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Immunity to human and bovine respiratory syncytial virus

Brief Review

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Summary. Human and bovine respiratory syncytial viruses resemble each other closely. During annual winter outbreaks, they cause similar respiratory tract disease in infants and calves. The disease is most severe in children and calves between 1 and 3 months old, when maternal antibodies against the virus are usually present. Reinfections, which are common, are accompanied by progressively milder illnesses in children, but are symptomless in calves. Because maternal antibodies suppress serum and mucosal antibody responses of all isotypes, the development of a vaccine that is effective in young children and calves with high levels of maternal antibodies has been severely hampered. Although virus administered intranasally to young calves with maternal antibodies does not evoke antibody responses, it can prime these calves for a protective memory response upon reinfection. Protection appears to be associated with the capacity to mount a mucosal memory IgA response. There are several indications that one or more immunopathologic mechanisms contribute to the disease. An Arthus reaction (type III) may have a role in the pathogenesis, because activated complement may cause most of the pathologic lesions, including edema and emphysema in uninfected parts of the lung. Lungs from calves with severe or fatal disease have depositions of complement component C3 and a low histamine content. The most immunogenic and protective antigen of the virus is the fusion (F) glycoprotein, which evokes a strong antibody response and is a target for cytotoxic T cells. On the F protein, epitopes that induce neutralizing and non-neutralizing antibodies, both of which may enhance complement activation, were identified. Immunity to the F protein may have beneficial and harmful effects.

Introduction

Human respiratory syncytial virus (HuRSV) and bovine respiratory syncytial virus (BRSV) are antigenically closely related and cause similar diseases. To-

gether with pneumonia virus of mice, they belong to the genus *Pneumovirus* of the family *Paramyxoviridae* [73]. HuRSV was discovered in the early 1950s. In the following decades its importance as a major cause of acute lower respiratory tract illness in children, particularly in the first year of life, was established. Infections occur worldwide and show a unique seasonal periodicity [133]. BRSV was first isolated in 1967 by Paccaud and Jacquier [108] in Switzerland. Serological studies in the 1970s and early 1980s clearly established that BRSV is one of the most important respiratory pathogens in older calves and yearlings [50, 131, 154]. Because maternal antibodies interfere with serological studies, BRSV was initially overlooked as a respiratory pathogen of calves younger than three months. When new diagnostic techniques were introduced, BRSV was found to be one of the most frequent and virulent causes of respiratory disease in young calves with maternal antibodies [30, 63, 66, 173]. The severity of HuRSV and BRSV-associated disease seems to be correlated with the level of exposure [42, 43, 46, 80, 121]. Stabling in combination with climatic conditions clearly seem to lead to numerous autumn and winter outbreaks in calves every year in Western Europe. How and where the virus survives between outbreaks is unknown.

Because there is no adequate animal model, progress in understanding the mechanisms of pathogenesis and immunity has been slow. Several species of animals, including calves, cotton rats, ferrets, and mice can be infected experimentally, but only mild clinical and pathological changes or none at all can be induced [27, 65, 112, 138]. Moreover, because the virus grows poorly in cell culture and is extremely labile, it is difficult to obtain large quantities of virus and viral proteins for experimentation. Because passively acquired maternal antibodies are of doubtful protective value, several investigators searched for other, more protective immune mechanisms. Indications for a role of the immune system in the pathogenesis of the disease in humans came from the enhanced disease that occurred when children, immunized with formalin-inactivated vaccine, underwent a subsequent natural infection [24, 85]. Because the vaccine-enhanced disease did not differ from the severe naturally occurring disease, a role of an immune-mediated mechanism was suggested, both in the vaccine-enhanced disease and in the naturally occurring disease. Striking pathological changes in children and calves with naturally occurring infections also suggested that immunological factors influence the development of the lesions [1, 67, 111]. The subject of the present review is the role of the immune system in protection against HuRSV and BRSV infections, as well as its role in the pathogenesis.

Properties of the virus

The genome of HuRSV is a single negative-sense strand of RNA composed of approximately 15,000 nucleotides. It contains one promoter, is transcribed as a single transcriptional unit, and encodes ten mRNAs each coding for a unique

protein. The order of transcription is 3′ 1 C-1 B-N-P-M-1 A-G-F-22 k-L 5′ [28, 29, 31]. The protein composition of HuRSV and BRSV strains is highly similar, with only minor differences in molecular weight between corresponding proteins [23, 77, 149, 174].

