (PEG)-conjugated antigen-binding (Fab') fragment, lacking an Fc domain, that inhibits CD40L signaling (**Figure 2**),[2] and is under investigation in individuals with SLE in a phase 3 clinical trial (PHOENYCS FLY; NCT04976322).
- Unlike full length IgG monoclonal antibodies that are associated with thrombotic complications in humans, anti-CD40L antibodies lacking an Fc domain do not induce thrombotic events in non-human primates and fail to activate platelets *in vitro*.[2]
- Post hoc analyses of the phase 2b RISE trial (NCT02804763) of DZP in SLE demonstrated that DZP suppresses the expression of genes involved in B and T cell activation, immunoglobulin production, and pro-inflammatory cytokines in patients with active SLE.[3,4]

Methods

- For antibody induction T follicular helper (Tfh)-B cell co-culture experiments, peripheral blood mononuclear cells (PBMCs) from healthy volunteers (N=6) were isolated by Ficoll-Paque. Naïve T cells (CD4+, CD45RA+, and CXCR5-), Tfh cells (CD4+, CD45RA-, and CXCR5+), and naïve B cells (CD19+, IgD+, and CD27-) were further purified by flow cytometric sorting. Naïve T cells or Tfh cells were then incubated with naïve B cells at a 1:1 ratio in the presence of 1 µg/mL Staphylococcal enterotoxin B (SEB) for 14 days with DZP or A33 Fab'-PEG isotype control (15 µg/mL).
  - Total or SEB-specific IgG and IgM were measured by MesoScale Discovery (MSD) and enzyme-linked immunosorbent assay (ELISA), respectively.
- For intracellular IFN-γ production experiments, whole blood samples from patients with SLE (N=12) were red blood cell lysed and cultured for 18 hours with 1 µg/mL SEB and DZP or A33 Fab'-PEG isotype control (100 µg/mL) in the presence of Brefeldin A and Monensin to prevent cytokine secretion.
  - Intracellular cytokine staining of CD4+ and CD8+ T cells was performed 18 hours after SEB antigen stimulation. Data were analyzed using a paired T test.
- For PBMC cytokine production experiments, whole PBMCs isolated by Ficoll-Paque from healthy volunteers (N=10) or patients with SLE (N=15) were cultured in the presence of 1 µg/mL SEB for 5 days with DZP or A33 Fab'-PEG isotype control (100 µg/mL).
  - Cytokine production, measured by LEGENDplex, was assessed after 5 days of culture with SEB.
  - Data were analyzed using a three-way ANOVA based on treatment, SEB-stimulation, and sample type. Comparisons between DZP and Fab'-PEG control were assessed at each combination of SEB-stimulation and sample type with t-tests using a pooled estimate of variance from the ANOVA.

Results

- Healthy volunteer Tfh cells were shown to drive increased naïve B cell antibody responses, increased IgG and IgA production, and class switching over naïve T cells in an *in vitro* co-culture assay (**Figure 3A**).
  - DZP inhibited production of both total and antigen-specific IgG antibody responses relative to the isotype control (**Figure 3B**).
- IFN-γ is a key pro-inflammatory cytokine involved in SLE pathogenesis; DZP reduced the induction of IFN-γ+ CD4+ and CD8+ T cells from patients with SLE after SEB stimulation relative to the isotype control (**Figure 4**).
- After culture of SEB-stimulated PBMCs isolated from both healthy volunteers and patients with SLE, DZP statistically significantly inhibited the release of a range of cytokines, including IL-1ß, IL-10, IL-12p40, and IL-6, relative to the isotype control (p<0.05; **Figure 5**).

Conclusions

DZP was shown to inhibit key T cell, B cell, and APC cell functions that drive SLE pathogenesis, leading to suppression of cytokines, and total and antigen-specific IgG production *in vitro*. These data support clinical and transcriptomic/proteomic findings from patients with SLE treated with DZP, where reductions of immunoglobulins and key pro-inflammatory cytokines have been observed.[4-6] Overall, the data indicate that DZP inhibits multiple pathways relevant to the pathogenesis of SLE.

Figure 1 DZP suppresses CD40-CD40L mediated immunological processes involved in SLE pathology

