EFFECTS OF TCDD ON LFA-1 MEDIATED MICE SPLEEN CELLS ADHESION

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Abstract

We examined whether the alternation in cell adhesiveness is associated with the modulation of LFA-1 expression and its second messengers following exposure to TCDD. *In vitro*, 10nM TCDD exposure suppressed splenocyte adhesion. In addition, the adhesiveness was reduced after *in vivo* exposure to TCDD (15μg/kg) for six weeks with one week interval and after additional *in vitro* stimulation with anti-CD3. The inhibition of adherence by TCDD was related to a decreased LFA-1 expression, and expression patterns of Rap1 following TCDD exposure correlated with those of LFA-1 expression. However, TCDD did not selectively alter LFA-1 and Rap1 expression in T cell subsets. TCDD caused apparent changes in PI 3-kinase expression levels and the expression patterns of H-Ras correlated with those of PI 3-kinase expression. These data suggest that TCDD exposure down-regulates the conformation and ligand binding affinity of LFA-1 by Rap1 and PI 3-kinase signaling pathways with the decreased expression of LFA-1, and consequently leads to a decrease in the LFA-1-mediated adhesion.

Introduction

TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) is potently immunotoxic in laboratory animals, with exposure primarily linked to alternations in cellular and humoral immunity[1]. Leukocyte migration across the endothelium is controlled by the activity of the intercellular adhesion molecules (ICAMs) and their receptors on the cell surface, the integrins[2]. LFA-1 (leukocyte function-associated antigen-1) is the most abundant and widespread in expression[2]. Therefore, together with ICAM-1, LFA-1 is of pivotal importance in adhesion events.

This study is a continuation of our previous investigations on the immunotioxic effect of TCDD exposure[3,4]. In this present study, we examined the alteration of cell adhesiveness after TCDD exposure and further investigated whether this change was associated with the modulation of LFA-1 and the second messengers, Rap1, Ras, and PI 3-kinase.

Materials and methods

Animals: C3H mice were used at 10-14 weeks of age. . Each group consisted of 5-10 mice.

Animal treatment *in vivo*: TCDD (Supelco) was diluted in olive oil (Sigma). The vehicle control consisted of an equivalent amount of toluene in olive oil. Mice were injected with vehicle or 15 μg/kg TCDD for six weeks with one week interval.

Culture conditions for *in vitro* experiments: Cell suspensions from pooled spleens from 5-10 mice were prepared from groups of age- and sex-matched mice. The cells were cultured at a $5x10^6$ cells/ml in complete medium. The cell suspensions were added to uncoated cell culture or plates coated with immobilized anti-CD3ε (BD Biosci., 10 μg /ml). The cell cultures were either treated with vehicle (DMSO, 10 μl/ml) or exposed to 10 nM TCDD, and incubated for 30 minutes in a humidified incubator containing 5% CO_2 at 37°C.

Cell adhesion assay: Adhesion assay using ICAM-1-coated plates were performed as described previously[5].

Immunoblot analysis: Proteins were transferred to PVDF-plus membranes (Osmonics) and blotted with peroxidase-conjugated antibodies as described elsewhere[5].

T cell subset selection: T cell subsets were selected using a direct selection method according to the manufacturer's protocol (MACS Microbeads). The level of purity in the cell preparation was analyzed by FACS using immunofluorescent staining with anti-mouse CD4, CD8, or B220 (BD Biosci.). Each T cell subset was resuspended at a concentration of $1x10^5$/ml in complete medium.

Quantitative PCR using real time system: The total cellular RNAs were extracted from the whole splenocytes, $CD4^+$ or $CD8^+$ T cells using the TRIzol Reagent (Invitrogen). Quantitative assays of LFA-1 and Rap1 mRNA expression were performed as described previously[6]. The primer sequences used are as follows: LFA-1(108bp): s-primer, CCGACAACTCCAACCAGTTT, as-primer, GCAATGCAACTTGCATT; Rap1(187bp): s-primer, GAGATCACTGAAGCCTGAGTCC, as-primer, GTGGAGACAGTGAAAGGACGTT.

