12-22-2011

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Recommended Citation

Hurd, T., Walker, J. and Whalen, M.M. (2012), Pentachlorophenol decreases tumor-cell-binding capacity and cell-surface protein expression of human natural killer cells. J. Appl. Toxicol., 32: 627-634. https://doi.org/10.1002/jat.1781

Published in final edited form as:
J Appl Toxicol. 2012 August ; 32(8): 627–634.

Pentachlorophenol Decreases Tumor-cell-binding Capacity and Cell-Surface Protein Expression of Human Natural Killer Cells

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Abstract

Pentachlorophenol (PCP) is an organochlorine pesticide that decreases the tumor-cell killing (lytic) function of human natural killer (NK) cells. NK cells defend against tumor cells and virally infected cells. They bind to these targets, utilizing a variety of cell surface proteins. This study examined concentrations of PCP that decrease lytic function for alteration of NK binding to tumor targets. Levels of PCP that caused loss of binding function were then examined for effects on expression of cell-surface proteins needed for binding. Exposure to 10 μM PCP for 24 h (which caused a greater than 70% loss of lytic function) decreased NK binding function (34.6%), and CD11a (21.7%) and CD56 (26.2%) cell-surface proteins. Both binding function and cell-surface proteins were decreased after longer exposures to lower concentrations of PCP. These data indicate that continuous exposures to PCP decreased binding function as well as cell-surface marker expression in NK cells and that these changes may in part explain the losses of lytic function seen with these exposures. PCP exposures have been shown to increase the incidence of blood and kidney cancers in humans. These data indicate that a possible explanation for this increased risk may be loss of NK lytic function, which is at least in part due to the loss of the ability of the NK cell to bind to tumor cells. These data also indicate that lost binding function may be due to loss of important cell surface proteins.

Keywords

pentachlorophenol; NK cells; binding function; CD16; CD56

INTRODUCTION

NK cells prevent the development as well as the metastases of tumors (Lotzova, 1993; Vivier et al., 2004). They also play a central role in defending against viral infections. Due to these functions, they are the primary immune defense against tumor cells and virally infected cells. Viral infection incidence has been shown to increase in individuals lacking NK cells (Fleisher et al., 1982; Biron et al., 1989). Importantly, it has been shown that a patient with a deficiency of NK cells suffered from both vulvar and cervical carcinomas (Ballas et al., 1990). Additionally, a correlation between the size of the tumor and a decrease in the number of NK cells was shown in breast cancer patients (Fulton et al., 1984). As these cells are the front line of immune response against tumor and virally infected cells (due to their ability to lyse target cells without prior sensitization), anything that interferes with their function could lead to an increased incidence of tumors or viral infections. It has been shown that exposure to the environmental contaminant dichlorodiphenyltrichloroethane

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(DDT) decreases both the number and cytotoxic function of human NK cells (Svensson et al., 1994; Eskenazi et al., 2009). In keeping with its effect on NK cell numbers and function. it has been found that DDT exposure at early ages (prior to age 14) causes and 5 fold increase in the risk of breast cancer (Cohn et al. 2007).

The NK cell must physically bind to a target cell (be it a tumor cell or virally infected cell) in order to destroy (lyse) the target. CD11a/CD18 form the functional LFA-1 adhesion complex shown to be required for NK binding to tumor targets (Nitta et al., 1989). CD56, a cognate of the neural cell adhesion molecule has also been shown to be important in NK binding to targets (Nitta et al., 1989; Lotzova, 1993). CD2, an NK cell adhesion molecule, has been implicated in activation of the cytotoxic signaling response (Lotzova, 1993). CD16 has a role as activating receptor of the NK lytic process with antibody-coated (Lotzova, 1993) and tumor targets (Mandelboim et al., 1999).

Pentachlorophenol (PCP) was at one time registered for use as an insecticide, fungicide, herbicide, molluscicide, algicide, disinfectant, and ingredient in antifouling paints (Cirelli 1978). Its use was restricted in 1984 (EPA, 2001). It is now mainly used as a wood preservative for power-line poles and fence posts. Approximately 80% of the PCP used in the U.S. is for the treatment of wooden utility poles (ATSDR, 2001). As with other organochlorine compounds PCP is quite stable. Due to its once widespread use and chemical stability, it is a very significant environmental contaminant. Humans are exposed to PCP through inhalation, absorption through the skin, and consumption of contaminated food and water (ATSDR, 2001). Measurable levels of PCP have been detected in human blood and urine (Atuma and Okor, 1985; CDC, 2005; Cline et al., 1989; Jones et al., 1986; Thornton et al., 2002; Uhl et al. 1986). Levels ranging from 0.26 -5 μM were found in the serum of individuals residing in PCP-treated log homes (Cline et al., 1989). Levels averaging about 0.15 μM were found in the serum of individuals with no known exposure (Cline et al., 1989; Uhl et al., 1986). PCP is associated with cancers of the blood and kidney in humans (Demers et al., 2006).

In previous studies we have shown that exposures to PCP decrease the lytic function of human NK cells (Reed et al., 2004; Taylor et al., 2005; Nnodu and Whalen, 2008). Levels ranging from 10 μM to 0.25 μM caused decreases in NK cell lytic function when cells were exposed continuously for 24 h, 48 h, and 6 days (Reed et al., 2004; Nnodu and Whalen 2008). A 1 hour exposure to 10 μM PCP followed by 6 days in PCP-free media also resulted in about an 80% decrease in the lytic function of NK cells (Taylor et al., 2005).

In the current study the effects of PCP exposures on the ability of NK cells to bind to target cells was examined. As mentioned earlier, binding is an essential step in the process by which NK cells lyse target cells. If PCP exposures were to decrease the ability of NK cells to bind to targets, this could explain (at least in part) the PCP-induced loss of lytic function. Certain cell surface proteins are needed for NK binding to target cells (Lotzova, 1993; Mandelboim et al., 1999). Five cell surface proteins that are important in NK cells binding to targets, CD2, CD11a, CD16, CD18, and CD56, were analyzed via flow cytometry to determine whether PCP interferes with cell surface protein expression.. Effects of both chronic and acute exposures to PCP were examined for their effects on binding and cell surface protein expression.

MATERIALS AND METHODS

Isolation of NK cells

Peripheral blood from healthy adult (male and female) volunteer donors was used for this study. Buffy coats (source leukocytes) obtained from Key Biologics, LLC (Memphis, TN)

were used to prepare NK cells. Highly purified NK cells were obtained using a rosetting procedure. Buffy coats were mixed with 0.6-0.8 mL of RosetteSep human NK cell enrichment antibody cocktail (StemCell Technologies, Vancouver, British Columbia, Canada) per 45 mL of buffy coat. The mixture was incubated for 25 min at room temperature (~ 25° C). Following the incubation, 7-8 mL of the mixture was layered onto 4 mL of Ficoll-Hypaque (1.077 g/mL) (MP Biomedicals, Irvine, CA) and centrifuged at 1200 g for 30-50 min. The cell layer was collected and washed twice with phosphate buffered saline (PBS) pH 7.2 and stored in complete media (RPMI-1640 supplemented with 10% heat-inactivated bovine calf serum (BCS), 2 m*M L*-glutamine and 50 U penicillin G with 50 μg streptomycin/ml) at 1 million cells/mL.

Chemical preparation

PCP was purchased from Fisher Scientific. PCP was dissolved in dimethylsulfoxide (DMSO) (Sigma-Aldrich, St. Louis, MO) to give a 100 mM stock solution. Desired concentrations of PCP were prepared by dilution of the stock into complete media. The final concentration of DMSO for PCP exposures did not exceed 0.01%. Appropriate DMSO controls were run.

