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RESEARCH ARTICLE

Reversal of perfluorooctanesulfonate-induced immunotoxicity by a glucan-resveratrol-vitamin C combination

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Abstract Perfluorinated compounds (PFCs) perfluoroctanic acid (PFOA) and perfluooctane sulphonic acid (PFOS) are environmentally widespread bioaccumulative chemicals known to induce profound effects on the immune system. In this study, two types of PFC, i.e., PFOS and PFOA, were administered orally (daily) a dose of 20 mg/kg/d. After exposure, all mice exhibited significant immunosuppressive effects upon both cellular (phagocytosis and NK cell activity) and humoral (antibody response) branches of their immune responses. The mice were then fed with a 4 mg/kg/d dose of a combination of resveratrol-glucan-vitamin C (RVB 300). The results showed that treatment with PFCs and RVB 300 resulted in significantly lower level of immunotoxic effects from PFCs. These outcomes suggest to us that RVB 300 can potentially be successfully used as a natural remedy against immunotoxicities induced by low-level exposure(s) to perfluorinated compounds.

Keywords Glucan · Immunotoxicity · Phagocytosis · Antibodies · PFOS · PFOA

Introduction

Perfluorinated hydrocarbons (PFC) are used in thousands of products. Two major classes are perfluorinated carboxylates like perfluoroctanic acid (PFOA) and perfluoroctane sulfonates such as perfluooctane sulfonic acid (PFOS). These compounds have an extremely long lifetime in the environment and are detectable in the blood of both animals and humans (Lau et al. 2007). A study of people with high exposure to perfluorinated compounds (as food contaminants) indicated that there was a significant reduction in humoral immune responses to immunizations (Grandjean et al. 2012). Animal studies noted severe suppression of both humoral and cellular immunity caused by exposure to these agents (Peden-Adams et al. 2007). Among the reactions suppressed by chronic exposure to this class of agents were: natural killer (NK) cell activity; lymphocyte proliferation and antibody responses (Dong et al. 2009; Brieger et al. 2011); release of tumor necrosis factor (TNF)-α (Brieger et al. 2011); and, secretion of interleukins (IL)-6 and −10 (Corsini et al. 2012). Wang et al. (2011) reported induction of thymic and splenic hypertrophy. Qazi et al. (2009) indicated there was an exposure-related decrease in levels circulating neutrophils. For a full review of the immunotoxicologic effects of PFC compounds, the reader is directed to De Witt et al. (2012).

While most of the current literature is focused on the description of immunotoxic effects of PFCs, there is little known about the possible blocking of these effects. Studies showing the strong potential of glucans to help overcome immunosuppressive effects induced by immuno- modulating processes such irradiation or chemotherapy (Patchen and MacVittie 1982; Vetvicka et al. 2007b; Vetvicka and Vetvickova 2009) led us to the hypothesis evaluated herein. Specific- ally, this study sought to first compare the types/degrees of immunosuppression caused by either PFOS or PFOA and to then to evaluate if any suppression could be reversed by a therapy that utilized a glucan-resveratrol-Vitamin C combination.

The choice of this particular combination to examine was based on our own earlier studies demonstrating significant stimulation of both cellular and humoral branches of the immune response using a combination of glucan and humic acid (Vetvicka et al. 2010). Another study showed that both glucan and resveratrol stimulated phagocytosis, increased splenic cell expression of CD4, and induced restoration of

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splenic population profiles after an experiment- ally-induced leukopenia (Vetvicka et al. 2007a). When effects of all of these substances on the expression of some important genes (i.e., *NF-κB2* and *Cdc42*) in breast cancer cells were measured, up-regulation of Cdc42 expression was evident only with the use of both immuno- modulators combined (Vetvicka et al. 2007b; Vetvicka and Vancikova 2010). Follow-up studies showed that, compared to each component alone, a glucan-resveratrol-Vitamin C combination caused the strongest inhibition of experimentally-induced lung and breast cancers (Vetvicka and Vetvickova 2012a).

Materials and methods

Animals

Female BALB/c mice (8-wk-old) were purchased from the Jackson Laboratory (Bar Harbor, ME). All mice were housed in a sterile animal facility that was maintained at 22 °C and at a 35 % relative humidity, with a 12-hr light/-dark cycle. All mice had ad libitum access to standard mouse chow and filtered water throughout the studies. All mice were allowed to accli- mate for a minimum of 10 day prior to any exposure being performed. All mice were weighed prior to the first dosing and again daily to assure that dosing volumes were constant over the course of the treatments. All animal work was done according to a University of Louisville IACUC protocol and with the approval of that committee IACUC #04050.

Reagents

RVB 300 mixture containing resveratrol, yeast-derived glucan #300, and Vitamin C (at a ratio of 2:2:1 [w/w/w]) was purchased from Restart Your Life (Lexington, SC). RPMI 1640 medium, Freund's adjuvant, tri-nitrophenol (TNP), bovine γ-globulin, MTT ([3-(4,5-dimethyl- thiazol-2-yl)-2,5-diphenyltetrazolium bromide]), bovine serum albumin (BSA), Concanavalin A (ConA), lipopolysaccharide (LPS), sodium dodecyl sulfate, perfluorooctanesulfonate (PFOS; 85 % purity), and perfluorooctanoic acid (PFOA; 96 % purity) were all purchased from Sigma (St. Louis, MO). Fetal calf serum (FCS) was obtained from Hyclone Laboratories (Logan, UT).

Treatment

Each treatment group in these studies contained five mice. Some mice received an oral (non-sedated gavage) dosing of PFOS or PFOA in phosphate-buffered saline (PBS; pH 7.4) at a dose of 20 mg/kg/day. Control mice received PBS only. Some mice (with and without any PFC treatments) also received 4 mg RVB 300/kg/d by oral gavage; these treatments occurred with the PFC or PBS instillation on each day. All treatments were for 7 day (except the antibody production) and all experiments were repeated three times. For all of the protocols outlined below (except those pertaining to antibody formation), 1 day after the final treatment, all mice were euthanized by CO_2 asphyxiation and their blood/organs (i.e., spleen, thymus) were aseptically removed for analyses. Blood was isolated from the orbital vein into 5 ml plastic tubes (Fisher, Pittsburgh, PA) and then placed at room temperature to allow for clotting. Serum was subsequently isolated and stored; the cells were then used in some of the protocols outlined below. The organs were blot dried, trimmed of extraneous tissue matter, and weighed; each value was normalized to body weight and reported as somatic index (i.e., 100 × organ weight/body weight).

Phagocytosis

The phagocytic activity of peripheral blood cells (phagocytes) in the blood samples iso- lated above was assessed using a technique described in Vetvicka et al. (1982, 1988). Briefly, peripheral blood cells (0.1 ml) were combined with 50 μl of a suspension of 2-hydroxyethyl methacrylate particles (HEMA; 5×10^8/ml) and then incubated 60 min at 37 °C, with intermittent gentle shaking. Thereafter, the samples were prepared as smears on glass slides that were, in turn, treated with Wright stain. Cells were then examined using a CK 30 microscope (Olympus, Pittsburgh, PA); cells with three or more HEMA particles were considered as positive for phagocytic activity. At least 200 cells in 60 high-power fields were examined per slide; five slides/animal were analyzed.

Lymphocyte proliferation assay

Splenocytes were prepared from each harvested organ using a standard protocol (Fernandez-Botran and Vetvicka 1995). Only preparations containing cells with viability >95 % were used in the experiments. Ultimately, isolated splenocytes were washed three times in RPMI 1640 medium supplemented with 10 % FCS and re-suspended at 5×10^6 cells/ml. An aliquot (100 μl) of each suspension was added to triplicate wells of a 96-well plate that contained either ConA or LPS (each at 10 μg/ml, final concentration after cell addition) or medium (negative control). The plates were then incubated for 48 h at 37 °C in a humidified 5 % CO_2 incubator. Thereafter, 10 μl of MTT (5 mg/ml) was added to each well, and the plates were incubated a further 4 h before 100 μl of 20 % sodium dodecyl sulfate (w/v) solution was added to each well. After incubation overnight at 37 °C, the plates were placed in an ELISA reader (Tecan, Research Triangle Park, NC) and the absorbance values in each well monitored at 570 nm (OD). The proliferation index was then calculated as: (OD_{ConA}-stimulated cells/OD non-stimulated cells) or (OD_{LPS}-stimulated cells/OD non-stimulated cells).

Ex vivo cytotoxicity assay

Spleen cells from the above preparations were also used for assessments of ex vivo cytotoxic activity. YAC-1 cells (obtained from ATCC; Manassas, VA) were grown in RPMI 1640 supplemented with 10 % FCS (without antibiotics) and then labeled as outlined in Vetvicka et al. (2007a). Splenocytes (10^6/ml stocks) were then placed as 100 μl aliquots into V-shaped 96-well microplates and incubated with 50 μl of the labeled YAC-1 target cells, at an effector- target ratio of 64:1. After gently centrifuging at 250 × g for 5 min, the plates were incubated for 4 h at 37 °C. The cytotoxic activity of the cells was then determined using a CytoTox 96 Non-Radioactive Cytotoxicity Assay (Promega, Madison, WI), following manufacturer instruc- tions.

Briefly, 10 μl of lysis solution was added to select wells (i.e., those for determination of maximum killing potential) 45 min before the end of the incubation period. The plates were then centrifuged at 250 × g for 5 min and 50 μl of culture supernatant from each well was then trans- ferred to corresponding wells of a flat-bottom 96-well microplate. Kit-provided reaction sub- strate (50 μl) was then added to each well, and the plate was covered and incubated (in dark) for 30 min at room temperature. The optical density (at 492 nm) in each well was then determined using the ELISA plate-reader. Specific cell-mediated cytotoxicity was ultimately calculated via the formula: Percent-specific killing (% cytotoxicity)=100 × [(OD_{exp} – OD_{spont})/(OD_{max} – OD_{spont})], where spontaneous release was obtained from target cells incubated with medium alone and maximum release was obtained from target cells lysed with the solution provided in the kit.

