NOVEL PHYTOCHEMICALS-BASED MEDIA SUPPORTS NK CELL EXPANSION USING NEITHER FEEDER CELLS NOR IL-2, WHILE ACHIEVING HIGH CYTOTOXICITY AGAINST CANCER CELLS


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Background Feeder-free NK cell expansion reduces cost and simplifies the approval of downstream therapies. Eliminating the use of IL-2 has the added benefit of reducing cellular exhaustion, leading to enhanced cytotoxicity. As previously reported, the novel phytochemicals-based media Enable-NK consistently exhibits enhanced proliferation and cytotoxic activity of NK cells compared to multiple standard medias for NK cells. Here, optimization of NK cell expansion using Enable-NK was achieved in the absence of IL-2 and feeder cells.

Methods NK cells from three different donors were activated by overnight culture in the presence of IL-12 (10 ng/ml), IL-15 (50 ng/ml), and IL-18 (50 ng/ml), and half were non-activated. In the first experiment, activated and non-activated cells were expanded in Enable-NK media with only IL-15; this was compared to an expansion protocol using both IL-2 (100 IU/ml) and IL-15 (10 ng/ml). In the second experiment, expansion in Enable-NK was compared to control medium following activation, with IL-15 (10 ng/ml) only. Additionally, phenotype was assessed using flow cytometry, and cytotoxicity against the neuroblastoma cell line CHLA-20 was assessed using an Incucyte imager. The anti-GD2 monoclonal antibody Ch14.18 was added to assess the antibody-dependent cellular cytotoxicity (ADCC) capacity of the expanded NK cells.

Results In the first experiment, activated NK cells achieved greater expansion than non-activated cells. The greatest proliferation was in the IL-15-only activated culture, which expanded 54-fold in 21 days. In the second experiment, activated cells from 3 separate donors cultured in Enable-NK expanded an average of 34-fold in 15 days, with the activated control culture only expanding 15-fold (figure 1). Activated NK cells expanded using Enable-NK media limited the growth of CHLA-20 to 30.3% (± 2.19 SEM) of what was observed with CHLA-20 alone over the course of 68 hours (figure 2). This growth was further limited to 21.1% (± 3.52 SEM) with the addition of the anti-GD2 monoclonal antibody. In comparison, activated control expanded NK cells limited growth to 50.2% (± 2.38 SEM) and 37.8% (± 5.64 SEM) (without and with the anti-GD2 antibody, respectively). On average, 25% of activated Enable-NK expanded cells expressed the memory associated marker CD57, and 80% retained expression of CD16, which correlates with observed ADCC.

Conclusions Here we report a novel NK expansion method without the use of feeder cells or IL-2. With this method we have achieved robust expansion over the course of 2 to 3 weeks with enhanced cytotoxicity, including retention of ADCC functionality.

REFERENCES

Ethics Approval This study obtained ethics approval from the institutional review board MRR IRB for the University of Wisconsin-Madison (ID number 2017-1070-CR006). All participants gave informed consent before taking part in this study.