Cell Treatments

NK cells (at a concentration of 1.5 million cells/ mL) were exposed to PCP or Control for 24 h, 48 h or 6 days. Following exposures, the cells were assayed for binding capacity or cell-surface marker expression. Additionally, NK cells were exposed to PCP for 1 h; following the 1 h exposure period, the PCP- containing or control media was removed and replaced with fresh PCP-free media and the cells were incubated in compound-free media for 24 h, 48 h or 6 days prior being assayed for binding function or cell-surface protein expression. The concentration range examined was 0.25- 10 μM PCP.

Cell Viability

Cell viability was determined by trypan blue exclusion to determine if the compound (at varying concentrations and lengths of exposure) was cytotoxic to the NK cells. Cell numbers and viability were assessed at the end of each exposure period. Viability was determined at each concentration for each of the exposure periods. The viability of treated cells was compared to that of control cells at each length of exposure (Whalen et al., 2003). Only those concentrations where viability was unaffected were used at a given length of exposure. Table 1 shows the viability of the cells after each length of exposure. It is important to note that the concentrations of PCP used in the studies were not cytotoxic to the NK cells at any of the lengths of exposure (compared to control NK cells), thus any changes in binding or cell surface protein expression were not due to NK cell death.

Conjugation Assay

The percentage of target cells with bound NK cells was determined at two effector to target ratios 12:1 and 6:1. The NK cells were treated as described above. Control and PCP exposed NK cells were then suspended at a concentration of 240,000/50 μL (for the 12:1 ratio) and 120,000/50 μL (6:1 ratios). The target cell was the NK-susceptible K562 cell (human chronic myelogenous leukemia). Target cells were suspended at a concentration of 20,000/50 μL. Tumor cells (50 μL) were then placed in the wells of microwell plates containing 50 μL of control or PCP exposed lymphocytes. Each condition was tested in triplicate. The plate was centrifuged at 300 g for 3 min and the cells were incubated at 37° C, air/CO_2, 19:1, for 10 min and then placed on ice. Following the incubation period, the cells were gently resuspended with a micropipette, placed in a hemacytometer and viewed under a light microscope. The number of target cells with two or more lymphocytes bound

to their surface was counted as well as the total number of targets to determine the percentage of tumor cells with lymphocytes bound. The minimum number of targets counted per determination was 50-100 (Whalen et al., 1992).

Flow Cytometry

NK cells were exposed to the appropriate concentrations of PCP for the appropriate length of time. Following exposure to PCP, the cells were washed and prepared for analysis on a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA). The cells were washed twice with ice-cold PBS and 100 μl of cell suspension (250,000-500,000 cells) was labeled with 10 μl of one of the following antibodies: anti-CD2, CD11a, CD16 , CD18, and CD56 (Pharmingen, San Diego, CA). Anti-CD2, CD11a, CD16, and CD18 were FITC-conjugated antibodies. Anti-CD56 was phycoerythrin (PE)-conjugated. Appropriate FITC- and PE-conjugated isotype control antibodies were used. Each antibody was a monoclonal antibody (mouse IgGκ) that was specific for the human cell surface protein, such as CD16. The antibody-containing cell suspension was incubated for a minimum of 30 minutes on ice, in the dark. Following the incubation period the cells were washed twice with ice-cold PBS (1 mL) and suspended in 500 μl of ice-cold 1% paraformaldehyde in PBS. Samples were analyzed using the the FACSCalibur flow cytometer from Becton Dickinson Immunocytometry Systems, Inc (BDIS), San Jose, CA. Instrument performance was standardized weekly using Calibrite beads (BDIS) and the same instrument settings were used for all acquisitions. The assays were sufficiently uniform to use the same forward scatter (FSC), side scatter (SSC), and fluorescence (FL) settings. The sensitivity of the instrument was constant. The acquisition and analysis software for flow cytometry data was CELLQuest Pro from BDIS running on an Apple computer.

Statistical Analysis

Statistical analysis of the data was carried out utilizing ANOVA and Student's t test. Data were initially compared within a given experimental setup by ANOVA. A significant ANOVA was followed by pair wise analysis of control versus exposed data using Student's t test, a p value of less than 0.05 was considered significant.

RESULTS

Effects of Exposures to PCP for 24 h, 48 h and 6 days on the Binding Function of NK Cells

NK cells were exposed to 2.5-10 μM PCP for 24 h, 2.5 and 5 μM PCP for 48 h, and 0.25-1 μM PCP for 6 days. Exposure to 10 μM PCP for 24 h caused a decrease in NK cell binding function (34.6% ± 8.7%). 5 μM PCP for 24 h caused a 15.7% ± 10% decrease in binding function while exposure to 2.5 μM PCP for 24 h caused no significant decrease (Figure 1) . A 48 h exposure to 5 μM PCP caused a decrease of 20.8% ± 15% (Figure 1). When NK cells were exposed to 1 μM PCP for 6 days, there was a significant decrease in tumor binding function of 46.6% ± 21%. (Figure 1). To combine results from separate experiments (using cells from different donors) the tumor binding caused by treated cells was normalized to that of control cells in a given experiment.

Effects of Exposures to PCP for 1 h Followed by 24 h, 48 h, and 6 Days in PCP-Free Media on the Binding Function of NK Cells

NK cells were exposed to PCP for 1 h after which the compound containing media was removed and the cells were washed two times with compound-free media. The NK cells were then suspended in PCP-free media for 24 h, 48 h or 6 days before assaying for tumor binding function. The only condition that caused a significant decrease in binding function

(26.0% ± 7.6%), was a 1 h exposure to 10 μM PCP followed by 6 days in PCP-free media (Figure 2).

Effects of Exposures to PCP for 24 h, 48 h and 6 Days on Cell-Surface Protein Expression

Levels of key cell surface molecules involved in the NK cell interaction with target cells were monitored using flow cytometry. Control and PCP-exposed cells were incubated with fluorescent-labeled antibodies for specific cell surface markers. The antibodies used were anti-CD2, CD11a, CD16, CD18, and CD56. A 24 h exposure to 5 μM PCP caused decreases in CD11a of 22.9% ± 8.9% and in CD56 of 21.8% ± 10% ($P < 0.05$) When NK cells were exposed to 10 μM PCP for 24 h, CD11a expression was decreased by21.7% ± 9.1% ($P < 0.05$) and CD56 expression was decreased by 26.2% ± 11.7% ($P < 0.05$) (as measured by mean fluorescence intensity). Figures 3A and B shows the shift in peak fluorescence intensity for a representative experiments of CD11a and CD56 at the 10 μM concentration. When NK cells were exposed to 2.5 μM PCP for 48 h there were decreases in the expression of CD16, CD18, and CD56 of 22.1% ± 16.7%, 15.9% ± 3.9%,and 28.7% ± 4.2%, respectively ($P<0.05$). A 48 h exposure to 5 μM PCP also decreased expression of CD16, CD18, and CD56. CD16 was decreased by 34.6% ± 16.6%, CD18 was decreased by 22.6% ± 5.7%, and CD56 was decreased by 37.7% ± 7.5% ($P<0.05$). Figures 4A-C show the shift in fluorescence intensity for each of these proteins from a representative experiment at the 5 μM concentration. A 6 day exposure to 0.5 μM PCP caused a significant decrease only in CD16 (21.2 ± 2.6%, $P<0.05$), while exposure to 1 μM PCP caused significant decreases in each of the cell surface markers that were tested with the largest decrease being in CD16 (73.3% ± 8.8%, $P<0.05$). CD2, CD11a, CD18, and CD56 were decreased by 40.2% ± 13.4%, 47.8% ± 1.9%, 32.1% ± 7.4%, and 55.1% ± 23.5%, $P<0.05$. Figures 5 A-E show the shift in fluorescence intensity for each of the proteins at the 1 μM PCP concentration from a representative experiment.