The Student's paired t-test was used to compare the treatment effects. A p-value <0.05 was considered significant.

Results and discussion

Effect on cell adhesion: A previous study showed that splenic dendritic cells from TCDD-treated mice expressed higher levels of ICAM, B7-2, CD24 and CD40, whereas the expression of LFA-1 was significantly reduced[7]. In first step, we investigated whether exposure to TCDD reduced cell adhesion to ICAM-1-coated plates. TCDD or anti-CD3+TCDD treatment reduced splenocyte adhesion to a purified ICAM-1-coated plate. Moreover, the relative percentage of TCDD/vehicle or anti-CD3+TCDD/anti-CD3+vehicle were 82.89 ± 12.88% and 67.31 ± 12.13%, respectively (Fig 1).

In next step, we determined whether the decreased cell adhesion ratio of splenocytes observed *in vitro* also occur *in vivo*. As shown in Fig 2, TCDD treatment *in vivo* also suppressed cell adhesion to ICAM-1 coated plate (TCDD/vehicle: 82.93%).

As further evaluation, we studied the changes of cell adhesiveness with stimulation after TCDD exposure. The cell adhesion pattern after anti-CD3 stimulation for 30 minutes was similar to those in the unstimulated group (TCDD/vehicle: 76.78%) (Fig 3).

Effects on LFA-1 and Rap1 mRNA expression: The stimulation of T cell receptor leads to rapid changes in the expression of adhesion molecule, which in turn activates LFA-1-dependent adhesion[8]. Therefore, we stimulated

the splenocytes with anti-CD3 in this experimental system. LFA-1 mRNA expression levels were suppressed by TCDD. In addition, the down-regulation of LFA-1 expression was more striking in the anti-CD3 stimulated splenocytes ($p<0.005$) than the unstimulated cells ($p<0.01$) (Fig 4).

Since Rap1 is a potent activator of the leukocyte integrins[9,10], we examined the change of Rap1 expression by TCDD. The expression pattern of Rap1 in the anti-CD3 stimulation group was similar to those of LFA-1 expression by TCDD ($p<0.05$) (Fig 5).

Next we investigated what kinds of T cell subsets were affected by TCDD. LFA-1 expression levels in $CD4^+$ and $CD8^+$ T cells were significantly suppressed by TCDD ($p<0.05$, respectively) (Fig 6). Interestingly, there was no significant difference on anti-CD3+TCDD/anti-CD3+vehcile between the T cells subsets.

As further investigation, we determined if the decrease in LFA-1 expression by TCDD may be related to the modulation in Rap1 expression in T cell subsets. The Rap1 expression patterns after anti-CD3+TCDD treatment in $CD4^+$ and $CD8^+$ T cells are similar to those in the LFA-1 ($p<0.05$ respectively). In addition, exposure to TCDD caused a similar pattern of effects on both T cell subsets as seen in LFA-1 mRNA expression (Fig 7).

Effects on PI 3-kinase and H-Ras expression: Since the activation of PI 3-kinase lead to activation of LFA-1-mediated adhesion in a variety of cell types and Ras plays an important role in signal transduction in multiple pathways[9,11], we investigated the levels of PI 3-kinase and Ras expression by western blotting. TCDD exposure caused a decrease of PI 3-kinase expression when compared with anti-CD3+vehicle treated group (84.41±8.00%) (Fig 8). However, TCDD had no obvious effect on Ras expression compared with the positive control (92.82±5.00%) (Fig 9).

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Fig 1.

