Anti-Leukemia Effect Associated with Down-Regulated CD47 and Up-regulated Calreticulin by Stimulated-Macrophages in Co-Culture

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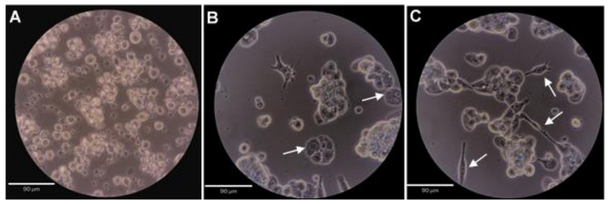
Highlight/précis

The AML cancer cells, not normal blood cells, were selectively eliminated by activated human macrophages that inhibit CD47 (don't eat me marker) and up-regulate calreticulin (CRT) in the co-culture model.

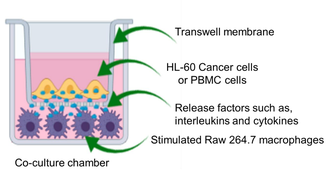
Differentiated macrophage from THP-1 cancer cells

Human THP-1 cells (image A below) were differentiated into macrophage-like cells (THP-1 macrophages) by incubation in the presence of phorbol 12-myristate 13-acetate (PMA, 5 ng/mL) overnight. Cell phenotype and morphological features, including both shape and size, were examined after the first four hours of incubation (B) and after overnight incubation (C).

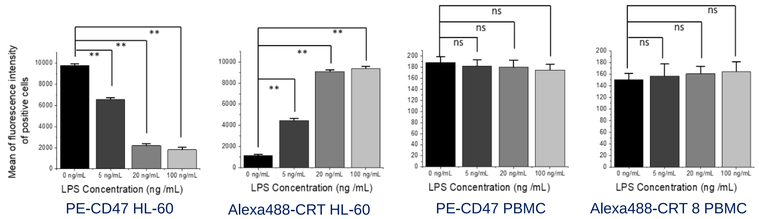


The PMA addition to THP-1 cells results in their differentiation into M1 macrophages, characterized by changes in morphology and increased cell surface expression of CD11b and CD14. These cells were then used as the human macrophage model to study the elimination of AML in co-culture.

Simultaneous down-regulation of CD47 and increased CRT on AML in co-culture



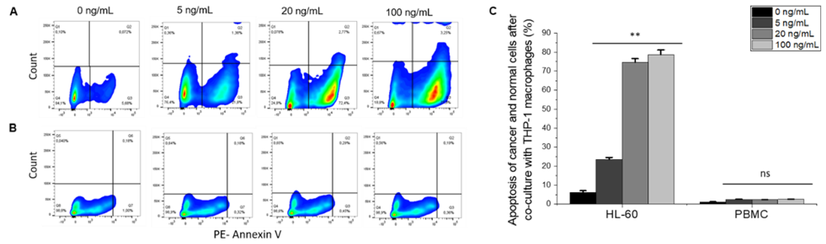
Human HL-60 cells were co-cultured with THP-1 macrophages in paired chambers. Lipopolysaccharide (LPS) at 0, 5, 20, and 100 ng/mL was added to the bottom chambers containing THP-1 macrophages and incubated for 16−18 h. All cells were then harvested and the levels of CD47 and CRT on HL-60 after co-culture were assessed using flow cytometry.



When HL-60 cells were cultured alone and treated with the same LPS concentrations used while co-cultured with THP-1 macrophages, CD47 and CRT levels did not show any significant changes compared to 0 ng/mL ($P > 0.05$). However, the change in the levels of both proteins was significant after co-culture, when compared to the levels in the absence of co-culture (** $P < 0.01$ at 5, 20, and 100 ng/mL), indicating the necessity of the co-culture model in order for this inhibition of CD47 and elevation of CRT to take place. Results on the PBMC normal cells revealed that the inhibition of CD47 and increase of CRT levels is selective for cancer cells.

