Extended Summary

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Diesel Exhaust Particles Modify Natural Killer Cell Function and Cytokine Release

This study is now published and can be found under "Müller et al.: Diesel exhaust particles modify natural killer cell function and cytokine release. Particle and Fibre Toxicology 2013 10:16."

Background

Natural killer (NK) cells make up about 10% of the lung lymphocyte population and are an important immune cell population in the human nasal mucosa. They link the innate and the adaptive immune response, play an important role in viral and bacterial infections and in tumor fighting. NK cells are found in the airways (lung, nasal mucosa), the spleen and in the blood. Their two main functions are direct cell-mediated cytotoxicity and the release of cytokines. Due to their location in the nasal mucosa and the lung, NK cells are likely exposed to inhaled pollutants, such as diesel exhaust (DE). DE exposure was shown to increase the susceptibility to viral infection, such as influenza [Hahon, Booth, Green et al. 1985; Harrod, Jaramillo, Rosenberger et al. 2003; Ciencewicki and Jaspers 2007]. It was also shown that NK cell cytotoxicity was reduced in postmenopausal, overweight women living and exercising near major roadways [Williams, Ulrich, Larson et al. 2009] and that DE suppresses the *in vivo* IFN-γ production by NK cells in mice [Finkelman, Yang, Orekhova et al. 2004]. Whether and how exposure to DE particles (DEP) affects NK cell function in the context of viral infections has not been investigated.

Methods

NK cells were isolated from peripheral blood obtained from normal healthy volunteers (Dynabeads Isolation kit for untouched human NK cells) and subsequently stimulated with the viral mimetic polyinosinic:polycytidylic acid (pI:C, 10μg/ml), DEP (10μg/ml), or pI:C+DEP (10μg/ml each) for 18-20 hours. NK cells were subsequently analyzed for changes in surface marker expression (CD56, CD16, NKG2D, NKp46M; using flow cytometry), cytokine production (Interleukin (IL)-1β, IL-2, IL-4, IL-5, IL-8, IL-10, IL-12p70, IL-13, IFN-γ, and TNF-α; using a Meso Scale Discovery Th1/Th2 10-plex tissue culture kit), gene expression changes (granzyme B, perforin; using real-time quantitative PCR) and the cytotoxic potential of NK cells to kill target cells (Cayman cell-mediated cytotoxicity assay, K562 cancer cell line as target cells).

Results

Cytokine release (Table 1): Stimulation of NK cells with pI:C and pI:C+DEP, but not DEP alone, increased the release of IL-1β, IL-2, IL-4, IL-8, IL-10, IL-12p70, IFN-γ and TNF-α. As compared to pI:C alone or pI:C+DEP, the release of IL-1β, IL-8 and TNF-α was significantly lower after DEP stimulation alone. IL-4 was significantly less released by pI:C&DEP compared to pI:C alone.

Table 1. Cytokine release of NK cells after stimulation with pI:C, DEP and pI:C&DEP.

	pI:C	DEP	pI:C&DEP
IL-1β	*193.9±111	#6.09±5.0	*111.7±61.6
IL-2	*2.11±0.44	1.13±0.16	*1.191±0.38
IL-4£	*5.99±2.35	1.58±0.55	*2.55±0.66
IL-5	108.6±104	1.56±0.62	6.26±3.21
IL-8	*10.2±3.08	#1.53±0.65	*9.53±2.87
IL-10	*21.0±9.4	5.01±2.8	*30.6±19.4
IL-12p70	*142±109	56.7±53.6	*82.0±60.7
IL-13	15.1±6.75	1.82±0.59	8.81±4.4
IFN-γ	*844±414	496±495	*520±241
TNF-α	*37.1±14.6	#3.08±1.87	*26.4±8.81

Data are presented as fold induction over control and shown as mean ± SEM. *,#,£p<0.05.
*significant different from untreated control
significant different from PIC and PIC & DEP
£ significant difference between pI:C and pI:C & DEP

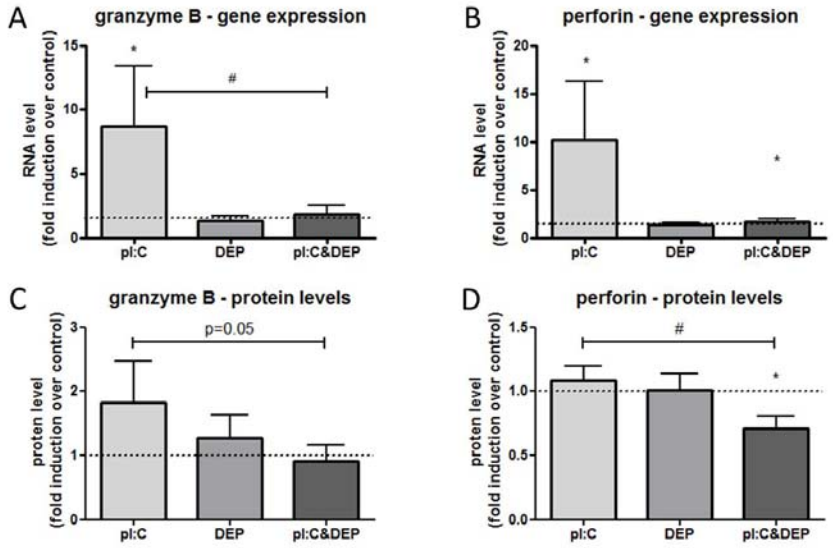

Figure 1. Cytotoxicity related factors in NK cells after stimulation with pI:C, DEP and pI:C & DEP. (A) Gene expression of granzyme B and (B) gene expression of perforin expressed as fold induction over untreated control. (C) Protein levels of granzyme B and (D) protein levels of perforin expressed as fold induction over untreated control. Data are presented as mean±SEM. *significant different from the untreated control, # significant different between each other (both tested with a Wilcoxon signed rank test, *,#p<0.05, n=9).

