Role of Endotoxin in Alterations of Hepatic Drug Metabolism by Diphtheria and Tetanus Toxoids and Pertussis Vaccine Adsorbed

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Administration of diphtheria and tetanus toxoids and pertussis vaccine adsorbed (DTP vaccine) or endotoxin (LPS) resulted in marked alterations in hepatic drug-metabolizing enzymes in endotoxin-responsive (R) and non-endotoxin-responsive (NR) mice. A single human dose (0.5 ml) of DTP vaccine increased hexobarbital-induced sleep times to 1.6- to 1.8-fold above those of controls in both strains of mice. This effect persisted for 7 days. In contrast, Bordetella pertussis LPS-treated mice showed an increase at 1 day (3.0-fold for R mice and 1.5-fold for NR mice), which returned to control levels by day 7. Furthermore, cytochrome P-450 levels were decreased 30 to 40% 24 h after DTP vaccine administration in both R and NR mice, while after LPS administration they were decreased 30% in R mice and less than 10% in NR mice. Both spleen and liver weights of R and NR mice were increased 7 to 14 days following DTP vaccine administration. However, LPS treatment had no apparent effect on liver weights, and spleen weights of R mice were elevated from days 3 to 7. Histopathologic tissue examination showed random, multifocal inflammation with hepatocyte necrosis after DTP vaccine administration to both R and NR mice and an absence of lesions in LPS-treated mice. Premixing LPS with polymyxin eliminated the increased sleep times, but premixing DTP vaccine with polymyxin did not affect the increased sleep times. Levels of tumor necrosis factor and interleukin-6 in plasma of R mice were markedly increased after DTP and LPS treatment, while NR mice had reduced increases. These results suggest that LPS contributes to the alterations in R and NR mice seen within the first 24 h of vaccine administration but that it is not likely to contribute to the effects observed at later time points.

There has been a great deal of concern about local and systemic adverse reactions to diphtheria and tetanus toxoids and pertussis vaccine adsorbed (DTP vaccine) in infants and children. Increased reporting of and publicity surrounding adverse reactions have been claimed to result in a drop in the number of children being immunized (10, 11). Concomitant with the decrease in immunizations has been a corresponding increase in the number of cases of pertussis (30, 34). The pertussis component of the DTP vaccine has been implicated as responsible for the adverse reactions, but the agents responsible for toxicity have not been identified. It has been suggested that endotoxin associated with the vaccine is the causative agent because of its role in fever induction and sepsis.

Several reports have demonstrated that administrations of DTP vaccine and other agents cause inhibition of hepatic drug-metabolizing enzymes in mice (2, 3). For humans, there are a number of reports on alterations of drug metabolism following immunization with influenza virus vaccine and administration of endotoxins and cytokines or cytokine-inducing agents (19, 23, 32). The relationship of these observations with animals to the reported adverse reactions in humans following immunization has not been established, nor has the mechanism for the inhibition of drug metabolism been identified. There is, however, the suggestion that the immune system may be involved. Administration of vaccines has been associated with an increase in interferon levels in sera of rodents and rabbits, and administration of interferon-inducing agents depresses hepatic drug metabolism (2, 3, 26, 27). In mice, the coadministration of interleukin-2 (IL-2) and alpha interferon decreases hepatic drug metabolism in a dose-dependent manner (2). Pretreatment of mice with gamma irradiation reduces the effects of the cytokines, which is consistent with a role for T cells. However, when irradiated mice were immunized with DTP vaccine, the alterations in hepatic drug metabolism were not changed from those of nonirradiated controls (2). This suggests that there may be an alternate pathway for vaccine toxicity.

Neither the mechanism by which vaccines inhibit drug metabolism nor the components of the vaccines responsible for these alterations have been established. Previous studies in our laboratory have shown that aluminum adjuvants, the preservatives, and diphtheria and tetanus toxoids are not the components responsible for the adverse reactions (3). Extensive studies with diphtheria and tetanus toxoids adsorbed (DT vaccine), which contains the same adjuvant and toxoid components as those present in the DTP vaccine, have failed to show any toxicity. Likewise, injection of DT vaccine mixed with pertussis toxoid or with Bordetella pertussis lipopolysaccharide (LPS) failed to induce the alterations noted below for DTP vaccine. In contrast, a nonadsorbed, whole-cell pertussis vaccine elicits the same response as DTP vaccine in our mouse model (3).

