Mechanism of Protection from Primary Bovine Viral Diarrhea Virus Infection I. The Effects of Dexamethasone

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ABSTRACT

A series of investigations was designed to study the role of cellular immunity and passive antibody in protecting neonatal calves from primary bovine viral diarrhea virus infection. Administration of corticosteroids (dexamethasone) in doses capable of suppressing cellular immunity markedly potentiated systemic bovine viral diarrhea virus infection in calves which lacked bovine viral diarrhea passive neutralizing antibody. Immunosuppressed calves did not form neutralizing antibody to bovine viral diarrhea virus and developed a fatal viremia. Calves with high levels of passive bovine viral diarrhea neutralizing antibodies were protected from the effect of corticosteroids. The results suggest an essential role for humoral passive antibody, but not for cellular immunity, in protection from primary systemic bovine viral diarrhea virus infection in calves.

RÉSUMÉ

Les expériences décrites dans le présent article visaient à étudier le rôle de l'immunité cellulaire et de l'immunité passive, relativement à la protection des veaux nouveau-nés contre une infection primaire par l'agent étiologique de la diarrhée à virus des bovins. L'administration de corticostéroïdes (déxaméthasone), à des doses capables de supprimer l'immunité cellulaire, aggrava considérablement l'infection systèmique par ce virus, chez les veaux dépourvus d'immunité passive à l'endroit de cette maladie. Les veaux dont on avait bloqué le système de défense n'élaborèrent pas d'anticorps neutralisants à l'endroit de l'agent étiologique de la diarrhée à virus des bovins et développèrent une virémie mortelle. Ceux qui possédaient une bonne immunité passive s'avérèrent protégés contre les effets néfastes de la déxaméthasone. Les résultats de ces expériences laissent supposer que, contrairement à l'immunité cellulaire, l'immunité passive jouerait un rôle essentiel dans la protection des veaux contre une première infection systémique par l'agent étiologique de la diarrhée à virus des bovins.

INTRODUCTION

The recovery of an animal from a primary viral infection has been extensively studied, but the relative importance of the several immune factors in this process has not been established. Recently, increased emphasis has been placed on the importance of cellular immunity and the relative lack of importance of humoral antibody in the recovery process (1,15). This impression is partially based on the observation that patients thought to have primarily defects in cellular immunity are much more susceptible to certain viral infections (3,15). In addition, it has recently been shown that administration of antithymocyte serum, antilymphocyte serum, or cyclophosphamide will significantly increase the mortality of mice infected with a number of viruses (4,5,10,11,16).

A series of investigations was designed to study the role of cellular immunity and passive antibody in protection from primary systemic bovine viral diarrhea (BVD)

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virus infection in neonatal calves. BVD virus was chosen because it has been shown to have profound suppressing effects on cellular immunity *in vivo* and *in vitro* (6, 7,8). It was planned to eventually use a number of immunosuppressing agents in the hope that deletions of the different components of the immune system would result in patterns of enhanced infection in the proportion to be importance of the immune factors. In the first of the experiments, dexamethasone was used as the immunosuppressive agent and its effect on primary BVD virus infection in calves with and without passive neutralizing antibodies was studied.

MATERIALS AND METHODS

ANIMALS

One to three week old colostrum-fed Holstein-Friesian calves were obtained from local dairy farms and the dairy herds at the University of Minnesota. Individual calf sera were assayed for neutralizing antibodies against BVD virus by standard were acclimated for three to five days prior virus neutralization methods. All animals to the initiation of the study.

BVD VIRUS STRAIN

BVD virus (strain BVDMJ) was isolated from a natural epizootic of BVD (14). The virus was characterized by neutralization and fluorescent antibody methods.[1] BVD virus was grown in embryonic bovine endocardial cells maintained in Eagle's Minimum Essential Medium supplemented with 10% bovine fetal serum, 0.5% lactalbumin hydrolysate, penicillin and streptomycin. The virus pool titered 10^6 $TCID_{50}$ per ml, and 30 ml was inoculated intratracheally into each selected calf.

RECOVERY OF VIRUS

Individual calf tissue samples or nasal swabs were homogenized, filtered through a 0.45 millipore filter and inoculated into a cell culture. BVD virus isolation was confirmed by examination of cell cultures for typical cytopathology and also by fluorescent antibody techniques. In addition, attempts were made to recover infectious bovine rhinotracheitis (IBR) and parainfluenza-3 (PI-3) virus. Tissue impressions were also prepared and examined by fluorescent antibody methods to detect the presence of BVD virus in tissues.

DEXAMETHASONE

Animals that received dexamethasone[2] were injected intramuscularly with 0.5 mg/kg/day for ten days.

