VIRAL–BACTERIAL SYNERGISTIC INTERACTION IN RESPIRATORY DISEASE

Lorne A. Babiuk, M. J. P. Lawman, and H. Bielefeldt Ohmann

Western College of Veterinary Medicine
Department of Veterinary Microbiology
and Veterinary Infectious Disease Organization
University of Saskatchewan
Saskatoon, Saskatchewan, Canada S7N 0W0

I. INTRODUCTION

Humans and animals are constantly being inoculated with various microorganisms resident in the upper respiratory tract and by inhaled aerosols, yet pneumonia is a relatively rare event. This implies the existence of very efficient defense mechanisms which are capable of eliminating the vast majority of microorganisms before they colonize and multiply to sufficient levels to result in clinical disease. In order to overcome this continuous barrage of microorganisms, there is a complex array of defense mechanisms present in the upper and lower respiratory tract capable of clearing these organisms. However, in individuals suffering from a variety of diseases, including virus infections, colonization occurs rapidly with subsequent development of pneumonia. Thus, it is estimated that 90% of bacterial pneumonias

develop after a viral infection. Furthermore, individuals suffering from a viral pneumonia have a 40% chance of developing bacterial pneumonia (Jakab, 1982). The reasons for the increased colonization of the lung by bacteria following virus infections has been shown to be related to the surface properties of epithelial cells lining the respiratory tract, the physiological environment of the respiratory tract, as well as the alteration of the specific and nonspecific defense mechanisms of the lung which occurs as a result of virus infection. These conditions allow greater numbers of bacteria to adhere to the surface of cells, possibly to replicate at a faster rate and overcome some of the nonspecific defense mechanisms present in the respiratory tract. In the present review, we will attempt to indicate how viruses enhance bacterial colonization and adherence to respiratory epithelial tissue, how viruses alter the specific and nonspecific defense mechanisms of the respiratory tract, which allow the newly adhered bacteria to replicate and further overcome some of these compromised defense mechanisms. In addition, we will discuss how the host defense mechanisms, in attempting to clear the viruses or bacteria, can themselves lead to immunopathology and further increase the replicative rate of the bacteria and aid in the development of pneumonia. Finally, we will discuss potential ways of reducing viral bacterial respiratory infections by immunization or the use of various chemotherapeutic and immunomodulators presently being developed. Although examples will be used from a variety of different human and animal respiratory infections, the major emphasis will be on attempting to provide a unifying hypothesis of how viruses modify the environmental conditions of the lung and alter host defense mechanisms to allow bacteria to replicate faster and produce pneumonia. Examples will be used to demonstrate specific events, but it should not be construed that every event occurs in every situation.

II. Virus Infections

During the past 15–20 years, considerable advances have been made in understanding the events which occur at the molecular level during initiation of respiratory disease (Fields, 1985). The sequence of events in respiratory infections, whether they are caused by rhinoviruses, orthomyxoviruses, paramyxoviruses, or adenoviruses, or coronaviruses, etc., is rather similar in that in order to initiate infection and produce illness the viruses must enter their host and come in contact with susceptible tissues and cells. This initial infection generally occurs in one of two ways: direct contact with droplets or secretions

containing virus or with aerosolized virus. The small aerosolized virus particles can enter various sections of the respiratory tract, including the lower respiratory tract. However, in general, the majority of the initial infections occur in the upper respiratory tract. Immediately upon contact with the respiratory epithelium the virus can encounter a variety of nonspecific defense mechanisms including mucus and nonspecific compounds inhibiting virus attachment to the host cell epithelium. If a specific interaction between the virus and host cell receptors occurs, the virus initiates its replication cycle and generally induces lytic infection of the respiratory epithelium. Since most viruses which infect the upper respiratory tract cause local infections and cytopathology, the incubation time is generally short.

One of the initial defense mechanisms in the respiratory tract is mucus, which may contain either glycoproteins similar to the receptor molecules on respiratory epithelial cells, or, indeed, secreted receptors. These glycoproteins may interfere with the initial attachment of the viruses to epithelial cells. Some viruses possess specific enzymes (neuraminidase) which may destroy some of these mucous glycoproteins to allow attachment and infection of epithelial cells. Furthermore, some proteases present within the mucus may result in cleavage of specific virus glycoproteins which are required for infectivity (Choppin and Scheid, 1980).

Once infection of the epithelial cells of the respiratory tract occurs, virus replication ensues with the production of a large number of progeny viruses, which can either bud from the plasma membrane of the epithelial cell or, in the case of naked viruses, result in lysis of the virus-infected cells. Upon release of virus from infected cells, they can spread throughout the respiratory tract either by cell-to-cell spread (slow), aerosolization into the lower tract during breathing, or spread in the mucus due to cilial action. As a result of this spread, virus infection of the sinuses, bronchi, lower trachea, and lung occurs (Chanock et al., 1963; Parrott et al., 1959). The result may be rapid vascular reactions and accumulation of transudates and exudates, cell debris, and inflammatory cells. As a result of cellular degeneration, environmental conditions are established which are conducive to bacterial cell attachment and growth (see Section III). In some cases, even if death of virus-infected cells does not occur, some of the host epithelial cells "luxury functions" may be reduced. These may include secretion of various glycoproteins, mucus, or bactericidal factors.

As a consequence of virus infection, interferon (IFN) is produced and released into the extracellular environment of the respiratory tract as well as systemically (Green et al., 1982; Babiuk et al., 1985). This family of antiviral molecules can limit the rate of virus replication and

modulate the host's defenses to both the virus and superinfecting bacteria (Babiuk *et al.*, 1985). In addition to the release of IFN, infected cells also release other viral components which can be detrimental to epithelial and leukocyte functions (see Sections V and VI).

III. BACTERIAL ADHERENCE

The binding or attachment of bacteria to various substrates within their environment is an important prerequisite in the process of colonization. Attachment of bacteria to the surface of cells is mediated through adhesins present on the bacterial cell wall. These adhesin proteins bind to specific glycolipid or glycoprotein receptors expressed on the surface of cells (Normark *et al.*, 1986). Not only is this attachment of bacteria to the mucosal surface an important initial step in colinization, it also forms the basis of infection and production of disease by pathogenic bacteria (Costerton *et al.*, 1981; Beachey, 1981; Sparling, 1983). The adherence of bacteria to the mucosal surface is a complex phenomenon involving virulence factors (including adhesins) of the pathogenic bacteria, as well as by the mucosal surface itself (local immunity, epithelial cell turnover, and the mucous lining or layer) (Babiuk, 1984; Reid and Sobel, 1986). The expression of these adhesin proteins can be modulated by the environmental conditions of the lung and are subject to both phase and antigenic variation. A single bacterial cell can give rise to progeny cells that express adhesin proteins that are different in structure, number, serology, and function depending on the environment. This variation in expression of adhesins is thought to be an important factor in the ability of the bacteria to adapt to its microenvironment and to evade the primary host defense mechanisms (Haas *et al.*, 1986). Although the type of adhesins varies with each bacteria, fimbrae, pilus, and lipoteichoic acid have been shown to be primarily involved in mediating adhesion (Ofek, 1984). Other factors have also been shown to be important adhesins, for example, the filamentous hemagglutinin and toxin of *Bordetella pertussis* (Twomanen and Weiss, 1985). In fact, these two adhesins have been reported to work in concert during the process of colonization (Twomanen and Weiss, 1985). The agglutinogens of *B. pertussis* have also been shown, *in vitro,* to be capable of acting as adhesins (Redhead, 1985).

The ability of bacteria to produce more than one adhesin-type protein is an added advantage for the bacteria during initial infection, since it is at this time that it is crucial for the bacteria to be able to become established and colonize (Ofek, 1984). As stated above, the

regulation of these factors is influenced by the phase variation and growth cycle of the bacteria. The microenvironment of the lung will therefore add selective pressure in determining whether or not the bacteria expresses one or more of the adhesins (Ofek, 1984). If virus infection favors bacterial growth and phase variation, the probability is high that colonization and subsequent pneumonia will ensue.

Structural variation, or phenotype, of bacteria also plays a role in bacterial adherence. Capsule production affects not only the hydrophobicity of the organism but alters its ability to interact with epithelial and phagocytic cells (Whitnack et al., 1981; Beachey, 1981; Ofek et al., 1982; Ofek, 1984). It has been suggested that the production of hyaluronic capsules interferes with adhesion. Thus, variation in phenotype, may be very important in pathogenesis in that noncapsulated bacteria can initiate the infection, as a result of their adherence capabilities, and, once established, they can become capsulated and thereby have a better chance of survival by preventing attachment to phagocytic cells (Ofek, 1984; Babiuk, 1984). Capsules composed of polysaccharide also have the ability to interfere with adhesion (Craven et al., 1980; Selinger and Reed, 1979; Glorioso et al., 1982). Ofek (1984) points out that the functional ability of bacteria to adhere to host cells is not always beneficial to the bacteria; that is, attachment of bacteria to the mucosal epithelium is essential for the initial survival of the pathogen, whereas attachment of bacteria to phagocytic cell does not, in most cases, benefit the bacteria but increases the survival of the host. The ability of the bacteria to change the expression of type and number of adhesin proteins and the production of substances capable of interfering with attachment may be of fundamental importance not only in the establishment of colonization and subsequent infection, but also as a means of circumventing the host's defense mechanisms.

