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Effects of lead and aluminum on the specific immune response of growing mice

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The aim of the present study was to evaluate the effects of lead and aluminum ions on the specific immune response of young mice infected by *Listeria monocytogenes*. Low-dose chronic exposure to lead (5 mg per 1 kg of body mass) and aluminum (2.5 mg per 1 kg of body mass) reduced the survival of animals from 83% in the control group to 72% in the lead-treated and to 76% in the aluminum-treated group. Moreover, both metals reduced the growth of infected animals. The number of *Listeria monocytogenes* infected mice-carriers was increased after 6 weeks of exposure to lead or aluminum ions. Both metals increased the delayed type of hypersensitivity to *Listeria monocytogenes* antigens. Lead- and aluminum-treated animals had a more than twofold lower blood serum antibody titer against *Listeria monocytogenes* antigens in comparison with the blood serum of control animals. These results suggest a significant damage of the T-system and B-system specific immunity in lead- and aluminum-treated (0.05 LD_{50}) mice.

Key words: lead, aluminum, mice, *Listeria monocytogenes*, immune response

INTRODUCTION

A common feature of two of the trace elements, lead and aluminum, regarding their impact on a living organism is that they both affect the immune system. Another similarity between these two metals, despite that they do not carry out any physiological function, is that a complete deficiency of them is equally harmful for the living organism.

Although lead is one the widespread toxic factors, latest data indicate that at low dietary intakes lead is an essential element (Nielsen, 1993). Its deficiency was shown to depress growth, disturb iron metabolism, alter activities of some enzymes, disturb the metabolism of cholesterol, phospholipids and bile acids (Hoekstra et al., 1974; Kirchgessner, Reychlmayr-Lais, 1981). On the other hand, excessive doses of this heavy metal cause pathologies in a living organism, among which are retardation in intellectual, sensorial, neuromuscular and psychological development of children (Seifert, Anke, 1999), chromosome aberrations (Wronka et al., 1999), etc. The immunodisturbing activity of lead belongs to the most dangerous effects of this pollutant (Heo et al., 1997).

Aluminum was considered as a non-toxic element until the 1970s when evidence came up indicating that aluminum induces morphological changes in the cells of the nervous system resembling those occurring as a consequence of Alzheimer's disease (Авцын и др., 1991). Now it is accepted that aluminum is associated with a variety of pathologies of the nervous system (Lechmann, 1992; Авцын и др., 1991). Another well-known target of aluminum is the immune system. It is known that aluminum stimulates some functions of the immune system (Brewer et al., 1999; Glynn et al., 1999). On the other hand, elevated concentrations of aluminum correlate with the immune system damages responsible for chronic renal failure after kidney transplantation (Tzanno-Martins et al., 1996).

This study was intended to investigate the impact of lead and aluminum on the antigen-specific immune response of experimental animals carrying bacterial infection. We present data which demonstrate that chronic exposure to both lead and aluminum significantly suppresses the antigen-specific immune response of experimental animals.

MATERIALS AND METHODS

Experiments were done on BALB/C mice weighing 17–24 g (Permission No. 0004 of the Lithuanian State Veterinary Service for the work with experimental animals). All animals were 4–6 weeks old at the beginning of experiment.

Mice were kept for 6 weeks under the conditions of chronic lead (36 mice) or aluminum (36 mice) poisoning by i.p. injections of 0.05 LD_{50} Pb $(CH_3COO)_2$ or $AlCl_3$ three times per week. The 0.05 LD_{50} was 5 mg of Pb^{2+} or 2.5 mg of Al^{3+} ions per 1 kg of body weight. Control animals (39 mice) received injections of the same volume of physiological solution.

Experimental infection was induced by a single i.p. injection of virulent serotype 1860 1a of *Listeria monocytogenes* (approximately 5×10^4 CFU per 20 g of body weight, which equals to 0.05 LD_{50}).

