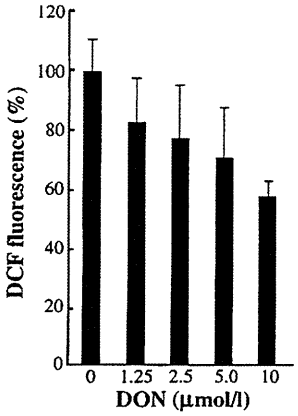

Fig. 2 Effects of DON on cellular oxidation in HepG2. HepG2 cells were treated with the indicated concentrations of DON for 24 h and ROS levels were measured using DCFH-DA. Values are presented as the means ± SEM from three independent experiments. A statistical analysis was performed using the unpaired Student's *t* test (*$P<.05$, vs. control)

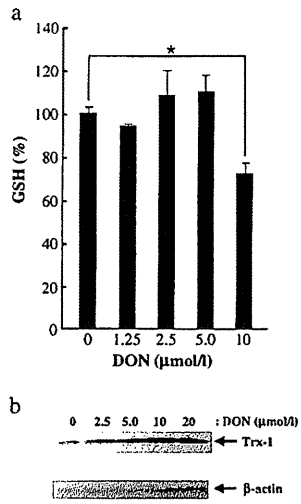

Fig. 3 Effects of DON on levels of GSH and Trx-1. a HepG2 cells were treated with the indicated concentrations of *DON* for 24 h and levels of *GSH* were measured. The GSH content in the absence of DON is expressed as 100%. Values are presented as the means ± SEM from three independent experiments. A statistical analysis was performed using the unpaired Student's *t* test (*$P<.05$, vs. control). b HepG2 cells were treated with the indicated concentrations of DON for 16 h. The cell lysates were prepared and analyzed for *Trx-1* and *β-actin* proteins by western blotting. The results are representative of three independent experiments

that acts as a reducing agent and thought to be the most abundant intracellular antioxidant (Sugiyama et al. 2000; Fath et al. 2011; Gaubin et al. 2000). The reducing environment of the intracellular redox state is maintained by the content of endogenous GSH (Fath et al. 2011). This study evaluated the intracellular GSH levels of HepG2 in the presence of DON to examine the involvement of intracellular GSH level in the reduced state of HepG2 cells treated with DON. Figure 3a shows that 1.25–5.0 μmol/l DON had no positive effect on intracellular GSH accumulation. Furthermore, intracellular GSH levels were significantly decreased by approximately 30% in HepG2 treated with 10 μmol/l DON in comparison to HepG2 control cells. Therefore, it appeared that the intracellular GSH content was not increased in HepG2 cells treated with non-cytotoxic levels of DON. Trx-1, which is a small ubiquitous protein (12 kDa), plays an important role in regulating cellular redox homeostasis (Tian et al. 2008). Trx-1 has a redox-active disulfide/dithiol bond within a highly conserved active site and shows scavenging activity for various ROS (Tian et al. 2008; Ohashi et al. 2006). The level of expression of Trx-1 in HepG2 cells treated with DON was measured by western blotting to examine the effect of DON on intracellular content of Trx-1. In contrast to GSH, the Trx-1 level in HepG2 cells was induced by DON in a concentration (2.5–10 μmol/l)-dependent manner, and DON-induced Trx-1 up-regulation was also observed in the presence of 20 μmol/l of DON (Fig. 3b). These results suggest that non-cytotoxic concentrations of DON maintain the intracellular redox state in HepG2 by up-regulating the Trx-1 expression level.

Up-regulation of Trx-1 by DON in HepG2 cells is suppressed by antioxidant treatment

The expression of Trx-1 in HepG2 cells treated with DON in the presence of antioxidants was evaluated to verify whether

