111. Use of COX2 knockout Monocytes and osteoclasts

(University of California – Los Angeles)

Asset Overview

Product Type	Cell therapy
Disease Area	Oncology
Indication	Cancer
Current Stage	Lead Optimization
Target	NK cells
MoA	A monocyte and/or an osteoclast deficient in one or more biomarkers are effective in activating NK cells
Brief Description	• Immunotherapy with NK cells has been limited due to inability to obtain sufficient numbers of highly functional NK cells, or an effective means to activate NK cells in vivo. Thus, there is a great need in the art to identify therapeutic compositions and methods for improved NK immunotherapy. • The present invention is based, at least in part, on the discovery that a cancer vaccine comprising cancer cells deficient in one or more biomarkers are effective in activating NK cells and inducing immune response. • Such deficiency results in de-differentiation of the cancer cells, and the proteins presented on their cell surface provide effective signals in activating various types of immune cells, including NK cells. Similarly, it has been determined herein that a monocyte and/or an osteoclast deficient in one or more biomarkers are effective in activating NK cells. The present invention provides compositions and methods to activate and expand large numbers of NK cells in vivo , in vitro , or ex vivo for use in immunotherapeutic strategies.
Intellectual Property	WO2022178138A1
Publication	Adoptive transfer of osteoclast-expanded Natural Killer cells for immunotherapy targeting cancer stem-like cells in humanized mice. Cancer Immunol Immunother, (2016) Differential cytotoxicity but augmented IFN-γ secretion by NK cells after interaction with monocytes from humans, and those from wild type and myeloid-specific COX-2 knockout mice. Front Immunol, (2015)
Inventors	Anahid Jewett

Highlights

- Increase immune responses to tumors by super-charging patient NK cells in vivo.
- Allows activation of NK cell population in vivo (avoids ex vivo expansion).
- Could apply to NK cell immunotherapy in caner patients
- Could apply to combination cancer immunotherapies