Granzyme B and perforin (Figure 1): Stimulation with pI:C alone increased the gene and protein expression of granzyme B and perforin, which was not found for DEP and was completely blunted by adding DEP to pI:C.

NK cell phenotype (Figure 2): NK cells were analyzed by flow cytometry for surface marker expression. Markers associated with NK cell activation (NKG2D and NKp46) or cytotoxic potential (CD16) were included. pI:C alone decreased the mean fluorescence intensity (MFI) of CD16 and addition of DEP further reduced the expression of CD16 in these cells. Conversely, the percentage of $CD16^-$ NK cells was increased by pI:C+DEP. NKG2D was not affected by pI:C or DEP, while the MFI of NKp46 was reduced by pI:C alone and DEP alone. DEP alone showed no effects on NKp46.

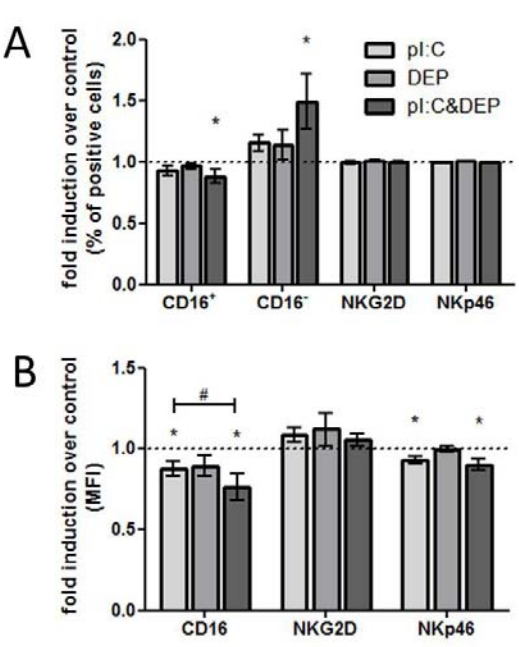

Figure 2. NK cell phenotypes assessed by flow cytometry after exposure to pI:C, DEP and pI:C & DEP. Surface markers presented as fold induction over untreated control of % of positive total NK cells (A) or mean fluorescecne intensity (MFI) (B). Data are presented as mean±SEM. *significant different from the untreated control, # significant different between each other (both tested with a Wilcoxon signed rank test, *,#p<0.05, n=9).

Cell-mediated cytotoxicity (Figure 3): Untreated control NK cells killed 53.3% of target cells on average (SEM 4.4, range of 22.2 to 82.6%). pI:C reduced the killing ratio slightly, but not significantly, while addition of DEP reduced the cytotoxic potential statistically significant compared to the untreated control and compared to pI:C alone (Figure 4F).

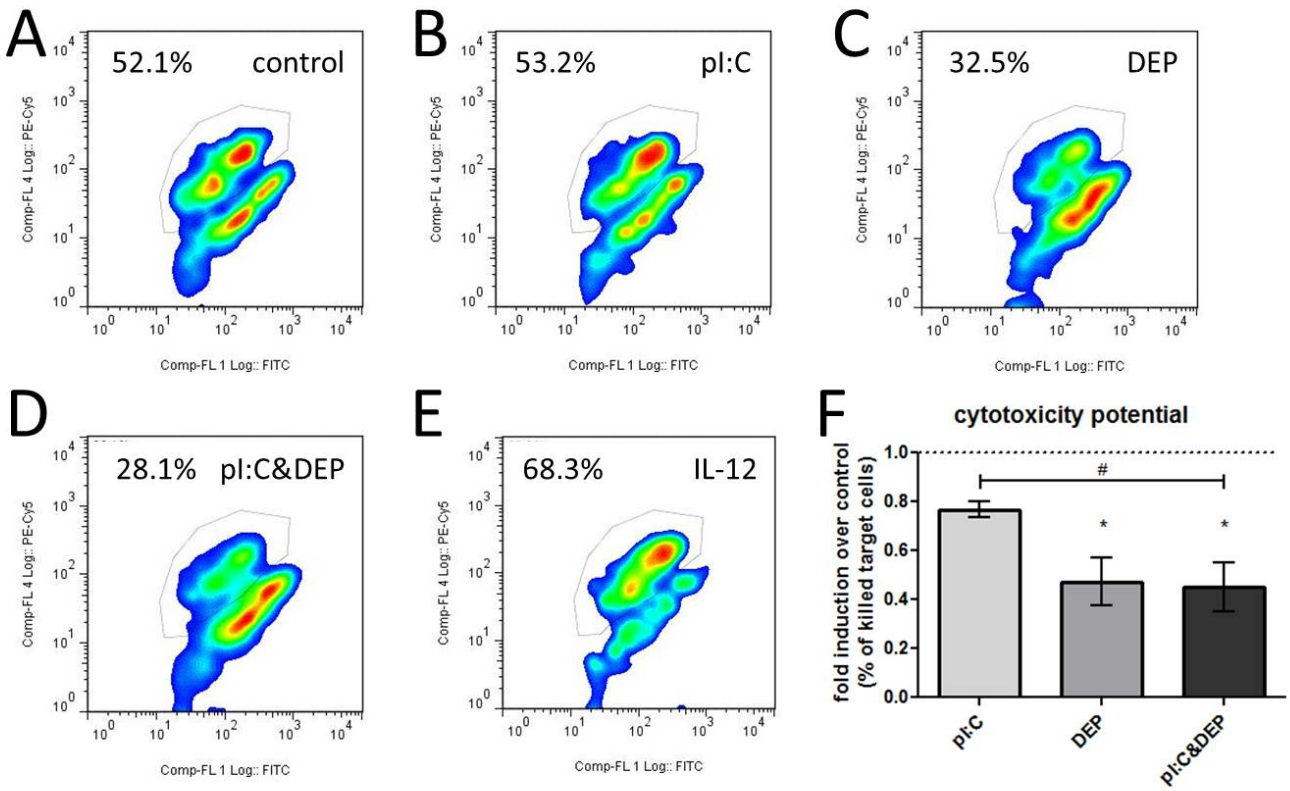

Figure 3. Cell-mediated cytotoxic potential of NK cells exposed to pI:C, DEP or pI:C & DEP. Results were assessed as % of killed target cells (K562 cancer cell line) and are expressed as fold induction over untreated NK cells. (A-E) Representative dot plots of all four conditions plus IL-12 stimulated NK cells to proof that the cytotoxicity can also be increased. (F) Summary of 5 individual experiments. Data are presented as mean±SEM. *significantly different from control, #significantly different between each other, *,#p<0.05.