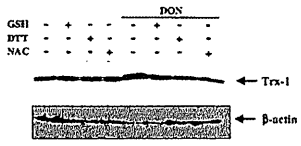

Fig. 4 Effects of antioxidants on the accumulation of Trx-1 in HepG2 treated with DON. HepG2 cells were treated without or with 10 μmol/l DON in the absence or presence of 1.0 mmol/l antioxidants (*NAC*, *DTT* and *GSH*) for 16 h. The cell lysates were prepared and analyzed for Trx-1 and β-actin proteins by western blotting. The results are representative of six independent experiments

the intracellular redox state might be associated with the DON-induced enhancement of Trx-1 levels in the cells. Figure 4 shows a western blotting analysis which demonstrated the up-regulation of Trx-1 induced by 10 μmol/l DON to be suppressed by treatment with 1.0 mmol/l antioxidants such as GSH, DTT or NAC. NAC is a synthetic precursor of GSH that is used to enhance the intracellular GSH content (Rahman and MacNee 1999). However, DTT cannot be used as a GSH precursor. Therefore, the antioxidant activity of GSH, DTT or NAC is thought to play an important role in the repression of DON-induced Trx-1 upregulation.

The current study found that non-cytotoxic concentrations of DON did not lead to a higher oxidation state in HepG2 cells. On the other hand, many studies have observed DON-induced oxidative stress including ROS generation (Krishnaswamy et al. 2010; Braicu et al. 2009; Zhang et al. 2009; Bouaziz et al. 2006, 2008). Indeed, HepG2 cells also produced ROS in the presence of 60 μmol/l DON (Zhang et al. 2009). This discrepancy may be due to the use of a range of toxin concentrations. Indeed, DON-induced cell death is accompanied by ROS formation, and the intracellular redox status shifts to more oxidative conditions (Braicu et al. 2009). Conversely, this means that HepG2 cells acquire adaptation mechanisms against oxidative stress induced by DON. HepG2 cells might be able to develop an adaptive response to oxidative stress induced by non-cytotoxic levels of DON. A non-oxidative intracellular environment may be needed for HepG2 cells to survive under these conditions. However, the current study showed that treatment with non-cytotoxic levels of DON did not increase intracellular GSH levels of HepG2 cells. On the contrary, intracellular GSH level was significantly reduced after exposure to 10 μmol/l DON. This suggests that GSH might be consumed by GST-mediated GSH conjugation during the detoxification processes of removing DON (Gouze et al. 2006). The present study observed that Trx-1, which is one of the major intracellular antioxidants in addition to GSH (Ago and Sadoshima 2006), was increased in HepG2 cells treated with nonlethal concentrations of DON. Therefore, it is suggested that the more reducing environment in HepG2 cells treated with non-cytotoxic concentrations of DON appeared to be due to the accumulation of Trx-1 caused by the mycotoxin. The vital biological activities of Trx-1 include acting as a cofactor in various processes (Watson et al. 2004). However, the DON-mediated up-regulation of Trx-1 may play an important role in maintaining or inducing a more reducing environment within the cells via its redox activity. This hypothesis is supported by the findings that the up-regulation of intracellular Trx-1 was suppressed by other antioxidants. In addition, (-)-epigallocatechin gallate (EGCG), the major green tea polyphenol, has protective ability against the cytotoxicity caused by DON in a macrophage cell line, suggesting that antioxidants can play an important role in reducing DON-induced cytotoxicity in leucocytes, because EGCG possesses stronger antioxidative properties (Sugiyama et al. 2011; Kagaya et al. 2002). Accordingly, it is thought that the antioxidant properties of Trx-1 could participate in regulating DON-induced cytotoxicity. Further studies are required to elucidate the exact mechanism underlying such responses and investigate the effect of DON on the activity of thioredoxin reductase, which is involved in the reduction of oxidized Trx-1, in order to understand the physiological significance of the up-regulation of Trx-1 by nonlethal concentrations of DON in HepG2 cells (Watson et al. 2004). Additionally, though DON-induced cytotoxicity evoked after oxidative stress including ROS production, more research is needed to investigate whether the up-regulation of Trx-1 by DON in HepG2 cells plays a role in protecting HepG2 cells from DON-induced cytotoxicity. Comparisons of antioxidant levels in hepatocytes and leucocytes are also important for further research to clarify the DON-sensitive mechanisms of leucocytes.

In conclusion, non-cytotoxic concentrations of DON, which caused cytotoxicity to THP-1 and KU812, did not induce a shift in the intracellular redox state of HepG2 cells to a more oxidizing environment, which might be caused by the up-regulation of the intracellular Trx-1 level. In addition, the DON-induced up-regulation of Trx-1 was suppressed by antioxidants, suggesting that antioxidative capacity may be closely related to development of resistance to DON-induced cytotoxicity.