Effects of Exposure to PCP for 1 h Followed by 24 h, 48 h, and 6 Days in PCP-Free Media on Cell- Surface Protein Expression

A 1 h exposure to 10 μM PCP followed by 6 days in PCP-free media was the only condition that caused any decrease in expression of any cell-surface protein. There was a slight decrease in the expression of CD16 of 15.7% ± 5%. All the other lengths of incubation at either 10 or 5 μM PCP caused no significant changes in any of the cell surface proteins examined.

DISCUSSION

In past studies we found that the organochlorine pesticide PCP, caused very significant decreases in the ability of human NK cells to destroy tumor target cells (Reed et al., 2004; Taylor et al., 2005). Even a brief exposure to 10 μM PCP (1 h) could induce processes that lead to dramatic decrease in lytic function in the 6 day period following the initial exposure to PCP. As mentioned earlier, PCP is now mainly used as a wood preservative for power-line poles and fence posts (ATSDR, 2001). However, due to past use in a wide variety of applications, including the treatment of logs used in log homes, significant numbers of people have been exposed to PCP. It has been found in the serum of humans living in PCP-treated log homes at levels of 0.26 -5 μM Cline et al., 1989) and levels averaging about 0.15 μM were found in the serum of individuals with no known exposure (Cline et al.,1989; Uhl et al., 1986). The levels of PCP found in human serum are in the range where we see effects on NK lytic function (0.25 -10 μM) (Reed et al., 2004; Taylor et al., 2005). As we have shown that PCP has negative effects on human NK immune function that can persist even after its removal, it is important to address further the mechanism of PCP-induced loss of NK lytic function. The purpose of the current study was to examine the effects of PCP on

the ability of NK cells to bind to their targets and to determine if there was a decrease in cell surface protein expression, that accompanied any loss of binding function. As NK cell binding to targets is the first step of achieving target lysis any interference with binding could, at least in part, explain the loss of lytic function seen with PCP exposures. Specific cell surface proteins are used by NK cells to recognize and bind to their targets, thus any decrease in binding could be explained by an accompanying decrease in specific cell surface protein expression, which was also examined.

The results of the current study indicate that the loss of NK lytic function that was seen in previous studies (Reed et al., 2004; Taylor et al., 2005) cannot be fully explained by loss of binding function. For instance a 24 h exposure to 2.5 μM PCP caused 48% decrease in lytic function but caused no loss of binding function (Figure 6A). Figure 6 plots the loss of lytic function seen in a previous study (Reed et al., 2004) with the loss of binding function seen in this study for ease of comparison. A 48 h exposure to 2.5 μM PCP caused a decrease in lytic function and 70% but no decrease in binding function (Figure 6B). After a 6 day exposure to 0.25 μM PCP the loss of lytic function was 73% but there was no decrease in binding function (Figure 6C). However, a 24 hour exposure to 10 μM PCP caused a decrease in lytic function of 76% and a decrease in binding function of 35%. Thus, it appears that the lack of ability of NK cells to bind to their target may be a contributing factor to the loss of lytic function seen at some lengths and concentrations of PCP exposure. However, loss of binding function does not explain the extent of loss of lytic function that has been seen with 24 h and 48 h continuous exposures to PCP. This same pattern has been seen with other compounds that are able to block the lytic function of human NK cells. These include the organtoins, tributyltin (TBT) and dibutyltin (DBT) (Whalen et al., 2002; Odman-Ghazi et al., 2003), the flame retardants tetrabromobisphenol A (TBBPA) (Kibakaya et al., 2009) and hexabromocyclododecane (HBCD) (Hinkson and Whalen, 2010) and the carbamate pesticide ziram (Taylor and Whalen, 2009).

NK cells utilize specific cell surface proteins to recognize and bind to their targets. Thus, any PCP exposures that caused loss of binding function were examined for their capacity to alter expression of several cell surface proteins. CD11a, CD18, CD16, and CD56 have all been shown to be important in binding to target cells (Lotzova, 1993: Mandelboim et al., 1999). The results indicated that there was a very significant loss of the cell surface proteins CD11a and CD56 in NK cells exposed to 10 μM PCP for 24 h. Exposure to 5 μM PCP for 24 h also caused a significant decrease in both CD2 and CD56. These exposures also caused significant decreases in binding function as seen in Figure 1. A 48 h exposure to 5 μM PCP caused decreases in CD16, CD18, and CD56 it also caused decreases in binding of about 21% (Figure 1). Finally, a 6 day exposure to 1 μM PCP caused the greatest decrease in binding (47%) (Figure 1) and also caused the greatest percentage decreases in all of the five cell surface proteins. Thus, there appears to be a significant association between loss of binding function and a decrease in one or more of the cell surface proteins needed for binding and lysis of tumor targets.

Studies examining the relationship between loss of NK binding function accompanying loss of cell-surface proteins have been carried out with other environmental contaminants, including butyltins, brominated flame retardants, and the carbamate pesticide (Whalen et al., 2002a,b; Odman-Ghazi et al., 2003; Taylor et al., 2009; Kibakaya et al., 2009; Hinkson an Whalen 2010; Hurd and Whalen, 2011). These studies, like the current study, have shown that there is an association of loss of binding function and a decrease of one or more cell-surface protein. The effects of TBT, DBT, TBBPA, HBCD, and ziram on the expression of cell surface proteins have also shown that there were very significant decreases in CD16 in NK cells under conditions where binding was decreased (Whalen et al., 2002; Odman-Ghazi et al., 2003; Kibakaya et al., 2009; Hurd and Whalen, 2011; Hinkson and Whalen, 2010;

Taylor et al., 2009). This is similar to what was seen with PCP exposures although PCP caused decreases in all of the cell surface markers at 6 days which was not seen with any of the other compounds tested.

Finally, the fact that PCP is found in human serum at levels that exceed those where NK lytic function (Reed et al., 2004; Taylor et al., 2005; Nnodu and Whalen, 2008), binding function, and cell surface protein levels are impaired, indicates that PCP may have the capacity to increase the potential for tumor development in humans. It has already been shown that individuals exposed to PCP have higher incidences of cancers (especially of the blood) (Demers et al., 2006) and this may be due to the inability of their NK cells to prevent tumor development due to PCP-induced decreases in NK function. Additionally, as mentioned in the introduction other contaminants such as DDT that are also capable of interfering with human NK function have been shown to increase the incidence of cancers in exposed individuals (Svensson et al., 1994; Eskenazi et al., 2009; Cohn et al. 2007).

In summary the current study shows: 1.) Exposure of NK cells to PCP interferes with ability of NK cells to bind to tumor target cells; 2.) PCP-induced loss of binding function does not appear to explain the extent of the loss of NK cell ability to lyse tumor cells under some conditions; 3.) PCP-induced loss of binding function can be accompanied by a significant decrease in the NK cell surface protein, which have roles in NK binding to certain target cells.

Acknowledgments

This research was supported by Grant 2S06GM-08092-35 from the National Institutes of Health.