One common component of many bacterial vaccines is endotoxin. Endotoxins are important factors in the pathogenicity of gram-negative organisms. The LPS of B. pertussis has been reported to account for most of the reported adverse reactions to the vaccine in humans (37). In mice, a single human dose (0.5 ml) of DTP vaccine increased hexobarbital-induced sleep times within 12 h and to a maximum

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at 7 to 10 days following immunization. In contrast, a single 50-μg injection of either *B. pertussis* or *Escherichia coli* endotoxin causes maximum sleep time increases on the first day following injection. Hexobarbital-induced sleep times returned to control levels by 7 days in these mice (3).

Endotoxins are known to induce a wide variety of biological activities. These studies were undertaken to further evaluate the role of endotoxin in the vaccine-induced alterations of hepatic drug metabolism. The effects of DTP vaccine, polyribosinosinic:polyribocytidylic acid (PIC), endotoxin, and other vaccines and components are evaluated with endotoxin-responsive (R) and non-endotoxin-responsive (NR) mice.

**MATERIALS AND METHODS**

PIC and *E. coli* endotoxin (*E. coli* LPS) (serotype O55:B5) were purchased from Sigma Chemical Co. (St. Louis, Mo.). *B. pertussis* endotoxin (*B. pertussis* LPS) was purchased from List Biological Laboratories (Campbell, Calif.). Vaccines were samples submitted to the Center for Biologics Evaluation and Research, Food and Drug Administration, for control testing. Endotoxin activity was determined by the Division of Product Quality Control, Food and Drug Administration, by using the *Limulus* amebocyte lysate (LAL) test and by rabbit pyrogenicity (20).

**Animals.** Female NR (C3H/HEJ) and R (C3H/HEN) mice (6 to 10 weeks old, 10 to 15 g) were purchased from the National Cancer Institute or Charles River Laboratories. They were provided free access to food and water and were maintained on a 12-h light-dark cycle. Animals were acclimated several days prior to the start of an experiment. All injections were administered intraperitoneally at a volume of 0.5 ml. PIC or LPS was dissolved in sterile, pyrogen-free saline prior to injection.

Sleep time. Hexobarbital sodium (Sigma Chemical Co.), 150 mg, was dissolved in 0.9 ml of 1 N NaOH and diluted to 30 ml in water immediately before use. Mice were weighed and injected intraperitoneally with a dose of 100 mg/kg of body weight. Sleep time was measured as the time elapsed from injection of hexobarbital to the return of the redressment reflex (22). A separate group of animals was used for each time point when sleep times were measured. The sleep time index refers to the mean sleep time of the experimental group divided by the mean sleep time of the control group.

**Enzyme assays.** Immediately after mice were sacrificed by cervical dislocation, livers were removed from mice, weighed, and homogenized in 3 volumes of ice-cold isotonic KCl. Cytosolic and microsomal fractions were prepared according to the method of Hodgeboom (21). Microsomes were resuspended in 2 ml of 0.1 M potassium phosphate (pH 7.4) containing 20% glycerol, frozen immediately in liquid nitrogen, stored at −70°C, and used within 1 week of preparation. No loss of activity was seen upon storage when samples were handled in this manner.

Cytochrome P-450 levels were measured according to the method of Omura and Sato (31) by using a model A4 50A Hewlett-Packard spectrophotometer.

Benzphetamine demethylase activity was assayed spectrophotometrically according to the method of Astrom et al. (5). Ethylmorphine was obtained from Alltech Applied Science (Deerfield, Ill.). Ethylmorphine demethylase activity was assayed by the method of Astrom et al. (5), with the following modifications: a total volume of 0.25 ml was used, to which an equal volume of trichloroacetic acid was added to stop the reaction. Formaldehyde production was determined according to the Nash procedure (29). Benzopyrene monooxygenase activity was determined by the method of DePierrre et al. (13). Benzopyrene was obtained from Aldrich, and [3H]benzopyrene (40 Ci/mmol) was obtained from Amersham. [3H]benzopyrene was purified before use as described previously (13).

