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Effects of DDT and Triclosan on Tumor-cell Binding Capacity and Cell-Surface Protein Expression of Human Natural Killer Cells

Tasia Hurd-Brown, Felicia Udoji, Tamara Martin, and Margaret M. Whalen*

*Departments of Biological Sciences and Chemistry, Tennessee State University Nashville, TN, 37209

Abstract

1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) and triclosan (TCS) are organochlorine (OC) compounds that contaminate the environment, are found in human blood, and have been shown to decrease 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 DDT and TCS that decrease lytic function for alteration of NK binding to tumor targets. Levels of either compound that caused loss of binding function were then examined for effects on expression of cell-surface proteins needed for binding. NK cells exposed to 2.5 μ M DDT for 24 h (which caused a greater than 55% loss of lytic function) showed a decrease in NK binding function of about 22%, and a decrease in CD16 cell-surface protein of 20%. NK cells exposed to 5 μ M TCS for 24 h showed a decrease in ability to bind tumor cells of 37% and a decrease in expression of CD56 of about 34%. This same treatment caused a decrease in lytic function of greater than 87%. These results indicated that only a portion of the loss of NK lytic function seen with exposures to these compounds could be accounted for by loss of binding function. They also showed that loss of binding function is accompanied by a loss cell-surface proteins important in binding function.

Keywords

DDT; Triclosan; NK cells; binding function; CD16; CD56

INTRODUCTION

NK cells are lymphocytes that are part of the innate immune system, they are able to destroy (lyse) tumor and virally infected cells and play a role in preventing the metastases of tumors (Lotzova, 1993; Vivier et al., 2004; Lanier, 2008). Due to their ability to lyse their target cells without a need for prior sensitization, 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). Their role in preventing cancer is illustrated by the fact that a patient with a deficiency of NK cells suffered from both vulvar and cervical carcinomas (Ballas et al., 1990). There is a correlation between the size of breast tumors and a decrease in the number of NK cells in breast cancer patients (Fulton et al., 1984). Families suffering from von Hippel-Lindau disease have non-functional NK cells and suffer from a variety of malignant tumors

(including hemangioblastomas of brain and spinal chord as well as renal adenocarcinomas) (Ortaldo et al., 1992). Additionally, individuals with genetic polymorphisms leading to inhibited NK function show elevated incidences of melanoma, leukemia, cervical neoplasia, and Hodgkin's lymphoma (Purdy and Campbell, 2009). Because of the important role of NK cells in destroying tumor and virally infected cells, agents that interfere with their function could lead to an increased incidence of tumors or viral infections.

NK cells directly bind to target cells (tumor cell or virally infected cell). This binding is required for the NK cell to lyse the target. Several cell surface proteins are involved in the binding of NK cells to targets (Lotzova, 1993). 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).

1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) and triclosan (TCS), are organochlorine (OC) compounds that contaminate the environment (Koplin et al., 2002; Singer et al., 2002; CDC, 2009). DDT was used to protect agricultural crops and to control mosquitoes (to prevent the spread of malaria). It has been banned for use in the USA but continues to be used in other countries (ATSDR, 2002; CDC, 2009). Although DDT has not been used in the US for many years, it can still be found in measureable quantities in blood samples in the US (Thornton et al., 2002; Patterson et al., 2009; CDC, 2009), which were highest in Mexican Americans (CDC, 2009). In Mexico, where DDT is still used, serum DDT levels as high as 23,169 ng/g of lipid (approximately 260 nM) have been found (Koepke et al., 2004; Trejo-Acevedo et al., 2009). An association between blood levels of DDT and decreased numbers of NK cells as well as decreased NK function has been reported (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 a 5 fold increase in the risk of breast cancer (Cohn et al. 2007). TCS is utilized as an antimicrobial and is in widespread use in antibacterial soap products as well as other personal hygiene products such as toothpaste (Bhargava and Leonard, 1996). It has been identified as an emerging pollutant in the environment and is present in some watersheds (Loganathan et al., 2009; Loganathan, 2012). It has been found in human blood at a level of about 14 ng/g of blood which is about 48 nM (Allmyr et al., 2006; 2008). It likely enters humans through absorption through the skin or by ingestion (Moss et al., 2000; Sandborgh-Englund, 2006).