Splenic and thymic cellularity

To determine cellularity in two key immune system organs in the mice, spleen and thymuses were suspended in RPMI 1640 and single cell suspensions were prepared using a steel mesh. The suspension was then washed twice with cold medium and any erythrocytes present were lysed with a 0.74 % (w/v) ammonium chloride lysing buffer. After washing via centrifuga- tion, the final cells present in the pellet were re-suspended in medium and counted in a hemo- cytometer. Viability of the final populations were assessed via trypan blue exclusion; only suspensions with a viability>95 % were analyzed.

Cells were stained with monoclonal antibodies in 12×75-mm glass tubes using standard techniques (Fernandez-Botran and Vetvicka 1995). For these analyses, fluorescein isothiocyanate (FITC)-labeled anti-CD4, -CD8, and -CD49b antibodies (BD Pharmingen, San Jose, CA) were employed. Briefly, aliqouts of 5×10^5 cells were combined with 10 μl of FITC-labeled anti- bodies (1–20 μg/ml in PBS) and placed on ice (in dark) for 30 min. Thereafter, the cells were diluted with ice-cold PBS, centrifuged to remove non-adherent antibody materials, and then re-suspended in PBS containing 1 % BSA and 10 mM sodium azide. Flow cytometry was then performed using a FACScan (Becton Dickinson, San Jose, CA) system. For each sample, a minimum of 10,000 events were analyzed using Flowjo software (Tree Star, Ashland, OR).

Antibody formation assay

Formation of antibodies was evaluated using ovalbumin (OVA) as antigen. Mice were injected twice (2 week apart, IP) with 0.1 mg of ovalbumin (in 100 μl volume) and the serum was collected 7 day after the second injection. Total level of specific antibodies against OVA was detected using an ELISA assay. As positive control, a combination of OVA and Freund's adjuvant (with no co-treatments with any PFCs) was used. All test groups here received daily administration of the PFCs (alone or in combination with RVB) or PBS for the entire 3-wk period of OVA exposures.

Measurement of IgM antibodies

Serum levels of IgM antibodies directed specifically against TNP hapten was measured by ELISA in 96-well plates with wells that had been coated with 100 μl TNP (10 μg/ml) conjugated to bovine γ-globulin by incubation overnight, followed by blocking with 1 % (w/v) BSA. Briefly, 100 μl of test serum was added to each well and the plate incubated for 120 min at 37 °C. After washing with PBS-Tween to remove non-adherent proteins, goat anti-mouse IgM conju- gated with alkaline phosphatase (1:2000, Sigma) was added and the plate incubated a further 120 min at 37 °C. After another round of washing to remove the detection antibody, *p*-nitrophenyl phosphate substrate (diluted according to manufacturer instruction; Sigma) was added and the absorbance in each well was measured using the ELISA plate-reader. Wells containing standard- ized amounts of mouse anti-TNP (IgM) antibody (BD Biosciences, San Jose, CA) were analyzed in parallel to generate standard curves from which levels of anti-TNP IgM in each test sample could then be extrapolated. All test groups here received daily administration of the PFCs (alone or in combination) or PBS for the entire 3-wk period of antigen exposures.

Statistics

All experiments were repeated three times with at least 5 mice/group (15 mice total). Initially, a one-way analysis of variance (ANOVA) was used to determine if there were signifi- cant differences between groups for a given measured endpoint. If significance was noted, a Student's *t*-test was then used to compare all treatment groups to the controls. Significance in each analysis was assigned when a *p*-value≤0.05 was obtained.

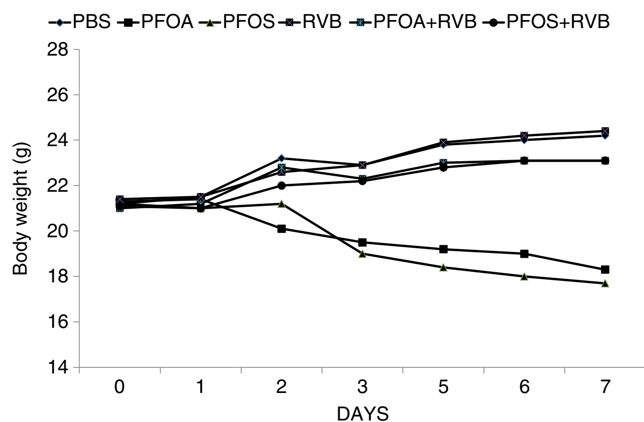

Fig. 1 Body weight changes in mice treated with the test substances. Body weights were measured daily. Values shown are mean ± SD

