# Th1/Th2 response after isopropyl methylphosphonofluoridate intoxication

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***Abstract***

*By blocking the enzyme, isopropyl methylphosphonofluoridate (IMPF) may affect the levels of acetylcholine (cholinergic neuromediator) and cause changes in the physiology of various cells (e.g. neurons, lymphocytes, macrophages, mast cells etc.). The aim of this study was to examine the influence of IMPF administration to mice on the level of selected cytokines (IL-2, IL-4, IL-5, IFN-γ) and immunoglobulins (IgA, IgE) in their sera, and to evaluate the direction of the immune response. The studies showed changes in the secretion of two factors: IL-4 and IL-5 (compared to control groups). In both cases, the statistically significant differences (increase of IL-4 and IL-5 decrease) were observed at day 7 after the IMPF intoxication. Similarly, the level of IgE (vs. control group) was significantly higher at 24 hours and 7 days from intoxication. Our results indicate that IMPF intoxication can produce changes in selected cytokines and immunoglobulins serum levels that may lead to the immune response disturbances.*

***Key words:*** *acetylcholinesterase irreversible inhibitors, cytokines, iResponse, in vivo model, mice.*

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## Introduction

Phosphoorganic compounds, widely used in various branches of the economy (agriculture, industry, medicine), are toxic chemicals that predominantly affect the nervous system. They act mostly by blocking acetylcholinesterase and therefore affect signal transmission through cholinergic pathways. Phosphoorganic compounds also include toxic warfare agents (TWA) of the class G and V, being mostly organic derivatives of phosphonic acid. They are characterized by particularly high toxicities and irreversible binding to acetylcholinesterase. Isopropyl methylphosphonofluoridate (IMPF) (Sarin) is one of the main representatives of this group [1, 2].

Terroristic attacks with this agent in the Japanese city of Matsumoto in 1994 resulted in about 200 intoxications, including seven casualties. One year later, similar attacks in the Tokyo underground resulted in 5000 intoxications and 12 deaths.

A variety of symptoms were observed amongst the Tokyo attack survivors, including persistent headaches, joint pain, weakness, fatigue, memory loss, and increased susceptibility to infections, probably resulting from an impaired immune status [3, 4]. Similar symptoms were described amongst soldiers who took part in the Gulf War [5, 6]. In the latter case, occurrence of these symptoms was, interpreted as a result of exposure to low doses of sarin or phosphoorganic insecticides, or prophylactic administration of acetylcholinesterase inhibitors – particularly pyridostigmine bromide, or perhaps as a consequence of stress and other factors [7]. Moreover, changes in certain immunity parameters were observed in Gulf War veterans, including elevations in such cytokines as: interleukin 2 (IL-2), IL-10, tumor necrosis factor α (TNF-α), and interferon γ (IFN-γ) [5],

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changes in the CD4 to CD8 lymphocyte ratio, along with elevation of B (CD19) lymphocyte fraction and the levels of certain autoantibodies [8]. These findings suggest that sarin intoxication may significantly alter the direction of the immune response and be crucial for its regulation.

The aim of this pilot study was to evaluate in mice the effects of IMPF intoxication on the levels of various cytokines and immunoglobulins in their sera. These results were further used to try determine the direction of IMPF-induced immune response.

## Material and methods

The study was performed using 6 to 8 week-old male BALB/c mice (5-11 animals per group). The selected parameters were determined in the serum obtained at 1, 7, 14 and 21 days following subcutaneous IMPF intoxication (100 µg/kg b.w.). Cytokine concentrations (IL-2, IL-4, and IL-5 and IFN-γ) were determined with Cytometric Bead Array test (CBA, BD Biosciences), whereas immunoglobulin levels (IgA and IgE) were determined with ELISA tests (Alpha Diagnostic Intl.). For comparison between intoxicated and control groups, the means (all samples are duplicated) were analysed with Student *t*-test for independent variables or with the Mann-Whitney U test. Statistical significance was defined as $p < 0.05$. All data were presented as the percent of control group ± SE.

The authors were granted permission by the Local Ethics Committee to use mice in this study.

