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MINIREVIEW

Evaluation of the Mouse Model for Study of Encephalopathy in Pertussis Vaccine Recipients

ERIK L. HEWLETT[1]* AND JAMES L. COWELL[2]

Departments of Medicine and Pharmacology, University of Virginia School of Medicine, Charlottesville, Virginia 22908,[1] and Bacteriology Research Department, Praxis Biologics, Inc., Rochester, New York 14623[2]

INTRODUCTION

From the onset of widespread administration of killed, whole-cell pertussis vaccine, there have been anecdotal case reports of adverse reactions associated with immunization (2, 3, 8, 10, 12). Fever and local reactions occur in >40% of the children receiving pertussis vaccine, and this rate is significantly higher than in recipients of diphtheria-tetanus toxoids (DT) (6). Furthermore, other whole-cell vaccines composed of gram-negative bacteria, such as cholera and typhoid vaccines, elicit similar reactions (9). Of greater concern are the neurologic events which have been reported to be temporally associated with pertussis vaccine administration, including collapse, convulsions, and encephalopathy with or without permanent sequelae (2, 3, 6, 8, 10, 12). The study by Cody et al. (6) in which the nature and rates of such reactions after DT or DT-pertussis vaccine (DTP) injections were compared did not have a sufficient sample size to provide an answer regarding the relationship between these neurologic events and the pertussis component of the DTP vaccine. Similarly, data provided by prospective case control evaluation of encephalopathies in Great Britain determined that the association between pertussis vaccine administration and encephalopathy is very rare but did not establish a cause and effect relationship between the two (14, 20, 21). Nevertheless, the existence of a temporal relationship even at the anecdotal level has resulted in interest, concern, and speculation about the mechanisms by which neurological reactions could possibly be produced by pertussis vaccine (4, 5, 13, 16, 21, 27, 28). The rarity of such events associated with pertussis vaccine administration in humans and the lack of an animal model have hampered progress in this area.

Several components of the pertussis organism, principally endotoxin and pertussis toxin (PT), have been considered as candidates for involvement should such reactions be caused by the vaccine. The majority of speculation has focused on PT, also known as histamine-sensitizing factor, lymphocytosis-promoting factor, islet-activating protein, and pertussigen. As the names indicate, this protein exotoxin possesses multiple biological effects when administrated to experimental animals (26). It has been designated a neurotoxin based upon its ability to alter the function of neuronal cells in culture (11, 17), but there is no evidence that it has such direct effects in animals or humans (25).

It was in this context that Steinman et al. (22) described a reaction in mice receiving bovine serum albumin (BSA) and killed *Bordetella pertussis* organisms which appeared to involve the central nervous system. The response was interpreted as representing an encephalopathy and was proposed as a model for vaccine-associated events in DTP recipients. Recently, three independent laboratories have studied the sequence of events involved in the induction of this lethal, "shock-like state" (23) and provided data indicating that it represents an acute type I hypersensitivity reaction with secondary, preterminal central nervous system manifestations (15, 18, 29). In this review, the characteristics of this animal system will be described and the conclusions of the investigators examined.

PROTOCOL FOR INDUCTION OF A LETHAL, SHOCK-LIKE CONDITION IN MICE

During studies of experimental allergic encephalomyelitis with *B. pertussis* organisms as adjuvant, Steinman et al. (22) noted serendipitously that control mice, which had received BSA and killed *B. pertussis* organisms, became ill and died after a final injection of BSA. The protocol for injecting the mice consisted of BSA (1 mg) given intraperitoneally on days 1 and 3 and killed *B. pertussis* (3×10^{10} organisms) given intravenously on days 2 and 4. The lethal, shock-like state was then induced by an injection of BSA (1 mg) intraperitoneally on day 8. The symptoms developing within 2 h included lethargy, tachypnea, seizures (myoclonic jerks of trunk and extremities), and death. Subsequently, other investigators (18) have demonstrated that an equivalent response can be elicited by administering the BSA intravenously and the *B. pertussis* organisms intraperitoneally.

