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THE PATHOLOGIES OF BOVINE VIRAL DIARRHEA VIRUS INFECTION

A Window on the Pathogenesis

Helle Bielefeldt-Ohmann, DVM, PhD

Because a *particular* RNA virus simply does not exist, a *particular* RNA virus disease does not exist either.

J. J. HOLLAND et al[75]

INTRODUCTION

The pathologic lesions caused by bovine viral diarrhea virus (BVDV) infections comprise a wide spectrum of both types and degrees, and are, like the clinical manifestations,[6, 102, 114] a result of the interactions of factors such as strain and biotype of the virus (see the article by Donis, this issue),[35, 36, 51–53, 60, 77, 113, 116, 121, 122] the host genotype, age, and immune status of the animal at the time of infection, the immune response induced, and intercurrent infections or other stress factor(s).[2, 29, 44, 45, 96] Some of the lesions are not specific for BVDV, reflecting the limitations in tissue reactions to noxae or parallels in pathogenic mechanisms. In other cases, only microscopic or submicroscopic changes are present, and, thus, not apparent in a routine postmortem examination. Other lesions, or rather simultaneous occurrence of a set of lesions, however, may be near pathognomonic for BVDV, and when combined with clinical manifestations and epidemiologic data, they can have conclusive diagnostic value. The latter may be the case for "typical" mucosal disease (MD) and for the occurrence of congenital defects of epidemic proportions. With the improvements in and wider application of virus diagnosis in clinical practice, however, it has become apparent that BVDV may be the cause of a much wider spectrum of clinical and

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pathologic phenomena, some of which in the past probably went undetermined.[96]

Traditionally, the pathologic (as well as clinical) manifestations of BVDV infection are presented under the categories (1) fetal infections, (2) acute virus diarrhea, (3) MD. This categorization will largely be maintained, although it should be emphasized that these groupings are only truly useful for didactic purposes, and that extensive overlap can be expected in the occurrence and severity of any particular type of lesion, between animals or outbreaks,[14, 18, 36, 113, 114] and that pathologic lesions can only be suggestive of a BVDV infection. A final diagnosis must rest on isolation of the virus and/or detection of specific viral antigen in appropriate samples.

In this section, special emphasis will be given to the histopathologic features and immunocytochemical analysis in persistently infected (PI) animals, whether clinically normal or suffering from MD, as a basis for a final discussion of pathogenic mechanisms, which, in addition to the virus replication per se (see articles by Donis and Bolin, this issue), may contribute to the varied picture of lesions in BVDV infections.

CONGENITAL INFECTIONS

Transplacental spread of BVDV during a clinically inapparent or apparent viremia of the dam may be the most crucial event in the entire BVDV complex (see articles by Moennig and Bolin, this issue). Considering the difficulties often encountered in field cases in retrospectively establishing the exact time for the fetal infection, much emphasis has been given to the experimental evidence of the gestational age-dependency of particular types of pathology. Although this temporal categorization will not be disputed and is used here, it should be remembered that the possibility that some BVDV strains may have relatively low pathogenicity; therefore, their effect may be more insidious or slow, resulting in morphologic and/or clinical manifest changes only after a *prolonged* effect on the germinal tissues.[14, 17, 111, 146] Additionally, lesions that have been examined and seem to be similar in the perinatal period of a full-term calf may be the endstage of basically different processes, e.g., interference with normal stem cell differentiation and tissue development versus repair processes after an inflammatory process.[12, 14, 41, 72] Conclusive differentiation between these possibilities based on morphologic criteria may not be possible.[14]

Fetal infections in the first trimester of gestation may result in embryo/fetal death followed by absorption, mummification, or abortion, with expulsion occurring at any time up to several months later. Pathologic and virologic examinations, therefore, are rarely informative if performed on isolated cases at the time of expulsion.[10, 105] As previously suggested, some of the congenital defects observed in full-term calves that often are ascribed to infections in the second trimester actually may have been caused by infections at the time of organ blastems

differentiation in the first trimester, whereas an infection in the second trimester is more likely to provoke an inflammatory response and result in reparatory rather than regenerative processes and, thus, organ defects.[14, 40, 41] The teratogenic lesions recognized as possible consequences of a intrauterine infection with BVDV are listed in Table 1.

The ocular and central nervous system (CNS) lesions have attracted special attention, both in studies of field cases[14, 34, 58] and in experimental work.[40, 41, 72, 127] The chorioretinopathy often consists of variable depigmentation and loss of neurons as well as cone- and rod-cells (Fig. 1). The lens may be affected by capsular cataract and degenerative changes in the lens fibers,[40] and there may be signs of inflammation of the cornea and gliosis in the optic nerve.[14]

The cerebellar hypoplasia may be constituted by subtle changes such as reduction of the molecular layer and granule cell numbers, as well as reduction and displacement of Purkinje cells,[14, 144] frank loss of overall structure, and total loss of Purkinje cells.[41, 58] In the latter cases, the remains of an inflammatory process such as leukocyte infiltration may still be evident.[41] In other cases, demyelination seems to be the most prominent histopathologic finding.[34, 58] So far studies of virus distribution in CNS lesions have been inconclusive with respect to pathogenic mechanisms.[14, 72, 144] It also remains unresolved whether these variations are the result of differences in virus strains (pathogenicity and tissue tropism), gestational stage at initial infection, host genotype, a combination of these, or some other factor(s), including the purely technical.[144] The fact that only one or a few of the possible teratogenic lesions previously listed are seen in any one field case/outbreak could be explained by several of these factors.

The thymus hypoplasia/atrophy has attracted much attention and

Table 1. CONGENITAL DEFECTS ASSOCIATED WITH FETAL BVDV INFECTION

Gross Pathology	References*
Micro-, hydran-, por-, and hydroencephaly	14, 72, 144
Cerebellar hypoplasia	14, 34, 41, 58, 78, 127, 144
Dysmyelination of the spinal cord	34, 58
Cateracts	14, 114
Chorioretinopathy or microphthalmia	14, 40, 78, 127
Optic gliosis or neuritis	14
Thymus hypoplasia or atrophy	14, 58, 127
Alopecia, hypotrichosis	6
Curly hair coat	88
Brachygnathism	114
Arthrogryposis	14
Deranged osteogenesis	11, 49
Pulmonary hypoplasia	6
Generalized growth retardation	14, 18, 58, 88

*List is not exhaustive. For further references see Baker,[6] Bielefeldt Ohmann,[14] and Murray.[105] See also Bielefeldt Ohmann[14] for references to other known bovine feto-pathogens and the differential diagnostic features.

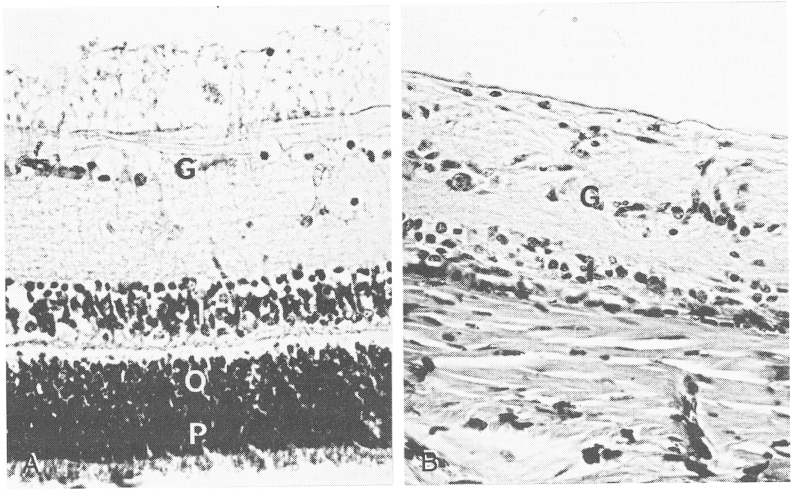

Figure 1. *A*, Retina from a normal bovine fetus, gestational age 7 months. P = Photoreceptor layer; O = outer granular layer; I = inner granular layer; G = ganglion cell layer. (Hematoxylin-eosin, original magnification, × 850). *B*, Severely atrophic retina in calf following congenital BVDV infection late in the first trimester of gestation. Only two layers can be distinguished. G = ganglion cell layer with few degenerated neurons; I = remnants of the inner granular layer (hematoxylin-eosin, original magnification, × 650).