Eight of the ten viral proteins are structural; only proteins 1 B and 1 C are nonstructural. The major glycoprotein (G), the fusion protein (F), and the 1 A protein are glycosylated. They, together with the 22 k protein, are expressed on the cellular membrane [28, 105, 124, 136]. The F protein is synthesized as a 68 k precursor molecule (F_0), which is proteolytically cleaved into disulphide-linked 48 k (F_1) and 20 k (F_2) polypeptide fragments [159]. After proteolytic cleavage, the F protein causes the virus or host cell membrane to fuse with the membrane of uninfected cells. The G protein is the attachment protein of the virus [78]; in contrast to other paramyxoviruses, no hemagglutinin or neuraminidase activity is associated with this protein [123]. The G protein has a protein backbone of approximately 33 k, but due to heavy O-linked glycosylation, it has an apparent weight of 90 k. Arumugham et al. [7] found that a portion of the G protein is linked to the F protein by disulfide bonds. Three proteins, the nucleocapsid protein (N), the phosphoprotein (P), and the large protein (L), constitute, together with the viral RNA, the nucleocapsid of the virus [for reviews, see 28, 133]. The functions of the matrix protein (M), the 22 k, 1 A, 1 B, and 1 C proteins are unknown.

Two broad subgroups (A and B) of HuRSV have been defined based on the reactivity of monoclonal antibodies (MAbs) with various viral proteins. The major antigenic differences between the subgroups are found in the G protein, but differences have also been detected in the F, N, M, and 22 k proteins [4, 39, 92, 114]. The two subgroups do not appear to differ in virulence or in epidemiologic behavior. The prevalence of the HuRSV subgroups varies both during outbreaks and from outbreak to outbreak [49, 94, 152]. There is no evidence that this variation allows reinfections to occur.

BRSV strains share considerable antigenic homogeneity with both HuRSV subgroups, except for the G protein, which is antigenically distinct [77, 107, 139, 149]. It is not clear whether BRSV strains have more than one antigenic type, because only few isolates have been analysed. Although outbreaks of BRSV-associated disease do vary in severity, there is otherwise no indication for clinically relevant strain variation.

Whether bovine isolates can infect humans is unknown. A human isolate, however, has been shown to be pathogenic for calves [56]. Bovine and human isolates both replicate in vitro in cells of bovine and human origin, but bovine strains replicate better in bovine than in human cells, whereas for human strains the reverse is true [81].

Viral target antigens

In HuRSV-infected children, antibodies were predominantly induced against the F and N proteins, and, according to some studies, against the G protein [40, 79, 96, 153, 165]. In BRSV-infected calves, the F and N proteins also were

found to be the most immunogenic proteins [174]. In both humans and cattle, antibodies may also be directed against the L, P, M, 22 k, and 1A proteins [79, 101, 165, 174]. The antigenicity of the 1B and 1C proteins is unknown.

Although the sensitivity of the assays used in several studies may be questionable, the G protein appears less immunogenic than the F protein [40, 79, 70, 153, 165, 174]. It has been suggested that the high carbohydrate content (65%) of the G protein may be responsible for the poor immune response [165]. However, Wagner et al. [157, 158] provided evidence that the G and F glycoproteins are both recognized by the human immune system as typical protein antigens, because both proteins elicited primarily IgG1 and IgG3 responses. Carbohydrate antigens primarily elicit IgG2 and IgG4 responses. The G protein is also poorly recognized by cytotoxic and helper T cells [10].

Immunizing mice or cotton rats with vaccinia recombinant viruses (VRV) and with purified proteins demonstrated that the F and G proteins are the major antigens that protect against challenge; these proteins also induce neutralizing antibodies [35, 72, 104, 125, 134, 162, 172]. Animals immunized with VRV that expresses the N protein (VRV-N) were less protected than those immunized with VRV that expresses either the F (VRV-F) or the G (VRV-G) protein [72], and those immunized with VRV-F were better protected than those immunized with VRV-G [104, 135]. Mice and cotton rats immunized with VRV-G that expresses the G protein of subgroup A HuRSV were protected against challenge with subgroup A, but not with subgroup B HuRSV. Those immunized with VRV-F that expresses the F protein of subgroup A HuRSV were protected against infection with either subgroup [135].

Several laboratories have identified four to five antigenic sites on the F protein, three to four of which are involved in neutralization [14, 107, 126, 147, 161]. MAbs that are directed against at least two of the antigenic sites involved in neutralization also inhibit fusion [14]. Using MAb-resistant mutants, several epitopes within the neutralization sites have been identified [14]. A peptide that was defined by amino acids 221–232 within the F_1 region of the F protein was identified as part of the binding site for neutralization and fusion-inhibiting MAbs [148]. The G protein of subtype A contains at least three antigenic sites and the G protein of subtype B at least two. Two of the antigenic sites of subtype A and both antigenic sites of subtype B were neutralizing sites, although not all MAbs directed to these sites neutralized the virus in vitro. One neutralizing site was shared by both subgroups [5, 151, 163]. However, neutralization of the virus is complex, and antibodies directed against different antigenic sites on the F or the G protein can act together to neutralize the virus (from partial or no neutralization to complete neutralization) [6, 163]. Effective neutralization is seen primarily with MAbs against the F protein [4, 136]. Using a series of overlapping synthetic peptides, Nicholas et al. [101] identified an antibody-binding site on the 1A protein (residues 51 through 60) that may be recognized during natural human infection.

