Lenalidomide alters the threshold for NK cell activation and augments NK cell effector functions

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Lenalidomide is an immunomodulatory drug (IMiD®) that is used to treat multiple myeloma. There is evidence to show that natural killer (NK) cells play an important role in the immune response against multiple myeloma. We are investigating the effect of lenalidomide on NK cell activation and immune synapse formation.

Introduction

- NK cells detect diseased and stressed cells and respond through direct cytotoxicity and by the production of cytokines such as IFN-γ.
- NK cells distinguish between healthy and diseased cells through a balance of signals from activating receptors (e.g. CD16 and NKG2D) and inhibitory receptors. How these signals are integrated has yet to be elucidated.
- Lenalidomide is a second generation IMiD and novel agent used to treat a variety of diseases, including multiple myeloma, mantle cell lymphoma and myelodyplastic syndromes
- It has been suggested that lenalidomide can enhance the function of NK cells although the molecular mechanisms are unknown.

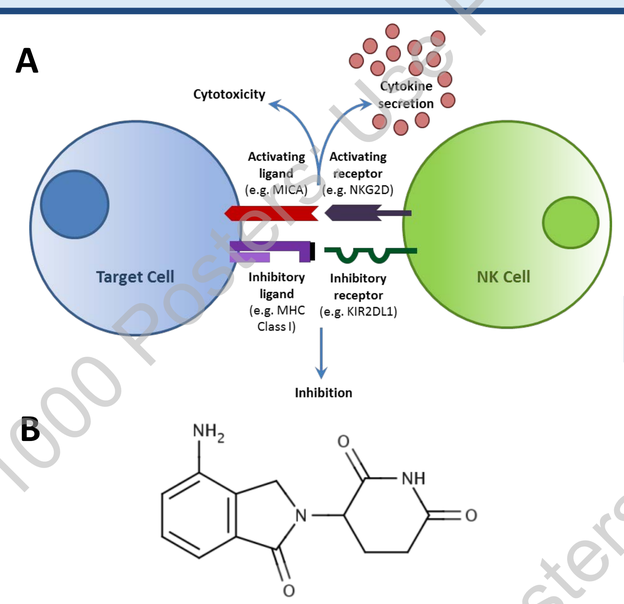

Figure 1 – (A) Schematic of NK cell synapse. **(B)** Chemical structure of lenalidomide

Main Findings

Here, we show that lenalidomide augments NK cell-mediated cytotoxicity and also increases the secretion of IFN-γ from primary human NK cells in conjugate with NK-sensitive target cells. The increase in IFN-γ secretion occurs after ligation of specific activating receptors, including CD16 and NKG2D. This establishes that lenalidomide acts downstream of different activating receptor signals.

When investigated in more detail, treatment with lenalidomide resulted in a two-fold increase in the proportion of primary NK cells producing IFN-γ, and a 10-fold increase in the amount of IFN-γ produced per cell, in response to ligation of CD16.

In addition, in the presence of Lenalidomide, NK cells were activated by lower concentrations of MICA used to coat slides, indicating that lenalidomide alters the threshold for NK cell activation. Taken together, these results show that lenalidomide can directly influence the NK cell immune response. We are now investigating the molecular basis by which lenalidomide has this effect on NK cells.

Lenalidomide affects both NK cell cytotoxicity and cytokine secretion

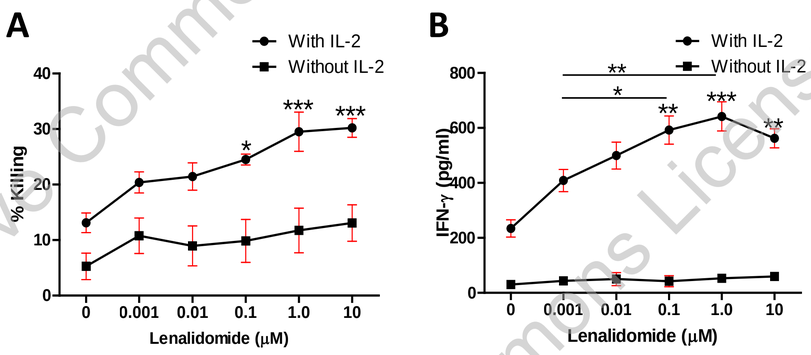

Figure 2 – (A) Radio-labelled Daudi B cells and IL-2 cultured primary NK cells were pre-treated for 24 hours with DMSO or 0.001-10 μM lenalidomide. The cells were then co-cultured for 5 hours. % Killing was assessed using a radioactive-release assay. **(B)** Daudi B cells and IL-2 cultured primary NK cells were co-cultured for 24 hours with DMSO or 0.001-10 μM lenalidomide. IFN-γ release was measured by ELISA. Mean of 3 donors ± SEM, analysed by one-way ANOVA