Alteration of adherence of bacteria may also come about by extrinsic factors such as the presence or absence of divalent cations. It has been shown that zinc levels are altered during bacterial infections and that zinc reduces the surface charges so that adhesion is enhanced (Sugarman et al., 1982). Viral infections appear to influence adherence and colonization of bacteria (Davison and Sandford, 1981; Fainstein et al., 1980; Sanford et al., 1978; Nugent and Pesanti, 1982). The mechanisms by which viruses alter bacterial adherence are many and include alteration of host cell surface membrane receptors and alteration of the microenvironment in which bacterial attachment occurs (Babiuk, 1984). Viral infections alter the levels of fibronectin production. Since colonization by bacteria has been correlated with low fibronectin levels (Hynes and Bye, 1974; Woods, 1987), this may be a very

important factor in bacterial colonization. Cell death caused by viruses also increases the release of proteases, which may be responsible for degrading fibronectin on the surface of innocent-bystander cells, thus favoring adherence. Bacteria themselves produce proteases which would be capable of further decreasing fibronectin levels and increasing colonization (Woods *et al.,* 1981; Yamada and Weston, 1974). Many medically important bacterial pathogens produce IgA proteases (Miluzzo and Delisle, 1984; Mulks and Plaut, 1978). Furthermore, many of these proteases are dependent on divalent cations for their activity (Labib *et al.,* 1978). If during a viral infection extensive hemorrhage and tissue damage occurs, an increase in levels of divalent cations will result. One other ion that has been shown to be very important in bacterial growth and pathogenesis is iron (Hatch *et al.,* 1981; Miles and Khimji, 1975). Certain bacteria produce iron chelators or iron-binding proteins capable of trapping the iron required for their growth. Furthermore, in some cases iron does increase the levels of adhesins or pili formation on bacteria (Babiuk, 1984). If a virus infects the respiratory tract and induces extensive cell damage and hemorrhages, this would increase the levels of iron and other divalent cations, which would favor both bacterial adhesion and growth. Furthermore, the amount of iron bound to transferrin has been shown to be critical in immune regulation (Matzner *et al.,* 1979). Thus, the level of iron can affect not only bacterial adhesion and growth, but also the host's ability to respond to the infection.

Viral infections have been implicated in alteration of epithelial cell secretion (Pijoan *et al.,* 1980). Some of these secretions have bactericidal activity. Therefore, their absence allows bacteria to gain a hold and initiate colonization. During infection of the upper respiratory tract, microcolonies are formed which can enter the lower respiratory tract either by aspiration (Johanson *et al.,* 1979) or by reduced mucociliary clearance (Jakab, 1982). Large microcolonies are difficult to phagocytize and may in fact cause phagocytic cells to discharge their enzymes; these contain granules which cause surrounding tissue damage (Slauson, 1982), thereby creating a better environment for bacterial replication and further adhesion. In addition, these bacteria can release cytotoxins which have an adverse effect on phagocytic cells (see Section VII), induce more cell death, and further improve the environment for bacterial growth.

IV. PULMONARY DEFENSE MECHANISMS

Defense of the normal lung against various microorganisms is mediated by a plethora of inflammatory and immune effector cells acting

in concert with various soluble factors. In the majority of cases, more than 90% of the cells obtained by bronchoalveolar lavage are macrophages and less than 1% are neutrophils (Hunninghake *et al.*, 1985). Based on these findings, it has often been considered that macrophages are the major cell type involved in maintaining sterility in the normal lung. However, it must be emphasized that part of the defenses begin in the upper respiratory tract where the initial interaction of the host and pathogen occurs, and the outcome of these interactions will determine whether the organism will ever enter the lung. The first defenses include anatomical barriers, mucociliary clearance mechanisms, reflex mechanisms, local secretory IgA, iron-containing proteins such as transferrin, and surfactant—even before alveolar macrophages become involved. These early surveillance mechanisms do not depend on the immunological status of the host, with the exception of immunoglobulin. When some of these mechanisms either individually or in concert fail to eliminate the microorganisms, then other more specific defense mechanisms must come into play to clear the organism. These include both humoral and cellular immune responses, as well as inflammatory responses best characterized by the influx of polymorphonuclear granulocytes (PMN; see Table I). In this review we will not discuss in detail all of the individual defense mechanisms that are functioning in the respiratory tract to prevent infection, but we will summarize the important defense mechanisms which can be altered as a result of virus infection and thereby increase the opportunity for bacteria to colonize the lower respiratory tract and cause pneumonia.

The advent of the bronchioalveolar lavage procedure has rapidly expanded our knowledge during the past 10 years of understanding the various secretory and cellular components of the lung. In normal individuals, a variety of secretory products are present at various levels in the respiratory tract. In many cases, these secretions contain complement components, transferrin, surfactant, fibronectin, and immunoglobulins. Mostly, these various humoral factors act in concert with specific immunoglobulin and cells to help destroy or reduce the replication of viruses and bacteria. Fibronectin, transferrin, and surfactant are three substances which, in addition to being able to potentiate nonimmune opsonic activity in host defense mechanisms, also are important in altering the rate of adherence and bacterial replication. For example, fibronectin or fragments thereof have been shown to be functionally important in enhancing macrophage–monocyte phagocytosis of particles, not only as a conventional opsonin, but also by stimulating the macrophages to ingest opsonized particles (Czop *et al.*, 1982; Pommier *et al.*, 1983). Transferrin has been demonstrated to have bacteriostatic effects on some bacteria which are strongly depen-

TABLE I

SOME IMPORTANT COMPONENTS OF LUNG DEFENSE

Defense component	Function
Surveillance mechanisms	
Ciliated epithelium	Removal of foreign pathogens; reduces
Mechanical barriers	probability of infection
Mucus	
Epithelium	
Humoral	
Surfactant	Alter surface charges; reducing macrophage surface tension; facilitate killing of bacteria by macrophages; direct antibacterial activity
Fibronectin	Enhance phagocytosis by macrophage–monocytes; alter bacterial attachment
Transferrin	Iron binding
Lysozyme	Bacterial activity
Immunoglobulin	Prevent adherence; opsonization
Cellular	
Macrophage/monocyte	Phagocytosis, killing of bacteria; release of chemotactic factors; release of soluble mediator (fibronectin, complement); antigen presentation
Lymphocytes	
T, B, NK, etc.	Cellular and humoral responses
Granulocytes	
Neutrophils, eosinophils, basophils	Phagocytosis and killing of bacteria; immunopathology, superoxide free radicals

dent on the presence of iron for their growth (Rankin and Reynolds, 1985). Although many studies have been conducted *in vitro,* very little information is available regarding the role of transferrin in lower respiratory tract infections and their effects there. Also lacking is information on whether the synergistic interaction between transferrin and secretory IgA has an effect similar to that of lactoferrin in breast milk (Stephens *et al.,* 1980). The action of surfactant in reducing alveolar surface tension has long been appreciated. Moreover, some evidence suggests that surfactant can also facilitate the killing of bacteria by alveolar macrophages (laforce, 1976). In addition to enhancing alveolar macrophage killing of bacteria, surfactant has also been shown to have antibacterial activity against gram-positive bacteria (Conrod and Yoneda, 1983). Thus, surfactant appears to be able to reduce bacterial growth by two mechanisms: by direct killing and by activation of cells with antibacterial activity.

In summary, the respiratory tract is endowed with many different components, all playing a crucial role in clearing various foreign organisms from the respiratory tract (Table I). In some cases, one factor is more important than another, both in relationship to the kinetics of the infection as well as to the specific organism involved. However, in many cases, these specific factors act in concert to eliminate the organism quickly, or if indeed the organism is not eliminated they may play an important role in immunopathology and enhancement of replication of the bacteria. Evidence for the interplay between these various factors is forthcoming from studies wherein a deficiency in one single component may predispose individuals to recurrent respiratory tract infections. More specifically, following virus infections not all of the specific and nonspecific defenses are compromised, yet superinfection with bacteria frequently occurs. The probability of bacterial superinfection will depend on the capacity of the lung to eliminate bacteria, which is determined by a large number of humoral or cellular factors (Table I), on the dose of virus or bacteria and the virulence of the organisms, and on the adequacy of the specific and nonspecific host defense mechanisms. In the following section, we will discuss how viruses alter various aspects of the host's defense mechanisms, which may then lead to a reduced threshold level of susceptibility to the bacteria.

V. VIRAL EFFECTS ON NONSPECIFIC HOST DEFENSE MECHANISMS

As stated above, pulmonary virus infections predispose animals and humans to secondary bacterial pneumonia (Jakab, 1981, 1982a; Loosli, 1973; Barber et al., 1985), and it is assumed that this is, at least in part, a result of virus-induced impairment of alveolar macrophage (AM) functions (Jakab, 1982a). In the lungs, distal to the ciliated airway epithelium (i.e., bronchioles and alveoli), the resident AM and monocytes, migrating into the alveolar interstitium and lumen from the blood, offer the first line of defense against invading microorganisms, partly by production and secretion of antimicrobial factors such as IFN (Bielefeldt Ohmann et al., 1984), reactive oxygen species (ROS), and enzymes, and, partly, by direct cytotoxic effector mechanisms, including phagocytosis and degradation of the microbial agents. In addition, the AM may have a proinflammatory function by producing factors that form part of or can activate other parts of the defense system, thereby inhibiting microbial spread or leading to pathological lesions (see below), or both (Slauson, 1982).

In experimental animal models using various respiratory viruses, it

has been demonstrated that the severity and duration of illness depends on the amount of virus reaching the lower respiratory tract (Yates *et al.*, 1983a; Jakab, 1982b). In the acute stages of the infection the epithelial cells of the airways appear to be the principal sites of viral replication (Yates, 1982; Jakab, 1982b), leading to direct destruction of the epithelium or at least obstruction of the "luxury functions" of cells (Oldstone *et al.*, 1982), such as production of pulmonary surface-active lipoproteins called surfactant. AM functions may thus be indirectly inhibited 1984, 1986). In addition, viral infection may have a direct suppressive (Schwartz and Christman, 1979) or its opsonizing effect (Juers *et al.*, 1976, 1976), which is important especially in AM phagocytosis of invading bacteria. The immediate tissue reaction to virus infection is the production of IFN, which can modulate various macrophage activities both *in vivo* and *in vitro* (Bielefeldt Ohmann and Babiuk, 1984; Bielefeldt Ohmann *et al.*, 1984, 1986). In addition, viral infection may have a direct suppressive effect on the AM population, affecting such functions as their immunological [Fc and complement (C')] and nonimmunological membrane receptor-binding activities and receptor-mediated phagocytosis, phagosome–lysosome fusion, intracellular killing, and bacterial degradation (reviewed by Jakab, 1982b), as well as production of neutrophil chemotactic factors (McGuire and Babiuk, 1983; Gadek and Hunninghake, 1980). Although these effects are clearly evident following *in vitro* infection of AM by viruses, there often appear to be discrepancies between *in vitro* and *in vivo* observations. This has been amply demonstrated in the bovine herpesvirus 1 (BHV-1) infection model. Bovine AM are susceptible to infection with BHV-1 *in vitro,* resulting in impairment of immune receptor functions and antibody-dependent cell-mediated cytotoxicity (ADCC) (Forman and Babiuk, 1982). In contrast, less than 0.1% of AM retrieved from experimentally infected calves are productively infected with BVH-1, and neither ADCC nor receptor functions appear to be altered or at least only transiently so (Forman *et al.*, 1982; Bielefeldt Ohmann and Babiuk, 1986). In fact it appears that selected stimulation of AM occurs after BHV-1 infection, rather than compromising functional activity (Bielefeldt Ohmann and Babiuk, 1986), as well as selective inhibition of some functions such as ADCC, chemotaxin production, and IL-1 generation (Bielefeldt Ohmann and Babiuk, 1986; McGuire and Babiuk, 1983). Whether this disparity in viral effect, directly or indirectly induced, is caused by selective effects on the activities of the AM population as a whole, or can be ascribed to a differential effect on subpopulations of AM (Bielefeldt Ohmann *et al.*, 1986b) varying in functional activities, remains to be investigated. Whatever the explanation might be, the functionally altered AM pop-

ulation may play both a beneficial role, as well as contribute to lung injury via its proinflammatory activities. AM and monocytes are avid prcducers of IL-1, complement components, tissue factor (McGee and Rothenberger, 1985), platelet-aggregating factor (Roubin et al., 1983), arachidonic acid derivatives (Larson and Henson, 1983), and neutral proteases (Unanue, 1976). These factors may contribute to increased vascular permeability, coagulation, fibrinolysis, and tissue damage (Bevilacagva et al., 1984; Till and Ward, 1986; Till et al., 1982; Slauson, 1982) (see below), thereby creating an environment which promotes secondary bacterial invasion and growth, and at the same time interferes with normal clearance mechanisms (Newhouse et al., 1976).