The cytosolic glutathione transferases were assayed with 1-chloro-2,4-dinitrobenzene and 1,2-dichloro-4-nitrobenzene as substrates (17). Dicoumarol-inhibitable quinone reductase was assayed according to the method of Ernst (14) with the modifications described by Benson et al. (7) by using 2,6-dichlorindophenol as the electron acceptor in a final volume of 1.0 ml.

Protein was determined with bicinchoninic acid (38), with bovine serum albumin as a standard.

Data were analyzed by Student's *t* test or analysis of variance by using Statview (MacIntosh).

**Plasma.** Blood was collected into heparinized tubes by retro-orbital bleeding immediately before sacrifice. Plasma was prepared by centrifugation and was stored at −20°C until used. No loss of activity was observed with samples stored for up to 1 month. The plasma samples were assayed (by Assay Research Inc., College Park, Md.) for tumor necrosis factor (TNF) by using a standard enzyme-linked immunosorbent assay (ELISA) kit (Genzyme). IL-6 assays were performed by bioassay according to the procedure of Aarden et al. by using B9 cells (1) as described in detail by Tosato et al. (39). The B9 cells were generously provided by G. Tosato. Activity is expressed in units per milliliter, where 1 U represents the dilution of standard necessary to give 50% of maximal activity. The standard was derived from the culture supernatant fluid of an IL-6-secreting cell line.

**Pathology.** Livers and spleens were removed from DTP vaccine- or endotoxin-treated mice at the times indicated, weighed, rinsed, and placed immediately in buffered formalin solution. Histologic examination of the tissues was performed by a veterinary pathologist.

**RESULTS**

Hexobarbital-induced sleep time 24 h after administration of vaccines or endotoxin. The abilities of DTP and pertussis vaccines to alter hexobarbital-induced sleep time were compared with those of *E. coli* and *B. pertussis* LPS preparations in R and NR mice. The dose of endotoxin used (50 μg) was chosen because it contained an endotoxic activity somewhat higher than that found in 0.5 ml of DTP vaccine when measured by the LAL test (typically 50,000 endotoxic units [EU]/ml for LPS versus 10,000 EU/ml for vaccine). Administration of either *E. coli* or *B. pertussis* endotoxin, at a dose of 50 μg, increased hexobarbital-induced sleep time in R mice 2.4- to 3.0-fold over that of controls, compared with a 1.2- to 1.5-fold increase in NR mice. Administration of DTP vaccine or pertussis vaccine adsorbed caused a 1.6- to 1.8-fold increase and a 1.4- to 1.5-fold increase, respectively, in sleep times in R and NR strains of mice (Fig. 1). DT vaccine administration did not significantly increase sleep times over that of controls for either strain (1.1- to 1.3-fold increase). Sleep time was also measured following administration of a single injection of cholera vaccine, a bacterial vaccine with an endotoxic activity similar to that of DTP vaccine (both have approximately 10,000 EU/ml by the LAL test). There was a 1.5-fold increase in sleep time in R mice but no increase in sleep time in NR mice at 24 h after cholera vaccine administration. By 4 days, the increased sleep time in R mice had returned to control levels. Administration of
PIC increased hexobarbital-induced sleep time 1.5- to 1.7-fold over that of controls in both strains of mice. No detectable endotoxic activity was present in the preparation as determined by LAL testing.

**Time course of hexobarbital-induced sleep time following vaccine or endotoxin administration.** Hexobarbital-induced sleep times were compared for up to 1 week following a single injection of B. pertussis LPS or DTP vaccine. Figure 2 shows the results of one experiment. At 24 h after a single injection of LPS, the sleep time index was 2.3 for R mice compared with 1.5 for NR mice. At 4 days postinjection, hexobarbital-induced sleep times in the NR mice were indistinguishable from control levels, while in the R mice, there was still a twofold increase over that of controls. By 7 days postinjection, however, both groups of mice had sleep times that were the same as that of controls. DTP vaccine-treated mice have sleep times at 24 h increased to 1.6-fold (NR mice) and 1.8-fold (R mice) above control levels. These increased sleep times persisted in both strains of mice for at least 1 week after DTP vaccine administration.