Using VRV-G, -N, and -F, it was shown that the N and F proteins are the major antigens recognized by cytotoxic T cells in mice and humans [11, 22, 72,

110] and by helper T cells in mice [106]. The 1A protein is also recognized by helper T cells in mice. The extracellular C-terminal domain of the 1A protein contains two overlapping epitopes which stimulate T helper cells and which can be distinguished by different class II MHC restriction elements [101, 102].

Protective immunity

Reinfections with HuRSV and BRSV occur readily. Children may have severe disease after the second infection with HuRSV, but subsequent infections gradually decrease in severity [48]. This partial protection is somewhat greater against viruses of the same subgroup [93]. HuRSV infection sometimes causes clinical signs of disease in adults [155] and can cause severe disease in the elderly [90]. Reinfected calves do not develop clinical signs of disease. Older cattle that contract primary infections can develop severe disease [55]. Interestingly, BRSV was first isolated from adult cattle with respiratory disease [108].

Antibodies in serum

Several field and laboratory studies of different species have failed to show a clear correlation between the level of actively or passively acquired serum antibodies (usually determined in virus neutralization tests) and protection. Age, species, and quantity of antibodies in serum seems to determine the level of protection [57, 66, 70, 76, 87, 89, 109, 115, 117, 132, 165]. Passive transfer of MAbs directed against the F and G proteins can reduce virus replication in the lungs of mice and cotton rates [115, 136, 139, 160]. MAbs directed against the F protein were more effective in neutralizing the virus in vitro and were more protective in vivo than MAbs directed against the G protein [4, 136, 139]. However, there was no correlation between neutralizing or complement-dependent cytotoxic activity of the passively transferred MAb and its protective effect, which suggests that other mechanisms may also be involved in protection [139]. In addition, not all neutralizing MAbs were protective and non-neutralizing MAbs may provide protection in vivo [139, 163]. Antibodies may act in association with effector cells in antibody-dependent cell-mediated cytotoxicity. Inhibition of cell fusion appears to be essential for the protective effect of MAbs directed against the F protein [136]. MAbs directed against the N protein did not protect [139]. Passively transferred antibodies that restrict virus replication in the lung have only a slight effect on virus replication in the nose [160, 175]. This suggests that the close contact between blood and alveolar lumina allows passive diffusion of protective serum IgG into the deeper airways, while the upper respiratory tract remains unprotected. Whether the observations made in mice and cotton rats can be extended to the natural hosts of HuRSV and BRSV is questionable. Reportedly, experiments are being conducted that will establish the protective capacity of neutralizing bovine MAbs directed against the F protein in calves [60].

Maternal antibodies

Both in infants and calves, maternal antibodies are universal, probably as a result of frequent reinfections in older individuals. Hornsleth et al. [51] determined that maternal antibody in children was entirely of the IgG1 isotype, although theoretically some IgG3 might have been present. Murphy et al. [96, 97] and Levine et al. [79] found that maternal antibodies of infants react with the F and G proteins. In calves, maternal antibodies are of the IgG1 isotype, they are only found in serum, and they have a half-life of 23 days. They are not actively transported to mucosal surfaces. Maternal antibodies of calves are predominantly directed against the F and N proteins [64, 65, 174]. Some calves also have maternal antibodies against the G protein. Calves, in contrast to humans, acquire maternal antibodies only via colostrum.

Maternal antibodies suppress serum and mucosal antibody responses of all isotypes, despite extensive replication of the virus. In calves, the IgM responses appeared the least sensitive to suppression [65, 173]. Murphy et al. [97] demonstrated that maternal antibodies may cause poor and irregular responses against the F and G proteins even in children as old as 8 months. Although the antibody response to whole virus is strongly inhibited by maternal antibodies, responses to individual viral proteins, notably the F and P proteins, can sometimes be detected [70, 79, 174]. In cotton rats, antibody responses to VRV-expressed F and G proteins were suppressed by passively transferred HuRSV immune serum, but antibody responses to the vaccinia virus antigens were not [98]. Bangham [10] demonstrated that passively acquired antibody in newborn mice may not only impair the antibody response but also the generation of specific cytotoxic T cell precursors.