Survival of *L. monocytogenes* in the liver and spleen of experimental animals was evaluated by the presence of bacteria colonies after the incubation of homogenate of these organs on agar plates at 30 °C for 24 h. The delayed type hypersensitivity was evaluated by the inflammatory response during the so-called "foot" test (Черкашин, 1978). Listeria protein solution was injected under plantare of lower aponeurosis of the rear foot of an experimental animal, while another foot received injection of physiological solution. After 24 h the level of inflammation was evaluated on the basis of foot weight increase. Accumulation of antibodies against *L. monocytogenes* in the blood serum of mice was estimated using a direct agglutination reaction. Series of blood serum samples from a single animal, differing in their dilution 2 times, were incubated at 37 °C for 24 h with a 24-h culture of *L. monocytogenes*, pretreated with 1% formaldehyde. Agglutination was evaluated visually using an agglutinoscope. Results were expressed as mean ± S.E.M.

RESULTS

It is known that the immune system of a living organism is rather sensitive to heavy metal poisoning. One of the ways to evaluate the state of the immune system is to check the ability of the organism to resist infection. In our study, we used experimental infection induced with *Listeria monocytogenes* bacteria, which had been introduced several decades ago as a model of bacterial infection, which is independent on antibodies (North et al., 1997).

The ability of an organism to resist infection is realized through two main defense systems – antigen-non-specific immune defense and antigen-specific immune response. Antigen-specific immune response to bacteria becomes involved starting from the 2nd–3rd day of infection, therefore we used experiments of chronic poisoning with 0.05 LD_{50} lead acetate or aluminum chloride. Animals were injected with lead acetate or aluminum chloride every 2–3 days for six weeks, and mice of the control group received injections of the same amount of physiological solution. At the day "zero" both groups were infected with the same amounts of *L. monocytogenes.* Figure 1 shows the survival of animals after experimental infection. It is obvious that chronic administration of lead or aluminum significantly reduces the survival of experimental animals. The survival of mice in the control group at the end of experiment was 83% as compared to 72% in the lead-treated group (Fig. 1A) or 76% in the aluminum-treated group (Fig. 1B). Simultaneously, the growth of the individuals from lead- or aluminum-exposed groups was compared with that of control. One can see (Fig. 2) that both metals caused growth suppression in the experimental animals. Within the first few days lead-treated animals were loosing about 10% of their body weight, which had a tendency to return to 100% in the third decade. On the other hand, listeria-infected animals of the control group were showing a more or less normal growth pattern, except for the first week when their body weight was held at 100%. The effect of aluminum was less severe on the growth of experimental animals – after an initial decrease in their weight by approximately 5–7% they were gaining weight, however, without catching up with the parameters of the control group.

Since these were long-term experiments, their results indicated a possibility that lead induces changes in the antigen-specific immune response of experimental animals. This assumption was confirmed by the results of the next experiment, where we examined liver and spleen of survived animals for the presence of *L. monocytogenis* six weeks after the experimental induction of infection. The results shown in Table indicate that chronic exposure to low-dose lead or aluminum markedly increase the number of infected animals still carrying bacteria in these two organs. Lead and aluminum increases the fraction of infection carriers very similarly, namely, by approximately 7-fold. This fact indirectly suggests that both metals affect the antigen-specific immune response of experimental animals.

Further experiments were carried out in order to examine directly some subsystems of antigen-specific immune response in listeria-infected animals under chronic exposure to lead or aluminum. Delayed type hypersensitivity response is a cell-mediated inflammation reaction of an organism to the antigen. Our results (Table) demonstrate that the extent of inflammation induced by listeria protein extract in both lead- and aluminum-treated mice is almost by 20% lower than in the control group, which was metal-free. These numbers indicate that the efficiency of the delayed type hypersensitivity response in lead- and aluminum-treated animals is significantly reduced.

Table. **Effect of chronic lead and aluminum poisoning on some parameters of *Listeria monocytogenes*-infected experimental animals**

Parameter	Metal			
	Lead		Aluminum	
	–	+	–	+
Percent of animals carrying *L. monocytogenes* in liver and/or spleen	5.3	37	5	36
Percent of foot weight increase in response to *L. monocytogenes* antigens	25.4 ± 1.7	20.6 ± 1.6	25.4 ± 1.7	20.28 ± 1.2
Mice blood serum antibody titer against *L. monocytogenes* antigens (times)	227 ± 14	105 ± 32	225 ± 13	136 ± 10

At the end of the experiment presented in Fig. 1 all survived animals were evaluated for the presence of *L. monocytogenes* in their liver and spleen; the "foot"-test was carried with them, and their blood serum was used for the agglutination experiments. Where applicable, differences were statistically significant.