speculation in the context of the purported immunosuppressive effect of BVDV (see the article by Potgieter, this issue).[5] Alternative results can be found elsewhere.[76, 87] Although BVDV antigen may be present in the thymus at the time of birth (Fig. 2), it does not always result in hypoplasia, as discussed in later sections. Although thymocytes seem to be responsive (or susceptible) to signals for apoptosis such as antigen at inappropriately high levels or presented in the absence of appropriate costimulatory signals, corticosteroids, or certain cytokines,[47, 90, 92, 97, 141] it may be questioned whether the BVDV infection is the cause of the atrophy or whether other events (parturition stress, neonatal infection, insufficient nutrition) around the time of delivery are the (additional?) cause(s) of an acute involution, which could have been reversible and inconsequential given time and appropriate care.[11, 68]

The generalized intra-uterine growth retardation, most often noted in PI calves (see section on persistent infection without overt clinical disease) may have significant clinical implications in relation to the "weak-calf syndrome," although the extent of the problem is not known because of the defect's subtle character (see the article by Baker, this issue).[114]

Intra-uterine infection in the last trimester is not usually thought to cause serious consequences for the fetus. At this stage, the fetus has attained immunocompetence,[30, 126] although still with some deficits,[67, 125] and will respond to the infection with BVDV-specific antibody production.[9, 10, 126] Whether the virus is eliminated in all cases,[10, 14, 30] and whether

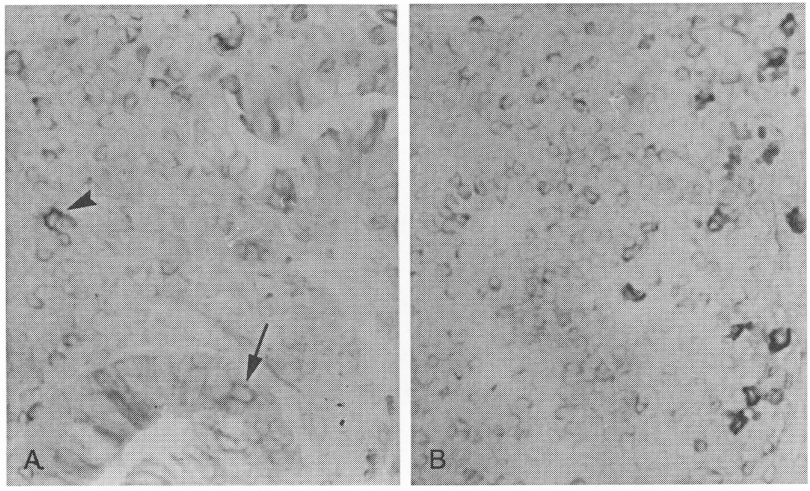

Figure 2. Demonstration of BVDV antigen by immunocytochemistry on cryosections in tissues of persistently viraemic, clinically healthy calves. *A,* ileum; virus antigen-positive cells mainly occur in the propria and comprise lymphocytes and macrophages *(arrowhead).* Few crypt epithelial cells also label weakly *(arrow)* (original magnification, × 100). *B,* thymus. Although most thymocytes label positively for BVDV antigen, the strongest antigen reactions are seen in macrophage-like cells in the cortico-medullary zone (original magnification, × 100).

an infection at this stage could potentially contribute to the problem of growth-retardation and/or weak calves does not seem to have been subjected to thorough investigation.

ACUTE VIRUS DIARRHEA

Despite being the first described clinical picture of BVDV infection,[110] acute virus diarrhea remains one of the two least well-characterized BVDV-associated syndromes (the other being respiratory disease, see later) mainly because of its perceived negligible long-term effect and mortality. Primarily described as a disease of cattle aged 6 to 24 months,[6] it remains unclear exactly what role BVDV plays in neonatal disease.[83, 84, 107] In either case the pathologic lesions usually are subtle and rather nonspecific, whether the infecting virus is of the noncytopathic (NCP) or cytopathic (CP) biotype,[107, 143] comprising flaccid, edematous intestines, occasionally with ecchymotic hemorrhages, and mild reaction in the mesenteric lymph nodes. Oral and esophageal lesions characterized by small erosions or shallow ulcerations have also been described.[61] Occasionally, lesions resembling those seen in MD (see section on mucosal disease and Figs. 3 and 4*A*) and consisting of linear erosions and hemorrhages, Peyer's patch-atrophy and hemorrhage, and erosion of the proximal colon occur.[36, 43] Virus antigen may be detected in the intestinal epithelium, as well as in tonsils, lymph nodes, and lungs.

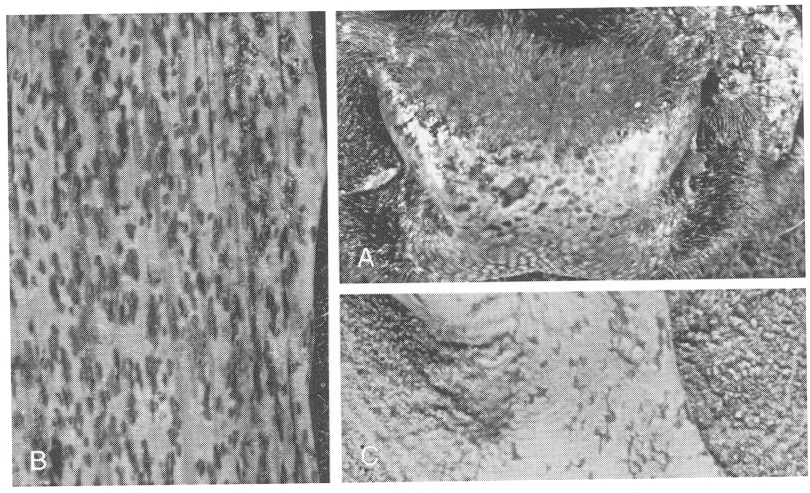

Figure 3. Gross pathologic lesions *A*, on the muzzle; *B*, in the eosophagus; and *C*, on a ruminal pillar in a typical case of MD.

ACUTE RESPIRATORY DISEASE

The importance of BVDV in bovine respiratory disease (BRD) remains another contentious subject.[3, 6, 113, 116] Clinical signs of respiratory distress are common in animals with both acute virus diarrhea[43, 59] and in MD. In many cases, however, these do not seem to be accompanied by gross pathologic changes, and are thought to be a consequence of high fever and/or abdominal pain.[114] In other cases, particularly in

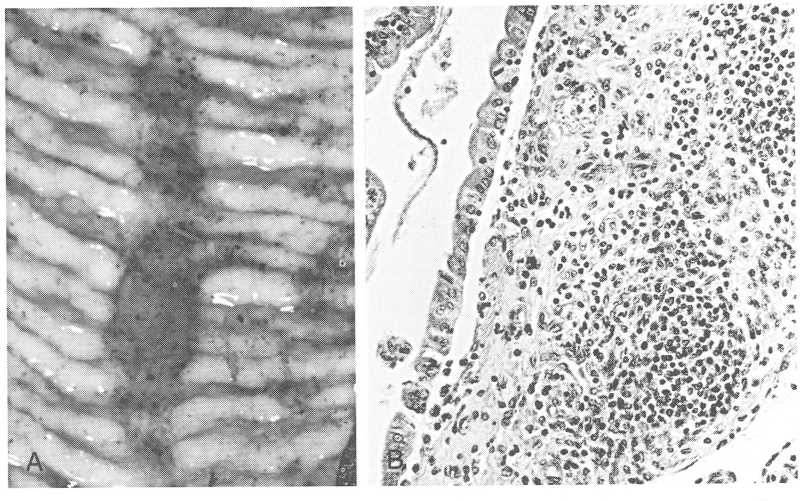

Figure 4. *A*, Gross and *B*, histologic appearance of Peyer's patch atrophy in the ileum in MD (hematoxylin-eosin, original magnification, ×650).

young animals, either PI or infected postnatally, BVDV is seen as a predisposing factor for bacterial infection, similarly to that of bovine herpesvirus-I (BHV-I), parainfluenza-3, and others.[3, 22, 29]

In PI calves, BVDV can be isolated from lung tissue, and virus antigen is widespread in bronchiolar and alveolar epithelial cells, however, without accompanying histopathologic changes (see section on persistent infection without overt clinical disease and Fig. 5).[17] Conversely, a proportion (the size varying with study) of cattle succumbing to BRD are positive for BVDV, by virus isolation from or antigen detection in lung tissue, but the ensuing pathologic changes cannot be distinguished from those of other viral pathogens in the BRD complex (perhaps with the exception of BHV-I[15]), and usually are dominated by the pathology caused by the secondary bacterial agent.[3, 29, 96] It is quite conceivable, although it remains to be shown experimentally, that the pathogenic mechanisms involved in the respiratory tract pathology of BHV-I plus bacteria also occur in BVDV-associated respiratory disease.[19, 22, 94, 115, 116] Whether strain differences exist with respect to pneumopathogenicity as it does for other aspects of the BVDV complex remains unresolved.[43, 113, 115, 116] Although of considerable interest in its own right and as a model-system for host-virus interactions,[20] an elucidation of this aspect may require an extensive experimentation.