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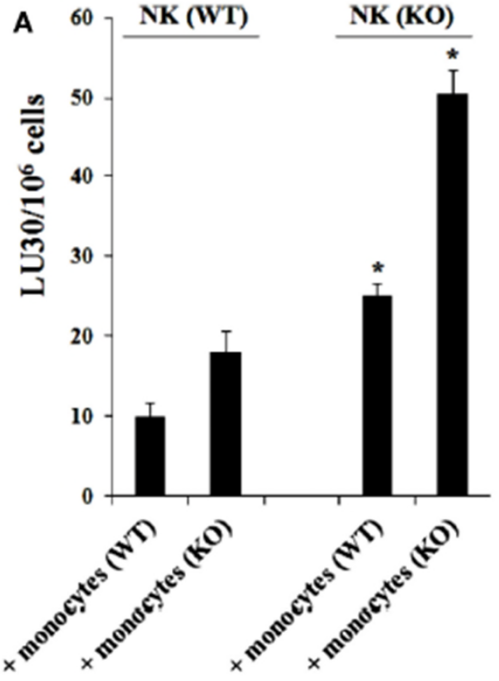
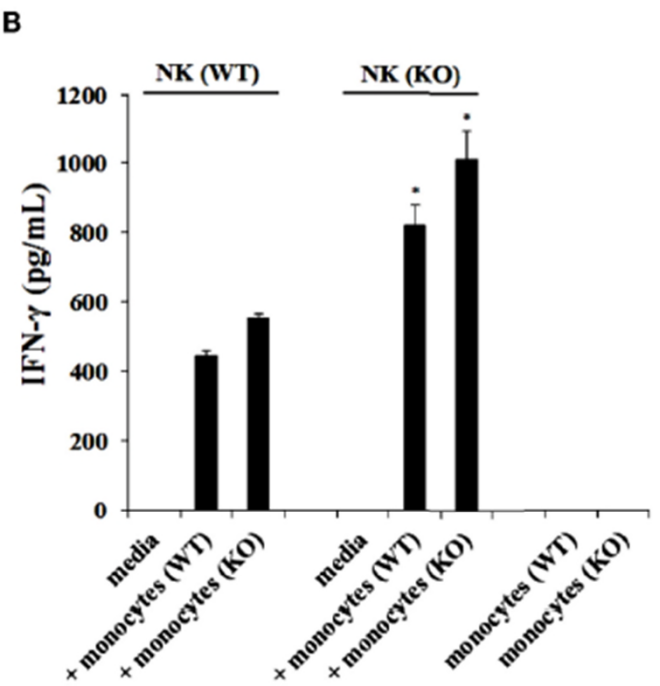
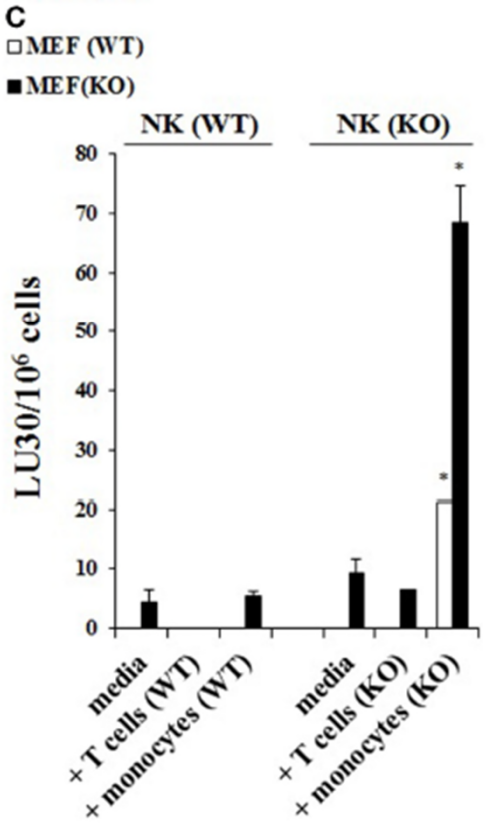
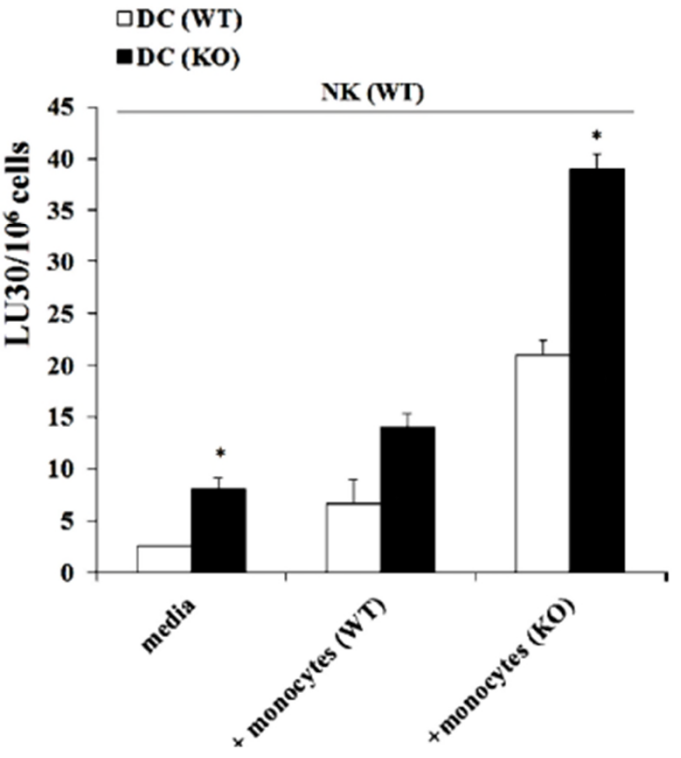
Key Data

Step Wise Increase in Cytotoxicity and IFN-γ Secretion









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5TH KDDF GLOBAL C&D TECH FAIR