## Results

### Serum levels of cytokines

In comparison to control groups, significant changes in the cytokine profiles (cytokine secretion) of intoxicated mice were observed only for IL-4 (Fig. 1) and IL-5 (Fig. 2). In both cases significant differences were found 7 days following IMPF intoxication. The most pronounced differences were observed for IL-4, the levels of which, compared to controls increased twofold at day 7 after intoxication. Following intoxication, less pronounced but still significant decreases were found for IL-5 concentrations.

The post-intoxication levels of the other two studied cytokines, both involved in the cellular Th1-mediated response did not significantly differ compared to the controls. However, an tendency to decrease in IL-2 was observed starting at day 7 day after sarin administration together with fluctuations in IFN-γ levels (Figs. 3 and 4).

Additionally, we analyzed the ratios of cytokines secreted by Th1 cells (IL-2 and IFN-γ) in comparison to IL-4 levels (as a main cytokine of Th2-mediated pathway). The values of these ratios are presented in Figures 5 and 6. A significant shift of immune response towards the humoral (Th2 mediated) pathway was observed at day 7 from intoxication for the IL-2/IL-4 ratio (Fig. 5) and for the IFN-γ/IL-4 ratio on days 1 and 7 (Fig. 6).

### Immunoglobulin levels

The percent of control group (along with their standard errors) of immunoglobulin A and E are presented in Figures 7 and 8, respectively. Significant increases were observed only in IgE levels at 1 and 7 day from IMPF intoxication.

## Discussion

The main mechanism of toxic interaction of organophosphate compounds, including IMPF, is the irreversible inhibition of acetylcholinesterase activity. By blocking the enzyme, this compound may affect the levels of acetylcholine (cholinergic neuromediator), causing changes in the physiology of neurons [1, 2].

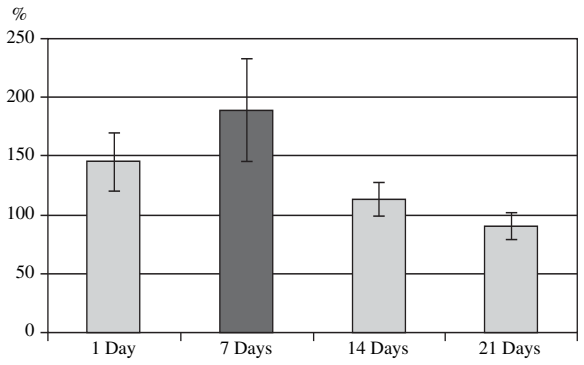


**Fig. 1.** Mean percent of IL-4 (± SE) in comparison with control group in the mice serum samples after IMPF administration. Total number of mice: 40
Statistically significant differences ($p < 0.05$) as against control group are marked as black bar

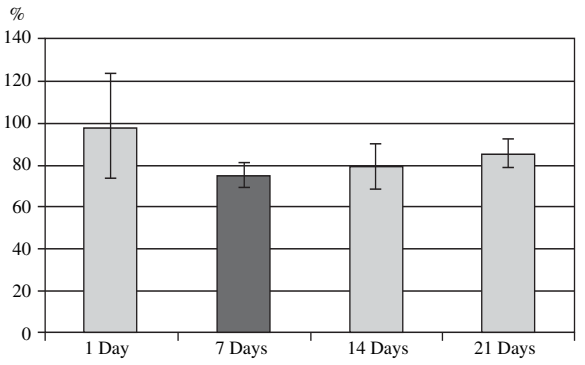


**Fig. 2.** Mean percent of IL-5 (± SE) in comparison with control group in the mice serum samples after IMPF administration. Total number of mice: 44
Statistically significant differences ($p < 0.05$) as against control group are marked as black bar