Results

Natural trans-resveratrol with a purity of 99.62 % (HPLC) was used in these experimens. Vitamin C was pharmaceutical grade, 1,3/1,6-D-β-glucan #300 was originally from Transfer Point and has a purity over 85 % (Vetvicka et al. 2007b; Vetvicka and Vetvickova 2012a). At the beginning of the experiment, there were no significant differences in body weights among the mice in the various groups (Fig. 1). However, from Day 4 and thereafter, there was a significant deterioration in body weights in mice in the PFOS and PFOA groups. Simultaneous addition of PFCs and RVB 300 produced no changes in body weight. On the last day of the treatment (i.e., Day 8), body mass was significantly decreased and liver mass significantly elevated in both the PFOS and PFOA groups (Table 1). Treatment with RVB 300 returned the body mass to normal levels and significantly improved the liver mass as well.

With regard to cellularity, 7 days of exposure to PFOA caused only an insignificant decrease in this value in the spleen, but a strong (i.e., >50 %) decrease in this parameter in the thymus. Exposure to PFOS resulted in a significant decrease in cellularity in both organs. Co-treatment with RVB 300 either returned the number of cells to normal levels (spleen and PFOS + RVB 300 in thymus) or at least significantly increased the number of cells in comparison to levels in PFOA-RVB 300 in thymus (Fig. 2). In light of this finding of altered cellularity in the spleen, any changes in CD4, CD8 and CD49b sub-populations were also evaluated (Fig. 3). Whereas there were no changes in CD4 or CD8 sub-population levels after any of the various test treatments, RVB 300 alone caused significant increase in levels of NK ($CD49b^+$) cells.

To evaluate the effects of the PFOS and PFO - and the possible mitigation of same by the test agents - on lymphocyte proliferation, mitogen-induced proliferation of B- and T-lympho- cytes was evaluated. Figure 4 illustrate how each PFC significantly suppressed proliferation of T-lymphocytes. PFOA exposure also inhibited proliferation of B-lymphocytes. In both cases, addition of RVB 300 returned cell proliferation activities to normal levels.

Effects of each PFC on phagocytosis (here, by peripheral blood neutrophils) were also evaluated using a well-established model of synthetic microspheres. Data in Fig. 5 reflect the fact that on one hand, both PFCs significantly inhibited phagocytosis. On the other hand, the stimulation of phagocytic activity caused by RVB 300 was strong enough not only return the 'reduced' phagocytosis to control values, but to a level even significantly higher than that noted with cells from the PBS control hosts.

Potential effects on cellular immunity were also evaluated by monitoring NK activity in cells harvested from the exposed hosts. The results of these studies showed almost the same results as with phagocytosis. Both PFOA and PFOS significantly inhibited NK cell activity and co-treatment with RVB 300 not only 'restored' activity, but increased it to levels above those of cells from the PBS-treated control mice (Fig. 6).

To assess effects on humoral immunity, an experimental model of mice immunized with ovalbumin (OVA) was employed. Mice were injected twice (14 days apart) with OVA and the serum was collected 7 days after the second

Table 1 Body mass change and organ mass change after PFOA, PFOS, and RVB 300 treatments

	Body mass change[a]	Spleen mass[b]	Thymus mass[b]	Liver mass[b]
PBS	1.74±0.24	0.44±0.03	0.29±0.01	6.12±0.10
PFOA	*1.11±0.20	0.42±0.03	0.30±0.01	*8.11±0.11
PFOS	*1.02±0.23	0.40±0.02	0.27±0.02	*9.73±0.14
RVB 300	1.75±0.27	0.45±0.04	0.28±0.01	6.13±0.12
PFOA + RVB 300	**1.60±0.22	0.44±0.05	0.29±0.02	**6.77±0.12
PFOS + RVB 300	***1.71±0.20	0.43±0.04	0.30±0.01	***6.40±0.11

[a] Final mass (g) – start mass (g)

[b] Calculated as: [organ mass (g)/body mass (g)]×100

* Significantly different at * p<0.05 from PBS values, ** p<0.05 from PFOA alone, *** p<0.05 from from PFOS alone