In some virus–host systems a temporal relationship between virus replication in the lungs, development of specific immunity, and suppression of pulmonary bactericidal activity has been noted. This has led to the suggestion that the depression of AM function during viral infection may be due to an immunopathological mechanism (Jakab, 1982a; Jakab and Warr, 1983; Astry and Jakab, 1984). That this is the case in some viral infections (e.g., influenza virus pneumonia) will not be challenged here, but it certainly is not applicable to all virus–lung defense relationships (Yates et al., 1983a).

PMN may play a crucial role in the clearance of bacteria from the lower respiratory airways. PMN recruitment is induced within hours after bacterial invasion, probably mediated by the combined effect of AM-, epithelial-, and endothelial-derived chemotactic factors; including complement fragments, thromboxanes, and leukotrienes (Davies et al., 1984; Larsen and Henson, 1983). However, prior lung infection with virus can delay this recruitment (McGuire and Babiuk, 1983), and thus, perhaps, give the bacteria enough time to multiply to numbers which eventually will overwhelm the various defense mechanism and/or cause severe inflammation which will progress into a circulus vitiousus. This delay in PMN immigration may be caused either by suppressed production of AM-derived chemotactic factors (McGuire and Babiuk, 1983), by a direct suppressive effect of the virus on the migratory activity of the PMN (Ruutu et al., 1977; Bultman and Gruler, 1983; Bultman et al., 1982), or, indirectly, via the induction and production of IFN or arachidonic acid derivatives by the various cell types in the lung (Bielefeldt Ohmann and Babiuk, 1984; Davies et al., 1984). Finally, it should be taken into consideration that the circulating PMN pool may already have been depleted of the readily migrating cells due to the virus-induced PMN migration into the alveoli (McGuire and Babiuk, 1983; Yourtee et al., 1982), by the time the secondary bacterial invasion occurs. This scenario seems conceivable

in light of the neutropenia that often occurs following virus infection of the respiratory tract (Yates, 1982; Bale et al., 1982).

Whether other functions of the PMN are affected by virus infection seems to vary among various virus–host systems. Influenza virus infection has been reported to cause depression of chemiluminescence, a measure of the respiratory burst, and bactericidal activities of PMN, whereas phagocytosis remained unaffected (Abramson et al., 1981, 1982a,b). In contrast, superoxide anion production by peripheral blood PMN is significantly increased during infection of cattle with BHV-1 (Bielefeldt Ohmann and Babiuk, 1985). Bovine viral diarrhea virus (BVDV) also is reported to suppress PMN-mediated bacterial defense (Roth et al., 1981; Reggiardo and Kaeberle, 1981; Roth and Kaeberle, 1983). In humans, except for delayed impairment of chemotaxis, no consistent abnormality in PMN function has been found during mild influenza (Martin et al., 1981). Whether more severe influenza infection in humans has similar suppressive effects as in other species remains to be determined. Furthermore, it cannot be excluded that once migrated into the lung, the antibacterial functions of the PMN may be suppressed by locally produced factors, such as IFN and prostaglandins (Bielefeldt Ohmann and Babiuk, 1984, 1985; Davies et al., 1984), or by partial autoinactivation by myeloperoxidase or ROS released from the PMN itself (Weber et al., 1983; Stendahl et al., 1984; Baehner et al., 1977; Mills et al., 1981; Kobayashi et al., 1982; Voetman et al., 1981).

VI. Viral Effects on Specific Host Defense Mechanisms

In addition to affecting nonspecific defenses, many viral infections also cause suppression of specific immune defense mechanisms. Although many of the mechanisms by which viruses suppress the immune response are not fully understood, attempts will be made in this section to discuss some alterations of the immune system which clearly are modified following virus infection. What is known is that in many cases suppression of the immune response is due to alteration in functional activity of the effector cell and that this impairment is caused either by a direct virus–effector cell interaction or indirectly by the release of suppressor molecules by a variety of cells in response to virus infection (Friedman et al., 1984). Although the exact role of lymphocytes in prevention or recovery from a primary viral bacterial infection in the lung is not clearly known, there is a considerable amount of evidence that viral infection does adversely affect lymphocyte functions. In studies using mitogen-driven lymphocyte prolifera-

tion assays, Viruses have been shown to reduce lymphocyte responsiveness. Maximal suppression often occurs at the time the host is most susceptible to secondary bacterial infection (Filion *et al.,* 1983). This loss of responsiveness may be due to a number of parameters including the induction of T-suppressor cells. If T-suppressor cells are to be a factor in suppression, they, like other T-cell subsets, are dependent on IL-2 to expand (Bensussan *et al.,* 1984). T-suppressor cells must therefore be able not only to suppress antigen-specific T-helper responsiveness to IL-2, but also to induce IL-2 production by T-helper cells (Herbert and Watson, 1986). In BHV-1, a respiratory virus of cattle, it has been observed that in cattle infected with BHV-1, IL-2 production peaks at a time of maximal immunosuppression (Babiuk *et al.,* 1987a). This observation makes the existence of BHV-1-specific T-suppressor cells highly probable. It should be pointed out that factor(s) other than and distinct from IL-2 have been isolated and have been shown to induce T-suppressor cell proliferation (Kasakura, 1983). Other human herpesviruses have also been shown to induce suppressor cell activity (Horohov *et al.,* 1986). Suppressor cells have also been implicated in the depression of cell-mediated immune responses following infection with influenza and respiratory syncytial virus (Roberts, 1982).

During BHV-1-induced respiratory infection, lymphocyte responsiveness to lectins becomes significantly suppressed and remains in this suppressed state for up to 9 days after BHV-1 challenge (Bielefeldt Ohmann and Babiuk, 1985; Filion *et al.,* 1983). Furthermore, these decreased lymphocyte responses cannot be restored by the addition of exogenous IL-2. As stated earlier, although animals appear to produce higher levels of IL-2 following infection with BHV-1, lymphocytes from these virus-infected animals are not responsive to IL-2. A number of reasons for this lack of IL-2 reactivity exist, namely, downregulation of IL-2 receptors; viral inhibition, steric or specific, of IL-2 binding, and margination of activated T cells to local drainage lymph nodes or sites of viral replication (Babiuk *et al.,* 1987b). Other studies have shown that T-cell proliferation can be inhibited by high antigen concentration, without affecting IL-2 production (Ceredig and Corradin, 1986).

The interaction of a virus with T lymphocytes generally occurs more readily when T cells are activated (Splitter and Eskra, 1986), as one would expect to be the case during an infection. Using both primary bovine T cells and T-cell clones, live BHV-1 decreases viability without replicating in these cells. This therefore supports the fact that viruses can alter T-cell functions without replicating within the cell and without the induction of antigen-specific T-suppressor cells. The alteration

of T-cell function may also be induced by virus-specific glycoproteins that are released during viral replication (Isfort *et al.*, 1986; Rea *et al.*, 1985). Thus, during viral infection the presence of viral products released from virus-infected cells can alter lymphocyte functions even if the virus does not replicate in the lymphocytes.

Indirect effects such as toxic soluble factors, induced by viruses that affect the functional integrity of lymphocytes, have been recorded (Wainwright *et al.*, 1979). Furthermore, viral infections also induce the production of IFN, which is known to have immunomodulatory activity (Bielefeldt Ohmann *et al.*, 1987b; Babiuk *et al.*, 1987a). IFN production in nasal passages is evident within 1–3 days after viral infection. Although very little free IFN is detectable in plasma following virus infection of the respiratory tract, it is possible that it is rapidly cell associated and thereby alters lymphocyte reactivity. Studies involving the pharmacokinetics of clearance of IFN clearly demonstrate that exogenous IFN is rapidly cleared (half-life approximately 2 hours). Coupled with the observation that IFN can dramatically alter lymphocyte function, it suggests that this possibility is highly likely (Bielefeldt Ohmann and Babiuk, 1985). IFN has been shown to inhibit lymphocyte proliferation *in vitro* and reduce the cells' responsiveness to IL-2 and PMA (Griebel *et al.*, unpublished observation). The observation that bovine IFN activity is enhanced at elevated temperatures (Letchworth and Carmichael, 1984) and that maximal immunosuppression correlates with peak temperature responses and IFN in nasal secretions, suggests that even low levels of serum IFN may play an important role in reduction of lymphocyte reactivity.

VII. Bacterial Effects on Host Defense Mechanisms

Bacteria, like viruses, can reduce the effectiveness of the immune response by a number of direct and indirect suppressive mechanisms. The indirect mechanisms include expression of virulence factors with reduced antigenicity, antigenic shift, production of IgA proteases, resistance to degradation by phagocytic cells, and the formation of cell wall-deficient L forms (Smith, 1984).