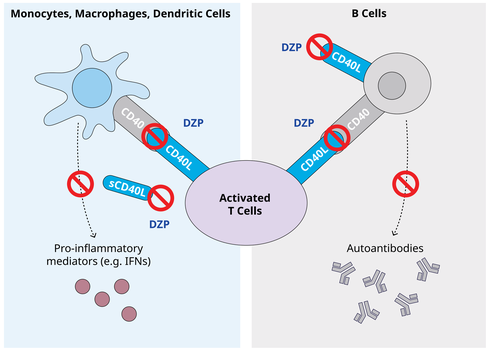


Figure 2 DZP is a novel PEG-conjugated Fab' fragment, lacking an Fc domain

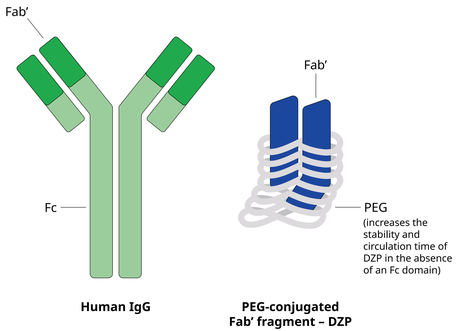
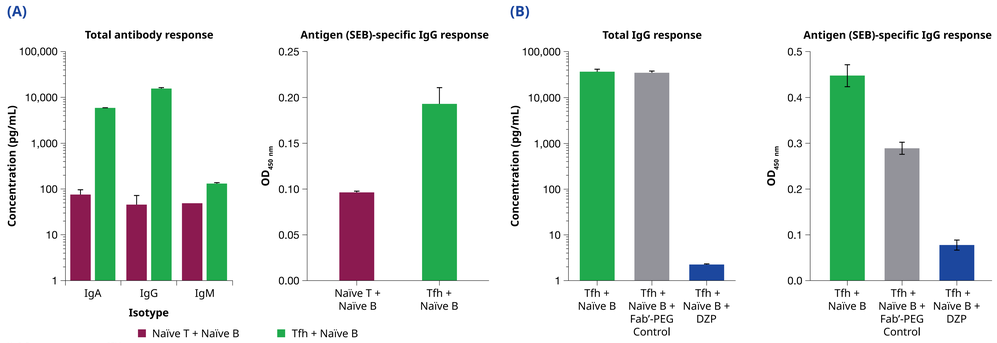
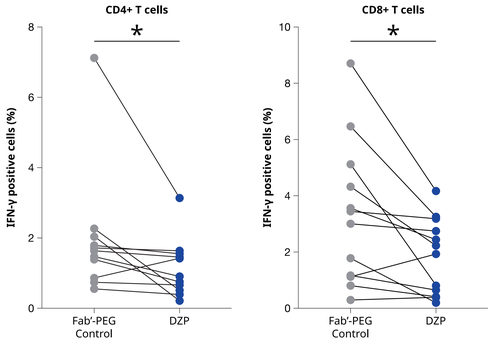


Figure 3 (A) Healthy volunteer Tfh cells drove naïve B cell antibody responses and class switching; (B) DZP inhibited total and antigen (SEB)-specific IgG responses



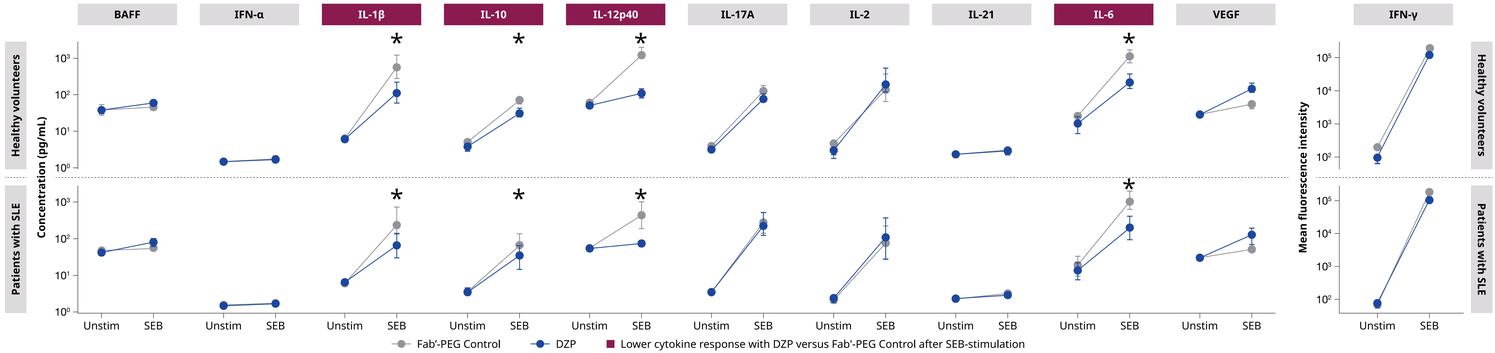
N=6. Bars represent mean ± SEM.

Figure 4 DZP reduced IFN-γ induction in CD4+ and CD8+ T cells from patients with SLE



N=12; p<0.05.

Figure 5 DZP modulated multiple cytokines in SEB-stimulated PBMCs from healthy volunteers and patients with SLE



Healthy volunteers: N=10; patients with SLE: N=15; *p<0.05. Data are presented as mean and interquartile range.

APC: antigen-presenting cell; **BAFF:** B-cell activating factor; **DZP:** dapirolizumab pegol; **ELISA:** enzyme-linked immunosorbent assay; **Fab':** antigen-binding fragment; **IFN:** interferon; **Ig:** immunoglobulin; **IL:** interleukin; **MSD:** MesoScale Discovery; **OD:** optical density; **PEG:** polyethylene glycol; **PBMC:** peripheral blood mononuclear cells; **SEB:** Staphylococcal enterotoxin B; **SEM:** standard error of the mean; **SLE:** systemic lupus erythematosus; **Tfh:** T follicular helper; **Unstim:** unstimulated; **VEGF:** vascular endothelial growth factor.

References: [1]Ramanujam M. Autoimmun Rev 2020;19:102668; [2]Shock A. Arth Res Ther 2015;17:234; [3]Cutcutache I. Arthritis Rheumatol 2023;75 (suppl 9); [4]Powlesland AS. Annals Rheum Dis 2024;83 (suppl 1):261; [5]Furie RA. Rheumatology (Oxford) 2021;60:5397–407; [6]Chamberlain C. Ann Rheum Dis 2017;76:1837–44.
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