**Cytochrome P-450 levels and enzyme activities.** Spectral cytochrome P-450 levels were measured 24 h after vaccine, LPS, or PIC administration. As can be seen in Table 1, both DTP vaccine and PIC decreased P-450 levels significantly, by 30 to 40% in R and NR mice. Administration of B. pertussis or E. coli LPS significantly reduced P-450 levels only in R mice by 30 to 45%. In NR mice, the decrease was about 10%. The cytochrome P-450 levels returned to control levels 2 days after LPS administration, while those of the DTP vaccine-treated mice remained reduced for 7 to 10 days (4).

The microsomal enzyme activities benzphetamine demethylase, benzo(a)pyrene monooxygenase, and ethylmorphine demethylase were also compared 24 h after vaccine or LPS administration. Figure 3 shows the results of one representative experiment. As was observed with P-450 levels, both DTP vaccine and PIC decreased benzphetamine demethylase activity about 40% in both strains of mice (Fig. 3A). LPS administration caused a marked decrease in benzphetamine demethylase activity in R mice (50% of control) but only a 20 to 30% decrease in NR mice. Benzphetamine monooxygenase activity was decreased to similar extents in both groups of mice after PIC and LPS administration (Fig. 3B). In DTP vaccine-treated mice, the benzphetamine monooxygenase activity was decreased by 30% in R mice, but the NR mice had a slight increase in activity. In contrast, ethylmorphine activity was decreased only in NR mice after DTP vaccine administration. Activity levels remained un-

![FIG. 1. Hexobarbital-induced sleep time index is shown for R and NR mice treated with DT vaccine (DT), DTP vaccine (DTP), pertussis vaccine adsorbed (PV), cholera vaccine (CHV), PIC, E. coli LPS (EC LPS), or B. pertussis LPS (BP LPS). Sleep times were measured 24 h after administration of agents. Control sleep times for each group were 33 ± 3 min. Each bar represents the mean sleep time for four animals per group divided by the mean sleep time for controls. Error bars are the pooled standard error for each treatment. Significant differences from control: *, P < 0.05; **, P < 0.005; *** P < 0.001.](http://iai.asm.org/)

![FIG. 2. Duration of hexobarbital-induced sleep time following administration of DTP vaccine or LPS. The sleep time index is shown for R (○, ○) and NR (□, □) mice after DTP vaccine (solid line) or LPS (dashed line) administration. Each point represents the mean results for four animals per group divided by the mean results for controls. If no error bar is shown, the standard error was smaller than the symbol on the graph. Results for one representative experiment of the four performed are shown in the graph. Sleep time of control animals was 30 ± 3 min. Significant differences from control: *, P < 0.05; **, P < 0.005; *** P < 0.001.](http://iai.asm.org/)
changed or slightly increased following all other treatments for both strains of mice (Fig. 3C).

Cytosolic enzyme activities were also measured 24 h after vaccine, PIC, or endotoxin administration. Consistent with our data with C57BL/6 mice (3), no changes in glutathione transferase or quinone reductase activity were evident at this time point (data not shown).

**Cytokine levels after administration of PIC, vaccine, or endotoxin.** Cytokines were measured in plasma 1 to 24 h after administration of vaccine, LPS, or PIC. These results are shown in Fig. 4 and 5 for TNF and IL-6, respectively. Both cytokines show increased levels in the plasma, which are transient. Each cytokine had returned to control values by 8 h; thus, the graphs extend only to 8 h. Basal levels of TNF following injection of saline are about 10 pg/ml, and those of IL-6 are about 10 to 20 U/ml. TNF was increased in R mice to more than 6,000 pg/ml 1.5 h following a single injection of *B. pertussis* LPS (Fig. 4). This was more than sixfold higher than the level of TNF measured after DTP vaccine administration (Fig. 4). In the NR mice, this dose of LPS induced TNF to levels of 500 pg/ml similar to results with both DTP and cholera vaccines in this mouse strain. PIC administration induced TNF to 500 pg/ml in both strains of mice. In contrast to the sharp peak of TNF after LPS administration, TNF levels were elevated over a period of 4 h in R mice after DTP vaccine administration. There was also a broader peak of TNF in both strains of mice after administration of PIC.