ACUTE SEVERE THROMBOCYTOPENIA AND HEMORRHAGIC DIATHESIS

Despite the generally accepted notion that infection of immunocompetent animals with either NCP or CP BVDV results in no apparent or

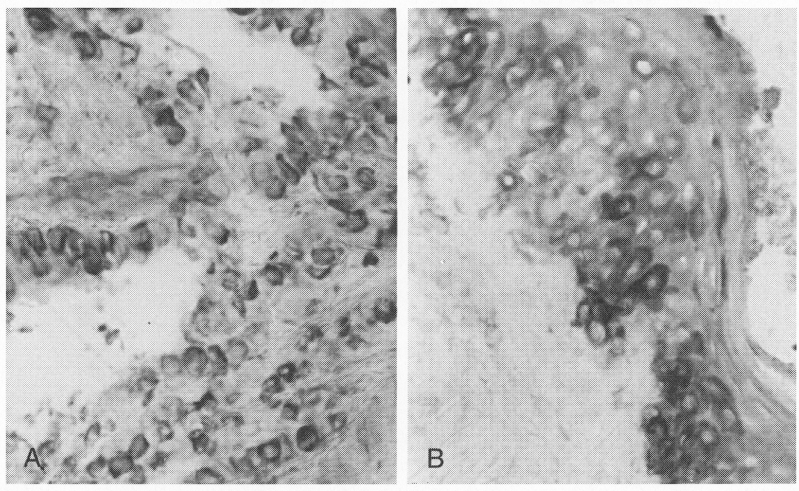

Figure 5. Demonstration of BVDV antigen in tissues of persistently viraemic, clinically normal calves. *A*, lung. Bronchiolar epithelial cells appear to label intensely for viral antigen (original magnification, ×100). *B*, Esophageal epithelium. Despite intense virus antigen labeling of cells in the basal layer, no inflammatory reaction is seen, and the epithelium seems to be morphologically intact (original magnification, ×100).

a relatively innocuous disease (see section on acute virus diarrhea), more severe outbreaks of diarrhea preceded by severe thrombocytopenia and widespread hemorrhages have been described.[52, 53, 96, 120] This syndrome, so far only described in the United States and Canada, is mainly, but not exclusively, seen in young calves.[96, 114, 120] At this stage it remains unclarified whether cases of hemorrhagic diathesis seen in cattle in England[56] and continental Europe,[137] as well as North American outbreaks of severe peracute-acute BVD with pneumonia, diarrhea, and abortions[43, 59] represent the same syndrome.

Peracute death with an overall lack of gross lesions or with hemorrhages of the Peyer's patches as the only grossly apparent lesion may be a frequent event in this syndrome.[7] Other gross pathologic findings may consist of petecchial and ecchymotic hemorrhages throughout the body, including on the sclera of the eyes, the margins and inner surfaces of the eyelids, the mucosal surfaces of the cheeks and gingiva, the tip of and ventrally on the tongue and soft palate, in the esophageal and ruminal epithelium and the mucosal surface of the abomasum and intestines, on the epicardium and subperiosteally on the calvaria. Additionally, some calves may have secondary bacterial pneumonia.[52, 53, 96, 120]

Although only likely to be diagnosed under clinic and experimental conditions, the cardinal sign seems to be megakaryocytosis, detected in bone marrow aspirates and core biopsies.[52] Although the syndrome has some superficial resemblance to dengue hemorrhagic syndrome in humans[68] and hog cholera lesions,[47] similarities in the pathogenic mechanisms have been ruled out,[53] leaving the possibility that it is a virus-strain specific phenomenon[36, 113] to be further explored.

PERSISTENT INFECTION WITHOUT OVERT CLINICAL DISEASE

PI calves born as a result of an intra-uterine infection most likely in the first trimester of gestation, may be indistinguishable from noninfected animals,[17, 100] or may seem to be growth retarded, at or soon postpartum.[6, 58] A large serologic survey of healthy adult cattle showed that up to 1% of such animals may be PI virus carriers.[100] It is not known how many of these animals would have had microscopic evidence of the infection. However, in other, more limited studies of PI animals (natural or experimental infections), histopathologic lesions were detected in some, but not all,[17, 18] clinically healthy animals, and comprised local interstitial infiltrations of mononuclear cells (MNC) in kidneys and local thickening of the glomerular basement membrane,[15, 54, 70] mild accumulation of MNC in liver triads, increased frequency of peribronchiolar lymphonoduli, and microfocal necrosis in the epithelium of the tongue and esophagus accompanied by moderate subepithelial MNC accumulation.[15] Conspicuously, in these same studies the lymphoid tissues of PI animals were indistinguishable from those of normal animals of comparable age and environmental exposure.[18, 54]

In animals with obvious growth retardation, both macroscopic and microscopic evidence of skin lesions may be present. Macroscopically, the changes may resemble those seen in chronic MD (see section on the gross pathology of acute and chronic MD) and consist of varying degrees of scurfiness and shallow erosive lesions around orifices and in the interdigital cleft. Microscopically, the changes may vary from slight attenuation of the rete pegs and mild subepithelial MNC infiltration (mainly macrophages) to more pronounced changes of parakeratotic and hyperkeratotic character, as seen in MD (see section on mucosal disease). Similar changes may be found in the keratinized epithelia of esophagus, rumen, reticulum, and omasum. Frank epithelial necrosis, however, is not common.[15] Mild retina lesions, of a type described in the section on congenital infections and not diagnosed clinically, may also occasionally become evident on histologic examination.

Despite the absence or very limited extent of histopathologic changes, virus occurrence seems to be widespread, with little variation between animals, regardless of the clinico-pathologic manifestations[17, 18, 91] (Table 2, and see Figs. 2 and 3). This distribution is similar to what is found in cases of MD, but differs with respect to extent, i.e., number of cells infected and/or virus antigen-labeling intensity, potentially reflecting virus-replication level and/or the biotype present.[66] This phenomenon is most notable in the gut in which in the fundi of the crypts of PI healthy cattle only few, often scattered, cells were found to be infected in comparison to more uniform infection in MD calves (Figs. 2 and 6).[13, 17, 91] Moreover, epithelial infection was not detected beyond the ileocaecal orifice. Below this anatomical site the alimentary infection was confined to intraepithelial lymphocytes and MNC in the propria.[17] Before euthanasia, virus was not or was only intermittently isolated from fecal

Table 2. BVDV ANTIGEN DISTRIBUTION IN TISSUES OF PI CATTLE WITHOUT OVERT CLINICAL DISEASE

Tissue	Cell Types*
Skin	Keratinocytes
Lungs	Bronchiolar and alveolar epithelial cells
Gingiva, labia, tongue	Keratinocytes, macrophages in submucosa
Esophagus, rumen, omasum	Keratinocytes
Abomasum	Mucosal epithelial cells, lymphoid cells in *lamina-propria*
Ileum	Scattered epithelial cells, lymphoid cells in *lamina propria*†
Colon, rectum	Lymphoid cells in *lamina propria*
Salivary glands	Acinar cells
Kidneys	Tubular epithelial cells
Tonsils	Keratinocytes, lymphoid cells
Thymus	Thymocytes, macrophages, interdigitating cells†
Spleen, lymph nodes, Peyer's patches	Lymphocytes, macrophages, interdigitating cells, follicular dendritic cells‡

*The predominant cell type(s) containing BVDV antigen as determined by immunocytochemistry.
†See Figure 2.
‡For the importance of this finding see the section on Mutifactorial Nature of MD Pathology.