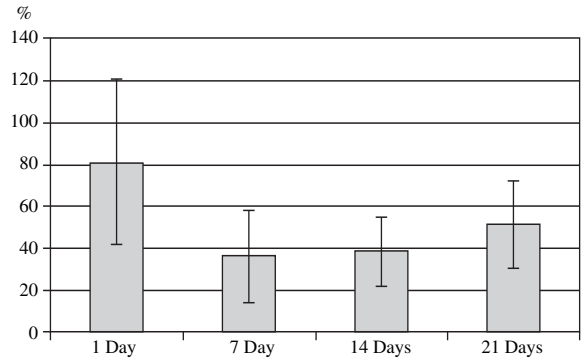


**Fig. 3.** Mean percent of IL-2 (± SE) in comparison with control group, in the mice serum samples after IMPF administration. Total number of mice: 40

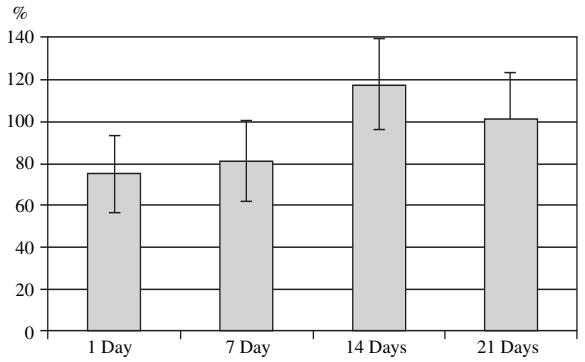


**Fig. 4.** Mean percent of IFN-γ (± SE) in comparison with control group in the mice serum samples after IMPF administration. Total number of mice: 43

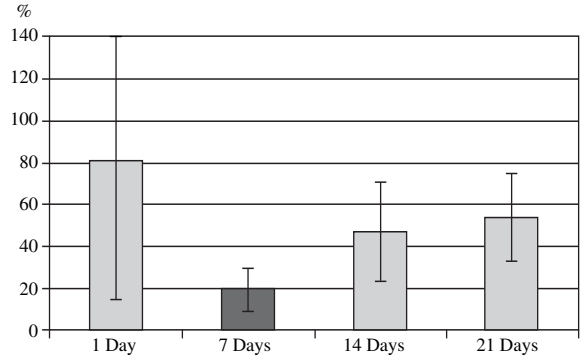


**Fig. 5.** Mean IL-2/IL-4 ratio (± SE) in comparison with control group in the mice serum samples after IMPF administration. Total number of mice: 35
Statistically significant differences ($p < 0.05$) as against control group are marked as black bar

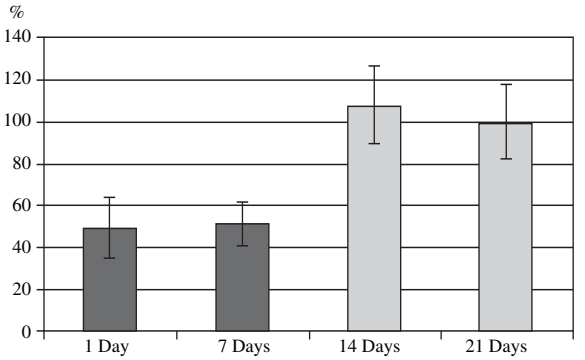


**Fig. 6.** Mean IFN-γ/IL-4 ratio (± SE) in comparison with control group in the mice serum samples after IMPF administration. Total number of mice: 41
Statistically significant differences ($p < 0.05$) as against control group are marked as black bars

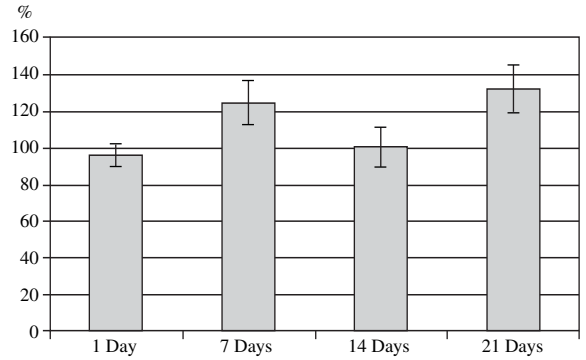


**Fig. 7.** Mean percent of IgA (± SE) in comparison with control group in the mice serum samples after IMPF administration. Total number of mice: 35

