Effects of Polybrominated Biphenyls (PBB) on Immune Response in Rodents

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Studies were performed to investigate the effects of FireMaster FF-1, a chemical fire retardant consisting of a mixture of polybrominated biphenyls (PBB), on immune functions in mice and rats. Animals received 22 daily treatments of 0.03, 0.3, 3.0, or 30 mg PBB/kg body weight in a period covering 30 days. PBB exposure severely depressed cell-mediated immunity in both mice and rats at the higher dosage levels as indicated by depressed responsiveness of splenic lymphocytes to mitogenic stimulation by polyclonal T-cell activators. Additionally, humoral immunity was depressed in mice at the 30.0 ppm dosage level. Assays for humoral immune functions included antibody production, serum immunoglobulin levels, and mitogenic stimulation of splenic lymphocytes to a polyclonal B-cell activator. These studies indicate that PBB exposure can lead to suppression of both humoral and particularly cell-mediated immune responses.

Immunosuppression by environmental chemicals in a developing concern in chronic toxicity assessment. As recently reviewed by Vos (1), a number of these chemicals have been found to severely suppress immune functions. For example, the organometallics, including triphenyltin and methylmercury as well as inorganic metals such as lead, mercury, and cadmium, suppress humoral immune functions. In contrast, the polychlorinated hydrocarbon, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) has been reported to suppress cell-mediated immune functions while polychlorinated biphenyl (PCB) and di-n-butyltin dichloride suppress both cellular and humoral immune functions.

In 1973 FireMaster PB-6, a chemical fire retardant consisting of a mixture of polybrominated biphenyls (PBB), was accidentally mixed with livestock feed in the State of Michigan. As a result, over 15 million livestock including 11,000 beef and dairy cattle had to be destroyed in 1974 alone (2). During the first 9 months following the accident, meat and dairy products containing PBB were widely consumed in Michigan. Subsequently, PBB was found in the serum and/or adipose tissues of dairy farmers in the state as well as many urban residents of Michigan (3,4). While many cattle died due to PBB-induced ailments and some pregnant animals may have aborted, there is no clear-cut evidence for similar effects in humans to date. However, Bekesi et al. (in press) recently indicated that Michigan dairy farm residents who ate farm products containing PBB following accidental contamination of animal feed in 1973, presumably a low dose chronic exposure, have significant alterations in their immune status. The studies reported here were undertaken to investigate the effects of an identical commercial preparation of PBB (FireMaster FF-1) on the immune response of mice and rats.

Materials and Methods

Animals

Male Fisher rats (CDF) and female mice (B6C3F1) were purchased from NCI (Fort Detrick, Md.). Animals were housed in hanging wire mesh cages and allowed free access to food and water. Separate animals were used for in vitro lymphocyte culture and antibody tests.

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PBB

FireMaster (FF-1) (lot #FF1312FT, Batch 03) was obtained from Midwest Research Institute. Mass spectral analysis indicated that the mixture was > 99% PBB with 56% of the mixture containing 2,4,5,2',4',5'-heptabromobiphenyl (HBB) and 27% consisting of 2,3,4,5,2',4',5'-heptabromobiphenyl (R. Hass, unpublished observations). The FireMaster was dissolved in corn oil and so diluted that mice received a total volume of 0.1 ml and rats received a total of 0.2 ml per dosage.

Dosage Regimen

Mice and rats were randomly grouped and treated with 0.03, 0.3, 3.0, or 30.0 mg PBB/kg body weight per day. Controls received corn oil alone. Animals were dosed by gavage 5 days per week until 22 doses were administered (a period covering 30 days). Functional immune studies were performed 26–34 days following the final dose in order to view clinical changes.

Cell Preparation

Animals used for in vitro lymphocyte cultures or antibody plaque assays were killed with CO₂ and their spleens removed aseptically. Cell suspensions were prepared as previously described (Luster and Faith, in press). Cell counts were determined with a Coulter Counter (Model ZB, Coulter Electronics, Inc., Hialeah, Fla.).

Cell Cultures

The lymphoid cells obtained from individual spleens were cultured as previously described (Luster and Faith, in press). Cell stimulation was measured by the rate of cellular DNA synthesis as determined by incorporation of ³H-thymidine into acid-insoluble material. In mice, the mitogens and concentrations in culture media employed were phytohemagglutinin-P (PHA) at 8 μg/ml, Concanavalin A (Con A) at 2 μg/ml and E. coli lipopolysaccharide 0127 (LPS) (Difco Lab.) at 25 μg/ml. In rats, the concentrations were 50 μg/ml PHA, 4 μg/ml Con A, and 100 μg/ml of pokeweed mitogen (PWM) (Gibco Lab.).