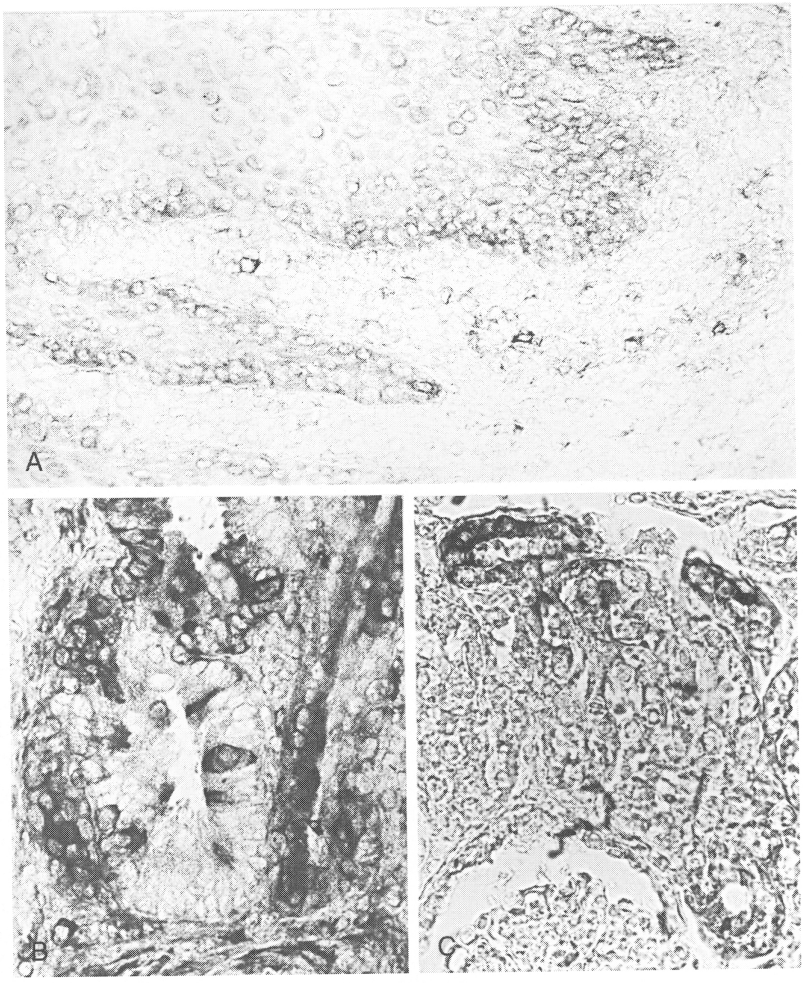

Figure 6. Demonstration of BVDV antigen by immunocytochemistry performed on acetone-fixed or ethanol-fixed cryosections in tissues of calves with MD. *A,* Esophageal epithelium. Virus-antigen positive cells occur in the reticular layer as well as in stratum basale and spinosum (original magnification, × 590). *B,* Ileum. Viral antigen is present in all epithelial cells in the atrophic crypt on the right, in scattered epithelial cells in a crypt not yet apparently affected morphologically, as well as in macrophages and lymphocytes in the propria (original magnification, × 675). *C,* In the kidneys, BVDV antigen occurs selectively in some tubular segments and is not (as in this case), or only rarely, giving rise to an inflammatory reaction (original magnification, × 625).

samples, whereas NCP BVDV was readily isolated from blood and nasal swabs.[15]

The clinically healthy PI animals also may differ from MD cases with respect to virus distribution in the CNS, although some discrepancies have been noted between published reports. In several studies, widespread occurrence of viral antigen in neurons, especially in the

deep lamina of the cerebral cortex, was described,[54, 64, 71, 144] whereas other studies have found that only microglia (macrophage-like cells) may be infected in PI clinically normal cattle, and large neurons only became virus-antigen positive in animals with MD.[15] Likewise, discrepancies exist regarding the presence of virus antigen in endothelial cells of small vessels, although the latter may be explained by the use, in some studies, of antibodies with nonspecific affinity for endothelia and/or adventitial cells.[27]

As an extension of PI cells of the lymphoid tissues, a proportion of peripheral blood MNC seems to contain BVDV antigen and replicating virus[16, 17, 24, 31, 37, 95, 132] with no or only minor apparent consequence for total leukocyte numbers, relative frequencies, or the fine structure of the cells, perhaps with the exception of slight "activation" of the blood monocytes.[24] Whereas there is agreement between reports as to the virus-carrier state of blood monocytes and T lymphocytes, there seems to be some discrepancy with regard to the B lymphocytes.[16, 31, 37, 95, 132] Whether this is ascribable to differences in methodology (including sensitivity and specificity of reagents), host genotype or virus strains involved remains unclarified, as does the importance of the infection of the leukocyte types to the pathogenic processes (see the section on Multifactorial Nature of MD Pathology).[13, 102]

MUCOSAL DISEASE

Although MD is a relatively uncommon form of the BVD spectrum of diseases, it has attracted much attention and continues to challenge the veterinary research community and virologists because of its elusive nature. The clinical manifestations and gross pathologic lesions of MD can be extremely varied, although this is mostly in degree rather than in type.[6, 9, 114] This has led a number of authors to distinguish an acute and a chronic form of MD.[6, 119] Whether this is entirely justified in terms of clinical entities must await clarification of the roles played by the previously mentioned factors such as the virus strains involved, host genotype, and the degree of "tolerance" and, therefore, host-reaction to the infecting virus(es) in the pathogenic processes (also see the section on Multifactorial Nature of MD Pathology).[17, 18, 42, 102]

Gross Pathology of Acute and Chronic MD

Classically, the gross pathologic lesions comprise necrotizing and erosive/ulcerative lesions on the muzzle and lips (see Fig. 3), the buccal mucosa, gingiva and the tongue, in the latter case particularly on the latero-posterior surfaces. Minor erosions may also occur on the external nares and in the nasal cavity. Elongated (.5–2 mm in width, 1–2 cm in length) necrotic-erosive lesions are common in the esophagus (see Fig. 3*A*), as are erosions on the rumenal pillars (see Fig. 3C), in the reticulum

and omasum. It should be emphasized, however, that especially in the chronic (protracted) cases of MD gross lesions in the upper alimentary tract may be completely absent or subtle, although histologic examination usually will show micro-lesions similar in type to those described in the section on PI without overt clinical disease (see Fig. 7*A*).

Erosive edematous and hemorrhagic lesions are seen in the abomasum. The enteritis can vary from catarrhal to hemorrhagic, fibrinonecrotic, and erosive/ulcerative. The Peyer's patches as well as the lymphoid tissue in the proximal colon may seem hemorrhagic and necrotic (see Fig. 4). The thymus is usually atrophic, and in many cases, only leaves a stringy fibro-epithelial, partly fat-infiltrated tissue. Peripheral lymph nodes may be enlarged, edematous, or hypoplastic, depending on degree of inflammation in the drainage bed, length of clinical disease course, and/or virus-host cell interactions in the organ.