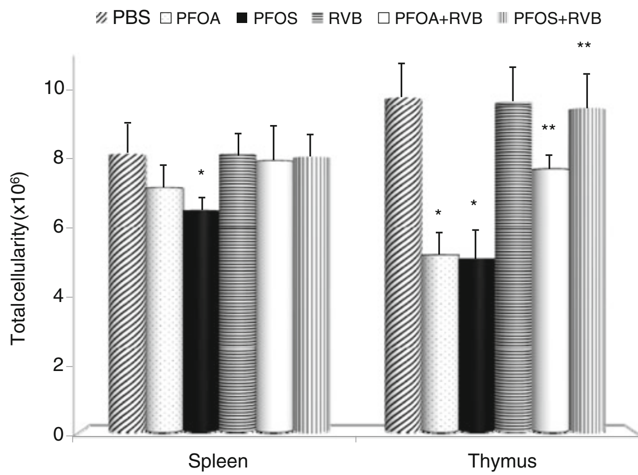

Fig. 2 Spleen and thymus cellularity after 7 days of exposures to the test substances. Values shown are mean ± SD. *Value significantly different from control (PBS only) at $p \leq 0.05$. **Significant differences between PFOA and PFOA + RVB group at $p \leq 0.05$

injection. In these studies, mice were being concur- rently injected with the various test materials over the entire 3-wk study period. The results indicate that both PFC caused significantly decreased antibody formation and that RVB 300 co- treatment helped to significantly mitigate only the reduction in antibody formation induced by PFOA (Fig. 7); RVB 300 led to an increase of 48 % vs. that from PFOS alone, but this change was not significant. These results were further confirmed by evaluation of the IgM formation using immunization with a different antigen (i.e., TNP). Once again, both PFOA and PFOS inhibited IgM formation and RVB 300 co-treatment more than doubled this production (Fig. 8).

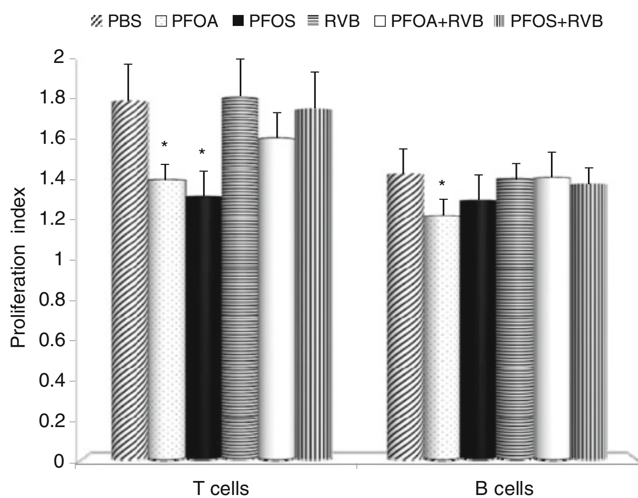

Fig. 3 Proliferation index of lymphocytes after 7 days of exposures to the test substances. Unstimulated data were not different between groups; therefore the data are presented as the proliferation index. *Value significantly different from control (PBS only) at $p \leq 0.05$

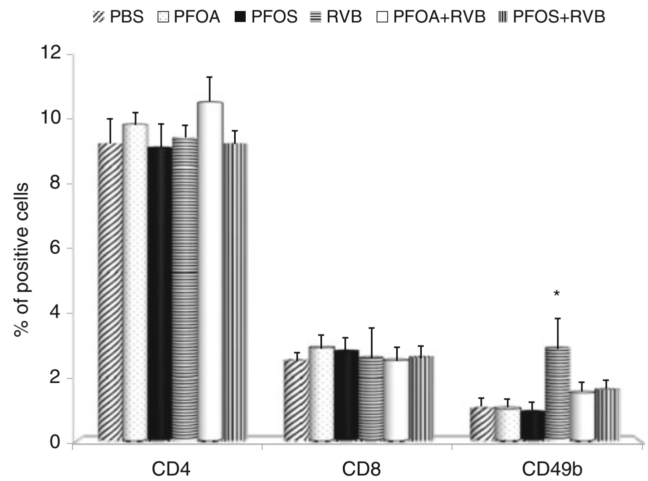

Fig. 4 Splenic populations in mice treated for 7 days. *Value significantly different from control (PBS only) at $p \leq 0.05$

Discussion

In the last decade, considerable attention has been paid to PFOS and related compounds, due to their presence in the environment and subsequently animal and human systems. Several important papers have discussed the immunosuppressive role of these compounds (Peden-Adams et al. 2007; Dong et al. 2009; Qazi et al. 2009; Zheng et al. 2009; Brieger et al. 2011; Corsini et al. 2012). Among immune system-related reactions that have been documented as adversely impacted by oral exposure to PFOS and PFOA are cell proliferation, antibody formation, inflam- mation, and cytokine production. Even more attention was focused on the toxic and immunosup- pressive effects of these compounds after reports surfaced of reduced humoral responses to routine immunization among children who had been exposed to PFCs (Grandjean et al. 2012).