Conclusions

In the context of viral infections, exposure to DEP reduces the cytotoxic potential of NK cells, but not the release of cytokines. This reduction in the potential ability of NK cells to kill virus-infected host cells may increase the susceptibility to viral infections after DEP exposure.

References

Ciencewicki, J. and I. Jaspers (2007). "Air pollution and respiratory viral infection." Inhal Toxicol **19**(14): 1135-1146.

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THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

Diesel Exhaust Particles Modify Natural Killer Cell Function and Cytokine Release

17th ETH Conference on Combustion Generated Nanoparticles

Zurich, Switzerland – June 26, 2013

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(from August 2013: University Children's Hospital Basel (UKBB), University of Basel, group of Philipp Latzin)

Why Natural Killer (NK) Cells & Diesel Exhaust Particles (DEP)?

Diesel & viral infections:
exposure to DE increases the susceptibility to viral infections, including influenza [Ciencewicki et al 2007, Harrod et al 2003, Hahon et al 1985]

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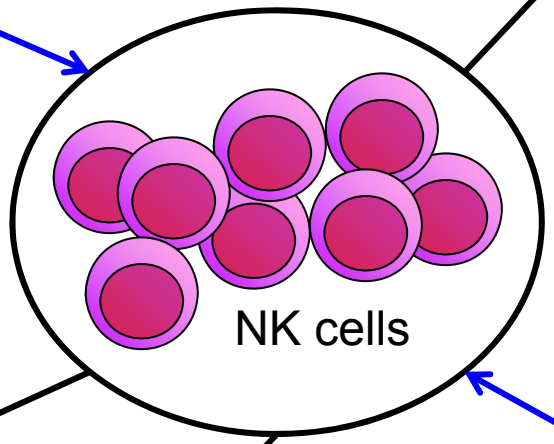


Role:
- Linking innate & adaptive immune response
- Important role in viral and bacterial infections
- Tumor fighting

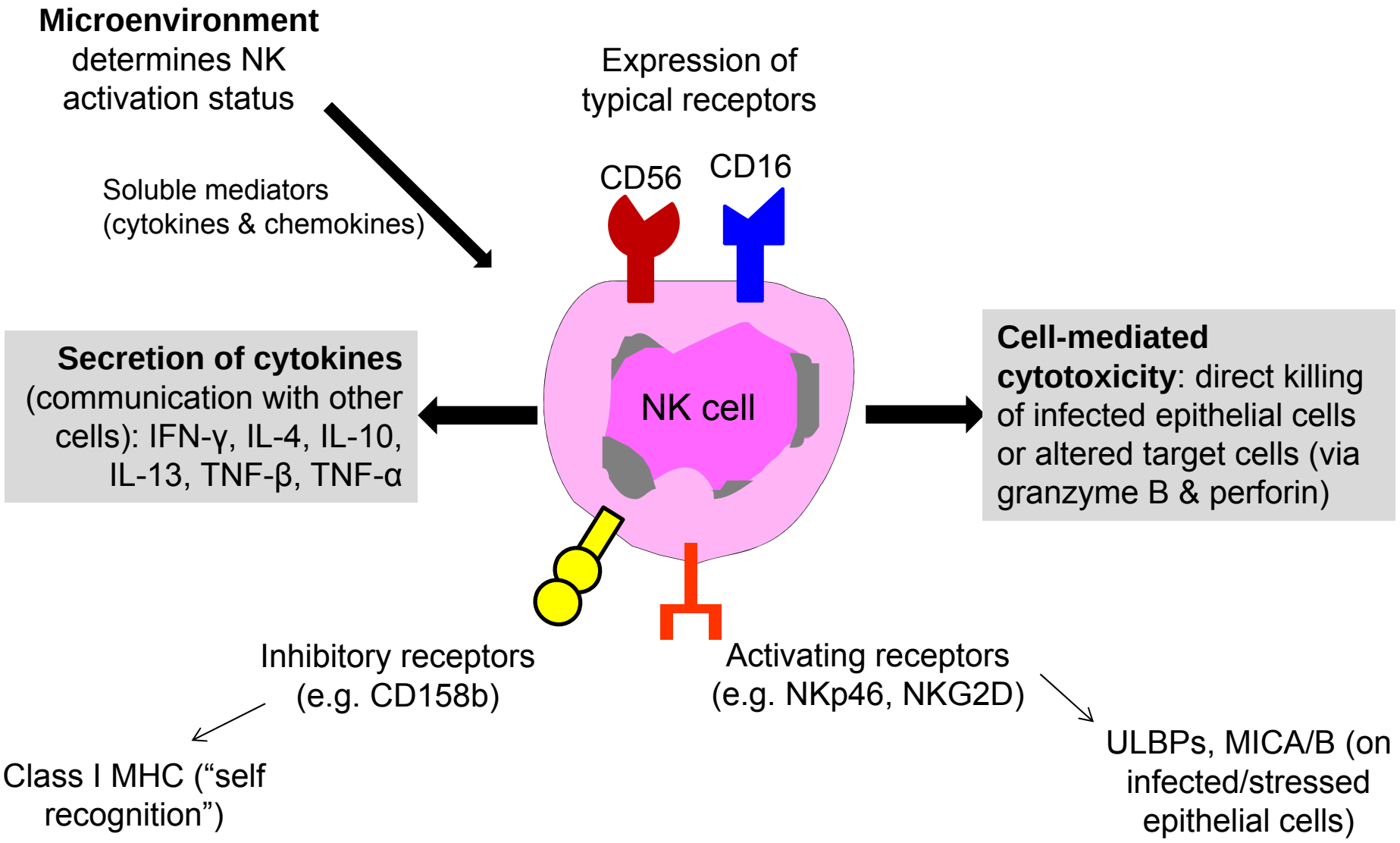
Localization:
- Airways (nasal mucosa, lung)
- Spleen
- Blood

