

Immunological Studies on Mice Exposed Subacutely to Methyl Isocyanate

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The immunotoxicity of methyl isocyanate (MIC) was evaluated in female B6C3F1 mice exposed via inhalation to 0, 1, or 3 ppm for 6 hr per day on 4 consecutive days. The antibody response to sheep erythrocytes and natural killer cell activity were found to be unaffected by MIC exposure. Although lymphoproliferative responses to mitogens were moderately suppressed by MIC, the differences were not statistically significant. The response of splenic lymphocytes to allogeneic leukocytes in a mixed leukocyte response (MLR) was suppressed in a dose-related fashion and was significantly different from the control response at the 3 ppm level. This effect was thought to be secondary and a result of general toxicity, rather than a direct effect of MIC on the immune system. Furthermore, resistance to the infectious agents *Listeria monocytogenes*, mouse malaria parasite, and influenza virus, or to transplantable tumor cells was not compromised by MIC exposure. Thus, the immune system does not appear to be a primary target for MIC toxicity.

Introduction

Although the lung is recognized as the primary target organ of methyl isocyanate (MIC), the toxicological evaluation recently undertaken by the National Toxicology Program included genetic toxicity, reproductive toxicity, and immunotoxicity studies. Toxicity in these organ systems could result from secondary, if not primary, effects of MIC. In order to maximize any potential of this highly reactive chemical to reach remote tissues, the specialized studies were conducted on animals exposed subacutely. The immunotoxicity studies reported here were composed of selected assays for immune function and host resistance following exposure of female B6C3F1 mice to 0, 1, or 3 ppm MIC for 6 hr periods on 4 consecutive days. With this exposure regimen, 3 ppm represents approximately one-half the LC₅₀. No deaths occurred at 3 ppm, although pulmonary pathology was present. General toxicity was indicated by the lethargic appearance of the mice following exposure, a significantly decreased body weight on the day following the last exposure, and significant decreases in spleen and thymus weights 7 days after the last exposure (1). All but certain lung changes were reversible within 28 days.

Methods

Exposure of Animals

The procedure for exposure of animals to methyl isocyanate (MIC) by inhalation has been described fully (3). Exposures were conducted on 3-11 to 3-14 and 6-17 to 6-20-85. Repeated dose studies were conducted in stainless-steel, wire-mesh exposure cages by exposing 10-week old B6C3F1 female mice 6 hr/day for 4 consecutive days to 0, 1, or 3 ppm MIC. During nonexposure periods, mice were housed in an adjacent room on a 12 hr light cycle with food and water *ad libitum*. Immunotoxicity tests were initiated within 5 days of the last exposure.

Immune Function Assays

Humoral immunity was measured by the response to sheep erythrocytes, a T-dependent antigen, in mice immunized 1 day after the last exposure. IgM plaque forming cells (PFC) were enumerated in spleen cells 4 days later using a modified Jerne plaque assay in agar (4). Natural killer (NK) cell activity of spleen cells was determined using YAC-1 tumor target cells in a ⁵¹Cr-release assay (5). Several effector:target (E:T) ratios were used, but data are reported only from the 100:1 ratio, which gave maximal lysis. Lymphocyte proliferation was measured by quantitating ³H-thymidine incorporation in splenic lymphocytes responding to the T-cell

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mitogens phytohemagglutinin (PHA) or concanavalin A (Con A), to the B-cell mitogen *E. coli* lipopolysaccharide (LPS), and to allogeneic leukocytes (mitomycin C-treated lymphocytes from DBA/2N mice) in a mixed leukocyte response (MLR). Mice were sacrificed 4 days after the last exposure for this assay. Conditions for this assay have been previously published (6). Although several concentrations of mitogens were run, only values from optimal mitogen concentrations are reported.