Most animals will have apparent skin lesions, although with considerable individual variations in degree and extent of distribution. Lesions are most common and most pronounced in the neck, shoulder, and perineal region. The changes present as an eczema resulting from crust-like dermatosis and hyperkeratosis and parakeratosis accompanied by hypotrichosis or alopecia. Underneath the dry scales, the corium seem hyperemic and occasionally thickened. Where the crustous formations are well-delineated, a hyperemic zone can be observed in the edge as well as local alopecia. In some cases, the dermatitis is of a generalized seborrhagic nature with hyperkeratinization and alopecia. Erosive lesions may be found in the perineal area, around the preputial opening

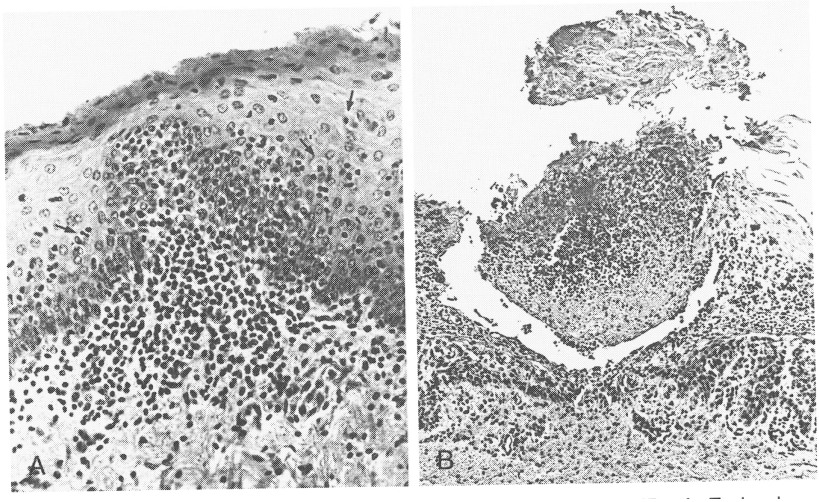

Figure 7. Microphotographs of lesions in keratinized epithelia in MD. *A*, Early changes characterized by massive infiltration of mononuclear leukocytes in the reticular stratum as well as intraepithelially. Singly scattered epithelial cells are undergoing degenerative changes *(arrows)* (hematoxylin-eosin, original magnification, × 650). *B*, Late changes with extensive epithelial necrosis and erosion of the epithelium (frenulum) (hematoxylin-eosin, original magnification, × 250).

or vulva. Erosive lesions with or without lichenification and exudation are also common in the interdigital cleft and around the skin-hoof junctions. Suppuration may occur due to secondary bacterial infection, although this is less common than might be expected. Generally, the skin lesions may be more pronounced in the protracted (chronic) cases, but there seems to be considerable variation between published descriptions—perhaps due to differences in the clinical categorization of acute versus chronic.

Conjunctivitis is an almost consistent finding. Bronchopneumonia and fibrinous pleuritis may occur, but can then usually be ascribed to secondary bacterial infection (often *Actinomyces bovis* or *Pasteurella* species).

Histopathology of MD

Despite the variation in the gross-pathologic appearance, the histologic picture in any one particular organ or organ-system is one of uniformity of type with variations ascribable to intensity and progression of the lesions, and perhaps modified by mechanically or, more rarely, bacterially induced secondary changes.

Keratinized Epithelia

The initial changes in keratinized epithelia (skin, the muzzle, and upper alimentary tract including rumen, reticulum, and omasum) is a focal infiltration of MNC in the papillar and reticular strata in propria/dermis, often with a tendency to periarteriolar and pericapillary localization, but never developing into distinct "cuffing," thus, probably reflecting migration in progress, rather than a reaction to vascular changes (see Fig. 7*A*). A thickening of propria occurs by widening and lengthening of the dermal papillae, often at the expense of the epithelial basal folds, which may appear lamella-like. The tissue may be hyperemic with diapedesis hemorrhage. In the epithelium degeneration and eosinophilic necrosis of scattered cells in stratum spinosum seems to constitute the initial changes, which progress to widespread cellular necrosis with disruption of the intercellular junctions. At the same time, MNC infiltration of the epithelium occur, often without apparent relation to the necrotic epithelial cells, but perhaps directed toward cells inapparently modified by virus infection.[18]

As this process progresses, well-delineated foci with a reticulated appearance arise in the stratum spinosum. They may remain covered by an intact stratum corneum. However, eventually this layer also loses integrity and erodes (Fig. 7*B*). The basal layer usually remains intact while the previously mentioned processes are in progression, but eventually necrobiotic and metaplastic changes may also occur. The basal cells may become squamous, but unless ulceration and/or secondary bacterial infection occur, the layer will remain uninterrupted.

In other areas, especially in the skin in the neck and shoulder

regions, the epithelial changes may be dominated by hyperkeratotic and parakeratotic processes, with formation of thick keratinoid masses, often 50 to 100 (cell) layers thick, interspersed with varying amounts of necrotic cell debris, keratin, and infiltrating MNC.

The hair follicles and sweat glands seem to become involved only relatively late in the processes of the skin, with initial periglandular and perifollicular MNC-infiltration and later degenerative and necrotizing changes of the follicles similar to those previously described. It is presumably at this stage that hypotrichosis and alopecia develops.

Intestines

In the intestines and abomasum pathologic changes are selectively localized in the fundi of the crypts. The initial changes occur in scattered single or small groups of neighboring cells, and consist of cytoplasmic condensation followed by vacuolation, rounding off, and desquamation. Electron microscopic examinations, supported by immunocytochemistry for virus antigen (see below), have shown that this cellular reaction occurs in cells with extensive viral replication, presumably CP virus.[9, 23, 66, 91] Cell debris and mucus accumulate in the crypt lumina that may become occluded and dilated with flattening of the epithelium and disappearance of microvilli.[23] Especially over the Peyer's patches these changes progress to a state of complete atrophy of crypts and villi, leaving a flat epithelium covering a lamina propria of fibrotic, almost acellular tissue (see Fig. 4). Although such areas seem especially prone to ulceration, it is worth noting that the "necrosis" described macroscopically in many, if not most, cases corresponds to areas with complete epithelial atrophy/metaplasia as seen in Figure 4.

In some cases, or some areas/parts of the intestine mucus cell metaplasia may dominate the crypt changes, and is accompanied by excessive mucus production and accumulation. The lamina propria reaction is usually dominated by massive infiltration of MNC, especially macrophages. Other changes may include edema in the subepithelial layers, hyperemia, and diapedesis.

Lymphoid Organs

Although there may be general agreement in the literature that clinical MD is accompanied by changes in the lymphoid tissues, there seems to be a conspicuous lack of attempts at staging the changes and correlating, if possible, these with functional characteristics of the "immune tolerance" and "suppression," presumed to be central to the pathogenesis of MD.[42, 102] As previously mentioned, no or very subtle morphologic changes occur in lymphoid tissues (thymus, spleen, lymph nodes, Peyer's patches) of PI clinically healthy animals despite widespread virus infection of all or most of the cellular elements.[17] Changes may occur (thymus atrophy, lymph node reactivity) in growth-retarded, weak calves, but may be caused by intercurrent infections and stress

rather than as an antecedent to secondary infections.[67, 125] Unfortunately, no thorough description of changes in lymphoid tissues of experimentally reproduced MD cases has yet appeared. The descriptions of changes in the thymus and Peyer's patches to follow, therefore, are based on natural cases of MD in which a staging was attempted based on clinical appearance and duration of the disease.[9]

In acute cases of brief duration (1–4 days) the overall structure of the thymus is retained, but a clear reduction occurs primarily affecting the cortex, which may be dominated by apoptotic and disintegrating thymocytes (Fig. 8*A*), prominent epithelial cells and large, actively phagocytosing macrophages. By electron microscopy degenerative changes are apparent in the epithelial cells, in particular in the cortical zone. Variable thymocyte depletion characterizes the medullary changes, but signs of frank cell death are rarely seen.

In subacute to distinctly chronic cases of MD, the thymus is characterized by progressive loss of zonal organization (Fig. 8*B*). The cortex may remain as a thin layer of "collapsed" epithelium, surrounding a fibro-reticular tissue with prominent Hassall's corpuscles and myoid cells. Few lymphocytes may persist in scattered islets, and fat infiltration may become prominent, especially in older animals.