Humoral Immune Function Tests

Serum IgG, IgM, and IgA levels were quantified on individual serum by the radial immunodiffusion technique (5) with the use of commercial heavy chain-specific antisera and standards (Meloy Lab., Springfield, Va.). IgM (19s) plaque forming cells (PFC) to sheep red blood cells (SRBC) were determined by the antibody plaque assay in mice (6). A microtiter (Cooke Engineering, Alexandria, Va.) technique was used to assay rat hemagglutinins to SRBC. Individual mice and rats were tested for their ability to produce an antibody response 4 days following intraperitoneal immunization with 0.1 ml of a 10% suspension of washed SRBC.

Statistical Analysis

For in vitro stimulation, the Mann-Whitney U test was employed to assess the significance of treatment effects (7). The significance of dose response trends was determined by Jonckheere’s test. For immunoglobulin quantitation and the antibody plaque assay statistical analysis was performed on the geometric means of transformed data employing analysis of variance procedures (8).

Results

All mice and rats treated with PBB survived the experimental period. Table 1 records body and selected organ weights of mice orally dosed with PBB at levels of 0, 0.03, 0.3, 3.0, and 30 mg/kg body weight for 22 days over a 30-day period. In mice, necropsied 30 days after the final exposure, an absolute and relative decrease in spleen as well as a slight reduction in thymus weight occurred at the 30 ppm dose level. Microscopic examination of the lymphoid organs at 30 days following the last exposure failed to reveal visible effects, although a slight decrease in the density of thymic cortex was noted at the 30 mg/kg dose level.

Body weights and selected organ weights of rats necropsied 30 days after the final exposure are given in Table 2. A marked reduction in body weight, spleen weight, as well as absolute and relative thymic weights were noted at the 30 mg/kg dose level. Reduced absolute spleen weights as well as relative spleen and thymus weights also occurred in the 3.0 mg/kg dosage group. Microscopically, minimal effects were observed in the spleen or thymus except for a slight decrease in the density of the thymic cortex in the 30 mg/kg group.

Figure 1 depicts the reactivity of splenic lymphocytes from PBB exposed mice to mitogen stimulation by the mitogens Con A, PHA and LPS as well as the antibody plaque-forming cell response to SRBC. The suppressive effect of PBB on mitogen responsiveness is indicated by a marked effect (p < 0.01) in the overall dose response trend to all
Table 1. Body and selected organ weights in mice exposed to PBB.*

| PBB dosage, mg/kg | Body weight, g | Spleen weight, g | Spleen/body weight × 10^3 | Thymus weight, g | Thymus/body weight × 10^3 |
|-------------------|----------------|-----------------|--------------------------|-----------------|--------------------------|
| 0                 | 24.37 ± 2.54b  | 0.104 ± 0.019   | 4.3 ± 0.7                | 0.041 ± 0.009   | 1.7 ± 0.5                |
| 0.03              | 25.97 ± 1.46   | 0.101 ± 0.006   | 3.9 ± 0.2                | 0.040 ± 0.003   | 1.6 ± 0.1                |
| 0.3               | 27.60 ± 3.11   | 0.096 ± 0.008   | 3.5 ± 0.2^c              | 0.047 ± 0.003   | 1.7 ± 0.3                |
| 3.0               | 25.33 ± 2.99   | 0.100 ± 0.013   | 4.0 ± 0.5                | 0.045 ± 0.003   | 1.8 ± 0.1                |
| 30.0              | 24.27 ± 0.80   | 0.079 ± 0.014d  | 3.3 ± 0.6^d              | 0.036 ± 0.010   | 1.5 ± 0.4                |

* At least three animals were observed in each dosage group.
^ The results are recorded as the mean ± S. D.
^ p < 0.05.
^ p < 0.10.

Table 2. Body and selected organ weights in rats exposed to PBB.*

| PBB dosage, mg/kg | Body weight, g | Spleen weight, g | Spleen/body weight × 10^3 | Thymus weight, g | Thymus/body weight × 10^3 |
|-------------------|----------------|-----------------|--------------------------|-----------------|--------------------------|
| 0                 | 309.45 ± 18.87b| 0.670 ± 0.039   | 2.2 ± 0.06               | 0.270 ± 0.021   | 0.9 ± 0.02               |
| 0.03              | 319.77 ± 21.54 | 0.663 ± 0.051   | 2.1 ± 0.03^c             | 0.305 ± 0.016^d | 1.0 ± 0.08^d             |
| 0.3               | 299.17 ± 8.00  | 0.633 ± 0.045   | 2.1 ± 0.12               | 0.264 ± 0.028   | 0.9 ± 0.12               |
| 3.0               | 315.07 ± 3.32  | 0.636 ± 0.034   | 2.0 ± 0.11^c             | 0.242 ± 0.013^c | 0.8 ± 0.03^d             |
| 30.0              | 243.17 ± 6.70^d| 0.527 ± 0.013^d | 2.2 ± 0.02               | 0.159 ± 0.008^d | 0.7 ± 0.02^d             |

* At least three animals were observed in each dosage group.
^ The results are recorded as the mean ± S. D.
^ p < 0.1.
^ p < 0.05.

three mitogens tested (Fig. 1A–C). The suppressed mitogenic reactivity was most clearly demonstrable in the 3.0 and 30.0 mg/kg dosage groups with the T-cell mitogens (PHA and Con A) and in the 30.0 mg/kg group with the B-cell mitogen (LPS); all mean values at these dosage groups being statistically significant (p < 0.10 or p < 0.01). Enumeration of IgM antibody plaque-forming cells to SRBC did not reveal any statistically significant differences between control and treated animals (Fig. 1D). However, the PFC response to SRBC in the 30.0 mg/kg dosage group with a geometric mean of 429 PFC/10^6 splenic lymphocytes was 27% lower than in controls with 584 PFC.

In Figure 2 the reactivity of splenic lymphocytes from PBB exposed rats to the mitogens PHA, Con A and PWM as well as the antibody response to SRBC is summarized. PWM rather than E. coli LPS was employed to evaluate B-cell reactivity due to the poor stimulation of normal splenic lymphocytes from Fisher rats to E. coli LPS (unpublished observations). A suppressive effect due to PBB was indicated by the overall dose response trend following mitogenic stimulation with the T-cell mitogens PHA (p < 0.01) and Con A (p < 0.1) (Fig. 2A, B). Marked depression of mitogenic stimulation, however, occurred only in the 3.0 and 30.0 mg/kg groups with PHA (p < 0.05) and in the 30.0 mg/kg group with Con A (p < 0.05). A dose-response trend was not found with PWM, a polyclonal T- and B-cell activator (Fig. 2C). The SRBC antibody titers were similar in treated and control rats (Fig. 2D).

Serum IgG1, IgG2, IgM, and IgA concentrations in PBB treated and control mice are depicted in Table 3. A decrease in serum levels of all three classes and subclasses tested occurred at the 30.0 mg/kg dosage group. However, only the decreases in serum IgM and IgG2 were statistically significant. Statistically significant differences in serum immunoglobulin concentrations in mice exposed to 0.03, 0.3, or 3.0 mg/kg concentrations of PBB were not found.

**Discussion**

In studies where toxicosis was induced by feeding PBB a variety of nonspecific clinical signs as well as specific repeatable pathologic tissue changes have been observed. The nonspecific clinical signs such as anorexia, dehydration, diarrhea, and excessive lac- rimation and salivation have been reported among accidentally contaminated farm animals in Michigan but not in controlled studies (2, 9). However, specific gross pathological changes including subcutaneous edema, atrophy of the thymus and fetal death with concomitant necrosis of cotyledons as well as his-
FIGURE 1. Effect of PBB exposure in mice on the responsiveness of splenic lymphocytes to mitogen stimulation and SRBC splenic plaque forming cells: (a) individual values; (*) mean value of the dosage group. The response of splenic lymphocytes to PHA (A) and Con A (B) were suppressed at the 3.0 and 30.0 mg/kg levels. The response to LPS (C) was suppressed at the 30.0 mg/kg level.

tological changes particularly in the kidneys, gall bladder, and eyelids have been present in animals initially contaminated with PBB (10). Based on the present studies immune function, particularly cell mediated immunity, also appears to be effected.

Mice exposed to PBB at 3.0 and 30.0 mg/kg levels and rats exposed to 30 mg/kg levels had impaired cell-mediated immune functions as evidenced by reduced mitogenic stimulation by the polyclonal T-cell activators PHA and Con A. In addition, there was a reduction of lymphoid organ weights at the 30 mg/kg level in both mice and particularly in rats. That immunosuppression may be a more subtle effect of exposure to PBB than either relative organ weights or microscopic examination is suggested by the fact that mice at the 3.0 mg/kg level were immunosuppressed while organ weights and histology were normal. In this respect, mice exposed to levels of TCDD, a potent immunosuppressive environmental chemical, that do not produce thymic atrophy were found to have increased susceptibility to infection with Salmonella bern, an infection which is dependent upon cell-mediated immunity (11).