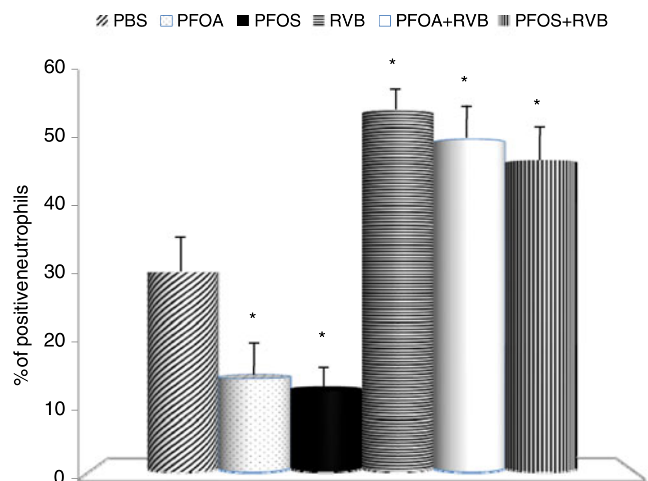

Fig. 5 Effects of 7 days of exposure to the test substances on phagocytosis by peripheral blood cells. Values shown are mean ± SD. *Value significantly different from control (PBS only) at $p \leq 0.05$

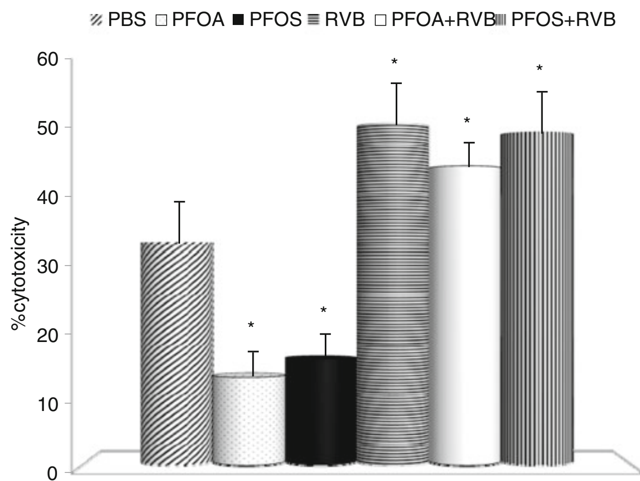

Fig. 6 Splenic NK cell activity following 7 days exposure to tested substances. Each value represents the mean ± SD. *Significant difference at p ≤0.05 from control (PBS) group

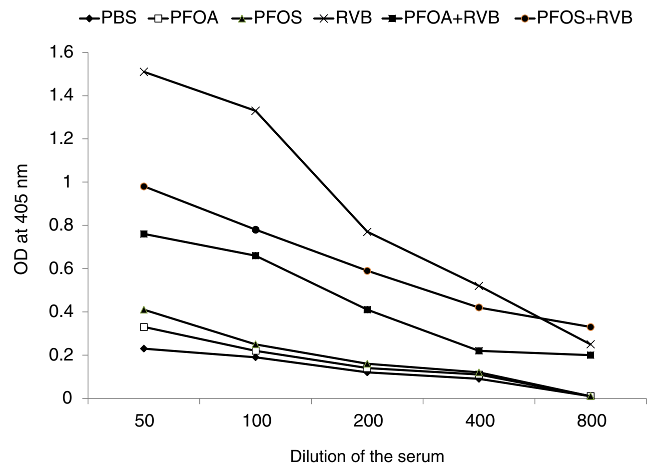

Fig. 8 Effects of tested substances glucans on formation of IgM antibodies against TNP. Mice were injected twice (2 weeks apart) with antigen and the serum was collected 7 days after the final injection. The level of specific antibodies against ovalbumin was detected using ELISA. *Value significantly different between control (TNP and Freund adjuvant) and samples at $p \leq 0.05$

However, despite all the current knowledge about immunosuppression caused by PFCs, no study about the possible reversal of these effects exists in the literature. This, together with our previous report showing that simultaneous treatment of mercury with glucan (which resulted in significantly lowered immunotoxic effects of the metal; Vetvicka and Vetvickova 2009) suggested to us that glucans can be used as a natural remedy against low-level exposures to immunosuppressants. Since those earlier observations, we have determined that a combination of glucan-resveratrol-Vitamin C had even superior effects to that derived from the glucan alone (Vetvicka and Yvin 2004). Among some of those observations, it was noted that both glucan and resveratrol stimulated phagocytosis of blood leukocytes, caused increased expression of CD4 on spleen cells, and potentiated a restoration of splenic 'normal' status after experimental induction leukopenia (Vetvicka et al. 2007a; Vetvicka and Vetvickova 2012b). Thus, the aim of this study was to determine if this combination could be used to overcome the immunosuppressive effects induced by PFCs.