In respiratory bacterial infections, virulence factors with poor immunogenicity include the production of capsular material which interferes with the process of phagocytosis and opsonization by antibody and complement. This is evident for capsular polysaccharides of pneumococcal bacteria and M protein of streptococci (Smith, 1977; Densen and Mandell, 1980). Bacteria can inhibit the bactericidal activity of macrophages or granulocytes by blocking both the oxygen-dependent

and oxygen-independent killing mechanisms, by resisting the toxic effects of the ROS or enzymatic action of lysosomal enzymes and proteases, or by escaping from the phagocytic vacuole into the cytoplasm. *Mycobacterium tuberculosis* is a good example of a bacterium capable of surviving in phagocytic cells. Its mechanism of survival has been related to prevention of phagosome–lysosome fusion (Lowrie *et al.,* 1979), or, if fusion occurs, to the resistance to lysosomal enzymes (Armstrong and Hart, 1975) and resistance to killing by H_2O_2 (Jackett *et al.,* 1981). The mechanisms by which *M. tuberculosis* resists intracellular killing have been related to the presence of sulfated glycolipids in the bacterium's cell walls (Draper, 1981). Other correlations have been found with increased levels of cyclic AMP (Lowrie *et al.,* 1979), presence of polyglutamic acids (Draper, 1981), and production of ammonia (Gordon *et al.,* 1980) or bacterial catalases (Walker and Lowrie, 1981).

Pasteurella haemolytica species are also able to circumvent the phagocytic effect of macrophages and neutrophils and to survive in an intracellular environment (Lawman *et al.,* unpublished observations). The reason for their survival is unclear; however, *Pasteurella* strains are capable of producing a cytotoxin during the log phase of growth that is detrimental to bovine leukocytes (Shewan and Wilkie, 1982). In *in vitro* bactericidal studies it is evident that phagocytes with ingested bacteria undergo morphological changes (i.e., cytoplasmic swelling with extrusion of the cytoplasm and nuclear swelling). The majority of these phagocytic cells are nonviable as assessed by trypan blue dye exclusion. Survival of bacteria in bovine phagocytes (neutrophils) has also been shown with *Haemophilus somnus* (Czuprynski and Hamilton, 1985).

Some bacteria that are pathogens of mucosal surfaces, including the respiratory tract (*Haemophilus influenzae, Streptococcus pneumoniae*) are able to produce and excrete IgA proteases (Kornfeld and Plaut, 1981). The ability of the bacterial IgA protease to cleave the immunoglobulin molecule into Fab and Fc fragments may be important in reducing the biological activity of the antibody (i.e., opsonization). In addition, the effectiveness (affinity) of antigen binding may be affected (Kornfeld and Plaut, 1981).

The generation of the immune response is a complex phenomenon involving cell–cell interaction and soluble factors. Therefore, in the generation of a specific immune response, there are many stages at which pathogenic bacteria may be able to induce suppression (Campa, 1984). The direct effects on the immune response involve the induction of specific suppressor cells (T lymphocytes) and activation of macrophages (Klimpell and Henney, 1979; Wadee *et al.,* 1980). Antiisotype

antibody responses have also been induced in response to antigen. Furthermore, this antiidiotypic response may be important in either binding to antigen receptors or activating suppressor cells (Siskind *et al.*, 1982; Eichman, 1975). Although we have provided some examples of how bacteria may evade the immune system and even cause immunosuppression, the mechanism of inducing specific immune suppression at the respiratory tract mucosal surface is not fully understood.

In addition to altering immune responses, bacteria can also aid in the replication of viruses. Thus, one of the prerequisites for infection by some of the myxoviruses and paramyxoviruses is cleavage of the surface glycoproteins (Lazarowitz *et al.*, 1973; Scheid and Choppin, 1974). Recent evidence suggests that colonization with *Haemophilus influenzae* results in enhanced virus infectivity due to the release of proteolytic enzymes by the bacteria which can cleave the viral hemagglutinin protein (Tashiro *et al.*, 1987). Once this occurs, the virus and bacteria benefit each other in that the virus allows better adherence of the bacteria, due to cell surface changes (Ramphal *et al.*, 1980) and the bacteria in turn release proteases to enhance virus infection of cells.

VIII. OTHER PREDISPOSING FACTORS IN RESPIRATORY DISEASE

Clinical observations in both humans and animals, as well as experimental studies in animals, have suggested that stress is associated with increased susceptibility to viral and bacterial pneumonia (Stephens, 1980; Pennington, 1977; Nugent and Pesanti, 1982; Filion *et al.*, 1984). Even though the exact mechanisms whereby increased susceptibility occurs have not been completely elucidated, stress does alter endocrine gland functions. An important response of the body to stress is the secretion of adenocorticotropic hormone (ACTH), which in turn stimulates the synthesis and secretion of cortisol (Stephens, 1980). High cortisol levels may have a deleterious effect on both the nonspecific and the antigen-specific defense mechanisms (Keller *et al.*, 1981; Hirschberg *et al.*, 1982; Forslid and Hed, 1982; Fauci, 1976; Crabtree *et al.*, 1980; Chretien and Garagusi, 1972; Zor *et al.*, 1982; Taylor *et al.*, 1981; Roth *et al.*, 1982; Pennington, 1977; Nugent and Pesanti, 1982; Nair and Schwartz, 1984; Bielefeldt Ohmann *et al.*, 1987b), thereby increasing the individual's susceptibility to invading microorganisms (Stephens, 1980; Filion *et al.*, 1984). In many cases, stress can be associated with crowding of individuals, exposure to new environments including new microorganisms, and increased infection rates. This occurs most frequently in army recruits and feedlot situations. Moreover, high cortisol levels may cause reactivation of latent

infections (Pastoret *et al.,* 1980; Davies and Duncan, 1974), either by a direct alteration of viral replication (Nutter and Doeherty, 1978; Costa *et al.,* 1974) or by suppression of immune functions controlling the state of latency (Wildy *et al.,* 1982; Babiuk and Rouse, 1979). Such reactivation of viral infection may in itself lower resistance to secondary bacterial infections by mechanisms dealt with in other sections of this review.

There is evidence to suggest that the peripheral nervous system can modulate immunological responses at mucosal surfaces (Payan and Goetzl, 1985). At present, it is not known how stress can modulate these responses, but the combination of stress with viral infections, and production of endorphins (Johnson *et al.,* 1982) can all play an important role in reducing resistance.

Pneumonia in aging individuals represents a clinical problem of increasing magnitude as the population ages. Thus, although the prevalence of viral bacterial infections in persons over 65 years of age is not much greater than in the normal population, a large percentage of these individuals require hospitalization once infection occurs, and despite extensive care, pulmonary infections are the leading cause of death in persons 65 years of age and older (Kovar, 1977). Furthermore, the average duration of hospitalization is much longer in the elderly with pneumonia than in younger individuals. At present very little is known about the differences in the immunobiology of the elderly as compared to middle-aged or younger individuals. Thus, very little information is available on the quantity and quality of the secretions in the respiratory tract of aged individuals versus younger people. Furthermore, it was not possible to correlate directly deficiencies of immune function or inadequacies of the inflammatory response with the occurrence of either colonization or pneumonia in the elderly (Phair *et al.,* 1971). Thus a considerable amount of investigation is required to elucidate why the elderly are more susceptible to life-threatening pneumonias following even mild virus infections than are other individuals. Suggestions have been made that some of this increased susceptibility is due to a decline in mucociliary transport and abnormalities of the ciliated epithelial cells lining the tracheobronchial tree, as well as the quantity, distribution, and viscoelasticity of the secretions coating the airways. However, these remain to be further investigated. One of the major factors which may be involved and enhance the severity of pneumonia is the patterns of respiration in elderly versus younger populations. Thus, it is suggested that the elderly are at a much greater risk of nocturnal aspiration. As a result, a much larger number of bacteria can enter the lower lung, and specific bacterial colonies may end up being aspirated into the lung where they

cause pneumonia. If this occurs, then even a minor viral infection, which causes minimal depression of specific and nonspecific defenses, may be sufficient to prevent elimination of the bacterial microcolonies aspirated into the lungs.

IX. IMMUNOPATHOLOGY

In the present context, the definition of immunopathology will be restricted to lung injury caused by immunological mechanisms induced by the invading microorganism(s), as the immunological defense per se has already been dealt with in preceding sections. The role of leukocytes in inflamed lungs, the contribution of the coagulation and fibrinolytic systems, as well as that of other mediators to inflammatory events in the lung, have been comprehensively reviewed by Slauson (1982), Ward (1986), and Malik and Staub (1982). On a more general basis, Larsen and Henson (1983) have reviewed the mediators of inflammation, Davies et al., (1984) the role of arachidonic acid derivatives in inflammation, and Roubin et al. (1983) the pathobiological effects of macrophage-derived platelet-activating factor. It is beyond our scope here to do more than highlight a few aspects of the pathogenesis of pulmonary immunopathology. Thus, for more comprehensive overviews the reader is referred to the above-cited reviews.

In viewing leukocyte functions in pulmonary defense, it must be taken into account that these cells not only have an obvious value in defending the lung against the microbial invaders, they are also one of the major mediators of tissue injury because they produce and release potent inflammatory mediators, such as complement factors, prostaglandins, IL-1 (Larsen and Henson, 1984; Unanue, 1976), lysosomal products, including acid hydrolases and neutral proteases (Klempner et al., 1978; Lonki and McCarren, 1983), and ROS (Ward, 1986; Gerberick et al., 1986). Such products may have an injurious effect in their own right through specific substrate cleavage, direct cytotoxicity, or cell activation/suppression (Holt, 1986), but can also have an amplifying effect in the developing pulmonary inflammatory response via their diverse effects on kinin generation, complement cleavage, controls over leukotoxins, and the generation of plasminogen-independent systems for intracellular and extracellular fibrinolysis (reviewed by Slauson, 1982).

The involvement of arachidonic acid derivatives in lung inflammation represents a very complicated picture, as various products with agonistic and antagonistic effects may be produced by the same cell types (AM, endothelial, or epithelial cells), and the resulting effect

may therefore reflect the degree of imbalance between the various agents (Davies *et al.*, 1984; Slauson, 1982). In addition, there appear to be considerable species variations in the effect of the thromboxanes, prostacyclins, and other arachidonic acid products, as well as variations in the amounts produced in response to any particular stimulus or virus (Higgs *et al.*, 1979; Davies *et al.*, 1984).