MOUSE STRAINS AND BACTERIAL COMPONENTS

In the initial description of the lethal, shock-like syndrome, Steinman et al. (22) suggested that susceptibility of different mouse strains was determined by the histocompatibility complex *H-2* genotype. Several inbred mouse strains from one colony were evaluated, and only those with an $H\text{-}2^d$ genotype experienced the lethal effect. This may have been due in part to the fact that $H\text{-}2^d$ strains are high responders to BSA (19). When BALB/c mice ($H\text{-}2^d$) from another colony were tested, however, they were not susceptible, suggesting that genes outside the *H-2* complex might also be involved (22).

Subsequent studies of this phenomenon by Munoz et al. (15) and Redhead et al. (18) have provided additional data. Munoz et al. (15) demonstrated that some non-$H\text{-}2^d$ strains which did not respond at day 8 did experience the lethal reaction when the final antigenic challenge was delayed until

* Corresponding author.

day 23. Redhead et al. (18) found that expression of the lethal syndrome was mouse strain dependent but not necessarily *H-2* type restricted. Thus, the cumulative data suggest a relationship between the antibody response to the sensitizing antigen and a sensitivity to the lethal reaction, rather than a strict association with *H-2* haplotype. This is illustrated by a passive transfer experiment in which resistant mice which had received the standard BSA-*B. pertussis* regimen could be made susceptible by intravenous injection of high-titer, anti-BSA serum immediately prior to the final injection of BSA on day 8 (23). These mice not only developed the lethal, shock-like syndrome but also were shown to have microscopic brain pathology, including hemorrhages (23).

By the use of a transposon-induced mutant of *B. pertussis* impaired in its ability to produce biologically active PT, it was determined that PT is a necessary component for the development of the syndrome (23, 24). As a consequence, studies of this phenomenon have been carried out in several laboratories with 100- to 400-ng doses of purified PT per mouse in place of killed *B. pertussis* organisms (15, 23, 24, 29). Redhead et al. (18) reported, however, that endotoxin-free PT was not adequate but that PT plus purified lipopolysaccharide (LPS) from *B. pertussis* or *E. coli* was effective in eliciting the response. They found that when 10 μg of *B. pertussis* LPS was injected with BSA on days 1 and 3 and 0.2 μg of PT was injected on days 2 and 4, the survival of sensitive mice was only 10% after BSA injection on day 8 (18). The LPS could also be given with the PT. Although the minimum amount of LPS required was not determined (18), it appears that contamination of either the BSA or the PT with LPS could account for the other reports of PT alone replacing whole *B. pertussis* organisms in inducing the syndrome (15, 23, 24, 29). The data of Redhead et al. (18) indicate that both PT and LPS are required for induction of the appropriate class and amount of antibody of the sensitizing antigen. In addition, Wiedmeier et al. (29) demonstrated that PT potentiation is required to elicit the pathological process which occurs after challenge with the sensitizing antigen on day 8.

SPECIFICITY OF SENSITIZING ANTIGEN

In the studies by Steinman et al. (22–24), it appeared that a combination of antibody against BSA and exogenously administered BSA as a final challenge were uniquely required to induce the lethal syndromes. Neither ovalbumin, DT, nor myelin basic protein could replace BSA as the sensitizing antigen in BALB/c mice. Using the same protocol and mouse strain, however, Redhead et al. (18) found that BSA was most effective but that bovine thyroglobulin and DT were also able to induce the syndrome in conjunction with PT and endotoxin. These investigators pointed out that quantitative or qualitative differences in the induced antibodies might explain the differences in results. The amount and class of antibody are probably influenced by the absolute quantities and the ratio of PT and LPS administrated in the form of whole organisms or as separate purified components. For example, when *B. pertussis* organisms are used for induction of the syndrome there appears to be strict genetic control, in contrast to only a relative genetic restriction when PT is used (15, 24). Also, although BSA is the most effective sensitizer with *B. pertussis* organisms, this is not true with purified PT. Munoz et al. (15) demonstrated that egg albumin was as effective in DBA/2 ($H\text{-}2^d$) mice but not in BALB/c and several other strains of mice. Munoz et al. (15) also found that when the challenge dose of antigen was delayed until day 21, both BSA and egg albumin were effective in all strains tested. This suggests again that the genetics of the mouse response is related to the regulation of foreign antigen recognition.