Although PBB appears to primarily effect cell-mediated (T-cell) immunity, humoral immune functions were also depressed in mice at the 30 mg/kg dose level. This was evidenced by statistically significant decreases in the in vitro mitogenic response to the polyclonal B-cell activator LPS, and serum IgG and IgM levels as well as slight reductions in relative spleen weights and the primary antibody response to SRBC. Reduction of relative spleen weights were also noted in rats at the 3.0 mg/kg level but functional humoral immune tests including antibody synthesis and in vitro mitogenic response to PWM did not indicate that immunosuppression had occurred. It is quite conceivable, however, that if more sensitive functional immune tests were available, suppression of humoral immunity would have been observed in rats. This reduction of humoral immune responsiveness appears analogous to earlier studies in guinea pigs fed hexabromobiphenyl (Fire-Master BP-6) for 45 days (12). In these studies a slightly reduced primary antibody response (50%) occurred in the 10 ppm group and significant reduction occurred at the 50 ppm group in animals im-

Figure 2. Effect of PBB exposure in rats on the responsiveness of splenic lymphocytes to mitogen stimulation and antibody titers to SRBC: (a) individual values; (*) mean value of the dosage group. The response of splenic lymphocytes to PHA (A) was suppressed at the 3.0 and 30.0 mg/kg levels and to Con A (B) at the 30 mg/kg level. Significant differences were not observed in the response to PWM (C) on SRBC immunization (D) between control and exposed animals.
munized against tetanus toxoid. The secondary (IgG) antibody response was significantly depressed in both groups.

Limited studies on the immunosuppressive effect induced by PCB suggests that PBB-induced immunosuppression is similar to that found with PCB. Feeding 50 ppm of Clophen A60 or Aroclor 1260, commercial PCB preparations, for 6 or 8 weeks in guinea pigs caused thymic atrophy and significantly reduced the antibody response to tetanus toxoid and the delayed hypersensitivity response to tuberculin (12, 13). However, part of the immunosuppressive effects caused by feeding PCB may have been due to the presence of chlorinated dibenzofurans as toxic impurities in commercial PCB preparations. In this respect, 2,3,7,8-tetrachlorodibenzofuran has recently been shown to cause severe thymus atrophy in a number of experimental animals (14).

It was noted that while high levels of PBB were generally immunosuppressive, lower levels tended to enhance immune activity. For example, in mice PHA responsiveness was slightly elevated at the 0.03 ppm level and enhanced responses to Con A occurred at both the 0.03 and 0.3 ppm dose levels. Similarly, enhanced PHA and Con A reactivity of splenic lymphocytes occurred in rats at the 0.03 and 0.3 ppm levels. Increases in absolute and relative thymic weights immediately following exposure of rats to 0.03 ppm of PBB lend support to these observations. In this respect, Bonnyns and Botonsky (15) reported that treatment of rats with low levels of PCB enhances PHA reactivity of lymphocytes. Additionally, low level exposure to heavy metals such as lead (16), or cadmium (17) results in enhanced antibody response while higher levels exert an immediate depressive effect (18, 19).

There is no evidence that environmental chemicals which induce immunosuppression do so through direct mechanisms. However, there are a wide variety of physiological and pathological conditions that, if altered by toxic chemicals, would indirectly alter the immune system. For example, hormones such as adrenal glucocorticoids (20) and estrogens (21) have been reported either to enhance or depress various cellular and humoral immune functions. In unrelated studies, feeding mice low levels of PCB resulted in a decrease in plasma corticoid levels (22) while higher doses of PCB increased serum corticoid levels (23). It is quite conceivable that low levels of PBB depress circulating corticosteroid levels resulting in enhanced immune responsiveness while high levels increase circulating corticosteroid levels causing an adverse effect on the immune system. Alternatively, α-fetoprotein which is synthesized in fetal liver, a primary target organ for PBB (24), may be induced or depressed. Recently, α-fetoprotein has been found to possess immunoregulatory capabilities (25). Liver pathology was noted in mice and rats at the 20 mg/kg levels (unpublished observations). Finally, it has been suggested that PBBs cause impairment in absorption and/or utilization of specific nutrients as evidenced by pair-feeding studies in Japanese quail and chickens following ingestion of 500 ppm of PCB-contaminated feed (26). Depression of humoral responses (27, 28) and depression (28) or enhancement (29) of cellular immune response have been observed in studies of experimental malnutrition. However, the immune alterations may be related to changes in serum levels of glucocorticoids as found in children with protein calorie malnutrition (30) and in undernourished young rats (31).

In conclusion, the mechanisms responsible for PBB-induced immunosuppression are uncertain. In any case, exposure to PBB may be more undesirable than previously thought, as it may lead to immune modulation in humans as in rodents. The mechanisms of immune modulation by PBB is being studied.

The authors wish to thank Ms. L. D. Lawson for valuable assistance, D. J. Haseman for statistical analysis, and Ms. S. Wilkins for typing the manuscript.

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