Complement factors derived via the alternative pathway of C′ activation have been shown to produce selective enzyme (lysosomal acid hydrolases) secretion from macrophages (Schorlemmer *et al.*, 1977a,b). This may have considerable importance in relation to lung infections, as many microorganisms can activate the alternative complement pathway locally or systemically (Fearon and Austen, 1980; Cooper and Oldstone, 1983; Bielefeldt Ohmann and Babiuk, 1985) and thereby indirectly cause selective enzyme release from AM, and, subsequently, tissue injury. In addition, systemic C′ activation has been shown to promote acute lung injury by inducing sequestration of PMN in lung interstitial capillaries, perhaps via involvement of arachidonic acid derivatives (Imasawa and Osifchine, 1983; Golditz and Movat, 1984). The activated PMN subsequently produce ROS which mediate acute lung microvascular injury (Martin, 1984; Till and Ward, 1986; Bass *et al.*, 1986; Fox *et al.*, 1981; English and Lukens, 1983), immediately followed by tissue reactions to the insult (Harlan and Callahan, 1984; Hoover *et al.*, 1984; Slauson, 1982). There is also growing evidence that macrophage-derived tumor necrosis factor (TNF-α) may be intimately involved in inflammatory and immunoregulatory reactions, either directly or indirectly by inducing IL-1 and other cytokines (Ruddle, 1987). It remains to be determined whether such mechanisms are operational during viral–bacterial infections of the lung and what their possible implications might be.

Although much still remains to be done to complete the very complex picture of the immunopathological scenario in the lungs, it is obvious that the possibilities for circumventing such events are limited once the complex processes are initiated. Thus, the best strategem seems to be to avoid infection in the first place, by induction of specific immunity and/or by modulating the nonspecific and specific immune defense mechanisms (Babiuk *et al.*, 1985).

X. Control

As stated above, it is important to avoid initiation of pneumonia because once it is initiated, the host itself may contribute to the immunopathology and severity of the disease. With this in mind, control can

be directed at a number of areas, such as immunization against the viral or bacterial pathogens, use of antiviral or antibacterial therapeutic agents, or use of immunomodulators which enhance either nonspecific or specific defense mechanisms. This is especially important in those viral infections where immunosuppression is one of the major underlying causes of increased susceptibility to secondary bacterial infections. In each of these strategies it is important to consider the timing of administration and delivery of the specific agents so as to ensure their efficacy. Thus, treatment with antibiotics after extensive lung damage and bacterial microcolony formation has occurred will be of little value, since the antibiotics cannot penetrate the microcolonies and the host's response to the infection will only lead to further immunopathology. If antibiotics are used, they should be selected for defined infections in a manner which results in cure, with reasonable cost and minimal damage to the environment. It is not recommended to use subclinical doses in animal feeds to prevent respiratory infections in animals. Unfortunately, this practice is often used.

A. Virus Vaccines

Since most of the viruses involved in respiratory infections enter via the respiratory tract and replicate in the respiratory epithelium, it is crucial to ensure local immunity at the site of entry. Thus, early intervention will greatly reduce the environmental alterations of the respiratory tract caused by the viruses, as well as the subsequent immunosuppression associated with some virus infections. Although immunity can be induced by intramuscular immunization with live or killed viral vaccines, it is often accepted that local immunization with attenuated vaccines is potentially more efficacious. Thus, currently inactivated vaccines given intramuscularly are between 60 and 90% effective against influenza viruses of the same antigenic type (Meyer et al., 1978). The potential advantages of a live attenuated vaccine include stimulation of both mucosal and systemic immunity. Recent advances in this area have included the production of deletion mutants, and temperature-sensitive or cold-adapted mutants (Johnson et al., 1986). In the case of segmented viruses such as influenza, it is possible to develop cold-adapted, temperature-sensitive mutants with temperature-sensitive lesions in a number of different segments of the virus. Cocultivation of the cold-adapted, temperature-sensitive mutant with wild-type virus of vaccine interest will result in a variety of different reassortants containing the hemagglutinin and neuraminidase of the virus of interest, but the temperature-sensitive segments from the attenuated virus vaccine. Thus, it is possible to isolate master strains containing

six RNA genes from the attenuated cold-adapted virus and two of the genes encoding the surface glycoproteins from the wild-type virus. These attenuated reassortant vaccines are infectious, immunogenic, relatively stable genetically, and often nonreactogenic when administered intranasally into humans (LaMontagne *et al.*, 1983).

Other examples of live attenuated vaccines against respiratory viral infections include those of bovine herpesvirus. BHV1 infection of cattle is one of the major initiators of secondary bacterial infections. It is possible with BHV-1, as with other herpesviruses, to induce deletions in the BHV-1 genome corresponding to the TK gene of the virus (Kit *et al.*, 1986). As a result of this deletion, the virus can still replicate in the respiratory epithelium of the animal; however, the degree of replication and systemic spread and latency is dramatically reduced (Stanberry *et al.*, 1985). Experiments need to be conducted in order to determine whether indeed there is minimal immunosuppression by this TK deletion mutant, or whether it can still predispose the animal to secondary bacterial infections, especially when it is stressed and vaccinated on entry into the feedlot.

The recent advances in recombinant DNA technology have great potential for the production of a wide variety of different subunit vaccines against respiratory infections. This approach appears to be especially relevant to producing vaccines against viruses which do not replicate well in culture or cannot be attenuated with ease. Furthermore, it is important for those viruses which may be genetically unstable and where reversion to virulence may be a problem with live attenuated vaccines. At present a large number of the important antigens or epitopes present on respiratory viruses have been identified and either synthesized using synthetic peptide technology or cloned into various expression systems (Bennink *et al.*, 1984; Norrby, 1987). Although most of these subunit vaccines will be injected intramuscularly, and therefore have the potential for inducing high levels of circulating antibody, they generally do not produce very good local immunity. In order to overcome this impediment, there is a considerable amount of effort being focused on delivering subunit vaccines to mucosal surfaces to be able not only to stimulate systemic immunity, but also to induce local immunity. It is our contention that within the next few years intramuscular vaccines will be administered in such a way that they will be able to induce local immunity as well as systemic immunity.

B. Antiviral Chemotherapy

At present a large number of compounds have been identified with potential antiviral activity *in vitro* against a number of the viruses

involved in initiating respiratory disease (Streissle *et al.*, 1985). Although few of these compounds have been either tested or proved to be effective *in vivo* against viruses of the respiratory tract (Gangemi *et al.*, 1987), the important advances made in this area over the last decade warrant serious consideration for the potential application of some of these drugs in the control of viral infections of the respiratory tract. In this section we will discuss the potential sites of activity of a selected number of drugs and speculate as to their application in respiratory tract infections. In addition, the limitations of using antiviral drugs in these infections will be addressed. The problems of delivery and maintenance of drugs at the site of infection so as to prevent initiation of infection rather than therapy will be emphasized.

In respiratory infections, as in other virus infections, the targets of antiviral drugs must be at sites that are unique to the virus and not have any physiological effects on the specific cells of the respiratory tract. As is the case in other virus infections, the antiviral drug must be directed at various stages of the virus replication cycle such as virus attachment, penetration, uncoating, macromolecular synthesis, and viral maturation and assembly. Regardless of the stage of virus replication to which the antiviral drug is targeted, the major problems with using antiviral drugs in controlling viral-induced respiratory infections is the rapidity with which respiratory diseases occur following local infection. Thus, prevention of viral activity at the early stages of infection appears to be the most promising approach, but also creates the most problems regarding delivery and maintenance of the antiviral drug for considerable periods of time within the respiratory tract environment during a pending epidemic.

In addition to the various chemicals that are used as antiviral drugs, the application of IFN as a broad-spectrum antiviral has continued to generate interest since Isaacs and Lindenmann first described this antiviral compound in 1957. The advent of recombinant DNA technology and production of recombinant forms of IFN has made it economical to use them as direct antiviral agents in the respiratory tract. These agents, as with many antiviral compounds, need to be present prior to challenge (Monto *et al.*, 1986; Turner *et al.*, 1986). It was initially felt that the economic production of IFN by recombinant DNA technology would greatly facilitate their use in respiratory infections. However, at the doses required for inhibition of some viruses in humans, symptoms of nasal irritation were observed following prolonged administration. Thus, there appear to be some unacceptable side effects in humans following administration of IFN-α. Since there are a variety of different types of IFN, it is possible that some of them may be less of a problem in inducing adverse clinical effects than others.

Thus, one should not discount the value of using IFN as an antiviral for local administration against respiratory infections. Certain host species are much more susceptible to the adverse side effects of local administration of IFN than are others. For example, cattle do not experience any intolerance to extremely high doses of IFN-α-administered intranasally. In addition to having direct antiviral effects, IFNs appear to have a very important role in reducing respiratory virus replication indirectly by stimulating the immune system (Babiuk *et al.*, 1985, 1987a).

C. Immunomodulators

Before effective manipulation of the immune system with various lymphokines can be achieved, some comprehension of the complexity of the interactions between lymphokines and the immune system is required. Thus, it is important to understand which specific arm of the immune system is required to be stimulated and which specific lymphokine or lymphokine combinations would best meet this need. Once this knowledge is available, it should be possible to regulate the immune system in such a way that they would be more resistant both to the viral infection and to secondary bacterial superinfection. However, before IFN or other lymphokines can be administered and used judiciously, more information regarding the types of IFN, dose, route of administration, and the mechanisms responsible for either the antiviral state or immunomodulation needs to be determined. Primary interest of the clinical investigations conducted with both bovine and human IFN-α have been concerned with the antiviral affects of compounds. However, it was found that the preventive treatment of calves with bovine IFN-α had a beneficial effect on clinical performance in experimental viral/bacterial pneumonia ("shipping fever") without significantly reducing virus replication in nasal passages (Babiuk *et al.*, 1985, 1987a). Therefore, it was assumed that IFN had an overall effect on the disease situation by modulating immune responses.

