7. BIOLOGICAL AGENTS

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Pulmonary immunotoxicity from microbial exposure is increasing in many aspects of modern society and presents new challenges to health professionals. The ability of microorganisms to cause disease through non-infectious mechanisms is well known and is the focus of this Chapter. The ability of microorganisms to cause pulmonary disease through infectious mechanisms is also well known, but will not be extensively reviewed in this treatise.

The pulmonary immune system is exquisitely designed to maintain a sterile oxygen exchange system. The interior surface of the lung represents the largest surface area of the body that is directly exposed to the external environment. The respiratory system uses ciliated cells, mucosal cells, mucus, antibodies, and alveolar macrophages in an attempt to protect this tremendous surface area (Quie, 1986). Despite this intricate system, inhaled microbial agents are occasionally able to circumvent the host's defenses and cause illness through infectious processes. Microbial agents are also able to cause illness through non-infectious means such as immune hypersensitivity reactions.

Microbial deposition in the lung may have different health outcomes based upon the type of microbe. For example, infectious agents, such as rhinoviruses that cause the common cold, are able to survive and replicate in susceptible hosts despite the body's attempt to prevent infection. Other agents are rapidly inactivated with no apparent effect on health. In simplistic terms, microbes are classified as pathogens, opportunists or non-pathogens (Salyers and Whitt, 1997). Generally, this classification is based upon the ability of a microbe to cause disease, not via an immunologic mechanism, but through an infectious process.

Microbial pathogens are able to circumvent the host's normal pulmonary defenses, multiply, and cause disease. Illness associated with inhaled microbes is generally attributed to pathogenic microbes such as *Mycobacterium tuberculosis*, the causative agent of tuberculosis. Opportunistic pathogens are microbes that are unable to cause disease in healthy immunocompetent people, but are able to cause disease in people with impaired immune defenses. Microbial opportunists may be

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able to multiply in a host, but they are only able to cause disease in immunocompromised hosts or when they are present in numbers sufficient to overwhelm normal defenses. An example of an opportunistic pathogen is *Pseudomonas aeruginosa* which is the predominant cause of mortality in persons suffering from burns. *P. aeruginosa* also causes significant morbidity and mortality in cystic fibrosis patients. In both cases, the immunocompromised host is unable to kill this ubiquitous microbe that multiplies and eventually causes disease. Sometimes, as in the case of the pathogen *M. tuberculosis*, disease is a result of damage induced by the body's own host defense mechanisms. Opportunists are able to replicate in the host and with assistance, evade the host's defenses. The difference between opportunists and non-pathogens was shown in a study where an opportunistic strain of *Pseudomonas* (*P. aeruginosa* AC869) survived at least 14 d in mice challenged via intranasal inoculation, while a larger dose of a non-pathogenic pseudomonad (*B. cepacia* AC1100) resulted in a shorter colonization period (only 7 d) (George et al., 1991). Non-pathogens are not able to cause infectious disease through replication or infection. Most microbes fall into this category. However, pathogens, opportunists and non-pathogens all may cause illness through adverse immunologic reactions.

Biologic agents have been associated with various immunologic diseases. Inhalation of thermophilic actinomycetes (Reijula, 1993; Nakagawa-Yoshida et al., 1997) has been associated with Farmer's Lung (Mundt et al., 1996). Similar lung diseases resulting in hypersensitivity pneumonitis are caused by inhalation of bacteria-associated products, such as lipopolysaccharide (LPS) (Burrel and Ye, 1990; Clapp et al., 1993; Zhiping et al., 1996). Incidence of asthma has also been associated with inhalation of microbes (Michel et al., 1992). Inhalation of certain fungi by sensitive individuals has also been associated with an illness termed sick building syndrome (Rautiala et al., 1996).