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FIGURE 3 | Monocytes, and not T cells, from *Cox-2*$^{flox/flox}$;*LysM*$^{Cre/+}$ mice enhanced the cytotoxic function of autologous NK cells and induced high levels of IFN-γ secretion. Wild type or *Cox-2*$^{flox/flox}$;*LysM*$^{Cre/+}$ derived NK cells were activated with IL-2 (1×10^4 U/million) and cultured with either wild type or *Cox-2*$^{flox/flox}$;*LysM*$^{Cre/+}$ monocytes for 7 days. Afterward, the cytotoxic function of NK cells against YAC-1 was determined using a standard 4 h ^{51}Chromium release assay. The lytic units $30/10^6$ cells were determined using inverse number of NK cells required to lyse 30% of the target cells × 100. $^*P < 0.05$ is for the difference in cytotoxicity against YAC-1 tumors between IL-2-treated NK cells from control and *Cox-2*$^{flox/flox}$;*LysM*$^{Cre/+}$ mice cultured with monocytes **(A)**. NK cells were prepared as described in (A) and then supernatants from NK cell cultures were harvested after co-incubation with monocytes for 7 days. Monocytes from wild type and *Cox-2*$^{flox/flox}$;*LysM*$^{Cre/+}$ mice were used as control. The levels of IFN-γ secretion were determined using specific ELISAs. $^*P < 0.05$ is for the difference in IFN-γ secretion between IL-2-treated NK cells from control and *Cox-2*$^{flox/flox}$;*LysM*$^{Cre/+}$ mice cultured with monocytes **(B)**. NK cells were treated with IL-2 (1×10^4 U/million) and cultured with either T cells from global COX-2 knockout mice or monocytes from wild type or *Cox-2*$^{flox/flox}$;*LysM*$^{Cre/+}$ mice for 7 days. Afterward, NK cells were used as effectors against wild type MEFs or MEFs with specific COX-2 deletion. The cytotoxic function of NK cells against MEFs was determined using a standard 4 h ^{51}Cr release assay. The lytic units $30/10^6$ cells were determined using inverse number of NK cells required to lyse 30% of the target cells ×100. $^*P < 0.05$ is for the difference in cytotoxicity between IL-2-treated NK cells from control and *Cox-2*$^{flox/flox}$;*LysM*$^{Cre/+}$ mice cultured with monocytes or T cells **(C)**. IL-2-treated (1×10^4 U/million) NK cells obtained from wild type mice were cultured with monocytes from wild type mice or *Cox-2*$^{flox/flox}$;*LysM*$^{Cre/+}$ mice for 7 days before the cells were used as effector cells in a standard 4 h ^{51}Chromium release assay. Monocyte-derived DCs from wild type or *Cox-2*$^{flox/flox}$;*LysM*$^{Cre/+}$ mice were prepared as described in Section "Materials and Methods" and used as target cells. The lytic units $30/10^6$ cells were determined using inverse number of NK cells required to lyse 30% of the target cells × 100. $^*P < 0.05$ was obtained for the difference in IL-2-treated NK cell-mediated lysis between DCs from control mice and from those of *Cox-2*$^{flox/flox}$;*LysM*$^{Cre/+}$ mice **(D)**. One of several representative experiments is shown in this figure.