Our studies have shown that both DDT and TCS inhibit the lytic function of highly purified human NK cells (Reed et al., 2004; Udoji et al., 2010). In the current study the effects of DDT and TCS exposures on the ability of NK cells to bind to target cells was examined. Binding to targets is an essential step in the NK lytic process. Decreased ability of NK cells to bind to targets, due to exposures to either DDT or TCS, could explain (at least in part) the DDT and TCS-induced loss of lytic function. As mentioned above, a number of cell surface proteins are needed for NK binding to target cells. 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 exposures to either DDT or TCS interferes with cell surface protein expression. Effects of both chronic and acute exposures to each of the compounds 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 the American Red Cross (Portland, OR) and 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 mM L-glutamine and 50 U penicillin G with 50 µg streptomycin/ml) at 1 million cells/mL.

Chemical preparation

TCS and DDT were purchased from Fisher Scientific. They were dissolved in dimethylsulfoxide (DMSO) (Sigma-Aldrich, St. Louis, MO) to give a 100 mM stock solutions. Desired concentrations of either DDT or TCS were prepared by dilution of the stock into complete media. The final concentration of DMSO for 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 the compound 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 the compound for 1 h; following the 1 h exposure period, the compound-containing or control media was removed and replaced with fresh compound-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 ranges examined was 0.5- 5 µM for DDT and 2.5 -10 µM for TCS.

Cell Viability

Cell viability was determined by trypan blue exclusion. Cell numbers and viability were assessed at the beginning and 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.

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 compound 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 compound 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/CO₂, 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 either DDT or TCS for the appropriate length of time. Following exposure to the compound, 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 μ l of cell suspension (250,000-500,000 cells) was labeled with 10 μ l 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 IgG κ) 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 μ l of ice-cold 1% paraformaldehyde in PBS. Samples were analyzed using 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 DDT for 24 h, 48 h and 6 days on the Binding Function of NK Cells

NK cells were exposed to 1 and 2.5 μ M DDT for 24 h and 48 h, and 0.5-2.5 μ M DDT for 6 days. 2.5 μ M DDT for 24 h decreased NK binding function by $22\% \pm 13\%$ (Figure 1). Exposure to 2.5 μ M DDT for 48 h caused a decrease in NK cell binding function of $35 \pm 11\%$ (Figure 1). NK cells were exposed to 2.5 μ M DDT for 6 days showed a significant decrease in binding function of $36\% \pm 18\%$. (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 DDT for 1 h Followed by 24 h, 48 h, and 6 Days in DDT-Free Media on the Binding Function of NK Cells

NK cells treated with DDT for 1 h were washed two times with DDT-free media and then given 24 h, 48 h, or 6 d in DDT-free media prior to assaying for their capacity to bind to tumor cells. A 1 h exposure to 5 μ M DDT followed by 24 h in DDT-free media caused a $45\% \pm 12\%$ decrease in the ability of NK cells to bind to tumor cells (Figure 2). 48 hours following a 1 h exposure to 5 μ M DDT, there was a $53\% \pm 14\%$ decrease in binding function (Figure 2). 1 h exposures to either 2.5 or 5 μ M DDT caused very significant decreases in

binding function ($55\% \pm 20\%$ with $5 \mu\text{M}$ and $54\% \pm 12\%$ with $2.5 \mu\text{M}$) when it was examined 6 d after the exposures (Figure 2).

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

NK cells exposed to $5 \mu\text{M}$ TCS for 24 h showed a decrease in NK binding function of $37\% \pm 19\%$ (Figure 3). A 48 h exposure to $5 \mu\text{M}$ TCS caused a decrease in NK cell binding function of $67 \pm 18\%$ (Figure 3). Exposure to $2.5 \mu\text{M}$ DDT for 6 days decreased binding function by $42\% \pm 21\%$ while $5 \mu\text{M}$ DDT for 6 days diminished binding function by $70\% \pm 23\%$ (Figure 3).

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

NK cells were treated with TCS for 1 h washed as described above for DDT and then given 24 h, 48 h, or 6 d in TCS-free media prior to assaying for their capacity to bind to tumor cells. Only the 1 h exposure to $10 \mu\text{M}$ DDT followed by 24 h in DDT-free media caused any decrease ($30\% \pm 11\%$) in the ability of NK cells to bind to tumor cells (Figure 4).