Although maternal antibodies play a crucial role in regulating the antibody response in young animals, remarkably little research has been done on this subject. In our studies, immunization via the respiratory tract did not prevent the priming for memory responses in calves with maternal antibodies [65, 70]. Thus, maternal antibodies did not completely prevent the processing of antigen, the recognition of antigen by B and T cells, or the induction of certain memory cells. Maternal antibodies may interfere with the forming of antibody-secreting B cells, possibly via suppressor or helper T cells [47, 120]. Other mechanisms may be the limiting of virus replication, the enhanced clearance of antigen, and idiotype interactions.

The immaturity of the immune system might also impair the immune response in young children [96, 109]. There are no indications that age affects the immune response of calves [65, 133].

The influence of maternal antibody on the outcome of disease

The influence of age and maternal antibodies on infection in young infants and calves has been investigated in several studies, but the results have often been conflicting [8, 45, 61, 65, 66, 76, 86, 89, 103, 165]. In hospitalized infants, the

severity of disease peaks in the second month of life, when maternal antibody is still universal [109]. In hospitalized children with severe disease there is no correlation between serum antibody level and severity of disease [15, 109]. There is, on the other hand, evidence that maternal antibody may provide some, yet incomplete, protection. Infants less than 3 weeks old, who have the highest levels of maternal antibody, are relatively spared from severe disease. Maternal antibody reduces virus shedding, and there is a correlation between the level of neutralizing maternal antibody at birth and the age at the time of infection [24, 44, 45, 76, 103, 165]. Ward et al. [165] found that a high level of maternal antibody directed against the N protein was associated with protection against disease.

During the seasonal circulation of the virus among cattle, disease can frequently be observed in calves of 2 weeks old and older. Most cases of severe disease develop in calves from 1 to 3 months old, nearly all of which still have maternal antibody [66]. However, both the incidence and severity of diseae in calves younger than 3 months were inversely related to the level of BRSV maternal antibodies. Thus, under field conditions, maternal antibodies do not effectively prevent BRSV-associated disease, but they do appear to mitigate it [66]. Under experimental conditions, calves with maternal antibodies can easily be infected [65, 89]. In newborn cotton rats, antibodies acquired via the placenta and from breast milk reduced the replication of virus in the lungs, but not in the nose [175].

Because most severe disease occurs both in calves and in children when maternal antibodies are present, it has been suggested that these antibodies may aggravate disease by an antigen – antibody reaction [66, 103]. Perhaps the ratio between antibodies directed against protective and nonprotective epitopes is a factor that determines the severity of disease (see below). Murphy et al. [98] found that in cotton rats the antibody response to neutralizing epitopes on the F protein was disproportionately more suppressed by passively transferred hyperimmune serum than the response to nonneutralizing epitopes on the same protein. Alternative explanations for the severe disease in children and calves with maternal antibodies may be that young age as such predisposes to severe disease, or that maternal antibodies suppress the immune response needed to clear the infection. No support was found for the latter possibility, however, after experimentally induced infection in calves [65, 70], but cotton rats, whose immune response were surpressed by passively transferred antibodies, were more susceptible to infection than control animals [98]. Both calves and infants can recover from infection without any detectable serum or secretory antibody response [64, 65, 83].

Some epidemiologic studies of HuRSV have suggested that breast feeding has a beneficial effect on infections, although conflicting evidence has also been reported [33, 42, 119, 137]. The possible protective mechanism of breast-feeding has not been identified with certainty, but may be related to interferon [25, 100, 133]. Colostrum and milk further contain abundant IgA, some of which

is HuRSV-specific and which may be excreted in nasal secretions of the newborn. HuRSV reactive lymphocytes are present in the colostrum of 30–40% of mothers [33, 129], but their significance is doubtful.

Antibodies at mucosae

Evidence for a beneficial effect of mucosal IgA in HuRSV infections is limited. The appearance of specific IgA and the disappearance of virus after the first infection were closely related in time [83, 84]. However, calves with maternal antibodies, which had completely suppressed mucosal IgA responses, did not shed virus any longer than calves without maternal antibodies [65]. Moreover, children that do not have a secretory antibody response because of suppressive maternal antibody can normally recover from infection [83]. In adult volunteers, resistance to infection and illness appeared to be correlated with high levels of neutralizing antibody in nasal wash at the moment of infection, but not with the level of neutralizing antibody in serum [88].