The Peyer's patches already seem to be severely depleted of lymphoid cells during the peracute to acute clinical phases, which especially affects the follicles as well as the interfollicular and subepithelial zones. The follicular changes comprise apparent hypertrophy followed by degeneration of the follicular dendritic cells (FDC) and replacement with a homogenous fibroid material (Fig. 9). The structure of the Peyer's

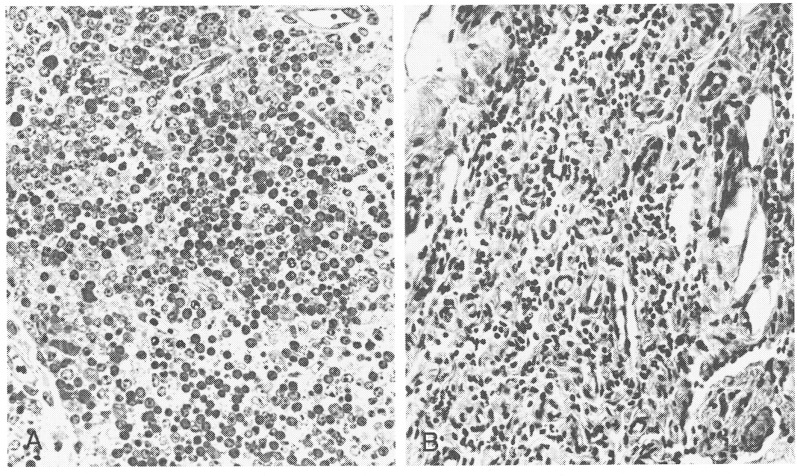

Figure 8. Microphotograph of changes in the thymus of calves with MD. *A*, Widespread apoptosis among thymocytes in the cortical zone. Peracute MD. Semithin Epon-section (Toluidinblue, original magnification, × 590). *B*, Complete thymus atrophy leaving a fibrotic tissue with indistinguishable cortex-medulla structure (hematoxylin-eosin, original magnification, × 590).

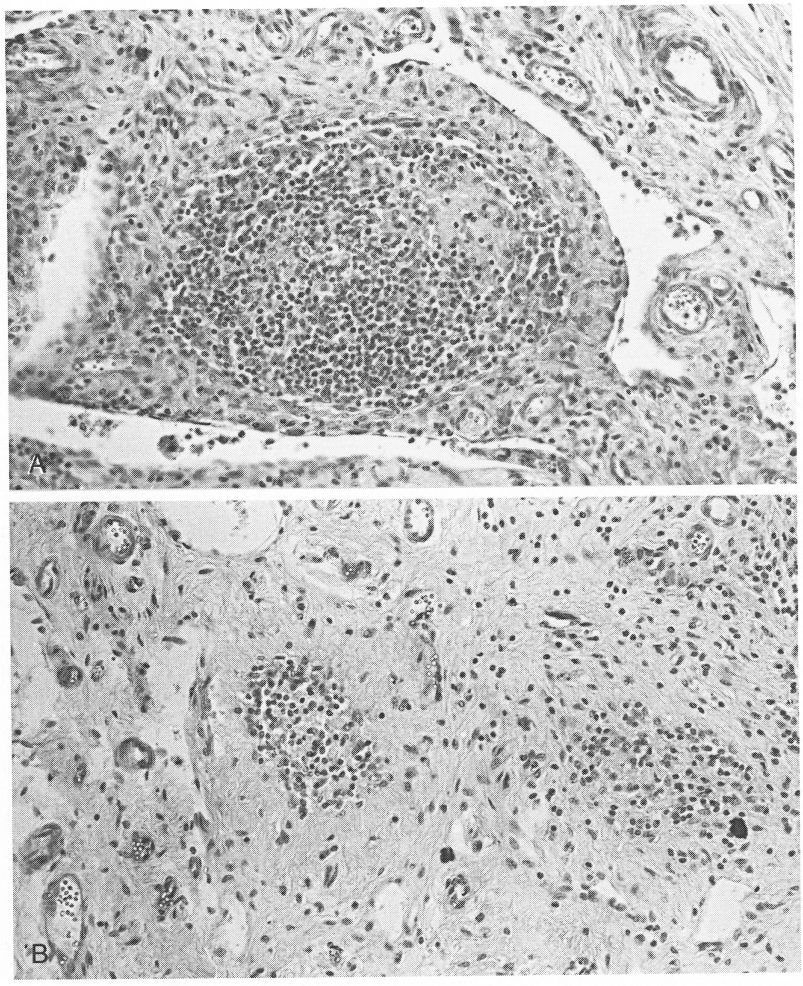

Figure 9. Photomicrographs of changes in Peyer's patches in ileum of calves with MD. *A*, Regression of the Peyer's patch and replacement by fibrotic tissue in acute MD (hematoxylin-eosin, original magnification, × 625). *B*, Barely distinguishable Peyer's patch follicles in ileum in a protracted (chronic) case of MD. The follicles consist of a concentric, contracted fibrotic tissue with a minute central collection of lymphocytes and few macrophages (hematoxylin-eosin, original magnification, × 625).

patches may be distorted further by extension of crypts into the submucosa and crypt-dilation followed by degeneration.

In more protracted (chronic) cases of MD, the Peyer's patches are characterized by atrophy. Often only a condensed/retracted (hyalinized) fibrotic tissue with small lymphoid islets remain (Fig. 9*B*), which are surrounded by concentric layers of fibrous tissue. The interfollicular zones comprise a narrow zone under the muscular layer with few lymphocytes, monocytes, and macrophages between fibroblasts and col-

lagen. The high-endothelial postcapillary venules (HEV) are no longer distinguishable.

In contrast to the thymus and Peyer's patches, a staging based on clinical criteria has not been possible for the parietal and mesenteric lymph nodes,[9] which not only vary in appearance between animals, but also vary within an animal. The most conspicuous changes comprise lymphocyte-depletion of the peripheral subsinusoid zone and regressive changes in primary and secondary follicles, the numbers of which seem to be comparable to that which occurs in conventionally raised noninfected or PI healthy calves. In secondary follicles, the germinal center may be replaced by a lightly stained eosinophilic, homogenous (structureless) cytoplasmic stroma, consisting of large dendritic cells, identified by electron microscopy as FDC. Distinct fibrosis, sometimes with a pericapillary origin, may be present. Scattered macrophages with large phagolysosomes may surround this area, partly embedded in an irregular and ill-defined lymphocyte corona. The central fibrosis may progress to a state in which the FDC have disappeared and the germinal center is completely replaced by an acellular, condensed hyalin fibroid mass that is surrounded by few scattered lymphocyte islets.

The paracortex may be depleted of lymphocytes to varying degrees; directly related to this event is a flattening of the endothelial cells of the HEV and decreased transendothelial migration of lymphocytes. The number of interdigitating cells may either remain within the normal range or decrease.

The medulla may be relatively or actually expanded. This is partly the result of a prominent sinus reaction characterized by accumulation of lymphocytes and typical monocytes, as well as large "activated" macrophages (Fig. 10). This is especially true for the more protracted cases and in the parietal lymph nodes, in which these cells may attain epithelioid cell characteristics, inclusive electron microscopically detectable Birbeck granules and micropinocytosis vermiformis (Fig. 11).

Changes in the bronchial and retropharyngeal lymph nodes may often be compounded by reactions to secondary respiratory bacterial infections and are not examined in this article. Changes in the tonsillar lymphoid tissue resemble those described for the Peyer's patches and lymph nodes, with a progression similar to that described for Peyer's patches. In the spleen, lymphocyte depletion of the periarteriolar lymphoid sheath and follicle changes that are similar to, albeit less pronounced, than those in the lymph nodes are characteristic findings.

Virus Antigen Distribution

Generally, BVDV antigen distribution in cattle with MD corresponds to that described for PI clinically healthy calves (see section on PI without overt clinical disease, Table 2; Figs. 6 and 12). A number of notable variations, however, should be examined. As the thymus involution progresses, the amount of detectable virus antigen also decreases

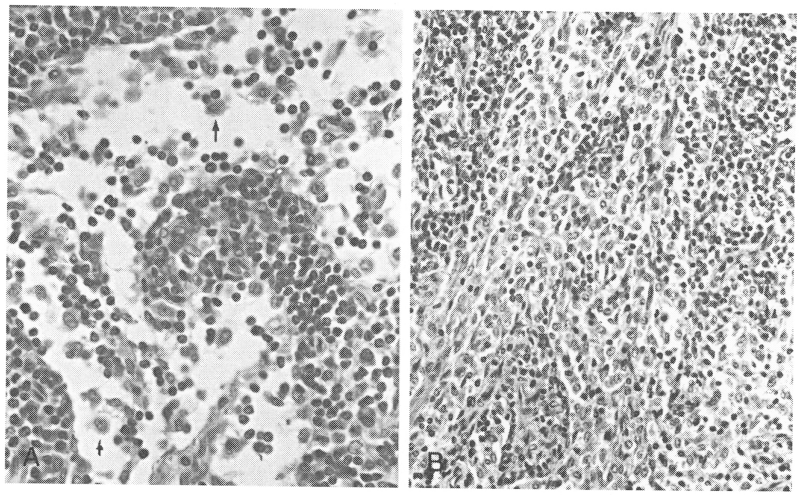

Figure 10. Microphotograph of lymph node sinus reaction. *A,* Acute MD. Small lymphocytes and monocyte-like cells (examples of the latter indicated by arrows) constitute the free cells in the sinus (hematoxylin-eosin, original magnification, × 940). *B,* In later phases of "acute" MD and in more protracted (chronic) cases of MD, the sinuses are completely congested by large macrophages with epithelioid cell characteristics (hematoxylin-eosin, original magnification, × 590).