Effector function:
- Release of cytokines
- Direct cell-mediated killing of target cells

???

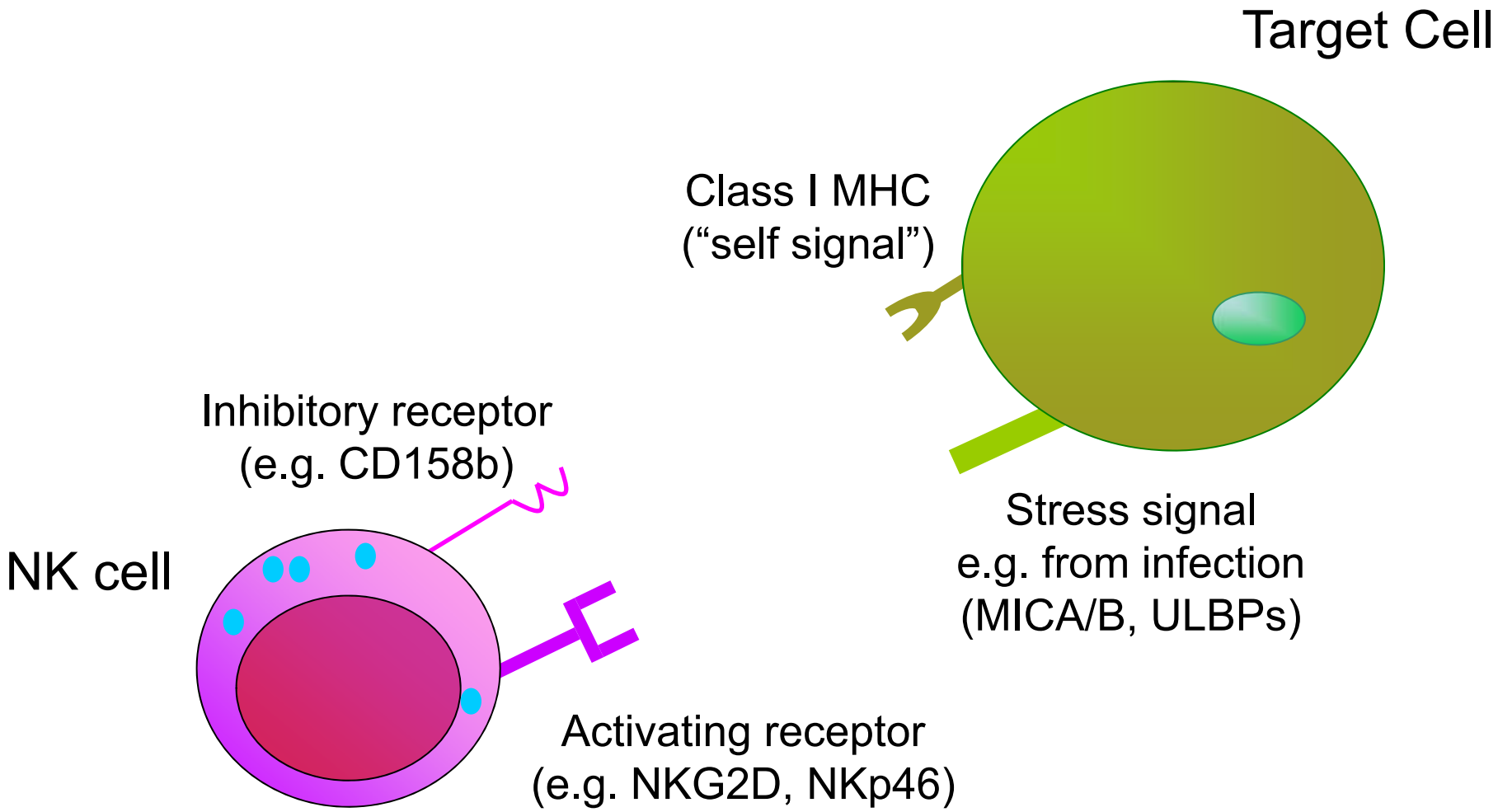
Effect of particles?
- reduced NK cell cytotoxicity in postmenopausal, overweight women living and exercising near major roadways [Williams et al 2009]
- diesel exhaust (DE) suppresses the *in vivo* IFN-γ production by NK cells in mice [Finkelman et al 2004]

Natural Killer (NK) Cells



[Farag&Caliguri, 2006 Blood Rev, Borchers et al Am J Physiol Lung Cell Mol Physiol 2006, Biassoni et al Curr Pharm Des 2009, Siren et al J Gen Virol 2004, Vivier et al Science et al 2011]