Host Resistance Assays

Mice were challenged with infectious agents 5 days after the last exposure to MIC. Resistance to bacterial challenge with *Listeria monocytogenes* (strain L242/73 type 4B) was tested by IV injection of 2×10^4 viable

bacteria, which approximates an LD₅₀ in control mice. Mortality was monitored for 14 days. A nonlethal strain of malaria, *Plasmodium yoelii* 17XNL, was administered by IV injection of 1×10^6 parasitized erythrocytes. The percentage of parasitized erythrocytes, determined by Diff-Quik staining of tail blood, was followed through the course of infection. The influenza challenge model used was developed by IIT Research Institute, Chicago, IL, under the direction of James Fenters and Peter Barbera (Report L06133-5 prepared for NIEHS contract N01-ES-1-5000). Influenza A2/Taiwan/64 virus was instilled intranasally, and mortality was monitored for 14 days. Resistance to syngeneic tumor cell challenge was determined by injecting 1×10^4 B16F10 tumor cells IV and determining tumor burden in the lungs 21 days later by quantitation of ¹²⁵I-iododeoxyuridine (7). Host resistance data from two separate studies was pooled for analysis.

Statistical Analysis

Data from immune function assays were analyzed by the RS/1 multicompare procedure using the Wilk-Shapiro test for normality, one-way analysis of variance, and Dunnett's test for multiple comparison with a control. Mortality data were analyzed by the chi-square test.

Results and Discussion

Toxicology

Based on previously reported data, mice subacutely exposed to 3 ppm of MIC exhibited signs of toxicity which included lethargy and decreases in body and certain organ weights, notably spleen and thymus (1). Hematological values, determined 5 days after the last exposure, were normal with the exception of hematocrit values, which were slightly higher in both of the MIC-exposed groups, perhaps reflecting some dehydration. The transient nature of the observed effects suggested a general rather than a specific systemic toxicological response. However, pathologic changes in the respiratory system were seen, and included erosion and necrosis of the epithelium of the nasal cavity, trachea, and bronchi, fibrosis in the walls of major bronchi, and fibroepithelial tissue projections into major airways (2).

Table 1. Plaque-forming cell response in MIC-treated mice.

Treatment ^b	PFC/10 ⁶ cells ^a	
	Experiment 1	Experiment 2
Control	1602 ± 112 ^c	1224 ± 245
1 ppm	1420 ± 96	1698 ± 162
3 ppm	1375 ± 172	1473 ± 197

^a The antibody response to sheep erythrocytes was determined by enumerating plaque forming cells (PFC) in splenic lymphocytes 4 days after primary immunization.

^b Seven mice per group.

^c Mean ± SEM; no values differ significantly from control.

Table 2. Natural killer (NK) cell activity in MIC-treated mice.

Treatment ^b	% Cytotoxicity ^a	
	Experiment 1	Experiment 2
Control	24 ± 1 ^c	14 ± 1
1 ppm	23 ± 2	15 ± 2
3 ppm	20 ± 3	16 ± 1

^a NK cytotoxicity against ⁵¹Cr-labeled YAC-1 target cells was measured at a 100:1 E:T ratio in two separate experiments. Cytotoxic activity was calculated from the formula:

$$\% \text{ cytotoxicity} = \frac{\text{cpmE} - \text{SR}}{\text{cpmT} - \text{SR}} \times 100$$

in which cpmE - SR is cpm of ⁵¹Cr release in the experimental minus spontaneous release, and cpmT - SR is cpm in total cells added (total release) minus spontaneous release.

^b Seven mice per group.

^c No values differ significantly from control.

Table 3. Splenic lymphocyte proliferative responses to mitogens and allogeneic leukocytes.

Treatment ^a	cpm ⁸ H - TdR (× 10 ⁻³)				
	No mitogen	LPS	PHA	Con A	MLR
Control	3.5 ± 0.7 ^b	35.5 ± 1.4	41.7 ± 3.2	55.6 ± 5.8	18.7 ± 0.9
1 ppm	4.4 ± 1.3	25.8 ± 2.4 (27)	34.4 ± 4.7 (18)	38.3 ± 1.8 (31)	16.4 ± 0.8 (12)
3 ppm	2.5 ± 0.6 (29) ^c	28.6 ± 4.3 (19)	35.5 ± 4.3 (15)	38.9 ± 7.3 (30)	12.7 ± 1.0* (32)

^a Seven mice per group.