Man has always been exposed to low levels of aerosolized bacteria. Microbes are present whenever the wind entrains dirt particles from the soil (Kwaasi et al., 1998). For reasons primarily associated with ecologic concerns, technologies such as biodegradation, biologic pesticides and genetically recombinant organisms are presenting added opportunities for microbial challenges of the pulmonary immune system. Grain elevators, indoor air conditioning, sprayed biopesticides, composting and recycling are some of the technologies leading to increased pulmonary exposure to microbes. Microbes have been recovered down wind of various types of treatment facilities, including wastewater treatment (Goff et al., 1973; Hickey and Reist, 1975; Fannin et al., 1976; Teltsch and Katzenelson, 1978), and composting (Brown et al., 1995). Aerosolized microbes are occasionally found in significant numbers in buildings with poorly maintained air conditioning systems. Legionnaire's disease was first recognized following pulmonary exposure of a large number of veterans in just such an environment (Weisse, 1992). Other examples of human exposure from newer technologies include contaminated cutting oils (Smith, 1970; Kreiss and Cox-Ganser, 1997; Robins et al., 1997), bioreactors of many designs (Feng et al., 1997; Cesario et al., 1998; Zhukov et al., 1998), microbial pesticides sprayed on crops, ultrasonic humidifiers (Alvarez-Fernandez et al., 1998), ice nucleation sprays used in enhancing snowfall or preventing frost damage to crops (Wolber, 1993), and bioremediation microbes sprayed on chemical spills (Brubaker and Exner, 1988).

A good example of increasing human exposure to microbes is the growing use of biologic pesticides (biopesticides). Biopesticides are microbial agents that have adverse effects on specific products such as bacteria, fungi, insects or plants. Biopesticides are an important addition to the world's anti-pest arsenal because they are generally much less toxic than the currently available chemical pesticides. Examples include insecticidal agents such as *Bacillus thuringiensis* var. *israelensis* (Bti), *B. thuringiensis* var. *kurstaki* (Btk), *B. cereus, Beauveria bassiana, Metarrhizium anisopliae, Paecilomyces* sp., and nuclear polyhedrosis viruses. Examples of fungicidal agents include *Trichoderma harzianum, Burkholderia cepacia, Pseudomonas fluorescens,* and *P. antimicrobica* (Table 1). More biopesticides, biofertilizers, and bioremediation microbes are discovered and developed each year.

Immunologic reactions in the lung may be classified as primary, secondary or tertiary responses (Bellanti, 1971). Primary immunologic responses involve immediate inflammatory reactions without extensive cellular processing and antigen recognition. Inflammation may consist of cellular and/or mucosal or lymphatic infiltration to the affected site. Microbes that cause physical damage may produce this type of response. For example, pulmonary instillation of large numbers of *B. thuringiensis* var. *kurstaki* (Btk) spores in mice causes mortality in less than 24 hr (Sherwood, unpublished observations). This response is not a result of an infectious process as Btk spores are incapable of replication in a mammalian host. Lung weights of dosed mice are significantly increased compared to controls indicating the presence of a significant inflammatory response. The crystal polypeptides produced by most strains of *B. thuringiensis* may cause the pulmonary inflammation seen in animal studies. Purified 28K crystal polypeptide and delta endotoxin of *B. thuringiensis* var. *israelensis* have been shown to be hemolytic and toxic in mice and rats (Armstrong et al., 1985; Mayes et al., 1989). Similar effects have been observed following intranasal instillation of more than 1 x 10^7 colony forming units (cfu) of *P. aeruginosa* in mice (George et al., 1991). In the case of *P. aeruginosa*, it is felt that the LPS of the Gram-negative *Pseudomonas* causes an overwhelming inflammatory response resulting in rapid mortality (less than 24 hr).

Studies performed in our laboratories with various strains of Bt have indicated that toxicity is strain dependent. Mice challenged once intranasally with vegetative or spore preparations of Btk, a crystal-minus Btk mutant, *B. cereus*, or *B. subtilis* had differing responses (Table 2). Both vegetative and spore preparations of *B. cereus* and crystal-minus Btk killed mice. However, heat inactivation of the preparations inactivated toxic factors in *B. cereus*, but not those of crystal-minus Btk. Vegetative, but not spore, preparations of Btk killed mice; no effects were caused by vegetative or spore preparations of *B. subtilis*. These data suggest that specific factors in microbes may result in adverse effects and that these factors are not always possessed by all members of the genus or species or indeed by all strains within the species.