Lenalidomide enhances IFN-γ secretion after NK cell activation via different receptors

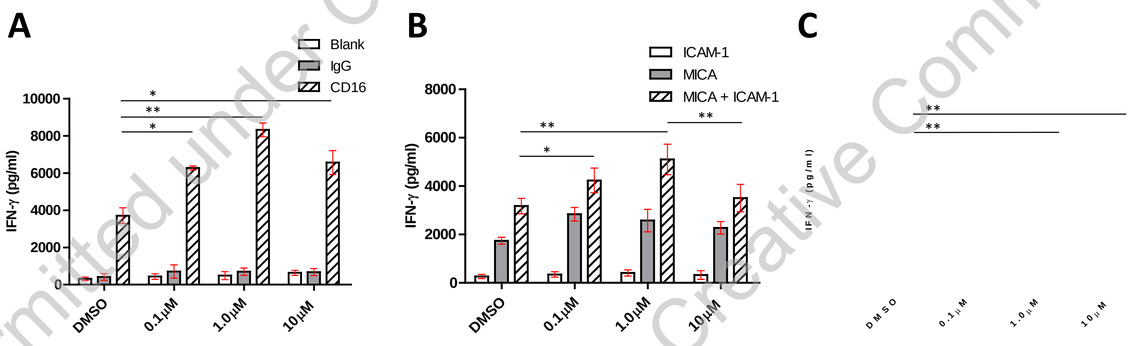

Figure 3 – Primary NK cells were treated with 0.1-10 μM lenalidomide for 24 hours in wells coated with **(A)** anti-CD16 antibody (3μg/ml), **(B)** recombinant MICA (2.5μg/ml) ± ICAM-1 (2.5μg/ml) or **(C)** anti-NKG2D and anti-2B4 mAbs (both at 3μg/ml) to stimulate the NK cells. IFN-γ release was measured by ELISA. Mean of 3 donors ± SEM, analysed by one-way ANOVA