Acknowledgements This work was supported by a Health and Labor Sciences Research Grant from the Ministry of Health, Labor and Welfare of Japan. The authors are also grateful to Rino Yamazaki for her excellent technical assistance.

Conflicts of interest None.

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...hydrocarbon receptor suppresses LPS-induced IL-6 production through ...n of histamine production in macrophages

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...d hydrocarbon receptor (Ahr) is a ligand-activated transcription factor. It is ...that Ahr is activated by such ligands as 2,3,7,8-tetrachlorodibenzo-p-dioxin ... and 3-methylcholanthrene (3MC). We have recently reported that Ahr is ...d in immune regulation. In particular, we demonstrated that Ahr forms a com... Stat1 and NF-kB in LPS-stimulated macrophages and that it negatively reg... LPS-induced pro-inflammatory cytokine production by inhibiting NF-κB ...on. In this study, we showed that Ahr-Sp1 complex, independent of Stat1, also ...ly controls another pathway of IL-6 production via histamine 1 receptor ...through suppression of histamine secretion in LPS-activated macrophages. ...d that Ahr inhibits the expression of LPS-induced histidine decarboxylase ...which produces histamine, and histamine secretion in macrophages activated ... Ahr combines with activated Sp1 that leads to the attenuation of Sp1 DNA ... activity in HDC promoter through inhibition of Sp1 phosphorylation on Ser ...

...well known that histamine is a chemical mediator that causes various phys... and pathological reactions, including smooth muscle contraction, gastric ...cretion, neurotransmission in the central nervous system, allergic reactions, ...mune regulation. These reactions are induced in various cell types via H1-...ch are G protein-coupled receptors. In particular, H1R activation enhances ...atory functions in immune cells. We also found that loratadine and chlor..., H1R antagonists, more critically impair the production of LPS-induced ...n that of other inflammatory cytokines, such as TNF-α, IL-12, and IL-10, in ...macrophages. This result suggests that there is a predominant pathway of ...duction via H1R in macrophages. Taken together, these results demonstrate ...r regulates IL-6 production via H1R signaling through suppression of LPS-... histamine production in macrophages.

...i:1016/j.cyto.2011.07.116

...lactoferrin counteracts toll-like receptor mediated activation signals in ...n presenting cells

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...ctoferrin (LF), a key element of mammalian immune system, plays pivotal roles ...defence against infection and excessive inflammation. LF exerts direct effects ...ogens but also has indirect activity on host cell components and immune sys... preventing systemic inflammation. In this study, we show that monocyte-... dendritic cells (MD-DCs) generated in the presence of bovine LF (bLF) fail ...go activation by up-modulating CD83, co-stimulatory and major histocom... complex molecules, and cytokine/chemokine secretion. Moreover, these ...ow an increased expression of molecules with negative regulatory functions ...3 and PD-L1), IDO, SOCS-3 and phospho-tyr-STAT3. Consistent with an ...d maturation, bLF-MD-DCs primed T lymphocytes exhibit a functional unre...ness characterized by reduced expression of CD154 and impaired secretion ...mma and IL-2.

...estingly, bLF induces IL-6 and CCL1 secretion in monocytes and bLF-MD-DCs ...s exposure of already differentiated MD-DCs completely fails to induce IL-6 ...hibit TRL agonist-induced activation. Cell-specific differences in bLF internal... likely account for the distinct response elicited by bLF in monocytes versus ...e MD-DC, providing a mechanistic base for its multiple effects. The receptors ...LR2 and TLR4 are significantly involved in bLF-induced secretion of IL-6 and ...t not in its internalization into these cells.

...ese results indicate that bLF exerts a potent anti-inflammatory activity by ...g monocyte differentiation into DCs with impaired capacity to undergo activa...d to promote Th1 responses. Overall, these bLF-mediated effects may represent ...gy to block excessive DC activation upon TLR-induced inflammation, adding ... evidence for a critical role of bLF in directing host immune function to protect ...microbial insults.