IL-6 levels in R mice were increased following administration of LPS, DTP and cholera vaccines, and PIC but not DT vaccine. The increases seen after administration of LPS and DTP vaccine were more similar in peak shape and magnitude than those of TNF (Fig. 5). The peak IL-6 level was measured at 4 h with LPS at 12,000 U/ml, while for DTP vaccine the peak was at 3 h and the level was 7,500 U/ml. In contrast, PIC induced IL-6 to 500 U/ml and cholera vaccine

**FIG. 3.** Microsomal enzyme activities 24 h following administration of DTP vaccine, PIC, or LPS. Each activity is depicted as the percentage of that of the control. This represents the mean result for the experimental group divided by the mean result for the control group. Each group contained three samples, and the experiment was performed three times. Pooled standard errors for each group are shown. Significant differences: *, P < 0.05; **, P < 0.01; +, P < 0.005; ++, P < 0.001. (A) Benzphetamine demethylase activity in R and NR mice. The mean control activity was 31.0 ± 0.4 for NR mice and 30.5 ± 2.2 for R mice. The activity is expressed in nanomoles per minute per milligram of microsomal protein. (B) Benzopyrene monooxygenase activity 24 h after administration of vaccine, PIC, or LPS. The mean control activity was 7.23 ± 0.34 for NR mice and 7.49 ± 0.14 for R mice. Activity was expressed as nanomoles per hour per milligram of protein. (C) Ethylmorphine demethylase activity in R and NR mice. The activity was expressed as micrograms of formaldehyde formed per hour per milligram of microsomal protein. Control activities were 14.7 ± 0.67 for NR mice and 19.2 ± 1.4 for R mice.

**FIG. 4.** TNF levels in plasma of mice after administration of *B. pertussis* LPS, vaccine, or PIC. TNF concentration was calculated by using a standard recombinant murine TNF. Results for one representative experiment of four are shown.
Effects of PXN on hepatotoxicity of DTP vaccine or LPS. DTP vaccine (0.5 ml) or B. pertussis LPS (50 μg) was mixed and incubated with 50 μg of polymyxin B sulfate (PXN) for 24 h before administration to mice. The LAL test-measurable endotoxic activity was reduced from 125,000 to 125 EU/ml after incubation of LPS with PXN. DTP vaccine was reduced from a rabbit endotoxic activity of 300,000 EU/ml to 19,200 EU/ml after mixing with PXN (a 16-fold reduction). These LAL tests were done with 1:100 dilutions of material because this dilution was necessary for the rabbit pyrogen test. Rabbit pyrogen testing showed a consistent decrease in pyrogenicity as a result of premixing the vaccine with PXN. The mean temperature increase for three rabbits was 1.4°C for a 1:100 dilution of the DTP vaccine and 0.7°C for the same dilution of the premixed PXN and DTP vaccine.

TNF and IL-6 were measured in R and NR mice 2 and 4 h after administration of LPS or DTP vaccine alone or after premixing with PXN as described above. These results are shown in Fig. 6 and 7 for TNF and IL-6, respectively. There is a twofold decrease in IL-6 units when the PXN-premixed DTP vaccine is administered to both NR and R mice. In contrast, PXN treatment of LPS reduced the increase in IL-6 by 5-fold in NR mice at 4 h and by more than 80-fold in R mice at 4 h.

Histologic examination of livers and spleens after administration of vaccine or LPS. Livers and spleens from R and NR mice treated with either DTP vaccine or LPS were examined for pathologic changes. Tissues were examined at days 1, 3, 5, 7, 10, and 14 postinoculation. The livers and spleens of the LPS-treated mice were indistinguishable from those of saline-injected controls at each time point. This was true for both R and NR mice. Administration of DTP vaccine resulted in a 1.5-fold increase in the liver weight and a 3-fold increase in the spleen weight at day 7 postinoculation in both R and NR mice. These two strains of mice exhibited similar
histopathologic changes of the liver and spleen following vaccine administration. By day 7, the livers of DTP-inoculated mice were characterized as having moderate to severe random multifocal inflammation, with associated hepatocyte necrosis. The inflammation was primarily neutrophilic, with fewer macrophages and lymphocytes (Fig. 8a and b). The capsular surface of the liver had multiple granulomas (Fig. 9). There were milder hepatic lesions beginning on day 3. Lesions began to resolve between days 10 and 14. Both R and NR mice treated with DTP vaccine had splenic hypertrophy of up to three times the normal spleen size. These affected spleens also had subcapsular aggregates of neutrophils and less severe capsular inflammation than that which appeared on the liver capsule (Fig. 10).

