POSTER 854

γc receptor antagonist, EQ102, prevents the NK and T cell-mediated responses driven by IL-15 and IL-21

Phoi Tiet[1], Valeria Marrocco[1], AJ Giovannone[1], Laith Al-Mawsawi[1], Dalena Chu[1], Jeanette Ampudia[1], Stephen Connelly[1], Cherie Ng[1]

[1] Equillium Inc., La Jolla, CA 92037

Introduction

- The common γc chain (γc, CD132) is a receptor subunit shared by a group of interleukin family members. These cytokines are crucial in regulating major immune responses and promote a range of inflammatory disorders.
- IL-15 and IL-21are two γc cytokines important in inflammation. IL-15 and IL-21 each promote cytolytic activity and IFN-γ production and can also enhance the function of other cytotoxic cytokines[1]. Synergistic signaling of IL-15 and IL-21 together is important in driving pathogenic T and NK cell responses in multiple inflammatory diseases, including celiac disease and other inflammatory gut and hepatic disorders.
- Blockade of both IL-15 and IL-21 is necessary to improve disease outcome in pathologic environments. While this can be achieved by blocking the γc, it is necessary to maintain signaling of the other γc family members (IL-2, IL-4, IL-7 and IL-9) to prevent non-specific and unnecessary immunosuppression.
- EQ102 is a peptide that was specifically designed to selectively block IL-15 and IL-21 signaling while preserving the signaling of other γc family members (FIGURE 1).
- Here, we sought to investigate the effect of synergistic signaling from both IL-15 and IL-21 on NK and T cell activities and the ability of EQ102 to inhibit these signals and resulting cellular response.

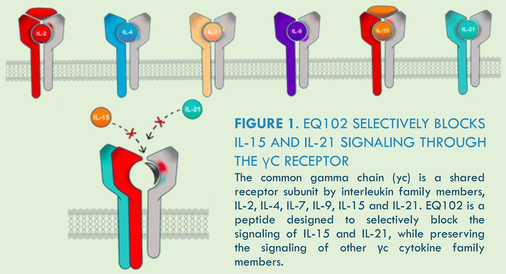


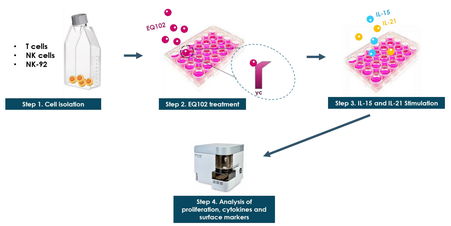
FIGURE 1. EQ102 SELECTIVELY BLOCKS IL-15 AND IL-21 SIGNALING THROUGH THE γC RECEPTOR

The common gamma chain (γc) is a shared receptor subunit by interleukin family members, IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21. EQ102 is a peptide designed to selectively block the signaling of IL-15 and IL-21, while preserving the signaling of other γc cytokine family members.

Methods

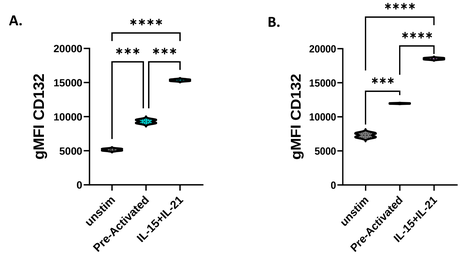
- **NK-92, NK and T cell Stimulation:** NK-92 cells were obtained and cultured in the presence of IL-2. Prior to stimulation, NK-92 cells were starved of IL-2 overnight. Primary NK and T cells were isolated from fresh PBMCs (peripheral blood mononuclear cells) by negative selection using magnetic isolation kits (STEMCELL). Prior to use in assays, T cells were pre-stimulated with anti-CD3 and anti-CD28 antibodies for 24hr at 37°C. After 24hr, cells were washed and rested overnight.
- **Proliferation Labeling:** After resting, cells were collected and stained with a proliferation dye (Cell Trace Violet (CTV)).
- **Blockade of γc-receptor:** Cells were plated after CTV staining and treated with PEG40 control and a titration of EQ102 for 1hr at 37°C, allowing the inhibitors to block the common gamma chain receptor.
- **Cytokine Stimulation:** After blocking, cells were stimulated with IL-15 alone, IL-21 alone and IL-15 and IL-21 together for 24hr and 72hr at 37°C.
- **Confirmation of inhibition:** Supernatant was collected for detection of NK and CD8-relevant cytokines and cells were analyzed for surface activation markers by flow cytometry.

FIGURE 2. SCHEMATIC OF INHIBITION ASSAY



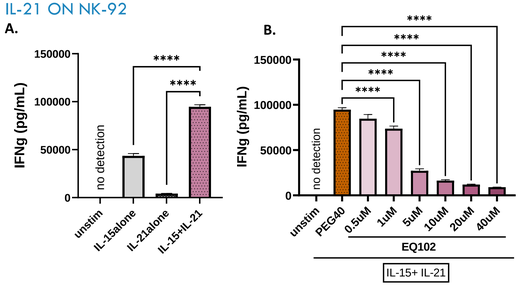
Results

FIGURE 3. γc EXPRESSION ON CD8 T CELLS AND CD56BRIGHT NK CELLS



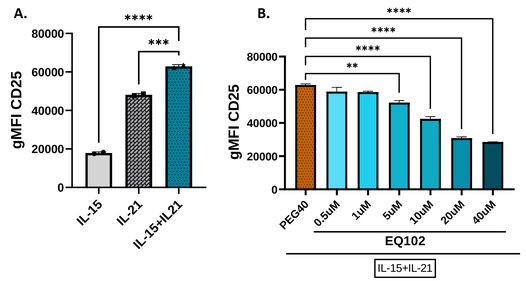
Expression of CD132 (γc) increased on both CD8 T cells (A) and CD56bright NK cells (B) following pre-activation with anti-CD3 and anti-CD28 and was further upregulated by IL-15 plus IL-21 stimulation.

FIGURE 4. EQ102 INHIBITS IFN-γ PRODUCTION DRIVEN BY IL-15 AND IL-21 ON NK-92



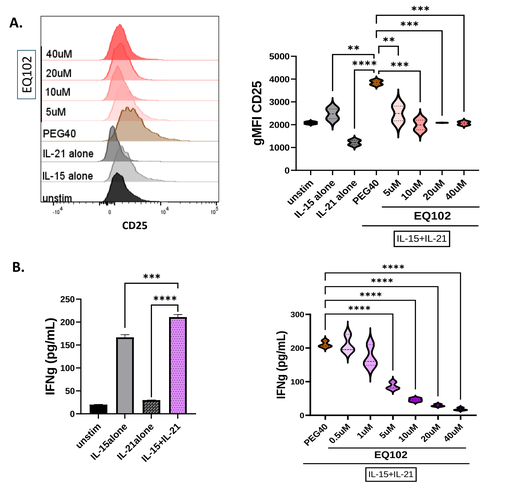
IFN-γ is known to play an important role in activating both the innate and adaptive immunes systems[1]. Here we collected cell supernatant from IL-15 and/or IL-21 stimulated NK-92 cells and examined for IFN-γ production by ELISA. Results showed that stimulation with IL-15 alone enhanced the production of IFN-γ, however, co-stimulation with IL-21 further increased the production of this pro-inflammatory cytokine (A). The synergistic effect of IL-15 and IL-21 on IFN-γ production was significantly downregulated with the introduction of EQ102 (B).

FIGURE 5. EQ102 DECREASES EXPRESSION OF CD25 ON NK-92 CELLS



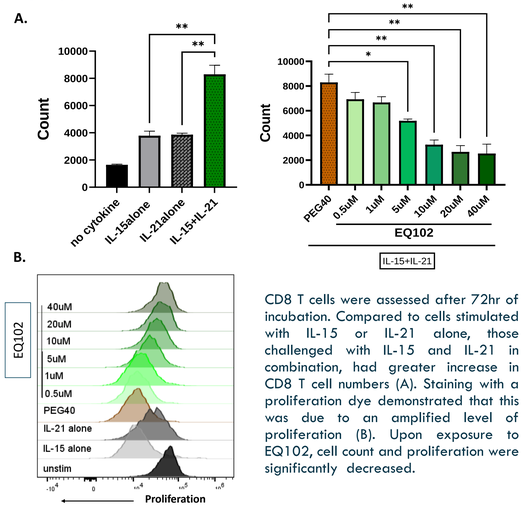
IL-15 and/or IL-21 stimulated NK-92 cells were examined for cell surface markers by flow cytometry. Geometric mean florescence intensity (gMFI) results of the activation marker, CD25, revealed inhibition potential by EQ102 on activation induced synergistically by IL-15 and IL-21 (A, B).