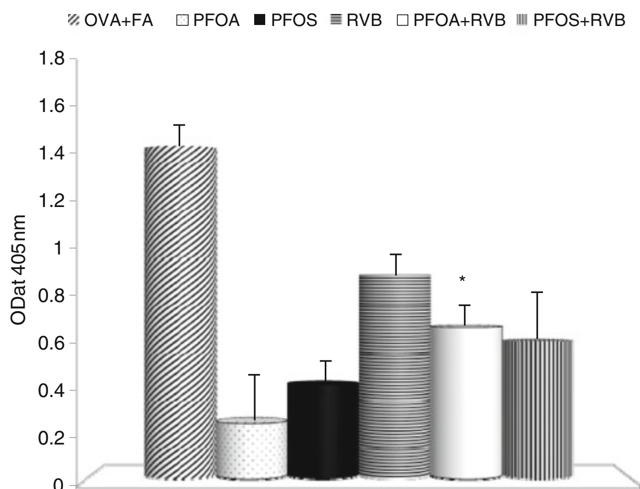

Fig. 7 Effects of tested substances glucans on formation of antibodies against ovalbumin. Mice were injected twice (2 weeks apart) with antigen and the serum was collected 7 days after the final injection. The level of specific antibodies against ovalbumin was detected using ELISA. *Value significantly different between control (ovalbumin and Freund adjuvant) and samples at $p \leq 0.05$

For the current study, a 7-day oral exposure to PFOS or PFOA - as originally described by Zheng et al. (2009) - was employed. As expected, decreases in body weight were followed by changes in organ mass (Dong et al. 2009). However, co-treatment with RVB 300 (the glucan- resveratrol-Vitamin C combination used here) 'returned' both weights body and liver mass to almost control levels. At the level of the primary immune organs, cellularity in the spleen and thymus was most impacted by PFOS. Again, co-treatment with RVB 300 'returned' the number of cells to near normal. In all instances, RVB 300 itself had no effects on cellularity in either organ.

The effects of each PFC on proliferation are less clear. Some studies documented suppression of ConA-induced T-lymphocyte proliferative activity (Zheng et al. 2009). Others, in contrast, showed an insignificant increase in T- and B-lymphocyte proliferative capacities (Peden-Adams et al. 2007). Here we demonstrated that, whereas the effects of both compounds on T-lymphocytes were profound, those on B-lymphocytes suggested a greater degree of resist- ance. No effects on the expression of surface markers CD4/CD8/CD49b were noted, suggesting that PFCs do not affect the migration of lymphocytes into the spleen or change the development and maturation of thymocytes.

In one portion of this study, the focus was upon the effects of the PFCs on cellular immunity. The effects on

phagocytic activity were assessed using 2-hydroxyethyl methacrylate particles that have only a slight negative charge and thus do not non-specifically adhere to the cell surface and significantly lowers the chance of false negative outcomes (Vetvicka et al. 1982). The results revealed that both PFOS and PFOA inhibited phagocytosis to a level equal to 30 % of normal values. The co-treatment with RVB 300, well known to strongly stimulate this activity, 'restored' phagocytosis by harvested cells to the levels noted in mice that received RVB 300 alone.

Some previous studies have noted a dose-dependent decrease in NK cell activity as a result of PFOS exposure (Zheng et al. 2009). However, as with the lymphocyte proliferative capacity analyses, other studies reported no effects at all (Nelson et al. 1992). This discrepancy sometimes can be ascribed to mouse strain or gender differences (as female $B_6C_3F_1$ mice were less sensitive than male mice; Peden-Adams et al. 2007). The results of the present study indicated that both PFOS and PFOA imparted strong inhibitory effects on the activity of NK cells in the hosts. As with many of the other endpoints analyzed here, the co-treatment with RVB 300 once again led to a significant restoration of this immune function.

In order to also assess the impact of each PFC (and any mitigating effects of the RVC 300) on humoral responses in the treated hosts, effects of PFOS and PFOA exposure on antibody responses were analyzed. Using two different antigens (i.e., ovalbumin and TNP), it was observed that both PFCs (particularly PFOA) imparted strong inhibitory effects on both total immunoglobulin formation and on IgM formation. Once again, RVB 300 helped to 'restore' some of the antibody formation activity; however, with neither PFC was the combination co-treatment able to fully restore the antibody formation capacities in the exposed hosts.

Conclusions

Two major conclusions were reached in this study. The first was that since both PFOS and PFOA significantly suppressed both branches of immune reactions even after short 7-d exposure - when taken together with findings of significant loss of body weight and hepato- megaly - these compounds clearly present a significant danger to exposed hosts. Our study, therefore, provides additional support to regulatory agencies seeking to eliminate/minimize PFOA and PFOS from pollutant emissions and other products. The second is that, for the first time, PFC-induced immunosuppression can be at least *partly* restored/mitigated by oral admini- stration of a combination of glucan-resveratrol-vitamin C. From our data, it could be imagined that RVB 300 might be used in the prophylactic treatment of PFC-based poisonings. Experi- ments evaluating the mechanisms of the RVB 300-based restoration of immunosuppression seen here are currently under way. Furthermore, the clinical importance of this observation deserves further study.

Declaration of interest The authors have no financial interests in any company mentioned in this study and report no conflicts of interest. The authors alone are responsible for the content of this manuscript.