**DISCUSSION**

The studies reported in this article were undertaken to assess the contribution of endotoxin to adverse reactions in mice immunized with DTP vaccine. Previous studies in our laboratory (2–4) have demonstrated that DTP vaccine alters hepatic drug-metabolizing enzymes in C57BL/6 mice. In this study, R and NR mice were used to further examine these observations. The rationale for this approach was that if the
alterations seen after administration of DTP vaccine were caused by endotoxin alone, then the hepatic changes observed would be similar to those caused by LPS in R and NR mice. If, on the other hand, the alterations were due to components other than LPS, then R and NR mice would show more similar responses to vaccine than to LPS.

As these studies demonstrate, endotoxin cannot fully reproduce the hepatic changes that follow DTP vaccine administration in mice. When LPS was administered to both strains of mice, there was a marked difference in hexobarbital-induced sleep times. LPS administration produced a maximum increase in hexobarbital-induced sleep time at 24 h to 2.4-fold above that of controls in R mice and an increase to 1.5-fold above that of controls in NR mice. By day 7

FIG. 9. Liver capsule showing granulomatous inflammation on the capsule. These foci have central cores of necrosis surrounded by a layer of neutrophils and an outer zone of histiocytes and fibroplasia. Magnification, ×10.

FIG. 10. Spleen from a DTP vaccine-inoculated mouse. Chronic inflammation on the capsular surface and subcapsular foci of neutrophilic infiltration are shown.
postinjection, the sleep times had returned to control levels. In contrast, DTP vaccine administration produced a 1.6- to 1.8-fold increase in sleep time in both R and NR mice at 24 h, which persisted at the same level for at least 7 days. Our previous studies with C57BL/6 mice had shown an increase in sleep time to 1.2- to 1.3-fold above that of controls at 24 h, which gradually increased to 2-fold at 7 to 10 days postinjection (3). Another strain of mice, B6F1, is completely refractory to DTP vaccine administration; its response to endotoxin is normal (unpublished observations). These strain differences are unexplained at this time.

Spectral cytochrome P-450 levels and microsomal enzyme activities also demonstrated differences in responses of R and NR mice to LPS and DTP vaccine administration. DTP vaccine administration reduced cytochrome P-450 levels at 24 h 30 to 40% in both strains of mice, whereas LPS (E. coli or B. pertussis) caused a significant reduction in P-450 levels only in R mice (Table 1). The observed effects of LPS administration are consistent with other reports of alterations in hepatic drug metabolism in rodents (8, 12, 15, 28, 35, 36). However, this is the first report describing alterations in hexobarbital-induced sleep time, cytochrome P-450 levels, and microsomal enzyme activities by LPS and vaccine administration in R and NR mice.

To further understand the differences between results with LPS and DTP vaccine, PIC was administered to R and NR mice. PIC is thought to inhibit drug metabolism by induction of alpha/beta interferon in response to its administration (33, 40). It has been well documented that administration of PIC decreases hepatic drug-metabolizing enzymes in several strains of mice. When hexobarbital-induced sleep time measurements and cytochrome P-450 levels were examined, PIC administration produced effects similar to those of vaccine administration. Sleep times were elevated 1.6- to 1.8-fold above controls for both strains of mice, while P-450 levels were decreased in a manner similar to those seen after DTP vaccine administration. This suggests that these two strains of mice have similar responses to other active compounds even though they differ dramatically in their responses to endotoxin.