^b Mean ± SEM.

^c Numbers in parentheses indicate percent suppression.

* Value differs from control at $p < 0.05$.

Immune Function

The results of two separate experiments evaluating the antibody response of MIC-exposed mice are shown in Table 1. No effect of MIC was detected on the PFC response to sheep erythrocytes. NK cell activity from two separate MIC exposures was also found to be unaltered by chemical exposure (Table 2). Splenic cellularity was also not altered by MIC exposure (data not shown).

The proliferative response of splenic lymphocytes from MIC-treated mice to B- and T-cell mitogens and allogeneic leukocytes is shown in Table 3. There was a trend toward suppression of the response to mitogens in treated mice and a statistically significant depression of the MLR at 3 ppm. A repeat study gave similar results, with the MLR again suppressed in a dose-related fashion (data not shown). The suppression was transient, however, as mice evaluated at the end of the 120-day observation period were found to be unaffected by chemical exposure (data not shown).

Host Resistance

Following chemical exposure, groups of mice were challenged with either *Listeria monocytogenes*, mouse malaria (*Plasmodium yoelli*), influenza virus, or B16F10 syngeneic tumor cells for evaluation of host resistance. The results, representing pooled data from the two separate exposures, are summarized in Figure 1. The *Listeria* and B16F10 studies included a positive control using cyclophosphamide at 20 mg/kg, which caused significantly impaired resistance in both cases (data not shown). There were no statistically significant

alterations in any of the host resistance models as a result of MIC exposure, although there was a trend toward susceptibility following intranasal challenge with influenza virus. This effect may be immunological in nature or a consequence of impairment of lung clearance mechanisms.

In conclusion, the immune system does not appear to be sensitive to MIC exposure in mice. The minimal changes in immune function that were observed are possibly a secondary consequence of the pulmonary or general toxicity observed in exposed mice. In light of the normal response of MIC-treated mice to challenge with infectious agents and tumor cells, the immune alterations must be considered biologically inconsequential. Although MIC did not appear to reach primary organs of the immune system, it could cause alterations in immune function as a secondary consequence to lung disease as has been seen in asbestosis in man (8) and in mice (9). This aspect of MIC toxicity was not evaluated in the present study.

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Details of these studies have been published [Luster et al., Immunotoxicity studies on mice exposed to methyl isocyanate. Toxicol. Appl. Pharmacol. 86, 140-144 (1986)].

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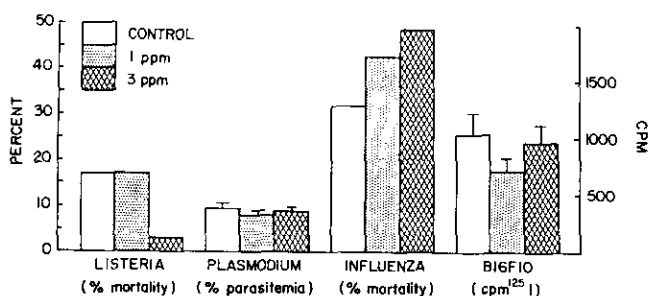


FIGURE 1. Effect of MIC on host resistance. The ability of mice treated with 1 or 3 ppm of MIC to resist bacterial, parasitic, viral, and tumor cell challenge was compared to control mice. Following infection with *Listeria*, mortality was monitored for 14 days and is given as percentage. There were 30 mice per treatment group in this assay. Infection with *Plasmodium* is indicated by percent parasitemia, which is the percent parasitized erythrocytes on the peak day of infection, day 10. There were 14 mice per group in this assay. Cumulative mortality for 14 days following infection with influenza virus is given as percentage, and there were 35 mice per group. Metastasis of B16F10 tumor cells was determined 21 days after injection and is expressed as cpm ¹²⁵I-iododeoxyuridine in the lungs using 14 mice per treatment group. No treatment values were significantly different from control in any of the assays.