While the overall effect of recombinant bovine IFN-α and IFN-γ on bovine macrophages and PMN might be characterized as activation, it appears that the influence of any particular function of these cell types is selection—that is, either enhancement, inhibition, or no detectable affects (Bielefeldt Ohmann *et al.*, 1987b). Similar effects are being observed with lymphokines from other species including humans. Thus, using the appropriate delivery systems and combinations of lymphokines, it should be possible to modulate selectively the arm of the immune system required to combat the infection, but not activate it in such a way as to result in immunopathology.
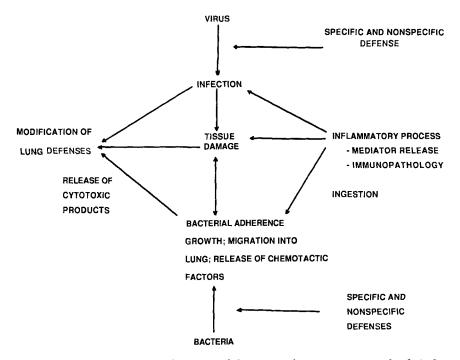
VIRUS

SPECIFIC AND NONSPECIFIC
DEFENSE

INFECTION

MODIFICATION OF

LUNG DEFENSES

TISSUE
DAMAGE

INFLAMMATORY PROCESS

- MEDIATOR RELEASE

- IMMUNOPATHOLOGY

RELEASE OF
CYTOTOXIC
PRODUCTS

INGESTION

BACTERIAL ADHERENCE

GROWTH; MIGRATION INTO

LUNG; RELEASE OF CHEMOTACTIC

FACTORS

SPECIFIC AND
NONSPECIFIC
DEFENSES

BACTERIA

FIG. 1. Schematic representing some of the events that occur as a result of viral–bacterial interaction in respiratory disease. From L. A. Babiuk, in "Applied Virology" (E. Kurstak, ed.), p. 431. Academic Press.

XI. SUMMARY

In the present review we have identified how viruses can alter the host's susceptibility to bacterial infections by altering both environmental conditions in the lung which favor bacterial replication as well as by suppressing the host's defense mechanisms which prevent clearance of the bacteria. In many instances, these interactions are extremely complex but similar for many viruses. If the virus can overcome the initial host defense mechanisms, which include local antibody and mucus, the virus initiates tissue damage as a result of direct replication within the epithelial cells lining the mucosal surfaces of the respiratory tract. As a result of virus infection, the host cells respond by producing a variety of mediators including various types of interferons, which can alter both virus replication and host response. Replication also produces by-products of virus infection capable of initiating an inflammatory process, which in turn, through release of other mediators, can further modify lung defense mechanisms and

encourage bacterial adherence and growth. The bacterium, in turn, releases chemotactic factors which encourage infiltration of specific effector cells into the lung. These effector cells can cause tissue damage and immunopathology, which encourage rapid bacterial growth and may result in death of the animal. In order to be able to control this complicated scenario, it is important either to prevent the initial infection with viruses or to reduce the degree of immunosuppression, so that bacterial clearance can occur rapidly before microcolony formation and extensive lung damage occur. Once a large amount of bacterial replication and lung damage is present, the use of antibiotics is generally of limited value. A schematic illustration of the complexity of the various interactions and counteractions occurring during virus–bacterial synergistic interactions is presented in Fig. 1.

REFERENCES

Abramson, J. S., Giebink, G. S., Mills, E. L., and Quie, P. G. (1981). *J. Infect. Dis.* **143**, 836–345.

Abramson, J. S., Lyles, D. S., Heller, K. A., and Bass. D. A. (1982a). *Infect. Immun.* **37**, 794–799.

Abramson, J. S., Mills, E. L., Giebink, G. S., and Quie, P. G. (1982b). *Infect. Immun.* **35**, 350–355.

Armstrong, J. A., and Hart, P. D. A. (1975). *J. Exp. Med.* **142**, 1–16.

Astry, C. L., and Jakab, G. J. (1984). *J. Virol.* **50**, 287–292.

Babiuk, L. A. (1984). *In* "Applied Virology" (E. Kurstak, ed.), pp. 431–443. Academic Press, New York.

Babiuk, L. A., and Rouse, B. T. (1979). *Can. J. Microbiol.* **25**, 267–274.

Babiuk, L. A., Bielefeldt Ohmann, H., Gifford, G., Czarniecki, C. W., Scialli, V. T., and Hamilton, E. B. (1985). *J. Gen. Virol.* **66** 2383–2394.

Babiuk, L. A., Lawman, M. J. P., and Gifford, G. A. (1987a). *Antimicrob. Agents Chemother.* **31**, 752–757.

Babiuk, L. A., Lawman, M. J. P., and Griebel, P. (1987b). *In* "Handbook on Viral Immunosuppression" (H. Friedman, S. Spector, and M. Bendinelli, ed.). Plenum, New York, in press.

Baehner, R. L., Boxer, L. A., Allen, J. M., and Davis, J. (1977). *Blood* **50**, 327–335.

Bale, J. F., Kern, E. R., Overall, J. C., and Glasgow, L. A. (1982). *J. Infect. Dis.* **145**, 525–531.

Barber, D. M. L., Nettleton, P. F., and Herring, J. A. (1985). *Vet. Rec.* **117**, 459–464.

Bass, D. A., Olbrantz, P., Szejda, P., Seeds, M. C., and McCall, C. A. (1986). *J. Immunol.* **136**, 860–866.

Beachey, E. H. (1981). *J. Infect. Dist.* **143**, 325–345.

Bennink, J. R., Yedell, J. W., Smith, G. L., Moller, C., and Moss, B. (1984). *Nature (London-* **322**, 578–579.

Bensussan, A., Acuto, O., Hussey, R. E., Milanese, C., and Reinherz, E. L. (1984). *Nature (London)* **311**, 565–567.

Bevilacqua, M. P., Pober, J. S., Majeau, G. R., Cotran, R. S., and Gimbrone, M. M. (1984). *J. Exp. Med.* **160**, 618–623.

Bevilacqua, M. P., Pober, J. S., Wheeler, M. E., Cotran, R. S., and Gimbrone, M. A., (1985). *J. Clin. Invest.* **76**, 2003.

Bielefeldt Ohmann, H., and Babiuk, L. A. (1984). *J. Interferon Res.* **4**, 249–263.

Bielefeldt Ohmann, H., and Babiuk, L. A. (1985). *J. Infect. Dis.* **151**, 937–947.

Bielefeldt Ohmann, H., and Babiuk, L. A. (1986). *Infect. Immun.* **51**, 344–347.

Bielefeldt Ohmann, H., Filion, L. G., and Babiuk, L. A. (1983). *Immunology* **50**, 189–197.

Bielefeldt Ohmann, H., Gilchrist, J. E., and Babiuk, L. A. (1984). *J. Gen. Virol.* **65**, 1487–1495.

Bielefeldt Ohmann, H., Davis, W. C., and Babiuk, L. A. (1986b). *Immunobiol.* **39**, 167–181.

Bielefeldt Ohmann, H., Baker, P. E., and Babiuk, L. A. (1987a). *Can. J. Vet. Res.* (in press).

Bielefeldt Ohmann, H., Lawman, M. J. P., and Babiuk, L. A. (1987b). *Antiviral Res.* **7**, 187–210.

Bultmann, B. D., and Gruler, H. (1983). *J. Cell Biol.* **96**, 1708–1716.

Bultmann, B. D., Heizner, I., Haferkamp, O., and Gruler, H. (1982). *Pathol. Res. Pract.* **175**, 308–311.

Campa, M. (1984). *In* "Bacterial and Viral Inhibition and Modulation of Host Defences" (G. Falcone, M. Campa, H. Smith, and G. M. Scott, eds.), pp. 136–157. Academic Press, New York.

Ceredig, R., and Corradin, G. (1986). *Eur. J. Immunol.* **16**, 30–34.

Chanock, R. M., Parrott, R. H., Johnson, K. M., Kapikian, A. Z., and Bell, J. A. (1963). *Am. J. Vet. Res.* **88**, 152–166.

Choppin, P. W., and Scheid, A. (1980). *Rev. Infect. Dis.* **2**, 40–61.

Chretien, J. H., and Garagusi, F. V. (1972). *J. Reticuloendothel. Soc.* **11**, 358–367.

Colditz, I. G., and Movat, H. Z. (1984). *J. Immunol.* **133**, 1269–2173.

Conrod, J. D., and Yoneda, K. (1983). *J. Clin. Invest.* **71**, 129–141.

Cooper, N. R., and Oldstone, M. B. A. (1983). *Immunol. Today* **4**, 107–108.

Costa, J., Yee, C., Troost, R., and Rabson, A. S. (1974). *Nature (London)* **252**, 745–746.

Costerton, J. W., Irvin, R. T., and Cheng, K. J. (1981). *Crit. Rev. Microbiol.* **8**, 303–338.

Crabtree, G. R., Gillis, S., Smith, K. A., and Munk, A. (1980). *J. Steroid Biochem.* **12**, 445–449.

Craven, D. E., Peppler, M. S., Frasch, C. E., Mocca, L. F., McGrath, P. P., and Washington, G. (1980). *J. Infect. Dis.* **142**, 556–568.

Czop, J. K., McGowan, S. E., and Center, D. E. (1982). *Am. Rev. Respir. Dis.* **125**, 607–609.

Czuprynski, C. J., and Hamilton, N. L. (1985). *Infect. Immun.* **50**, 431–436.

Davies, D. H., and Duncan, J. K. (1974). *Cornell Vet.* **64**, 340–366.

Davies, P., Bailey, P. J., and Goldenberg, M. N. (1984). *Annu. Rev. Immunol.* **2**, 335–357.

Davison, V. E., and Sanford, B. A. (1981). *Infect. Immun.* **32**, 1118–1126.

Densen, P., and Mandell, G. L. (1980). *Rev. Infect. Dis.* **2**, 817–838.

Dinarello, G. A. (1984). *Rev. Infect. Dis.* **6**, 51–95.

Dinarello, G. A., Cannon, J. G., Wolff, S. M., Bernheim, H. A., Beutler, B., Cerami, A., Figari, I. S., Palladino, M. A., and O'Connor, J. V. (1986). *J. Exp. Med.* **163**, 1433–1450.

Draper, P. (1981). *In* "Microbial Perturbation of Host Defenses" (F. O'Grady and H. Smith, eds.), pp. 143–164. The Beecham Colloquia Academy Press, London.

Eichman, K. (1975). *Eur. J. Immunol.* **5**, 511–516.

English, D., and Lukens, J. N. (1983). *J. Immunol.* **130**, 850–856.

Fainstein, V., Musher, D. M., and Cates, T. R. (1980). *J. Infect. Dis.* **141**, 172–176.

Fauci, A. S. (1976). *Clin. Exp. Immunol.* **24**, 54–62.

Fearon, D. T., and Austen, K. F. (1980). *N. Engl. J. Med.* **303**, 259–263.