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

Levels of CD2, CD11a, CD16, CD18, and CD56 were examined following exposures to DDT. A 24 h exposure to $2.5 \mu\text{M}$ DDT caused a decrease in CD16 of $20\% \pm 11\%$ ($P < 0.05$). Exposure of NK cells to $2.5 \mu\text{M}$ DDT for 48 h also decreased CD16 ($17 \pm 13\%$) ($P < 0.05$). None of the other cell surface proteins examined showed significant decreases in expression at either time point. There was no decrease in expression of any of the proteins after 6 d exposures to DDT. Figure 5A shows the shift in peak fluorescence intensity for a representative experiments of CD16 at the $2.5 \mu\text{M}$ concentration after 24 h. Figure 5B shows the shift in fluorescence intensity for CD16 after a 48 h exposure to $2.5 \mu\text{M}$ DDT from a representative experiment.

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

A 1 h exposure to $5 \mu\text{M}$ DDT followed by 24 h in DDT-free media decreased CD16 expression by $39\% \pm 8\%$ ($P < 0.05$). NK cells exposed to $2.5 \mu\text{M}$ DDT followed by 24 h in DDT-free media also exhibited decreased CD16 expression ($26\% \pm 8.5$, $P < 0.05$). 48 h after a 1 h exposure to $2.5 \mu\text{M}$ DDT, there was a decrease in the expression of CD16 of $23\% \pm 7\%$ ($P < 0.05$). A 1h exposure to $5 \mu\text{M}$ DDT followed by 6 d in DDT-free media resulted in a $30\% \pm 4\%$ decrease in CD16 ($P < 0.05$). Figure 6A shows the change in intensity for a representative experiments at the $5 \mu\text{M}$ concentration after 24 h in DDT-free media and Figure 6B shows the shift in fluorescence intensity for CD16 48 h following a 1h exposure to $2.5 \mu\text{M}$ DDT from a representative experiment. Figure 6C is a representative experiment for the change in CD16 mean fluorescence intensity 6 d after a 1h exposure to $5 \mu\text{M}$ DDT.

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

A 24 h exposure to $5 \mu\text{M}$ TCS caused decreases in CD11a ($16\% \pm 8\%$, $P < 0.05$) and CD56 ($34\% \pm 9\%$, $P < 0.05$). Exposure of NK cells to $5 \mu\text{M}$ TCS for 48 h decreased the expression of CD11a ($24\% \pm 12\%$), CD16 ($39\% \pm 25\%$), CD18 ($21\% \pm 10\%$), and CD56 ($48\% \pm 9\%$), ($P < 0.05$). A 6 d exposure to $5 \mu\text{M}$ TCS caused a $34\% \pm 8\%$ ($P < 0.05$) decrease in CD11a and a $74\% \pm 9\%$ decrease in CD16. Figure 7A shows the shift in peak fluorescence intensity for a representative experiment at $5 \mu\text{M}$ TCS for CD11a and Figure 7B shows a representative experiment for CD16 at 24 h. Figures 8A-D show the decreases in peak fluorescence intensity for representative experiments for CD11a, CD16, CD18, and CD56 with NK cells exposed to $5 \mu\text{M}$ TCS for 48 h. The decrease in mean fluorescence intensity for

representative experiments for CD11a and CD16 at 5 μ M TCS for 6 d is shown in Figures 9A and 9B.

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

Exposures of NK cells to concentrations of TCS as high as 10 μ M for 1 hr, followed by 24 hr, 48 hr, or 6 d incubations in TCS-free medium, caused no significant decreases in expression of any of the cell surface proteins that were examined in these studies (data not shown).

DISCUSSION

Previously, we demonstrated that both DDT and TCS were able to diminish the ability of human NK cells to destroy tumor target cells (Reed et al., 2004; Udoji et al., 2010). Both of these compounds have been found in human serum (Allmyr et al., 2006; 2008; Koepke et al., 2004; Trejo-Acevedo et al., 2009). As mentioned earlier, exposure to DDT correlates with increased incidences of several cancers: liver, pancreatic, and breast (Eskenazi, 2009). The current study examines whether exposures to DDT and TCS, that are known to decrease NK lytic function, have effects on the ability of NK cells to bind to their targets. In addition, levels of several cell-surface proteins important in the binding of NK cells to targets were examined in NK cells exposed to either DDT or TCS. NK cell binding to targets is a necessary step in their lysis of target cells and any interference with binding could, at least in part, explain the loss of lytic function seen with exposures to DDT and TCS. The cell-surface proteins, CD2, CD11a, CD16, CD18, and CD56, are all important in the recognition and binding of target cells by NK cells (Lotzova, 1993; Mandelboim et al., 1999; Nitta et al., 1989). Thus any decrease in binding could be explained by an accompanying decreases of one or more of these proteins on the cell surface, and this was also examined.