References

Brieger A, Bienefeld N, Hasan AR, Goerlich R, Haase H (2011) Impact of perfluorooctanesulfonate and perfluorooctanoic adic on human peripheral leukocytes. Toxicol In Vitro 25:960–968

Corsini E, Sangiovanni E, Galbiati V, Viviani B, Marinovich M, Galli CL et al (2012) In vitro characterization of the immunotoxic potential of several perfluorinated compounds (PFC). Toxicol Appl Pharmacol 258:248–255

De Witt JC, Peden-Adams MM, Keller JM, Germolec DR (2012) Immunotoxicity of perfluorinated compounds: recent development. Toxicol Pathol 40:300–311

Dong GH, Zhang YH, Zheng L, Liu W, Jin YH, He QC (2009) Chronic effects of perfluorooctanesulphonate exposure on immunotoxicity in adult male C57BL/6 mice. Arch Toxicol 83:805–815

Fernandez-Botran R, Vetvicka V. (Eds.) 1995. Flow cytometry. In: *Methods in Cellular Immunology*, Boca Raton, FL. CRC Press, pp. 29–46.

Grandjean P, Andersen EW, Budtz-Jergensen E, Nielsen F, Molbak K, Weihe P et al (2012) Serum vaccine antibody concentrations in children exposed to perfluorinated compounds. JAMA 307:391–397

Lau C, Anitole K, Hodes C, Lai D, Pfahles-Hutchens A, Seed J (2007) Perfluoroalkyl acids: a review of monitoring and toxicological findings. Toxicol Sci 99:66–94

Nelson DL, Frazier DE, Ericson JE, Tarr MJ, Mathes LE (1992) The effects of perfluorodecanoic acid (PFDA) on humoral, cellular, and innate immunity in Fischer-344 rats. Immunopharmacol Immunotoxicol 14:925–938

Patchen ML, MacVittie TJ (1982) Use of glucan to enhance hemopoietic recovery after exposure to Co^{60} irradiation. Adv Exp Med Biol 155:267–272

Peden-Adams MM, EuDaly JG, Dabra S, EuDaly A, Heesemann L, Smythe J et al (2007) Suppression of humoral immunity following exposure to the perfluorinated insecticide sulfluramid. J Toxicol Environ Health 70:1130–1141

Qazi MR, Bogdanska J, Butenhoff JL, Nelson BD, DePierre JW (2009) High-dose, short-term exposure of mice to perfluorooctanesulphonate (PFOS) or perfluoro- octanoate (PFOA) affects the number of circulating neutrophils differently, but enhances the inflammatory response of macrophages to lipopolysaccharide (LPS) in a similar fashion. Toxicology 162:207–214

Vetvicka V, Vancikova Z (2010) Synergistic effects of glucan and resveratrol. Afr J Biochem Res 4:105–110

Vetvicka V, Vetvickova J (2009) Effect of glucan on immunosuppressive action of mercury. J Med Food 12:1098–1104

Vetvicka V, Vetvickova J (2012a) Combination of glucan, resveratrol and Vitamin C demonstrates strong anti-tumor potential. Anticancer Res 32:82–88

Vetvicka V, Vetvickova J (2012b) Comparison of immunological properties of various bioactive combinations. Biomed Papers (In press)

Vetvicka V, Yvin JC (2004) Effects of marine β-glucan on immune reaction. Int Immunopharmacol 4:721–730

Vetvicka V, Fornusek L, Kopecek J, Kaminkova J, Kasparek L, Vranova M (1982) Phagocytosis of human blood leukocytes: a simple micromethod. Immunol Lett 5:97–100

Vetvicka V, Holub M, Kovaru H, Siman P, Kovaru F (1988) α-Fetoprotein and phagocytosis in athymic nude mice. Immunol Lett 19:95–98

Vetvicka V, Dvorak B, Vetvickova J, Richter J, Krizan J, Sima P et al (2007a) Orally administered marine (1→3)-β-D-glucan phycarine stimulates both humoral and cellular immunity. Int J Biol Macromol 40:291–298

Vetvicka V, Volny T, Saraswat-Ohri S, Vashishta A, Vancikova Z, Vetvickova J (2007b) Glucan and resveratrol complex – possible synergistic effects on immune system. Biomed Pap Med Fac 151:41–46

Vetvicka V, Baigorri R, Zamarreno AM, Garcia-Mina JM, Yvin JC (2010) Glucan and humic acid: synergistic effects on the immune system. J Med Food 13:863–869

Wang Y, Wang L, Liang Y, Qui W, Zhang J, Zhou Q et al (2011) Modulation of dietary fat on the toxicological effects in thymus and spleen in BALB/c mice exposed to perfluoroooctane sulfonate. Toxicol Lett 204:174–182

Zheng L, Dong GH, Jin YH, He QC (2009) Immunotoxic changes associated with a 7-day oral exposure to perfluorooctane sulfonate (PFOS) in adult male C57BL/6 mice. Arch Toxicol 83:679–689