Other examples of the specific reactions caused by various inhaled microbes are shown in studies performed by Onofrio and colleagues (1981, 1983). Studies performed with *Staphylococcus aureus* demonstrated that the host response changed as the pulmonary dose increased. Low numbers of bacteria were rapidly cleared without neutrophil influx while higher numbers took longer to clear and initiated a significant influx of neutrophils (Onofrio et al., 1983). Additional studies were done

TABLE 1. MICROBES USED IN ENVIRONMENTAL APPLICATIONS

Microbe	Use	Reference
B. thuringiensis *var.* kurstaki	Insecticide	Damgaard et al., 1996
Bacillus cereus	Fungicide	Silo Suh et al., 1994
Bacillus thuringiensis var. *israelensis*	Insecticide	Armstrong et al., 1985
Beauveria bassiana	Insecticide	Wraight et al., 1998
Burkholderia cepacia	Bioremediation, Fungicide	Govan et al., 1996
Colletotrichum gloeosporioides	Herbicide	Rikkerink et al., 1994
Comamonas testosteroni	Bioremediation	Bae et al., 1996
Entomophaga grylli	Insecticide	Bidochka et al., 1996
Metarrhizium anisopliae	Insecticide	Burgner et al., 1998; De Garcia et al., 1997
Nuclear polyhedrosis viruses	Insecticides	Castro et al., 1997; Andrews et al., 1980; McClintock et al., 1991
Paecilomyces sp.	Insecticide	Wraight et al., 1998
Pediococcus pentosaceus	Hay preservative	Duchaine et al., 1996
Pseudomonas syringae	Ice-nucleation	Goodnow et al., 1990
Pseudomonas antimicrobica	Fungicide	Walker et al., 1996
Pseudomonas fluorescens	Fungicide	Laville et al., 1998
Pseudomonas putida	Bioremediation	Ronchel et al., 1995; Guerin and Boud, 1995
Rhizobia	Bioremediation	Damaj and Ahmad, 1996
Rhizobium meliloti	Fertilizer	Dammann et al., 1996
Trichoderma harzianum	Fungicide	Grondona et al., 1997; Flores et al., 1997
White rot fungi	Bioremediation	Barr and Aust, 1994

TABLE 2. EFFECT OF SINGLE INTRANASAL CHALLENGE WITH VARIOUS BACILLUS FORMULATIONS ON MORTALITY OF FEMALE CD-1 MICE

Strain	% Spore	CFU Administered Per Mouse (Log10)	Dead/Total	MTD[a]
B. thuringiensis var. *kurstaki* HD73	0	5.96	1/10	-
		6.96	7/10	1
		7.96	9/10	2
		(7.96)[b]	0/10	-
	100	5.97	0/10	-
		6.97	0/10	-
		7.97	0/10	-
		(7.97)	0/10	-
B. thuringiensis var. *kurstaki* HD31 (crystal -)	0	6.36	7/10	1
		7.36	10/10	1
		8.36	10/10	0
		(8.36)	1/10	1
	100	5.61	0/10	-
		6.61	2/10	1
		7.61	8/10	1
		(7.61)	4/10	2
B. cereus 14579	0	4.98	0/10	-
		5.98	1/10	1
		6.98	10/10	1
		7.98	10/10	0
		(7.98)	0/10	-
	100	3.88	0/9	-
		4.88	0/10	-
		5.88	6/10	1
		6.88	9/10	1
		(6.88)	0/10	-
B. subtilis 6051	0	6.40	0/10	-
		7.40	0/10	-
		8.40	0/10	-
		(8.40)	0/10	-
	100	6.49	0/10	-
		7.49	0/10	-
		8.49	0/10	-
		(8.49)	0/10	-

[a]MTD = mean time to death (days)
[b]( ) – number of heat-killed bacteria

with *Streptococcus sanguis, S. salivarius* and *Neisseria catarrhalis,* bacteria that are commonly found in the human pharynx. These studies showed that *S. sanguis* was cleared more easily from the lung than *S. salivarius*, with *N. catarrhalis* taking the longest. Further, the predominant pulmonary cell responsible for bacterial clearance was the alveolar macrophage for *S. sanguis*, while *S. salivarius* and *N. catarrhalis* caused a significant influx of neutrophils (Onofrio et al., 1981).