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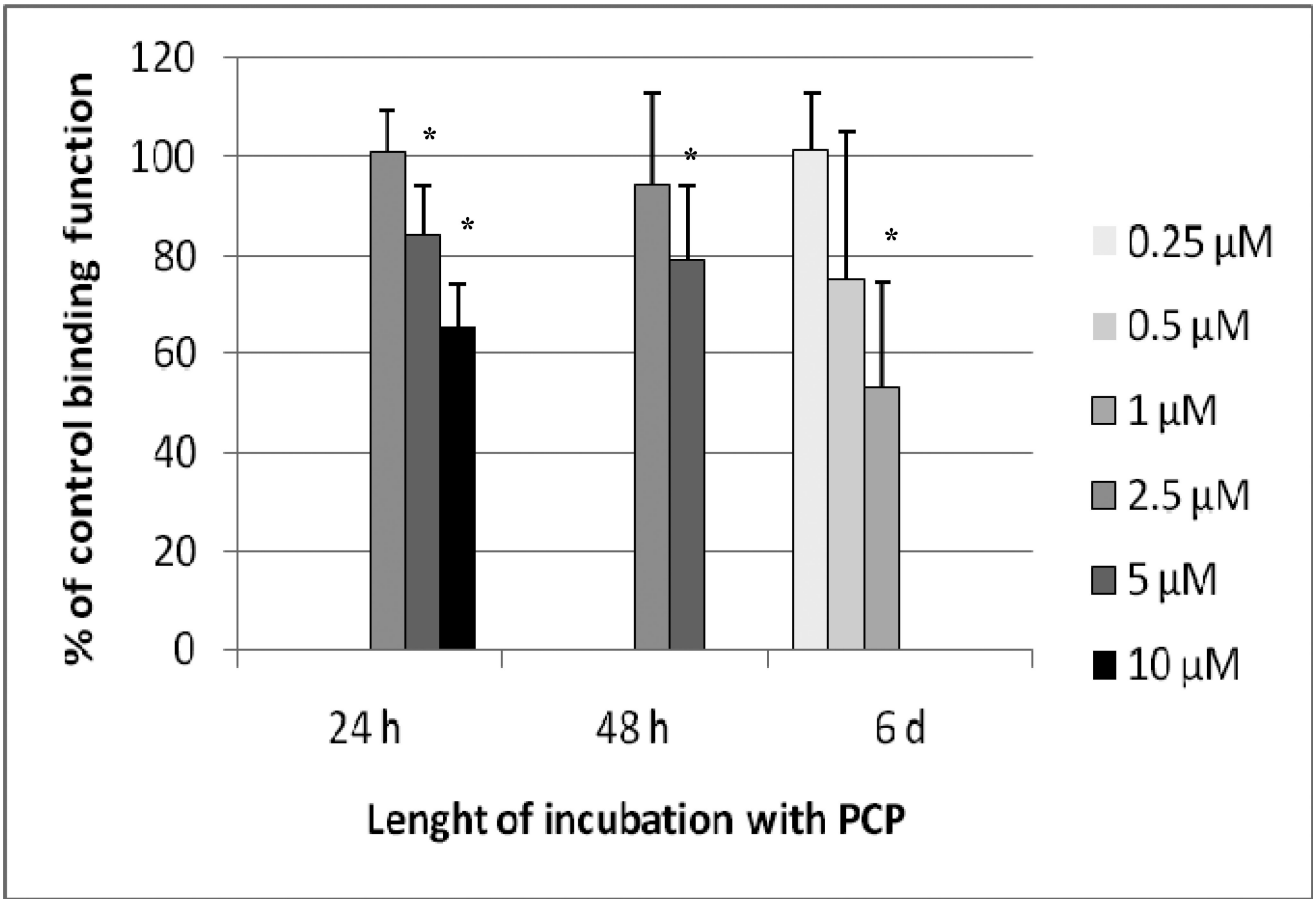


Figure 1.

Effects of 24 h, 48 h and 6 day continuous exposures to PCP on the ability of NK cells to bind K562 tumor cells. Very light gray bars = NK cells exposed to 0.25 μM PCP; light gray bars = NK cells exposed to 0.5 μM PCP; medium gray bars = NK cells exposed to 1 μM PCP; Dark gray bars = NK cells exposed to 2.5 μM PCP; Very dark gray bar = NK cells exposed to 5 μM PCP; Black bars = NK cells exposed to 10 μM PCP. Results are mean ±S.D. (n=12 for 24 h, 48 h and 6day exposures). * indicates that the decrease in binding was statistically significant ($p<0.01$).

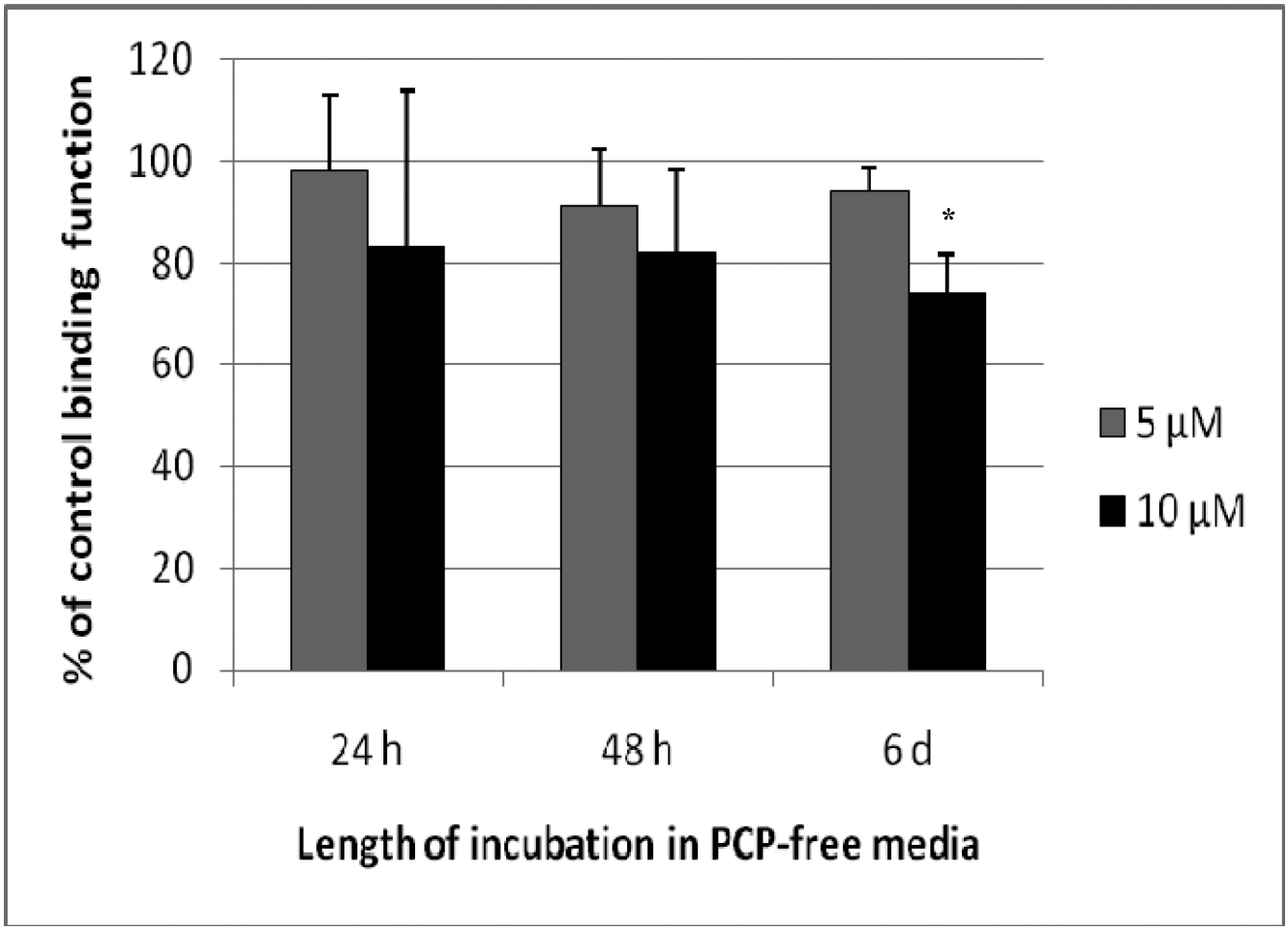
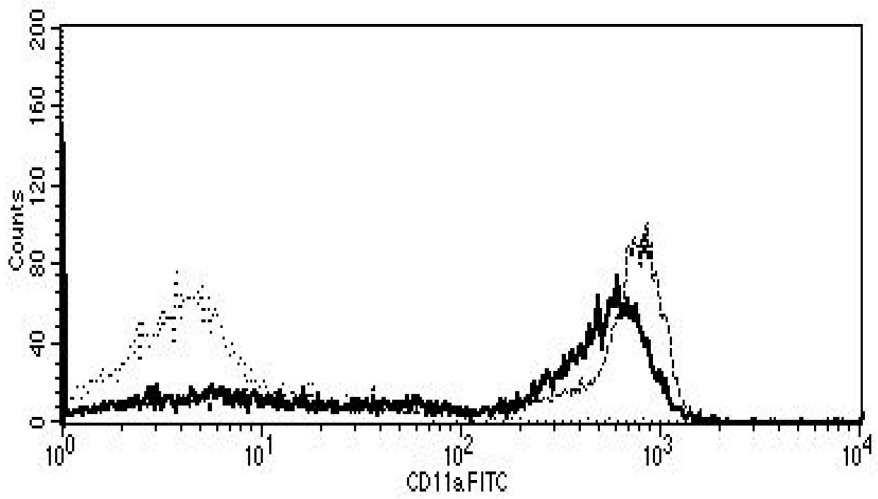