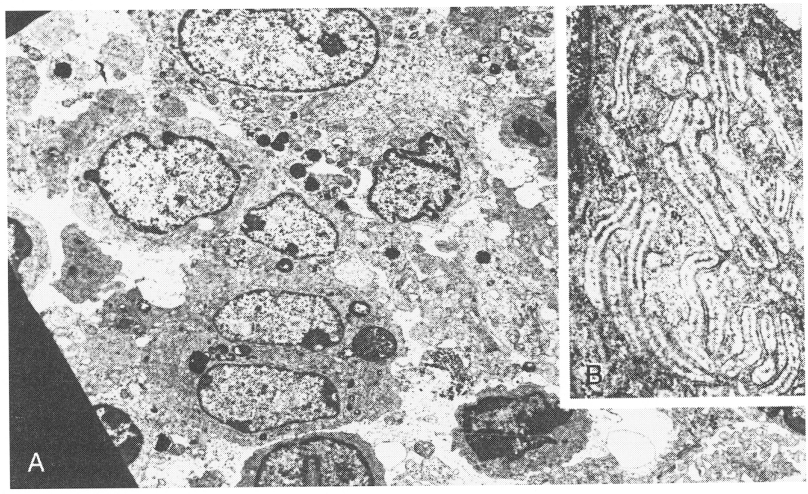

Figure 11. *A,* Electron micrograph of epithelioid-like macrophages in lymph node sinus of calf with MD, characterized by large organelle-rich cytoplasm, many phagolysosomes, and tubular formations (original magnification, × 240). *B,* Micropinocytosis vermiformis (original magnification, × 19,800).

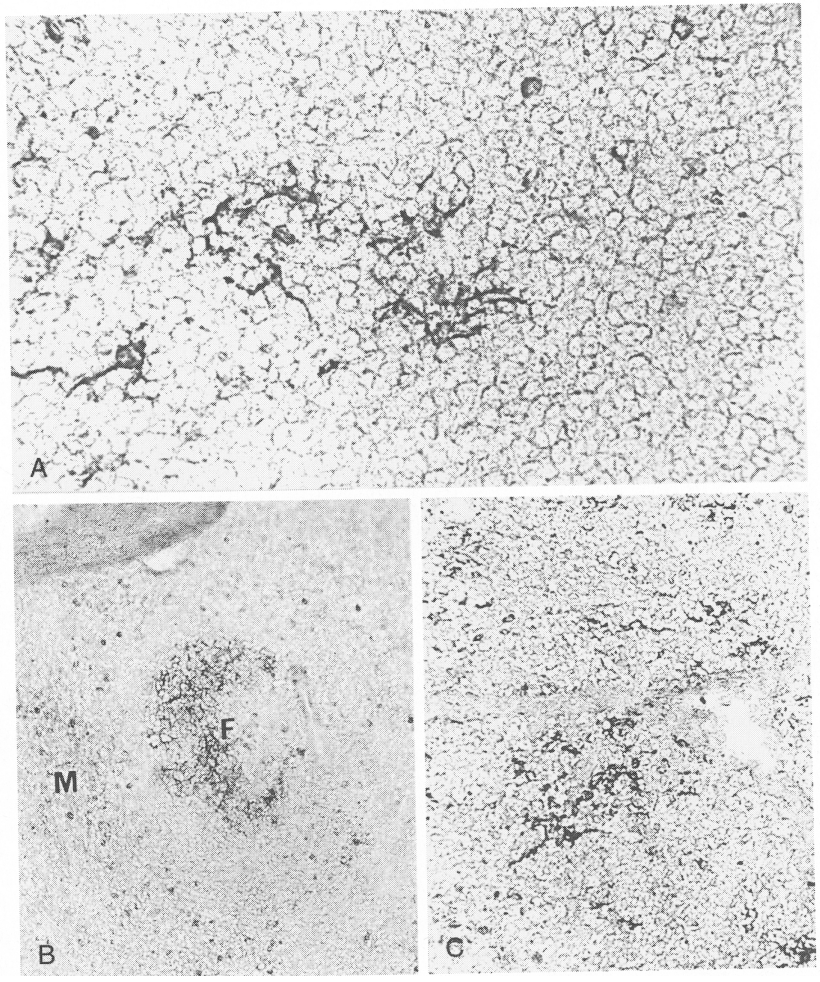

Figure 12. Demonstration of BVDV antigen by immunocytochemistry on acetone-fixed cryosections in tissues of calves with MD. *A,* Lymph node. The virus antigen labeling seems particularly strong in the interdigitating cells, less prominent in the lymphocytes, perhaps reflecting either less virus-replication in the latter, antigen uptake in the former (in addition to viral replication), a difference in cytoplasmic volume or a combination of these (original magnification, × 470). *B,* Spleen. During "acute" MD, BVDV antigen occurs particularly prominent in the "dark" zone of the follicles (F) and in marginal zone-macrophages (M), but also is seen in scattered lymphocytes, interdigitating cells, and macrophages in other zones (original magnification, × 260). *C,* Thymus. During "acute" MD, BVDV antigen occurs most prominently in macrophages and dendritic cells in the corticomedullary zone; however, the majority of thymocytes also seem to label positively for virus antigen (original magnification, × 190).

and eventually is limited to MNC in the adventitia of blood vessels and scattered macrophages. The same occurs to the secondary follicles in peripheral lymphoid organ. As the germinal centers become depleted of FDC and are replaced by acellular matrix, the virus antigen disappears. In contrast, the occurrence of virus antigen in lymph node medullary cord and sinus cells increases with protraction of the clinical course. Most of these cells seem to be macrophages, as determined by double-labeling for virus antigen and phenotypic (CD)-markers. The degeneration of HEV is notably *not* accompanied by virus antigen presence in the endothelial cells.

In the skin, virus antigen-positive cells occur as fairly evenly scattered cells in the basal lamina, inclusive of hair follicles. They may be more concentrated and the labeling more intense in areas of early and progressed subepithelial inflammatory reactions. Additionally, some of the infiltrating macrophages and T cells are virus antigen positive (see Fig. 6).[18] In the oral cavity, esophagus, and keratinized forestomachs, the antigen distribution tends to be more focal, even in areas without visible inflammatory reactions or frank erosion, although considerable variation between different anatomical sites of the same animal is usual.[13]

In the intestines of cattle with MD, more widespread occurrence of virus antigen-positive cells occur in the crypts compared with PI healthy calves, and the cells tend to label more intensely (compare Figs. 2 and 6).[13, 17, 18, 66, 91] This is the case in the epithelium associated with submucosal lymphoid tissues (Peyer's patches in ileum and in the proximal colon). Additionally, many macrophages in the lamina propria are virus antigen positive (see Fig. 6), although it remains unknown whether this is due to productive infection in all cases, or if it reflects extensive uptake of cellular debris from surrounding infected and lysed cells in some cells.

In Situ Characterization of MNC in Mucosal Tissues

In animals succumbing to MD, the previously described hyperkeratotic and parakeratotic changes, as well as necrotizing lesions, are accompanied by massive infiltration of MHC class II antigen-positive macrophages and cells with dendritic morphology. T lymphocytes (predominantly $CD8^+$ cells) also occur, whereas B lymphocytes are rare. Using macrophage differentiation markers as immunocytochemical target antigens, it was determined that the majority of macrophages in the infiltrates may be relatively undifferentiated but highly activated,[18, 28, 33] and this may reflect a highly dynamic process with continuous influx from the blood circulation and either premigratory or postmigratory activation.[33]

Macrophages of all maturation stages as well as T lymphocytes occur in the lamina propria of the digestive tract (inclusive in Peyer's patches) with occasional focal concentration of $CD8^+$ cells perivenularly.