Elimination of AML by macrophages in co-culture

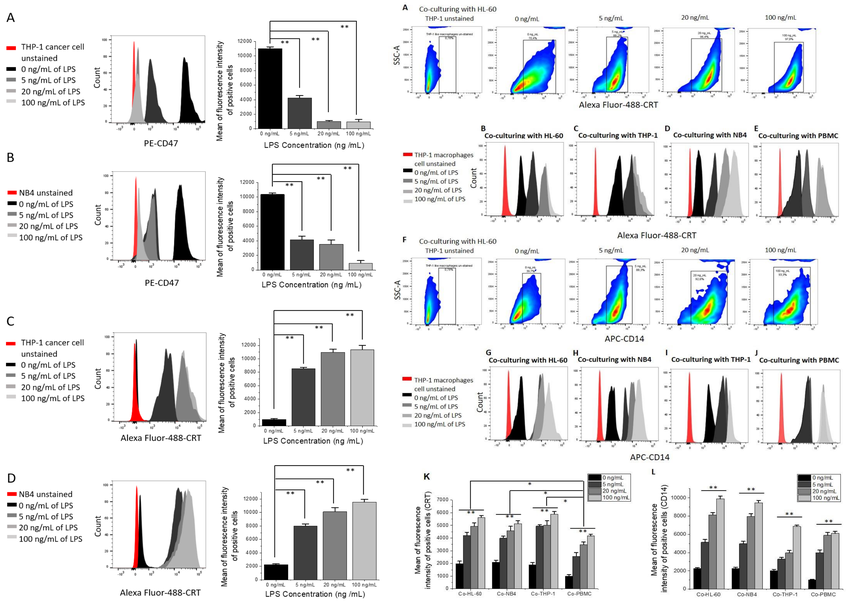
To further test our hypothesis and to investigate the elimination of cancer cells by macrophages, the apoptosis rate of HL-60 and PBMC (as the control) after co-culture with macrophages was determined. PE Annexin V apoptosis based detection followed by flow cytometric analysis was performed.



The elimination of cancer cells by macrophages as a result of LPS activation is selective to cancer cells and that the co-culture model is necessary for this cancer cell elimination to take place.

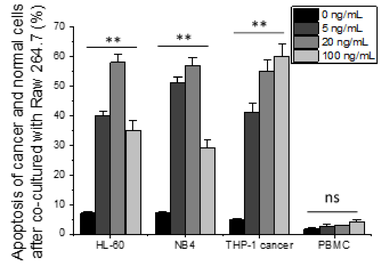
Down-regulation of CD47 & increased levels of CRT and CD14 on macrophages in co-culture

Other types of leukemia cells such as NB4 and THP-1 cancer cells were responsive to the co-cultured macrophages. Furthermore, the inhibition of CD47 and increased CRT expression levels were observed in all cancer cells, suggesting the macrophage elimination of leukemia cells only happens when co-culturing together.



Cancer cell elimination in co-culture using murine macrophage model Raw 264.7

Cancer cells (HL-60, NB4, and THP-1), as well as normal cells (PBMC) were each co-cultured with Raw 264.7 cells that were activated with the same concentrations of LPS. CD47 down regulation went from 41% to 64% at 5 and 20 ng/mL of LPS, respectively; and CRT levels increased at the same LPS concentrations in HL-60 cells. HL-60 and NB4 cells showed similar trends of decreased CD47 and increased CRT levels. However, at 100 ng/mL both cells showed a significant reduction in CD47 down regulation and increase of CRT levels compared to the same concentration of LPS (100 ng/mL) when both cell lines were co-cultured with THP-1 macrophages.

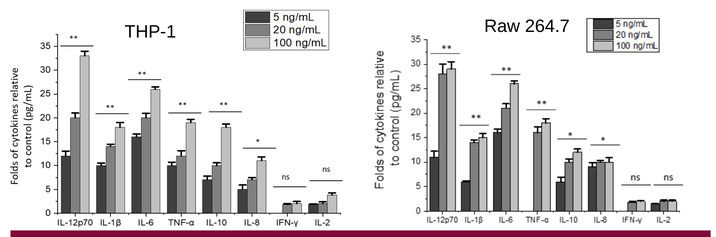


When investigating the apoptosis rate in all cancer cells mentioned above, we found similar CD47 and CRT level changes. In HL-60 and NB4 cells, the apoptosis rate increased as the LPS concentration increased compared to the control (0 ng/mL), except at 100 ng/mL, it drops from 58% at 20 ng/mL to 35% at 100 ng/mL for HL-60, and from 57% at 20 ng/mL to 29% at 100 ng/mL for NB4.

As the CD47 down-regulation and CRT increase levels were the highest in THP-1 cancer cells at 100 ng/mL of LPS, the apoptosis rate was also the highest with around 60% of THP-1 cancer cells being killed. This suggested that THP-1 cancer cells might be more sensitive to the elimination by Raw 264.7. PBMC cells did not show any significant increase of the apoptosis rate at all LPS concentrations used for activation when compared to the control (0 ng/mL).

Increased levels of M1 macrophage cytokines

The CD47 inhibition methods result in increased production of certain cytokines and stimulate phagocytosis. We also found that LPS stimulation resulted in significant productions of IL-12p70, IL-6, TNF-α, IL-1β, IL-10 and IL-8, compared to the control in a dose-dependent manner. All these cytokines are secreted from type M1 macrophages as a result of mainly LPS stimulation.



Conclusions

Our findings demonstrate the successful use of human macrophage model to inhibit CD47 and up-regulate CRT in AML cancer cells to increase their elimination in co-culture. Moreover, our data showed that this model is selective to AML cells, and does not affect normal blood cells, implicating the use of this model in drug screening targeted against AML.