Microbial opportunists vary in their ability to colonize or cause disease. Colonization capacity is not common to all members of a genus and indeed varies between strains. Mice challenged via intranasal instillation with strains of *P. aeruginosa* (AC869) and *B. cepacia* (AC1100) had varying pulmonary clearance rates (George et al., 1991). Clearance of microbes from the lung is dependent upon the host response and the microbe. Strains of *B. thuringiensis* act as particulates and are cleared in a biphasic manner by the pulmonary defense system. Cells are first phagocytized and then removed via the lymphatics or via the mucociliary escalator to the stomach. While most non-pathogenic microbes are killed in less than 7 d, *B. thuringiensis* strains often remain viable in the lung for at least 30 d and sometimes over 3 mo (Sherwood, unpublished observations; Siegel et al., 1987). Other microbes, such as *S. aureus*, cause virtually no inflammation at low doses and are easily cleared by the alveolar macrophage, which is the basic pulmonary cellular defense mechanism (Goldstein et al., 1974). Higher initial pulmonary doses overwhelm the ability of the alveolar macrophage to respond and cause a polymorphonuclear (PMN) cellular infiltration (Onofrio et al., 1983). Still other microbes, such as *Streptococcus zooepidemicus*, are rapidly cleared from the lung upon initial challenge, but small numbers escape detection and create foci of infection. If the host immune response is unable to combat these foci that become filled with PMN cells and fluid, the infection escapes to the bloodstream and the animals succumb to septicemic infection (Sherwood et al., 1981, 1988). In yet another mechanism of evading the host defenses, *P. aeruginosa* strains produce a polysaccharide glycocalyx that inhibits phagocytosis. The cellular rhamnolipids of *P. aeruginosa* have also been shown to inhibit phagocytosis (McClure and Schiller, 1996).

Secondary immune responses result in stimulation of humoral and cell-mediated cells and production of antibodies and activated T-lymphocytes. This, of course, is the body's normal response to foreign materials and our bodies regularly respond to inhaled biologic materials with no adverse response. Only the rare exposure that results in illness is recognized.

Tertiary immune responses may be produced whenever antigen excess drives the secondary immune response to hypersensitivity. Four types of immunologically -mediated hypersensitivity disease occur: Types I, II, III, and IV. Three of the these types (i.e., Types I, III and IV) have been associated with microbial exposure.

Type I disease, also known as immediate hypersensitivity, is caused by antigenic elicitation of IgE antibody in humans. IgE binds to basophils causing release of preformed mediators such as histamine. Based upon the location and severity of the response, the immediate hypersensitivity disease is classified as allergic rhinitis, asthma, urticaria, or angioedema (Bellanti, 1971). Several types of microbes have been implicated in causation of type I hypersensitivity disease, including several types of fungi (*Fusarium vasinfectum*, *Aspergillus fumigatus*, *Cladosporium* sp, and *Altemaria* sp.) and LPS associated with Gram-negative bacteria (Table 3).

TABLE 3. MICROORGANISMS IMPLICATED IN CAUSATION OF PULMONARY TYPE I HYPERSENSITIVITY DISEASE

Microbe	Disease	Reference
Aspergillus fumigatus	Asthma (Allergic bronchopulmonary aspergillosis)	Zhaoming and Lockey, 1996; Greenberger, 1997; Chauhan et al., 1997
Fusarium vasinfectum	Asthma	Saini et al., 1998
Gram-negative bacteria	Organic dust toxic syndrome	Zhiping et al., 1996
Gram-negative bacteria	Asthma	Michel et al., 1992
Molds (*Cladosporium, Alternaria, Aspergillus*)	Asthma	Cross, 1997