We studied the mucosal antibody response of calves in detail [64, 65, 70]. BRSV-specific IgM appeared 8–10 days after a primary infection in blood, and in samples collected from the eye, nose, lungs, and even the intestine; shortly afterward, IgA appeared [65]. The antibodies remained detectable for various lengths of time. BRSV-specific IgG1 and IgG2 appeared later and were only detected in serum. In maternally immune calves, antibody responses were undetectable or detectable only for short periods and at low titers. All calves, with or without maternal antibodies, excreted virus in about equal amounts and for the same period of time. After reinfection, 3 to 4 months later, memory responses were observed in serum and on the mucosae in calves with or without maternal antibodies. Memory responses were characterized by strong and rapid increases (from day 6 after inoculation) in mucosal and serum IgA as well as increases in serum IgG1 and IgG2. Also, strong mucosal, but not serum, IgM responses were observed, but they did not develop faster than they did after primary infection. Memory responses were even detected in calves that had not developed an antibody response after the primary infection. Maternal antibodies, present at the time of priming, adversely affected the maximum antibody titers after challenge, either because priming was inefficient or because the immune response continued to be suppressed. After reinfection, none of the calves, with or without maternal antibodies at the time of priming, excreted virus.

Virus administered intramuscularly to seronegative calves failed to induce a mucosal antibody response, but did prime for a mucosal memory response [70]. This finding indictates that immune cells circulate, before or after challenge, from peripheral lymph nodes to the mucosae, as well as circulating between the mucosae. The memory response in intramuscularly immunized calves, however, started somewhat later than in calves primed intranasally and did not prevent virus excretion. Because virus excretion peaks on day 5 or 6 after inoculation [65, 70], rapidity of the IgA memory response may be im-

portant for protection. The mucosal memory response may be more rapid after intranasal priming because local memory cells are activated. Parenteral immunization may be effective because it primes for mucosal memory. In the same study, seronegative calves were immunized with inactivated virus via the respiratory tract and maternally immune calves were immunized with live virus intramuscularly. These calves were the least effectively primed for mucosal memory and also excreted virus after challenge [70]. The following conclusions were drawn [70]:

- Protection against virus excretion was not so much associated with the presence of IgA on the mucosae at the time of challenge, as with the capacity to mount a mucosal memory response. The presence of local IgA does indicate that the mucosae have been primed; but even when local IgA is not present, mucosae may still be primed.
- Intranasal immunization with live virus can prime the mucosae of calves (even those with maternal antibodies) for antibody memory responses.
- Intramuscular immunization with live virus, which probably results in limited replication of virus at the site of inoculation [75, 113, 176], can prime mucosae, but the memory response after challenge appeared somewhat delayed and did not prevent virus excretion.
- In contrast with intranasal immunization, intramuscular immunization with live virus did not prime for mucosal memory in calves with maternal antibodies.

We could not conclude whether IgA alone protects, or whether other mechanisms, such as cytotoxic T cells or killer cells, work in conjunction with it. Mazanec et al. [82] has demonstrated that monoclonal IgA directed against neutralizing epitopes on the haemagglutinin-neuraminidase molecule of Sendai virus protected mice against the virus when administered to the respiratory tract. We and others could not demonstrate neutralizing activity of IgA, which may reflect low avidity or differences in the sensitivity of the assays [64, 83]. IgA may be active in antibody-dependent cell-mediated cytotoxicity, but probably plays no role in complement-mediated cell lysis [69].

T cell-mediated immunity

Little is known about the role of T cells in the recovery from and protection against disease. Proliferating T cell responses have been detected after infection of calves and infants [128, 141], but their function and role in protection is unknown. Virus-specific MHC-restricted cytotoxic T cells have been detected in mice, cotton rats, and humans [9, 10, 75, 140]; helper T cells have been detected in mice [106]. Murine helper and cytotoxic T cells appear to be partly subgroup-specific [12, 22, 106, 110]. Non MHC-restricted natural cytotoxicity has been detected in cotton rats [75]. T cells seem to be beneficial because infants and mice with a defective cell-mediated immune response are unable to eliminate a HuRSV infection [37, 140]. Moreover, immunization with VRV-N limits virus replication in cotton rats [72]. Transfer of primed T cells can

clear persistent HuRSV infection in immunodeficient mice [20, 110]. Transfer of specific cytotoxic T cell lines and clones into infected mice also resulted in virus clearance, but was associated with a lethal respiratory disease, characterized by hemorrhage and neutrophil influx [21]. It is unclear whether this phenomenon has anything to do with naturally occurring disease.