Listeriosis belongs to the type of bacterial infection which is independent on antibodies, nevertheless, antibodies against *L. monocytogenes* are produced in infected animals (North et al., 1997). This fact allowed us to evaluate the effects of lead and aluminum on the state of humoral immune response of infected mice. Our data (Table) demonstrate that chronic treatment with lead or aluminum results in an approximately two-fold lower mice blood serum antibody titer against *L. monocytogene*s antigens in comparison with the blood serum of control animals. These results suggest a significant reduction of the humoral immune response in both lead- and aluminum-treated experimental animals.

DISCUSSION

Data presented in this study show that lead and aluminum markedly suppress the antigen-specific immune response of experimental animals, which is demonstrated by several lines of evidence. Indirectly, it can be assumed from the results showing a significantly lower survival of *L. monocytogenes*-infected and lead- or aluminum-intoxicated animals as compared with mice carrying infection without lead treatment (Fig. 1). Besides, animals of the metal-exposed groups had a disturbed pattern of their increase in weight during 1.5 months compared to the metal-free control groups (Fig. 2). In both cases, at the same chronic exposure to an 0.05 LD_{50} dose, lead had a slightly more severe impact than aluminum. Surprisingly, both metals very similarly (by approximately 7-fold) increased the number of individuals that still were carriers of the *L. monocytogenes* 6 weeks after the infection (Table). These evidences suggesting the suppression of antigen-specific immune response by lead and aluminum were supported by a direct demonstration that metal exposure causes a significant damage to the constituent parts of antigen-specific immune response, possibly, to the Th_1 and the Th_2 systems (Table).

Taken separately, most of our observations find support in the literature, especially in the case of lead. Thus, a decrease in delayed-type hypersensitivity reactions has been found in lead-exposed rats (Miller et al., 1998). Delayed type hypersensitivity of mice sensitized with sheep red blood cells was found to be

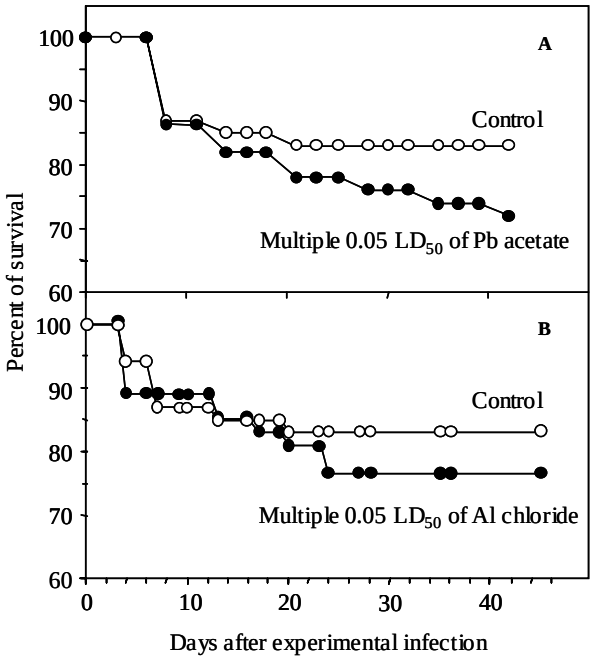

Fig. 1. Effect of lead (A) or aluminium (B) on the survival of *L. monocytogenes*-infected animals. At the day "zero" animals were injected with bacteria as described in Methods. Three days later one group received the first injection of 0.05 LD_{50} of Pb acetate or Al chloride and the second got the same volume of physiological solution. Such injections were done three times per week every 2–3 days