In past studies, 2.5 μ M DDT was shown to decrease NK lytic function by about 60% after a 24 h exposure and 1 μ M DDT decreased lytic function by about 20% (Udoji et al. 2010). When NK cells were exposed to DDT under these same conditions, binding function was not decreased by 1 μ M DDT and was decreased by 22% at 2.5 μ M DDT. Decreases in binding function were about 35% when NK cells were exposure to 2.5 μ M DDT for 48 h or 6 days, however the decreases in lytic function at these same time points were 70% and 85%, respectively (Udoji et al., 2010). A 6 day exposure to as little as 0.25 μ M DDT caused a decrease in lytic function of 19%. These results indicate that the loss of NK lytic function that was seen with DDT exposures in previous studies (Udoji et al., 2010) cannot be fully explained by loss of binding function.

Effects on NK lytic function can be compared to effects on binding function with TCS exposures. We see that for a 24 h exposure to 5 μ M TCS there is a decrease in lytic function of 87% (Udoji et al., 2010) and decrease in binding function of 37%. Exposure to 2.5 μ M TCS for 24 h decreases lytic function by greater than 60% (Udoji et al, 2010) but has no effect on binding function. Forty eight hour and 6 day exposures to 5 μ M TCS caused decreases in lytic function of around 85% (Udoji et al., 2010) and decrease binding function by nearly 70%. However, 48 h exposures to 2.5 μ M TCS decreased lytic function by 65% (Udoji et al., 2010) but had no effect on binding function and 6 day exposures to 1 μ M TCS decreased lytic function by over 35% (Udoji et al., 2010) but had no effect on binding function. Decreases in binding function can account for a significant portion of the losses of lytic function seen at certain exposures to TCS, but they cannot fully explain loss of lytic function. Decreased NK lytic function that is partially associated with decreased binding function has been seen with other environmental contaminants: tributyltin (TBT), dibutyltin (DBT) (Whalen et al., 2002; Odman-Ghazi et al., 2003), tetrabomobisphenol A (TBBPA)

(Kibakaya et al., 2009), hexabromocyclododecane (HBCD) (Hinkson and Whalen, 2010), and ziram (Taylor and Whalen, 2009). Another organochlorine (OC) compound, pentachlorophenol (PCP), has also been shown to block the lytic function of human NK cells under conditions where binding function is unaffected (Hurd et al., 2012).

The association of decreased NK binding function and decreases in specific cell-surface proteins with exposures to DDT and TCS were also examined. The results indicated an association between decreased binding function and expression of CD16 after a 24 h and 48 h exposure to 2.5 μ M DDT. For instance, binding was decreased by 22% after 24 h and expression of CD16 was decreased by 20%. However, decreased binding at 6 days did not associate with a significant decrease in CD16 expression. When NK cells were treated with DDT for 1 h and then given 24 h, 48 h, or 6 days in DDT-free media following the exposures, there was an association between the loss of binding function and the loss of CD16 expression. As an example, 24 h after a 1 h exposure to 5 μ M DDT there was a decrease in binding function of 45% and a decrease in CD16 expression of 39%. Thus, there appears to be a significant association between loss of binding function and a decrease in CD16 when NK cells are exposed to DDT. Other categories of environmental contaminants, butyltin (BTs) (TBT and DBT), brominated flame retardants (BFRs) (TBBPA and HBCD), and a carbamate (ziram), have also been shown to cause 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).

There was also an association between decreased NK binding function and expression of cell-surface proteins when NK cells were exposed to TCS. A 24 h exposure to 5 μ M TCS decreased binding function by 37% while decreasing the expression of 2 cell surface proteins CD11a (16%) and CD56 (34%). NK cells exposed to 5 μ M TCS for 48 h showed a decrease in binding function of 67% which was associated with decreases in CD11a (24%), CD16 (39%), CD18 (21%), and CD56 (48%) and those exposed for 6 days had decreased binding of 70% and decreases in CD11a of 34% and CD16 of 74%. As mentioned above, several other environmental contaminants have shown associations of binding function and cell-surface protein expression and there is also an association between binding and cell-surface protein expression with TCS exposures. However, the pattern for TCS exposures is somewhat distinct from that seen with most of the other contaminants tested, including the other OC examined in this study, DDT. TCS affected more cell-surface proteins than DDT and had an even stronger association between decreased binding function and decreased cell-surface proteins than DDT. Another OC, PCP, showed very similar effects on cell-surface protein expression and binding (Hurd et al., 2012). Nonetheless, the loss of CD16 is common to nearly all conditions that decrease binding function regardless of the category of contaminant being examined.

CD16 has been shown to be important both in binding and lysis of antibody coated targets (Lotzova, 1993) as well as binding and lysis of tumor targets (Mandelboim et al., 1999). Thus, it is not surprising that compounds that interfere with binding of the NK cell to its target would in most cases decrease the expression of CD16. This information is important in beginning to sort out the molecular basis for the interference in NK function that has been reported for a range of environmental contaminants, including (but not limited to) the BTs, BFRs, and OCs (Whalen et al., 2002; Odman-Ghazi et al., 2003; Kibakaya et al., 2009; Hinkson and Whalen, 2010; Udoji et al., 2010). Clearly disruption of binding is not the entire explanation for loss of lytic function but it is a critical component.

DDT exposure has been correlated with decreased NK cell number and function and increased incidences of liver, pancreatic, breast, testicular cancers, and leukemia (Svensson

et al., 1994; Eskenazi et al., 2009; Cohn et al. 2007). We see decreases in NK function at levels of DDT that are present in some individuals (260 nM) (Koepke et al., 2004; Trejo-Acevedo et al., 2009) (decreased lytic function was seen at 250 nM after 6 days of exposure (Udoji et al., 2010)). The data presented here indicate that the losses of lytic function seen at 250 nM were not due to decreased binding function or decreased cell surface protein expression. Furthermore, our studies showing decreases in lytic function were done *in vitro* and it would be expected that the *in vivo* effects will be far more complex. However, we have shown that DDT does interfere with NK function. NK function, as mentioned in the introduction, is an important aspect of preventing tumor development. Thus, it is important to understand how DDT and other environmental contaminants interfere with this essential immune protection against cancer development.

In summary the current study shows: 1.) Exposure of NK cells to DDT interferes with ability of NK cells to bind to tumor target cells; 2.) Exposure of NK cells to TCS interferes with ability of NK cells to bind to tumor target cells; 3.) Compound-induced loss of binding function explains, in part, the loss of NK cell ability to lyse tumor cells under some conditions; 4.) DDT-induced loss of binding function can be accompanied by a decreased expression of CD16; 5.) TCS-induced loss of binding function is accompanied by decreased expression of several cell-surface proteins.

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Figure 1.

Effects of 24 h, 48 h and 6 day continuous exposures to DDT on the ability of NK cells to bind K562 tumor cells. Medium gray bars = NK cells exposed to 0.5 μM DDT; dark gray bars = NK cells exposed to 1 μM DDT; black bars = NK cells exposed to 2.5 μM DDT. Results are mean ± S.D. (n=9 for all exposures). * indicates that the decrease in binding was statistically significant (p<0.05).

Figure 2.

Effects of 1 h exposures to DDT followed by 24 h, 48 h and 6 days in DDT-free media on the ability of NK cells to bind to K562 tumor cells. Medium gray bars = NK cells exposed to 1 μM DDT; dark gray bars = NK cells exposed to 2.5 μM DDT; black bars = NK cells exposed to 5 μM. Results are mean ± S.D. (n=9 for all exposures). * indicates that the decrease in binding was statistically significant (p < 0.05).

Figure 3.

Effects of 24 h, 48 h and 6 day continuous exposures to TCS on the ability of NK cells to bind K562 tumor cells. Dark gray bars = NK cells exposed to 2.5 μ M TCS; black bars = NK cells exposed to 5 μ M. Results are mean $\pm$ S.D. ($n=9$ for all exposures). * indicates that the decrease in binding was statistically significant ($p < 0.05$).