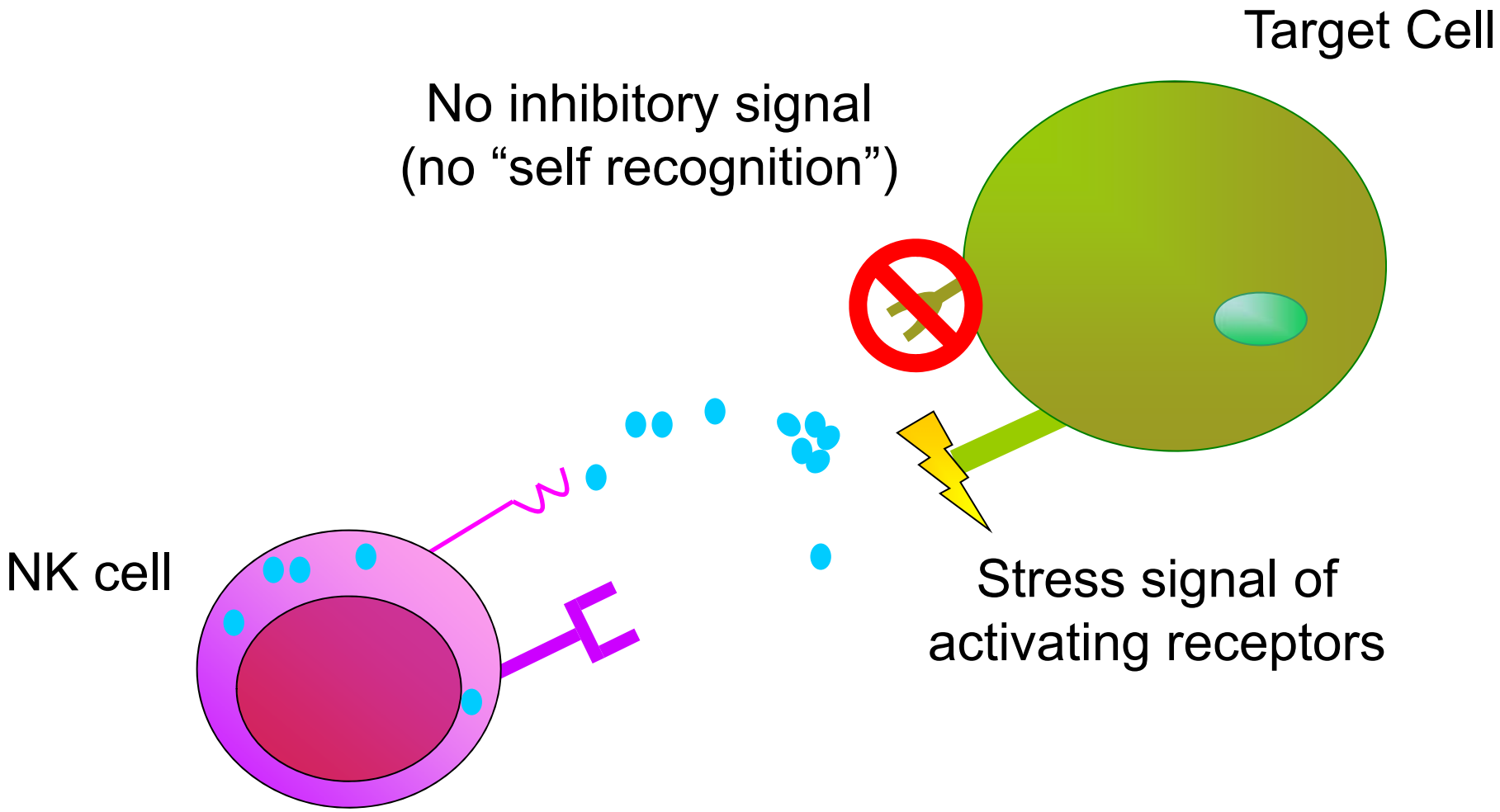
NK cell cytotoxicity = balance between activation & inhibition.