From the findings in natural cases of MD and with the use of only

a limited set of lymphocyte surface markers, detection of consistent selective losses of any one particular subset of lymphocytes from lymphoid tissues, or significant changes over time in the composition of cell infiltrates in other tissues has not been possible.[18] With the availability of a larger panel of CD-specific reagents and the possibility of reproducing MD experimentally, however, it would now be feasible to conduct such studies in a controlled and sequential manner.

A MULTIFACTORIAL NATURE OF MD PATHOLOGY: THE IMPORTANCE OF TARGET CELL DISTRIBUTION AND CYTOKINES IN THE PATHOGENIC PROCESS(ES)

The role of cytokines in the pathogenesis and pathology of BVDV infections in general and in MD in particular, so far has only attracted limited attention,[21, 25, 33] but deserves thorough investigation. Lesions comparable to those seen in the intestines, lymphoid tissues, and cutaneous epithelia can be provoked by either overexpression, overproduction, or gene-disruption of several cytokines, including TNFα,[26, 73] GM-CSF,[85] TGFβ,[129] and IL-6.[32, 74] The lesions characteristic of BVD-MD are likely to have a multifactorial cause: by a combination of virus-cpe and specific and nonspecific immune responses, with the latter comprising *local* cytokine production by nonlymphoid cells as well as macrophages and perhaps $CD8^+$ T cells and "null" cells in response to cell injury or other cytokines. In the unveiling of such mechanisms, however, it will be crucial to take into account the temporal relationships.[22, 57, 112] Liebler et al have begun such studies, but much remains to be done.[91]

Another aspect of the pathogenesis of MD that needs clarification is the tissue/cell location for the events leading to a biotype change.[1, 98, 99, 136] A number of studies have documented that flavivirus genome mutations and selection of variants only occur in certain cell types.[50, 93] It is notable that the CP virus seems to have a somewhat restricted distribution,[66, 91] and it is even more notable that this includes FDC, tissue macrophages, and other dendritic cells. A similar, although not identical, distribution was described in lymphocytic choriomeningitis virus (LCMV) infections in which specific types of mutations in the viral genome caused the virus to become either macrophage-tropic, lymphocytotropic, or both, i.e., amphotropic.[1, 80] Likewise, in visna virus infection tissue tropism is determined by a specific genome segment.[131] Although the noted distribution for CP-BVDV cannot be taken to imply that these are the types of cells in which the change(s) occurs, the preferential replication of CP-BVDV in professional antigen presenting cells (APC) makes it possible that the cleavage products of the 125kDa protein, after genomic changes, are processed and presented differently to the immune system than NCP-virus products.[106] The tolerant state may be "broken" with the subsequent development of immunopathologic, perhaps even autoimmune-like, disease.[109, 123] That a measurable antibody (AB) response does not always occur,[11, 15, 62] which might other-

wise be expected,[65, 147] does not contradict this hypothesis, because such a response would not only depend on a time-factor, i.e., survival time, but also on the type of T-cell response induced.[4, 103, 104, 112, 142] If this is preferentially a $CD8^+$ T-cell response, which might be inferred from the predominance of $CD8^+$ to $CD4^+$ cells in inflammatory foci, submucosae, and lymphoid tissues,[18] and is followed by destruction of antigen-positive cells, including the FDC (see Figs. 9*B* and 12),[108] the B-cell response would be abrogated.[79, 117] Such mechanisms have been demonstrated to occur in LCMV infection,[108] and are thought to take place in HIV infections.[63]

If at the same time as the APC are presenting "new" virus-protein epitopes to the immune system one or more of their accessory functions, such as expression of costimulatory surface molecules or production of cytokines, have been altered directly or indirectly due to the virus infection,[1] apoptosis may be the outcome rather than stimulation of a proliferative T-cell response.[89, 141] Such a scenario could be invoked to explain the apparent "acuteness" of thymocyte depletion (see Fig. 8*A*), as well as lymphocyte depletion of peripheral lymphoid tissues.[117] In addition, factors such as corticosteroids and sudden excessive release of certain cytokines may contribute to the depletion by directly inducing apoptosis in lymphocytes.[48, 90] The resulting disruption of lymph node, Peyer's patches, and tonsil architecture would result in an abnormal (even if in some cases subtle) distribution of lymphocytes within these tissues. This reaction combined with the productive virus infection of both lymphocytes, macrophages, and dendritic cells (see Figs. 2 and 12) may profoundly impair normal cell-cell interactions.[16, 17, 31, 66]

Such mechanisms may help to explain why neither T nor B lymphocytes are particularly prominent in the inflammatory processes in epithelial lesions.[18] Furthermore, the absence of *de novo* expression of MHC class II antigens on endothelia and epithelia suggest that either IFNγ is not produced locally to any significant extent,[22, 25] or the IFNγ effect is antagonized by other cytokines. Because inflammatory macrophages in the lesions express high levels of MHC class II antigen, however, this would suggest that cytokines such as IL-10 and TGFβ may not play major roles in the inflammatory reactions,[55, 139, 140] despite at least the latter's known involvement in skin pathology.[38, 129] Instead, to explain the inflammatory process in the cutaneous epithelia the role of IL-1α and IL-1β may be invoked. Keratinocytes are known to express large amounts of intracellular pro-IL-1α and pro-IL-β.[82, 101] Cell injury, due to virus-CPE or a cell-mediated cytotoxic response, would result in release of the cytokines. Because pro-IL-1α is biologically active, its release could initiate the inflammatory reaction by induction of adhesion molecules on endothelia and the production, by keratinocytes and macrophages, of chemokines and other proinflammatory cytokines such as CSFs, IL-6, TNFα, and fibroblast and keratinocyte growth factors.[81] The process may start with the damage of only a few keratinocytes, and thereby create the impression of MNC-infiltration preceding the epithelial lesions (see

section on histopathology of MD and see Fig. 7*A*). Furthermore, it is possible that such a process could become self-perpetuating.[81]

An alternative, although not a mutually exclusive possibility, is that the cutaneous lesions in MD are initiated by CP-BVDV replication in Langerhans cells. These cells are potent APC and cytokine producers,[86] and the latter function could be envisioned to be upregulated on infection, in particular of such pro-inflammatory mediators as IL-1, IL-6, TNFα, and chemokines, which subsequently could stimulate the keratinocytes as well as attract macrophages. Another possibility is virus-induced depletion of Langerhans cells, directly or indirectly,[128] with subsequent loss of an important immunoregulatory component of the skin.[4, 8, 124, 133] Thus the question of distribution and density variation of epidermal and mucosal Langerhans cells as a determining factor in the distribution and/or severity of the lesions in cutaneous epithelia, such as has been described in other dermatologic reactions,[39, 130, 138, 145] becomes pertinent. So far the cutaneous lesions characteristic for MD have received comparatively little attention; further study in this area is needed.

Despite the impressive progress in the unveiling of the molecular phenomena involved in the biotype-change, from NCP to CP, of BVDV[98, 99, 118, 135, 136] (see the article by Donis and Bolin, this issue), the biological significance of the p80 protein at the level of host cell-virus interaction(s) remains unresolved.[134] It is by no means clear that the p80 protein is *the* cytopathogenic factor, and the possibility remains that this protein represents only an epiphenomenon or convenient marker for other more crucial events in the host-pathogen relationship.

Thus, before jumping to (easy) conclusions about virus-CPE as the main, if not sole, cause of tissue pathology and ultimate death of the animal,[42, 98, 136] it might be prudent to remember a number of characteristic features of the MD pathology and to place these in context of what is currently known about inflammatory processes and induction of pathomorphologic changes in lymphoid tissues, as well as the possible role of target cell distribution. It also should be remembered that many of the lesion types characteristic of MD can also be found in acute outbreaks of BVD in which the causative strain is NCP.[43, 56, 96, 114] A broader and more integrated approach may be needed to succeed in unraveling the enigmas of the pathogenesis of MD and of BVDV infections in general.

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