Role of immunity in pathogenesis

The role of immune mechanisms in the pathogenesis of HuRSV disease has been the subject of numerous studies [133, 170]. These studies were spurred not only by the high incidence of severe disease in children with maternal antibodies, but also because formalin-inactivated vaccine actually enhanced the disease. Evidence has been provided that formalin destroys epitopes that bind neutralizing and fusion-inhibiting antibodies on the F or G protein or both [95, 99, 116]. Formalin-inactivated vaccine induces predominantly "non-functional" antibodies, which can bind virus, but cannot neutralize infectivity or inhibit cell fusion. "Non-functional" antibodies present at the time of infection or accelaratively produced after infection, may enhance disease by causing an Arthus reaction (type III). The role of these antibodies in complement activation or in complement-mediated cytotoxicity was, however, not examined. The F protein in particular seems capable of inducing "non-functional" antibodies [14, 125]. Some MAbs that are directed against the F protein and neutralize a given strain bind to other strains, but fail to neutralize them [14]. Prince et al. [116] enhanced the disease in cotton rats by using fomalin-inactivated vaccine and found histologic evidence for an Arthus reaction 24 h after challenge. A second influx of neutrophils and lymphocytes 4 days after challenge suggested that also a delayed type hypersensitivity reaction (type IV) may develop.

Some clinical findings suggest that antibody is also involved in the pathogenesis of BRSV disease in calves. Calves with maternal antibodies have a high frequency of severe disease [66, 133]. Severe disease also occurs, however, in older calves that are seronegative at time of infection. These calves show the characteristic abdominal breathing just before a vigorous IgM, IgG1 and IgA response can be measured. At that time, low levels of neutralizing antibodies can already be detected [50, 64, 67, 111, 166]. Thus, if disease is indeed enhanced by antigen – antibody interaction, this may occur at low antibody levels. There are no indications for prior sensitizing infections, because only calves that have not been exposed to earlier periods of virus circulation become ill [50, 154]. One case of naturally occurring BRSV infection indicated that vaccination with modified live vaccine during the infection may have also enhanced the severity of disease [71].

Welliver et al. [169] have postulated that an anaphylactic hypersensitivity reaction (type I) may develop in children with HuRSV infections. Anaphylaxis is mediated by cytophilic antibodies on mast cells, which degranulate on binding of antigen. They assumed that a defect in the suppressor cells may lead to

uncontrolled IgE synthesis, mast cell degranulation, and bronchoconstriction. Two groups of investigators found an association between early and high anti-HuRSV IgE antibody levels in secretions and serum and the severity of lower respiratory tract disease [17, 169, 170]. However, not all children hospitalized for a HuRSV infection have detectable antiviral IgE [169, 171], and it is questionable whether IgE actually reaches high levels in the early stages of primary infection. Unfortunately, virus-specific IgE assays have been difficult to develop, and their results have not been confirmed in other laboratories [144].

In cattle, anaphylaxis can be mediated by IgG1 and probably also by IgE [19, 142]. Stewart and Gershwin [130] failed to find a clear correlation between BRSV-specific IgE concentration in serum and clinical signs of disease after experimentally induced infection. They noted that, in contrast to the naturally occurring situation, clinical signs of disease after primary and secondary infections were similar. This finding suggests that hypersensitivity to cell culture components might have occurred. Unfortunately, IgE responses have not yet been examined in calves with severe natural disease.

Antibody may possibly also enhance disease by facilitating the infection of monocytes and macrophages. Antibody may bind with the virus and then bring the virus in contact with Fc receptors on these cells [41, 74]. The Fc receptor-bearing cells are not the primary target cells of HuRSV, but these cells can be infected in vitro, and HuRSV antigen has been found in circulating monocytes after naturally occurring infections [32, 74]. When cells bearing Fc receptors become infected or interact with the virus, they may release leukotrienes and platelet-activating factor, which could induce bronchoconstriction [36, 146].

It has also been proposed that a cell-mediated immune reaction might contribute to the pathogenesis of the disease, but firm evidence was not provided [62, 167].

Lesions

In naturally infected calves with respiratory distress or that succumbed, severe lesions were observed: consolidation of the cranioventral (CV) lung, severe edema and emphysema throughout the lung, and signs of severe dyspnea, such as cyanosis and widespread hemorrhages [67, 111]. The emphysema appears to be caused by widespread bronchoconstriction. The edema and emphysma likely cause the severe dyspnea. Hyaline membranes accompanied by lung parenchymal necrosis were often detected in both the CV and caudodorsal (CD) lung. The virus, however, was only detected in the CV lung [67]. Thus, a major question is what causes the severe changes in blood vessels and smooth muscles in parts of the lung where no virus is detected? In the CV lung, viral cytopathologic changes were detected in bronchiolar and alveolar epithelium and were accompanied by inflammation. Some few eosinophilic leukocytes were found in both the CV and CD lung. Dyspnea probably develops late in the infection: it usually lasted only a short time, whereas the inflammation in the