ASSAY OF CELLULAR IMMUNITY

Cell-mediated immunity was assayed by the *in vitro* stimulation of peripheral blood lymphocytes with phytohemagglutinin. Blood samples were taken prior to the study and at 48-hour intervals following administration of dexamethasone and this protocol was continued until the animal died or results returned to normal. Methodologies have previously been described in detail (7,8,9).

EXPERIMENTAL DESIGN

Calves were divided into four groups as follows: 1) calves without passive neutralizing antibody to BVD virus, which received only BVD virus, 2) calves without passive neutralizing antibody to BVD virus, which received dexamethasone and were inoculated with BVD virus on the fifth day of dexamethasone treatment, 3) calves with passive neutralizing antibody toward BVD, which received dexamethasone and were inoculated with BVD virus on the fifth day of dexamethasone treatment and 4) calves with passive neutralizing antibody to BVD virus, which received only dexamethasone.

All animals were followed in this study until either 1) there was complete recovery or 2) the disease terminated in a fatal viremia. All animals which died were examined for gross and histological lesions.

[1]Fluorescent anti-BVD reagents and control cell lines were obtained from the Veterinary Services Laboratories, National Animal Disease Center, Ames, Iowa.

[2]Union Veterinary Products, Los Angeles, California.

RESULTS

Effect of dexamethasone on BVD virus infection

The results presented (Table I) demonstrate that dexamethasone treatment potentiates a fatal viremia in BVD virus-infected calves which are lacking neutralizing antibodies. Three calves which received just BVD virus developed no clinical disease and developed neutralizing antibodies (data not shown). Dexamethasone treatment alone produced a fatal *E.* coli infection in one calf and no effect on seven other calves.

Three calves with high levels of passive neutralizing antibody to BVD virus, which were infected with BVD virus received dexamethasone, developed no clinical diease. One calf with low levels of passive BVD virus-neutralizing antibodies developed a fatal disease (Table I). Dexamethasone-treated calves formed no neutralizing antibodies to BVD virus, while control calves studied developed normal levels of neutralizing antibody.

Virus isolation

All animals without passive antibody to BVD virus, which received dexamethasone and BVD virus, developed a fatal viremia and BVD virus was isolated from several tissues (spleen, thymus, lymph nodes, Peyers' patches, lung and bone marrow). BVD virus could not be isolated from nasal exudate from animals with high levels of passive antibody. In one calf with low levels of passive antibody (which died), virus could not be isolated but was detected in the lungs by direct fluorescent antibody methods. IBR and PI-3 virus were not isolated.

Effect of dexamethasone on cell-mediated immunity

Administration of dexamethasone drastically suppressed *in vitro* lymphocyte responses to phytohemagglutinin as early as 48-72 hours posttreatment. Lymphocyte responses remained suppressed for several days, at which time they returned to normal. This shows that in the dose used dexamethasone can profoundly affect lymphocyte function and parameters of cell-mediated immunity. Complete results on the effect of dexamethasone on *in vitro* lymphocyte responses have been published elsewhere (9).

TABLE I. Effects of Dexamethasone on Primary BVD Virus Infections in Calves With and Without Passive Antibody

Animal Number	Treatment		Level of Passive Antibody	Isolation of Virus From Tissues[a]	Death
	Dexamethasone	BVD Virus			
Group I					
333	−	+	Undetectable	ND	−
342	−	+	Undetectable	ND	−
343	−	+	Undetectable	ND	−
Group II					
326	+	+	Undetectable	+	+
327	+	+	Undetectable	+	+
328	+	+	Undetectable	+	+
Group III					
334	+	+	1:128	ND	−
335	+	+	1:2	−[c]	+
337	+	+	1:128	ND	−
339	+	+	1:64	ND	−
341	+	+	1:128	ND	−
Group IV					
329	+	−	1:64	ND[d]	−
330	+	−	1:32	ND	+[b]
331	+	−	1:8	ND	−
332	+	−	1:32	ND	−

[a]Virus isolation attempted from spleen, thymus, lymph nodes, lung and bone marrow
[b]Calf 330 died of *E. coli* septicemia
[c]Virus was not isolated but was detected in lungs by direct fluorescent antibody techniques
[d]ND = Not determined

CLINICAL AND PATHOLOGICAL EXAMINATION

Calves receiving BVD and dexamethasone developed varying degrees of diarrhea and moderate elevation of body temperature to 103-104°F. This persisted two to five days, at which time body temperature dropped to normal or subnormal and the animals died. Animals receiving BVD alone developed only a transient febrile response by day 3, which lasted one to three days, at which time the temperature returned to normal. No other clinical signs were observed. Calves receiving dexamethasone only had no increased body temperature and developed no abnormal clinical signs with the exception of the single animal, which died of *E. coli* bacturemia.

