Effect of Methylmercury on Humoral Immune Responses in Mice Under Conditions Simulated to Practical Situations

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INTRODUCTION

Human hair has been employed as a biological indicator of mercury pollution more than a decade and recent analyses suggest that an average Japanese is exposed to mercury to a much greater extent than an inhabitant of other industrialized countries (Hosono et al. 1966, Doi 1975). In fact some of the hair samples, especially the ones obtained from those whose consumption of fish is greater than average showed mercury levels equivalent to the concentrations noted among the patients with Minamata disease (Nishima et al. 1974). Since dietary methylmercury, held mainly responsible for the elevated levels in the hair among Japanese, originates from fish meat and also since transfer of this substance takes place via placenta and to a lesser extent via lactation (Mansour et al. 1973), exposure to methylmercury starts early in the life of Japanese. This fact demands an appreciation of various biological effects which relatively low levels of methylmercury exert on the rapidly proliferating cells such as those of fetus. Though pathological observations made during the outbreak of Minamata disease attested to the suppression of the hematopoietic and lymphoid organs where active cell divisions take place (Takeuchi, 1970), little is known as regards its influence on immune response.

The purpose of our experiment is to see the effect of methylmercury on humoral immune mechanism when 1) mice were dosed with relatively large amount of methylmercury for a short time period, 2) animals were exposed to methylmercury from the very early stage of life until they attained sexual maturity and 3) adult mice were fed subchronically relatively low levels of dietary methylmercury.

MATERIALS AND METHODS

Experiment 1

Three groups of six week old male ICR mice

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(all the animals were obtained from Nippon Clea Co. Ltd. Tokyo) were administered orally with a metal tube respective doses of 0, 0.34, 3.4 mg/kg of methylmercury chloride (MMC) (Wako Chemical Co., Tokyo) for 5 consecutive days. LD_{50} of MMC was previously determined as 34 mg/kg. MMC was dissolved in salad oil and a daily dose was adjusted to be 0.1 ml/10 g of body weight.

Twenty four hours after the last dose all the animals were intraperitoreally innoculated with 4×10^8 sheep erythrocytes (SRBC). Assay of primary plaque-forming cell (PFC) response (19 S antibody) was conducted on days 3 to 6 after the first innoculation and secondary response (7 S antibody) on days 12 to 14 following the second innoculation on day 10. Twenty mice from each group were killed for each assay by cervical dislocation; the spleen was immediately removed; five of them were pooled, cut into pieces and forced through a steel mesh into sterile cold Eagle's medium. The value of each PFC response was expressed as a mean of the counts of four pooled spleens (total of 20 spleens). Statistical analysis was made by student's t test. The cells were washed; a stock dilution of 1 spleen equivalent per 2 ml was made and cell count was performed in a hemocytometer. Appropriate dilutions (1 : 10, 1 : 100) of the stock suspensions were made in Eagle's medium for the count of PFC in a chamber. The number of PFC was measured according to the method of Cunningham and Szenberg (1963). The mixture of the 0.4 appropriately diluted spleen cell suspension, 0.05 ml 50% SRBC suspension and 0.05 ml (1 : 50) complement (Toshiba Chemical Ind., Tokyo) was delivered with a syringe into Cunningham chambers consisting of three small (0.1 ml) sections. The chambers were made duplicate for each determination. They were sealed with paraphine and incubated at 37°C for 1 hour. For the assay of the secondary response, goat antiserum (Hyland, Calif. USA) was added to the spleen cell suspension prior to mixing with complement and SRBC.

Experiment 2

Three groups of eight week old female ICR mice were put on diets containing 0.5, 5 ppm MMC respectively at the time of mating. They were maintained on the same diet during pregnant and lactating period. The weaning male mice were maintained on the same diet for further six weeks, thereupon they were innoculated with SRBC and the primary and secondary responses were assayed described as above.