...1016/j.cyto.2011.07.117

PS1-087

Trichothecene mycotoxins inhibit MyD88-independent pathways of Toll-like receptors

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Trichothecne mycotoxins are secondary metabolites produced by several fungi, including Fusarium, Mycothecium, Trichoderma, Trichothecium, Stachybotrys, Verticimonosporium, and Cephalosporium species, and then more than 150 trichothecenes and their derivatives have been identified. The occurrence of trichothecene mycotoxins in food is a potentially serious problem related to food safety. Trichothecene mycotoxins cause immune dysfunction, thus leading to diverse responses to infection. However, mechanisms involved in the immunotoxic effects of these mycotoxins has not been well elucidated. We have evaluated the effect of trichothecene mycotoxins on Toll-like receptor signaling. We found that the type B trichothecenes deoxynivalenol (DON) and nivalenol (NIV) which are the most common trichothecenes in the world inhibited lipopolisaccharide (LPS)-induced nitric oxide (NO) production by mouse macrophage cell line, RAW264. Moreover, it is appeared that the expression and transcription of inducible NO synthase (iNOS) mRNA were also inhibited. Since IFN-β produced in response to LPS is involved in the expression of iNOS, we examined the effects of DON and NIV on LPS-induced IFN-β promoter reporter activity, and found that the promoter activity was repressed in a concentration-dependent manner. IFN-β expression can be induced by TLR4-mediated MyD88-independent signaling pathway. Therefore, we examined the effect of DON on IFN-β promoter reporter activity induced by TLR3 ligand Poly I:C. We also found that DON inhibited the reporter activities. In addition, T-2 and HT-2 toxin belonging to the group of type A trichothecenes reduced LPS-induced IFN-β promoter reporter activity in a dose dependent manner. These results suggest that trichothecene mycotoxins inhibit MyD88-independent pathways of Toll-like receptors and the inhibition of these factors may be involved in the immunotoxic effects of these mycotoxins.

doi:10.1016/j.cyto.2011.07.118

PS1-088

Involvement of CD23 overexpression in inflammatory Bowel Disease Pathogenesis: Immunomodulatory effect of interleukine-10 and retinoic acid

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Background: Inflammatory bowel diseases (IBD) are chronic inflammatory diseases of the gastrointestinal tract, which clinically present as one of two disorders, Crohn's disease (CD) and ulcerative colitis (UC). CD23 is a multifunctional molecule expressed on various cells. It is known as the low-affinity receptor for the Fc portion of IgE. It expression at the cell surface of phagocytic cells has been associated with the development of many inflammatory processes. In a previous study, we have shown correlation between NO production and intestinal tissue damages in patients reached by Crohn disease and ulcerative colitis. In this study, we investigate in vitro the induction of nitric oxide production by Cross linking of CD23 by monoclonal antibody in the absence or presence of interleukin-10 (IL-10) or retinoic acid in cultures of peripheral blood mononuclear cells (PBMC) and colonic mucosa from patients (CD and UC). We also evaluated CD23 expression in inflamed colonic mucosa. *Patients and methods:* Twenty Patients with active UC, patients (n=10) with active CD and healthy controls (n=10) were enrolled in this study. Freshly isolated (PBMCs) were resuspended in RPMI 1640 culture medium. Multiple colonic biopsies were taken from patients who underwent colonoscopy. Colonic biopsies were immediately placed in DMEM culture medium. The cultures were stimulated by anti-CD23 in the absence or presence of IL-10 or retinoic acid. Cultures supernatants were harvested after 24h of incubation; NO measurement was performed by modified Griess. CD23 expression was evaluated by standard immunohistochimical procedure. *Results:* Our results show that cross linking of CD23 by monoclonal antibody up regulated NO production by PBMC and colonic mucosa.Retinoic acid and exogenous IL-10 down regulated the CD23 induced NO production in vitro for the two groups of patients. A marked increase CD23 expression was seen in the inflamed colonic mucosa from active UC and CD patients. In particular, CD23 expression markedly increased in samples from active UC patients. *Conclusion:* These results suggest that CD23 play a pivotal role in IBD pathogenesis disease through nitric oxide pathway. Collectively our study suggests that IL-10 seem to be a useful tool for development of therapeutic strategies in IBD.

doi:10.1016/j.cyto.2011.07.119