Pulmonary diseases associated with microorganisms and type III hypersensitivity include farmer's lung, malt worker's lung, wheat weevil disease (Bellanti, 1971), machine operator's lung (Bernstein et al., 1995), organic dust toxic syndrome (Zhiping et al., 1996). New diseases are also included such as composter's lung (Brown et al., 1995), humidifier lung, air-conditioner disease (Ando and Suga, 1997), and sump bay fever (Anderson et al., 1996). Adult respiratory distress syndrome (ARDS) may be triggered by pulmonary challenge with significant amounts of biologic products such as LPS (Simpson and Casey, 1989; Burrell and Ye, 1990). Exposure to microbes associated with cutting oils has also been associated with illness (Bernstein et al., 1995; Kriebel et al., 1997). Similar illnesses have been reported in personnel working in waste sorting (Poulson et al., 1995a and b). Type III hypersensitivity disease is characterized by production of antibody-antigen complexes and subsequent localization of those complexes. The complexes bind complement causing vascular permeability with resultant edema and influx of neutrophils. Release of cellular enzymes from the activated cells causes tissue damage (Bellanti, 1971). A wide variety of microbes have been implicated in causation of this disease including Gram-positive bacteria (*Bacillus pumilus*, *Staphylococcus capitis*, *Rhodococcus*, and *Pediococcus*), Gram-negative bacteria (*Pseudomonas fluorescens*), actinomycetes (*Thermoactinomyces*), and fungi (*Penicillium, Aspergillus*, and *Saccharopolyspora*) (Table 4).

Type IV hypersensitivity caused by microbes is classically associated with species of *Mycobacteria* and specifically *M. tuberculosis*. Because the manifestation of type IV hypersensitivity generally occurs 24 - 48 hr after induction, type IV hypersensitivity is also known as delayed-type or cellular hypersensitivity. This immune disease is characterized by activation of T-lymphocytes that recognize specific proteins or protein-hapten conjugates. After antigen recognition, many T-lymphocyte factors are released such as transfer factor, macrophage activating factor, skin reaction factors, chemotactic factors, mitogenic factors, lymphotoxin, and inter-

TABLE 4. MICROORGANISMS IMPLICATED IN CAUSATION OF PULMONARY TYPE III HYPERSENSITIVITY DISEASE

Microbe	Disease	Reference
Aspergillus fumigatus	Hypersensitivity pneumonitis	Hinojosa et al., 1996; Madan et al., 1997
Aspergillus niger	Machine operator's lung	Bernstein et al., 1995
Aspergillus spp.	Hypersensitivity pneumonitis	Moreno-Ancillo et al., 1997
Bacillus pumilus	Machine operator's lung	Bernstein et al., 1995
Epicoccum nigrum	Hypersensitivity pneumonitis	Hogan et al., 1996
Micropolyspora faeni	Hypersensitivity pneumonitis	Hinojosa et al., 1996
Pediococcus pentosaceus	Farmer's lung	Duchaine et al., 1996
Penicillium brevicompactum	Farmer's lung	Nakagawa-Yoshida et al., 1997
Penicillium olivicolor	Farmer's lung	Nakagawa-Yoshida et al., 1997
Pseudomonas fluorescens	Machine operator's lung	Bernstein et al., 1995
Rhodococcus sp.	Machine operator's lung	Bernstein et al., 1995
Rhodotorula spp.	Hypersensitivity pneumonitis	Alvarez-Fernandez et al., 1998
Saccharopolyspora rectivirgula	Farmer's lung	Mundt et al., 1996; Gudmundsson et al., 1998; Reijula, 1993
Staphylococcus capitis	Machine operator's lung	Bernstein et al., 1995
Thermoactinomyces vulgaris	Farmer's lung	Reijula, 1993
Thermoactinomyces vulgaris	Hypersensitivity pneumonitis	Hinojosa et al., 1996

feron. Release of these factors results in an intense cellular infiltrate consisting of activated mononuclear cells whose byproducts cause swelling, pain and tissue destruction. Although *M. tuberculosis* has typically been implicated in causation of tuberculosis, many species of *Mycobacteria* are now recognized as causative agents of tuberculosis, particularly in immunocompromised individuals. Examples include, *M. avium* complex, *M. kansasii*, *M. chelonei*, and *M. flavescens* (Table 5).