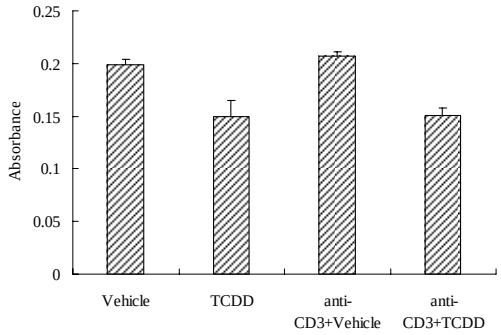


Fig 2.

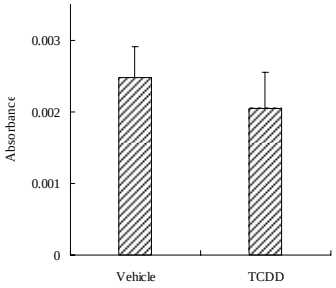


Fig 3.

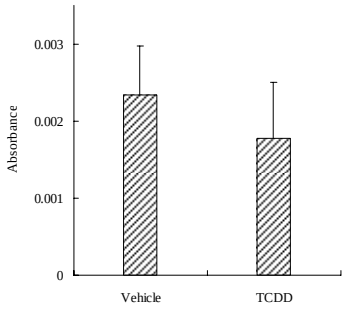
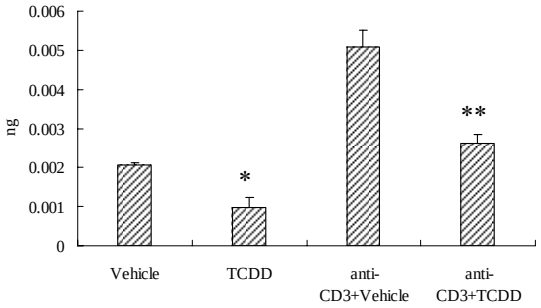


Fig 4



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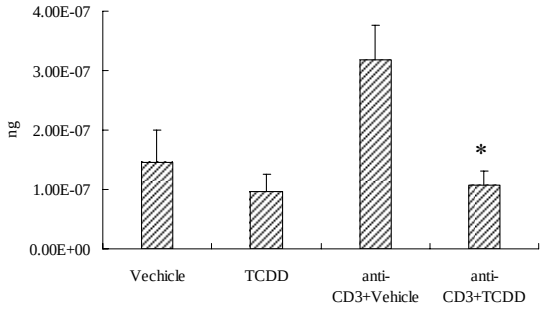
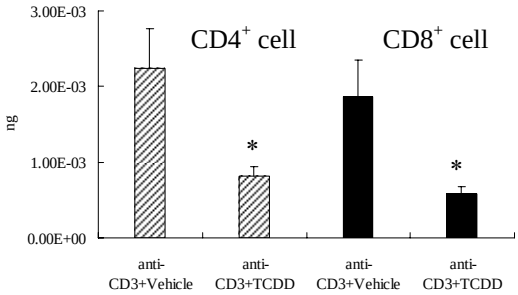


Fig 6.



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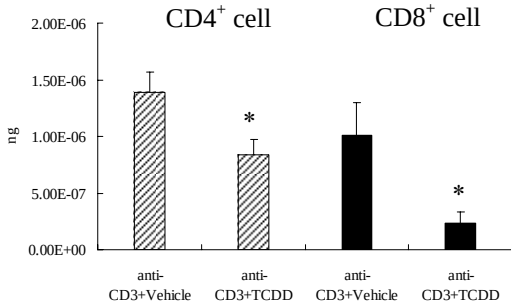

Fig 8.

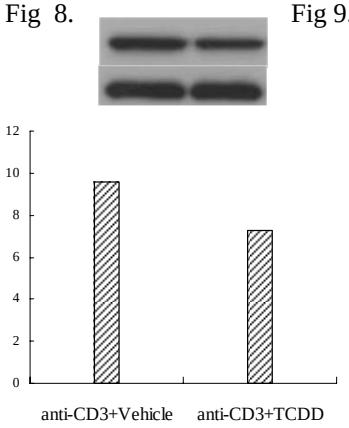

Fig 9.