Gross pathological examination of animals dying from BVD and dexamethasone treatment revealed erosions and hemorrhages throughout the digestive tract (tongue, rumen, abomasum, Peyers' patches, ileocoecal valve, and colon). No erosions were seen in the esophagus. The dependent lobes of the lungs were congested in two animals, and one calf had petechial hemorrhages of the urinary bladder, adrenal and brain. The one animal which died following dexamethasone treatment showed severe acute pneumonia and enteritis. No animals with BVD alone died.

Histopathological examination of tissue from animals in the BVD and dexamethasone group are as described below. Erosions in the digestive tract showed a definite inflammatory reaction, which included hyperemia, edema and infiltration of leukocytes. In the intestines, there was depletion of lymphoid follicles in the Peyers' patches, hemorrhage and dilitation of the mucous glands of crypts characteristic of BVD virus infection. Lymph nodes showed depletion of lymphocytes, especially in the cortex, and there was an absence of germinal centers. In the medullary areas, there was evidence of an inflammatory reaction with granulocytes and macrophages.

The thymus showed a generalized decrease of thymocytes in both central and peripheral areas of lobules. Cells were smaller and did not stain as intense as do cells of a normal thymus. Spleen exhibited an irregular depletion of lymphocytes in the red and white pulp. Granulocytes were present in follicles and lymphocytes appeared smaller in size. Pathological examination of the group receiving BVD or dexamethasone alone was not determined.

DISCUSSION

The present results indicate that administration of dexamethasone in amounts sufficient to suppress cellular immunity (9) markedly potentiated primary systemic BVD virus infection in calves. Dexamethasone treated calves did not form detectable neutralizing antibody to BVD virus and developed a fatal viremia. Passive BVD neutralizing antibody reversed the enhancing effect of dexamethasone on BVD virus infection.

These results suggest that neutralizing antibody prevents BVD viremia, and in this manner antibody plays a critical role in limiting the primary BVD virus infection. Earlier studies by Robson *et al* demonstrated a correlation between the level of neutralizing antibody and immunity to BVD virus (12). The severe fatal viremia in immunosuppressed calves apparently resulted in more extensive dissemination of BVD virus to target organs, particularly lymphoid tissue. Our results do not exclude the possibility that cellular immunity plays a role in recovery from primary BVD virus infection, but because of a lack of an adequate direct test for cellular immunity to BVD virus in calves, it is more difficult to reach a conclusion concerning the importance of cellular immunity in the recovery process. Indirect evidence indicates that dexamethasone did suppress cellular immunity under the present experimental conditions (9). It is apparent that the degree of suppression of cellular immunity to BVD virus was significant but not enough to explain the potentiation of the infection, since the effect of dexamethasone was entirely reversed by high levels of passive neutralizing antibody.

In recent years it has been demonstrated the immune response is critical in recovery from a number of experimental primary viral infections (2,4,5,10,13). There are several mechanisms by which the immune response might contribute to recovery during primary virus infection. Neutralizing antibody could control viremia, as appeared to be the case in our experiments and thus prevent further virus dissemination. Cellu-

lar immunity could play a role in primary virus infection by helping control viremia by unknown mechanisms or by destruction of virus infected cells. These possibilities are not mutually exclusive.

It is quite clear that different immunological processes are required to stop different types of infections based on the mechanism of virus spread. Recently, Notkins (11) proposed three different mechanisms of virus spread: Type 1 viruses, illustrated by vaccinia virus are immunologically controlled by neutralization of extracellular virus by specific antibody. Type 2 viruses, illustrated by most of the herpes viruses, can spread in the presence of high levels of antibody and may require both cellular and humoral components of the immune system acting in conjunction to halt virus spread. In some cases, a nonspecific inflammatory response is beneficial. Type 3 infections, illustrated by viruses which pass during the course of cell division from parent to progeny cells, or where the virus genome becomes integrated into the host cell, require complex cellular and humoral interaction to halt virus infection. These studies suggest that BVD virus may represent a type 1 virus.

It is clear that much more knowledge is needed to gain a better understanding of the mode of spread of viruses. This knowledge will help in determining which immune system components are essential to achieve immunity. In conclusion, the relative contribution of antibody, cellular immunity and nonspecific inflammatory responses is currently debated; it is becoming increasingly clear to these authors that all components of the immune system may be relevant and the relative importance varies on different virus-host interactions.

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