CV lung often already had chronic characteristics, such as epithelial hyperplasia, fibrosis, and bronchiolitis obliterans. In addition, all calves that died or were killed during severe dyspnea had antiviral IgG 1 or IgM or both [67]. These antibodies were directed against the same viral proteins as antibodies of calves that recovered [174]. BRSV-specific IgA was usually not detected, either in serum or in lung lavage fluid. Whether the absence of specific IgA is an immunologic defect is unknown. A deficiency in IgA has also been reported in children with fatal infections [3]. Chronic lesions may develop in calves because it takes time to produce sufficient viral antigen or antibody or both to initiate an immune-mediated reaction. Sometimes two stages of disease are observed shortly after each other, which may be in agreement with this suggestion [8, 50]. Because a few very young calves had only acute bronchiolar lesions [67], severe disease may develop earlier in calves with high levels of maternal antibodies. The lesions detected in the CV lung of calves closely resemble those in infants with fatal HuRSV disease [1]. Widespread alveolar lesions in parts of the lungs that are not infected have not been reported in children, however.

Complement component 3 (C3) was detected in the CV lung, but although antigen and antibody were both present, immune complexes were not found [68]. Fixation of C3 to exfoliated airway epithelial cells was also found in children with HuRSV infection [58]. A low histamine content of both the CV and CD lung, together with small numbers of mast cells and mast cell granules, indicated mast cell degranulation [68]. Complement and mast cell activation are probably linked, because activated complement components C3a and C5a (anaphylatoxins) are known to liberate histamine and other mast cell mediators. Virus infections can probably further enhance the release of histamine through interferon [18, 54]. Evidence has been provided that several secondary mediator systems are recruited by anaphylatoxins, including vasoamines, prostaglandins and leukotrienes. The activity of these mediators is synergistic. In nasopharyngeal samples from HuRSV-infected infants leukotriene C4 has been demonstrated [156]. Anaphylatoxins enhance vascular permeability, smooth muscle contraction, and chemotactic attraction of neutrophils. Anaphylatoxins can mediate acute, often fatal, lung injury, after either intravascular or intrabronchial instillation. Characteristics of the response are prolonged bronchospasm, hyperinflation of the lungs, increased vasopermeability, and cellular infiltration [52, 53].

In conclusion, the extent to which the virus itself, complement, mast cell mediators, or other mechanisms contribute to the final disease is unclear. Anaphylaxis and delayed type hypersensitivity might be involved in the pathogenesis but these mechanisms do not explain the widespread lesions and mast cell degranulation detected even in the absence of antigen; there are no histological indications for delayed type hypersensitivity. We may postulate an important role for an Arthus reaction, because activated complement would explain the following findings:

- pulmonary edema and emphysema,

– lesions outside the focus of infection, assuming circulation of activated complement components,
– aggravation of disease by IgG1 or IgM. This would also explain the severe disease in advanced stages of infection when chronic lesions and antibodies are present.
– severe disease thanks to the amplification loop of the alternative pathway of complement,
– release of mast cell mediators,
– neutrophil influx in infected parts of the lung.

Activation of complement

In the absence of antibody, HuRSV- and BRSV-infected cells activate more complement than uninfected cells. Antibodies increase complement activation and cooperation of antibody and complement is required to induce complement-mediated lysis [59, 69]. In a homologous bovine system, complement activation was enhanced by specific IgG1 and IgM, but not by IgA and IgG2. Antibody-enhanced complement activation was largely mediated by the alternative pathway and caused cell lysis [69]. Murine MAbs directed against neutralizing and non-neutralizing epitopes on the F protein can also enhance complement activation. One MAb of the IgG1 isotype and directed against a non-neutralizing epitope on the F protein enhanced C3 binding to infected cells, but did not induce complement-mediated lysis [69]. Thus, IgG1 or IgM antibodies directed against this epitope may activate the adverse inflammatory effects of the complement system without inducing beneficial lysis and without neutralizing the virus. Once more is known about epitopes that induce protective or non-protective responses, it would be worthwile to measure epitope-specific immune responses. Individuals may respond differently to different epitopes, as demonstrated for hemagglutinin of influenza virus [164].

Whether antibodies directed against other surface proteins (i.e., the G, 22 k, and 1A proteins) can also enhance complement activation is unknown. During the acute stages of the disease, however, anti-G antibodies are probably not important in activating complement. The G protein seems less immunogenic and antibodies against the G protein were usually not detected in sera of calves collected during the acute phase or postmortem [174].

Besides having an inflammatory effect, complement activation may also help in recovery from the disease. Complement activation, whether in the presence or absence of antibody, may lead to the interaction of neutrophils bearing complement receptors with infected cells and thus cause the destruction of these cells [59].