Figure 4.

Effects of 1 h exposures to TCS followed by 24 h, 48 h and 6 day in TCS-free media on the ability of NK cells to bind to K562 tumor cells. dark gray bars = NK cells exposed to 5 μ M TCS; black bars = NK cells exposed to 10 μ M TCS. Results are mean $\pm$ S.D. (n=9 for all exposures). * indicates that the decrease in binding was statistically significant ($p<0.05$).

Figure 5.

Histograms from representative experiments showing effects of 24 h, 48 h and 6 day exposures to DDT on CD16 expression in NK cells. A.) 24 h exposure to 2.5 μ M DDT: Dashed line = IgG control; thin solid line = control NK cells stained with anti-CD16 antibody; bold line = DDT-exposed cells stained with anti-CD16 antibody; y axis = cell number; x axis = fluorescence intensity . B.) 48 h exposure to 2.5 μ M DDT: Dashed line = IgG control; thin solid line = control NK cells stained with anti-CD16 antibody; bold line = DDT-exposed cells stained with anti-CD16 antibody; y axis = cell number; x axis = fluorescence intensity.

A

B

C

Figure 6.

Histograms from representative experiments showing effects of 1 h exposures to DDT followed by 24 h, 48 h, and 6 days in DDT-free media on CD16 expression in NK cells. A.) 24 h following a 1 h exposure to 5 μ M DDT: Dashed line = IgG control; thin solid line = control NK cells stained with anti-CD16 antibody; bold line = DDT-exposed cells stained with anti-CD16 antibody; y axis = cell number; x axis = fluorescence intensity. B.) 48 h following a 1 h exposure to 2.5 μ M DDT: Dashed line = IgG control; thin solid line = control NK cells stained with anti-CD16 antibody; bold line = DDT-exposed cells stained with anti-CD16 antibody. C.) 6 days following a 1 h exposure to 5 μ M DDT: 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.

Figure 7.

Histograms from representative experiments showing effects of 24 h exposures to 5 μ M TCS on CD11a and CD56 expression in NK cells. A.) 24 h exposure to 5 μ M TCS: Dashed line = IgG control; thin solid line = control NK cells stained with anti-CD11a antibody; bold line = TCS-exposed cells stained with anti-CD11a antibody; y axis = cell number; x axis = fluorescence intensity. B.) 24 h exposure to 5 μ M TCS: Dashed line = IgG control; thin solid line = control NK cells stained with anti-CD56 antibody; bold line = TCS-exposed cells stained with anti-CD56 antibody; y axis = cell number; x axis = fluorescence intensity.

Figure 8.

Histograms from a representative experiments showing effects of 48 h exposures to 5 μ M TCS on CD11a, CD16, CD18, and CD56 expression in NK cells. A.) Dashed line = IgG control; thin solid line = control NK cells stained with anti-CD11a antibody; bold line = TCS-exposed cells stained with anti-CD11a antibody; y axis = cell number; x axis = fluorescence intensity. B.) Dashed line = IgG control; thin solid line = control NK cells stained with anti-CD16 antibody; bold line = TCS-exposed cells stained with anti-CD16 antibody; y axis = cell number; x axis = fluorescence intensity. C.) Dashed line = IgG control; thin solid line = control NK cells stained with anti-CD18 antibody; bold line = TCS-exposed cells stained with anti-CD18 antibody; y axis = cell number; x axis = fluorescence

intensity. D.) Dashed line = IgG control; thin solid line = control NK cells stained with anti-CD56 antibody; bold line = TCS-exposed cells stained with anti-CD56 antibody.

Figure 9.

Histograms from a representative experiments showing effects of 6 day exposures to 5 μ M TCS on CD11a and CD16 expression in NK cells. A.) Dashed line = IgG control; thin solid line = control NK cells stained with anti-CD11a antibody; bold line = TCS-exposed cells stained with anti-CD11a antibody; y axis = cell number; x axis = fluorescence intensity. B.) Dashed line = IgG control; thin solid line = control NK cells stained with anti-CD16 antibody; bold line = TCS-exposed cells stained with anti-CD16 antibody; y axis = cell number; x axis = fluorescence intensity.