TABLE 5. MICROORGANISMS IMPLICATED IN CAUSATION OF PULMONARY TYPE IV HYPERSENSITIVITY

Microbe	Disease	Reference
Mycobacterium avium complex	Tuberculosis	Martinez Moragon et al., 1996
Mycobacterium chelonei	Tuberculosis	Martinez Moragon et al., 1996
Mycobacterium flavescens	Tuberculosis	Martinez Moragon et al., 1996
Mycobacterium kansasii	Tuberculosis	Martinez Moragon et al., 1996
Mycobacterium tuberculosis	Tuberculosis	Dannenberg, 1989

Mycobacteria cause disease almost completely through host immunologic reactions. *M. tuberculosis* and related microbes evade host inactivation because of their unique ability to evade the macrophage phagolysosome. They then replicate within the cytoplasm of the host macrophage. In order to prevent the spread of infection, the host forms thick granulomatous structures called tubercles that wall up the microbes and prevent spread. At the same time the host develops a strong cellular immune response to the microbe. A complex lipopolysaccharide called wax D is the mycobacterial component contained by these bacteria that is often implicated in activation of cellular immunity (Hamamoto et al., 1981). Tubercles occasionally rupture, thus exposing cellular antigens to the host's highly active immune system. The subsequent inflammatory response causes significant tissue destruction (Dannenberg, 1989). Release of tumor necrosis factor from interferon-activated macrophages is one factor that has been associated with tissue destruction in tuberculosis (Rook et al., 1987). Although this disease is not currently of great concern in most developed countries, it still is the leading cause of mortality from infectious disease in the world with over 2 - 3 million deaths per year (WHO, 1992; Murray and Lopez, 1997).

Virtually all microbes are nonpathogenic. However, exposure to nonpathogenic microbes may not be as innocuous as sometimes thought. In a study performed in our laboratories we found that daily low level pulmonary challenge ($\approx 1 \times 10^3$ viable bacteria) with nonpathogenic microbes (once per day for 5 d) enhanced susceptibility to concurrent infection with the pathogenic microbe, *Streptococcus zooepidemicus* (Tables 6 and 7). Daily pulmonary challenge with higher levels of nonpathogenic microbes ($\approx 1 \times 10^6$ viable bacteria) was protective to concurrent *Streptococcus* infection even when challenged with a higher infectious dose (68% mortality vs. 15% in controls) (Table 8).

TABLE 6. EFFECT OF INTRANASAL CHALLENGE OF MICROBES ON *STREPTOCOCCUS* MORTALITY IN CD-1 MICE

Group[a]	Daily Dose[b]	# of Mice	Percent Mortality	MST[c]	MTD[d]
Saline	0	20	15	13.3	10.0
Vegetative *B. thuringiensis* var. *kurstaki* HD31	1.60×10^3	19	32	11.5	7.0*
	1.60×10^6	20	0	14.0	-
B. thuringiensis var. *kurstaki* HD31 Spores	2.31×10^3	20	75*	7.9*	6.9*
	2.33×10^6	5	0	14.0	-

[a]Mice challenged i.n. daily on Days –2 to 2 relative to infectious challenge on Day 0 with *Streptococcus*
[b]Viable colony forming units per animal
[c]Mean survival time (days)
[d]Mean time to death (days)
*p<0.05 vs saline control; chi-square (% mortality) and Dunnett's test (MST and MTD)

TABLE 7. EFFECT OF INTRANASAL CHALLENGE OF MICROBES ON *STREPTOCOCCUS* MORTALITY IN CD-1 MICE

Group[a]	Daily Dose[b]	# of Mice	Percent Mortality	MST[c]	MTD[d]
Saline	0	21	5	14.0	14.0
Pseudomonas aeruginosa AC869	1.40×10^2	17	41*	12.1	9.7
	1.40×10^5	16	56*	7.3*	3.0*
Burkholderia cepacia AC1100	2.23×10^3	20	55*	10.2	8.0*
	2.21×10^6	20	0	14.0	-

[a]Mice challenged i.n. daily on Days –2 to 2 relative to infectious challenge on Day 0 with *Streptococcus*
[b]Viable colony forming units per animal
[c]Mean survival time (days)
[d]Mean time to death (days)
*p<0.05 vs. saline control; chi-square (% mortality) and Dunnett's test (MST and MTD)