Courtesy to Michael Henderson

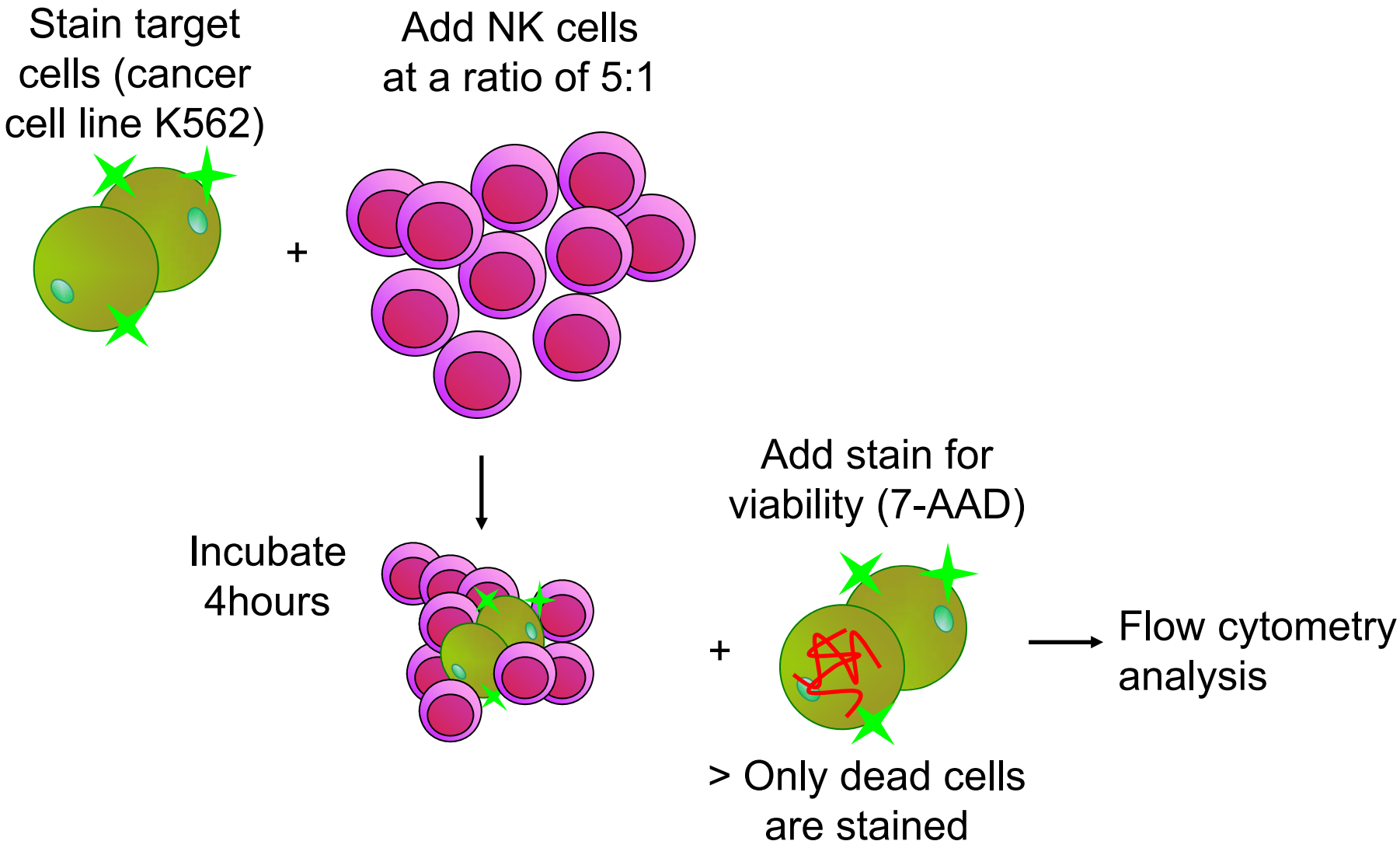
Granzyme B & perforin

NK cell cytotoxicity = balance between activation & inhibition.



Courtesy to Michael Henderson

Assay for NK cell functionality



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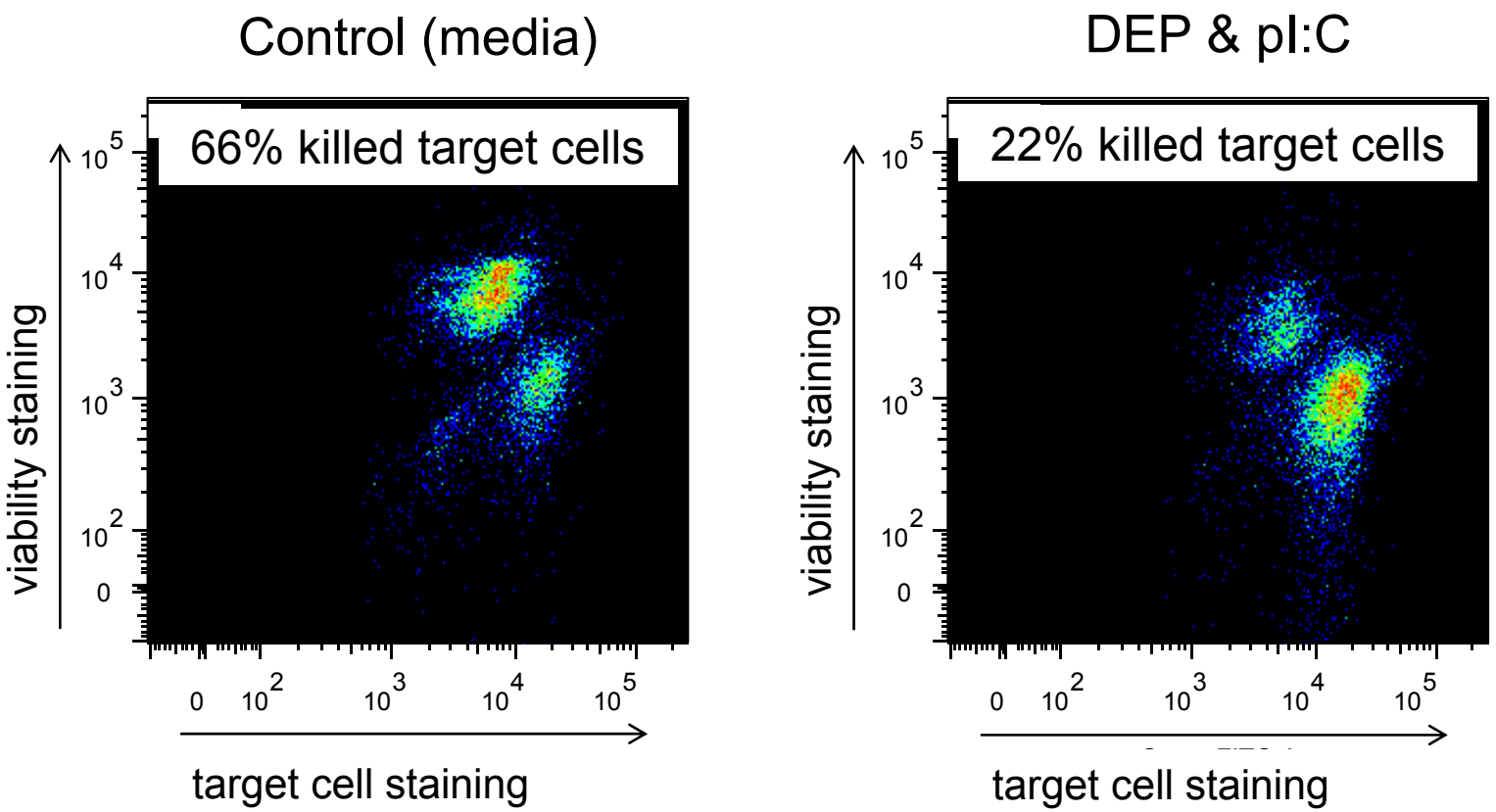
Assay for NK cell functionality

pI:C = polyI:C = polyinosinic:polycytidylic acid = viral mimetic



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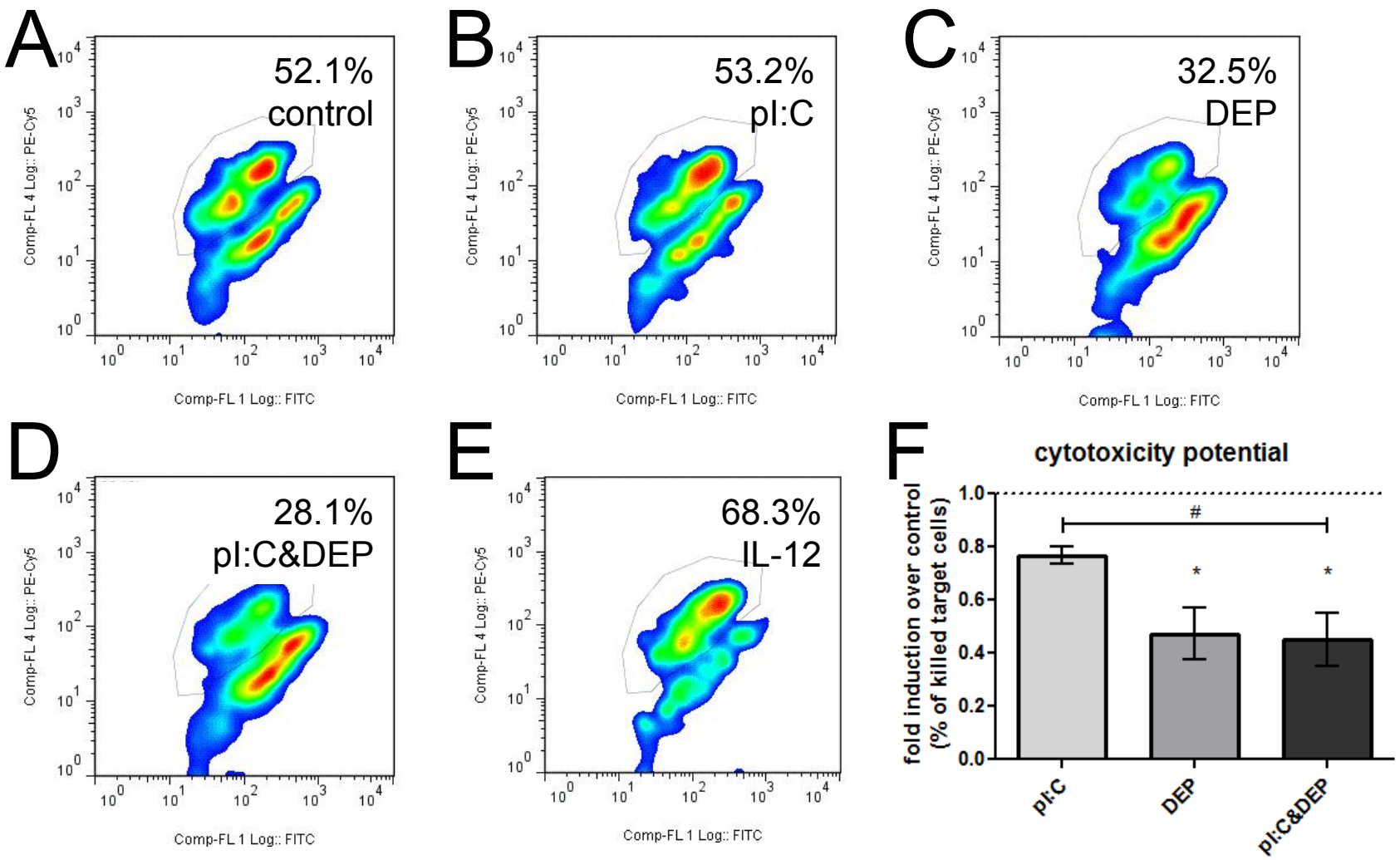
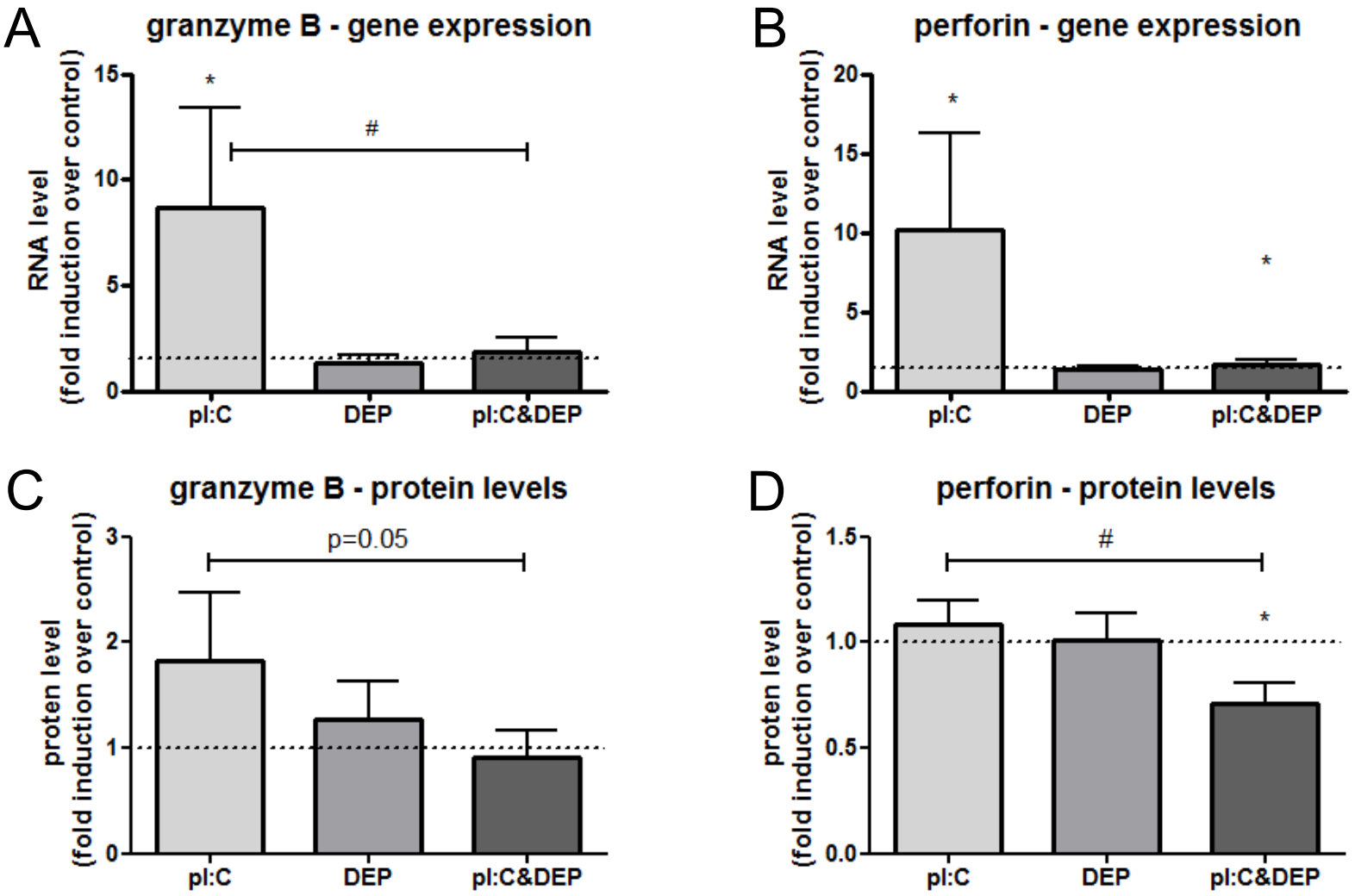
DEP reduce NK cell cytotoxicity