FIG. 1

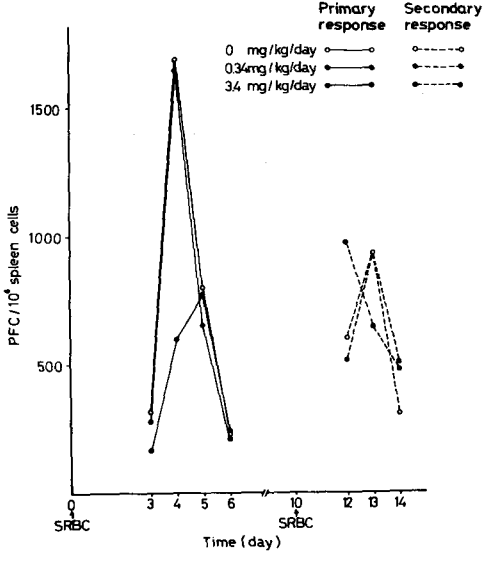


FIG. 2

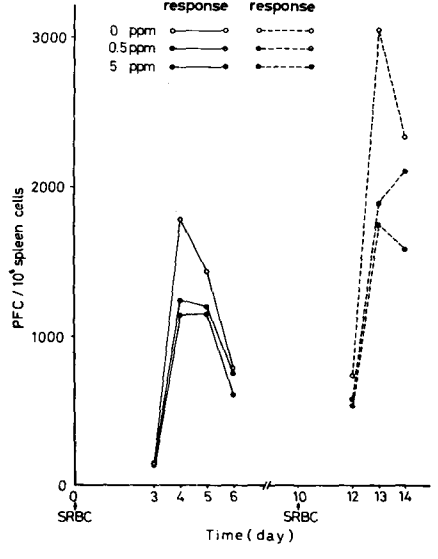
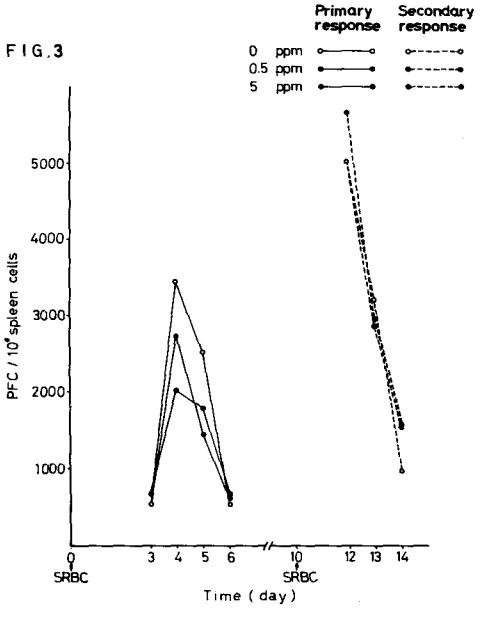


FIG. 3



Experiment 3

Three groups of six week old male ICR mice were put upon diet containing 0, 0.5, 5 ppm MMC respectively and maintained for twelve weeks. At the end of this period, they were innoculated SRBC and the primary and secondary responses were assayed.

In these three experiments, all the animals were weighed and examined weekly for neurological manifestations. The weight of the thymus and the spleen was also measured when these organs were removed. A few organs in each experiment were sampled for histological evaluation.

RESULTS

The exposure to methylmercury in these three experiments did not cause significant differences in the weight or in the histological findings of thymus and spleen between the dosed and the control groups. Slight reduction in the body weight was noted toward the end of the experiments 2 and 3 among the mice group on 5 ppm MMC. Only two in the 5 ppm group in experiment 3 showed neurological signs, e. g. crossing of hind legs toward the end of the experimental period.

Fig. 1 shows that administration of 34 mg/kg MMC for 5 days caused suppression of primary response ($P < 0.01$) at day 4 as well as a delay in reaching its peak. On the other hand, this group shows earlier peak response than other groups in the secondary PFC response.

Fig. 2 shows PFC responses when the animals were exposed to MMC from the very beginning of embrionic stage till nine weeks of age. It appears that counts of PFC tend to be depressed in both primary and secondary responses ($P < 0.05$) for 5 ppm group on day 14, but this is not signfficantly "suppressed" from our laboratory criteria. Shown in Fig. 3 are PFC responses of the mice which started on MMC treated diet at the age of six weeks and continued on it for twelve weeks. The primary response tends to be depressed but again not significantly suppressed. Thus, subchronic administration of rather low levels of MMC appears less suppressive on humoral immunity than dosing sublethal but relatively large amount within a short time period.

DISCUSSION

Though inorganic mercury and some other metals were reported to have immuno-suppressive effect

(Koller 1973), effect of methylmercury on humoral immunity has not been delineated. During the outbreak of Minamata disease, Takeuchi noted hypoplastic hematopoietic tissue and decrease in the number of lymphoid cells in the spleen of patients (1970). Klein et al observed depleted small lymphocytes in the atrophic malphigian corpuscle of the spleen in the rats with acute methylmercury poisoning (1972). Not only morphological changes in the lymphoid tissue, methylmercury causes prolongation of generation time and interferes with cell division in Tetrahymena pyriformis (Thrasher and Adams 1972), chromosomal aberration (Ramel and Magnusson 1969, Umeda et al. 1969, Fiskejo 1970) and disturbance in spermatogenesis (Sakai 1972). The suppression and delay in reaching the peak in the primary response in acute intoxication (experiment 1, Fig. 1) is in accord with those observations and can be explained on the basis of disturbance of cell division. However, the total dose we administered in our experiment was 17 mg/kg in 5 days whereas in Klein's series 70 mg/kg of methylmercury hydroxide was given to rats over the period of 7 days which showed atrophy of the malphigian corpuscles of the spleen. This dose difference probably accounts for the absence of histological changes in the spleen in our series. The dosage of MMC ingested by the animals in experiment 3 is also estimated as approximately 70 mg/kg in 12 weeks, and the absence of notable pathological change can, in this case, be attributed to the extended time period in relation to the consumed dose.

In view of the fact that human fetuses in Japan are more heavily contaminated by methylmercury than their mothers (Suzuki et al 1971), it is of note that the secondary responses of the groups which started their exposure to methylmercury at the very beginning of the life tended to be depressed while the animals which had attained full immunological development at the commencement of their exposure to methylmercury did not seem to get affected at all (Figs. 2 & 3). However, the difference in the absolute peak height of secondary responses noted between these two experiments may be explained by the difference of immunological maturation (9 week old in Fig. 2, 18 weeks in Fig. 3) as demonstrated by Makinodan and Peterson (1966).

SUMMARY

A suppression and delay in reaching the peak of primary humoral response in mice was noted when relatively large dosage of methylmercury was administered in a short time period, suggesting the interference with the initial multiplications of antibody

producing cells. On the other hand, subchronic administration of low levels of methylmercury in diet (0.5 and 5 ppm) caused no definite suppression on either primary or secondary responses, though they tended to be depressed.

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