Fields, B. N. (1985). *In* "Virology." Raven, New York.

Filion, L., McGuire, R., and Babiuk, L. A. (1983). *Infect. Immun.* **42**, 106–112.

Filion, L. G., Willson, P. J., Bielefeldt Ohmann, H., Babiuk, L. A., and Thomson, R. G. (1984). *Can. J. Comp. Med.* **48**, 268–274.

Forman, A. J., and Babiuk, L. A. (1982). *Infect. Immun.* **35**, 1041–1047.

Forman, A. J., Babiuk, L. A., Baldwin, P., and Friend, S. C. E. (1982). *Am. J. Vet. Res.* **43**, 1174–1179.

Forslid, J., and Hed, J. (1982). *Infect. Immunol.* **38**, 811–816.

Fox, R. B., Hoidal, J. R., Brown, D. M., and Repine, J. E. (1981). *Am. Rev. Respir. Dis.* **123**, 521–533.

Friedman, H., Specter, S., and Bendinelli, M. (1984). *In* "Bacterial and Viral Inhibition and Modulation of Host Defenses" (G. Falcone, M. Campa, H. Smith, and G. Scott, eds.), pp. 119–133. Academic Press, New York.

Gadek, J. E., and Hunninghake, G. W. (1980). *Am. Rev. Respir. Dis.* **121**, 723–733.

Gangemi, J. D., Nachtigal, M., Barnhart, D., Krech, L., and Jani, P. (1987). *J. Infect. Dis.* **155**, 510–517.

Gerberick, G. F., Jaffi, H. A., Willoughby, J. B., and Willoughby, W. F. (1986). *J. Immunol.* **137**, 114–121.

Glorioso, J. C., Jones, G. W., Rush, H. G., Pentler, L. J., Darif, G. A., and Coward, J. E. (1982). *Infect. Immun.* **35**, 1103–1109.

Golditz, I. G. and Movat, H. Z. (1984). *J. Immunol.* **133**, 2168–2173.

Gordon, A. H., Hart, P. D. A., and Young M. R. (1980). *Nature (London)* **286**, 79–80.

Green, J. A., Charette, R. P., Yeh, T. J., and Smith, C. B. (1982). *J. Infect. Dis.* **145**, 837–841.

Haas, R., Nickel, P., Stern, A., and Meyer, T. F. (1986). *FEMS Symp.* **31**, pp. 81–89.

Harlan, J. M., and Callahan, K. S. (1984). *J. Clin. Invest.* **74**, 442–448.

Hatch, G. E., Slade, E., and Boykin, E. (1981). *Am. Rev. Respir. Dis.* **124**, 167–173.

Herbert, A. G., and Watson, J. D. (1986). *Immunol. Today* **7**, 72–76.

Higgs, G. R., Moncada, S., and Vane, J. R. (1979). *Adv. Inflammation Res.* **1**, 413–418.

Hirschberg, H., Hirschberg, H., Neuslanen, H., Braathen, L. R., and Jaffe, E. (1982). *Clin. Immunol. Immunopathol.* **23**, 577–581.

Holt, P. G. (1986). *Clin. Exp. Immunol.* **63**, 261–270.

Hoover, R. L., Karnovsky, M. J., and Austen, K. F. (1984). *Proc. Natl. Acad. Sci. U.S.A.* **81**, 2191–2193.

Horov, D. W., Wyckoff, J. H., Moore, R. N., and Rouse, B. T. (1986). *J. Virol.* **58**, 331–338.

Hunninghake, G. W., Fick, P. B., and Nugent, K. M. (1985). *In* "Advances in Host Defense Mechanisms" (J. I. Gallin and A. S. Fauci, eds.), Vol. 4, pp. 89–114. Raven, New York.

Hynes, R. A., and Bye, J. M. (1974). *Cell* **3**, 113–120.

Imasawa, D. K., and Osifchine, N. E. (1983). *Proc. Natl. Acad. Sci. U.S.A.* **80**, 6647–6651.

Isaacs, A., and Lindenmann, J. (1957). *Proc. Ret. Soc. London Ser. B* **147**, 258–267.

Isfort, R. J., Stringer, R. A., Kung, H. J., and Velicer, L. F. (1986). *J. Virol.* **57**, 464–474.

Jackett, P. S. Aber, V. R., Mitchison, D. A., and Lowrie, D. B. (1981). *Br. J. Exp. Pathol.* **62**, 32–40.

Jakab, G. J. (1981). *Clin. Chest Med.* **2**, 59–66.

Jakab, G. J. (1982a). *Adv. Vet. Sci. Comp. Med.* **26**, 155–171.

Jakab, G. J. (1982b). *Am. Rev. Respir. Dis.* **126**, 776–782.

Jakab, G. J., and Warr, G. A. (1983). *Bull. Eur. Physiopathol. Respir.* **19,** 173–178.

Johanson, W. G., Wood, D. E., and Chaudhuri, T. (1979). *J. Infect. Dis.* **139,** 667–673.

Johnson, D. M., Smith, E. M., Torres, B. A., and Blalock, J. E. (1982). *Proc. Natl. Acad. Sci. U.S.A.* **79,** 4171–4174.

Johnson, D. W., and Muscoplat, C. C. (1973). *Am. J. Vet. Res.* **34,** 1139–1141.

Johnson, P. R., Feldman, S., Thompson, J. M., Mahoney, J. D., and Wright, P. F. (1986). *J. Infect. Dis.* **154,** 121–127.

Juers, J. A., Rogers, R. M., and McCurdy, J. B. (1976). *J. Clin. Invest.* **58,** 271–275.

Kasakura, I. (1983). *J. Immunol.* **130,** 2720–2726.

Keller, S. E., Weiss, J. H., Schleifer, S. J., Miller, N. E., and Stein, M. (1981). *Science* **213,** 1397–1399.

Ketelsen, A. T., Johnson, D. W., and Muscoplat, C. C. (1979). *Infect. Immun.* **25,** 565–568.

Kit, S., Kit, M., and McConnell. (1986). *Vaccine* **4,** 55–64.

Klempner, M. S., Dinarello, C. A., and Gallin, J. I. (1978). *J. Clin. Invest.* **61,** 1330–1336.

Klimpel, G. R., and Henney, C. S. (1979). *J. Immunol.* **121,** 749–754.

Kobayashi, M., Tanaka, T., and Usui, T. (1982). *J. Lab. Clin. Med.* **100,** 896–907.

Kornfeldt, S. J., and Plaut, A. G. (1981). *Rev. Infect. Dis.* **3,** 521–533.

Kovar, M. D. (1977). *Public Health Rep.* **92,** 9–19.

Labib, R. S., Calvanico, C., and Tomasi, T. B. (1978). *Biochim. Biophys. Acta* **526,** 547–552.

Laforce, F. M. (1976). *J. Lab. Clin. Med.* **88,** 691–699.

LaMontagne, J. R., Wright, P. F., Clements, M. L., Maassab, H. F., and Murphy, B. B. (1983). *In* "The Origin of Pandemic Influenza Viruses" (W. G. Laver, ed.). pp. 243–258. Elsevier, New York.

Larsen, G. L., and Henson, P. M. (1983). *Annu. Rev. Immunol.* **1,** 335–339.

Lazarowitz, S. G., Compans, R. W., and Choppin, P. W. (1973). *Virology* **52,** 199–212.

Letchworth, G. J., and Carmichael, L. E. (1984). *Arch. Virol.* **82,** 211–221.

Lonki, S. A., and McCarren, J. (1983). *Am. Rev. Respir. Dis.* **127,** 59–515.

Loosli, C. G. (1973). *Medicine (Baltimore)* **52,** 369–384.

Lowrie, D. B., Aber, V. R., and Jackett, P. S. (1979). *J. Gen. Microbiol.* **110,** 431–441.

McCarthy, J. P., Bodrochy, R. S., Jahbling, P. D., and Sobocinsky, P. Z. (1980). *Infect. Immun.* **30,** 824–832.

McGee, M. P., and Rothenberger, H. (1985). *Am. Rev. Respir. Dis.* **131,** 331–336.

McGuire, R. L., and Babiuk, L. A. (1983). *Vet. Immunol. Immunopathol.* **5,** 259–271.

Malik, A. B., and Staub, N. C. (1982). *Ann. N.Y. Acad. Sci.* **384,** 269–358.

Martin, W. J. (1984). *Am. Rev. Respir. Dis.* **130,** 209–213.

Martin, R. R., Couch, R. B., Greenber, S. B., Kate, T. R., and Warr, G. A. (1981). *J. Infect. Dis.* **144,** 279.

Matzner, Y., Hershko, C., Polliack, A., Konijn, A. M., and Izak, G. (1979). *Br. J. Hematol.* **42,** 345–355.

Meyer, H. M., Hopps, H. E., Parker, P. D., and Ennis, F. A. (1978). *Am. J. Clin. Pathol.* **70,** 146–152.

Miles, A. A., and Khimji, I. (1975). *J. Med. Microbiol.* **8,** 477–490.

Mills, E. L., Debets-Ossenkopp, Y., Verbrugh, H. A., and Verhoef, J. (1981). *Infect. Immun.* **32,** 1200–1205.

Miluzzo, F. H., and Delisle, G. H. (1984). *Infect. Immun.* **43,** 11–19.

Monto, A. S., Shope, T. C., Schwartz, S. A., and Albrecht, J. K. (1986). *J. Infect. Dis.* **154,** 128–133.

Mulks, M. H., and Plaut, A. G. (1978). *N. Engl. J. Med.* **299,** 973–979.

Nachman, R. L., Najjar, K. A., Silverstein, R. L., and Dinarello, C. A. (1986). *J. Exp. Med.* **163,** 1595–1600.

Nair, M. P. N., and Schwartz, S. A. (1984). *J. Immunol.* **132,** 2876–2882.

Nawroth, P. P., Bank, I., Handlex, D., Cassimeris, J., Chess, L., and Stern, D. (1986a). *J. Exp. Med.* **163,** 1363–1375.

Nawroth, P. P., Handley, D. H., Esmon, C. T., and Stern, D. M. (1986b). *Proc. Natl. Acad. Sci. U.S.A.* **83,** 3460–3464.

Newhouse, M., Sanchis, J., and Bienenstock, J. (1976). *New Engl. J. Med.* **295,** 990–998.