TABLE 8. EFFECT OF INTRANASAL CHALLENGE OF MICROBES ON *STREPTOCOCCUS* MORTALITY IN CD-1 MICE

Group[a]	Daily Dose[b]	# of Mice	Percent Mortality	MST[c]	MTD[d]
Saline	**0**	**19**	**68**	**9.4**	**8.0**
Vegetative *B. thuringiensis* var. *kurstaki* HD31	**1.40×10^2**	**18**	**44**	**10.5**	**7.0**
	1.40×10^5	**16**	**0 ***	**14.0***	**-***
***Burkholderia cepacia* AC1100**	**2.23×10^3**	**20**	**55**	**9.4**	**6.5**
	2.21×10^6	**20**	**0***	**14.0***	**-***

[a]Mice challenged i.n. daily on Days –2 to 2 relative to infectious challenge on Day 0 with *Streptococcus*
[b]Viable colony forming units per animal
[c]Mean survival time (days)
[d]Mean time to death (days)
***p<0.05 vs. saline control; chi-square (% mortality) and Dunnett's test (MST and MTD)**

Daily pulmonary exposure to low levels of nonpathogenic microbes caused no change in numbers or types of cells isolated by bronchopulmonary lavage (data not shown). However, daily exposure to higher levels of nonpathogenic microbes caused significant increases in numbers of neutrophils. Similar effects have been reported with *Hartmanella vermiformis* enhancing disease caused by *Legionella pneumophila* (Brieland et al., 1996). This effect does not appear to be consistent for all infectious microbes or even for all routes of exposure as daily intraperitoneal challenge with nonpathogenic microbes had no effect on concurrent intraperitoneal infectious challenge with *Listeria monocytogenes* (Table 9). These data suggest that repetitive pulmonary challenge with levels of microbes that fail to elicit an immune response may distract the normal functioning of the pulmonary immune system and render the host more susceptible to infectious disease. Thus, the increasing use of microbes may have a detrimental effect on health if the low-level effect on the immune response is not considered.

Pulmonary exposure to microbes can cause disease through infectious and immunologic mechanisms. Modern technology is creating many new opportunities for significant human pulmonary exposure to microbes and microbial products. Because of the possible adverse consequences in some people, efforts need to be increased to better understand immunologic disease mechanisms and methods for alleviating immunologic disease caused by inadvertent exposure to microbes or microbial products.

TABLE 9. EFFECT OF INTRAPERITONEAL CHALLENGE OF MICROBES ON *LISTERIA* MORTALITY IN $B_6C_3F_1$ MICE

Group[a]	Daily Dose[b]	# of Mice	Percent Mortality	MST[c]	MTD[d]
Saline	**0**	**20**	**10**	**9.6**	**7.0**
Vegetative *Bacillus thuringiensis* var. *kurstaki* HD31	**1.60×10^3**	**20**	**0**	**10.0**	**-**
	1.60×10^6	**20**	**10**	**9.6**	**6.5**
***Bacillus thuringiensis* var. *kurstaki* HD31 spores**	**2.31×10^3**	**20**	**5**	**9.8**	**7.0**
	2.33×10^6	**20**	**75***	**5.4***	**4.9***
***Pseudomonas aeruginosa* AC869**	**1.40×10^2**	**20**	**0**	**10.0**	**-**
	1.40×10^5	**20**	**10**	**9.7**	**7.5**
***Burkholderia cepacia* AC1100**	**2.23×10^3**	**20**	**0**	**10.0**	**-**
	2.21×10^6	**20**	**0**	**10.0**	**-**
***Beauveria bassiana* 48023**	**2.75×10^3**	**20**	**0**	**10.0**	**-**
	2.75×10^3	**20**	**0**	**10.0**	**-**

[a]Mice challenged i.n. daily on Days –2 to 2 relative to infectious challenge on Day 0 with *Listeria*
[b]Viable colony forming units per animal
[c]Mean survival time (days)
[d]Mean time to death (days)
***p<0.05 vs. saline control; chi-square (% mortality) and Dunnett's test (MST and MTD)**

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