Lenalidomide lowers the threshold for NK cell activation through NKG2D

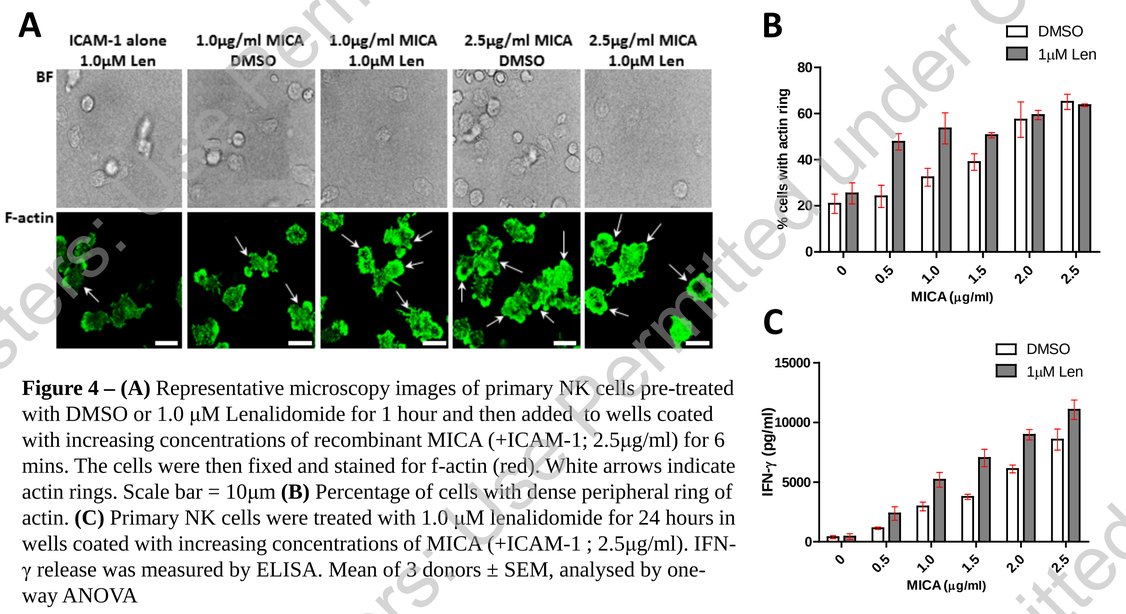

Figure 4 – (A) Representative microscopy images of primary NK cells pre-treated with DMSO or 1.0 μM Lenalidomide for 1 hour and then added to wells coated with increasing concentrations of recombinant MICA (+ICAM-1; 2.5μg/ml) for 6 mins. The cells were then fixed and stained for f-actin (red). White arrows indicate actin rings. Scale bar = 10μm **(B)** Percentage of cells with dense peripheral ring of actin. **(C)** Primary NK cells were treated with 1.0 μM lenalidomide for 24 hours in wells coated with increasing concentrations of MICA (+ICAM-1 ; 2.5μg/ml). IFN-γ release was measured by ELISA. Mean of 3 donors ± SEM, analysed by one-way ANOVA

Lenalidomide increases both the proportion of NK cells expressing IFN-γ and the amount of IFN-γ produced by each cell

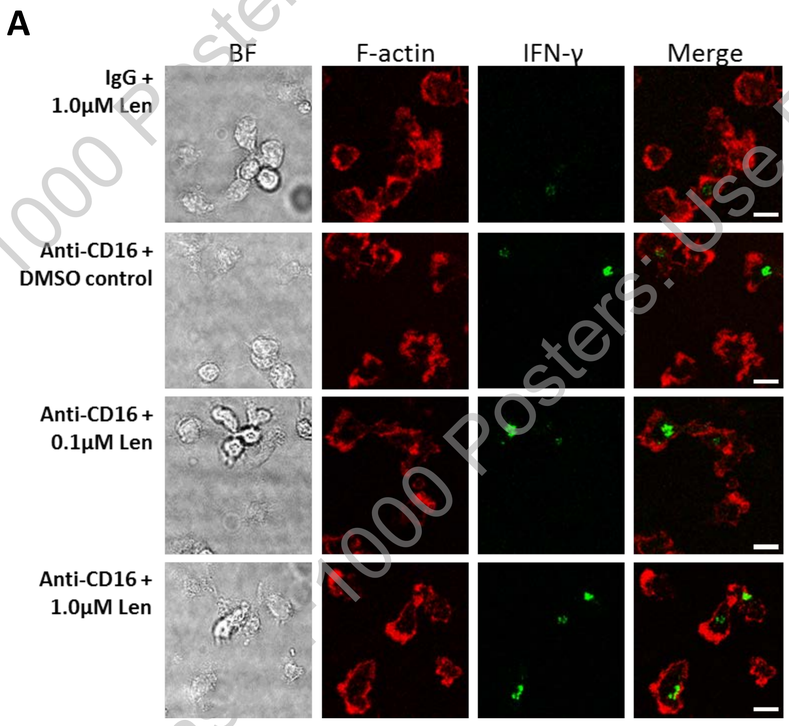

Figure 5 – (A) Representative microscopy images of primary NK cells treated with DMSO or 0.1-10 μM lenalidomide for 2 hours in wells coated with anti-CD16 antibodies and then fixed and stained for IFN- γ (green) and F-actin (red). Scale bar = 10μm **(B)** Proportion of primary NK cells expressing IFN-γ (mean±SEM). **(C)** Number of IFN-γ vesicles per cell (identified using the image analysis program Imaris) **(D)** Total IFN- γ fluorescence per cell. Each dot represents one cell. Bars show mean ± SEM). All data analysed by one-way ANOVA with Bonferroni post-test.

Conclusions

(1) Lenalidomide treatment increases both cytotoxicity and IFN-γ production by activated NK cells

(2) Lenalidomide augments NK cell function triggered by different activating receptors.

(3) Lenalidomide increases both the proportion of NK cells expressing IFN-γ and the amount of IFN-γ produced by each NK cell.

(4) Lenalidomide lowers the threshold for NK cell activation through NKG2D, resulting in NK cells responding to lower levels of MICA.

Taken together, these results demonstrate that lenalidomide has a direct effect on NK cell function, after the cells have been stimulated, by augmenting both cytotoxicity and IFN-γ secretion.