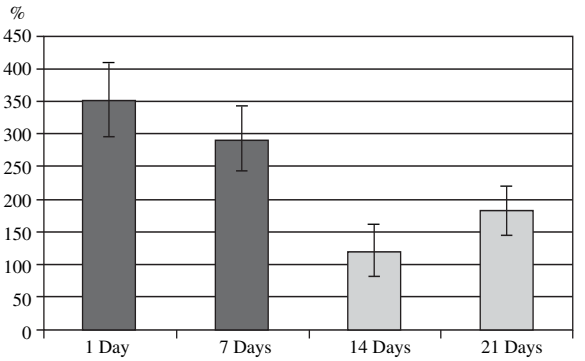


**Fig. 8.** Mean percent of IgE (± SE) in comparison with control group in the mice serum samples after IMPF administration. Total number of mice: 25
Statistically significant differences ($p < 0.05$) as against control group are marked as black bars

The presence of multiple elements of the cholinergic system on many other cells, including immune competent cells was documented for T cells [9-11], macrophages [12, 13] and endothelial cells [14, 15]. It is worth to notice that those cells do not only respond to acetylcholine, but they can also produce this mediator [15, 16].

The network of interactions through receptors and the cholinergic mediator was named a *non-neuronal cholinergic system* [17]. Acetylcholine can influence many cellular processes, including:

- migration of endothelial cells [18] and expression of certain adhesion proteins on these cells [15];
- production of certain cytokines by lymphocytes and macrophages; NK cells cytotoxicity [19, 20];
- dose-dependent and time-dependent increase of the monocytes chemotactic activity [12].

Many papers indicate that acetylcholine may be also involved in other processes, such as: proliferation, differentiation, cytoskeleton organisation, production of proinflammatory cytokines and nitric oxide, even in mobilisation of precursor cells [16, 21].

It has been observed that the changes observed in animals after intoxication with IMPF lead not only to disturbances in the "cytokine network", but also to the changes in the T cell activity. This was confirmed by earlier studies, which indicate inhibition of T cell proliferation activity in mice subjected to IMPF intoxication [22-24]. However, the exact mechanism of the IMPF influence upon T cells activity is unknown. Involvement of the autonomic nervous system (ANS) has been proposed. Kalra *et al.* [25] showed that subclinical doses of IMPF inhibit proliferation activity of T cells in response to concanavalin A independently of hypothalamic-pituitary-adrenal axis (HPA). The corticosteroids levels in serum of rats subjected to intoxication with this compound were also significantly lower in comparison to control animals. These observations suggest that immunosuppression induced by IMPF is independent from HPA activation, but involves autonomic nervous system. Furthermore, this was confirmed by chlorizondamine action, which blocks transmission in ANS, abolishing effects of IMPF action. Other studies showed that chemical disruption of sympathetic nerve transmission influences T cell-dependent antibody production, IL-2 production, T cell cytotoxic activity and late hypersensitivity [26, 27].

On the basis of the produced cytokines, helper T cells can be divided into two basic subpopulations [28]: Th1 – producing cytokines involved in cellular response, such as IL-2, IFN-$\gamma$, and Th2 – producing cytokines involved in humoral response – IL-4, IL-5, IL-6, IL-10, IL-13 and IgE production.

This study showed the increase in IL-4 production together with a decrease in IL-5 production after IMPF intoxication (Figs. 4 and 5). Interestingly, as previously mentioned, both of these cytokines (IL-4, IL-5) participate in the immune mechanisms of the Th2 mediated pathway. Interleukin 4 exhibits a variety of actions including strong stimulation of B lymphocytes towards immunoglobulin E synthesis (Fig. 8). Moreover, IL-4 modulates Th0 lymphocytes (by positive feedback), inducing their differentiation into Th2 cells. Interleukin 5 is secreted by Th2 lymphocytes and regulates basophil and eosinophil granulocyte differentiation. Moreover, it may affect B lymphocytes by enhancing secretion of IgM and IgA antibodies.

## Conclusions

The results of this study showing changes in selected cytokine (IL-4 and IL-5) concentrations and immunoglobulin E levels suggest that the exposure to IMPF may switch the immune response towards its humoral pathway.

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