ABSTRACT

NK cells’ immediate cytolytic response renders them promising candidates for cancer immunotherapy. However, insufficient effector potency and toxicity of systemic IL-2 applications limited traditional approaches. Novel GMP-compliant culture conditions involving IL-15, a potentially more tolerable cytokine, were investigated to generate powerful NK. NK from 2 donors expanded on average 26-fold in LAK AIM-V/IL-2 cultures, but 63-fold in AIM-V/IL-15, within 28 days. Replacing AIM-V/IL2 with RPMI-1640/IL-2 yielded only 12±10-fold vs. 22±15-fold NK expansion within 22 days for 3 donors tested. AIM-V-generated LAK were highly cytotoxic against K562 compared to RPMI-LAK. Strikingly, CellGro/IL-2-cultured LAK expanded on average 191-fold and NK 738-fold. Cell-expansion with IL-15 was similar, on average 235-fold and NK 753-fold until day 21. Furthermore, NK were identified as the main cytotoxic effectors in expanded cultures. These data indicate that NK determines overall efficacy of LAK. Expression of KIR, NKG2A/C/D and NCRs were significantly upregulated after CellGro/IL-15 expansion, CD27 was unchanged and NK became CD56bright/CD16bright. Such effectors targeted K562 (MHC I+ CML), KU812 (MHC I+ CML) and MOLT-4 (T-ALL), but not autologous PBMC. 14-day expansion of NK with CellGro/IL-15 seems promising in generating highly potent NK cells for adoptive immunotherapy against haematological malignancies and other cancers. (195 words)

INTRODUCTION

Biologic approaches like immunotherapy involving NK cells could overcome limitations of typical cancer treatments including chemotherapy and surgery. NK cells play an integral part of the innate immune system and can naturally eradicate pathogen infected and neoplastic cells. NK express an array of activating and inhibitory receptors on their surface, which engages cognate ligands expressed by target cells. NK therefore can respond very quickly and target many types of malignancies. However, traditional approaches using short-term expanded IL-2 stimulated LAK cells (Rosenberg, 1989) were not as clinically effective as anticipated likely due to insufficient numbers and cytotoxicity of NK effectors. Furthermore systemic IL-2 application is highly cytotoxic. We aim to develop and evaluate improved culture conditions using the novel, more tolerable cytokine IL-15. (Oei, 2009)
MATERIALS AND METHODS

Blood was obtained from consented donors in the BloodBank. NK were long-term expanded with IL-2 (1000-2000 U/ml) or IL-15 (500-1000 U/ml) within stimulated PBMC or PBL (LAK) cultures, RPMI-1640 or AIM-V, 5% AB-HS, 5% hiFBS, or CellGro®-SCGM, 10% hiFBS; viability was assessed by trypan blue-exclusion-assays and cytotoxicity against K562 (E:T = 3:1, 4hr) by flow cytometry. After determining the optimum culture condition to be 500UIL-15 with CellGro SCGM expanded for 14 days, the NK cells were characterised. Based on the optimised culture conditions, NK cells were expanded as previously optimised. NK cells were surface stained on day 0 and day 14 for CD16, CD27, KIRs, NKG2D/A/C and NKp30/44/46 and interrogated by flow cytometry to determine any changes in expression of these receptors. Cytotoxicity assays against MOLT-4, KU812, K562 and PBMCs were carried out and analysed by flow cytometry.

RESULTS

We first compared AIM V/IL-2 conditions for three donors to traditional RPMI and found that NK cell numbers increased 4-8 fold as compared to only 1-2 fold in RPMI as shown in figure 1. Furthermore, AIM V NK was significantly more cytotoxic than RPMI NK, max. 94% vs 54%. Interestingly, long-term expansion had resulted in CD56bright/CD16bright cells. However additional studies are necessary to better characterise such not very well understood cell type. Data are representative for 3 donors tested.

Figure 1: AIM V conditions resulted in greater NK expansion and increased cytotoxicity compared to RPMI.

After long-term LAK expansion in AIM V, cultures consisted mainly NK (CD56+CD3-) and NKT (CD56+CD3+) cells. We magnetically purified the populations and tested them in cytotoxicity assays against K562. Figure 2 showed that NK cells were 2-5 times more cytotoxic than NK T cells. Data are representative for 3 donors tested.

We then further studied differences between IL-2 and IL-15 stimulation. As shown in figure 3, we used different doses of IL-15, 100U/ml, 500U/ml and 1000U/ml and compared to 1000U/ml IL-2 (high dose). NK fold expansion and cytotoxicity were generally comparable (donor variability), however, we found that the percentage cytotoxicity ranged 5-20% (slightly) higher for IL-15 at all doses for 4 donors tested.

We aimed to further improve NK expansion since in AIM V conditions we obtained a maximum of only 50 fold. It was previously shown that the novel media CellGro SCGM/IL2 could produce almost 1000 fold NK expansion within 14 days (Alici, 2008). We tested, if we could replace IL-2 with the less toxic novel cytokine IL-15. As shown in figure 4 IL-15
stimulation of PBL have comparable effects on NK expansion and cytotoxicity with IL-2 for 4 donors tested. Both conditions generally led to 65-90% LAK toxicity at 1:1 E:T ratio against K562, exceeding IL-2 cultures by 1-20%.

Figure 2: NK are the main effectors in long-term expanded LAK cultures.

Figure 3: IL-15 stimulation of PBL has comparable effects on NK expansion and cytotoxicity with IL-2 in AIM V.

Figure 4: IL-15 stimulation of PBL has comparable effect in NK expansion and cytotoxicity with IL-2 in CellGro SCGM.

Cytotoxicity of LAK cells grown in CellGro SCGM/IL-15 against K562 peaked at Day 14 of culture. To further characterise such potent effectors we studied expression of activating and inhibitory KIRs, inhibitory NKG2A, activating NKG2C/D, NCR (NKp30/44/46), CD16 and CD27. Data demonstrated that these cells had a significant increase in expression of most of these receptors, in particular NKp44 and NKG2A for 5 donors tested. Furthermore, these killer
cells were highly cytotoxic against target cell lines other than K562 (CML), including MHC+ KU812 (CML) and MOLT-4 (T-ALL). However, effectors did not attack autologous PBMC which indicates that these highly potent killer cells seem to be safe for clinical application.

Figure 5: CellGro/IL-15 long-term expanded NK showed significant increase in expression of surface receptors and were highly cytotoxic against K562, KU812 and MOLT-4 but not PBMCs

DISCUSSION

Our results showed that culturing NK cells within LAK cultures in GMP compliant AIM V conditions including IL-2 or IL-15 might be useful for long-term expansion (28 days) and generated highly cytotoxic effectors. However, maximum expansion of only 50 fold was achieved. A much more powerful media recently published for NK cells is CellGro SCGM. Here we show that the novel cytokine IL-15 can be used in such cultures as a very promising way to generate highly cytotoxic adoptive immunotherapy effectors. Interestingly, we observed an overall increase of NK receptor expression after CellGro SCGM/IL-15 culture as similarly previously published (de Rham, 2007). We also found that NK displayed a novel, not well-characterised CD56 bright/CD16 bright phenotype. Future studies will show significance of these findings. We already demonstrated that such effectors were highly cytotoxic against several tumour target cell lines without targeting healthy donor tissues and we aimed to investigate the cytotoxicity against primary tumour tissues including AML, CML, ALL as well. This work forms the basis for future clinical studies to show efficacy in cancer therapy.

REFERENCES