FIGURE 6. DOWNREGULATION OF ACTIVATION AND IFN-Y PRODUCTION ON CD56BRIGHT NK CELLS BY EQ102



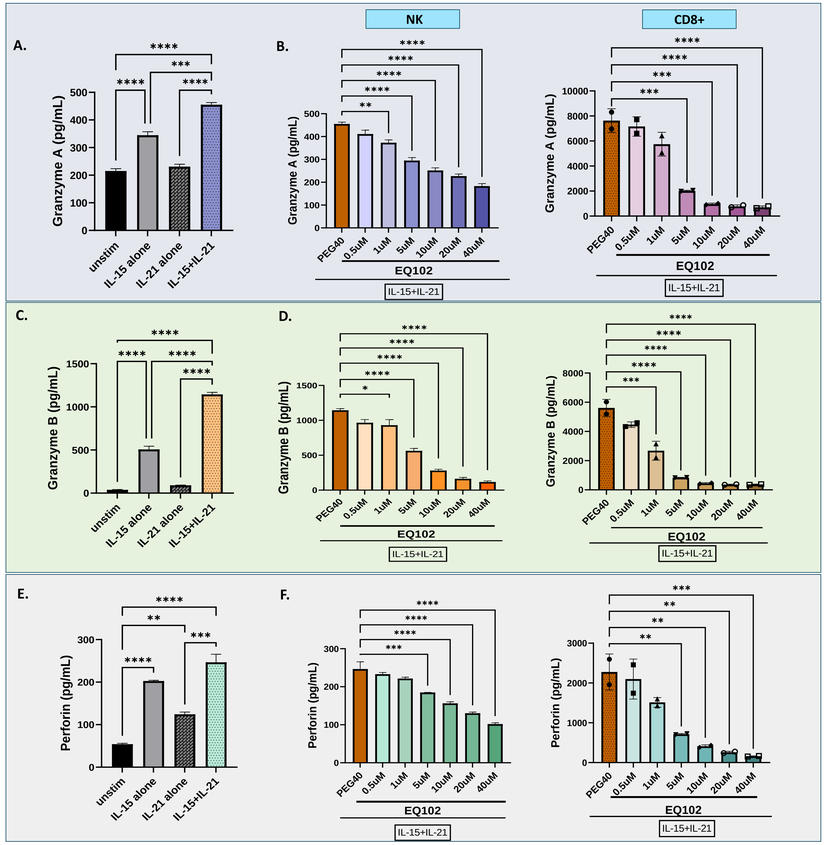
CD56bright NK cells are effective producers of cytokines following combined IL-15 and IL-21 stimulation and function primarily in immunomodulation[2]. Primary NK cells were stimulated with IL-15 and/or IL-21 under similar conditions as NK-92 cells. This resulted in elevated levels of IFN-γ production upon stimulation with the cytokine combo in comparison to IL-15 or IL-21 alone (B). Additionally, CD56bright NK subpopulation also increased expression of CD25 (B). Introduction of EQ102 reduced the cytotoxic activities of this NK subpopulation by downregulating activation and cytokine production (A,B).

FIGURE 7. EQ102 DECREASES PROLIFERATION OF CYTOTOXIC CD8 T CELLS



CD8 T cells were assessed after 72hr of incubation. Compared to cells stimulated with IL-15 or IL-21 alone, those challenged with IL-15 and IL-21 in combination, had greater increase in CD8 T cell numbers (A). Staining with a proliferation dye demonstrated that this was due to an amplified level of proliferation (B). Upon exposure to EQ102, cell count and proliferation were significantly decreased.

FIGURE 8. EQ102 DOWNREGULATES CYTOLYTIC ACTIVITY OF PRIMARY CD8 AND NK CELLS



Primary NK and CD8 T cells were stimulated with IL-15 and IL-21, and then granzyme A, granzyme B and perforin were quantified from cell supernatant. Stimulation with Il-15 or IL-21 alone increased secretion all 3 cytolytic enzymes by both NK and CD8 T cells (A, C, D), however, these levels were significantly heightened by the synergistic signaling of both IL-15 and IL-21. Blockade of IL-15 and IL-21 signaling by EQ102 resulted in significant dose-dependent decreases in production of granzyme A (B), granzyme B (D) and perforin (F) for both cytotoxic NK and CD8 T cells.

Discussion

- Stimulation with IL-15 increases proliferation and pro-inflammatory cytokine production among NK, NK-92 and CD8+ T cells, where IL-21 had a modest effect. However, co-stimulation of IL-15 and IL-21 enhanced proliferation, activation, and IFN-γ, Granzyme A, Granzyme B, and Perforin production above single cytokine conditions.
- EQ102 treatment effectively inhibits the IL-15/IL-21 co-stimulatory cytolytic responses of these cell populations.
- These results demonstrate that the use of selective blockade of IL-15 and IL-21 by EQ102 inhibits the synergistic signaling that mediates NK and CD8+ T cells responses in multiple disorders.

References

1. Strengell et al. 2003
2. Wagner et al. 2017
3. Kim et al, 2009

Disclosures

This study was funded by Equillium, Inc.
Phoi Tiet, AJ Giovannone, Laith Al-Mawsawi, Dalena Chu, Jeanette Ampudia, Stephen Connelly and Cherie Ng are currently employees and stockholders of Equillium. Stephen Connelly is currently employee, stockholder, and officer of Equillium.

Additional Information: To request permission or to ask any questions about the poster, please contact medinfo@equilliumbio.com

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