Source: Front Immunol. 2015 Jun 9;6:250. Fig.3

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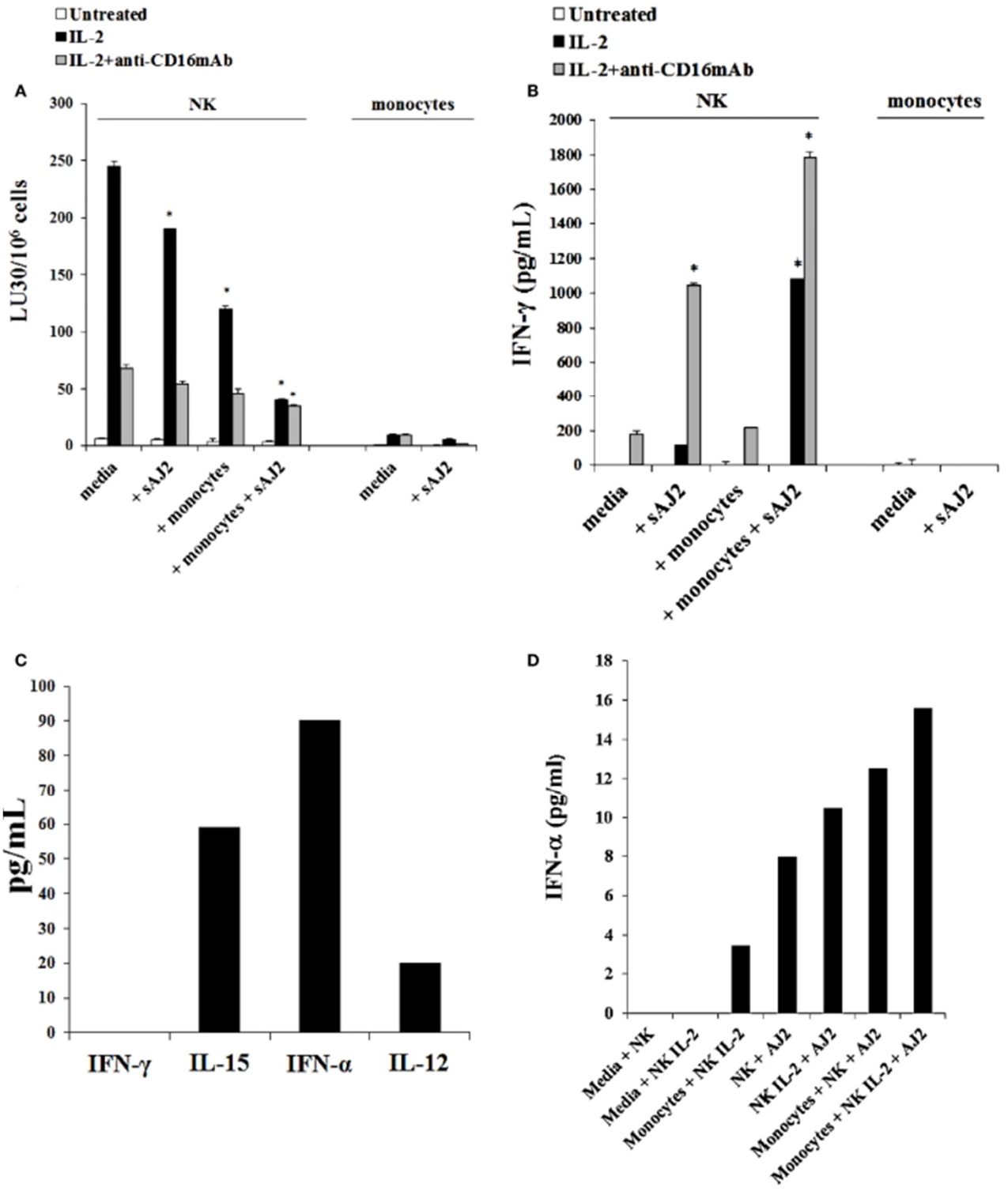
Split anergy induced by sAJ2 and monocytes also occurred in Human NK cells



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Split anergy induced by sAJ2 and monocytes also occurred in Human NK cells

FIGURE 8 | Split anergy induced by sAJ2 and monocytes also occurred in Human NK cells. Human NK cells were purified from healthy donors and left untreated or treated with IL-2 (1000 U/mL) or the combination of IL-2 (1000 U/mL) and anti-CD16mAb (3 μg/mL) in the presence of sAJ2 (NK cell:sAJ2, 1:3), autologous monocytes (NK cell:monocytes, 1:1) or the combination of sAJ2 (NK cell:sAJ2, 1:3) and autologous monocytes (NK cell:monocytes, 1:1) for 24–48 h. Afterward, the cytotoxicity against OSCSCs cells was assessed using a standard 4 h ^{51}Chromium release assay. Percent cytotoxicity was obtained at different effector to target ratio and the lytic units $30/10^6$ cells were determined using inverse number of NK cells required to lyse 30% of the tumor cells X100 **(A)**. NK cells were prepared as described in **Figure 6A** and after the treatment period, the supernatants were removed from the co-cultures and the levels of IFN-γ cytokine were measured with specific

(Continued)

FIGURE 8 | Continued
ELISA. $^*P < 0.05$ was obtained for the differences in cytotoxicity and IFN-γ secretion between human NK cells cultured in media and those treated with sAJ2, monocytes or the combination of sAJ2 and monocytes. One of several representative experiments is shown in this figure **(B)**. Purified NK cells were cultured with autologous monocytes (NK cell:monocytes, 1:1). After an overnight incubation, the supernatants were collected and the levels of IFN-γ, IL-15, IFN-α, and IL-12 were determined by ELISAs in a multiplexed format using Luminex technology **(C)**. Untreated and IL-2 stimulated NK cells were treated with monocytes and sAJ2 as described in (A). Afterward, the supernatants were collected and the level of IFN-α was determined by ELISAs in a multiplexed format using Luminex technology **(D)**.

Source: Front Immunol. 2015 Jun 9;6:250. Fig.8