Figure 2.
Effects of 1 h exposures to PCP followed by 24 h, 48 h and 6 day in PCP-free media on the ability of NK cells to bind to K562 tumor cells. Dark gray bar = NK cells exposed to 2.5 µM PCP; Black bars = NK cells exposed to 5 µM PCP. Results are mean±S.D. (n=9 for all lengths of exposure). * indicates that the decrease in binding was statistically significant ($p<0.01$).

A



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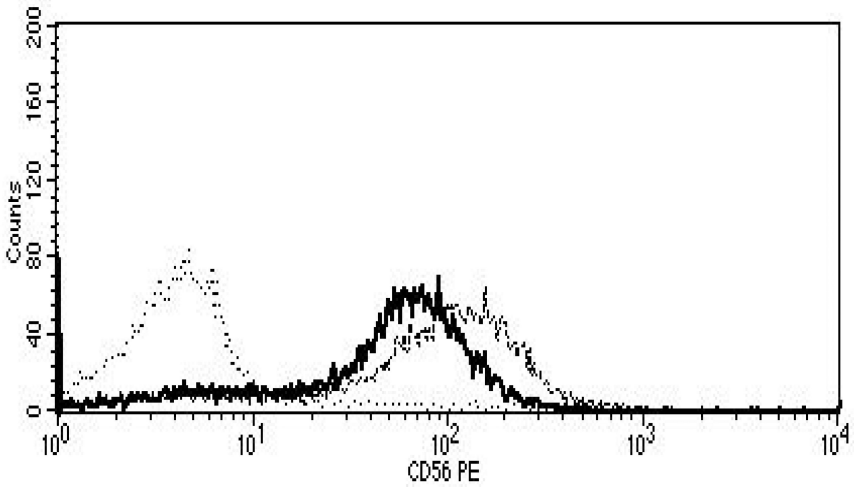
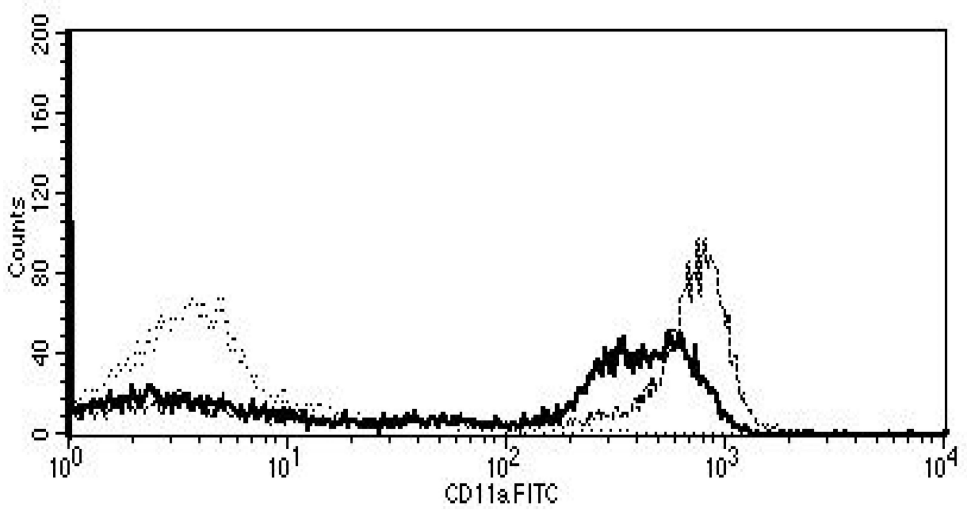


Figure 3.

Histograms from representative experiments showing effects of 24 h exposures to 10 μM PCP on CD11a and CD56 expression in NK cells. A.) 24 h exposure to 10 μM PCP: Dashed line = IgG control; thin solid line = control NK cells stained with anti-CD11a antibody; bold line = PCP-exposed cells stained with anti-CD11a antibody; y axis = cell number; x axis = fluorescence intensity . B.) 24 h exposure to 10 μM PCP: Dashed line = IgG control; thin solid line = control NK cells stained with anti-CD56 antibody; bold line = PCP-exposed cells stained with anti-CD56 antibody; y axis = cell number; x axis = fluorescence intensity.

