

METHOD FOR THE TREATMENT OF PERTUSSIS WITH AMINO GUANIDINE

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BACKGROUND OF THE INVENTION

The present invention relates to a method for the treatment of pertussis.

Despite the availability of a highly effective vaccine, whooping cough (pertussis) remains a global problem and a persistent clinical challenge. Fears about vaccine safety have spawned recent epidemics in industrialized nations like Japan, Great Britain, and Sweden; even in the U.S., with vaccine acceptance approaching 95%, new estimates of the annual number of pertussis cases are as high as 125,000 (Sutter et al., *JAMA* 26-7, 386-391 (1992)). Much of pertussis research has centered on the development of an acellular vaccine with a lower side effect rate, but it is unlikely that the pertussis burden in well-vaccinated countries will decrease. Infants and children hospitalized with pertussis still must endure frequent violent coughing episodes that continue for weeks after antibiotics have eliminated the bacteria. Complications range from the encephalopathy (presumably from anoxia) to secondary pneumonia, the latter being the most frequent cause of pertussis-related mortality [Olson, *Medicine* 54, 427-469 (1975)]. Currently, there is no therapy to relieve the debilitating symptoms of pertussis, shorten its duration, or reduce the frequency of sequelae.

The causative agent of pertussis is *Bordetella pertussis* (and, less frequently, *B. parapertussis*), which specifically colonizes and then destroys the ciliated cells lining the large airways [Mallory et al., *J. Med. Res.* 27, 115-123 (1912)]. The consequences of this cytopathology are severe, since ciliary activity is normally the sole means of transporting mucus out of the respiratory tract. As mucus, multiplying bacteria, and inflammatory debris accumulate, coughing becomes the only remaining means of airway clearance.

Of the various toxins and virulence-related factors produced by *B. pertussis*, only one has been demonstrated to reproduce the specific respiratory tract cytopathology of the pertussis syndrome. That molecule is tracheal cytotoxin (TCT), a low molecular weight glycopeptide released by *B. pertussis* during normal growth.

It is known that the destruction of ciliated cells can be duplicated by TCT (Goldman et al., *Infect. Immun.* 36, 782-794 (1982)), and this toxin has been subsequently purified [Cookson et al., *Infect. Immun.* 57, 2223-2229 (1989)] and chemically characterized [Cookson et al., *Biochemistry* 28, 1744-1749 (1989)]. TCT is enzymatically processed from *B. pertussis* cell wall peptidoglycan and accumulates at micromolar levels in the culture supernatant during log-phase growth. It is a 921 dalton disaccharide-tetrapeptide and is illustrated in the accompanying FIG. 1.

BRIEF DESCRIPTION OF THE INVENTION

In accordance with the present invention, a method is provided for the treatment of pertussis. The method preferably comprises treating a mammalian host susceptible to pertussis with aminoguanidine in a small but

effective amount for inhibiting the toxic effects of TCT released by *Bordetella pertussis*.

The peptidoglycan-derived structure of TCT identifies it as a member of the "muramyl peptide" family. Muramyl peptides are responsible for a wide variety of biological activities, including adjuvanticity, pyrogenicity, and somnogenicity. There are many similarities between the biological activities of muramyl peptides and those of the inflammatory mediator interleukin-1 (IL-1) [Dinarello and Krueger, *FASEB Journal* 45, 2545-2548 (1986)]. IL-1 may also be the central factor linking the pathology of pertussis to muramyl peptide effects, including immunopotentiality, fever, and sleep (Nixon et al., *Abst. Gen. Meet. Am. Soc. Microbiol.*, p. 61, abst. no. B-216 (1991)). In these other activities, the responses to muramyl peptides have been correlated to the production of IL-1 as an intercellular mediator; in contrast, as shown herein, muramyl peptides may also act directly on their target cells through induction of intracellular IL-1 activity. This mechanism represents a unique enlistment by *B. pertussis* of a natural host cytokine to trigger cell-specific pathophysiology.

One mechanism by which cytokines can cause macrophage-mediated destruction of target cells is through the production of nitric oxide (NO) [Hibbs et al., *Science* 235, 473-476 (1987)]. NO is a free radical derived from the guanidino nitrogen atom of L-arginine through the action of nitric oxide synthase (NOS). NO complexes with iron in heme-containing proteins and in enzymes containing iron-sulfur centers; while this inhibits the activity of most such enzymes, one target, soluble guanylyl cyclase, is activated to produce high levels of CGMP. The rate limiting enzyme in DNA synthesis, ribonucleotide reductase, is another non-heme iron-containing enzyme that is a target of NO. In addition NO can react with superoxide anion to form peroxynitrite, which decays to form highly reactive hydroxyl radical.

Cytokines have been shown to activate an inducible isoform of NOS that is distinct from the constitutive NOS responsible for effects on vascular tone and neurotransmission [Moncada et al., *Pharmacol. Rev.* 43, 109-142 (1991)]. This inducible NOS is implicated herein as a key element in TCT-triggered pathology. In accordance with the present invention, aminoguanidine, a selective inhibitor of inducible NOS (Corbett et al., *Diabetes* 41, 552-556 (1992)), is able to interfere with TCT (or IL-1) toxicity for respiratory epithelial cells. All of the results herein point to NO-mediated damage for destruction of ciliated cells and for halting proliferation of HTE cells (perhaps through the inhibition of ribonucleotide reductase). Treatment with aminoguanidine greatly reduces the toxic effects of TCT.

It will be appreciated by the person skilled in the art that other selective inhibitors of inducible NOS can similarly be used in the method of the invention in place of aminoguanidine.

DETAILED DESCRIPTION OF THE INVENTION

While the specification concludes with claims particularly pointing out and distinctly claiming the subject matter regarded as forming the present invention, it is believed that the invention will be better understood from the following detailed description of preferred embodiments taken in conjunction with the accompanying drawings in which: