INFLUENCE OF COLOSTRAL ANTIBODIES TO
THE INFECTION WITH AVIRULENT
TESCHEN DISEASE VIRUS (Z STRAIN)*

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Colostrum-deprived pigs are known to be hypo-γ-globulinemic. They have been shown to have no detectable antibodies against porcine enterovirus9) or give no measurable antibody response7), while colostrum-receiving pigs have been reported to respond to the virus infection7). In contrast to the results described above, colostrum-deprived pigs born from an immunized sow were demonstrated to have a very small amount of antibodies and to respond strongly to the antigenic stimulus18). Some workers18~20) mentioned that a small amount of antibodies or thymic extract administered with antigens provided a sufficient stimulus for antibody production. It was also remarked that an appropriate antigenic stimulus would be provided not by an antigen alone, but by an antigen-antibody complex20,25).

It was suggested that local infection and multiplication of porcine enteroviruses might take place in the intestinal tract23), and that colostral antibodies might retard the excretion of the viruses into feces25). It required a very large dose of a general porcine enterovirus or avirulent Teschen disease virus for colostrum-receiving pigs to manifest clinical signs8,13). On the contrary, colostrum-deprived pigs presented clinical signs and histological lesions when administered with a small dose of such virus8,10).

In the present study, antibodies transferred transplacentally or through colostrum were investigated for influence on the multiplication of avirulent Teschen disease virus.

MATERIALS AND METHODS

Virus

The Z strain, which belongs to subgroup 2 of Teschen disease virus described by Mayr in 196114), was isolated from the brain of an apparently healthy pig in Moravia, Czechoslovakia, and supplied for the present study by V. Mádra of the Bioveta Laboratory, Czechoslovakia. It had been passaged 438 times in porcine kidney cell cultures. It had a titer of 10^8.3 tissue culture infective doses (TCID)_{50}/0.1 ml.

Antiserum

Antiserum was prepared from rabbits inoculated with the virus-infected cell culture fluid in the same manner as mentioned by Watanabe (1971).

Cell culture

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Primary monolayer cultures were prepared from the kidneys of young pigs.

Virus titration

Serial tenfold dilutions of test samples were made in Earle’s buffered salt solution (BSS) without serum. Each dilution was inoculated into 3 tubes of cell culture. The tubes were examined for cytopathic effect (CPE) over a period from the 4th to 7th day of infection. From the number of tubes of such dilution as showing a typical CPE, TCID\textsubscript{50} was calculated by the method of Reed and Muench\textsuperscript{16}. The titers were expressed in TCID\textsubscript{50}/0.1 ml.

Animals

Colostrum-deprived group: Eight pigs were obtained by hysterectomy and reared in isolation chests. Of them, seven were inoculated with the virus at 2 days of age, and one was set for control. All the pigs were housed in isolation chests in such manner as to avoid contamination with any microorganism. The chests were kept under the conditions of strict isolation and at a proper temperature during the experimental period.

Colostrum-receiving group: Seven newborn pigs were nursed by their mother, receiving sow’s colostrum under the conventional conditions. They were inoculated with the virus at 5 days of age.

Infection procedure

All the pigs were anesthetized with ether. A ginslet was pushed into the brain through the left frontal bone immediately. Then it was withdrawn, and a fine hypodermic needle was inserted through the resulting hole to a depth of 1.5 cm. Half ml of the virus-infected cell culture fluid was injected into the brain with a syringe. Pigs were observed daily for clinical signs.

Tissue collection

At the time of delivery of baby, sow’s milk was collected, as well as blood from the umbilical cord of the baby (colostrum-receiving group).

Each one pig of the colostrum-deprived group was killed for examination 1, 3, 4, 6, 8, and 11 days after infection (d.a.i.). One control pig was killed on the 11th day. One pig of colostrum-receiving group was killed every other day from 1 to 9 d.a.i. and 12 and 14 d.a.i.. Autopsy was carried out immediately on all the killed pigs. At slaughter, blood was collected from the axillary artery, defibrinated, and centrifuged. The resulting supernatant was stored at $-20^\circ\text{C}$.

At necropsy, rectal feces were collected for virus isolation. An approximately 10% suspension of feces was prepared in Earle’s BSS containing a final concentration of 1,000 U/ml of penicillin, 1,000 µg/ml of streptomycin, and 500 U/ml of mycostatin. It was frozen and thawed once, centrifuged at 4,000 rpm for 30 minutes, and stored at $-20^\circ\text{C}$.

Samples were collected aseptically from the motor cortex of the cerebrum, the thoracic part of the spinal cord, and the mesenteric lymph node. Then an approximately 10% suspension was prepared from each sample by grinding for the titration of the viruses. The following organs were removed: the motor cortex of the cerebrum, the olfactory bulb, the cerebellum, the thalamus, the cervical and the lumbar parts of the spinal cord, two parts of the lung, the mesenteric lymph node, two parts of the duodenum, the colon, and the rectum. They were divided into portions which were examined immediately for indirect fluorescent antibody (FA).

Virus isolation

Serum and organ suspensions were subjected directly to virus titration. Meanwhile, the fecal suspension which had once undergone blind passage in cell culture was used for virus detection.
Neutralization tests
Virus neutralizing antibodies were titrated in porcine kidney cell cultures. The antibody titer was expressed as the reciprocal of the serum dilution which had prevented the viral CPE from appearing in 50% of the cultures. Fluorescent antibody technique
Tissue blocks were fixed in acetone at 4°C overnight and embedded in paraffin by the method of Saint-Marie. They were cut into sections, which were stained for indirect FA examinations in the same manner as mentioned in the previous experiment. Observation was made under the fluorescent microscope, type ML-2, made in the USSR, equipped with a dark field condenser.

RESULTS

Clinical signs
No obvious clinical signs were observed in the pigs of both groups, except one which excreted yellowish soft feces 3 d.a.i.

Recovery of virus
Colostrum-deprived group: The virus was demonstrated in the cerebrum, spinal cord, mesenteric lymph node, serum, and rectal feces 1 d.a.i.. The highest virus titer, $10^{2.5}\text{TCID}_{50}/0.1\text{ml}$ of 10% organ suspension, was recorded in the cerebrum, spinal cord, and mesenteric lymph node some time between 1 and 4 d.a.i.. There was a decrease in virus titer thereafter. The virus was constantly recovered from the cerebrum and feces during the experimental period, while it was not isolated from the spinal cord, mesenteric lymph node, or serum on the last day of experiment. Viremia was recognized 1 and 3 d.a.i. (Fig. 1).

No virus was recovered from the samples of an uninfected control pig examined 11 d.a.i..

Colostrum-receiving group: No virus was recovered from any sample 1 d.a.i.. The virus injected might have been diffused and highly diluted in organ tissues. Two days later, the virus which must have multiplied in some organs was recovered from the brain and spinal cord of some pigs. The virus titer in the cerebrum was higher than that in the spinal cord. The virus was always recovered from the cerebrum over a period from 3 to 14 d.a.i.. The highest titers of the cerebrum, $10^{2.5-4.8}\text{TCID}_{50}/0.1\text{ml}$, were reached 9 and 14 d.a.i.. The highest titer of the spinal cord was $10^{1.5}\text{TCID}_{50}/0.1\text{ml}$, appearing 5 d.a.i.. There were large differences among the virus titers in each organ of every pig. No virus was recovered from the mesenteric lymph node, serum, or rectal feces of any animal of this group during the experimental period (Fig. 2).

All the viruses isolated from the pigs of both groups were identified as the Z strain by neutralization tests.

Development of antibody
Colostrum-deprived group: When serum sample was collected from sow just after hysterectomy, it showed a neutralizing antibody titer of 1:128 against the Z strain. No antibodies were detected from any newborn young till 3 d.a.i.. Thereafter, the titer rose to 1:30 6 d.a.i. and exceeded 1:500 11 d.a.i. (Fig. 1).

Colostrum-receiving group: Serum and milk were collected from sow just after the parturition and proved to have antibodies at a titer of 1:32 and 1:192, respectively. No antibodies were detected from the blood of the umbilical cord of any newborn young. The serum titer already rose to 1:961 d.a.i. when the pigs were 6 days old. It fluctuated from 1:64 to 1:192 during an experimental period of 14 days. No obvious fluctuation
was recognized in antibody titer (Fig. 2).

Gross changes in organs

No distinct gross changes were noticed in the pigs of either group, except a mild congestion of the intestine, a very small amount of gas in the stomach, and a slightly edematous aspect of the brain.

Occurrence of virus-specific fluorescence

No specific fluorescence was found in the lung, mesenteric lymph node, or intestine of the pigs of either group during the experimental period. Specific fluorescence was observed mainly in capillary endothelial cells and the ground substance of the central nervous system (CNS) in the pigs of both groups, though no specific fluorescence or vacuolation was recognized in the neurons of the CNS at all.

Colostrum-deprived group: The most abundant specific fluorescence occurred in capillary endothelial cells of the CNS, except the olfactory bulb, 1 d.a.i. It was accompanied by some specific fluorescent granules in the surrounding ground substance. Specific fluorescent capillaries were consistently fewer in this group than in the colostrum-receiving group. The ubiquity in distribution of capillaries showing specific fluorescence was not so prominent as in the colostrum-receiving group. The specific fluorescent capillaries decreased rapidly in number in the CNS, especially in the cerebellum, on the
Influence of Colostral Antibodies to Infection

Table 1. Specific Fluorescence in Colostrum-Deprived Pigs Infected Intracerebrally with Z Strain

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<th>Organ</th>
<th>Days after infection</th>
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<td>Central nervous system(^1)</td>
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<tr>
<td>Olfactory bulb</td>
<td>—</td>
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<td>Cerebral cortex</td>
<td>2+</td>
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<tr>
<td>Cerebellum</td>
<td>2+</td>
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<tr>
<td>Thalamus</td>
<td>2+</td>
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<tr>
<td>Spinal cord(^2)</td>
<td>2+</td>
</tr>
<tr>
<td>Lung(^2)</td>
<td>—</td>
</tr>
<tr>
<td>Mesenteric lymph node</td>
<td>—</td>
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<tr>
<td>Small intestine(^3)</td>
<td>—</td>
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<tr>
<td>Large intestine(^3)</td>
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\(^1\) Negative. 1+ to 3+: Faint to strong fluorescence respectively.
\(^2\) The greater part of specific fluorescence was observed in capillaries and the smaller part in the ground substance.
\(^3\) Two parts were observed.

Table 2. Specific Fluorescence in Colostrum-Receiving Pigs Infected Intracerebrally with Z Strain

<table>
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For legends see Table 1.

following days. During a period beginning with 6 d.a.i., a rather faint specific fluorescence characterized by granulation was often found in capillary endothelial cells in the CNS, especially in the cerebrum and thalamus (Table 1). The number of specific fluorescent capillaries and the intensity of specific fluorescence tended again to increase in some parts of the CNS on the last day of experiment. A faint nonspecific fluorescence was seen in some lymphoid cells in the mesenteric lymph nodes 3 d.a.i.

No specific fluorescence was observed in any sample collected from the control pig.

Colostrum-receiving group: Capillaries showing the most abundant specific fluorescence were observed in the brain 3 and 5 d.a.i. and decreased rapidly in number on the following days. The existence of specific fluorescent capillaries in the brain was apparently characterized by a remarkable mal-distribution. Specific fluorescent capillaries existed not in the marginal part of the parenchyma of the brain, but in a very few relatively deep parts of the parenchyma. The specific fluorescence in capillaries
was characterized by granulation from 9 d.a.i. (Table 2). Only faint specific fluorescence was found in some parts of the CNS on the last day of experiment.

DISCUSSION

Virus-specific fluorescence was detected from some neurons of the CNS of conventional 4-week-old pigs infected with virulent Teschen disease virus (KNM strain)\textsuperscript{22}. Whereas no specific fluorescence was found in any neuron of colostrum-deprived or colostrum-receiving pigs infected with the Z strain in the present experiment. The finding common to both experiments with the KNM and Z strains was the occurrence of specific fluorescent capillaries in the brain. Capillary endothelial cells may be the most universal target tissue for each strain of Teschen disease virus. The virulence of the virus may be determined more or less by the ability of the virus to multiply in neurons.

It should be noted that no specific or nonspecific fluorescence was found in the mesenteric lymph node or intestine of either group of pigs infected with the Z strain, though the virus was isolated constantly from the organs of the pigs of the colostrum-deprived group. In 4-week-old pigs infected with the KNM strain, abundant nonspecific and/or specific fluorescence was shown in macrophages, and lymphoid and reticular cells in the mesenteric lymph node and intestine; the virus was also isolated. It is necessary to carry out further studies to explain the difference in fluorescent appearance and virus isolation between both experiments. It may be possible for the authors to give the following supposition: Phagocytic cells in baby pigs might phagocytize a very few virions, and those in 4-week-old pigs many. A number of virions phagocytized in these pigs were digested in the cells, and some of them might multiply. The authors could find digested and/or multiplying cells with viral antigens by means of abundant fluorescence.

An abundant specific fluorescence appeared in the CNS rather earlier than the time when a high titer of Z strain virus was recovered from the CNS. Soluble antigens might exist in capillary endothelial cells before release of complete virions, as Koestner et al.\textsuperscript{11,12} observed by an electron microscope.

Two sows had been infected naturally with the virus of the same antigenicity as Z strain virus. The antibody titer of sow’s colostrum was higher than that of sow’s blood, as described by Izawa et al.\textsuperscript{8}) A pig of the colostrum-receiving group already had antibodies 1 d.a.i. No fluctuation of antibodies was recognized in the group. This result indicates that the pigs obtained antibodies from the colostrum.

Beran et al.\textsuperscript{2}) described that colostral antibodies retarded excretion of porcine enteroviruses. In the present experiment, no virus was isolated from blood, mesenteric lymph node, or rectal feces, or no specific fluorescence was found in the mesenteric lymph node or intestine of any pig of the colostrum-receiving group. The colostral antibodies transported from the intestine into the circulating blood might repress the virus not only in the intestinal tract but also in blood and mesenteric lymph node. Virus multiplication was further delayed in the CNS of the colostrum-receiving group. Some colostral antibodies which passed through the blood-brain barrier might have more or less influence on the virus multiplication in the CNS.

The antibody response in colostrum-deprived or colostrum-receiving pigs was reported by many workers\textsuperscript{4,5,7,15,18,19}. It was suggested from experimental results that antibodies might be produced not by the stimulus of antigen alone or of antigens mixed with too large a quantity of antibodies, but by antigens mixed with colostral factors or
with a small amount of antibodies or with glycopeptide moieties\textsuperscript{5,15,18\textendash}20,24. On the other hand, Myers and Segre\textsuperscript{15} proved a transplacental transfer of antibody globulins with the concentrated globulin fraction in colostrum-deprived pigs. In the present experiment, colostrum-deprived pigs responded to the initial antigenic stimulus. Therefore, it is presumed that these pigs may have accepted maternal antibodies of undetectable level transplacentally, and that these antibodies may have helped the pigs to produce enough antibodies against the virus injected.

**SUMMARY**

Two sows showed a relatively high antibody titer against avirulent Teschen disease virus (the Z strain). The babies farrowed by them were divided into two groups, a colostrum-deprived and a colostrum-receiving, and inoculated with the virus intracerebrally. No obvious clinical signs were observed in either group of pigs.

In the colostrum-deprived group, an abundant virus-specific fluorescence was found in the capillary endothelial cells of the central nervous system (CNS) in the first half of the experimental period. The virus of relatively high titer was detected from the CNS, mesenteric lymph node, serum, and rectal feces. The serum titer began to rise rapidly in this group 6 days after inoculation. In the colostrum-receiving group of pigs, virus multiplication was retarded in the CNS. A high titer of humoral antibody appeared in this group 1 day after inoculation and was maintained later. The viruses were entirely repressed in the mesenteric lymph node, serum, and rectal feces during the experimental period. Specific fluorescent capillaries were characterized distinctly by ubiquity in distribution in a few parts of the parenchyma of the CNS.

**ACKNOWLEDGMENTS**

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弱毒チッセング病ウイルス (Z 株) 感染に対する初乳移行抗体の影響

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母豚2頭は、既に弱毒チッセング病ウイルス (Z 株) に対する比較的高力価の血清抗体を有していた。各母豚から得た colostrum-deprived および colostrum-receiving 初生豚の群に、該ウイルスを脳内接種したが、両群とも臨床症状を示さなかった。

colostrum-deprived 群において、実験初期に、豊富な特異蛻光を中枢神経系毛管内皮細胞内に認めた。その後比較的高力価のウイルスを、中枢神経系、腸間膜リンパ節、血清、直腸内容から分離し得た。また、血清抗体価は、6日目から急激に上昇した。

一方、colostrum-receiving 群においては、中枢神経系でのウイルス増殖の遅れるのが認められた。また、移行抗体と考えられる高力価血清抗体は、1日目から持続し、腸間膜リンパ節、血清、直腸内容中のウイルスは、完全に抑制された。特に目だったのは、特異蛻光陽性細胞が、中枢神経実質のごく一部にのみ集会していたことであった。