A



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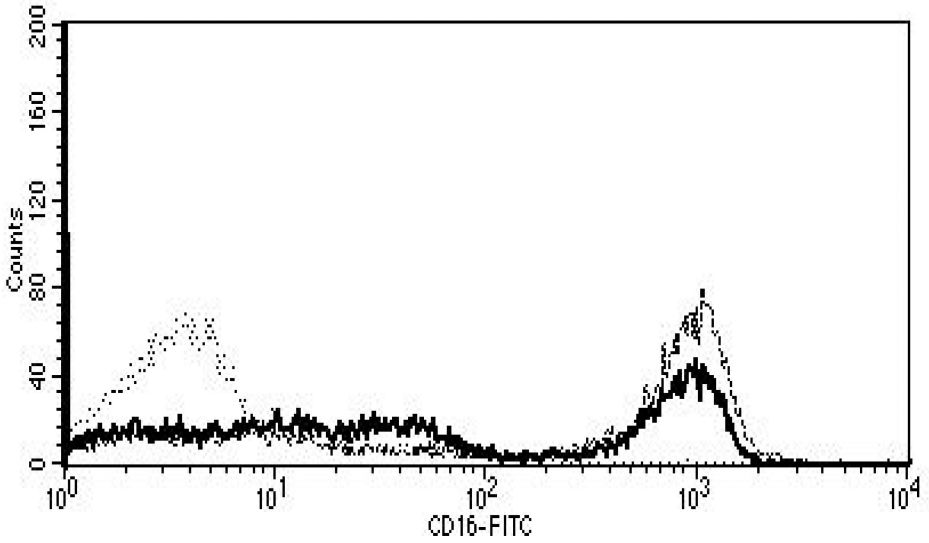

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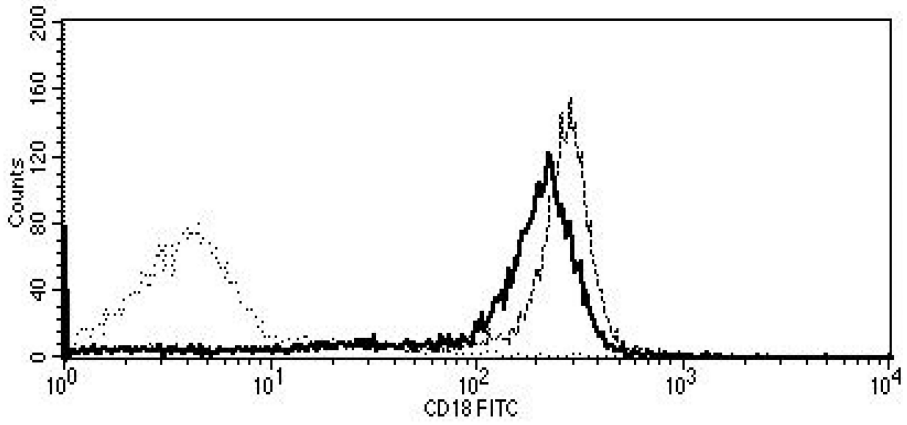

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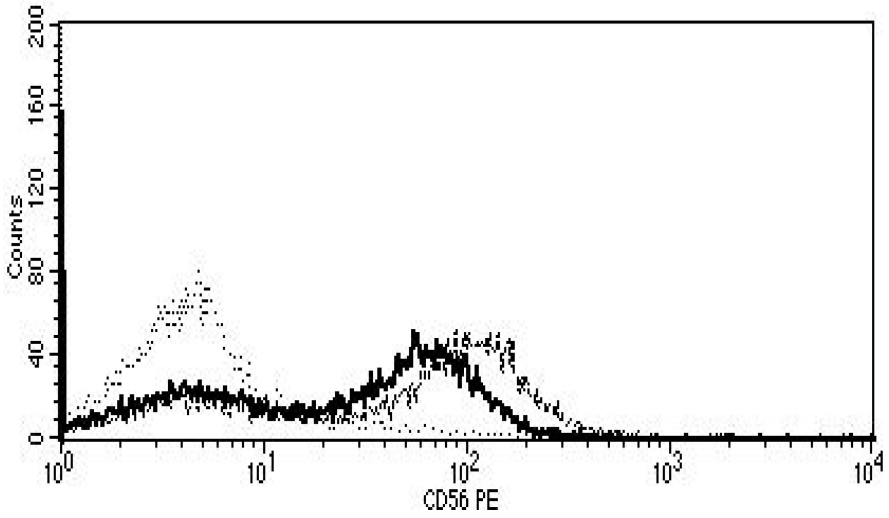
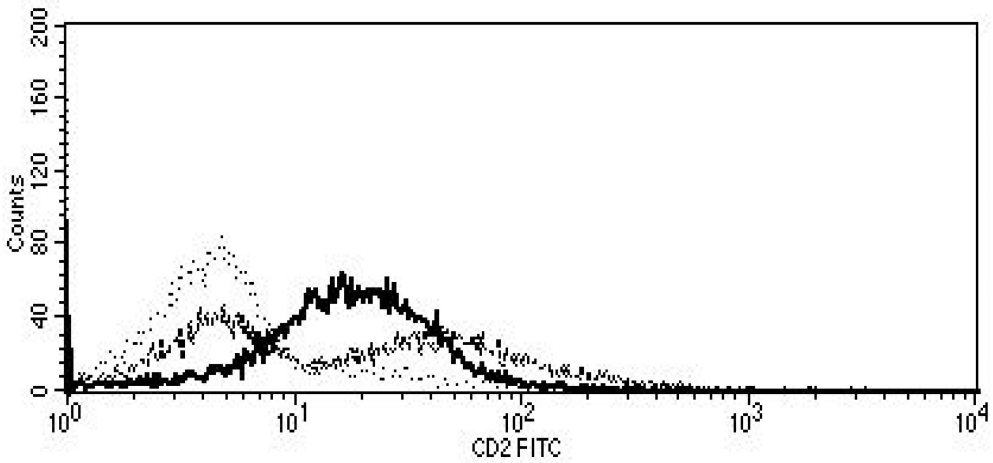


Figure 4.

Histogram from a representative experiment showing effects of 48 h exposures to 5 μM PCP on CD16, CD18, and CD56 expression in NK cells. A.) 48 h exposure to 5 μM PCP: Dashed line = IgG control; thin solid line = control NK cells stained with anti-CD16 antibody; bold line = PCP-exposed cells stained with anti-CD16 antibody; y axis = cell number; x axis = fluorescence intensity . B.) 48 h exposure to 5 μM PCP: Dashed line = IgG control; thin solid line = control NK cells stained with anti-CD18 antibody; bold line = PCP-exposed cells stained with anti-CD18 antibody. C.) 48 h exposure to 5 μM PCP: Dashed line = IgG control; thin solid line = control NK cells stained with anti-CD56 antibody; bold line

= PCP-exposed cells stained with anti-CD56 antibody. ; y axis = cell number; x axis = fluorescence intensity.

A



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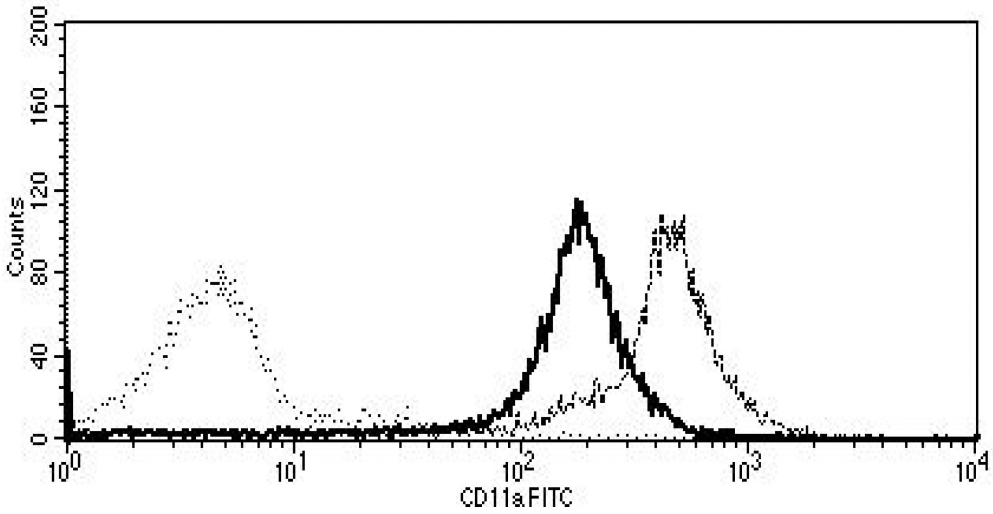