Cytolytic Enzymes in NK cells



Cytokine release

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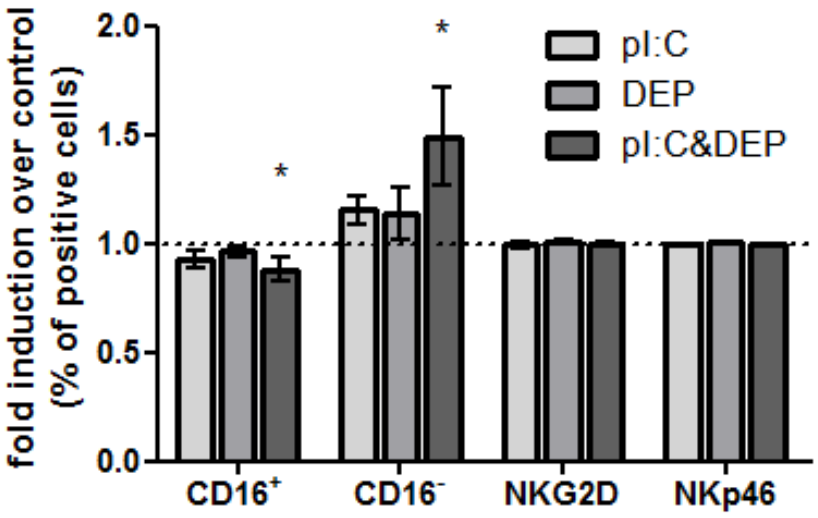
*,#,£p<0.05.
*significant different from untreated control
significant different from pI:C and pI:C & DEP
£ significant difference between pI:C and pI:C & DEP

pI:C increased cytokine release (compared to untreated control)

pI:C&DEP increased cytokine release

DEP lowered the cytokine release compared to pI:C and pI:C&DEP

NK cell phenotype – flow cytometry analysis



DEP&pI:C

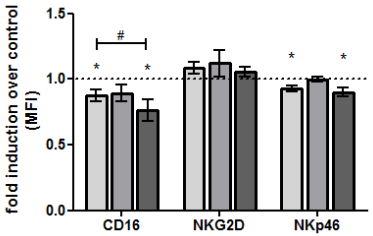
- Less NK cells with high CD16 expression
- More NK cells with low CD16 expression

> Reduced cytotoxicity

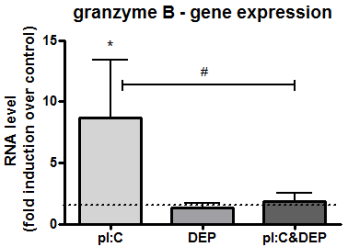
Summary

In the context of stimulation with the viral mimetic pI:C, Diesel Exhaust Particles (DEP):

- Decrease markers of cytotoxic function, such as CD16 expression



- Reduce the expression of cytolytic enzymes, such as granzyme B and perforin



- Have minor effects on cytokine release

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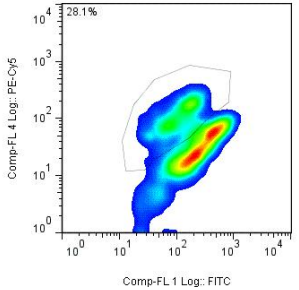
significant different from pI:C and pI:C & DEP
^ significant difference between pI:C and pI:C & DEP

- Suppress the ability of NK cells to kill target cells



Conclusion

- Impaired antiviral host defense responses seen after exposure to DEP may be related to the **direct effects** these pollutants have **on NK cell function**

- DEP-induced reduction of the cytotoxic potential in NK cells may play a role in the **increased susceptibility to viral and bacterial infections** seen after exposure to particulate matter and more specifically, DE.

- **Mechanisms** by which DEP impair the NK cell function needs to be **further investigated**

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