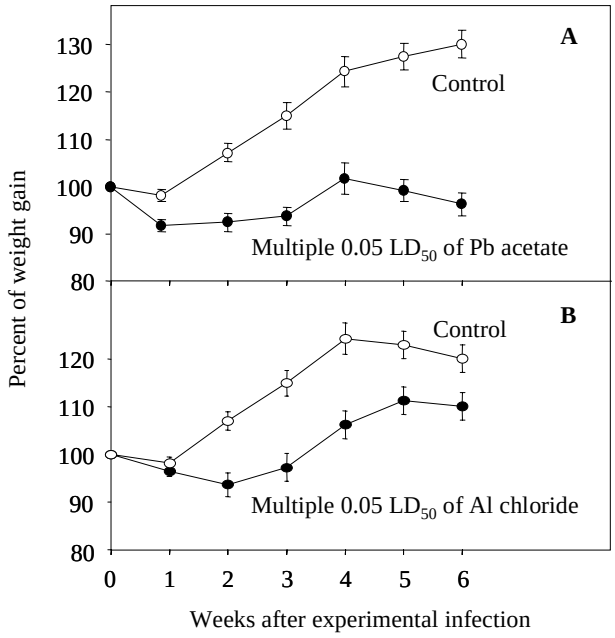

Fig. 2. Body weight gain of the lead- (A) or aluminum-treated (B) *L. monocytogenes*-infected animals. Shown are body weight changes of animals described in the legend to Fig. 1

markedly suppressed in lead-intoxicated mice (McCabe et al., 1999). Lead distorts proper development of some subclasses of T-cells, increasing the amount and activity of Th_2 cells with a concomitant decrease in the number and activity of Th_1 cells (Heo et al., 1998; Sata et al., 1998). Lead-induced decrease in the agglutination titers of serum antibodies against *L. monocytogenes* was observed in sheep (Pistl et al., 1995). The levels of some classes of immunoglobulins were significantly decreased in blood of workers occupationally exposed to lead (Anetor, Adeniyi, 1998). Much fewer reports can be found on aluminum effects on the immune system, besides, most of them deal with the adjuvant properties of aluminum compounds (Brewer et al., 1999). These data indicate that at a very low concentration aluminum stimulates both T- and B-systems of antigen-specific immune response. However, the concentrations of aluminum used in this study were much higher, and it explains why this metal displayed a strongly negative impact on the antigen-specific immune response. This is in good agreement with the observation that the impairments of the immune system responsible for the chronic renal failure correlate with aluminum intoxication (Tzanno-Martins et al., 1999).

In conclusion, the results presented here demonstrate that the trace elements lead and aluminum should be regarded as strong suppressors of the antigen-specific immune response in mammals.

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ŠVINO IR ALIUMINIO ĮTAKA AUGANČIŲ PELIŲ SPECIFINIAM IMUNINIAM ATSAKUI

S a n t r a u k a

Šio tyrimo tikslas – įvertinti švino ir aliuminio jonų įtaką augančių pelių, užkrėstų *Listeria monocytogenes* bakterijomis, specifiniam imuniniam atsakui. Darbe pateikti rezultatai rodo, kad dėl ilgalaikio apsinuodijimo švinu (5 mg/kg kūno masės) arba aliuminiu (2,5 mg/kg kūno masės) sumažėja gyvūnų išgyvenamumas nuo 83% kontrolinėje grupėje iki 72% švinu ir 76% aliuminiu apnuodytų pelių grupėse. Be to, abu metalai sulėtina pelių augimą. Tiek švino, tiek aliuminio jonai padidina gyvūnų, išliekančių *Listeria monocytogenes* nešiotojais, skaičių po 6 savaičių nuo pelių užkrėtimo. Abu metalai patikimai sumažina eksperimentinių gyvūnų uždelsto tipo hiperjautrumo bakterijų antigenams reakciją. Švinu arba aliuminiu apsinuodijusių pelių kraujo serumo antikūnų titras prieš *Listeria monocytogenes* antigenus buvo beveik dvigubai mažesnis negu kontrolinės grupės pelių. Gauti rezultatai rodo, kad švino arba aliuminio jonai (0,05 LD_{50}) gali sukelti gyvūnų specifinio imuniteto T- ir B-sistemų pažeidimą.

Raktažodžiai: švinas, aliuminis, pelės, *Listeria monocytogenes*, imuninis atsakas