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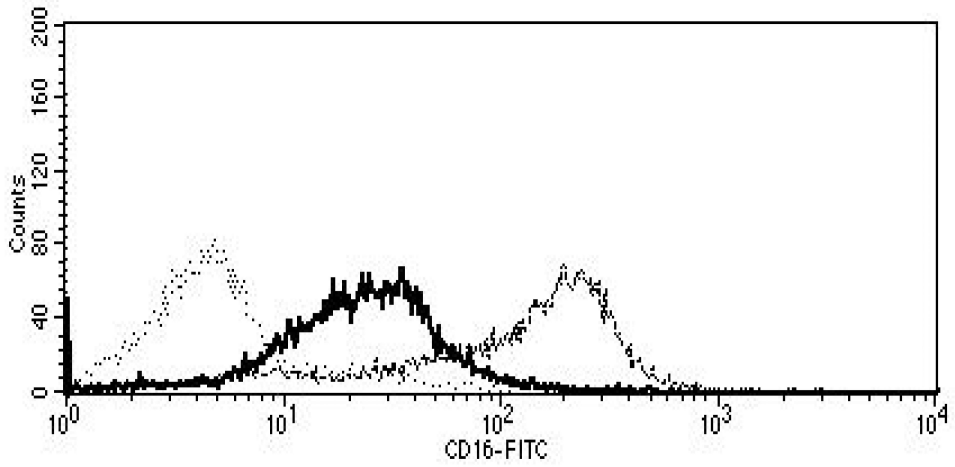

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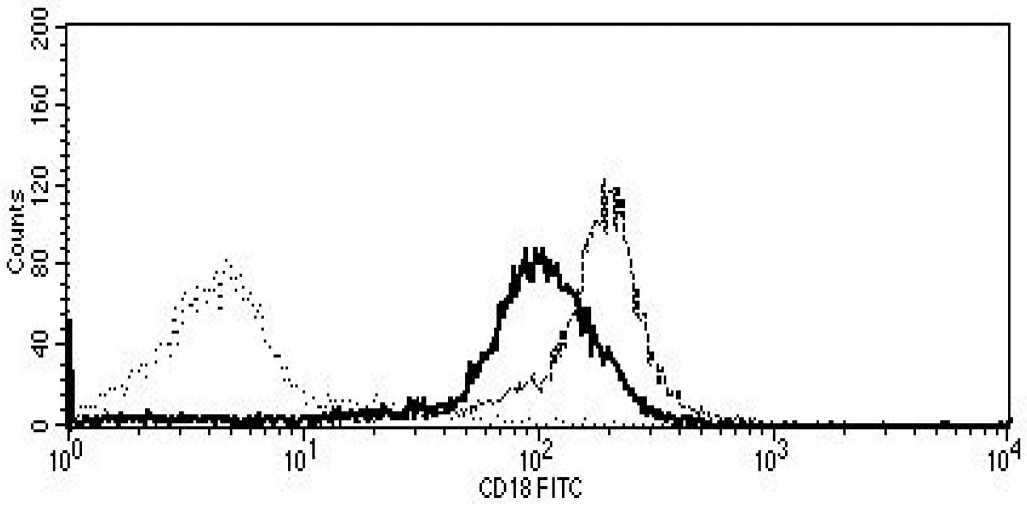


E

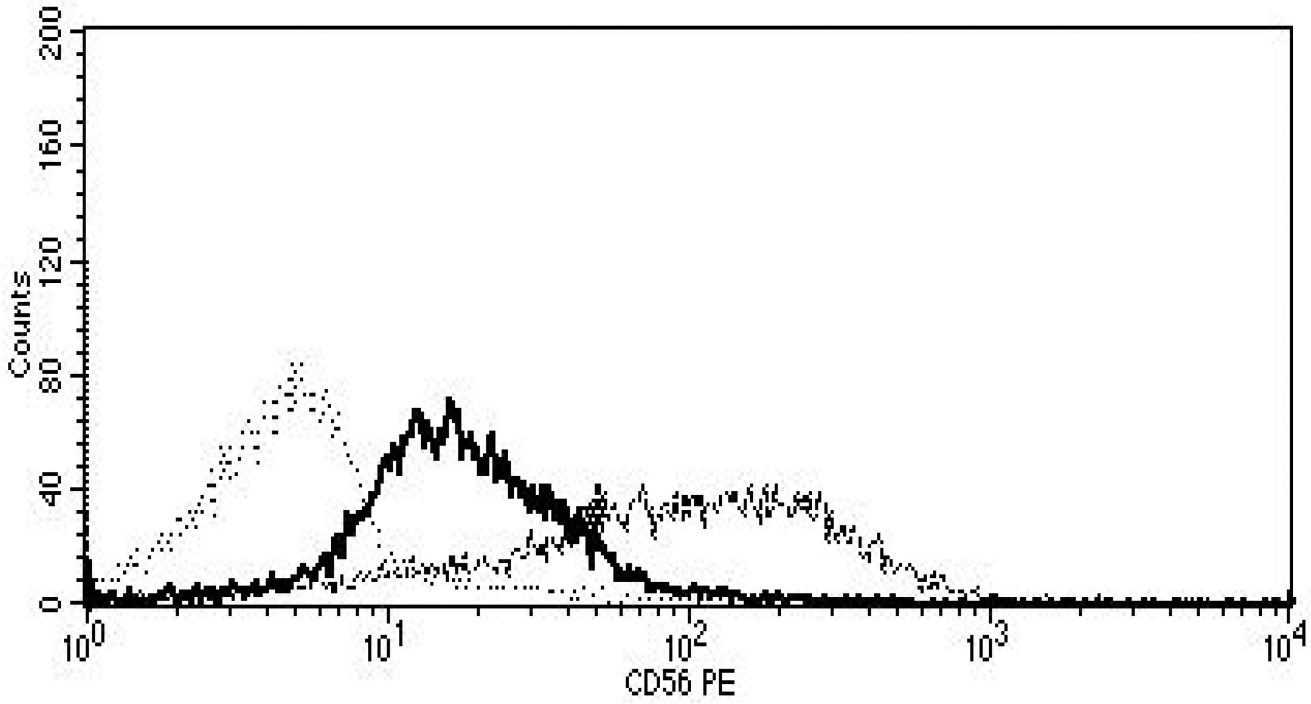
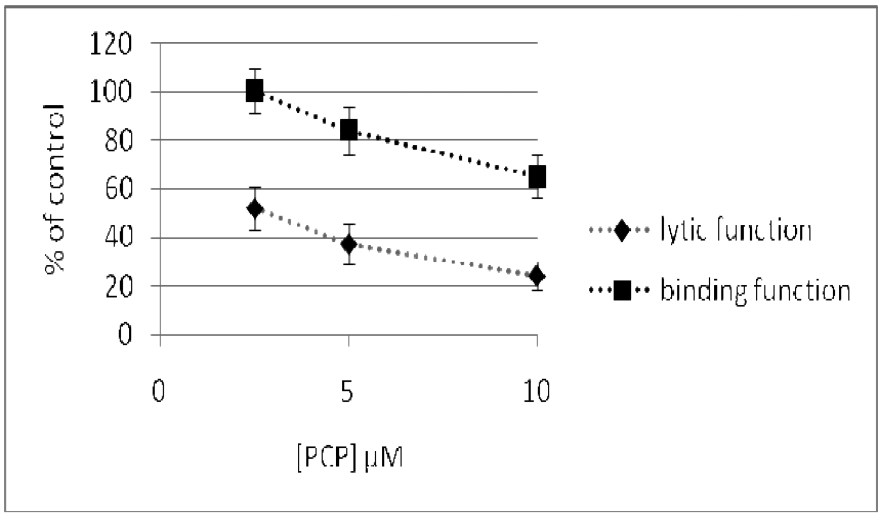


Figure 5.