Studies with PXN were designed so that neutralization of the endotoxin present in the DTP vaccine would allow for a straightforward evaluation of the other components in the vaccine. Unfortunately, as described in the text, while PXN completely neutralized soluble LPS, a significant amount of detectable LPS remained in the DTP vaccine. This is consistent with earlier studies in which unadsorbed pertussis vaccine mixed with PXN failed to show a reduction in endotoxic activity at doses of PXN comparable to those used in this study (6). Although the PXN treatment should reduce the endotoxic activity, the failure to completely eliminate measurable endotoxin complicates the interpretation of the sleep time data. It is not possible to rule out the possibility that residual LPS contributes to the failure of DTP vaccine premixed with PXN to reduce sleep time to control levels, as seen when LPS is premixed with PXN. Hexobarbital-induced sleep times were measured for mice injected with DT vaccine premixed with LPS to simulate bound endotoxin. This failed to cause increases in sleep time that persisted for longer than 24 h (as seen with LPS alone). Other whole-cell vaccines, such as cholera vaccine, only increased sleep time for 24 h, while fluid pertussis vaccine caused increased sleep times similar to that caused by DTP vaccine (reference 3 and unpublished observations).

Histopathologic examination supported the thesis that endotoxin alone does not account for the alteration in hepatic function caused by DTP vaccine. Purified LPS caused no lesions in either R or NR mice. Additionally, these mouse strains had lesions similar in severity and type of inflammation at 7 days following vaccine administration. Both R and NR DTP vaccine-inoculated mice had extensive multiple foci of inflammation in the liver, which consisted of mainly neutrophils accompanied by hepatic necrosis. The extent of the lesions was severe enough to result in the loss of the ability to metabolize, thus resulting in increased sleep times. The severity of lesions at day 7 postinoculation coincides with our finding that sleep times remained elevated in DTP vaccine-inoculated mice at 7 days. The livers from mice at days 10 and 14 after DTP vaccine inoculation showed gradual resolution of lesions. Again, this coincides with the return of normal sleep times.

The observed differences in the responses of R and NR mice following LPS and vaccine administration strongly suggest that LPS is not the sole component responsible for the hepatic alterations to the DTP vaccine. NR mice differ from R mice at a single gene locus on chromosome 4 (28). The most recent evidence suggests that the LPS locus is composed of genes which encode cellular components involved in signal transduction (28). It is also possible that R and NR mice have normal levels of LPS-binding proteins and CD14 receptors (16, 18, 24). Thus, if one bypasses the receptor for LPS and introduces a toxic substance that exerts its effects later in the pathway, the results with R and NR mice would be expected to be similar (36). This is apparently the case when DTP vaccine and PIC are administered. In vitro, similar results have been obtained by using phorbol myristate acetate in macrophages derived from R and NR mice. This protein kinase C activator induces similar changes in these strains of mice (34).

TNF, IL-1, and IL-6 levels are known to be increased following administration of endotoxin in animals (25) and following sepsis in humans (9). TNF and IL-6 levels were measured to determine the role these cytokines might play in toxicity. In contrast to the hexobarbital-induced sleep time results, LPS, DTP vaccine, and cholera vaccine caused marked increases in TNF and IL-6 levels in R mice. NR mice showed only a small increase in these cytokines in response to these agents. DT vaccine administration did not increase TNF or IL-6 levels in either mouse strain, and PIC induced small increases in both cytokines in both strains of mice. The similarity in the inductions of TNF and IL-6 by LPS and DTP vaccine in R mice suggests a role for endotoxin in the early response of these mice to vaccine administration. The shape of the TNF curve following DTP vaccine administration was unexpected. It may be that the adjuvant prolongs stimulation of TNF release. This effect was not seen with cholera vaccine or the other agents tested.

Neutralization of the endotoxin by PXN reduced the elevated levels of cytokines in the plasma of mice treated with LPS as well as in the plasma of LPS-treated mice. The cytokine levels were not reduced as much when PXN was premixed with DTP vaccine as when PXN was premixed with LPS. Again, this could be due to incomplete neutralization of the endotoxin in the vaccine. However, the finding of reduced levels of cytokines in the plasma of mice treated with PXN premixed with DTP vaccine further supports a role for endotoxin in the early stage of vaccine-induced alteration of hepatic drug metabolism.

Further studies are in progress to identify the components of the DTP vaccine which contribute to the alterations in hepatic drug metabolism described in this article as well as to identify ways to more effectively neutralize the endotoxin.
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