BRAIN HISTOPATHOLOGY

In attempting to determine whether the central nervous system involvement in this syndrome is secondary or primary, brain histopathology is an important consideration. Using the injection protocol with BSA and killed *B. pertussis* organisms, both Steinman et al. (22) and Redhead et al. (18) reported that brains of treated, susceptible (BALB/c) mice had diffuse vascular congestion with some focal hemorrhages. Redhead et al. (18) noted similar pathology without the hemorrhages in treated, syndrome-resistant NIH mice, whereas Steinman et al. (22) reported no brain pathology in syndrome-resistant BALB/c mice. Interestingly, in the study carried out by Munoz et al. (15) with BSA and purified PT in BALB/c mice, there was no brain pathology observed, suggesting the possibility that the LPS or other components of the whole organism might be contributing to the histopathology independent of effects on the lethal shock-like syndrome. In other studies using PT rather that *B. pertussis* organisms, brains were not examined for histopathologic lesions (23, 24, 29).

PATHOPHYSIOLOGIC MECHANISM OF THE LETHAL, SHOCK-LIKE SYNDROME

As the cumulative data on this phenomenon are reviewed, it is important to recall that two different sensitizing protocols have been used. The first, as originally described by Steinman et al. (22), used whole *B. pertussis* organisms in conjunction with BSA. In light of the data subsequently presented by Steinman et al. (23, 24) implicating PT as the critical component of the pertussis organism, subsequent studies were carried out using purified PT (15, 29) or PT plus LPS (18). In the characterization of the lethal, shock-like state that occurred, Steinman et al. (22) described it as encephalopathy but recognized that the kinetics of the reaction were suggestive of anaphylaxis. The presence of brain pathology, usually not seen in anaphylaxis in most animal species, seemed to justify the use of the term encephalopathy. The additional studies with this protocol, however, have provided data which do not support that conclusion (15, 18, 29).

The data collected with purified PT in the protocol support the concept that the subject animals die from an anaphylactic reaction to the final dose of challenge protein. They include observations that (i) sensitivity to the final BSA challenge can be passively transferred by immune serum from animals sensitized with BSA and PT, but only if the recipient has also received a dose of PT (29); (ii) administration of serotonin antagonists (29) or adrenalin and betamethasone (18) to sensitized animals prior to BSA challenge can protect against the lethal reaction; and (iii) physical manifestations and death of the sensitized animals can be prevented by intravenous administration of saline following BSA challenge (15). Although the details of the interactions among PT, LPS, and the sensitizing antigen in promoting the antibody response necessary for this reaction are not understood, the cumulative data indicate that the protocol induces an acute anaphylactic reaction and does not cause an encephalopathy.

SUMMARY AND CONCLUSIONS

With one exception (1), the data from other investigations (15, 18, 29) confirm the observations by Steinman et al. (22–24) that a protocol consisting of injections of BSA and *B. pertussis* organisms followed by BSA alone can elicit a consistent and reproducible lethal syndrome in mice. An equivalent sequence of events can be produced when PT and LPS are used in place of the *B. pertussis* organisms (18). There are two phases of the reaction which have been identified: (i) an initial period during which PT, LPS, and the sensitizing antigen are required to induce the appropriate class and quantity of antibody; and (ii) a separate phase during which PT sensitizes the animal prior to the final antigenic challenge (29). Investigators other than Steinman et al. were able to produce the syndrome with antigens other than BSA (15, 18). In light of all the data available, it is clear that the lethal, shock-like event being studied is an acute, type I hypersensitivity reaction which can be ameliorated or prevented by appropriate therapeutic intervention.

Since there is no consistent clinical syndrome of neurologic events temporally associated with pertussis vaccine administration and no histopathologic pattern has been demonstrated (5, 7, 21), it is difficult, if not impossible, to validate any response in animals as a model for the hypothetical phenomenon. It is certainly reasonable to continue to study the details and mechanisms of the sensitization occurring in mice treated by the protocols described by Steinman et al. (22–24). In fact, consideration of whether such an acute hypersensitivity reaction could occur in vaccine recipients will be important. It is not appropriate at present, however, for this phenomenon to be considered a "model for pertussis vaccine encephalopathy" or for this protocol to be used to test the "encephalopathic potential" of pertussis vaccines (23).

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