Histogram from a representative experiment showing effects of 6 day exposures to 1 μM PCP on CD2, CD11a, CD16, CD18, and CD56 expression in NK cells. A.) 6 day exposure to 1 μM PCP: Dashed line = IgG control; thin solid line = control NK cells stained with anti-CD2 antibody; bold line = PCP-exposed cells stained with anti-CD2 antibody; y axis = cell number; x axis = fluorescence intensity. B.) 6 day exposure to 1 μM PCP: Dashed line = IgG control; thin solid line = control NK cells stained with anti-CD11a antibody; bold line = PCP-exposed cells stained with anti-CD11a antibody; y axis = cell number; x axis = fluorescence intensity. C.) 6 day exposure to 1 μM PCP: Dashed line = IgG control; thin solid line = control NK cells stained with anti-CD16 antibody; bold line = PCP-exposed cells stained with anti-CD16 antibody; y axis = cell number; x axis = fluorescence intensity. D.) 6 day exposure to 1 μM PCP: Dashed line = IgG control; thin solid line = control NK cells stained with anti-CD18 antibody; bold line = PCP-exposed cells stained with anti-CD18 antibody. E.) 48 h exposure to 1 μM PCP: Dashed line = IgG control; thin solid line = control NK cells stained with anti-CD56 antibody; bold line = PCP-exposed cells stained with anti-CD56 antibody. ; y axis = cell number; x axis = fluorescence intensity.

A



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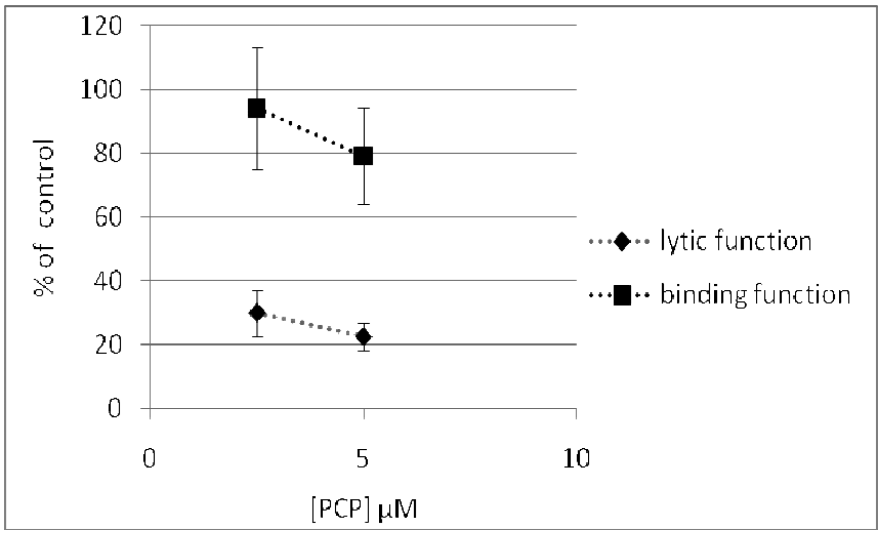

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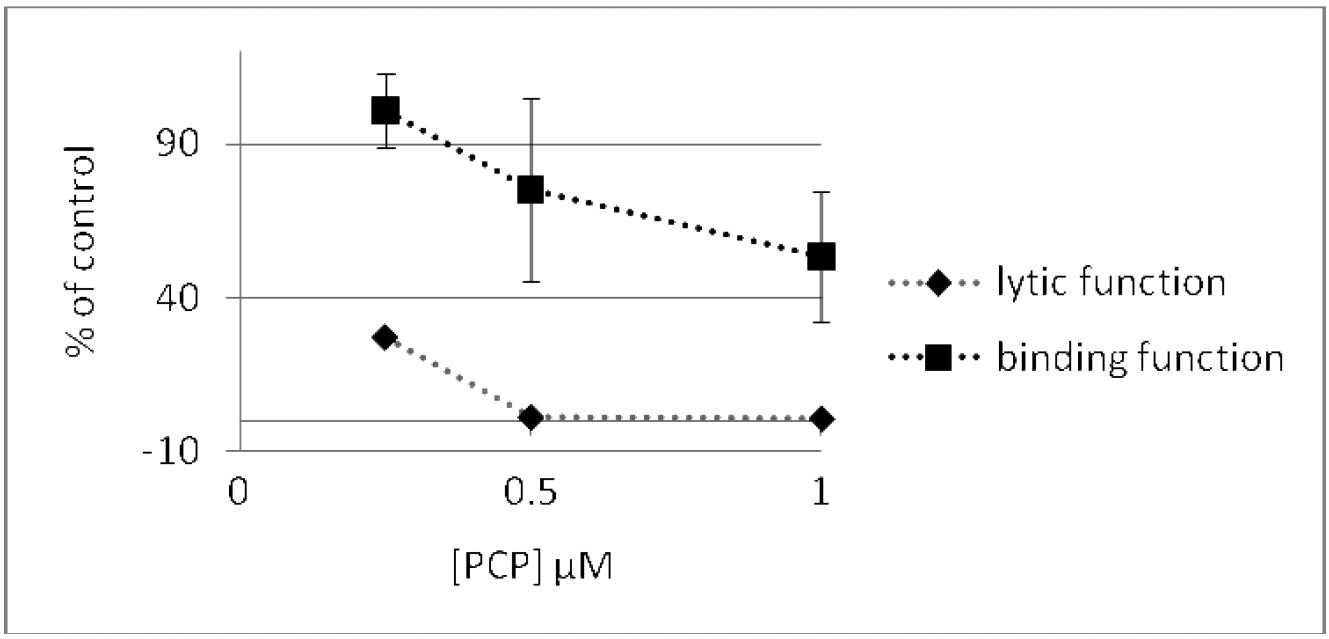

Figure 6.
Effects of PCP exposure on lytic function (◇) and binding function (■) (plotted on the same graph). (A) 24 h exposure to PCP, (B) 48 h exposure to PCP, (C) 6 days exposure to PCP.

Table 1

Effect of 24 h, 48 h, and 6 d exposures to PCP or 1 h exposures followed by 24 h, 48 h, or 6 d periods in PCP-free media on human NK cell viability.

	% NK cell viability of PCP Exposed NK cells						
Length of exposure to PCP	Concentration of PCP (μM) in exposure						
	10	5	2.5	1	0.5	0.25	0
24 h	98.0±1.7	99.0±1.2	99.1±0.6	99.1±1.1			98.6±1.0
48 h		97.6±2.8	98.2±2.0	99.0±1.3			97.4±2.8
6 d				87.0±5.5	92.6±6.6	88.8±12.6	89.6±6.6
1 h/24 h*	99.6±1.1	99.3±0.6					99.1±0.9
1 h/48 h*	98.2±0.6	99.6 ±0.6					98.5±0.6
1 h/6 d*	95.4±1.8	96.8 ±2.1					95.1±0.6

*1h/24h, 1h/48h, 1h/6d indicate a 1 h exposure to PCP followed by 24 h, 48 h, or 6 days in PCP-free media