Normark, S., Lindberg, F., Lund, B., Baga, M., Ekback, G., Goransson, M., Morner, S., Norgren, M., Marklund, B-I., and Lih Lin, B. E. (1986). *In* "Minor Pilus Components Acting as Adhesions in Protein–Carbohydrate Interactions" (D. L. Lark, ed.), pp. 3–12. Academic Press, New York.

Norrby, E. (1987). *Adv. Virus Res.* **32,** 1–34.

Nugent, K. M., and Pesanti, E. L. (1982). *Am. Rev. Respir. Dis.* **125,** 5173–5185.

Nutter, M. F. D., and Doeherty, J. J. (1978). *J. Med. Virol.* **2,** 247–252.

Ofek, I. (1984). *In* "Bacterial and Viral Inhibition and Modulation of Host Defenses" (G. Falcone, M. Campa, H. Smith, and G. M. Scott, eds.), pp. 7–24. Academic Press, New York.

Ofek, I., Whitnack, A., and Beachey, E. H. (1982). *J. Bacteriol.* **149,** 426–433.

Oldstone, M. B. A., Sinha, Y. N., Blount, P., Tishon, A., Rodriguez, M., von Wedel, R., and Lampert, P. W. (1982). *Science* **218,** 1125–1127.

Parrott, R. H., Vargosko, A., Luckey, A., Kim, H. W., Cumming, C., and Chanock, R. (1959). *Am. J. Public Health* **52,** 907–917.

Pastoret, P. P., Babiuk, L. A., Misra, V., and Griebel, P. (1980). *Infect. Immun.* **29,** 483–488.

Payan, D. G., and Goetzl, E. J. (1985). *J. Immunol.* **135,** 783–796.

Pennington, J. E. (1977). *Am. Rev. Respir. Dis.* **116,** 885–893.

Phair, S. P., Kauffman, C. A., and Bjornson, A. (1971). *J. Reticuloendothel. Soc.* **23,** 397–405.

Pijoan, C., Campos, M., and Ocha, G. (1980). *Rev. Latin Am. Microbiol.* **22,** 69–72.

Pommier, C. G., Inada, S., Fries, L. F., Takahashi, T., Frank, M., and Brown, E. J. (1983). *J. Exp. Med.* **157,** 1844–1854.

Ramphal, R., Small, P. M., Shands, J. W., Fischlschweiger, W., and Small, P. A. (1980). *Infect. Immun.* **22,** 614–619.

Rankin, J. A., and Reynolds, H. Y. (1985). *In* "Advances in Host Defense Mechanisms" (J. I. Gallin and A. S. Fauci, eds.), Vol. 4, pp. 115–140. Raven, New York.

Rea, T. J., Timmins, J. G., Lony, G. W., and Post, L. E. (1985). *J. Virol.* **54,** 21–29.

Redhead, K. (1985). *J. Med. Microbiol.* **19,** 99–108.

Reggiardo, C., and Kaeberle, M. L. (1981). *Am. J. Vet. Res.* **42,** 218–221.

Reid, G., and Sobel, J. D. (1986). *Rev. Infect. Dis.* **9,** 470–487.

Roberts, N. J. (1982). *Infect. Immun.* **35,** 1142–1146.

Roth, J. A., and Kaeberle, M. L. (1983). *Am. J. Vet. Res.* **44,** 2366–2372.

Roth, J. A., Kaeberle, M. L., and Griffith, R. W. (1981). *Am. J. Vet. Res.* **42,** 244–250.

Roth, J. A., Kaeberle, M. L., and Hsu, W. H. (1982). *Am. J. Vet. Res.* **43,** 412–416.

Rothberger, H., and McGee, M. P. (1984). *J. Exp. Med.* **160,** 1880–1890.

Rothberger, H., McGee, M. P., and Lee, T-K. (1984). *J. Clin. Invest.* **73,** 1524–1529.

Roubin, R., Tence, M., Mencia-Huerta, J. M., Arnoux, B., Ninio, E., and Benvenisie, J. (1983). *Lymphokines* **8,** 249–276.

Ruddle, W. H. (1987). *Immunol. Today* **8,** 129–130.

Ruutu, P., Vaheri, A., and Kosunen, B. (1977). *Scand. J. Immunol.* **6,** 897–906.

Sanford, B. A., Shelorou, A., and Ramsay, M. A. (1978). *J. Infect. Dis.* **137,** 176–181.

Scheid, A., and Choppin, P. W. (1974). *Virology* **57,** 475–490.

Schorlemmer, H. U., Bitter-Suermann, D., and Allison, A. C. (1977a). *Immunology* **32,** 929–940.

Schorlemmer, H. U., Edwards, J. H., Davies, P., and Allison, A. C. (1977b). *Clin. Exp. Immunol.* **27,** 198–207.

Schwartz, L. W., and Christman, C. A. (1979). *Chest* **75,** 2845–2885.

Selinger, D., and Reed, W. P. (1979). *Infect. Immun.* **23,** 455–458.

Shewan, P. E., and Wilkie, B. N. (1982). *Infect. Immun.* **35,** 91–94.

Siskind, G. W., Schrater, A. F., Thorbecke, G. J., Weksler, M. E., and Goidl, E. A. (1982). *Cell. Immunol.* **66,** 34–40.

Slauson, D. O. (1982). *Adv. Vet. Sci. Comp.* **26,** 99–154.

Slauson, D. O., Lay, J. C., Castleman, W. L., and Neilsen, N. R. (1987). *J. Leuk. Biol.* **41,** 412–420.

Smith, H. (1977). *Bacteriol. Rev.* **41,** 475–500.

Smith, H. (1984). *In* "Bacterial and Viral Inhibition and Modulation of Host Defenses" (G. Falcone, M. Campa, H. Smith, and G. M. Scott, eds.), pp. 171–190. Academic Press, New York.

Sparling, P. F. (1983). *Rev. Infect. Dis.* **5** (Suppl), S637–646.

Splitter, G. A., and Eskra, L. (1986). *Vet. Immunol. Immunopathol.* **11,** 235–250.

Stanberry, L. R., Kit, S., and Meyers, M. G. (1985). *J. Virol.* **55,** 322–328.

Stendahl, O., and Coble, B. I. (1984). *J. Clin. Invest.* **73,** 366–373.

Stephens, D. B. (1980). *Adv. Vet. Sci. Comp. Med.* **24,** 179–210.

Stephens, S., Dolby, J. M., Montreuil, J., and Spik, G. (1980). *Immunology* **41,** 597–603.

Streissle, G., Paessens, A., and Oediger, H. (1985). *Adv. Virus Res.* **30,** 83–138.

Sugarman, B., Epps, L. R., and Stenback, W. A. (1982). *Infect. Immun.* **37,** 1191–1199.

Tashiro, M., Ciborowski, P., Renacher, M., Pulverer, G., Klenk, H. D., and Rott, R. (1987). *Virology* **157,** 421–430.

Taylor, C. E., Welser, W. Y., and Bang, F. B. (1981). *J. Exp. Med.* **153,** 732–737.

Till, G. O., and Ward, P. A. (1986). *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **45,** 13–18.

Till, G. O., Johnson, K. J., Kunkel, R., and Ward, P. (1982). *J. Clin. Invest.* **69,** 1126–1132.

Turner, R. B., Felton, A., Kosak, K., Kelsey, D. K., and Meschievitz, C. K. (1986). *J. Infect. Dis.* **154,** 443–447.

Twomanen, E., and Weiss, A. (1985). *J. Infect. Dis.* **152,** 118–125.

Unanue, E. R. (1976). *Am. J. Pathol.* **83,** 396–422.

Unanue, E., Beller, D. I., Calderon, J., Kiely, J. M., and Stadecker, M. J. (1976). *Am. J. Pathol.* **85,** 465–478.

Voetman, A. A. (1981). *J. Clin. Invest.* **67,** 1541–1549.

Voetman, A. A., Weening, R. S., Hamers, M. N., Meerhof, L. J., Bot, A. M., and Roos, D. (1981). *J. Clin. Invest.* **67,** 1541–1549.

Wadee, A. A., Sher. R., and Rabson, A. R. (1980). *J. Immunol.* **125,** 1380–1386.

Wainwright, W. H., Beltri, R. W., and Sprinkle, P. M. (1979). *J. Infect. Dis.* **140,** 22–32.

Walker, L., and Lowrie, D. B. (1981). *Nature (London)* **293,** 69–70.

Ward, P. A. (1986). *J. Allergy Clin. Immunol.* **78,** 373–376.

Weber, L., Peterhans, E., and Wyler, R. (1983). *Vet. Immunol. Immunopathol* **4,** 397–412.

Wewers, M. D., Rennard, S. I., Hance, A. J., Bitterman, P. B., and Crystal, R. G. (1984). *J. Clin. Invest.* **74,** 2208–2213.

Whitnack, E., Bisno, A. L., and Beachey, E. H. (1981). *Infect. Immun.* **31,** 985–991.

Wildy, P., Field, H. J., and Nash, A. A. (1982). *In* "Virus Persistence" (B. W. J. Mahy, A. C. Minson, and G. K. Darby, eds.), pp. 133–167. Cambridge Univ. Press, London.

Woods, D. E., Strauss, D. S., Johanson, W. G., and Bass, J. A. (1981). *J. Infect. Dis.* **143,** 784–790.

Woods, D. E. (1987). *Rev. Infect. Dis.* **9,** 5386–5390.

Worthen, G. S., Haslott, C., Smedley, L. A., Rees, A. J., Gumbay, R. S., Henson, J. E., and Houson, P. M. (1986). *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **45,** 7–12.

Yamada, K. M., and Weston, J. A. (1974). *Proc. Natl. Acad. Sci. U.S.A.* **71,** 3492–3496.
Yates, W. D. G. (1982). *Can. J. Comp. Med.* **46,** 225–232.
Yates, W. D. G., Babiuk, L. A., and Jericho, K. W. F. (1983a). *Can. J. Comp. Med.* **47,** 257–264.
Yates, W. D. G., Jericho, K. W. F., and Doige, C. E. (1983b). *Can. J. Comp. Med.* **47,** 57–63.
Yourtree, E. L., Bia, F. L., Griffith, B. P., and Root, R. K. (1982). *Infect. Immun.* **36,** 11–16.
Zor, U., Ben-Dori, R., Maoz, I., Wallach, D., and Gurari-Rotman, Z. D. (1982). *J. Gen. Virol.* **63,** 359–363.