Role of antibodies in diagnosis

The standard method of diagnosing HuRSV infections is to isolate virus in cell culture. Nasal washes give more successful results than nasal or throat swabs, or tracheal aspirates [145]. Because antiviral therapy requires a more rapid

diagnosis than is possible with cell culture, several groups developed methods for rapid identification of viral antigen by immunoassay [2, 26, 38, 170]. The specificity and sensitivity of these assays is usually good, and antigen may even be identified in samples that are negative in cell culture. Attempts to isolate virus from nose swabs collected from calves has rarely been successful [16, 34, 111, 143, 166], perhaps because the virus mainly replicates in the lung [67]. Detecting virus or viral antigen in material collected by lung lavage has improved BRSV diagnosis, especially in calves with maternal antibodies [63].

Because of suppressive maternal antibody, classic serodiagnostic techniques are quite insensitive for diagnosing infection in infants and calves younger than 3 months [66, 122, 127, 168]. Some maternally immune children and calves that do not have an increase in serum IgG level upon infection maintain IgG titers for several months [103, 173]. Although IgA and IgM responses confirmed infection, maintained IgG titers do not seem feasible for routine diagnostic use. In a small study of infants from 1 to 3 months old, increases were more often detected in IgG3 antibodies than in IgG1 antibodies [51]. This finding indicates that IgG3 may be valuable in diagnostics, although it has only a brief half life (seven days).

Detecting specific serum IgM proved to be useful in diagnosing infection in calves with and without maternal antibodies [66, 173]. In calves older than 3 months from herds with BRSV-associated disease, 80 per cent showed an increase in IgG titer in paired sera against BRSV and 77 per cent had specific serum IgM. In contrast, only 10 per cent of the calves younger than 3 months showed an increae in IgG titers, while 51 per cent had specific IgM. In calves older than 3 months, BRSV-specific IgM was detected for 12 to 37 days [66]. However, sampling in the acute stage of disease may be too early, and in calves younger than 3 months, the IgM response may be so short-lived that it is easily missed [65, 173]. HuRSV-specific IgM responses have also been evaluated for the diagnosis of acute infection in humans. Though easily detected in infants of more than 3 months, IgM was only found in a minority of HuRSV-infected children between 1 and 3 months [170, 171]. More frequent sampling may increase the detecting of IgM responses in this age group. Specific IgA has not been evaluated for diagnostic use on a large scale [65, 96]. For diagnosing infection in calves, specific IgM has appeared to be superior to IgA, although this finding may be the result of different sensitivities of the assays [65].

Epilogue

An effective and safe vaccine that can be administered to very young children and calves with high levels of maternal antibodies is clearly needed. The live attenuated vaccines available for calves are ineffective in calves with maternal antibodies. The WHO has given high priority to the development of a HuRSV vaccine [118]. The lack of a good laboratory animal model severely hampers vaccine development, as it does the study of the pathogenesis. If continued

efforts to improve animal models fail, vaccine candidates should be tested in cotton rats, calves, or nonhuman primates before being tested in the field; virus contents of lungs or lung lavage fluid and the development of lesions can be used as parameters to measure protection. Because naturally infections do not induce protection against reinfection in either humans or cattle [50, 88, 132], attempting to prevent infection by vaccination is probably unrealistic. The goal of vaccination must therefore be to prevent clinical disease. A live vaccine administered intranasally may give the best results. Mucosal immunization not only seems more effective than parenteral immunization, it may also be effective in the presence of maternal antibody, and leads to IgA antibodies, which are less likely to contribute to immune-mediated pathogenesis than IgG1 or IgM. Recombinant DNA technology may be able to create such a vaccine, but classical methods of vaccine development should not be neglected. Another possibility, at least in cattle, is the use of inactivated antigen in an immunogenic form and in combination with an adjuvant. Examples are immunostimulating complexes (ISCOMs), made from viral surface proteins absorbed to the adjuvant Quil A, and glutaraldehyde-fixed infected cells [132, 150].

Vaccine may be developed by constructing a vector organism that expresses one or more antigens of the virus. Candidate antigens are not only the F and G proteins, which have been shown to be important for protection, but also the N protein. The N protein has been shown to afford a significant degree of protection against HuRSV in mice and has also been demonstrated to be a target protein for murine and human cytotoxic T cells [13, 35, 72, 104, 125, 135, 139]. The safety of such a vaccine may be a problem, because the F and G proteins might alter host and tissue tropism of the vector and virulence [135]. Recombination with wild-type variants of the vector may be another risk. Vaccine may also be developed by the introduction of (small) deletions or mutations in the genome to reduce the virulence of the virus without losing its protective ability. Such an approach might also establish the function of viral structures [91]. Because the F protein functions both in protection and pathogenesis (fusing cells and mediating complement activation), it would particularly be interesting to examine the effect of specific deletions or mutations in the F gene. Mutations that decrease the cleavability of the F protein might decrease virulence. Deletion of one or more non-neutralizing epitopes, which in particular are suspected to participate in immunopathology, might also decrease virulence.

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